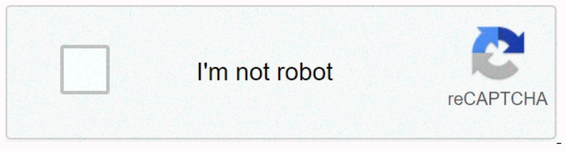

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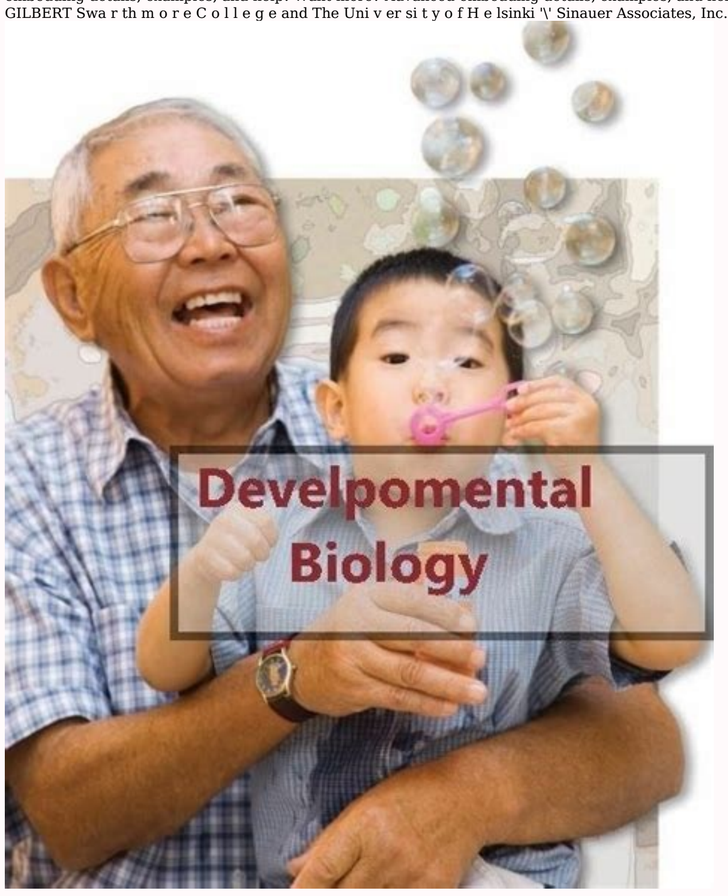

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. The Cover Three-day-old embryos of Costa Rican red-eyed tree frogs (Agalychnis callid,.yas). TI,e female frog lays her eggs on a leaf overhangmg a pond, so that when the tadpoles hatch (normally in about 7 days), they wiggle out and fall into the pond. The tadpoles' development is exquiSitely tuned to their environ ment, and the larvae place their branching gills near the oxygen-rich egg ,.,r ' ⬚' surface. Tadpoles respond rapidly to the presence of infectious fungi or predaceous snakes, hatching early (often at 5 days) and taking their chances in the pond rather than succumbing to predation (see Chapter 18). Photograph courtesy of Karen Warkentin, Boston University. '., Developmental Biology, 9th Edition Copyright © 2010 by Sinauer Associates, Inc. All rights reserved.
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QL955.G48 2010 571.8-dc22 Printed in USA. 5 4 3 2 2010009918 To Daniel, Sarah, and David Brief Contents PART I QUESTIONS Introducing Developmental Biology 1 CHAPTER 1 Developmental Anatomy CHAPTER 2 CHAPTER 3 5 Developmental Genetics 31 Cell-Cell Communication in Development 69 PART II SPECIFICATION Introducing Cell Commitment and Early Embryonic Development 109 CHAPTER 4 Fertilization 121 CHAPTER 5 Early Development in Selected Invertebrates CHAPTER 6 159 The Genetics of Axis Specification in Drosophila 203 CHAPTER 7 Amphibians and Fish CHAPTER 8 Birds and Mammals 241 287 PART III THE STEM CELL CONCEPT Introducing Organogenesis 323 CHAPTER 9 The Emergence of the Ectoderm 333 CHAPTER 10 Neural Crest Cells and Axonal Specificity CHAPTER 11 Paraxial and Intermediate Mesoderm 413 CHAPTER 13 Development of the Tetrapod Limb 485 CHAPTER 15 Postembryonic Development 541 373 CHAPTER 12 Lateral Plate Mesoderm and the Endoderm CHAPTER 14 Sex Determination CHAPTER 16 445 511 The Saga of the Germ Line 583 PART IV SYSTEMS BIOLOGY Expanding Developmental Biology to Medicine, Ecology, and Evolution 617 CHAPTER 17 Medical Aspects of Developmental Biology CHAPTER 18 Developmental Plasticity and Symbiosis 625 659 CHAPTER 19 Developmental Mechanisms of Evolutionary Change 683 Contents PART I QUESTIONS Introducing Developmental Biology 1 CHAPTER 1 CHAPTER 2 Developmental Anatomy 5 Developmental Genetics 31 The Cycle of Life 6 Evidence for Genomic Equivalence 32 A Frog's Life 6 • SIDELIGHTS & SPECULATIONS Developmental Genetics Gametogenesis and fertilization 6 Organogenesis 10 Metamorphosis and gametogenesis 11 "How Are You?" 1 2 Anatomy of the gene: Active and repressed chromatin 36 Anatomy of the gene: Exons and introns 37 Anatomy of the gene: Promoters and enhancers 39 Comparative embryology 12 Transcription factor function 42 Epigenesis and preformation 12 Naming the parts: The prim a ry germ layers and early organs 14 The four principles of Karl Ernst von Baer 16 Keeping Track of Moving Cells: Fate Maps and Cell Lineages 17 Fate maps 19 19 Dye marking 19 Genetic labeling 20 Transgenic DNA chimeras 22 Evolutionary Embryology 23 Embryonic homologies 24 Medical Embryology and Teratology 27 Genetic mallormations and syndromes 27 Disruptions and teratogens 28 The Basic Tools of Differential Gene Transcription 35 Cleavage and gastrulation 7 Direct observation of living embryos 32 • SIDELIGHTS & SPECULATIONS. 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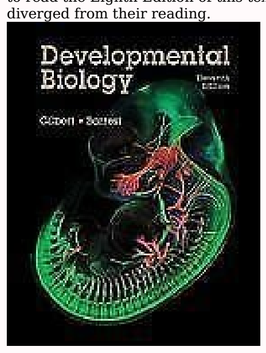
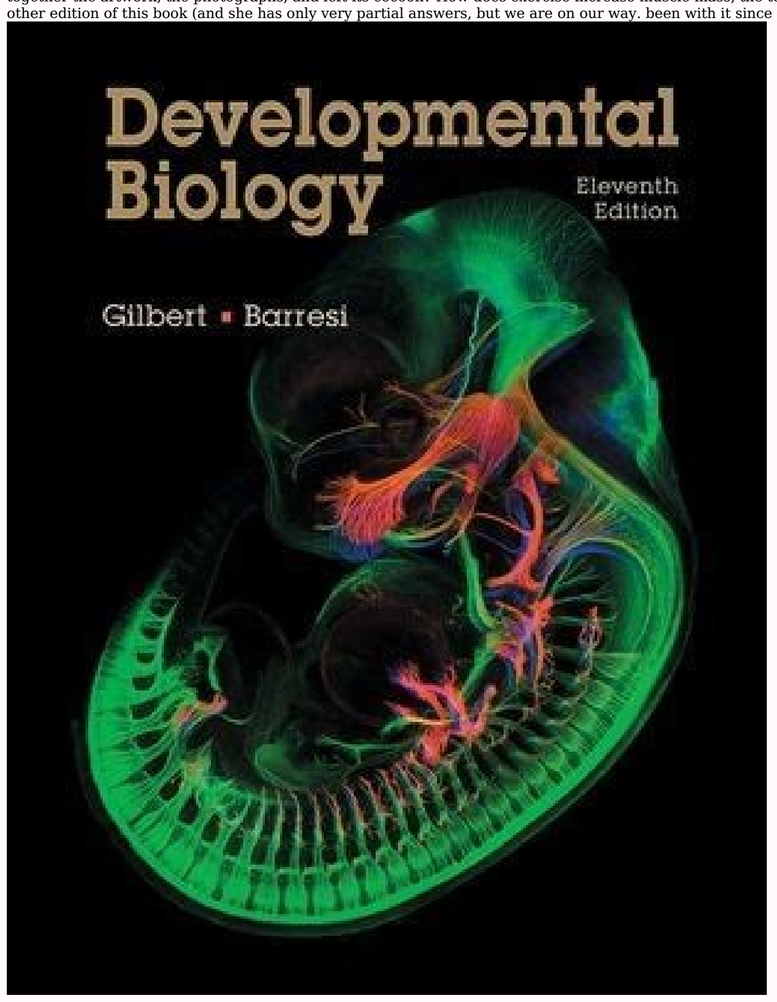
Germination Shoot development Shoot development Leaf development The Vegetative-to-Reproductive Transition Juvenility Floral signals Inflorescence development Floral meristem identity Senescence -- ---- - • • . • Preface It has become increasingly embarrassing for me to ask stu in the entire field of developmental biology. We are seeing dents to read the Eighth Edition of this textbook. It's so, an inversion of relationships within the biological sciences. well, 2006. Developmental biology has progressed so rap Genetics is more and more becoming a subset of develop idly in the past four years that my lectures have fundamen ment. Similarly, the dynamic of evolution is being studied tally diverged from their reading.



My "big" lecture on tran as a question of gene expression as well as gene frequen scription now focuses on the ability of transcription factors cies. And developmental biology may be on the threshold to reprogram cell fates; and my lectures on stem cells and of changing medicine as mud, as microbiology did at the clomng have scrapped the notion of therapeutic cloning turn of the twentieth century. altogether, focusing instead on induced pluripotent stem I began U,e Preface of the last edition with a quotation cells. In both instances, w e discuss what this means for from the Grateful Dead, recalling "What a long, strange understanding normal development, as well as what impli trip it's been." The epigra• for this ecUtion might be cations these technologies have for the future of medicine. Eminem's "Be careful what you wish for. " We may achieve Neither induced pluripotential stem cells nor "transdiffer biological powers that are " tenfold" what we had hoped entiation" was established when the last edition of this to have. And i t is axiomatic for this generation that "with book was published. great power comes great responsibility." Even my most basic lectures have changed. The lecture I hope this Ninth Edition of Developmental Biology pres on fertilization has to cover the new da ta on mammalian ents a better way of teaching and learning (and question egg activation. My lectures on sea urchin development ing) developmental biology. The introductory section has an area of study that has been fundamental to develop been streamlined from six chapters to three-one each on mental biology for over a century-now include systems developmental anatomy, the mechartisms of gene regula theory operations involving double-negative gates and tion during differentiation, and cell--cell communication feedforward loops, and my evo-devo talks have led to dis during morphogenesis. Another new feature is the addi cussions of mathematical modeling and parasitism. I can't tion of short part-opening "chaplets" that address key talk about limb development without including the vari concerns in developmental biology. These provide an ations seen in dachshlUlds and bats, and I can't discuss sex introduction to the subsequent chapters, placing the forth determination without using the •-catenin model for mam coming information into a specific context. Each chapter malian ovary production. None of these areas were cov ends with a guide to web-based resources relevant to that ered in earlier editions of my book. So this is really a very chapter's content, and the Ninth Edition is the first to new ecUtion. My editor tells me it has close to 700 new ref include an extensive glossary of key terms. erences; she only wishes I had deleted a t least that many old ones. During the writing of this edition, I re-read some of the papers written by the first generation of experimental Developmental biology is in a state of rapid metamor embryoJogists, scientists who were experiencing a Gestalt phosis. And, as in insect and amphibian metamorphosis, change as important as what we are experiencing today. some old tissues remain the same, some get substantially What impressed me was not necessarily their answers remodeled, and some old tissues perish altogether; and (although some of them were remarkably good even by all the while, new tissues are forming new structures. I today's standards); rather, it was their asking the "right" hope that I have gotten these correct, and that the added questions. Some of their research did not give us any new material will stand the test of time. I have tried to answers at all. But the results told the next generation of remodel the retained material into new narratives that are biologists what questions to ask. These embryologists more inclusive of the data, and to appropriately jettison stood in awe of the complexity of the embryo; yet they the inlormation that was needed for earlier stages of the began to remove, transplant destroy, and recombine cells book's development but which is no longer needed by in order to find out just how the fertilized egg could give undergraduates. rise to a structured body composed of different cell types. Embryologist' John Fallon once wrote me that new data change the story one tells. It is, he said, like putting togeth They had faith that these were scientific questions and that science would eventually be able to answer them. er a picture puzzle. At first, you think the structure in front The glory of developmental biology is that we now have of you is a sailboat; but you add another piece, and-no, interesting answers to many of their questions. But muner wait-it's a mountain. Psychologists call these alterations "Gestalt changes," and I think that we are seeing these changes in both our day-to-day interpretations of data and ellS questions that were asked a century ago still lack anSW('l'S. How does the human brain become organized so that we can think, plan, reca l l , interpret, hate, and love? xviii How PREFACE is the development of plants and insects timed so that Small and Janice Holabird of Sinauer's production depart the flower opens at the same time when its pollinator has ment have put together the artwork, the photographs, and left its cocoon? How does exercise increase muscle mass, the text into a format that is both informative and pleas and how does our face corne to resemble those of our par ing. For a book of this size, this is a heroic undertaking. ents more than any one else's? To these questions, we have And, more than any other edition of this book (and she has only very partial answers, but we are on our way. been with it since its inception), this incarnation has been a Developmental biology presents a nascent scientist with a host of fascinating questions that are worth solving.



And that's the invitation this book offers. One can enter devel collaborative effort with my editor Carol Wigg. The book I espeCially wish to thank Dr. Hannah Galantino-Homer seems to have become a full-time job for both of us. opmental biology through many portals-genetics, cell of the University of Pennsylvania School of Veterinary biology, embryology, physiology, anatomy-and with Medicine, who took it upon her shoulders to compile a many valid motivations.

This is a field that needs the help glossary for this book. Numerous people have said that of people with all sorts of competencies and talents. It is they wanted such a glossary for their students, and put an old field that is itself undergOing metamorphic change ting one together is an extremel y dillicult task, involving and emerging as a new field that welcomes newcomers an enormous amount of thought and expertise. with open arms-full of questions. This textbook officially entered the "electronic age" in the mid 90s, and its web segment, www.devbio.com. has grown more important with each subsequent edition. With Acknowledgments this edition, the Tn addition to the remarkable reviewers listed below, whose candid and thorough criticisms of early chapter drafts made this book so much better, there are some peo ple whose help was absolutely critical. In particular, David McClay and Bill Anderson gave me many suggestions that were outstandingly important in constructing this edition. I also appreciate enormously the cooperation from all those vade mecum) companion that debuted in not only created vade mecum3, with its laboratory sections 2002 is also on the web.

Mary Tyler and Ron Kozlowski and its introductions to model animals, they have also pro duced interviews and filmed the techniques of several developmental biologists. You really have to see these films to realize what a valuable resource these are. I am blessed by teaching some remarkable students who scientists who sent me their photographs, and who even have not been shy about offering constructive criticism. told me about others they had seen. The graphiCS of this Their suggestions will, book are truly amazing, and this is due to the community students. of developmental biologists. The book's beauty and success is also the result of Andy I hope, benefit the next round of And finally, this revising process has taken much longer than expected. I apologize to my wife, Anne Raunio, who Sinauer's vision and the hard work of the immensely tal has had to put up with me through it all, and to my friends, ented staff he has assembled at Sinauer A.sociates. David who may have wondered where I've been. I'll be back. Mclntyre's ability to find appropriate photographs from the public and private databases is almost uncanny. Chris SCOTT F. GILBERT Reviewers OF THE N I NTH E D I T I O N It is no longer possible (if it ever was) for one person to comprehend this entire field. As Bob Seger so aptly sings, "['ve got so much more to think about . . . what to leave in, what to leave out." The people who help me leave in and take out the right things are the reviewers. Their expertise in particular areas has become increasingly valuable to me. Their comments were made on early versions of each chapter, and they should not be held accountable for any errors that may appear. Arkhat Abzhanov, Harvard University Kelly McLaughlin, Tufts University Radwan Abu-Issa, University of Michigan, Dearborn William Anderson, Harvard University Sarah Millar, University of Pennsylvania Blanche Capel, Duke University Mary Mullins, University of Pennsylvania Bradley Davidson, University of Ariwna Barry Shur, Emory University Carol Erickson, University of California, Davis Ana Soto, Tufts University Susan Ernst, Tufts University Claudio Stern, University College London Mindy George-Weinstein, Lankenau Institute for David Stocum, Indiana University-Purdue University, Medical Research Kathleen Molyneaux, Case Western Reserve University Indionapolis Stuart A. Kauffman, Santa Fe Institute Kathleen Sulik, University of North Carolina Daniel Kessler, University of Pennsylvania Nicole Theodosiou Napier, Union College Laura Lee, Vanderbilt University Lien Van Speybroeck, Ghent University Karen Liu, King's College London Steven Vokes, University of Texas Thomas Maynard, University ofNorth Carolina Gary Wessel, Brown Universihj David McClay, Duke University

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For more for laboratory exercises. It also includes excerpts from the Differen tial Expressions series of videos, ltighlighting some major concepts in developmental biology, famous experi ments, and the scientists who performed them. Developmental Biology: A Guide for Experimental Study, Third Edition MARY S.

TYLER (Included i n DevBio Laboratory: vade mecu"") Tltis lab manual teaches the student to work as an inde pendent investigator on problems in development and pro vides extensive background information and instructions for each experiment. It emphasizes the study of living material, intermixing developmental anatomy ill an enjoy able balance, and allows the student to make choices in their work. information, please visit www.cou rsesmart.com. For the I nstructor For the Student (Available to qualified adopters) Companion Website Instructor's Resource Library www.devbio.com The Developmelltal Biology, Available free of charge, this website is intended to supple ment and enrich courses in developmental biology. It pro vides more information for advanced students as well as historical, philosophical, and ethical pcrspectives on issues links, updates, and more. Refer in developmental biology. Included are articles, movies, interviews, opinions, Web ences to specific website topics are included throughout each chapter as well as at the end of each chapter. DevBio Laboratory: vade mecum 3: An Interactive Guide to Developmental Biology MARY S. TYLER and RONALD N. KOZLOWSKI ew for Version 31 DevBio Laboratonj: vade meCll",.3 is now online. Access to the program is included with every new inth Edition Instructor 's Resource Library includes a rich collection of visual resources for use i n preparing lectures and other course materials. Thc lRL includes: • All textbook figures (including photos) and tables in jPEG (high and low resolution) and PowerPoint'" for mats • A collection of videos illustrating key developmental processes • Chick embryo cross-sections and chick embryo whole mounts from DevBio Laboratory: vade mecum3 (Power Point" format) • Video segments from DevBio Labomto/y: vade mecum' • Instructor's Reference Guide for Differelltial Expressions' MEDIA AND S U PPLEMENTS Also Available The following titles are available for purchase separately or, in some cases, bundled with the textbook.

Please contact Sinauer Associates for more information. Integrating Epigenetics, Medicine, and Evolution A Dozen Eggs: Scott F. Gilbert and David Epel Paper, 460 pages · ISBN 978-0-87893-299-3 Rachel Fink DVD • ISBN 978-0-87893-329-7 Ecological Developmental Biology: Time-Lapse Microscopy of Normal Development Bioethics and the New Embryology: Fly Cycle' Springboards for Debate Mary S. Tyler and Ronald N. Kozlowski DVD • ISBN 978-0-87893-849-0 Scott F. Gilbert, Anna Tyler, and Emily Zackin Paper, 261 pages · ISBN 978-0-7167-7345-0 From Egg to Tadpole: Differential Expressions': Early Morphogenesis in Xenopus Key Experiments in Developmental Biology Jeremy D. Pickett-Heaps and Julianne Pickett-Heaps DVD • ISBN 978-0-97752-224-8 Mary S. Tyler, Ronald N. Kozlowski, and Scott F. Gilbert 2-DVD Set · UPC 855038001020 xxi ;J • PART :J • :J • QU ESTIONS Introducing Deve lopmental Bio logy apter 1 Between ferti lization and birth, the developing organism is known as an Developmental anatomy embryo. The concept of a n embryo is a staggering one, and forming an embryo Developmental genetics yourself from a single cell. You had to respire before you had lungs, digest napter 2 'lapter 3 Cel l-c e l l com m u n ication i n development is the hardest thing you w i l l ever do. To become an embryo, you had to build before you had a gut, build bones when you were pulpy, and form orderly arrays of neurons before you knew how to think. One of the critical differences between you and a machine is that a machine is never required to function until after it is built. Every animal has to function even as it builds itself. For animals, fungi, and plants, the sole way of getting from egg to adult is by developing an embryo. The embryo mediates between genotype and pheno type, between the in her ited genes and the adult organism. Whereas most fields of biology study adult structure and function, developmental biology finds the study of the transient stages leading up to the adult to be more i n teresting. Developmental biology studies the i n itiation and construction of organisms rather than their maintenance. It is a science of becoming, a science of process. This development, this formation of an orderly body from relatively homo geneous material, provokes profound and fundamental questions tha t Homo sapiens have been asking since the dawn of self-awareness: How does the body form with its head always above its shoulders? Why is the heart on the left side of our body? Why do we have five fingers on each hand and not more or fewer? Why can't we regenerate limbs? How do the sexes develop their differ ent anatomies? Why can only females have babies? 2 PART I Our answers to these questions must respect the complexity of the inquiry and must form a coherent causal network from gene through functional organ . To say that xx mammals are usua l l y females and that XV mammals are usually males does not explain sex determination to a developmental biologist, who wants to know how the XX genotype produces a female and how the XV genotype pro duces a male. Simi larly, a geneticist might ask how globin genes are transmitted from one generation to the next, and a physiologist might ask about the function of globin proteins i n the body. But the developmental biologist asks how it is that the globin genes come to be expressed only in red blood cells, and how these genes become active only at specific times in development.

(We don't know the answers yet.) Each field of biology is defined by the questions it asks. Welcome to a wonderful set of importa nt questions! The Questions of Developmental Biology Development accomplishes two major objectives. First, it generates cellular diver sity and order within the individual orga n ism; secondly, it ensures the continuity of l ife from one generation to the next. Put another way, there 'are two fundamental questions in developmental biology. How does the ferti lized egg give rise to the adult body? And how does that adult body produce yet another body? These two huge questions can be subdivided i n to seven general categories of questions scrutinized by developmental biologists: o • T he question of differentiation. A single cell, the fertilized egg, gives rise to hundreds of different cell types-muscle cells, epidermal cells, neurons, lens cells, lymphocytes, blood cells, fat cells, and so on. The generation of this cellular diversity is called differentiation. Since every cell of the body (with very few exceptions) contains the same set of genes, how can this identical set of genetic instructions produce different types of cells? How can a single cell, the fertilized egg, generate so many different cell types?" The question of morphogenesis. How can the cells in our body organize themselves i nto functional structures?

Our differentiated cells are not ran domly distributed. Rather, they become organized into intricate tissues and organs. During development, cells divide, migrate, and die; tissues fold and separate. Our fingers are always at the tips of our hands, never in the mid dle; our eyes are always i n our heads, not in our toes or gut. This creation of ordered form is called morphogenesis, and it involves coord inating cell growth, cell m igration, and cell death. o The question of growth. If each cell in our face were to undergo just one more cell division, we 'would be considered horribly malformed. If each cell i n our arms underwent just one more round of cell division, we could tie our shoelaces without bending over. How do our cells know when to stop dividing?

Our arms are generally the same size on both sides of the • body. How is cell division SO tightly regulated? The question of reproduction. The sperm and egg are very special ized cells, and only they can transmit the instructions for making an organism *There are more than 2 1 0 different cell types recognized in the adult human, but this number has l i ttle or no significance. There are many tra n sien t cell types that are formed during development but are not seen i n the adull. Some of these embryonic cells are transitional stages or precu rso rs of ad u l t cell types. Other embryonic cell types perform pafticular functions in constructing an organ and then undergo pro grammed cell death after completing their tasks. QUESTIONS from one generation to the next.

How are these germ cells set apart from the cells that are constructing the physical structures of the embryo, and what are the i nstructions i n the nucleus and cytoplasm that allow them to form the next generation? • The question of regeneration. Some organisms can regenerate their entire body. Some salamanders regenerate their eyes and legs, and many reptiles can regenerate their tails. Mammals are generally poor at regeneration, and yet there are some cells i n our bodies-stem cells-that are able to form new structures even in adults. How do the stem cells retain this capacity, and can we harness it to cure debil itating diseases? • The question of evolution.

Evolution involves i nherited changes in devel opment. When we say that today's one-toed horse had a five-toed ancestor, we are saying that changes in the development of cartilage and muscles occurred over many generations in the embryos of the horse's ancestors. How do changes in development create new body forms? Which heritable changes are possible, given the constraints imposed by the necessity that the organism survive as it develops? • The question of environmental integration. The development of many (perhaps all) organisms is influenced by cues from the environment that sur rounds the embryo or larvae. The sex of many species of turtles, for i nstance, depends on the temperature the embryo experiences while in the egg. The formation of the reproductive system in some insects depends on bacteria that are transmitted inside the egg. Moreover, certa in chemicals i n the environment can disrupt normal development, causing malformations in the adult. How is the development of an organism i ntegrated into the larger context of its habitat? The study of development has become essential for understanding all other areas of biology.

Indeed, the questions asked by developmental biologists have also become critical in molecular biology, physiology, cell biology, genetics, anatomy, cancer research, neurobiology, immunology, ecology, and evolutionary biology. In turn, the many advances of molecular biology, along with new techniques of cell imaging, have finally made these questions answerable. This makes developmental biologists extremely happy; for, as the Nobel Prize-winning developmental biologist Hans Spe mann stated in 1 927, We stand i n the presence of riddles, but not without the hope of solving them. And riddles with the hope of solution-what more can a scientist desire? So, l i ke the man i n the ca rtoon, I come bearing questions. They are questions bequeathed to us by earlier generations of biologists, philosophers, and parents. They are questions with their own history, questions discussed on an anatomical level by people such as Aristotle, William Harvey, St. Albertus Magnus, and Charles Darwin. More recently, these questions have been addressed on the cellular and molecular levels by men and women throughout the world, each of whom bri ngs to the labo ratory his or her own perspectives and training.

For there is no one way to become a developmental b iologist, and the field has benefitted by having researchers trained i n cell biology, genetics, biochemistry, immunology, and even anthropology, engineer ing, physics, h istory, and art. You are now i nvited to become part of a community of question-askers for whom the embryo is a source of both wonder and the most inter esting questions in the world. The next three chapters outline some of the critical framework needed to answer these questions. Chapler 1 discusses organisma' concepts, including life cycles, the 3 4 PART I three germ layers that form the organs, and the migration of cells during develop men t Chap ter 2 concentrates on the genetic approach to cell differentiation and out l i nes the pri n c ipl e of differential gene expression (which explains how different pro teins can be made in different cells from the same set of inherited genes). Cha pter 3 focuses on the cellular approach to morphogenesis, showing how communication between cells is critical for their formation into tissues and organs. Thus, you will be intro duced to development at the orga nisma l, genetic, and cellular, and much of the textbook thereafter will show how these levels are integrated to produce the remark .

able panoply of animal development. - - • • Developmental Anatomy ACCORDING TO ARISTOTLE, the first embryologist known to history, science begins with wonder: "It is owing to wonder that people began to philosophize, and wonder remains the beginning of knowledge" (Aristotle, Metaphysics, ca. 350 BeE). The development of an animal from an egg has been a source of won der throughout history. The simp le proced ure of cracking open a chick egg on each successive day of its 3-week incubation period provides a remarkable expe rience as a thin band of cells is seen to give rise to an entire bird. Aristotle per formed this procedure and noted the formation of the major organs.

Anyone can wonder at this remarkable-ye t commonplace-phenomenon, but it is the sci entist seeks to discover how development actually occurs. And rather than dis sipating wonder, new Wlderstand.ing increases it. Multicellubr organisms do not spring forth fully formed. Rather, they arise by a relatively slow process of progressive change that we call development. In nearly all cases, the development of a multicellular organism begins with a sin gle cell-the fertilized egg, or zygote, which divides mitotically to produce all the cells of the body. The study of animal development has traditionally been called embryology, after that phase of an organism that exists between fertiliza tion and birth. But development does not stop at birth, or even at adulthood. Most organisms never stop developing.

Each day we replace more than a gram of skin cells (the older cells being slo ughed off as we move), and our bone mar row sustains the development of millions of new red blood cens every minute of our lives. In addition, some ar:-imais can regenerate severed parts, and many species lffidergo metamorphosis (such as the transformation of a tadpole into a frog, or a caterpillar into a butterfly). Therefore, in recent years it has become customary to speak of developmental biology as the discipline tha t studies embryonic and other developmental processes. As the introduction to Part T notes, a scientific field is defined by the ques tions it seeks to answer. Most of the questions in developmental biology have been provided to it by its embryological heritage. We can identify three major approaches to studying embryology: • Anatomical approaches • Experimental approaches • Genetic approaches Each of these traditions has predominated during a different era. However, although it is true that anatomical approaches gave rise to experimental approach es, and that genetic approaches built on the foundations of the earlier two approaches, all three traditions persist to this day and continue to play a major role in developmental biology. The basis of aU research in developmental biolo gy is the changing anatomy of the organism. Today the anatomical approach to It is a most beautiful thing to s tudy the different changes of life, from the microscopic changes of conception to the more apparent ones of maturity and old age. FRANKLIN MALL (CA. 1 890) The greatest progressive minds of embryology have not looked for hypo th eses; they have looked at em bryos. JANE OPPENHEIMER (1 955) 6 CHAPTER 1 development is continually expanded and enhanced by rev olutions in microscopy, computer-aided graphical recon structions of three-dimensional objects, and methods of applying mathematics to biology. Many of the beautiful photographs in this book reflect this increaSingly impor S. In many species, the organism that hatches from the egg or is born into the world is not sexually mature. Rather, the organism needs to undergo metamorphosis to become a sexually mature adult. In most animals, the young organism is a called a larva, and it may look sig tant component of embryology. nificantly different from the adult. In many species, the The Cycle of life brief stage whose sole purpose is to reproduce. In silk-

One of the major triumphs of descriptive embryology was the idea of a generalizable animal life cycle. Each animal, whether earthworm or eagle, termite or beagle, passes through similar stages of development. The stages of devel opment between fertilization and hatching are collectively called embryogenesis. Throughout the animal kingdom, an incredible variety of embryOnic types exist, but most patterns of embryogen esis are variations on six fundamental processes: fertiliza tion, cleavage, gastrulation, organogenesis, metamorpho larval stage is the one that lasts the longest, and is used for feeding or dispersal. [n such species, the adult is a worm moths, for instance, the ad ults do not have mouthparts and cannot feed; the larvae must eat enough so that the adult has the stored energy to Sur vive and mate. Indeed, most female moths mate as soon as they eclose from their pupa, and they fly only once to lay their eggs. Then they die. 6. In many species, a group of cells is set aside to produce the next generation (rather than forming the current embryo). These cells are the precursors of the gametes. The gametes and their precursor cells are collecti vely sis, and gametogenesis. called germ cells, and they are set aside for reproduc 1.. Fertilization involves the fusion of the mature sex cells, somatic cells. This separation of somatic cells (w.hlch the sperm and egg, which are collectively called the gametes. The fusion of the gamete cells stimulates the egg to begin development and initiates a new individ ual. The subsequent fusion of the gamete nuclei (both of which have only half the normal number of chromo somes characteristic for the species) gives the embryo its genome, the collection of genes that helps instruct the embryo to develop in a manner very similar to that of it parents. 2. Cleavage is a series of extremely rapid mitotic divisions that immediately follow fertilization. During cleavage, the enormous volume of zygote cytoplasm is divided into numerous smaller cells called blastomeres. By the end of cleavage, the blastomeres have usually formed a sphere, known as a blastula. 3. After the rate of mitotic division slows down, the blas tomeres undergo dramatic movements and change their positions relative to one another.

This series of exten sive cell rearrangements is called gastrulation, and the embryo is said to be in the gastrula stage. As a result of gastrulation, the embryo contains three germ layers that will interact to generate the organs of the body. tive function. All the other cells of the body are called give rise to the individual body) and germ cells (which contribute to the fonnation of a new generation) is often one of the first differentiations to occur during animal development. The germ cells eventually migrate to the gonads, where they differentiate into gametes. The development of gametes, called gametogenesis, is usu ally not completed until the organism has become phys ically mature. At maturity, the gametes may be released and participate in fertilization to begin a new embryo. The adult organism eventually undergoes senescence and dies, its nutrients often supporting the early embryogenesis of its offspring and its absence allowing less competition. Thus, the cycle of life is renewed. A Frog's life All animal life cycles are modifications of the generalized one described above. Figure 1 . 1 shows the development of the leopard frog, Rana pipiens, and provides a good start ing point for a more detailed discussjon of a representa tive life cycle. 4. Once the germ layers are established, the cells interact with one another and rearrange themselves to produce tissues and organs. This process is called organogene sis. Many organs contain cells from more than one germ layer, and it is not unusual for the outside of an organ to be derived from one layer and the inside from anoth er. For example, the outer layer of skin (epidermis) comes from the ectoderm, whereas the inner layer (the dermis) comes from the mesoderm. Also during organo genesis, certain cells undergo long migrations from their place of origin to their finaI location. These migrating cells include the precursors of blood cells, lymph cells, pigment cells, and sex cells. --- -- Gametogenesis and fertilization The end of one life cycle and the beginning of the next are often intricately intertwined. Life cycles are often controlled by environmental factors (tadpoles wouldn't survive if they hatched in the fall, when their food is dying), so in events. A combination 01 photoperiod (hours of daylight) most frogs, gametogenesis and fertilization are seasonal and temperature informs the pituitary gland of the mature female frog that it is spring. The pituitary then secretes hor mones that stimulate her ovary to make the hormone estro gen. Estrogen then instructs the liver to make and secrete yolk proteins, which are then transported through the - • DEVELOPMENTAL ANATOMY Oocyte ./ Sperm Ge!m plasm (maleg•• FERTILI0 •••:• gamete) ZATION GAMETOGENESIS (- a Location of germ ce s CLEAVAGE Sexually a tur adult "" Gonad GASTRULATION MATURITY Metamorphosi (in some species)s LARVAL STAGES • Immature •stages larval Morul ll - Im e 7 ORGANOGENESIS t t)ng (bir Ha ch i •• FIGURE 1.1 Developmental history of the leopard frog, Rana pipiens. The stages from fertilization through hatch i ng (birth) are k n own col lecti ve ly as embryogenesis. The region set aside for pro• m cells is shown in dueing ge r purple. Gametogenesis, which is completed in Ihe sexu al ly mature adult, begins at different times during development, depending on the species. (The sizes of the varicolored wedges shown h ere are arbitrary and do not corre spond to the proportion of the l ife cycle spent in eac h stage.) h •--.. - Fertilization accomplishes several things. First, it allows the haplOid nucleus of the egg (the female pronucleus) to merge with the haploid nucleus of the sperm (the male pronucl e us) to form the diploid zygote nucleus. Second, fertilization causes the cytoplasm of the egg to move such that different parts of the cytoplasm find themselves in new locations (Figure 1 .20). This cytoplasmic migration blood into the enlarging eggs in the ovary. The yolk is will be important in determining the three embryonic axes vegetal he misp h ere , where it will serve as food for the transported into the bottom portion of the egg, called the of the frog: anterior-posterior (head-tail), dorsal-ventral 1 .2A). The upper half of the egg those molecules necessary to begin cell cleavage and gas developing embryo (Figure is called the animal hemisphere.· Sperm formation also (back-belly), and right-left. Third, fertilization activates trulation (Rugh 1950). occurs on a seasonal basis. Male leopard frogs make sperm during the summer, and by the time they begin hiberna tion in the fall they have produced all the sperm that will Cleavage and gastrulation be available for the following spring's breeding season. During cleavage, the volume of the frog egg stays the same, In most species of frogs, fertilization is external. The male frog grabs the female's back and fertilizes the eggs as but it is divided into tens of thousands of cells (Figure 1 .2E-H) .

The cells in the animal hemisphere of the egg 1 .28). Some species lay divide faster than those in the vegetal hemisphere, and the 1.2Q. Other species er the more vegetal the cytoplasm. Meanwhile, a fluid-filled float their eggs into the center of the pond without any cavi ty, the blastocoel, forms in the animal hemisphere (Fig the female releases them (Figure their eggs in pond vegetation, and the egg jelly adheres to the plants and anchors the eggs (Figure cells of the vegetal hemisphere become progressively larg 1 .21).

This cavity will support. So the first important thing to remember about ure life cycles is that they are often intimately involved with movements to occur during gastrulation. environmental factors. be important for allowing cell Gastrulation in the frog begins at a point on the embryo surface roughly 180 degrees opposite the point of sperm "The use of the terms anima! and vegetal for the upper and lower hemispheres of the early frog embryo reflect the division rates of . of the lower half (hence "animated"), while the yolk-fiUed cell.. the cells. The upper cells divide rapidly and become actively mobile were seen as being immobile (hence like plants, or "vegetal"). entry with the formation of a dimple, called the blastopore. This dimple (which will mark the future dorsal side of the embryo) expands to become a ring, and cells migrating through the blastopore become the mesoderm (Fig u re 1.3A--C). The cells remaining on the outside become the ecto- 8 CHAPTER 1 (A) FIGURE 1.2 Early development of the frog Xenopus /aev/s. (A) As the egg matures, i t accumu lates yolk (here stained yellow and green) in the vegetal cytoplasm. (B) Frogs mate by amplexus, the male grasping the female around the belly and fertilizing the eggs as they are released. (C) A newly laid clutch of eggs. The brown area of each egg is the pigmented animal hemisphere. The white spot in the middle of the pigment is where the egg's nucleus resides. (O) Cytoplasm rearra ngement seen during first cleav age. Compare with the initial stage seen in (A). (E) A 2-cell embryo near the end of its first cleavage. (F) An 8-cell embryo.

(G) Early blastula.

Note that the cells get smal ler, but the volume of the egg remains the same. (H) Late blastula. (I) Cross section of a late blastula, showing the blastocoel (cavity). (A-H courtesy of Michael Danilchik and Kimberly Ray; I cou rtesy of J . Heasman.) (F) (G) (H) (I) Blastocoel DEVELOPMENTAL A N ATOMY FIGURE 1.3 Continued development of Xenopus laevis (A) Gastrulation begins with an invagination, or slit, in the future dorsal (top) side of the embryo. (B) This slit, the dorsal blastopore lip, as seen from the ventral surface (bonoml of the embryo. (0 The slit becomes a circle, the blastopore. F u ture mesoderm cells m igrate into the interior of the embryo along the blastopore edges, and the ectoderm (future epidermis and nerves) migrates down the outside of the embryo. The remaining part, the yolk-filled endoderm, is eventually encircled. (0) Neural folds begin to form on the dorsal surface. (E) A groove can be seen where the bottom of the neural tube will be. (F) The neural folds come together at the dorsal midline, creating a neural tube. (G) Cross section of the Xenopus embryo at the neurula stage. (H) A pre-hatching tadpole, as the protrusions of the forebrain begin to induce eyes to form. (I) A mature tadpole, having swum away from the egg mass and feeding independently. (Courtesy of Michael Oanilchik and Kimberly Ray.) . Dorsal blastopore lip (B) (E) (e) Yolk plug (AI (D) Blastopore Neural folds (G) (F) Notochord Open neural tube Neural groove (ectoderm) (I) S e Brain m ites Gill Tailbud 9 Dorsal (back) Ventral (belly) /1',"u;rru tube 10 CHAPTER 1 (B) (A) (C) (D) (E) FIGURE 1.4 Metamorphosis of the frog. (A) Huge changes are obvious when one con trasts the tadpo le and the adu lt bullfrog. Note espec ial l y the differences in jaw structure and limbs. (8) Premetamorphic tadpole. (C) Prometamorphic tadpole, showi ng hindlimb growth. (0) Onset of metamorphic cl imax as forelimbs emerge. (E,F) Climax stages . (A to Patrice CeiselNisuals Unlimited.) derm, and this outer layer expands to enclose the entire (F) embryo. The large, yolky cells that remain in the vegetal Organogenesis hemisphere Organogenesis begins when the n otochord-a rod of (W1til they are encircled by the expanding ecto derm) become the endoderm. Thus, at the end of gastrula mesodermal cells in the most dorsal portion tion, the ectoderm (precursor of the epidermis, brain, and embryo*-signals the ectodermal cells above it that they of the nerves) is on the outside of the embryo, the endodenm (pre cursor of the gut and respiratory systems) is On the inside of the embryo, and the mesoderm (precursor of the connec tive tissue, blood, heart, skeleton, gonads, and kidneys) is behveen them. the Introduction-Le., cells that are important for constructing the *The notochord consists of cells such as those mentioned on p.

2 of embryo but which, having performed their tasks, die. Although critical for establishing the fates of the ectodermal cells above it, as adult vertebrates do not have notochords. this embryonic organ is we shall see in Chapters 7-9. Meiosis I: Separation of homologous chromosomes Iuterphase DNA repl icates Earl y prophase] Mid prophase I Late prophase I Metaphase I The nuclear envelope breaks down and homologous chromosomes (each chromosome being douhle, with the chromatids joined at the kinetochore) align in pairs.

Chromosomal rearrangements can occur between the four homologous chromatids at this time --- - . -...-,"" DEVELOPMENTAL ANATOMY are not going to become epidermis.

Instead, these dorsal ectoderm cells form a tube and become the nervous sys tem. At this stage, the embryo is called a neurula. The neu ral precursor cells elongate stretch, and fold into the embryo, forming the neural tube (Figure 1 .3D-F); the future epidermal cells of the back cover the neural tube. Once the ne ural tube has formed, it and the

notochord induce changes in their neighbors, and organogenesis con tinues. The mesodermal tissue adjacent to the neural tube and notochord becomes segmented into somi tes (Figure l.3G,H), the precursors of the frog's back muscles, spinal vertebrae, and dermis (the iImer por tion of the s kin) The embryo develops a mouth and an anus, and it elongates iIlto the familiar tadpole structure (Figure 1 .31). The neu rons make their connections to the muscles and to other neurons, the gills form, and the larva is ready to hatch from its egg jelly. The hatched tadpole will feed for itself as soon as the yolk supplied by its mother is exhausted. , . See VADE MECUM The amphibian life cycle Metamorphosis and gametogenesis rod of of the lat they ' p. 2 of ing the)Ugh rgan js '"e it, as Metamorphosis of the fully aquatic tadpole larva into an adult frog that can live on land is one of the most striking transformations in all of biology. In amphibians, metamor phosis is initiated by hormones from the tadpole's tllyroid gland. (The mechanisms by which thyroid hormones accomplish these changes will be discussed in Chapter IS.) In frogs, almost every organ is subject to modification, and the resulting changes in form are striking and very obvi ous (Figu re 1 .4). The hindlimbs and forelimbs the adult will use for locomotion dillerentiate as the tadpole's paddle tail recedes. The cartilaginous tadpo le skull is replaced by the predominantly bony skull of the young frog. The horny teeth the ta dpole uses to tear up pond plants disappear as the mouth and jaw take a new shape, and the fly-catching tongue muscle of the frog develops. Meanwhile, the tad pole's lengthy intestine-a characteristic of herbivores shortens to suit the more camivorous diet of the adult frog. The gills regress and the lungs enlarge. The speed of meta morphosiS is carefully keyed to environmental pressures. In temperate regions, for instance, Rana metamorphosis must occur before ponds freeze in winter. An adult leop ard frog can. burrow into the mud and survive the winter; its tadpole cannot. As metamorphosis ends, the development of the germ cells begins. Gametogenesis can take a long time. In Rana pipiens, it takes 3 years for the eggs to mature in the female's ovaries. (Sperm take less time; Rona males are often fertile soon after metamorphosis.) To become mature, the germ cells must be competent to complete meiosis. Meiosis (Figure 1 .5) is one of the most iInportant evolu tionary processes characteristic of eukaryotic organisms. It makes fertilization pOSSible and is critical in recombin ing genes from the two parents. Genetics, development, and evolution throughout the aninlal kingdom are p redi cated on meiosis. We will discuss meiosis more thorough ly in Chapter 16, but the most iInportant tllings to remem ber about meiosis are: 1. The cllrDmosornes replicate prior to cell division, so that each gene is represented four tinles.
2. The replicated chromosomes (each called a chromatid) are held together by their kinetochores (centromeres), and the four homologous chroma tids pair together. FIGURE 1.5 Summary of meiosis. The DNA replicates during i nterp h ase. During first m ei oti c p rop hase the nuclear envelope breaks down and the homologous chromosomes (eac h chromo some is double, with its two chromatids joi n ed at th e kin etochore) al ign together Chromosome rearrangements (l cross i n g over") can occur atthis stage. After the first m etaphase, the kinetochore remains unsplit and the p airs of homologous chromosomes are sorted into different ce lls . During the second meiotic division, the kintochore splits and the sister chromatids are moved into sepa rate cells, each with a haploid set of chromosomes.
, . i Meiosis II: Separation of the chromatids ase I Anaphase 1 Telophase I The two original homo logous chromosomes are segregated into different ceUs 11 Metaphase II Anaphase II Telophase 1I The kinetochore splits Each new cell has one copy of each chromosome CHAPTER 1 12 3. Ex ovo omnia ("All from The first meiotic division separates the chromatid pairs mammals-originate from eggs. from one another. the egg") was the motto on the frontispiece of Harvey's On 4. The second meiotic division splits the kinetochore such that each chromatid becomes a chromosome. 5. The result is four germ cells, each with a haploid nucleus. the Generation of Living Creatures, and this precluded the spontaneous generation of animals from mud or excre ment. This statement was not made lightly, for Harvey knew that it went against the views of Aristotle, whom Having undergone meiosis, the mature sperm and egg Harvey still venerated.
(Aristotle had thought that men nuclei can unite in fertilization, restoring the diploid chro stru al fluid formed the material of the embryo, while the mosome number and ini tia ting the events that lead to development and the continuation of the circle of life. semen gave it form and animation.) Harvey also was the first to see the blastoderm of the chick embryo (the small region of the egg containing the yolk-free cytoplasm that gives rise to the embryo), and he was the first to notice that "How Are You? " "islands" of blood tissue form before the heart does. Har The fertilized egg has no heart. It has no eye. No limb is found in the zygote. So how clid we become what we are? What part of the embryo forms the heart? How do the celis that form the eye's retina migrate the proper clistance from the cells that form the lens? How do the tissues that form a b ird' s wing relate to the tissues that form fish fins or the human hand? What organs are affected by mutations in p art ic ular genes? These are the types of questions asked by developmental anatomists. Several strands weave together to form the anatomical approaches to development . The first strand is compara tive embryology, the study of how anatomy cllanges dur ing the development of different organisms. The second strand, based on the first, is study of how changes in evolutionary embryology, the develop ment may cause evolu- tionary change and of how an organism's ancestry may constrain the types of ellanges that are possible. The third strand of the anatomical approaell to developmental biol ogy is teratology, the study of birth defects. Comparative embryology vey also suggested that the anlniotic fluid might function as a "shock absorber" for the embryo. As might be expected, embryology remained little but speculation until the invention of the microscope allowed detailed observations. In 1672, Marcello Malpighi pub lished the first microscopic account of chick development. Here, for the first time, the neural groove (precursor of the neural tube), the muscle-forming somites, and the first cir culation of Ille arteries and veins-to and from the yolk were identified (Figure 1.6). Epigenesis and preformation Wi!b Malpighi begins one of the great debates in embry ology : the controversy over whether the organs of the embryo are formed de novo ("from scratell ") at each gen eration, or whether the organs are already present, in miniature form, within the egg (or sperm). The first view, called epigenesis, was supported by Aristotle and Harvey. The second view, calJed preformation, was reinvigora ted with Malpighi's support. Malpighi showed that the unin cuba ted' chick egg already had a great deal of st ructure, The fir st known study of comparative developmental and this observa tion provided him with reasons to ques anatomy was undertaken by Aristotle in the fourth centu BCE. In The Generation of Animals (ca. 350 BCE), he noted tion epigenesis. According to the preformationist view, all ry the organs of the adult were prefigured in miniature with some of the variations on the life cycle themes: some ani mals are born from eggs (ovipari ty, as. in birds, frogs, and most invertebrates); SOme by live birth (vi vi p arity, as in not seen to be "constructed" but rather "wuoUed . " placental mammals); and some by producing an egg that teenth-century science, religion, and philosophy (Gould hatelles inside the body (ovoviviparity, as in certain rep tiles and s ha rks) . Aristotle also identified the two major in the sperm or (more usually) the egg. Organisms were The preformationist hypothesiS had the backing of eigh 1977; Roe 1981; Pinto-Correia 1997). First, if all organs were prefigured, embryonic development merely required the cell division patterns by which embryos are formed: the growth of existing structures, not the formation of new holoblastic p attern of cleavage (in which the entire egg is ones. No extra mysterious force was needed for embryon divided into smaller cells, as it is n i frogs and mammals) and the meroblastic pattern of cleavage (as in chicks, wherein only part of the egg is destined to become the embryo, while the other portion-the yolk-serves as nutrition for the embryo). And should anyone want to know who first figured out the functions of the placenta and the umbilical cord, it was Aristotle. There was remarkably little progress in embryology for the two thousand years following Aristotle. It was only in 1651 that William Harvey concluded that all ani.maJs-... ic development. Second, just as the adult organism was prefigured in the germ cells, another generation already existed in a prefigured state within the germ cells of the first prefigured generation. This corollary, called emboit ment (encapsulation), ensured that the species would *As pointed out by Maitre·Jan in 1722, the eggs Malpig.hi examined may teclmically be called "unincubated," but as they were left sit· ting in the Bolognese sun in August, they were not unheated. Such eggs would be expected to helve developed into chicks. _ . -- DEVE LOPME NTAL ANATOMY (A) 13 (B) Ic) ! (D) Metencephalon vesicle Mesencephalon Heart FIGURE 1.6 Depi ct ion s of chick developmental anatomy. (A) Dorsal view (looking "down" at what will become the back) of a 2-day chick embryo, as depicted by Marcello Malpighi in 1 672. (B) Ventra l view (looking "up" at the prospective belly) of a chick embryo at a s i milar stage, seen through a dissecting microscope and rendered by F. R. Lillie in 1 908. (C) Eduard d' Alton's depi c tion of a later stage 2-day chick embryo in Pander (1 8 1 7). (D) Modern rendering of a 3-day chick embryo. Details of the anato my will be discussed in later c hapters . (A from Mal p ighi 1 672; B from Lillie 1 908; C from Pander 1 8 1 7, courtesy of Ernst Mayr library of the Museum of Comparative Zoology, Harvardi D after Carlson 1 9 8 1 .) remain constant. Although certain microscopists claimed to see fully formed human miniatures within the sperm or egg, the major proponents of this hypothesis-Albrecht von Haller and Charles Bonnet-knew that organ systems develop at different rates, and that structures need not be in the same pl ace in the embryo as they are in the newborn. The preformationists had no cell theory to provide a lower limit to the size of their preformed organisms (the Diencephalon Liver rudiment Forelimb bud Somite Hindl im b ud b " vir,ell;',. artery bud cell theory arose in the mid-1S00s), nor did they view mankind's tenure on Earth as potentially infinite. Rather, said Bonnet (1764), "Nature works as small as it wishes," and the human species existed in that finite time between Crea tion and Resurrection. This view was in accord with the best science of its time, conforming to the French math ematician-philosopher Rene Descartes' principle of the infi nite divisibility of a mechanical nature initiated, but not interfered with, by God. It also conformed to Enlighten ment views of the Deity. The scientist-priest Nicolas Male- 14 . CHAPTER 1 branche saw in preform ationism the fusion of the rule-giv ing God of Christianity with Cartesian science (Churchill 1991; Pinto-Correia 1997).The embryological case for epigenesis was revived at the same time by Kaspar Friedrich Wolff, a German embry ologist working in St. Petersburg.
By carefully observing the development of chick embryos, Wolff demonstrated that the embryonic parts develop from tissues that have no counterpart in the adult organism.
The heart and blood vessels (which, according to preformationism, had to be present from the beginning to ensure embryonic growth) could be seen to develop anew in each embryo. Similarly, the intestinal tube was seen to arise by the folding of an originally flat tissue. This latter observation was explicitly detailed by Wolff, who proclaimed in 1767 that "When the formation of the intestine in this manner has been duly weighed, almost no doubt can rema in, I believe, of the truth of epigenesis," To explain how an organism is creat ed anew each generation, however, WoUf had to postulate an unknown force-the vis essentinlis ("essential force") which acting according to natural laws in the same way as gravity or magnetism, would organize emb ryoniC development. A reconciliation between preformationism and epigen esis was attempted by the German philosopher Immanuel Kant (1724-1804) and his colleague, bi ologis t Johann Friedrich Blumenbach (1752-1840). Attempting to construct a scientific theory of racial descent, Blumenbach postulat ed a mechanical, goal-directed force he called Bildungstrieb (" developmental force"). Such a force, he said, was not theoretical, but could be shown to exist by experimentation. A hydra, when cut, regenerates its amputated parts by rear ranging elUsting elements (see Chapter 15). Some purpose ful organizing force could be observed in operation, and this Bildungstrieb wa s a property of the organism itself, thought to be inherited through the germ cells. Thus, development could proceed through a predetermined force inherent in the matter of the embryo (Cassirer 1950; Lenoir 1980). Moreover, this force was believed to be susceptible to change, as demonstrated by the left-hanoled variant of snail coiling (where left-coiled snails can produce right-coiled progeny).
In this hypothesis, wherein epigenetic develop ment is directed by preformed instructions, we are not far from the view held by modern biologists that most of the *Prelormation was a conservative theory, emphasizing the lack of change between generations. Its prinCipal failure was its inability to account for the variations revealed by the limited genetic evidence of the time. It was known, for instance, that matings betw"een white and black parents produced children of intermediate skin color, all. impossibility if inheritance and development were solely through either the sperm or the egg. In morc controlled experiments, the German botanist Joseph Kolreuter (1766) produced hybrid tobacco plants havmg the characteristics of both species. Moreover, by mat ing the hybrid to either the male or femaJe parent, KOlreuter was able to "revert" the hybrid back to one or the other parental type after several generations. Thus, inheritance seemed to arise from a mixture of parental components. - - instructions for forming the organism are already present in the fertilized egg.Naming the parts: The primary germ layers and early organs The end of preformationism did not come until the 1820s, when a combination of new staining techniques, improved microscopes, and institutional reforms in German univer sities created a revolution in descriptive embryology.
The new techniques enabled microscopists to document the epigenesis of anatomical structures, and the institutional reforms provided audiences for these reports and students to carry on the work of their teachers. Among the most tal ented of this new group of microscopica lly inclined inves tigators were three friends, born within a year of each other, all of whom came from the Baltic region and stud ied in northern Germany.
The work of Christian Pander, Karl Ernst von Baer, and Heinrich Rathke transformed embryology into a specialized branch of science. Pander studied the chick embryo for less than two years (before becoming a paleontologist), but in those 15 months, he discovered the germ l ayerst three distinct regions of the embryo that give rise to the differentiated cells types and specific organ systems (Figure 1.7). - • The ectoderm generates the outer layer of the embryo. It produces the surface layer (epidermis) of the skin and forms the brain and nervous system. • The endoderm becomes the innermost layer of the embryo and p rod uces the epithelium of the digestive tube and its associa ted organs (including the lungs). • The mesoderm becomes sandwiched between the ecto derm and endoderm. It generates the blood, heart, kid ney, gonads, bones, muscles, and connective tissues.
c These three layers are found in the embry os of all triplo blasti ("three-layer") animals. Some phyla, such as the poriferans (sponges) and c tenophores (comb jellies) lack a true mesoderm and are considered diploblastic animals. Pander and Rathke also made observations that weight ed the balance in favor of epigenesis. Rathke followed the intricate development of the vertebrate skull, excretory sys tems, and respiratory systems, showing that these became increasingly complex. He also showed that their complex ity took on different trajectories in different classes of ver tebrates. For instance, Rathke was the first to identify the "But, as we shall see, not all the instructionr:; there. Later m this book, we will see that temperature, diet, predators, symbionts, crowding, and other environmental agents normally regulate gene expression in the embryo and can cause particular phenotypes to occur. tFrom the same root as genn;,wlion, the Latin germe'l means "sprout" or " bud:' The names of the three germ layers "rc from the Greek: ectoderm from cktos ("outside") plus derma ("skin"); meso derm from mesos ("middle"); and endoderm from endoll ("within"). - -- -• DEVELOPMENTAL ANATOMY f I Ectoderm (outer layer) I Zygote Blastula Gastrull a I Mesoderm (miI ddle layer) I I Germ1 ceUs I !Endoderm (internal layer) I Respi r.gestIr.ve Di atmy nervous I surface system crest Dorsal Paraxial IntermedIate Lateral Head tube -••.- 15 fl§X i y® Faciale Stomach Sperm Egg Pigment NotoBone Tubulof thee blRedood muscl Thyroi d Lung cell celEpilsdofermal skin Neuron of chord ti s sue cel l cel l cel l cel l kidney cells brain (mel (alcelveoll) ar ocyte)an FIGURE 1.7 The dividing cells of the fertilized egg form three distinct embryonic germ layers. Each of the germ layers gives rise to myriad differentiated cell types (only a few representatives are shown here) and distinct organ systems. The germ cells (precur sors of the sperm and egg) are set aside early in development and do not arise from any particular germ layer. pharyngeal arches (Figure 1.8). He showed that these same embryonic structures became gill supports in fish and the jaws and ears (among other things) in mammals. Pander demonstrated that the germ layers did not form their respective organs autonomously (Pander 1817). Rather, each germ layer "is not yet independent enough to indi cate what it truly is; it still needs the help of its sister trav elers, and therefore, a lthough already designated for dif (B) (A) (C) Squamosal (0) ferent ends, all three influence each other collectively until each has reached an appropriate leveL" Pander had dis- Upper jaw Braincase Gill arches Lower jaw Nasal Sqnarnosal (temporal bone) _---.-_1 FIGURE 1.8 Evolution of pharyngeal arch structures in the vertebrate head. (A) Pharyngeal arches lalso called branch ial arches) in the embryo of the salamander Ambysloma mexicanum.
The surface ecto derm has been removed to permit visuali zation of the arches Ihighl ighted i n color) as they form. IB) In adult fish. pharyngeal arch cells form the hyomandibular jaws and gill arches. In amphibians, birds, and reptiles (a crocodile is shown here), these same cells form the quadrate bone of the upper jaw and the articular bone of the lower jaw. (D) I mammals, the qua drate has become internalized and forms the incus of the middle ear. The articular bone retains its contact with the quadrate, becoming the malleus the middle ear. IA c u rtes of P. Falck and L. Olsson; B-O aher Zanger! and Williams 1 975.) (C) o y n of 16 CHAPTER 1 (A) and left halves and which instructs the ectoderm above it to become the nervous system (Figure 1 .9). He also discov ered the mammalian egg, that long-sought cell that every one believed existed but no one before von Baer had ever seen,· In 1828, von Baer reported, "I have two small embryos preserved in alcohol, that I forgot to label. At present I am unable to determine the genus to which they belong. They may be lizards, small birds, or even mammals." F igure Unsegmented mesoderm Hensen's node V Pnmlt1ve streak (0) Somite (source of muscles. spine. Lateral plate mesoderm (source of heart, blood vessels) embryos (fish, reptiles, amphibians, birds, and mammals) begin with a basically similar structure. From his detailed stud y of chick development and his comparison of chick embryos with the embryos of other vertebrates von Bae r derived four generalizations Now often referred to as "von . Baer's laws," they are stated here with some vertebrate _ _ - Area opaca examples.
1. The ge"eral features of a large group of a"imals appear earlier in development than do the specialized features of a smaller group. All developing vertebrates appear very N u al g e r 1 .10 allows u s to appreciate his quandary. All vertebrate roove similar right after gastrulation. It is only later in devel opment that the specia l features of class, order, and Notochord Intermediate mesoderm (source of kidneys, gonads) Endoderm (gut, lungs) "von Baercould hardly believe that he had at last found what so many others-Harvey, de Graaf, von Hallel Prevost, Dumas, and even Purkinje-had searched for and failed to find. "I recoiled as if struck by lighten ing . .
. r had to lTy to relax a while before 1 could wOrk up enough courage to look again, as I was afraid I had been deluded by a phantom. 1s it not strange that a sight which is expect ed, and indeed hoped for, shouJd be frighteuing when i t eventually materializes?" FIGURE 1.9 No toc ho rd in chick devel opm ent. The notochord separates vertebrate embryos into r igh t and left halves and instructs the ectoderm above it to become the nervous system. (A) Dorsal view of the 24-hour chick embryo (B) Cross secti o n through the trunk region shows the notochord and developing ne ura l tube_ By comparing this illustration ard F igure 1 .6, you can see the remarkable changes between days 1 , 2, and 3 of chick egg incubati on . (A after Patten 1 95 1 .) . covered the tissue interactions that we now call induction_ No tissue is able to construct organs by itself; it must inter act with other tissues. (We will discuss the principles of induction more thoroughly in Chapter 3.) Thus, Pander showed that preformation could not be true, since the organs come into being through interactions between sim pler structures. The four principles of Karl Ernst von Baer Karl Ernst von Baer extended Pander's studies of the cruck embryo. He discovered the notochord, the rod of dor sa Lmost mesoderm that separates the embryo into right Human Opossum Chicken Salamander (axolotl) Fish (gar) FIGURE 1 .1 0 Simi larities and differences among vertebrate embryos as th ey p roceed th ro ugh devel op ment . Each species' em bryos begin with a basically similar structure, although they acq ui re this structure at different ages and sizes. As they deve l op, the species become less l ike each other. (Adapted from Richard son et a l . 1 998; p hotograp h courtesy of M. R i c h ardson .) DEVELOPMENTAL ANATOMY finally species emerge. All vertebrate embryos have gill arches, a notochord, a spinal cord, and primitive kid neys. 2.
Less general characters develop from the more general, until finally the most specialized appear. All vertebrates initial ly have the same type of skin.
Only later does the skin develop fish scales, reptilian scales, bird feathers, or the hair, claws, and nails of mammals. Similarly, the early development of limbs is essentially the same in all ver tebrates. Only later do the differences between legs, wings, and arms become apparent. 3. The embryo of a given species, instead of passing through the adult stages of lower animals, departs more and more from them.' The visceral defts of embryonic birds and mam mals do not resemble the gill slits of adult fish in detail. Rather, they resemble the visceral clefts of embryonic fish and other embryonic vertebrates. Whereas fish preserve and elaborate these clefts into true gill sUts, mammals convert them into structures such as the eustachian tubes (between the ear and mouth). 4. Therefore, the early embryo of a higher animal is never like a lower animal, but only like its early embryo. Human embryos never pass through a stage equivalent to an adult fish or bird. Rather, human embryos initially share characteristics in common with fish and avian embryos. Later, the mammalian and other embryos diverge, none of them passing through the stages of the others. von 8aer also recognized that there is a common pattern to all vertebrate development: each of the Iluee germ lay ers generally gives rise to the same organs, whether the organism itself is a fish, a frog, or a chick. Comparative embryonic anatomy remains an active field of research today, although it is now done in an evo lutionary context. What embryonic interactions, for instance, cover the squirrel's tail with fill but provide scales on the rat's tail? The author's own research concerns how turtles get their shells-a skeletal feature generally com posed of 59 bones Illat no other vertebrate possesses. What is Ille relationship of these 59 bQJJes to the bones found in alligators and prehistoric marine reptiles? What changes in the "typical" development of the vertebrate skeleton allowed these unique bones to form? Jack Horner and Hans Larsson are looking at the similarities between the developmental anatomy of chick and dinosaur embryos and have found that the embryonic chick, unlike the dinosaur, regresses its tail. They are conducting experi ments to block this regression, and actually hope to obtain a chick that more closely resembles its dinosaur ancestors (Horner and Gorman 2009). Keeping Track of Moving Cells: Fate Maps and Cell Lineages By the late 1800s, the cell had been conclusively demon strated to be the basis for anatomy and phYSiology. Embry olOgiSts, too, began to base their field on the cell. But unlike those who studied the adult, developmental anatomist found that cells do not stay still il1 the embryo. Indeed, one of the most important conclusions of developmental anatomists is that embryonic cells do not remain in one place, nor do they keep the same shape (Larsen and McLaughlin 1987).
Early embryologists recognized that there are two major types of cells in the embryo: epithelial cells, which are tightly connected to one another in sheets or tubes; and mesenchymal cells, which are unconnected to one anoth er and operate as independent units. Morphogenesis is brought about tluough a limited repertoire of variations in cellular processes witrun these two types of arrangements (Table 1 . 1): • Direction and number ofcell divisions. Think of the faces of two dog breeds-say, a German shepherd and a poodle. The faces are made from the same cell types, but the number and orientation of the cell divisions are differ ent. Think also of the legs of a German shepherd com pared with those of a dachshund.
The skeleton-forming cells of the dachshund have undergone fewer cell divi sions than those of taller dogs (see Figure 1.21). • ry of evolution. "Lower animals" would be those having simpler anatomies.
Cell shape changes. Cell shape change is a critical part of not only of development but also of cancer.
In develop ment, change in the shapes of epithelial cells often cre ates tubes out of sheets (as when the neural tube (arms); and a shape change from epithelial to mesenchymal is critical when cells migrate away from the epithelium (as when muscle cells are formed).
This same type of epithe lial-to-mesenchymal change allows cancer cells to migrate and spread from the primary twnor to new sites.
• Cell movement. Cell migration is critical to get cells to their appropriate places. The germ cells have to migrate into the developing gonad, and the primordial heart cells meet in the middle of the vertebrate neck and then migrate to the left part of the chest. • Cell growth. Cells can change in size. This is most appar ent in the germ cells: the sperm eliminates most of its cytoplasm and becomes smaller, whereas the develop ing egg conserves and adds cytoplasm, becoming com paratively huge. Many cells undergo an "asymmetric" cell division that produces one big cell and one small cell, each of which may have a completely different fate. • "von Baer formulated these generalizations prior to Darwin's theo 17 Cell death. Death is a critical part of life. The cells that in the womb constitute the webbing between our toes and fingers die before we are born. So do the cells of our tails. The orifices of our mouth, anus, and reproductive glands all form through cells dying at particular times and places. 18 CHAPTER 1 TABLE 1 .1 Summary of major morphogenic processes regulated by mesenchymal and epithelial cells Process MESENCHYMAL CELLS Condensation Mesenchyme becomes epithelium Cartilage mesenchyme Cell division Mitosis produces more ceUs (hyperplasia) Limb mesenchyme Cell death CeUs die Interdigital mesenchyme Migration Cells move a t particular times and places Heart mesenchyme Matrix secretion and degradation Synthesis or removal of extracellular layer Growth CeJls get larger EPITHEUAl CELLS Dispersal Fat cells 0 · · · ·· Epithelium becomes mesenchyme (entire Epithelium becomes mesenchyme (part of structure) Shape change or growth CeJls remain attached as morphology is altered Cell migration (intercalation) Rows of epithelia merge to form fewer rows Cell division Mitosis within row Matrix secretion and degradation Sy nthesis or removal of extl·aceUular matrix Migration Formation of free edges 1.1.14013·1.1 ·· Neurulation _ 1.1.1.1.1.1 .1.1 1 ! ' ['l q '! 'I '1 Vertebrate gastrulation I •m .J C::;;; Q :C; J::''J Vertebrate gastrulation · tim ··· · • Changes in the composition of the cell membrane or secreted products. C ell membranes and secreted cell products .. . ------ . .
. ..
-------,.-- m·· . . . . . .
. Vertebrate organ formation Chick ectoderm allow the migration of their neighboring cells. Extracel lular matrices made by other cell types will prohibit the migration of the same set of cells.
In this way, "paths and influence the behavior of neighboring cells. For instance, extracellular matrices secreted by one set of cells will _ Chick hypoblast 1:1:1:I -+- \.l.p\' 4\. -+- G-;r;r;r;r;) or coJumn _ Mtilierian duct degeneration '.." structure) Delamination Cartilage mesenchyme - (hypertrophy) .-. Example Morphology Action, guiderails" are established for migrating cells. . - - ...- Fate maps Given such a dynamic situation, one of the most important programs of descriptive embryology became the tracing of cell lin eages: following individual cells to see what those cells become. In many organisms, resolution 01 individual cells is not possible, but one can label groups 01 embryonic cells to see what that area becomes in the adult organism. By bringing such studies together, one can construct a late map. These diagrams "map" larval or adult structures onto the region 01 the embryo from which they arose. Fate maps constitute an important loundation for experimental embry ology, providing researchers with inlormation on which portions of the embryo normally become which larval or adult structures. Figu re 1 .1 1 shows late maps 01 some ver tebrate embryos at the early gastrula stage. Fate maps can be generated in several ways, and the technology has changed greatly over the past lew years. Construction of these maps is an ongoing research pro- Zebrafish Frog Epidermal ectoderm graml and some of the results can be controversial. Recent examples of controversial fate maps include the map for tile region 01 the lrog embryo that specifies heart and blood ceU precursors (Lane and Sheets 2006), and that for the region 01 the embryonic turtle that becomes the bones 01 the plastron (Cebra-Thomas et al. 2007). As we will see later in this book, researchers are currently constructing, relin ing, and arguing about the fate maps 01 mammalian embryos. Direct observation of living embryos Some embryos have relatively lew cells, and the cytoplasm in each 01 the early blastomeres has a different pigment. In such fortunate cases, it is actually possible to look through the mkroscope and trace the descendants of a particular cell into the organs they generate. E. G. Conklin patiently followed the fates of each early cell 01 Styela partita, a tuni cate (sea squirt) that resides in waters off the coast 01 Mass achusetts (Conklin 1905). The muscle-Iorming cells 01 the embryo always had a yellow color, derived lrom a region 01 cytoplasm fowld in the B4.1 blastomere (Figu re 1 .1 2). Conklin's fate map was confirmed by cell-removal exper inlents. Removal of the B4.J cell (which according to Con klin's map should produce aU the tail musculature) in lact resulted in a larva with no tail muscles (Reverberi and Min ganti 1946).
See WEBSITE 1 .1 Conklin's art and science Yolk See VADE MECUM The compound microscope Endoderm p p Mouse GlUck A Epidermal ectoderm A FIGURE 1 .11 Fate maps of vertebrates at the early gastrula stage. AI! are dorsal surface views (looking "down" on the emhryo at what will become its back).
Despite the different appearances of the adult animals, fate maps of these four vertebrates show numerous similarities among the embryos. The cells that will form the notochord occupy a central dorsal position, while the precur sors of the neural system lie immediately anterior to it. The neural ectoderm is surrounded by less dorsal ectoderm, which will form the epidermis of the skin. A indicates the anterior end of the emhryo, P the posterior end.
The dashed green lines indicate the sHe of ingression-the path cells will follow as they migrate from the exterior to the interior of the embryo. Dye marking Most embryos are not so accommodating as to have cells 01 diflerent colors. In the early years of the twentieth cen tury, Vogt (1929) traced the lates 01 different areas of amphibian eggs by applying vita l dyes to the region of interest. Vital dyes stain cells but do not kill them. Vogt mixed such dyes with agar and spread the agar on a micro scope slide to dry. The ends of the dyed agar were very thin. Vogt cut clups from these ends and placed them on a frog embryo. After Ule dye stained the cells, he removed the agar chips and could lollow the stained cells' move ments within the embryo (Figure 1 .1 3). One problem with vital dyes is that as they become more diluted with each cell division, they become difficult to detect. One way around this is to use fluorescent dyes that are so intense that once injected into individual ceUs, they can still be detected in the progeny of these cells many divisions later. Fluorescein-conjugated dextran, for exam ple, can be injected into a single cell of an early embryo, and the descendants of that cell can be seen by examining the embryo under ultraviolet light (Figure 1 .1 4). See VADE MECUM Histotechniques 20 CHAPTER 1 (A) (B) Animal pole Ectoderm Nervous system Anterior Notochord Endoderm Mesenchyme Vegetal pole (C) 2-cell stage 4-cell stage FIGURE 1.12 Fate map of the tunicate embryo. (A) Confocal sec tion through a larva of the tunicate Oona savignyi.
The notochord cells are stained green; the cell boundaries are stained white. The endoderm is blue, the muscles red, the neural tube)'ellow, and the epidermis magenta . (B) Zygote of Styela partita (left), shown shortly before the first cell division, with the fate of the cytoplas mic regions indicated. The 8-cell embryo on the right shows these regions after three cell divisions.
(C) A linear version of the S. partita fate map, showing A3 the fates of each cell f the embryo. we will use color o Throughout this book, the conventions of developmental anatomy: B-cell st age M.I Anterior .5.3 Nishida .4.2 and Nishida 1 987.) One way of permanently marking cells and following their lates is to create emb ryos in which the same organisUl contains cells with different genetic constituhons. In the 19205, the German embryologists Hilde Mangold and Hans Spemann perlormed some of the most important experiments in the his tory of embryology when they trans planted embryonic tissues from one species of newt into the embryo of a different newt species.
These chimeric embryos-,mbryos made from tissues of more than one ge ne tic SOUfce enabled Mangold and Spemann to tell which structures arose from donor tis sue and which from host tissue (see Fig u res 7.16 and 7.17). Vegetal AS.2 blue for ectoderm, red for mesoderm, and yellow for endoderm. (A fro m Veeman et al. 2008; B after 1 987 and Reverberi and Minganti 1 946; C after Conklin 1 905 Genetic labeling {AS.I 16-cell stage AB2 H.lf-embryo 84. 1 '--" 1l3 Animal { .5.4 85. 1 Veget.1 { 85.2 Posterior b4.2 b5.3 Animal bS.4 E ndoder m Mesenchyme 32-cdl stage 64-cell stage Derivative { A6. 1 A6.2 -c A7 . 1 Endoderm Endoderm -{]��• toch= ord A7.2 { ���'•••••3 @!i { - C { -C A6.3 A 7.4 A7 5 Brain stem Endoderm A7.B Brain 7. .6.5 rt . 9 L A7.10 .7.
1 t Palps .6.6 ... a7. 1 2 Epidermis = ... a7.13 Sense organ .6.7 .7.14 Epidermis .7. 1 5 Epidermis .6.8 .7.16 Epidermis B7. 1 Endoderm 86.1 1l7.2 Endoderm Mesenchyme A6.4 ({ { ::: { { 1l6.4 b6.S b6.6 b6.7 b6.B -C ,...=.,,... . -::.
"J".:-;;,.:.... Muscle 9 -C bb7. 7.10 -C -C Epidermis b7. t t Epidermis b7.12 Epidermis b7. t 3 Epidermis b7. t 4 Epidermis b7.1S Epidermis b7. 16 Epidermis D EVELOPMENTAL ANATOMY Agar chips with dye (A) 1 2 (B) Embryo ;;:..
"\ 8 9 10 ":0 II 11 10 9 (E) 7 Dye stains on embryo - -------.. 6 " Dorsal lip J , FIGURE 1.13 Vital dye staining of amphibian embryos. (A) Vogrs method for marking spec ific cells of the embryonic surface with vital dyes. (8-0) Dorsal surface views of stain on successively later embryos. (E) Newt embryo dissected in a medial sagittal 5ec ticn to show the stained cells i n the interior. (After Vogt 1 929.) One of the best examples . Of blastoP (where cells begm to enter the embryo) (D) (C) C J , 5 21 : Section plane : of view (El 1 II J can be performed on an embryo while it is still inside the egg, and the chick that hatches will have quail cells in par ticular sites, depending on where the graft was placed. Quail cells differ from chick cells in two important ways. First, the quail nucleus has condensed DNA matin) (heterochro concentrated around the nucleoli, making quail nuclei easily distinguishable from chick nuclei. Second, cell-specific antigens that are quail-specific can be used to cells. In this way, fine-struc ture maps of the chick brain and skeletal system have been f.ind individual quail cells, even if they are "hidden" with in a large population of chick of this technique is the con struction of chimeric embryos by grafting quail cells inside produced (Figure 1 . 1 5; Le Douarin 1969; Le DOllarin and Teillet 1973). a chick embryo. Chicks and quail embryos develop in a In addition, the chick-quail chimeras dramatically con similar manner (especially during the early stages devel firmed the extensive cell migrations taken by neural crest opment), and the grafted quail cells become integrated into cells during vertebrate development.
Mary Rawles (1940) the chick embryo and participate in the construction of the showed that the pigment cells (melanocytes) of the chick originate in the neural transient band of cells that various organs. The substitution of quail cells for chick cells crest, a (C) (A) (B) Ventral AnimaJ __•.:t. ___ viewed FIGURE 1 .14 Fate mapping using a fluorescent dye. (A) Specific cell s of a zebrafish embryo were injected with a fluorescent dye that will not diffuse from the cells. The dye was then activated by laser in a small region (about 5 cel l s) of the late cleavage stage embryo. (8) Aher formation of the central nervous system had begun, cells that expressed the active dye were visualized by fluorescent light. The fluorescent dye is seen in pa rticu lar cells that generate th e forebrain and midbrain. (C) Fate map of the zebrafish central nervous system. Dye was injected into cel ls 6 hours aher fertilization (lehl, and the results are color-coded onto the hatched fish (rightl . Overlapping colors indicate that cells from these regions of the 6-hour embryo contribute to two or more regions. (A,B from Kozlowski et al.
1 998, photographs courtesy of E. Wei nberg; C aher Woo and fraser 1 995.) Anterior Posterior forebrain forebrain 22 CHAPTER 1 (TI) (A) Quail embryo 24 h (donor) Chick embryo 24 h (host) FIGURE 1 .15 Genetic markers as cell l i neage tracers. (A) Crahing experiment where i n the cells from a particular region of a l -day Chick embryo with region of quail ceUs on the neural tube quail embryo have been placed into a simi lar region of a l -day chick embryo. Aher several days, the quail cells can be seen by using an antibody to quail-specific proteins. This region of the 3day embryo produces cells that populate the neural tube. (B) Chick and quail cells can also be distinguished by the heterochromatin of their nuclei. The quail cells have a single large nucleolus (dense purple), distinguishing them from the diffuse nuclei of the chick. (From Darnell and 5choenwolf 1 997, courtesy of the authors.) joins the neural tube to the epidermis. When she trans planted small regions of neural crest-containLng tissue from a pigmented scrain of chickens into a similar position in an embryo from an unpigmented strain of chickens, the migrating pigment cells entered the epidermis and later entered the feathers (Figure 1 .1 6). Ris (1941) used similar techniques to show that while almost all of the external pigment of the chick embryo came from the migrating neu plant cells from a gene tically modified organism. In such a te dutique the genetic mudification can then be traced ral crest cells, the pigment of the retina formed in the reti fish. It emits bright green fluorescence when exposed to ultraviolet light and is widely used as a transgenic label for cells in develop mental and other research. na itself and was not dependent on the migrating neural crest cells. This pattern was confirmed in chick-quail in whlch the quail neural crest cells produced their hybrids, own pigment and pattern in the chick feathers. , only to those cells that express it. One version is to infect the cells of an embryo with a virus whose genes have been altered such that they express the gene for a fluorescently active protein such as GFP.' (This type of altered gene is . *GFP-green fluorescent protein-occurs naturally in certain jelly See VADE MECUM Chick-quail chimeras Transgenic DNA chimeras In most animals, it is difficult to meld a chimera from two species.
One way of cin:urnventing this problem is to traJ1S- FIGURE 1 .16 Chick resulting from transplantation of a trunk neural crest region from an embryo of a pigmented strain of chickens into the same region of an embryo of an unpig mented strain. The neural crest cells that gave rise to the pigment migrated into the wing epidermis and feathers. (From Ihe archives of B. H. Willier.) DEVELOPMENTAL ANATOMY (A) (B) 23 every cell of the Xenopus embryo. In this way, Gross and colleagues generated tad poles and adult frogs in which every cell glowed green when placed under ultra violet light (Figure 1 .17A) .
They then removed cells from the neural tube and neural crest of a GFP-transgenic embryo and transplanted the cells into a similar Chimera region of a normal Xenopus embryo (Figure 1 .1 7B). Once the host emb ryos had meta morphosed into adult frogs, the biologists looked at the lower jaw, a structure wh

form, whereas others migrate from their original sites and differentiate in their tually, usually after adulthood is reached, the mature new locations. Migratory cells include neural crest gametes are released to unite during fertilization. The cells and the precursors of germ cells and blood resulting new generation then begins development. 3. Epigenesis happens. New organisms are created de cells. S. "Community of embryonic structure reveals com novo each generation from the relatively disordered munity of descent" (Charles Darwin, cytoplasm of the egg. Species). 4. Preformation is not found in the anatomical struc On the Origin of 9. Homologous structures in different species are those tures themselves, but in the genetic instructions that organs whose similarity is due to sharing a cornmon instruct their formation. The inheritance of the fertil ancestral structure.
Analogous structures are those ized egg includes the genetic potentials of the organ organs whose Similarity comes from serving a simi ism. These preformed nuclear instructions include lar function (but which are not derived from a com the ability to respond to environmental stimuli in mon ancestral structure) . specific ways. 5. The three germ layers give rise to specific organ sys tems. The ectoderm gives rise to the epidermis, nerv ous system, and pigment cells; the mesoderm gener ates the kidneys, gonads, muscles, bones, heart, and blood cells; and the endoderm forms the lining of the digestive tube and the respiratory system. 6. Karl von Baer's principles state that the general fea tures of a large group of animals appear earlier in the embryo than do the speCialized features of a 10. Congenital anomalies can be caused by genetic fac tors (mutations, aneuploidies, translocations) or by environmental agents (certain chemicals, certain viruses, radiation). 1 1 . Syndromes consist of sets of developmental abnor malities that "run together." 12. Organs that are linked in developmental syndromes share either a common origin or a cornman mecha nism of formation.
For Further Reading Complete bibliographical citations for all literature cited in this chapter can be found at the free-access website www.devbio.com Cadieu, E. and 19 others.
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Winter, R. M. 1996. Analyzing human developmental abnormalities. BiDEssays 18: 965-971. Woo, K and S. E. Fraser. 1995. Order and coherence in the fate map of the zebrafish embryo. De-aelapmellt 121: 2595-2609.
30 CHAPTER 1 Go Online WEBSITE 1.1 Conklin's art and science. The plates from Conklin's remarkable 1905 paper are online. Looking a t theJn, one can see the precision o f his observations and how he constructed his fate map of the tunicate embryo. VADE MECUM Chick-quail chimeras.
We are fortunate to present here a grafts. You will be able to see how these grafts are ac tua lly movie made by Dr. Nicole Le DOllarin of her chick-quail done. The compound microscope. The compound microscope has been the critical tool of developmental anatomists. Mastery of microscopic techniques allows one to enter an entire world of form and pattern. Histotechniques. Most cells must be stained in order to see them; different dyes stain different types of molecules . Instructions on staining cells to observe p articular struc tures (such as the nudeus) are given here. - . Developmental Genetics CYTOLOGICAL STUDIES DO E AT THE TURN OF THE TWENTIETH CENTURY estab But wilateller the immediate opera lished that the chromosomes in each cell of an organism's body are the mitotic [ions of the genes wrn out to be, descendants of the chromosomes established at fertilization (Wilson 1896; Boveri 1904). In other words, each somatic cell nucleus has the same chromosomes This funda and therefore the same set of genes-as all the other somatic nuclei. mental concept is called genomic equivalence. Given this concept one of the major questions faCing biologists of the early twentieth century was how nuclear genes could direct development when these genes are the same in every cell type (Harrison 1937; Just 1939). If every cell in the body contains the genes for hemo globin and insulin proteins, why is it that hemoglobin proteins are made only in red blood cells, hlSUlin proteins are made only in certain pancreas cells, and neither protein is made Based on the embryological evidence for genomic equivalence (as well as on 19605 that the this question lies in differential gene ex pression . The three postulates of d iHerential gene expression are: • Every cell nucleus contains the complete genome established • egg. 1n molecular terms, the DNAs of aU differentiated cells are identical. in the fertilized The unused genes in differentiated ceUs are neither destroyed nor mutated, but retain the potential for being expressed. • Only a small percentage of the genome is expressed in each cell, and a portion of the RNA synthesized in each cell is specific for that cell type. Gene expression can be regulated at several levels such that different cell types synthesize different sets of proteins: • Differential gene transc.ription regulates which of scribed into nuclear RNA. • the nuclear genes are tran Selective nuclear RNA processing regulates whicl1 of the transcribed RNAs (or which parts of such a nuclear RNA) are able to enter into the cytoplasm and become messenger RNAs. • Select ive messenger RNA transl a tion regt.lates which of the mRNAs in the cytoplasm are translated into proteins. • Di fferen ti al protein modification regula tes which proteins are allowed to remain and/or function in the cell. Some genes (such as those coding for the globin proteins of hemoglobin) are regulated at all these levels. c. H . WADDINGfON (1 956) We flalle en tered the cell, the mansion of our birth, and have started the inI!enwry of our acquired wealth. ALBERT ClAUDE in the kidneys or nervous system? bacterial models of gene regulation), a consensus emerged in the answer to [hey [he category of developmental pmcesses and thus belong to [he prollince of embryology. mosl cenainly belong to (1974) 32 CHAPTER 2 When Giemsa dyes allowed such observations to be made Evidence for Genomic Equivalence Until the m id-twent ieth century, genomic equivalence was not so m u ch proved as it was asswned (because every cell is the mitotic descendant of the fertilized egg . One of the first tasks of developmental genetics was to dete rmine whether every cell of an organism indeed does have the same genmtte-t ha t is, the same set of genes-as every o ther cell . Evidence that every cell in t he body has the same genom e originally came from the analysis of Drosoph.ila chromosomes, in which the DNA of certain larval tissues undergoes numerous rounds of DNA replication without separation, such that the structure of the chro moso me s can be seen. In these polytene (Gr. "many strands") chro mosomes, no structural differences were seen between cells; but different regions were seen to be "puffed up" at di ffe rent times and in different cell types, suggesting that these areas were activ ely making RNA (Beerman 1952). See WEBSITE 2.1 Does the genome or the cytoplasm direct developmentl See WEBSITE 2.2 The origins of developmental genetics in malnmaJian chromosomes, it was also found that no chromosomal regions were lost in most cells. These obser vations, in tum, were confirmed by nucleic acid hybridiza tion studies, which (for instance) found globin genes in pancreatic tissue, which does not m ake globin proteins. But the ultimate test of whether the nucleus of a differ entiated cell has undergone irreversible functional restric tion is to have that nucleus generate every other type of differentiated cell in the body. If each cell's nucleus is iden tical to the zygote nucleus, then each cell's nucleus should also be capable of directing the entire development of the organism when transplanted into an activated enucleated egg.
As early as 1895, the embryologist Yves Delage pre dicted that "If, without deterioration, the egg nucleus could be replaced by the nucleus of an ordinary em bryonic cell, we should probably see this egg developing without changes" (Del age 1895, p. 738). In 1952, Briggs and King demonstrated that blastula cell nuclei could direct the development of com plet e tad poles when transferred into the cytoplasm of an activat ed enucleated frog egg. This procedure is called somatic nuclear transfer or, more commonly, cloning. Nuclei from adult frogs, however, were not able to generate adult frogs. How ever, ad ult nuclei (fro m skin cells.. for instance) were SIDEL IG H TS SPECULATIONS The Basic Tools of Developmental Genetics DNA analysis Embryol ogist Theodor Boveri (1 904) wrote that to discover the mechanisms of development, it was " not cell nuclei, not even individual chromosomes, but certain parts of certain chromosomes from certain cells that must be isolated and collected in enormous quantities for ana lysis ." This analysis was finally made possible by the techniques of gene cloning, DNA sequencing, South ern bl otti ng, gene knockouts, and enhancer traps. In add ition, techniques fo r showing which enhancers and pro moters are methylated and which are u nmethy l ated have become more importan t, as investigations of differen tial gene transc rip tio n have focused on these elements. For discussions of these te chniques, see Website 2.3. RNA analysis Bioinformatics Cre-lox analysis (which allows the message to be made or destroyed i n particular cell types) a n d C h I P-o n-C hip techniques (which enable on e to local ize active ch romatin) . Modern developmental genetics often involves comparing DNA sequences (especially regulatory units such a s enhancers a n d 3 ' UTRs) a n d looki ng at spec i fi c genomes to determine how genes are being regu lated . "High throug hput" RNA a n alysis by micro and mac roarrays enables researchers to com pa re thousands of mRNAs, and computer-aided synthetic techn iqu es can predict interactions between pro teins and mRNAs. Various free web sites enable researchers to use the tools that allow such comparisons. Other sites are organism- or organ speci fic and are used by researchers studyi ng that particular organ or organi sm .
For discussions of these techniques; see Website 2.4. For more about these sites and links to them, see Website 2.5 Differential ge ne transc ription is criti cal in development. In order to know the time of gene expression and the place of gene expression, one has to be able to have procedures that actual ly locate a pa rtic u lar type of messenger RNA. These techniques include north ern blots, RT-PC R, in situ hybri diza tion, and array tech no logy. To ascertain the function of these mRNAs, new techniques have been formulated, which include antisense and RNA i nterference (which destroy messages), DEVELOPMENTAL G EN ETICS IAl OOCYTE DONOR (Scottish blackface strain) �� Udder cells removed Udder cells placed in culture, grown in G1 stage. V ,--) Y1 T>" ' ,,)(U K Remove spindle Micropipette) . 0 · ,#:· 0 or #. Transfer cell into enucleated egg O   · t o t g and Celi fused with electric current Embryo cultured 7 days ".-- Embryo transferred to surrogate mother (Scottish blackface) (B) r__-----. NUCLEAR DONOR (Finn-Dorset strain) Eggs removed @ Meiotic spindle , ;;tleated 33 Birth of Dolly (Finn-Dorset lamb genetically identical to nuclear donor) able to direct the development of all the organs of the tad poles (Gurdon et a1. 1975). Although the tadpoles all dled prior to feeding, their existence showed tha t a single dif ferentiated cell nucleus still retained incredible potencies. FIGURE 2.1 Cloned mammals have been created using nuclei from adult somatic cells. (A) Procedu re used for cloning sheep. IB) Dolly, the adult sheep on the left, was derived by fusing a mammary gland cell nucleus with an enucleated oocyte, which was then implanted in a surrogate mother (of a different breed of sheep) that gave birth to Dolly. Dolly l ater gave birth to a lamb (Bonnie, at right) by normal reproduction. (A after Wilmut et al. 2000; B, photograph by Roddy Fi e ld © Roslin Institute.) , A nucleus of a skin cell could produce all the cells of a young tadpole.
In 1997, Ian Wilmut announced that a sheep had been cloned from a somatic cell nucleus from an adult female sheep. This was the first time an adult vertebrate had been successfully cloned from another adult.
Wilmut and his colleagues had taken cells from the mammary gland of an adult (6-year-old) pregnant ewe and put them into culture (Wilmut et a1. 1997; Figure 2.1A). The culture medium was formulated to keep the nuclei in these cells at the intact diploid stage (G1) of the cell cycle. This cell-cycle stage turned out to be critical.
The researchers then obtained oocytes from a different strain of sheep and removed their nuclei. These oocytes had to be in the second meiotic metaphase (the stage at which they are usually fertilized). Fusion of the donor cell and the enucleated oocyte was accomplished by bringing the two cells together and send ing electric pulses through them, destabilizing the cell membranes and allowing the cells to fuse. The same elec tric pulses that fused the cells activated the egg to begin development. The resulting embryos were eventually transferred into the uteri of pregnant sheep. CHAPTER 2 34 ((8) Of the 434 sheep oocytes originally used in this exper 2.1 B). DNA iment, only one survived: Dolly ' (Figure analysis confirmed that the nuclei of Dolly's cells were derived from the strain of sheep from which the donor nucleus was taken (Ashworth e t al. 1998). 1998; Signer e t al. Cloning of adult mammals has been confirmed FIGURE 2.2 The kitten "CC" (AI was a clone produced using somatic nuclear transfer from IIRainbow," a female calico cat (B). The two do not appear identical because coat pigmentation pat tern in calico cats is affected by the random inactivation of one X chromosome in each somatic cell (see Sidel ights & Speculations, p. 50). Their behaviors were also quite different. (Photographs courtesy Coliege of Veterinary Medicine, Texas A&M University.) of in guinea pigs, rabbits, rats, mice, dogs, cats, horses, and oped debilitating diseases as they matured (Humphreys cows. In 2003, a cloned mule became the first sterile ani et al. mal to be so reproduced (Woods et aJ. will shortly see, this problem is due 2003). Thus i t appears that the nuclei o f vertebrate adult somatic cells 2001; Jaenisch and Wilmut 2001; Kolata 2001).
As we in large part to the dif ferences in methylation between the chromatin of the contain all the genes needed to generate an adult organ zygote and the differentiated cell. Second, the phenotype ism. No genes necessary for development have been lost of the cloned animal is sometimes not identical to that of or mutated in the somatic cells.t the animal from which the nucleus was derived. There is Certain caveats must be applied, however. First, variability due to random chromosomal events and the although it appears that all the organs were properly effects of environment. The pigmentation of calico cats, for formed in the cloned animals, many of the clones devel- instance, is due to the Yalldom inactivation of one or the other X chromosome (a genetic mechanism that will be discussed later in this chapter) in each somatic cell of the female cat *The creation of Dolly was the result of a combination of scientific and social circumstances. These circumstances involved job securi ty, people with different areas of expertise meeting each other, chil dren's school holidays, international politics, and who sits near whom in a pub. The complex interconnt;'ctions giving rise to Dolly are told in The Secotld Crefltior! (Wilmut et aL 2000), a book that should be read by anyone who wants to know how contemporary science acrually works. As Wilmut acknowledged (p. 36), "The story may seem tAlthough cloning humClns does not seem feasible at present each cell of the human body (with just a few exceptions, such CIS lymphO cytes) does appear to contain the same genome as every other cell. As we will see in Ch£lpter 17, adding certain activated transcription factors to ordinary skin fibroblasts will convert them into embryon ic stem cells that are indeed capable of generating entire embryos, CIt least in mice. embryo. Therefore, the coat color pattern of the first cloned cat, a calico named "CC," were different from those of "Rainbow," the adult calico whose cells provided the implanted nucleus that generated "CC" (Figu re 2.2). The same genotype gives rise to multiple phenotypes in cloned sheep as well. Wilmut noted that four sheep cloned from blastocyst nuclei from the same embryo "are genetically identical to each other and yet are very different in size and temperament, showing emphatically that an animal's genes do not 'determine' every detail of its physique and personality" (Wilmut et at 2000, p. 5). Wllmut concludes that for this and other reasons, the "res urrection" of lost loved ones by cloning is not feasible.
SEE WEBSITE 2,6 Cloning and nuclear equivalence DEVE LOPMENTAL G E N ETICS FIGURE 2.3 Nucleosome and chromatin struc ture. (A) Model of nuclcosomc structure as seen by X-ray crystallography at a resolution of 1 .9 A. Histones H2A and H2B are yellow and red, respectively; H3 is purple and H4 is green. The DNA helix (gray) winds around the protein core. The histone "tails" that extend from the core are the sites of acetylation and methylation, which may disrupt or stabi lize, respectively, the forma tion of nucleosome assemblages. (8) Histone H l can draw nucleosomes together into compact forms. About 1 40 base pairs of DNA encircle each histone octamer, and about 60 base pairs of DNA link the nucleosomes together. iO Model for the arrangement of nucleosomes in the highly com pacted solenoidal chromatin structure. Histone "tails" protruding from the nucle050me subunits a llow for the attachment of chemical groups. (D) Methyl groups condense nuclcosomes more tight ly, preventing access to promoter sites and thus preventing gene transcription.
Acetyla tion loosens nucleosome packing, exposing the DNA to RNA poly H2B tail merase and transcription fac tors that will activate the genes. (A after Davey et al. H2A taii 2002) (B) (A) 35 (e) Nucleosome H2A taIl H4 tail H2B tail H4 tail (D) Condensed nucleosomes: Histone tails largely methylated :;;g:;/lJ-':l;Irj H3 tail Methyl groups / bind H3 and H4 Uncondensed nucleosomes: Histone tails largely un me thylated and acetylated Differential Gene Transcription genes is that eukaryotic genes are contained within a com plex of DNA and protein called chromatin. The protein So how does the same genome give rise to different cell component constitutes about half the weight of chromatin types? To understand this, one needs to understand the and is composed largely of histones. The nucleosome is anatomy of the genes. One of the fundamental differences the basic unit of chromatin structure (Figure 2.3). It is com d i stinguishing most eukaryotic genes from prokaryotic posed of an octa.mer of histone proteins (two molecules 36 CHAPTER 2 1 1 FIGURE 2.4 Histone methyl ations on his tone H3. The tail of h istone H3 (its amino most sequence, at the beginning of the pro Transcriptional Tran cri tjonal tein) sticks out from the nucleosome and is m' 0ry actlv on capable of being m ethyl ated o r acetylated. Here, Iysines can be m ethyl ated and recog n ized by particular proteins. Methylated lysine residues at pos itions 4, 38, and 79 are a ssoci ated with gene activation, whereas methyl ated lysines at positions 9 and 27 re associated 9 H3 tail with repression. The proteins binding these sites (not shown to scal e) are represented above the methyl group. (Aher Kouzarides and Berger 2007.) Transcriptional Silent elongation heterodnomatin Histone octamer \ f Cell cy le regU lon 'f y w Gl) 27 4 a Enzymes known as histone acetyltransferases place acetyl groups on histones (especially on lysines in H3 and H4), ily. As might be expected, then, enzymes that remove acetyl destabiliZing the nucleosomes so that they come apart eas each of histones H2A, H2B, H3, and H4) wrapped with groups-hjstone deacetyJases--stabilize the nucleosomes two loops containing approximately 140 base pairs of and prevent transcription. DNA (Kornberg and Thomas 1974). Histone HI is bound Histone methylation, the addition of methyl groups to to the 60 or so base pairs of "linker" DNA between the histones by histone methyltransferases, can either acti nucleosomes (Weintraub 1984). There are 14 points of con vate Or further repress transcription, depending on the tact between the DNA and the histones (Luger et al. 1997). amino acid being methylated and the presence of other methyl or acetyl groups in the vicinity (see Strahl and Allis Anatomy of the gene: Active and repressed chromatin 2000; Cosgrove et a1. 2004). For instance, acetylation of the "tails" of H3 and H4 along with methylation of the lysine at position 4 of H3 (i.e., H3K4; remember that K is the Whereas classical geneticists have likened genes to "beads abbreviation for lysine) is usually associated with active on a string," molecular geneticists liken genes to "string ly transcribed chromatin. In contrast, a combined lack of on the beads," an image in which the beads are nucleo acetylation of the H3 and H4 tails and meU1ylation of the somes. Most of the time, the nucleosomes are wound into lysine in the ninth position of H3 (H3K9) is usually asso tight "solenoids" that are stabilized by histone HI (Figure eiated with highly repressed chromatin (Norma et a1. 2001). 2.3C). This HI-dependent conformation of nucleosomes Indeed, lysine methylalions at H3K9, H327, and H4K20 are inhibits the transcription of genes in somatic ceUs by pack often associated with highly repressed chromatin.
Figure ing adjacent nucleosomes together into tight arrays that 2.4 shows a schematic drawing of a nucleosome, with the prevent transcription factors and RNA polymerases from histone H3 tail having on it some residues wlJOse modifica gaining access to the genes (Thoma et a1. 1979; Schlissel tion can regulate transcription. and Brown 1 984). It is generally thollght, then, that the As might be expected, if methyl groups at specific places "default" condition of cluomatin is a repressed state, and on the histones repress transcription, then getting rid of that tissue-specific genes become activated by local inter these methyl moieties should permit transcription. This ruption of this repression (Weintraub 1985). has been shown in the activation of those genes responsi ble for specifying the posterior halves of vertebrate bod HISTONES AS AN ACTIVATION SWITCH The histones are crit ies. These genes, called Hox genes, encode transcription ical because they are responsible for maintaining the factors that are critical in giving ceUs their identities along repression of gene expression. This repression can be local the anterior-posterior axis.
In early development, Hox ly strengthened (so that it becomes very difficult to tran genes are repressed b y H3K27 trimethylation (the lysine scribe those genes in the nucleosomes) or relieved (so that at position 27 having three methyl groups). However, in transcribing them becomes relatively easy) by modifying differentiated cells, a demethylase that is specific for the histones (Figure 2.3D) . Repression and activation are H3K27me3 is recruited to these promoters and enables the controlled to a large extent by modifying the tails of his gene to be transcribed (Agger et a1. 2007; Lan et a1. 2007) . tones H3 and H4 with two small organic groups: methyl The effects of methylation in controlling gene transcrip (CH,.) and acetyl (CaCH3) residues. In general, histone tion are extensive. So far, we have documented transcrip acetylation-the addition of negatively charged acetyl tional regulation by histone methylation. Later in this chap groups to histones-neutralizes the basic charge of lysine ter we will discuss the exciting research on the control of and loosens the his tones. This activates transcription. transcription by DNA methylation. DEVELOPMENTAL GENETICS In addition to regula ting H I STONE REGULATION OF TRANSCRIPTIONAL ELONGATION the initiation of the transcription al complex (i.e., getting RNA polymerase on the promot er), nucleosomes also appear to regula te the progression of RNA polymerase and the elongation of the mRNA. Indeed, recent evidence suggests that i t is rela tively Com mon for RNA polymerase to be poised at the promoters, ready to go. For transcription to occur, these nucleosomes need to be modified, and it is possible that the acetylation of histone H3 at positions 9 and 14, coupled with the trimethylation of that histone at position 4, is critical for allowing elo ngation of the message (Guenther et al. 2007; Li et a1. 2007). lation is the 5' untranslated region, often called the 5' UTR or leader sequence. The 5' UTR can determine the rate at which translation is initiated. The second difference between prokaryotic and eukaryot ic genes is that eukaryotic genes are not co-linear with their peptide products Rather, the single nUclek acid strand of eukaryotic mRNA comes from noncontiguous regions on the chromosome. Between exons-the regions of DNA that code for a protein-·are intervening sequences called introns that have nothing whatsoever to do with the amino acid sequence of the protein. The structure of a typical eu karyotic gene can be illustrated by the human P-globin gene (Figure 2 .5). Thi s gene, which encodes part of the hemoglob in protein of the red blood cells, consists of the following elements: . region, which is responsible for the binding of RNA polymerase and for the subsequent initiation of ITansc ription The promoter region of the human p-glo bin gene has three distinct units and extends from 95 to 26 base pairs before (up stream from ")! tbe transcrip tion initiation site (i.e., from -95 to -26). • A promoter . " • The transcri ption initiation site, which for human Il-glo bin is ACATTTG.
This site is often called the cap sequence because it represents the 5' end of the RNA, whic h will receive a "cap" of modified nucleotides soon after it is transcribed. The spe.c ific cap sequence varies among genes. • The translation initiation site, ATG. This codon (which becomes AUG in mRNA) is located 50 base pairs after the transcription initiation site in the human P-globin gene (although this distance differs grea tly among dif ferent genes). The sequence of 50 base pairs intervening between the initiation points of transcription and trans- -The term txon refers to a nucleotide sequence whose RNA "exits" the nucleus. It has taken on the functional definition of A protein· encoding nucleotide sequence. Leader sequences and 3' UTR sequences cue also derived from exons, even though they are not tra.nsl t By convention, upstream, downstream,S', and 3' directions are specified in relation to the RNA. Thus, the promoter is upstreAm of the gene, near its 5' end. which contains 90 base pairs coding for acids 1-30 of human p-globin prote in. • The first exon, amino • • • Anatomy of the gene: Exons and introns 37 An intron containing 130 base pairs with no coding sequences for -globin. However, the structure of this intron is important in enabling the RNA to be processed into mRNA and exit from the nucleus. An exon containing 222 base pairs coding for amino acids 31-104.
A large intron-850 base pairs-having nothing to do with globin protein structure. • A trans la tion termination codon TAA. This codon • An exon containing 126 base pairs co ding for amino acids 105-146 of the protein. at this codon and the protein is released. becomes UAA i n the mRNA. The ribosome dissociates • A 3' un trans lated region (3' lITR) that, although tran scribed, is not translated into protein. This region includes the sequence AATAAA, which is needed for pOlyadenylation, the ins ert ion of a "tail" of some 200-300 adenylate residues on the RNA transcript, about 20 bases downstream of the AAUAAA sequence. This polyA tail (1) confers stability on the mRNA, (2) allows the mRNA to exit the nucleus, and (3) permits the mRNA to be tra nsla ted into p rote in , .
• A transc rip ti on termination seq ue nce . Transcription co ntin ues beyond the AATAAA s i te for about 1000 nucleo tides before being terminated. The original transcription product is ca lled nuclear RNA (n R NA), sometimes called Ite/erogelleous >IlIciear RNA A (hnRNA) or pre-messenger RNA (pre-mRNA). uclear contains the cap sequence, the 5 UTR, exons, introns, and the 3' UTR (Figure 2.6). Both ends of these transcripts are modified before these RNAs leave the nucleus. A cap con Sis ting of methylated gua nosine is placed on the 5' end of the RNA in opposite polarity to the RNA itsel f 1l.is means there is no free 5' phosphate group on the nRNA. The 5' cap is necessary for the binding of mRNA to the ribosom e and for subsequent translation (Shalkin 1976). The 3 ' ter minus is usually ruodified in the nucleus by the addition of a polyA tail.
The adenylate resid ues in this tail are put toge ther enzymatically and are added to the transcript; they are not part of the gene sequence. Both the 5' and 3' modifications Lnay protect the mRNA from exonucleases that would otherwise digest it (Sheiness and Darnell 1973; Gedamu and Dixon 1978). The modifications thus stabi lize the message and its precu rsor.
As the nRNA leaves the nucleus, its introns are removed and the remaining exons spliced together In this way the coding regions of the mRNA-i.e., the exons-are brought together to form a single transcrip t, and this transcript is translated into a protein.
The p rote in can be further mod ified to make it functional (see Figure 2.6). .
. 38 CHAPTER 2 (A) Promoter region ())))) '--y-J Translation Transcription initiation codon (Amino acid 1) initiation (cap sequence) 30 ,------ }k '-v--' I--Exon 1 TATA Upstream box promoter region (B) I)\ , I i PolyA Translation terminator codon addition site Amino acid numbers 31 \ ;;;OJ \ 105 104), I Intron I I-Exon 2 -1) I Intron 2 , \ 146 l.il Transcription termination lJ 1 --- Exon 3 - 1 Leader sequence (5' UTR) 3' UTR , Upstream promoter Pro o te r elements Cap sequence / ccctgtggagccacaccctagggttggcca ctactcccaggagcagggagggcaggagccagggctgggcataaaa 5' UTR Translation initiation codon alHisLeuThrProGluGluLysSerAlaValThrAlaLeuTrpGlyLysVaiAsnValAspGlu ValGlrGlyGlu Exon 1 gtcagggcagagccatcta ttgcttlCAmGCITClGACACAACIGTGTICACIAGCAACCICAAACAGACAC I\!CACCIGA(Jl:CIGAG!i.\GMlijkfUGG:ffiGID fiCmJJ:.\ClQQ:qGLG IiG.G!:MGJi1GMQ:1G G.\1GM.G leuLeuValValTyr ACfCTfGGGTlTCTGATAGGCACTGACTcrcrCTGCCTATIGL'TCTATITTCCCACCCITAGGCTGCTGGTGGTCTAC ProTrpThrGlnArgPhePheGluPheGlyAspLeuSerThrProAspAlaValMetGlyAsnProLys Val Lys Ex on 2 CCTTGGACCCAGAGGTTCTTTGAGTCCTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAG AlaHisGlyLysLysValLeuGlyAlaPheSerAspGlyLeuAlaHisLeuAspAsnLeuLysGlyThrPheAlaThr GCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACA LeuSerGluLeuHisCysAspLysLeuHisValAspProGluAsnPheArg = _ CTGAGTGAGCTGCACTGTGACAAG CTG CACGTGGATCCTGAGAAClTCAGGGTGAGTCTATGGGA@CC TrG ATGrrrTI CITrCCCCITGn:rTCfATGGTIAAGTTCATGTCATAGGAAGGGGAGAAGTAACAGGGTACAC'rl-rAGAATGGGAAC AGACGAATAGATTGCATCAGTGTGGAAGTCTCAGGATCGTITfAGItiLl!IfATTTGCfGTTCATMCAAITGTITIC TTTTGTTTAATTCTTGCTTICIIIIIIICTOCGCAATTT IICII CTATTATACTTAATGCCTAGTACATTAtrATT TTA AACAAAAGGAAATATCTCTGAGATACATTAAGTAACITAAAA AA ACITTACACAGTCTGCCTAGTACATTACrAIT TGGAATATATGTGTGCTTATTTGCATATTCATAATCTCCCTACTTTATTTTCTTTTATTTTTAATTGATACATAATCA TTATACATATTTATGGGTTAAAGTGTAATGTITTAATATGTGTACACATATTGACCAAATCAGGGTMTTTTGCATT TCTAATTITAAAAAGCTTTCTTCITTIAATATAC1T1TITGTITATCTrATTTCTAATACTIT AT CCTAATCTCTTT AGGCAATAGCAATATTTCTGCATATAAATATTTCTGCATATAAATTGTAACTGATG,\AAGAGGTTTCATATTGCTAA ClTTCAGGGCAATAATGATACAATGTATCATGCcrcrITGCACCATTCTAAAGAATAACAGTCATAATTTCTGUGTTA TAGCAGCTACAATCCAGCTACCATTCTGCTCTTTTATITIATGGTTGGGATAAGGCTGGATTATTCTGAGTCCAAGCTAG LeuLenGlyAsnValLeuValCysValLeuAla Exon 3 GCCCTIITGCTAATC."GTICATACCfCIT6ICITCCTcCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTC(;fAGCC HisHisPheGlYLysGluPheThrProProValGlnAlaAlaTyrGlnLysValVaWaGlyVaWaAsnAlaLeu CATCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCIATCAGAAAGTGGTGGCTGGTGTGGCIAATGCCCTG Translation - termination codon AlaHiSLysTyrHis , GCCCACAAGTATCAT
GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTAC TAAACIGGGGGATATFATGAAGGGCCTTGAGCATCIGGATTCTGC FIGURE 2.5 CAmATmCATTGC PolyA addition site Nucleotide sequence of the human p-globin gene. Schematic representation of the locations of the promoter region, transcription initiation s ite (cap sequence), 5' untranslated regi on (leader sequence), exons, introns, and 3' untranslated region. Exons are shown in color; the n umbers flanking them indi cate the am i no acid positions each exon encodes in globin. (8) The nucleotide sequence shown from the 5 ' end to the 3' end of the RNA. The co lors correspond (0 their diagrammatic representa tion in (A). The promoter sequences are boxed, as are th e t ransla- (A) 3' UTR tion initiation and termination codes ATG and TAA. The large cap ital letters boxed in color are the bases of the exons, with the amino ac i ds for which they code abb rev iated above them. Small er capi ta l letters indicate the intran bases. The codon s after the translation termination ste ex i st in -globin mRNA but are not translated into proteins.
Withi n this group is the sequence thought to be needed for polyadenylation. By convention, onl y the RNA like strand of the DNA double helix is shown. (B a fter Lawn et al. 1 980.) DEVELOPMENTAL G E N ETICS Promoter region (RNA polymerase binding) GENE (DNA) ())))))) FOR TATA -GLOBrN box NUCLEAR RNA (nRNA) Transcription initiation r--Exon 1- I)1 Translation initiation codon (ATG) I-Exon 2 _I I Intron 1 I)L)t?ead '---y----J Leader seqnence 1---1 r---Exon I m7 GpppAC -+! !f ("Cap") 1 lntron I • I rExo l -rExon 2 n m? GpppAC -0 ("Cap") -GLOB!N PROTEIN ! Leader !!\ \ f ! T Iorron 2 Transcription rExon 2l AUG MESSENGER RNA (mRNA) 1) PolyA Translation terminator codon addition (TAA) site Intron 2 ! Processing £xon 3 UAA -' W I -Exon 3) I 39 Transcription terminates -1 1 I Q U ;;) - -Exon 3 -1 ! 1ft! "" >-- AAAA .. .AQ["Tail" t..-1 r--AAAA ...AOH "Tail" Translation HzN .il••••I•••••••••••••••• COOH HEMOGLOBIN Heme fiGURE 2.6 Summary of steps involved in the production of .g lob in and hemoglobin.
Transcription of the gene creates a nuclear RNA containi ng exons and introns, as well as the cap, ta il , 3' a n 5' untranslated region.s. Processing the nuclea r and d Anatomy of the gene: Promoters and enhancers In addition to the protein-encoding region of the gene, there are regulatory sequences that can be located on either end of the gene (or even within it). These sequences-the promoters and enhancers-are necessary for controlling where and when a particular gene is transcribed. Promoters are the sites where RNA polymerase binds to the DNA to initiate transcription. Promoters of genes that syntheSize messenger RNAs (i.e., genes that encode proteins*) are typically located immediately upstream from the site where the RNA polymerase initiates transcription. Most of these promoters contain the sequence TATA, to RNA into messenger RNA removes the introns. Translation on ribosomes uses the mRNA to encode a protein. The protein is inactive until it is modified and complexed with a-globin and heme to become active hemoglobin (bottom). which RNA polymerase will be bOlmd. This site, known as the TATA box, is usually about 30 base pairs upstream from the site where the first base is transcribed. Since this sequence will appear randomly in the genome at more places than just at promoter sites, other regions flanking it are also important. Many TATA box regions are flanked by *There are several types of RNA that do riot encode proteins.
These include the ribosomal RNAs and transfer RNAs (which are used in protein synthesis) and the small nuclear RNAs (which are used in RNA processing). In addition, there are regulatory RNAs (such as the microRNAs that we will discuss later in this chapter), which are involved In regulating gene expressi and are not translated into peptides . on 40 CHAPTER 2 TFIID O The TFIID complex binds to the TATA box through its TBP subunit epG islands, regions of DNA rich in those two nucleotides (Down and Hubbard Euka ryo tic RNA 2002).
polym erases will not bind t o the "naked" TATA sequence; they require the presence of addi tional proteins to place the polymerase properly on the pro the TATA-binding pro tein (TBP), which forms a complex (TFUD) with other proteins to create a "saddle" upon which the RNA poly merase sits; and TFITB, which recruits RNA polymera se to the TBP and positions it in such a manner that it can read the DNA c odons (Kostrewa et aJ. 2009). Other p rote ins (TFIIA and TFIIH) stabilize the complex. In addition, aux iliary transcription-associated factors (TAFs) stabilize the RNA polymerase on the promoter and enable it to initiate transcription. These TAFs are bound by upstream promot er elements (some times called proximal promoter sites), which are DNA sequences near the TATA box and us ua l ly upstream hom it. Eventually, TFIIH will phosphorylate the carboxy terminal of RNA polymerase, releasing it hom TFIIA moter (Figure 2.7). Two of these are -i Tran sc ri ption initiation site 8 TFIID is stabilized by TFllA TFIIA C) TFII B and TFIIH join the complex on tll e TATA box; TFIIE and TFIIF associate with RNA polymerase 11 TFIIH the saddle so that it can transcribe the mRNA. An e nhancer is a DNA sequence that controls the effi ciency and rate of transcription from a specific promoter. In other words, enhancers tell where and when a promot much of the gene product to make. Enhancers bind specific transcription fadors, pro teins that activate the gene by (1) recruiting enzymes (such as histone acetyltransferase s) that break up the nucleo SOmes in the area or (2) stab ilizing the transcription iJtiti ation complex as describ ed above Enhancers can activate only cis-linked promoters (i e., promoters on the same chromosome*); therefore they are er can be used, and how . . o RNA polymerase II is positioned by TFIIB, and its RNA sometimes caned cis[egulatory elements.
However, carboxy-terminaJ domain is bound by TFIID because of DNA folding, enhancers can regulate genes at great distances (some as great as a million bases away) from the promoter (Visel et aI.
2009). Moreover, enhancers do not 5' (upstream) side of the gene; they can be at the 3' end, or even in the introns (Maniatis et al. 1987). The human p-globin gene has an enhancer in its 3' UTR. This enhancer sequence is necessary for the temporal- and tissue-specific expression of the p-globin gene in ad ult red blood cell precursors (Trudel and Constantini 1987). One of the principal methods of identifying enhancer sequences is to clone DNA sequences flanking the gene of in terest and fuse them to re po rter genes whose products are both readily id entifia ble and no t usuall y made in the need to be on the ·Os· and traits-regulatory elemen ts arc so named by analogy with E. coli genehcs and organic chemistry. There, cis-elements are regula tory elements Ulat reside on the same slTand of DNA (cis-, "on the same side as"), while trans-elements are those that could be supplied from another

Trithorax and Poly PIONEER TRANSCRIPTION FACTORS: BREAKING THE SILENCE comb families. When bound to the nucleosomes of active Finding a promoter is not easy, because the DNA is usual genes, Trithorax proteins keep these genes active, where ly so wound up that the promoter sites are not accessible. as Polycomb proteins, which bind to condensed nueleo Indeed, more than 6/eet of DNA is packaged into chromo somes, keep the genes in an inactive state . somes of each human cell nucleus (Schones and Zhao The Polycomb proteins fall into two categories tha t act 20(8). sequentially in repression.
The first set has histone methyl How can a transcription factor find its binding site, transferase activities that methylate lysines H3K27 and given that the enhancer might be covered by nucleo H3K9 to repress gene activity. In many organisms, thit,; somes? Several studies have identified certain transcrip repressive state is stabilized by the activity of a second set tion factors that penetrate repressed chromatin and bind of Polycomb factors, which bind to the methylated tails of to their enhancer DNA sequences (Cirillo et a1. 2002; DEVELOPMENTAL G E N ETICS FIGURE 2.15 Model for the role of the "pioneer" tran scrip ti on factor Pbx in aligning th e muscle-specific transcription factor MyoD on DNA (A) Pbx protein recogn izes its DNA bi nding site eTG.A.T), even within nucleosome-rich c hromatin . Pbx probably binds to transcriptional inhibitors. (8) MyoO, com pl exed with its E'I2 cofactor, is able to bind to Pbx, replacing the transcriptional (A) r7 MyoD-U TGAT inhibitors. MyoD then binds to its recognition element on the DNA, (e) The MyoD/E1 2 complex can then recruit the histone acetyltransfcrases and nucleosome remodeling compounds that make the chro ati n accessible to other transcription factors (Mef3 and Mef2) and to RNA polymerase.
(AncrTapscott 2005.) M3 m Berkes e t al. 2004). They have been called " pioneer " Transcriptional tran scription factors, and they appear to be critical in estab lishing certain cell lineages. One of these transcription fac tors is FoxAl, which binds to certain enhancers and opens lip the chromatin to allow other transcription factors access to the promoter (Lupien et al. 2008). FoxA proteins remain bound to the DNA during mitosis, providing a mechanism to re-establish normal transc ription in pre sumptive liver cells (Zaret et a l. 2008). Another pione er transcription factor is the Pax? protein mentioned above. It activates muscle-specific gene transcription in a popu rtition sequence and being stabilized there by dimethylat (C) 47 ◆ M", IL◆ _ M3 M2 !..OJ M2 ,,, ◆ ◆ M3 M2 d r Transcription J lation of muscle stem cells by binding to its DNA recog sequence, given the name neural restrictive silencer ele ed H3K4 on the nucleosomes. It then recruits the histone ment methyl transferase that converts the dimethyla ted H3K4 whose expression is limited to the nervous system: those Another pioneer transcription factor in muscle devel binds to the NRSE is a zinc finger transcription factor called into the trimethylated H3K4 associated with active tran scription (McKinnell et al. 2008). opment is Pbx. Members of the Pbx family are made in (NRSE), has been found in several mouse genes encoding synapsin I, sodium channel type II, brain-derived neurotrophic factor, Ng-CAM, and L l .
The protein that neural restrictive silencer factor (NRSF). (It is also called in every cell that is every cell, and they are able to find their appropriate sites REST). NRSF appears to be expressed even in highly co mpacted chromatin. Pbx appears to b e 1101 a mature neuron (Chong et al. 1995; Schoenherr and used a s a "molecular beacon" for another muscle-deter Anderson 1995). To test the hypothesis that the NRSE sequence is neces mining transcription factor, MyoD (mentioned earlier). MyoD is critical for initiating muscle development in the sary for the normal repression of neural genes embryo, activating hundreds of genes that are involved ral cells, transgenes were made by fusing a ◆-galactosidase with estab lishing the muscle phenotype. However, MyoD is not able to bind to DNA without the help of Pbx pro sequence recognized by MyoD (Figure 2.15A). Berkes and teins, which bind to DNA elements adjacent to the DNA in non-neu (lacZ) gene with part of the L1 neural cell adhesion gene. (Ll is a protein whose function is critical for brain clevel opment as we will see l L1 in later chapters.) In one case, the gene, from its promoter through the fourth exon, was have shown that My aD (when com fused to the lacZ sequence. A second transgene was made plexed with another transcription factor, E12) can bind to just like the first, except that the NRSE was deleted from the Pbx protein and align itself on its target DNA sequence (Figure 2. 1 5 B) . Once bound there, the E12 protein recruits the L1 promoter. The two transgenes were s◆parately inserted into the pronuclei of fertilized oocytes, and the complexes to open up the DNA on those genes (Figure dase expression (Kallunki et al.
1995, 1997). In embryos 2.15C). receiving the complete transgene (which included the St LENCERS Silencers are DNA regulatory elements that actively repress the transcription of a partic ular gene. They (Figure 2.16A) . In mice whose transgene lacked the NRSE colleagues (2004) histone acetyltransferases and nucleosome remodel ing resulting transgenic mice were analyzed for ◆-galactosi NSRE), expression was seen only in the nervous system can be viewed as "negative enhancers." For instance, in the mouse, there is a DNA sequence that prevents a pro moter's activation in any tissue except ne urons. This sequence, however, expression was seen in the heart, the limb mesenchyme and limb ectoderm, the kidney meso denn, the ventral body wall, and the cephalic mesenchyme (Figure 2.16B). CHAPTER 2 48 (A) promoler its importance for transcription. Now we look at how the DNA itself can be methylated to regulate transcription.
sequence Gene,rally speaking, the promoters of inactive genes OL_o..L)=),-mj_) IT -=) .◆=D NRSE LI become methylated at certain cytosine residues, and the resulting methylcytosine stabilizes nucleosomes and pre vents transcription factors from binding. It is often asswned that a gene contains exac tly the same lacZ nucleotides whether it is active or inactive; that is, a ◆-glo bin gene that is activated in a red blood cell precursor has the same nucleotides as the inactive p-globin gene in a fibroblast or retinal cell of the same animal.
However, it (B) promoter "--",,,"-w/..»='IT Ll -) LI Nosequenc NRSEe Hotchkiss discovered a "fifth base" in DNA, 5-methylcy tosine. In vertebrates, this base is made enzymatically after ':;J lacZ h tums out there is in fact a subtle difference. In 1948, R. D, DNA is replicated, At this time, about 5% of the cytosines in mammalian DNA are converted to 5-methylcytosine (Figure 2.17A). This conversion can occur only when the cytosine resid ue is followed by a guanosine-in other words, at a CpG sequence (as we will soon see, this restric tion is important). Numerous studies have shown that the n degree to which the cytosines of a gene are methylated can control the level of the gene's transcription. C ytosine methylation appears to be a major mechanism of transcrip FIGURE 2.16 Silencers. A alys i s of ◆-gal actos idase staining pat terns in 1 1 .S-ciay embryonic mice. (A) Embryo containing a trans gene composed of t e L 1 prom oter, a porti on of the L1 gene, and a lacZ ge e fused to the second exon (which conta i ns (he NRSE region), (B) Embryo containing a similar transgenc but lacking the NRSE seq uence. The dark areas reveal the presence of ◆-ga lac tosi dase (the lacZ product). (Photographs from Kallunki et a l . 1 997.) n tional regulation among vertebrates; however, some other species (Drosophila and nema todes among them) do not methylate their DNA.
In vertebrates, the presence of methylated cytosines in a gene's promoter correlates with the repression of tran scription from that gene. In developing hwnan and chick red blood cells, for example, the DNA of the globin gene promoters is almost complctely uJ1lnethylated, whereas the same promoters are highly methylated in cells that do not DNA Methylation and the Control ofTranscription produce globins. Moreover, the methylation pattern How does a pattern of gene transcription become stable? lated promoters in the genes encoding the £-globins changes during development (Figure 2.1 76). The cells that produce hemoglobin in the human embryo have urunethy How can a lens cell continue to remain a lens cell and not ("embryonic globin chains") of embryonic hemoglobin, activate muscle-specific genes?
How can cells undergo These promoters become methylated in the fetal tissue, as than the embry rounds of mitosis and still main tain their differentiated the genes for fetal-specific y-globin (rather characteristics? The answer appears to be DNA methyla onic chains) become activated (van der Ploeg and Flavell tion. We have already discussed 1980; Groudine and Weintraub 1981; Mavilio et a1. 1983). histone methylation and Methyl a ted Unmethyl ated (A) promoter om te NH2 A o n gene I e-glo◆in gen◆ CH, NJ:: Me◆tion 17' :x Weeks I bL◆=====◆L-◆◆◆,◆◆=- ◆◆◆◆ DNA Inactive o◆) Active O◆ N o n Demelhyl a tion H I DNAI DNA U Cytosine ylcytosi.ne Weeks [d l)):◆::◆:}J))=;= ;; =)Dl:;:;:;

Drosophila. . It appears that microRNAs are able to bind to the nuclear RNA as it is being transcribed, and form a com plex with the methylating and deacetylating enzymes, th u s repress i ng the gene (Kato et a l . 2005; Schramke et a l .
2005). If synth et ic microRNA made complementary to specific promoters is added to cul tured human cells, that microRNA is able to induce that promoter's DNA to become methylated.
Lysine 9 on his tone H3 also becomes methylated around the promoter, and transcrip tion from that gene stops (Kawasaki and Taira 2004; Mo rris et a l . 2004). This appears to be the mechanism by which NRSF (see page 47) functions. NRSF prevents gene expression in non neural cells by repress i ng microRNAs that would otherwise recruit histone acetyltransferases to activate genes that promote neuron production. In the presence of NRSF, these miRNAs are not present, and so histone deacetylases and methyltransferases are recruited to the chromatin instead. The resulting methylation produces conglomeration s of nucleosomes l i n ked together by hete rochromatin p rotei n- l (HP1), thereby stabilizing the conglomera te and pre venting transcription of the neu ron pro- of miR430 goes up very rapidly. This nticroRNA has hun dreds of ta rget s (about 40% of t h e maternal RNA types), and when it binds to the 3' UTR of these target mRNAs, these mRNAs lose their polyA tails and are degra ded (Giraldez et aI. 2006). Slightly later in developm ent this same microRNA is used in the fish embryo to fine-tune the expression of Nodal mRNA (Choi et a!. 2007). The conse quence of this latter use of miR430 is the determina tion of The l ymp hoi d precursor cell can generate B cel ls (lymphocytes that make antibodies) orT cel ls (lymphocytes that kill vira lly infected cells). This differentiation depends on the organ in which they reside. The regulation of the l i n eage pathway is controlled i n part by levels of the microRNA mill 181. The lym phocyte precursor cell has l i U le mi1l 181.
A B cell has high levels of miR 1 8 1 , w ereas T cells do not appear to have any. If lymp ho cyte precursor cells are virally transfected with miR 181, they pref eren tial ly generate B cells at the expense of T cells. " Thus, microRNA directed against the 3' end of mRNA may be able to shut down gene ex pression on the translational level, while microRNA di rected at the promoters of genes may be able to block gene expression at the tra n scr i pti ona l level. The thera peutic value of these RNAs in cancer therapy is j u st beg i n n i ng to be explored (see Gaur and Rossi 2006). X chromosome inactivation i n mammals is also directed b y small noncoding RNAs, albeit not the canonical microRNAs. Although DNA methylation is responsible for keeping one of the two X chromosomes inac tive, the choice of which X ch romo some to activate a rises from the physi cal interactions between the two X c h rom oso mes and their expressing several small, noncoding RNAs. The mech a n i sms of differentia l expression of small RNAs between the two X ch rom oso mes is under intensive inves tigation (Augu i et a l . 2007; Migeo n 2007; Ogawa et a l . 2008).
, Lymphoiceld l precursor V ◆ Low miRJ81 2.36 h " how many cells become committed to the endoderm and how many become committed to be mesoderm. Althou gh the microRNA is usually about 22 bases long it recognizes its target primarily through a "seed" reg ion , FIGURE meting genes hidden within it (Ooi and Wood 2007; Yoo et al. 2009.) A sin gl e silencer protein bound to the DNA can prevent the gene's expression. I \ @ cell @cell Lymphoiy d T miRJ81 @) ◆ Number cells l I 1 i R 223 miR18J of (no miR223) (no miRIBJ) enhanced B precursor cell + v i Tall activated B ofNumber cells reduced T 64 CHAPTER 2 FIGURE 2.37 The miRNA complex, including numerOus pro· teins that bind to the miRNA (miR PI, can block translation in several ways. These include (A) blocking the binding or the mRNA to initiation factors or ribosomes; (B) recruiting endonu· cleases to chew away the polyA tail or the mRNA, thereby caus ing its destruction; and (C) recru i ti ng protein-digesting enzymes that destroy the nascenl protein. (After Filipowicz et al. 2008.) of about 5 bases in the 5' end of the microRNA (usually at positions 2-7). This seed region recognizes targets in the 3' UTR of the message. What happens, then, if an mRNA has a mutated 3' UTR? Such a mutation appears to have given rise to the Texel sheep, a breed with a large a nd weJl-defined musculature that is the dominant meat-pro- ducing sheep in Europe. We have already seen that a mutation in the myoslalin gene that prevents the proper splicing of the nRNA can produce a large-muscled phe notype. Another way of reducing the levels of myos tatin involves a mutation in its 3'UTR sequence (see Fi gu re 2.30). Genetic techniques mapped the basis of the sheep's meaty phenotype to the myoslatin gene. In the Texel breed, there has been a G-to-A transition in the 3' VIR of the gene for my o sta tin, creating a target for the mirl and ",ir206 microRNAs that are abundant in skeletal muscle (Clop et al. 2006). This mutation causes the depletion of myostatin messages and the increase in muscle mass characteristic of these sheep. The binding of microRNAs to the 3' UTR can regulate translation in several ways (Figure 2.37; Filipowicz et al. 2008). First, they can block initiation of lPanslation, pre venting the binding of initiation factors or ribosomes. Sec ond, they can recruit endonucleases that digest the rnRNA, usually starting with the polyA tail. In a third mechanism, they allow translation to be initiated, but recruit proteolyt ic enzymes that digest the protein as it is being made. It is also possible that some microRNAs use more than one method, and it has been proposed (Mathonnet et al. 2007) that the microRNAs may first inhibit translation initiation and then consolidate mRNA silencing by causing the d igestion of the message. Control of RNA expression by cytoplasmic localization Not only is the time of mRNA translation regulated, but place of RNA expression. A majority of mRNAs (about 70% in Drosophila embryos) are localized to specif ic places in the cell (Lecuyer et al. 2007). Just like the selec tive repression of rnRNA translation, the selective 10caUza tion of messages is often accomplished through their 3' UTRs. There are three major mechanisms for the localiza tion of an rnRNA (Figure 2.38; see Palacios 2007): so is the • DiffUSion and local anchoring. Messenger RNAs such as nanos diffuse free l y in the cytoplasm. Howe v er, when they diffuse to the pos terior pole of the Drosophila oocyte, (A) Initiation block --- Initiation com plex --◆--"" 3' UTR (B) Endonuclease digestion (de-adenylation) Coding mRNA (C) Proteolysis Degradation of nascent peptide they are trapped there by proteins that reside particular ly in these regions .
These pr o teins also activate the mRNA, allowing it to be translated. • • Localized prolection. Messenger RNAs such as those encoding the Drosophiln heat shock protein hsp83 (which helps protect the emb ryos from thermal extremes) also float freely in the cy top la sm . Like nanos mRNA, hsp83 accumulates at the posterior pole, but its mechanism for getting there is different. Throughout the embryo, the protein is degraded. However, proteins at the poste rior pole protect the hsp83 mRNA from being destroyed. Active tral1sport along the cytoskeletol1. This is probably the most widely used mechanism for mRNA localization . Here, the 3' VIR of the rnRNA is recognized by proteins that can bind these messages to "motor proteins" that trav el along the cytoskeleton to their final destination.
These motor proteins are usuany ATPases such as dynein Or kinesin that split ATP for their motive force. For instance, in Drosophila oocytes, the bicoid messages (which instruct the formation of the head) are localized to one end of the oocyte. The 3' VIR of bicoid mRNA aUows its message to bind to the microtubules through its association wiU, two other proteins (Swallow and Staufen). If the bicoid 3' VIR is attached to some other message, that mRNA will also be bound to the anterior pole of the oocyte (Driever and Niisslein-Volhard 1988a,b; Ferrandon et al. 1994). The 3' VIR of the bicoid message binds the Staufen protein that connects it to dynein . Dynen i travels along the micro t ub u l es in the "mmus" direction, that is, toward the si te where tnicrotubules begin. In this way, the bicoit! mRNA is localized to U,e future anterior p a rt of the oocyte. Other mRNAs, such as the Oskar message, in contrast, appear to DEVELOPMENTA L GEN ETICS (A) D iffusion and local anchoring Anterior \ I --! -- ◆ Posterior I◆/◆ 1/ v' ◆- \ c;:)::= nanos mRNA ◆ o Anchor being translated = nanos RNA ------- (B) Localized protection \ 0 ':, I ------- Q ◆ ◆------- = hsp83 mRNA o Protector protein complex ◆ Deadenylase complex (degrades hsp83) (C) Active transport along cytoskel eton .
---"◆ Microtubules g Dynein fiGURE 2.38 Localization of mRNAs. (A) Diffusion and local ancho ring . Nanos mRNA diffuses through the Drosophila egg and is bound (in part by the Oskar protein, whose message is described i n the text) at the posterior end of the oocyte. Th is anchoring allows the nanos mRNA to be translated. (B) ocal ized protection. The mRNA for Drosophila heat shock protein (hsp83) will be degraded unless it binds to a protedor protein (in this case, also at the poste rior terminal of the oocyte). (C) Active transport on the cytoskele ton, ca u sing the accumulation of mRNA at a parti cu lar site. Here, bicoid mRNA is transported to the anter ior of the oocyte by dynein and kinesin motor proteins. Meanwhile, Oskar mRNA is brought to the posterior pole by transport along microtubules by kinesin ATPases. (After Pa lac ios 2007.) L mRNAs often bind to other cytoskeletal proteins (such as actin microfiJaments). Stored mRNAs in brain cells c?% / 01 65 mRNA Q=;a " Ki esin bicoid n mRNA bind to the kinesin motor protein, and it is taken toward the "plus" end of the microtubules, at the tip of their assetnbl y. It is thereby taken to the posterior end of the Drosophila oocyte. Once transported to their destinations, One of the most important areas of local translational reg ulation may be in the brain. The storage of long-term mem ory requires new protein synthesis, and the local transla tion of mRNAs in the dendrites of brain neurons has been proposed as a control point for increasing the strength of synaptic connections (Martin 2000; Klann et al. 2004; Wang and Tiedge 2004). The ability to increase the strength of the connections between neurons is critical in fOrming the orig inal architecture of the brain and also in the ability to learn. Indeed, in recent studies of mice, Kelleher and coHeagues (2004) have shown that neuronal actiVity-dependent mem ory storage depends on the activation of eIF4E and other components of protein synthesis. Several mRNAs appear to be transported along the cytoskeleton to the dendrites of neurons (the "receiving por tion" of the neuron, where synapse connections are fonned with the other neurons). These messages include those mRNAs encoding receptors for neurotransmitters (needed to transmit the signals from one neuron to another); activi ty-regulated enzymes; and the cytoskeletal components needed to build a synapse (Figure 2.39). As we will see in later chapters, one of the proteins responSible for construct ing specifiC synapses is brain-derived neurotrophic factor, or BONF. BDNF regulates neural activity and appears to be critical for new synapse formation. Takei and colleagues (2004) have shown that BDNF induces local translation of these neural messages in the dendrites. Another indication of the importance of dendritic mRNA translation comes from studies of a leading cause of human mental retardation, fragile X syndrome. Fragile X syndrome is caused by loss-of-function mutations in tl,e X-linked FMRI gene. The FMRI protein appears to pre vent the translation of several mRNAs that are being trans ported to the dendrites along microtubules in response to stimulation by glutamic acid (Oictenberg et al. 2008; Wang et al. 2008b). In the absence of functional FMRl, these rnRNAs are expressed in the wrong amounts, leading to signaling abnormalities that are believed to cause the prolr CHAPTER 2 66 FIGURE 2.39 A brain-specific RNA in a cultured mammalian neuron. BCl RNA (stained white) appears to be clustered at specific sites i n the neuron (stained light blue), especially in the dendrites. (From Wang and liedge 2004, courtesy of the authors.) Cell body 25 !!m lems in cognition and learning. Thus, translational regula Some newly synthesized proteins are inactive without tion in neurons might be important not only for their ini the cleaving away of certain inhibitory sections. This is tial development but also for their contiinued ability to what happens when insulin is made from its larger pro learn and change. tein p recursor. Some proteins must be "addressed" to their Posttranslational regulation of gene expression teins are often sequestered in certain regions of the cell, When a protein is synthesized, the story is still not over.
Some proteins need to assemble with other proteins in Once a protein is made, it becomes part of a larger level of order to form a organization. For instance, it may become part of the struc the microtubule, and the ribosome are all examples of tural framework of the cell, or it may become involved in numerous proteins joining together to form a functional specific intracellular destinations in order to function. Pro such as membranes, lysosomes, nuclei, or mitochondria. hmctional unit. The hemoglobin protein, one of the myriad enzymatic pathways for the synthesis unit. And some proteins are not active unless they bind an or breakdown of cellular metabolites. ion (such as Ca'·), or are modified by the covalent addi In any case, the indi vidual protein is now part of a complex "ecosystem" that tion of a phosphate or acetate gro up. The importance of integrates it into a relationship with numerous other pro this last type of protein modification will become obvious teins. Thus, several changes can still take place that deter in Chapter 3, since many of the critical proteins in embry mine whether or not the protein will be active. onic cells just sit there until some signal activates them. Snapshot Summary: Developmental Genetics 1. Differential gene expression from genetically identi 3. Eukaryotic genes contain promoter sequences to cal nuclei creates different cell types. Differential which RNA polymerase can bind to initiate tran gene expression can Occur at the levels of gene tran scription.
To accomplish this, the eukaryotic RNA scription, nuclear RNA processing, mRNA transla polymerases are bound by a series of proteins called tion, and protein modification. Notice that RNA pro transcription-associated factors, or TAFs. cessing and export occur while the RNA is still being transcribed from the gene. 2. Genes are usually repressed, and activating a gene often means inhibiting its repressor. This fact leads to thinking in double and triple negatives: Activa tion is often the inhibition of the inhibitor; repression is the inhibition of the inhibitor of the inhibitor. 4. Eukaryotic genes expressed in specific cell types contain enhancer sequences that regulate their tran scription in time and space. 5. Specific transcription factors can recognize specific sequences of DNA in the promoter and enhancer DEVELOPMENTAL G E N ETICS regions. These proteins activate or repress transcription from lhe genes to which they have bound. 6. Enhancers work in a combinatorial fashion. The binding of several transcription factors can act to promote or inhibit transcription from a certain promoter. ln some cases transcription is activated only if transcription is activated if either factor A or factor B both factor A and factor B are present; in other cases, is present. 7.
A gene encoding a transcription factor can keep itself activated if the transcription factor it encodes also activates its own promoter. Thus, a transcription factor gene can have one set of enhancer sequences to initiate its activation and a second set of enhancer sequences (which bind the encoded transcription factor) to maintain its activation. 8 Often, the same transcription factors that are used during the differentiation of a particnlar cell type are also used to activate the genes for that cell type's specific products. 9.
Enhancers can act as silencers to suppress the transcription of a gene in inappropriate cell types.
10. Transcription factors act in different ways to regulate RNA synulesis. Some transcription factors stabilize RNA polymerase binding to the DNA; some disrupt nucleosomes, increasing the efficiency of transcription. 11. Transcription correlates with a lack of methylation on the promoter and enhancer regions of genes. Methylation differences can account for examples of genomic imprinting, wherein a gene transmitted through the sperm is expressed differently than the same gene transmitted through the egg. 67 12. Dosage compensation enables the X chromosomederived products of males (which have one X chromosome per cell in fruit flies and mammals) to equal the X chromosome-derived products of females (which have two X chromosomes per cell). This compensation is accomplished at the level of transcription, either by accelerating transcription from the lone X chromotranscription from each X chromosome by 50% (C elegans), or by inactivating a large portion of one of the two X chromosomes in females (mammals). some in males (Drosophila), decreasing the level of 13. Differential nuclear RNA selection can allow certain transcripts to enter the cytoplasm and be translated while preventing other transcripts from leaving the nucleus. 14. Differential RNA splicing can create a family of related proteins by causing different regions of the nRNA to be read as exans' or introns. VVhat is all exon in one set of circumstances may be an intron in another. 15. Some messages are translated only at certain times. The oocyte, in particular, uses translational regulation to set aside certain messages that are transcribed during egg development but used only after the egg is fertilized. This activation is often aCCOffiplished either by the removal of inhibitory proteins or by the polyadenylation of the message.
16. M.icroRNAs can act as rranslational inhibitors, binding to the 3' UTR of the RNA. 17. Many mRNAs are localized to partiCllar regions of the oocyte or other cells. This localization appears to be regulated by the 3' UTR of the mRNA. For Further Reading Complete bibliographical citations for aU literature cited in this chapter can be found at the free-access website www.devbio.com Clop, A. and 16 others. 2006. A muta tion creating a potential iUegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nature Genet. 38: 813-81S. Davidson, E. H. 2006. The Regulatory Genome. Academic Press, New York. Migeon, B. R. 2007. Females Are Mosaics: X Inactivation and Sex Differences ill Dis ease. Oxford University Press, New York. Palacios, I. M. 2007. How does an mRNA find its way? Intracellular local ization of h·anscripts. Sem. Cell Oev. BioI. 163-170. Strahl, B. D. and C. D. Allis. 2000.
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P., C. Girardot, J. Gagneur, M. Braun and E. E. Furlong.
2009. Com binatorial binding predicts spatio temporal Cis-regulatory activity. Nature 462: 65-70. 68 CHAPTER 2 Go Online WEBSITE 2.1 Does the genome or the cytoplasm direct WEBSITE 2.6 Cloning and nuclear equivalence. The sev development?
The geneticists versus the embryologists. Geneticists were certain that genes controlled development, whereas embryologists generally favored the cytoplasm. Both sides had excellent evidence for their positions. eral entries here address WEBSITE 2.2 The origins of developmental genetics. WEBSITE 2.7 Silencing large blocks of chromatin.
The inactivation or the elimination of entire dU'omosomes is not uncommon among invertebrates and is sometimes used as a mechanism of sex determination. Moreover, among mammals, random X chromosome inactivation may provide females with health benefits-as long as the process occurs flawlessly. The first hypotheses for differential gene expression came from C.
H. Waddington, Salome Gluecksohn-Waelsch, and other scientists who understood both embryology and genetics. WEBSITE 2.3 Techniques of DNA analysis. The entries of this website describe crucial laboratory skill including gene cloning, DNA sequencing, Southern blotting, "knock outs" of specific genes, enhancer traps, and identification of methylated sites.
WEBSITE 2.4 Techniques of RNA analysis. Techniques described here include northern blots, RT-PCR, in situ hybridization, microarray technology, antisense RNA, interference RNA, Cre-Iox analysiS, and ChIP-on-Chip. WEBSITE 2.5 Bioinformatics. This entry provides links to various free web sites with tools that enable researchers to compare DNA sequences and specific genomes with the aim of further illuminating the various mechanisms of gene regulation. the issues of cloning and whether or not the entire genome is the same in each cell of the body. As it turns out, lymphocytes make new genes during their development and their genomes are not identical. WEBSITE 2.8 So you think you know what a gene is? Different scientists have differen t definitions, and nature has given us some problematic examples of DNA sequences that may or may not be considered genes. Vade Mecum in Drosopllila. These movies describe Ernst Hadorn's discovery of transdetermination and Walter Gehring's pioneering study of homeotic mutants, changing body parts into eyes through transcrip tion factors. Transdetermination Cell-Cell Com m unication in Development THE FORMATION OF ORGANIZED ANIMAL BODIES, or morphogenesis, has been one of the great sources of wonder for humankind.
Indeed, the "miracle of life" seems just that-inanimate matter becomes organized in such a way that it lives. The twelfth-century rabbi and physician Maimonides (1190) framed the ques tion of morphogenesis beautifully when he noted that the pious men of his day believed that an angel of God had to enter the womb to form the organs of the embryo. This, the men say, is a miracle. How much more powerful a miracle would life be, Maimonides asked, if the Deity had made matter such that it could generate SUdl remarkable order without a matter-molding angel having to inter vene in every pregnancy? The problem addressed today is the secular version of Maimonides' question: How can matter alone construct the organized tissues of the embryo? The idea of angelic intervention remained prevalent in the embryology of the Renaissance. By the eighteenth century, however, scientific advances had allowed In the learned to dispense with the necessity of involving heavenly beings in human conception and development, even though the process remained a mystery. 1782, the Eniightenment essayist Denis Diderot posed the question of morpho genesis in the fevered dream of a noted physicist. This character could imagine that the body was formed from myriad "tiny sensitive bodies" that collected together to form an aggregate, but he could not envision how this aggregate could become an animal.
Diderot's "tiny sensitive bodies" are what we call cells, and we can break his problem into at least five questions that confront modern embryologists who study morphogenesis: 1. How nre sepnrnte tissues formed from populatiolls of cells ? For example, how do neural retina cells stick to other neural retina cells rather than becoming part of the pigmented retina or the iris cells next to them? How are the different cell types found in the retina (the three distinct layers of photoreceptors, bipolar neurons, and ganglion cells) arranged such that the retina is functional? 2. How are organs constructed from tissues? The retina of the eye forms at a pre cise distance behind the cornea and the lens. Tile retina would be useless if i t developed behind a bone or i n the middle of the kidney. Moreover, neurons from the retina mllst enter the brain to innervate the regions of the brain cor tex that analyze visual information. All these connections must be precisely ordered. 3. How do organsform in particular locations, and how do migrating cells reach their destinations? What causes there to be two-and usually only two-kidneys, and how do their ducts form SO that they can collect urine made by the filter- All that you touch You Change. All that you Change Changes you. The on.ly lasting tntth Is Change. OCfAVIA BurLER (1 998) The behaviour of a cell in an embryo depends on the exten. t to which it lis tens to its mother aT its neighborhood. The size and nature of the noise, the way in which it is heard, and the response are unpredictable and can only be discovered by experimenul.tion. JONATHAN BARD (1997) 70 CHAPTER 3 Just (1939) and Johannes Holtfreter (Townes and Holtfreter ing tissues of the nephron? Some cells-for instance, the 1955) predicted that embryonic cells could have differences in their cell membrane components which would enable the formation of organs. In the late tvventieth century, these precursors of our pigment cells, germ cells, and blood cells-must travel long distances to reach their final des tinations. How are cells instructed to travel along cer- membrane components-the molecules by which embry tain routes in our embryonic bodies, and how are they onic cells adhere to, migrate over, and induce gene expres told to stop once they have reached their appropriate sion in neighboring cells-began to be discovered and destinations? 4. described. And presently, these pathways are being mod- How do organs and their cells grow, and how is their growth coordinated throughout development? The cells of all the eled to understand how the cell integrates the information from its nucleus and from its surroundings to take its place tissues in the eye must grow in a coordinated fashion if one is to see. Some cells, including most neurons, do not in the community of cells. divide after birth. Ii) contrast, the intestine is constantly of an embryo are either epithelial cells or mesenchymal As we discussed in Chapter 1 (see Table 1.1), the cells shedding cens, and new intestinal cells are regenerated each day. The mitotic rate of each tissue must be care cells. The epithelial cells can form tubes and sheets while it sloughed off, it could produce tumorous outgrowths. mal cells often migrate individually and form extensive fully regulated. If the intestine generated more cells than remaining adhered to one another, whereas the mesenchy extracellular matrices that keep the individual cells separate. If it produced fewer cells than it sloughed off, it would This chapter will discuss the mech◆sms of truee behav soon become nonfunctional. What controls the rate of iors requiring cell-cell communication: cell adhesion, cell mitosis in the intestine? 5. How do organs achieve polarity?
If one were to look at a migration, and cell signaling. cross section of the fingers, one would see a certain organized collection of tissues-bone, cartilage, mus Cell Adhesion cle, fat, dermis, epidermis, blood, and neurons. Look ing at a cross section of the forearm, one would find the Differential cell affinity same collection of tissues. But they are arranged very Many of the answers to our five questions about morpho differently. How is it that the same cell types can be genesis involve the properties of the cell surface. The cell arranged in dillerent ways in different parts of the same surface looks pretty much the same in all cell types, and structure, and that fingers are always at the end of the many early investigators thought that the cell surface was arm, never in the middle? not even a living part of the cell. We now know that each Answers to these questions came slowly and are still com type of cell has a different set of proteins in its cell mem ing. In the 1850s, Robert Remak (1852, 1855) formulated the cell theory and showed that the fertilized egg divides to brane, and that some of these differences are responsible for forming the structure of the tissues and organs during produce the myriad "tiny sensitive bodies"-cells-need development. Observations of fertilization and early ed to form an embryo. In the mid-twentieth century, E. E. embryonic development made by E. E. Just (1939) suggest- Reaggregation of cells from amph ib ian neurulae. Presumptive epider mal cells from pigmented embryos and neural plate cells from unpigmented embryos are dissociated and mixed together. The cells reaggregate so that one type (here, the presumptive ep idermis) covers the other. (After Townes and H ol tfreter 1 955.) FIGURE 3.1
/-◆ / epidermal cells " y------.... ◆ ◆ Presumptive (◆ VJ ,---. \ .
◆ ., r . " ◆◆ . .
.
Dissociation ◆ . . Neural plate cells Spontaneous reaggregation < . ◆◆◆◆ _-,6◆3illttBi'0 Outside Neural cells Epidermal cells Cross section CELL-CELL COMMUNICATION I N DEVELOPMENT (B) (AI (C) + Epidermis Mesoderm mesoderm + endoderm + endoderm mesoderm (El (D) Epidermis + 71 Neural plate + Neural plate axial mesoderm + + epide r mis epidermis Neural plate Epider m is ' Neural plate ◆◆ Epidermis Mesoderm Neural plate the experiments of Townes and Holtfreter in 1955. Taking fiGURE 3.2 Sorting out and reconstruction of spatial relation ships in aggregates of embryonic amphibian cells. (Aher Townes and H o ltlreter 1 955.) advantage of the discovery that aJ;Ilphlbian tissues become to the inside ed that the cell membrane differed among cell types, but the experimental analysis of morphogeneSiS began with dissociated into single cells when placed in alkaline solu (Figure 3.1). In no case do the recombined cells remain randomly mixed; in most cases, one tissue type tions, they prepared single-cell suspensions from each of completely envelops U,e other. the three genm layers of amphlbian embryos soon after the suspensions could be combined in Various ways. When the final positions of positions in the embryo. TI,e reaggregated mesoderm migrates central of the solution was normalized, the cells adhered to ly with respect to the epidermiS, adhering to the inner epi one another, forming aggregates on agar-coated petri dish 3.2A). The mesoderm also migrates centrally with respect to the gut or endoderm (Figure 3.2B). neural tube had formed. Two or more of these Single-cell pH Second, the researchers found that the the reaggregated cells reflect their respective dermal surface (Figure es. By using embryos from species having cells of differ ent sizes and colors, Townes and Holtfreter were able to However, when the three germ layers are mixed together, follow the behavior of the recombined cells. the endoderm separates from the ectoderm and mesoderm The results of their experiments were slriking. First, they ed. That is, instead of two cell types remaining mixed, each and is then enveloped b y them (Figure 3.2C). In the final iound that reaggregated cells become spatially segregat configuration, the ectoderm is on the periphery, the endo type sorts out into its own region. Thus, when epidermal between them. (ectodermal) and mesodermal cells are brought together in a mixed aggregate, the epidermal cells move to the periphery of the aggregate and the mesodermal cells move derm is internal, and the mesoderm lies in the region Holtfreter interpreted this finding in terms of selective affinity.
The inner surface of the ectoderm has a positive affinity for mesodenmal cells and a negative affinity for the 72 CHAPTER 3 (A) endoderm, while the mesoderm has positive affinities for both ectodermal and endodermal cells.
Mimicry of normal embryonic structure by cell aggregates is also seen in the recombination of ep iderm is and ne ural pla te cells (Figure 3.2D; also see Figure 3.1). The presumptive epidemlal cells migrate to the p eriphery as before; the neural plate cells migrate inward, forming a structure reminiscent of the neu ral tube. When axial mesoderm (no tochord) cells are added to a suspension of presump tive epidermal and presump tive neural cells, cell segregation results in an external epi dermal layer, a centra lly located neural tissue, and a layer of mesodermal tissue between them (Figure 3.2E). Some how, the cells are able to sort out into their proper embry Olue p osi tions. The third conclusion of Holtfreter and his colleagues was that selective affinities change during development. Such changes should be expected, because embryonic cells do not retain a single stable relationsh ip with other cell types. For development to occur, cells must interact differ ently with other cell populations at speci fic times. Such changes in cell affinity are extremely important in the processes of morphogenesis. When tissues from later-stage mammalian and chick embryos were made into single cell suspensions (using the enzyme trypsin, which split the proteins connecting the cells together), the cells reaggre gated to form tissuelike arrangements (Moscona 1961; Giu dice and Just 1962). Aggregates formed by mixing 7-day chick embryo neural retina (unpigmented) cells with pigmented retina cells. (A) Five hours after the s ingle-cel l suspensions are mixed, aggregates of andom ly distributed cells are seen. (B) At 1 9 hours, the pig mented retina cells are no longer seen on the periphery. (C) At 2 days, a great majority of the pigmented retina cells are located in a central internal mass, surrounded by the neural retina cells. (The scattered pigm ented cells are probably dead cells.) (From FIGURE 3.3 The thermodynamic model of cell interactions Cells, then, do not sort randomly, but can actively move to create tissue organiz ation .
What forces direct cell move ment during morphogenesis? In 1964, Malcolm Steinberg proposed the differential adhesi on h yp o thesis, a model that sought to exp la in patterns of cell sorting based on thermodynamic principles. Using cells derived from r Armstrong 1 989. courtesy of P. B. Armslrong.) trypsinized embryonic tissues, Steinberg showed that cer tain cell types migrate cenlTally when combined with some cell types, but migrate peripherally when combined with facial free energy. In other words, the ceUs rearrange them others . Figure 3.3 illustrates the interactions between pig selves into the most thermodynamically stable pattern. If mented retina cells and neural retina cells. When single cell suspensions of these two cell types are mixed together, they form aggregates of randomly arranged cells.
Howev er, after several hours, no pigmented retina cells are seen on the periphery of the aggregates, and after 2 days, two distinct l aye rs are seen, with the pigmented retina cells lying internal to the neural retina cells. Moreover, such interactions form a hierarchy (Steinberg 1970). If the final position of ce ll type A is internal to a second cell type B, and the final p osition of B is inte mal to a thi rd cell type C, then the final position of A will always be internal to C. For example, pigmented retina cells migrate internally to neu ral retina cells, and heart cells migrate inte rna lly to pig mented retina cells. Therefore, heart cells migrate internal ly to neural retina cells . This observation led Steinberg to propose that cells cell types A and B have different strengths of adhesion, and interact so as to form an aggregate with the smallest inter- if the strength of A-A connections is greater than the strength of A-B or B-B connections, sorting will occur, with the A ce lls becoming cen tra l . On th e other hand, if the strength of A-A connections is less than or equal to the strength of A-B connections, then the aggregate will remain a s a random mix of cells. Finally, if the slTength of A-A con nections is far greater than the strength of A-B connec tions◆in other words, if A and B cells show essentially no adhesivity toward one another-then A cells and B cells will form separate aggregates.
According to this hypothe sis,. the early embryo can be viewed as existing in an equi librium state until some change in gene activity changes the cell surface molecules. The movements that result seek to restore the cells to a new equilibrium configuration. All that is required for sorting to occur is that cell types differ in the strengths of their adhesion. CELL-CELL COMMUNICATION I N DEVELOPMENT Tiss ue Limb bud (green) Surface tension (dyne/em) £quilibriurn configuration 73 ference in the number of stable contacts made between cell types. In a more specific version of this model, the thermo dynamic differences could be caused by different types of adhesion molecules (see Moscona 1974). When Holtheter's studies were revisited llSing modem techniques, Davis and colleagues (1997) fOlmd that the tissue surface tensions of the individual germ layers were precisely those required for the sorting patterns observed both in vitro an.d in vivo. See VADE MECUM The differential adhesion hypothesis Cadherins and cell adhesion Heart (yellow) 'Recent evidence shows that boundaries between tissues can indeed be created by different cell types having both different types and different amounts of cell adhesion mol ecules. Several classes of mol e◆ules can mediate cell adhe sion, but the major cell adhesion molecules appear to be the cadhe rins As their name suggests, cadherins are calcium-depend ent adhesion molecules. They are critical for establishing and maintaining intercellular connections, and they appear to be crucial to the spatial segregation of cell types and to the organization of animal form (Takeichi 1987). Cadherins are transmembrane proteins that interact with other cad herins on adjacent cells. The cadherins are anchored inside the cell by a complex of proteins called c ateni ns (Figure 3.5A), and the cadherin-catenin complex forms the classic adherens junctions that help hold epithelial cells together, Moreover, since the cadherins and the catenins bind to the actin (microfilament) cytoskeleton of the cell, they integrate the epithelial cells into a mechanical Lmit. Interfering with cadherin function (by univalent antibodies against cad herin or morpholinos against cadherin mRNA) can pre vent the formation of tissues and cause the cells to disag gregate (Figure 3.5B; Takeichi et al. 1979). Cadherin proteins perform several related functions.
First, their external domains serve to adhere cells togeth er. Second, cadherins link to and help assemble the actin cytoskeleton, thereby providing the mechanical forces for forming tubes. Third, cadherins can serve as signaling mol ecules that change a cell's gene expression. In vertebrate embryos, several major cadherin types have been identified. E-cadherin is expressed on all early mammalian embryonic cells, even at the zygote stage. Later in development, this molecule is restricted to e pithe lial tissues of embryos and adults. P-cadherin is found pre dominantly on the p lacenta where it helps the placenta stick to the uterus (Nose and Takeichi 1986; Kadokawa et al. 1989). N-cadherin becomes highly expressed on the cells of the developing central nervous system (Hatta and Take ichi 1986), and it may play roles in mediating neural sig nals. R-cadherin is critical in retina formation (Babb et al. 2005). A class of cadherins called protocadherins (Sano et al. 1993) lack the attachment to the actin skeleton through catenins. Expressing similar protocadherins is an inlpor. Liver (blue) 1.6 Neural retina FIGURE 3.4 H ierarchy of cell sorting in order of decreasing sur face tensions.
The equilibrium configuration reflects the strength of ce ll cohesion, with the ce l l types hav i ng the greater cell cohe sion segregating inside the cel ls with less cohesion. The images were obtained by section i ng the aggregates and assigning colors to the cell types by computer The black areas represent cells whose signal was edited out in the progra m of image optimiza tion. (From Foty et al 1 996, cou rtesy of M. S.
Steinberg and R. A. foty,) . s k U 'r . [n 1996, Poty and his colleagues in Steinberg's labora tory demonstrated that this was indeed the case: the cell types that had greater surfa ce cohesion migrated central ly compared with those cells that had less surface tension (F i g u re 3.4; Poty et aL 1996). In the simplest form of this model, all cells could have the same type of "glue" on the cell surface. The amount of this cell surface product, or the cellular architecture that allows the substance to be differ entially distributed across the surface, could cause a dif- , 74 CHAPTER 3 (B) " FIGURE 3.5 Cad herin-med i ated cell adhesion. (A) Si mpl i fied scheme of cadherin linkage to the cytoskeleton via caten i ns. (B) When an oocyte is injected with an an◆isense ol i gon ucleot i de against a maternally inherited cadherin mRNA (thus preventing the synt hes i s of the cadherin), the inner cells of the resulting embryo dispe rse when the animal cap is removed (left). In control embryos (right), the inner cells remain together. (A after Takeichi 1 99 1 ; B from Heasman et al. 1 994, courtesy of J. Heasman.) Cadherin-cadherin binding tant means of keeping migrating epithelial cells together; and expressing dissimilar protocadherins is an important way of separating tissues (as when the mesoderm fomting the notochord separates from the surrounding mesoderm that will form somites).
Differences in cell surface tension and the tendency of cells to bind together depend on the strength 01 cadherin interactions (Duguay et aJ. 2003). This strength can be achieved quantitatively (the more cadherins on the appos ing ceJl surlaces, the tighter the adhesion) or qualitatively (some cadherins win bind to dillerent cadherin types, whereas other cadherins will not bind to different types). The ability to sort cens based on the amount of cadherin was first shown when Steinberg and Takeichi (1994) col laborated on an experiment using two cell lines that were identical except that they synthesized different amounts of P-cadherin.
When these two groups 01 cens, each expressu1g a different amount of cadherin, were mixed, the cells that expressed more cadherin had a higher surface cohesion and migrated internally to the lower-expressing group 01 cells. Foty and Steinberg (2005) demonstrated that this cad herin-dependent sorting directly correlated with the aggregate surlace tension (Figure 3.6). The surface ten- sions 01 these aggregates are linearly related to the amount of cadheru1 they are expressilng on the cell surlace. The cell sorting hierarchy is strictly dependent on the cadherin interactions between the cells. Moreover, the energetic value of cadheri.n-cadherin binding is remarkably strong-about 3400 kcal/mole, or some 200 tilmes stronger than most metabolic protein-pro tein interactions. Tills free energy change associated with cadheriln function could be dissipated by depolymerizing the aclin skeleton. The underlying actin cytoskeleton appears to be crucial in organizing the cadherins in a man ner that allows them to form remarkably stable linkages between cells (Foty and Steinberg 2004). Qualitative interactions are also important. Duguay and colleagues (2003) showed, for ilnstance, that R-cadherin and p-cadherin do not bind well to each other, and in these interactions the type 01 cadherln expressed becomes ilmpor tan1. In another example, the expression of N-cadherin is important in separating the precursors of the neural ceUs from the precursors of the epidennaJ cells. All early embry onic cells originally contain E-cadherin, but those cells des tined to become the neural tube lose E-cadherin and gain N-cadheru1. If epidermal cells are experimentally made to express N-cadherin, or if N-cadherin synthesis is blocked in the prospective neural cells, the border between the nerv ous system and skin fails to lorm properly (Figure 3.7; Kint ner 1993). 111e timing 01 particular developmental events can also depend on cadherin expression. For instance, N-cadherin appears in the mesenchymal cells of the developing chick legjust before these cells condense and form nodules of CELL-CELL COMMU N I CATION I N DEVELOPMENT (A) 6 • Eu 5 ◆ N ◆ 8 C 75 (B) 4 " -'" /' .◆./. 3 "i! ◆ v u «:◆ ◆ 2 -'" 0 0 • 50

factor is Fgf8, a Signal that induces the appearance of the L-Mal transcription factor (Ogino and Yasuda 1998; Vogel-Hop ker et al. 2000).
As we saw in Chapter 2, the combination of Pax6, Sox2, and L-Maf in the ectoderm is needed for the production of the lens and the activation of lens-specific factor (the receptor for Branchless is the Breathless protein) and the ability respond to a specific inductive signal is called competence (Waddington 1940). Even if receptor proteins are present, not every tissue type is competent to respond to an inducer's signal . For instance, if the optic vesicle (the presumptive retina) of a Xenopus laevis genes such as &-crystallin. to respond to the signal. The abiEty to Cascades of induction: Reciprocal and sequential inductive events Another feature of induction is the reciprocal nat"re of many inductive interactions. To continue the above example, embryo is placed in an ectopic location once the lens has formed, it induces other tissues. One of underneath the head ectoderm (i.e., in a different part of these responding tissues is the optic vesicle itself; thus the the head from where the frog's optic vesicle normally inducer becomes the induced. Under the influence of fac occurs), it will induce that ectoderm to form lens tissue. tors secreted by the lens, the optic vesicle becomes the optic Only the optic vesicle appears to be able to do this; there if the optic vesicle is placed cup and the wall of the optic cup differentiates into two fore, it is an inducer. However, layers, the pigmented retina and the neural retina (Figure beneath ectoderm in the flank or abdomen of the same 3.15; Cvekl and Piatigorsky 1996; Strickler et al. 2007). Such organism, that ectoderm will not be able to form lens tis interactions are called reciprocal inductions. sue. Only head ectoderm is competent to respond to the sig nals from the optic vesicle by producing a lens (Figure 3.1 3; Saha et al. 1989; Grainger 1992). At the same time, the lens is inducing the ectoderm above it to become the cornea . Like the lens-forming ecto derm, the cornea-forming ectoderm has achieved a partic Often, one induction will give a tissue the competence ular competence to respond to inductive signals, in this to respond to another inducer. Studies on amphibians sug case the Signals from the lens (Meier 1977; Thut et al. 2001). gest that the first inducers of the lens may be the foregut Under the influence of the lens, the corneal ectoderm cells endoderm and heart-forming mesoderm that underlie the become columnar and secrete multiple layers of collagen. lens-forming ectoderm during the early and mid gastrula Mesenchymal cells from the neural crest use this collagen stages Oacobson 1963, 1966). The anterior neural plate may matrix to enter the area and secrete a set of proteins produce the next signals, including a signal that promotes (including the enzyme hyaluronidase) that further differen the synthesis of Pax6 transcription factor in the anterior tiate the cornea. A third Signal, the hormone thyroxine, ectoderm (Figure 3 .14; Zygar et aI. 1998). Pax6 is important dehydrates the tissue and makes it transparent (Hay 1980; in providing the competence to respond to the inducers Bard 1 990). Thus, there are sequential ind uctive events, from the optic cup (Fujiwara et al. 1 994). Thus, although and multiple causes for each induction. CELL-CELL COMMU N I CATION I N DEVELOPMENT FIGURE 3.14 Lens induction in amphibians. (Al Additive effects of inducers, as shown by transplanta tion and extirpation (removal) experiments on the newt Taricha torosa. The ability to produce lens tissue is first induced by foregu t endoderm, then by cardiac mesoderm, and finally by the optic vesicle. The optic vesicle eventually acquires the abil ity to induce the lens and retain its differentiation. (8) Sequence of induction postulated by similar experiments performed on embryos of the frog Xenopus laevis. Unidentified inducers (possibly from the foregut endoderm and car diac mesoderm) cause the synthesis of the Otx2 tran scription factor in the head ectoderm during the late gastrula stage. As the neural folds rise, inducers from the anterior neural plate (including the region that will form the retina) i nduce Pax& expression in the anterior ectoderm that can form lens tissue. Expression of Pax& protein may constitute the competence of the surface ectoderm to respond to the optic vesicle during the late neurula stage. The optic vesicle secretes fadors (probably of the BMP family) that induce the synthesis of the Sox transcription factors and initiate observable lens formation. (A after Jacobson 1 966; B after Grainger 1 992.) (B) Ectoderm Dorsal Presumptive mesoderm retina be i ng neocalized ti ssue Presnmptive lens ecto derm Mid gastrula stage Otx2 expressed in presumptive lens ectoderm l , &#65533; --'.O---:: (A) Fiber PlaCOde _ _ -- Vesicle R 81 esponse in head ectoderm ___ } f d/ /OPtic CODtlDDed differentiation, growth, and maintenance Endoderm Cardiac mesoderm Inducer tissue vesicle retina f---/fj} ---' D orsal mesoderm Late gastrula stage Another principle can be seen in such reciprocal induc tions: a structure does not need to be fully differentiated in order to have a function. As we will detail in Chapter 9, the optic vesicle induces the lens placode before it becomes the retina; the lens placode (the prospective lens) recipro- cates by indUCing the optic vesicle before the lens forms its characteristic fibers. Thus, before a tissue has its "adult" functions, it has critically important transient functions in building the organs of the embryo. Otx2 and Pax6 expressed in presumptive lens ectoderm \ Larva Tailbud stages Neural plate &#65533; Early neurula stage Adult S ox3 expressed in Otx2, Pax6, and presumptive lens ectoderm L O ptic vesicle Late neurula stage derm and causes that region of the ectoderm to fonn a lens, that is an instructive interaction. The second type of inductive interaction is permissive interaction. Here, the responding tissue has already been specified, and needs only an environment that allows the expression of these traits.
For instance, many tissues need a solid substrate containingfibronectin or laminin in order to develop. The fibronectin or laminin does not alter the type of cell that is produced, but it enables what has already been determined to be expressed.' Instructive and permissive interactions Howard Holtzer (1968) distinguished two major modes of inductive interaction.
In instructive interaction, a signal from the inducing cell is necessary for initiating new gene expression in the responding cell. Without the inducing cell, the responding cell is not capable of differentiating in that particular way.
For example, when the optic vesicle is experimentally placed under a new region of the head ecto- *It is easy to distinguish permissive and instructive interactions by an analogy with a more familiar situation. nus textbook is made possible by both permissive and instructive interactions. A reviewer can convince me to change the material in the chapters. nus is an instructive interaction, as the information expressed in the book is changed from what it would have been.
However, the information in the book could not be expressed at all without permissive inter actions with the publisher and printer. CHAPTER 3 82 (B) (A) (C) (D) (E) Lens Future optic nerve - Pigmented layer of retina space ectoderm (F) Early gastrula t Late gas r u la Early ueurula Mid to late neurula Chordamesoderm Prospective lens ectoderm I ----'----I.&#65533; Cornea FIGURE 3.15 Schematic diagram of induction of the mouse lens. (A) At embryonic day 9, the optic vesicle extends toward the sur face ectoderm from the forebrain. The lens placode (the prospec tive lens) appears as a local thickening of the surface ectoderm near the optic vesicle. (B) By the middle of day 9, the lens pla code has enlarged and the optic vesicle has formed an optic cup.
(C) By th e middle of day 1 0, the central portion of the lens-form ing ectoderm invaginates while the two layers of the retina become distinguished.
(D) By the middle of day 1 1 , the lens vesi cle has formed. (E) By day 13, the lens consists of anterior cuboidal epithelial cells and elongating poslerior fiber cells. The cornea develops in front of the lens. (F) Summary of some of the i nductive interactions during eye development. (A-E after Cvekl and Piatigors ky 1 996.) I interactions are among the most impo rtant phenomena in 3.1 . nature.
Some examples are listed in Table REGIONAL SPECIFICITY OF INDUCTION Using the induction of cutaneous (skin) structures as our examples, we will look at the properties of epithelial-mesenchymal interac tions. The first of these properties is the regional speCifici ty of induction. Skin is composed of two main tissues: an outer epidermis (an epithelial tissue derived from ecto derm), and a dermis (a mesenchymal tissue derived from mesoderm) . The chick epidermis secretes proteins that sig nal the underlying denna] cells to fonn condensations, and the condensed dermal mesenchyme responds by secreting factors tha t cause the epidermis to form regionally specif 3.16; Nohno et a1. 1995; 1996). These structures can be the broad feathers of the wing, the narrow feathers of the ic cutaneous structures (Figure Epithelial-mesenchymal interactions Some of the best-studied cases of induction invoLve the interactions of sheets of epithelial cells with adjacent mes enchymal cells. All organs consist of an epithelium and an associated mesenchyme, so these epithelial-mesenchymal Ting-Berreth and Chuong thigh, or the scales and claws of the feet. is responsible for the regional specificity of induction in As Figure 3.17 demonstra tes, the dermal mesenchyme the competent epidermal epithelium. Researchers can sep- CELL-CELL COMMU N I CATION IN DEVELOPMENT (A) 83 FIGURE (B) 3 . 1 6 Feather induction in th e chick. (A) Feath e r tracts on the dorsum of a 9-day chick embryo. Note tha t each feather primordium is located between the primordia of adjacent rows. (8) In situ hybridiza tion of a 1 a-day chick embryo s hows So ni c hedgehog expression (dark spots) in the ectoderm of the developing feathers and scales.
(A co urte sy of P. S engal ; B courtesy of w.-S. Kim and J. F. Fall on.) arate the embryonic epithelium and mesenchyme from each other and recombine them in different ways (Saun GENETIC SPECIFICITY OF INDUCTION The second proper ty of epithelial-mesenchymal interactions is the genetic ders et a1. 1957). The same epithehum develops cutaneous specificity of induction. Whereas the mesenchyme may structures according to the region from which the mes instruct the epithelium as to what sets of genes to activate, enchyme was taken. Here, the mesenchyme plays an the responding epithelium can comply with these instruc instructive role, calling into play different sets of genes in tions only so far as its genome permits. This property was the responding epithelial cells. discovered through experiments involving the transplan tation of tissues from one species to another.
TABLE 3.1 interactions Some epithelial-mesenchymal Mesenchy ma l component Epithelial component Cutaneous structures (hair, feathers, sweat glands, mammary glands) Epidermis (ectoderm) Dermis (mesoderm) Limb Epidermis (ectoderm) Mesenchyme (mesoderm) Gut organs (liver, pancreas, salivary glands) Epithelium (endoderm) Mesenchyme (mesoderm) Foregut and respiratory associated organs (lungs, thymus, thyroid) Epithelium (endoderm) Mesenchyme (mesoderm) Kidney Ureteric bud epithelium (mesoderm) Mesenchyme (mesoderm) Tooth Jaw epithelium (ectoderm) Mesenchyme (neural crest) Organ " , i - - n , n - , , d 'g f- 5;)e le ,e in p- Source of dermal mesenchyme Wing epidermal epithelium Specific indnction Wing Thigh Thigh leather Foot FIGURE Scales, claw 3.1 7 Regi o nal specificity of induction in the chick.
When cells from different reg ions of the dermis (mesenchyme) are recombined with the epidermis (epithe lium), the type of cuta neous structure made by the epid ermal epithelium is determined by the ori gi nal source of the mesenchyme. (After Saunders 1 980.) 84 CHAPTER 3 In one of the most dramatic examples of interspecific induction, Hans Spemann and Oscar Schotte (1932) trans planted flank ectoderm from an early frog gastrula to the region of a newt gastrula destined to become parts of the newt gastrula into the presumptive oral mouth. Similarly, they placed presumptive flank ectoder mal tissue from a regions offrog embryos.
The structures of the mouth region differ greatly between salamander and frog larvae. The salamander larva has club-shaped balancers beneath its mouth, whereas the frog tadpole produces mucus-secret ing glands and suckers (Figure 3.18) . The frog tadpole also has a horny jaw without teeth, whereas the salamander has a set of calcareous teeth in its jaw. The larvae resulting from the transplants were chimeras.
The salamander larvae had froglike mouths, and the frog tadpoles had salamander teeth and balancers. In other words, the mesenchymal cells instructed the ectoderm to make a mouth, but the ectoderm responded by making the only kind of mouth it "knew" how to make, no matter how inappropriate.* Thus the instructions sent by the mesenchymal tissue can cross species barriers. Salamanders respond to frog inducers, and chick tissue responds to mammalian induc ers. The response of the epithelium, however, is species specific. So, whereas organ-type specificity (e.g., feather or claw) is usually controlled by the mesenchyme, species specificity is usually controlled by the responding epithe- lium.
As we will see in Chapter 19, major evolutionary changes in the phenotype can be brought about by chang ing the response to a particular inducer. Paracrine Factors: The Inducer Molecules How are the Signals betv.Jeen inducer and responder trans mitted? While studying the mechanisms of induction that DONOR &#65533;_ H ---.... OArea presumptive °tectoderm ST of &#65533; RESULT o o &#65533; folt) Sucker \ Newt Frog gastrula gastrula Newt wie suckers th frog tadpol h&#65533; &#65533; . /"\.
o o &#65533; FrOg Newt gastrula gastrula Bal(:J't ancer\ : r Frog newttabalpancerse with d ol FIGURE 3.1 8 Genetic specificity of induction in amphibians.
Rec ip ocal transplantation betvveen the presumptive oral ecto l derm regions of salamander and frog gastrulae leads to newts with tadpole suckers and ta d po es with newt balancers. (After Ham burgh 1 970.1 consider paracrine interactions first, returning to juxtacrine Whereas endocrine factors* (hormones) travel through interactions later in the chapter. produce the kidney tubules and teeth, Grobstein (1956) and the blood to exert their effects, paracrine factors are secret others (Saxen et a1. 1976; Slavkin and Bringas 1976) found ed into the immediate spaces around the cell producing that some inductive events could occur despite a filter sep them. These proteins are the "inducing factors" of the clas arating the epithelial and mesenchymal cells. Other induc sic experimental embryologists. There is considerable tions, however, were blocked by the filter. The researchers debate as to the distances at which paracrine factors can therefore concluded that some of the inductive molecules operate. The proteins Nodal and activin, for instance, can were soluble factors that could pass through the small diffuse over many cell diameters and induce different sets pores of the filter, and that other inductive events required of genes at different concentrations (Gurdon et a1. 1994, physical contact between the epithelial and mesenchymal 1995). The Wnt, Vg1, and BMP4 proteins, however, prob cells. When cell membrane proteins on o.ne cell surface ably work only on their adjacent neighbors (Jones et a1. interact with receptor proteins on adjacent cell surfaces, 1996; Reilly and Melton 1996). These factors may induce these events are called juxtacrine interactions (since the the expression of other short-range factors from these cell membranes are juxtaposed). When proteins synthesized by one cell can diffuse over small distances to induce neighbors, and a cascade of paracrine inductions can be initiated. changes in neighboring cells, tIle event is called a paracrine estrogen, un l interaction. A we saw earlier, this type of interaction is mediated by paracrine factors and their receptors. We will p a 'you a your of mouth; that.'" own *S em nn is reported to have put it this way: "The ectoderm says to the in_ducel&#65533; tell me to make mouth; all right, I'll do so, but I can't make kind I can make my and I'll do (Quoted in Harrison 1933.) th i *Some endocrine factors: are active in development; these include the hormones testosterone, and yrox ne . They affect many tissues sim lta eous y and often coordinate development throughout the body (as in metamorphosis or the morphogenesis sexual phenotypes).
These hormones work directly, binding to a dormant transcription factor ("receptor"), thereby activating the transcription f c or nd llowing it to enter the nucleus and bind to DNA at a a of CELL-CELL COMMU N I CATION I N DEVELOPMENT In addition to endocrine, paracrine, and juxtacrine inter actions, there are also autocrine intera&#65533;tions, Autocrine interactions occur when the same cells that secrete paracrine factors also respond to them. In other words, the cell syn thesizes a molecule for which it has its own receptor. o domain __ 85 Ligand (paracrine factor) Extr-acellular AJthough autocrine regulation is not common, it is seen in placental cytotrophoblast cells; these cells synthesize and secrete platelet-derived growth factor, whose receptor is on Cytoplasm Inactive responding protein \ the cytotrophoblast cell membrane (Goustin et a1. 1985). The result is the explosive proliferation of that tissue. Signal transduction cascades: The response to inducers Active responding protein The induction of numerous organs is actually effected by a relatively small set of paracrine factors. The embryo inherits a rather compact genetic "tool kit" and uses many of the same proteins to construct the heart, kidneys, teeth, eyes, and other organs. Moreover, the same proteins are used throughout the animal kingdom: the factors active in creating the Drosophila eye or heart are very similar to those used in generating mammalian organs, Many of the paracrine factors can be grouped into one of four major FIGURE 3.19 Structure and function of a receptor tyrosine ki nase. The bi nding of a paracrine factor (such as Fgf8) by the extracellular portion of the receptor protein activates the dormant tyrosine kinase, whose enzyme activity phosphorylates spec ific tyrosi n e residues of certain p roteins . families on the basis of their structure: 1. The fibroblast growth factor (FGF) family 2.
The Hedgehog family catalyze reactions that phosphorylate other proteins, and 3. The Wnt family 4. The TGF-&#65533; superfamily, encompassing the TGF-&#65533; fam the Vgl family, and sever ily, the activin family, the bone morphogenetic proteins (BMPs), the Nodal proteins, al other related proteins. Paracrine factors function by binding to a receptor that ini- this phosphorylation in turn activates their latent activi ties. Eventually, the cascade of phosphorylation activates a dormant transcription factor or a set of cytoskeletal proteins. tiates a series of enzymatic reactions within the cell. These Fibroblast growth factors and the RTK pathway enzymatic reactions have as their end point either the reg The f ib roblast growth factor (fGF) family of paracrine ulation of transcription factors (such that different genes factors comprises nearly two dozen structurally related are expressed in the cells reacting to these paracrine fac members, and the FGF genes can generate hundreds of tors) or the regulation of the cytoskeleton (such that the protein isoforms by varying their RNA splicing or initia cells responding to the paracrine factors alter their shape tion codons in different tissues (Lappi 1995). Fgfl protein or are permitted to migrate).
Th&#65533;se pathways of respons is also known as acidic FGF and appears to be important es to the paracrine factor often have several end points and are called signal transduction cascades. during regeneration (Yang et al. 2005); Fgf2 is sometimes called basic FGF and is very important in blood vessel for The major signal transduction pathways all appear to mation; and Fgf7 sometimes goes by the name of ker be variations on a common and r.ather elegant theme, atinocyte growth factor and is critical in skin development. 3.1 9. Each receptor spans the cell Although FGFs can often substitute for one another, the membrane and has an ex tracellular region, a transmem expression patterns of the FGFs and their receptors give brane region, and a cytoplasmic region. When a ligand them sepaIate functions. In (here, the paracrine factor) binds to its receptor 's extracel FGF protein . exemplified in Figure Drosophila, Breathless is an lular domain, that ligand induces a confonnational change One member of this family, Fgf8, is especially impor in the receptor's structure. This shape change is transmit tant during limb development and lens induction. Fgf8 is ted through the membrane and alters the shape of the usually made by the optic vesicle that contacts the outer recep tor's cytoplasmic domains. This conformational change in cytoplasmic domains gives those domains enzy- ectoderm of the head (Figure 2000). After contact with the outer ectoderm occurs,fgf8 3.20; Vogel-Hopker et al. matic activity-usually a kinase activity that can use ATP gene expression becomes concentrated in the region of the to phosphorylate specific tyrosine residues of particular presumptive neural retina-the tissue directly apposed to proteins. Thus, this type of receptor is often called a recep the presumptive lens. Moreover, if FgfS-containing beads tor tyrosine kinase are placed adjacent to head ectoderm, this ectopic FgfS will (RTK). The active receptor can now 86 CHAPTER 3 (C) Contact with optical vesicle .. --r Contact \(;:l:..,with Fgf8 bead induce this ectoderm to pro duc e ectopic lenses and to express the lens-associated transcription factor t-Maf (see Figure 3.20B). FGFs often work by activating a set of recep tor tyrosine kinases called the fibroblast growth factor receptors (FGFRs). The Branchless protein is an PGP recep tor in Drosophila. When an FGFR binds an FGF (and only when it binds an FGF), the dormant kinase is activated and phosphory lates certain proteins (including other FGFRs) within the responding cell. These proteins, once activated, can per form new functions. The RTK pathway was one of the first signal transduction pathways to unite various areas of developmental biology (Figure 3.21). Researcl1ers study ing Drosophila eyes, nematode vulvae, and human cancers found that they were all studying the same genes. The pathway begins at the cell surface, where an RTK binds its specific ligand. Ligands that bind to RTKs include the fibroblast growth factors, epidermal growth factors, platelet-derived growth factors, and stem cell factor. Each RTK can bind only one or a small set of.these ligands. The RTK spans the cell membrane, and when it binds its lig and, it undergoes a conformational change that enables it to dimerize with another RTK. This conformational change activates the latent kinase activity of each .RTK, and these receptors phosphorylate each other on particular tyrosine residues (see Figure 3.19). Thus, the binding of the ligand to the receptor causes the autophosphorylation of the cyto plasmic domain of the receptor. The phosphorylated tyrosine on the receptor is then rec ognized by an adaptor protein. The adaptor protein serves as a bridge that links the phosphorylated RTK to a power ful intracellular signaling system.
While binding to the phosphorylated RTK through one of its cytoplasmic domains, the adaptor protein also activates a G protein, such as Ras. Normally, the G protein is in an inactive, GDP bound state. The activated receptor stimulates the adaptor protein to activate the guanine nucleotide releasing fac- FIGURE 3.20 Fgf8 in the developing chick. (A) Fgf8 gene expres sion pattern in the 3 -day chick em bryo, shown by in situ hybridization. Fgf8 protein (dark areas) is seen in the distalmost limb bud ectoderm (1); in the somitic mesocerm (the seg m ented blocks of cells along the anteri o r- posterior axis; 2); in the branchial arches of the neck (3); at the boundary between the midbrain and hindbrain (4); in the developing eye (5); and in the tail (6). (B,C) Fgf8 function in the developing eye. (B))n situ hybridization of fgra in the optic vesicle. The fgfB mRNA (pu rple) is localized to the presumptive neural retina of the optic cup and is i n d irect contact with the outer ectoderm cells that will becom e the lens. (C) Ectopic expression of l-Maf in competent ectoderm can be induced by the optic vesicle (above) and by an Fgf8-con taining bead (below). (A courtesy of E. Laufer, c.-Y. Yeo, and C. Tabin; B,C courtesy of A. Vogel - Hopker.) tor (GNRP). This protein exchanges a phosphate from a GTP to transform the bound GDP into GTP. The GTP bound G protein is an active form that transmits the sig nal to the next molecule. After the signal is delivered, the GTP on the G protein is hydrolyzed back into GDP. This catalysis is greatly stimulated by the complexing of the Ras protein with the GTPase-ac tivating protein (GAP). In tills way, the G protein is rerurned to its inactive state, where it can await further signaling. Without the GAP protein, Ras protein carmot catalyze GTP well, and so remains in its active configuration (Cales et aI. 1988; McCormick 1989). Mutations in the RAS gene account for a large proportion of cancerous human tumors (Shih and Weinberg 1982), and the mutations of RAS that make it oncogenic all inhibit the binding of the GAP protein. The active Ras G protein associates with a kinase called Raf. The G protein recruits the inactive Raf protein to the cell membrane, where it becomes active (Leevers et al. 1994; Stokoe et a1. 1994). The Raf protein is a kinase that activates the MEK protein by phosphorylating it. MEK is itself a kinase, which activates the ERK protein by phos phorylation. In turn, ERK is a kinase that enters the nucle us and phosphorylates certain transcription factors. The RTK pathway is critical in numerous developmen tal processes. Moreover, it can be activated by paracrine factors other than those of the FGF family. (And FGF fam- CELL-CELL COMM U N I CATION IN DEVELOPMENT !! t Ligand t 87 Extracellular Cytoplasm RTK t GNRP Adaptor protein (50S) RAS t RAF t t MEK ERK t Transcription factor t Transcription I Modulation of transcription FIGURE 3.21 The widely used RTK signal transduction pathway. The receptor tyrosi ne kinase is dimerized by the l igand, which causes the autophosphorylation of the receptor. The adaptor protein recognizes the phosphorylated tyrosines on the RTK and activates an intermediate protein, GNRp, which activates the Ras G protein by allowing the phosphoryla tion of the GDP·bound Ras. At the same time, the GAP protein stimulates the hydrolysis of this phosphate bond, returning Ras to its inactive state. The active Ras activates the Raf pro tein kinase C (PKC), which in turn phosphorylates a series of kinases.
Eventually, the activat ed kinase ERK alters gene expression in the nucleus of the responding cell by phosphorylat ing certain transcription factors (which can then enter the nucleus to change the types of genes transcribed) and certain translation factors (which alter the level of protein synthesis). In many cases, this pathway is reinforced by the release of calcium ions.
A simplified version of the pathway is shown on the left. In the migrating neural crest cells of humans and mice, the pathway is innportant in activating the microphthalmia transcription factor (MITF) to produce the pigment cells (Figure 3.2 2). MITF, whose mechanism of action was described in Chapter 2, is transcribed in the ily proteins can activate other Signaling pathways, depend ing on the receptor.) their pigment cells have failed to migrate. Could it be that all three genes (Mit!, Steel, and White) are on the same In 1990, several laboratories demonstrated that the Steel developmental pathway? gene encodes a paracrine protein called stem cell factor pigment-fOrming melanoblast cells that migrate from the Kit receptor tyrosine kinase encoded by the White gene of the pigmented retina. But we have not yet discussed binding of stem cell factor to the Kit. RTK dinnerizes the Kit neural crest into the skin and in the melanin-forming cells (see Witte 1990). Stem cell factor binds to and activates the (Spritz et al. 1992; Wu et a1. 2000; also see Chapter 1). The '.vhat proteins signal this transcription factor to become protein, causing it to become phosphorylated. The phos active. The clue lies in two mouse mutants whose pheno phorylated Kit activates the pathway whereby phospho types resemble those of mice homozygous for microph thaLmia mutations. Like Mitfmutant mice, homozygous IYhite mice and homozygous Steel mice are white because rylated ERK is able to phosphorylate the MITF transcrip tion factor (Hsu et al. 1997; Hemesath et al. 1998). Only the phosphorylated form of MITF is able to bind the p300/CBP 88 CHAPTER 3 histone acetyltransferase protein that enables it to activate transcription of the genes encoding tyrosinase and other proteins of the melanin-formation p ath wa y (see Figure 3.22; Price et al.
1998). 1996, 2001). The STAT pathway is very important in the reg ulation of human fetal bone groWtJl. Mutations that prema turely activate the STAT pathway have been implicated in some severe forms of dwarfism, such as the lethal thanatophoric dysplasia, wherein the growth plates of the rib and limb bones fail to proliferate.
The short-limbed new born dies because its ribs cannot support breathing. The genetic lesion responsible is in tile gene encoding fibroblast growth factor receptor 3,or FgfR3 (F igu re 3.24; Rousseau et al. 1994; Shiang et al. 1994). This protein is expressed in the cartilage precursor cells-known as chondrocytes-in the growth plates of the long bones. Normally, the FgfR3 pro tein (a receptor tyrosine kinase) is activated by a fibroblast growth factor, and it signals the chondrocytes to stop c1ivid ing and begin differentiating into cartilage. This Signal is mediated by the Statl protein, which is phosphorylated by activated FgfR3 and then translocated into the nucleus. Inside the nucleus, Statl activates the genes encoding a cell cycle inhibitor, the p21 protein (Su et al. 1997). Thus, the mutations causing thanatophoric dwarfism result from a gain-of-function phenotype, wherein the mutant FgfR3 is active constitutively-that is, WitJ10Ut the need to be acti vated by an FGF (Deng et al.
1996; Webster and Donoghue The JAK-STAT pathway Fibroblast growth factors can also activate the JAK-STAT cascade. This pathway is extremely important in the dif ferentiation of blood cells, the growth of limbs, and the acti vation of the casein gene during milk production (Figure 3.23; Briscoe et al. 1994; Groner and Gouilleux 1995). Here, the ligand is bound by receptors tha t are linked to J AK Uanus kinase) proteins. The binding of ligand to the recep tor phosphorylates the STAT (signal transducers and acti vators of transcription) family of transcription factors (Ihle (A) (C) protein (SOS) (B) (. GD&#65533; ib! == == '"' &#65533;I 1&#65533; ! AAF FIGURE 3.22 Activation of MITF transcription factor through the binding of stem cell factor by the Kit RTK protein. The information received at the cell membrane is sent to the nucleus by the RTK signal transduction pathway. (A,B) Demonstration that Kit protein and MITF are present in the same cells.
Antibodies to these pro teins stain the Kit protein (red) and MITF (green). The overlap is yel low or yellow-green. Both proteins are present in the migrating melanocyte precursor cells (melanoblasts). (A) Migrating melanoblasts can be seen in a wi ld-type mouse embryo at day 1 0.5. (8) No melanoblasts are Visible in a microphthalmia mutant embryo of the same age. The lack of melanoblasts in the mutant is fi 1.1 """ "" Q &#65533; ®&#65533; &#65533; &#65533;r-----&#65533; i ,j I Transcription due to the relative absence of MITF. (C) Signal transduction path way leading from the cell membra e to the nucleus. When the receptor domain of the Kit RTK protein binds the stem cell factor, Kit dimerizes and becomes phosphorylated. This phosphorylation is used to activate the Ras G protein, which activates the chain of kinases that will phosphorylate the MITF protein. Once phospho rylated, MtTF can bind the cofactor p300/CBP, acetylate the nucle osome histones, and initiate transcription of the genes needed for melanocyte development. (A,B from Nakayama et al. 1 998, courtesy of H. Arnheiter; C aher Pr ice et al. 1 998.) n CELL-CELL COM M U N I CATION I N DEVELOPMENT Prolacti n r ecept&#65533;s Dimeri:red. Prolactin CJ), 89 t recep o rs .. Ligand t t Receptor I JAK t STAT STAT dimerization I Transcription I . i&#65533; 1Ianscription initiation +1 ,--J®WC?-r'&#65533;1 GR .&#65533;-- Casein gene promoter - 1600 ... - 85 FIGURE 3.23 A STAT pathway: the casein gene activation path way activated by prolactin. The casein gene is activated during the last (lactogenic) phase of mammary gland development, and its signal is the secretion of the hormone prolactin from the anterior pituitary gland. Prolactin causes the dimerization of prolactin receptors in the mammary duct epithelial cells. A particular JAK protein Oak2) is "hitched" to the cytoplasmic domain of these receptors. When the receptors bind prolactin and dimerize, the JAK proteins phosphorylate each other and the dimerized recep tors, activating the dormant kinase activity of the receptors. The Premature activation of FgfR3 kinase Cartilage growth stops before birth FGF receptor 3 (FgiR3) Mutation Phosphorylated Star) Narrow chest.
extremely short limbs - 24 F , .0 activated receptors add a phosphate group to a tyrosine residue (V) of a particular STAT protein-in this case, StatS.
This allows StatS to dimerizel be translocated into the nucleus, and bind to particu Jar regions of DNA. In combination with other transcription factors (which presumably have been waiting for its arrival), the StatS pro tein activates transcription of the casein gene. GR is the glucocorti.
coid receptorl OCTl is a general transcription factor, and TBP is the TATA-binding protein (see Chapter 2) responsible for binding RNA polymerase. A simplified diagram is shown to the right. (For details, see Groner and Gouilleux 1 995.) Thanatophoric dysplasia FIGURE 3.24 A mutation in the gene for FgfR3 causes the premature constitutive activation of the STAT pathway and the production of phos phorylated Stall protein_ This tran scription factor activates genes that cause the premature termination of chondrocyte cell division. The result is thanatophoric dysplasial a condi tion of failed bone growth that results in the death of the newborn infant because the thoracic cage cannot expand to allow breathing_ (Aher Gilbert-Barness and Opitz 1 996.) 90 CHAPTER 3 1996). The chondrocytes stop proliferating shortly after they are formed, and the bones fail to grow. Other muta tions that activate FgfR3 prematurely but to a lesser degree produce achondroplasic (short-limbed) dwarfism, the most prevalent of the human dominant syndromes (Leg eai-Mallet et al.
2004). See WEBSITE 3.1 FGF receptor mutations The Hedgehog family The proteins of the Hedgehog family of paracrine factors are often used by the embryo to ind uce particular cell types and to create boundaries between tissues. Hedgehog pro teins are processed such that only the amino-terminal two thirds of the molecule is secreted; once this takes place, the protein must become comp1exed with a molecule of cho lesterol in order to function. Vertebrates have at least three homologues of the Drosophila hedgehog gene: sonic hedgehog (5I1h), desert hedgehog (dhh), and il1dian hedgehog (ihh). The Desert hedgehog protein is expressed in the Sertoli cells of the testes, and mice homozygous for a null allele of dhh exhibit defective spermatogenesis. Indian hedgehog pro tein is expressed in the gut and cartilage and is important in postnatal bone growth (Bitgood and McMahon 1995; Bitgood et a1. 1996). Sonic hedgehog* has the greatest number of functions of the three vertebrate Hedgehog homologues. Among other important functions, this paracrine factor is responsible for assuring that motor neurons corne only from the ventral por tion of the neural tube (see Chapter 10), that a portion of each somite forms the vertebrae (see Chapter 12), that the feathers of the chick form in their proper places (see Figure 3.16), and that our pinkies are always our most posterior digits (see Chapter 14). Sonic hedgehog often works with other paracrine factors, such as Wnt and FGF proteins. THE HEDGEHOG PATHWAY Proteins of the Hedgehog fam ily fWlction by binding to a receptor called Patched. The Patched protein, however, is not a -signal transducer.
Rather, it is bound to a signal transducer, the Smoothened protein. The Patched protein prevents Smoothened from functioning.
In the absence of Hedgehog binding to *Yes, it is named after the Sega Genesis character. The original hedgehog gene was fo und in Drosophila, in which genes are named after their mutant phenotypes-the Joss-oi-function hedgehog muta tion causes the fly embryo to be covered with pointy dentides on its cuticle, so it looks like a hedgehog.
The vertebrate Hedgehog genes were discovered by searching vertebrate gene libraries (chick, rat, zebra fish) with probes that would find sequences similar to that of the fruit fly hedgehog gene. Riddle and colleagues (1993) discovered three genes homologous to Drosophila hedgehog. Two were named after existing species of hedgehog; the third was named after the animated character. Two other Hedgehog genes, {mUld only in fish, are named echidllil hedgehog (after the spiny Austr Patched, Smoothened is inactive, and the Cubitus inter ruptus (Ci) protein (or the homologous Gli protein in ver tebrates) is tethered to the microtubules of the responding cell While on the microtubules, it is cleaved in such a way that a portion of it enters the nucleus and acts as a tran scriptional repressor. When Hedgehog binds to Patched, the Patched protein's shape is altered such that it no longer inhibits Smoothened. Smoothened acts (probably by phos phorylation) to release the Ci protein from the micro tubules and to prevent its being cleaved. The intact Ci protein can now enter the nucleus, where it acts as a tran scriptional activator of the same genes it used to repress (Figure 3.25; Aza-Blanc et a1. 1997; Lum and Beachy 2004). The Hedgehog pathway is extremely important in ver tebrate limb development, neural differentiation, and facial morphogenesis (McMahon et a1. 2003). When mice were made homozygous for a mutant allele of sonic hedgehog, they had major limb and facial abnormalities. The midline of the face was severely reduced and a single eye formed in the center of the forehead, a condition known as cyclop iat (Figure 3.26; Chiang et al. 1996). In later development, Sonic hedgehog is critical for feather formation in the chick embryo and hair formation in mammals (Harris et al. 2002; Michino et a1. 2003). While mutations that inactivate the Hedgehog pathway can cause malformations, mutations that activate the path way ectopically can cause cancers. If the Patched protein is mutated in somatic tissues such that it can no longer inhib it Smoothened, it can cause tumors of the basal cell layer of the epidermis (basal cell carcinomas). Heritable mutations of the patChed gene cause basal cell nevus syndrome, a rare autosomal dominant condition characterized by both devel opmental anomalies (fused fingers; rib and facial abnor malities) and multiple malignant tumors such as basal cell carcinoma (Halm et a1.
1996; JolUlson et al. 1996). One remarkable feature of the Hedgehog signal trans duction pathway is the importance of cholesterol. First, cholesterol is critical for the catalytiC cleavage of Sonic hedgehog protein. Only the amino-terminal portion of tlle protein is ftmctional and secreted. The cholesterol also binds to tlle active N-terminus of the Sonic hedgehog pro tein and allows this paracrine factor to diffuse over a range of a few hundred &#65533;m (about 30 cell diameters in the mouse limb). Without this cholesterol modification, tlle molecule diffuses too quickly and dissipates into the surrounding space. Indeed, Hedgehog proteins probably do not diffuse as single molecules, but they are linked together through their cholesterol-containing regions into lipoprotein pack ets (Breitling 2007; Guerrero and Chiang 2007). Second, the Patched protein that binds Sonic hedgehog also needs cho lesterol in order to function. Some human cyclopia syn dromes are caused by m u tations in genes that encode either Sonic hedgehog or the enzymes that synthesize chot This pathology, which is named for the one-eyed Cyclops of Homer's Odyssey, will be discussed again in Chapter 9. CELL-CELL COMMUN ICATION IN DEVELOPMENT 1 1 Smoothened Hedgebog (A) Smoothened 91 (B) Patched t Ci protein t made activator \ Microtubule Transcription I inhibits Smoothened FIGURE 3.25 Hedgehog Signal transduction pathway. Patched pro tein in the cell membrane is an inhi bi tor of the Smoothened protein. (A) In the absence of Hedgehog bind i ng to Patched, the Ci protei n is tethered to the microtubules by the Cos2 and Fused proteins.
This bind No trans&#65533;ription of ing al lows the PKA and Slimb pro Hedgehog-responsive teins to cleave Ci into a transcrip genes tional repressor that blocks the transcription of particular genes. IB) When Hedgehog binds to Patched, its conformation changes, releasing the inhibition of the Smoothened protein. Smoothened then releases Ci from the (A) microtubules (probably by adding more phosphates to the Cos2 and Fused protei ns) and inactivates the cleavage proteins PKA and 5limb. The Ci protein enters the nucleus, binds a CBP protein, and acts as a transc ri ptiona l activator of particular genes. (After John son and Scott 1 998.) les terol (Kell ey et aL 1996; Roessler et aL 1996). Moreover, certain chemicals that induce cyclopia do so by interfering with the cholesterol biosynthetic enzymes (Beachy et a L 1997; Cooper et a L 1998). Two ter.. togens known to cause cyclopia in vertebrates are jervine and cyclopamine. Both substances are found in the plant Veratrum cnlifornicum, and both block the synthesis of cholesterol Keeler and Binns 1968). (see Figure 3.26; See VAD

for either caspase-J or caspase-9 d i e around birth from massive cell overg rowth i n the nervous system (Figure 3.32; Kuida et al. 1 996, 1 998; Jacobson et al.
1 997). Mice homozygous for targeted deletions of Apaf7 have simi larly severe craniofa cial abnormalities, brain overgrowth, and webbing betvveen their toes .
There are instances where cell death is the normal state unless some Figure 3.32 D isru ption of normal brain development by block ing apoptosis. In mice i n which the genes for caspase-9 have been knocked out, normal neural apoptosis fails to occur, a nd the over pro liferation of brain neu rons is obvious. (A) 6-day embryonic wild-type mouse. (B) A caspase-9 knockout mouse of the same age. The enlarged brain protrudes above the face, and the limbs are still webbed. ((,0) This effect is confirmed by cross sections through the forebrain at day 13.5. The knockout exhibits thick ened ventricle walls and the near-obliteration of the ventricles. (From Kuida et al. , 998.1 ligand "rescues" the cells. I n the chick neural tube, Patched protein (a Hedge h og receptor) wi II activate caspases. The binding of Sonic hedgehog (from the notochord and ventral neu ra l tube cells) suppresses Patched, and the cas pases are not activated to sta rt apopto sis (Thibert et al. 2003). Such "depend ence receptorsN probably prevent n eural cells from prol i ferati n g outside the proper tissue, and th e loss of such receptors is associated with cancers (Porter and Dhakshinamoorty 2004). Moreover, we will soon see that cer ta i n epithel ia l cells must be attached to the extracellular matrix i n order to function.
If the cell is removed from the matrix, the apoptosis pathway is activated and the cell dies Gan et al. 2004). This, too, is probab ly a mecha nism tha t prevents cancers once cells have lost their adhesion to extracellu lar matrix proteins.
See WEBSITE 3.2 The uses of apoptosis (A) caspase-9+1+ (Wild type) (B) caspase-9-1- (Knockout) (C) Wild type (D) Knockout 98 CHAPTER 3 ergoes a confonnational change that enables a part of its cytoplasmic domain to be cut off by the presenilin-l pro und J uxtacrine Signaling In juxtacrine interactions, proteins from the indUCing cell tease. The cleav ed portion enters the nucleus and binds to interact with receptor proteins of adjacent responding cells withou t d iffusing from the cell producing it, Two of the bound to the Notch protein, the CSL transcription factors most widely used families of juxtacrine factors are the Notch proteills (whlch bind to a family of ligands exempli Schweisguth 1998; Schroeder et al.
1998; Struhl and Adachl a dormant transcription factor of the CSL family. When activate their target genes (F i gu re 3.33; Lecourtois and fied by the Delta protein) and the eph receptors and their 1998). Thls activation is thought to involve the recruitment ephrin ligands, When the ephrin on one cell binds with of histone acetyltransferases (Wallberg et a!. 2002). Thus, the eph receptor on an adjacent cell, signals are sent to each Notch can be considered as a transcription factor tethered of the two cells (Davy et al. 2004; Davy and Soriano 2005), These signals are often those of either attraction or repul sion, and ephrins are often seen where cells are being told to the cell membrane. When the attachment is broken, Notch (or a piece of it) can detach from the cell membrane and enter the nucleus (Kopan 2002). where to migrate or where boundaries are fonning. We will Notch proteins are involved in the formation of numer see the ephrins and the ep h receptors functioning in the ous vertebrate organs-kidney, pancreas, and heart-and formation of blood vessels, neurons, and somites. For the they are extremely important receptors in the nervous sys moment, we will look at the Notch proteins and their lig tem, In both the vertebrate and Drosophila nervous systems, the binding of Delta to Notch tells the receiving cell not to become neural (Chitnis et al. 1995; Wang et al. 1998). In the ands. vertebrate eye, the interactions between Notch and its lig The Notch pathway: juxtaposed ligands and receptors ands seem to regulate which cells become optic neurons and which become glial cells (DoISky et a!. 1997; Wang et r While most known regulato s of induction are dillusible a!. 1998). Notch proteins are also important in the pattern inducing cell surface. In one such pathway, cells express ing of the nematode vulva. The vulval precursor cell clos in their cell mem est to the anchor cell becomes the central vulva cell, and branes activate neighboring cells that contain Notch pro tral vulval cells by signaling to them through its Notch tein in their cell membranes. Notch extends through the homologue, the LIN-12 receptor (BeISet et a!. 2001), proteins, some inducing proteins remain bound to the ing the Delta, Jagged, or Serrate proteins cell membrane, and its external surface contacts Delta, Jagged, or Serrate proteins extending out from an adja cent this cell is able to prevent its neighbors from becoming cen See WEBS ITE 3.3 Notch mutations cell. When complexed to one of these ligands Notch , (B) (A) FIGURE 3.33 Mechan i sm of Notch activity.
(A) Prior to Notch signaling, a CSL transcription factor (such as Suppres sor of hairless or CBF1) i s on the enhancer of Notch regulated genes. The CSl binds repressors of transcription. (B) Model for the activation of Notch. A ligand (Delta. Jagged. or Serrate protein) on one cell binds to the extracellular domain of the Notch protein on adjacent cel l. This binding causes 1\ shape change the intracellular domain of Notch, which acti vates a protease, The protease cleaves Notch and allows the i ntracel lular region of the Notch prolein to enter the nucleus and bind the CSL transcription factor. This intercellular regio of Notch displaces the repressor proteins and binds activators of transcription, including the histone acetyl transferase p300.
The activated CSl can then transcribe its target genes. (After . Koziol·Dube. Pers. Comm.) Signaling cell - n in Receiving ce l e l ;;;;;;;;;=l c R pressor ==' ='==' = ◆)i .
Target gene ◆ No transcription t Transcription CELL-CELL COMMU N I CATION I N DEVELOPMENT 99 SIDEL IG H TS SPECULATIONS J uxtacrine Signaling and Cell Patterning I nduction does indeed occur o n the cell-to-cell level, and one of the best examples is the formation of the vulva in the nematode worm Caellorhabditis e/egans. Remarkably, the signal transduction pathways involved turn out to be the same as those used in the formation of retinal receptors in Drosophila; only the tar geted transcription factors are different.
In both cases, an epidermal growth factor-like inducer activates the RTK pathway. induction in C. elegans individuals are her maphrodites. In their early develop ment, they are male and the gonad produces sperm, which is stored for Vulval Most C. e/egans (A) (B) (C) Basement Gonad '-.J.(Anchor cell ◆ IIn VPCs (vulval -precursor ceUs) " , ◆ '-' ,- , , , Gonad cell destroyed (or if the Iin-3 gene is mutat ed), the VPCs will not form a vu Iva, but instead become part of the hypodermis (skin) (Kimble 1 98 1). The six VPCs i nfl uenced by the anchor cell form an equivalence group. Each member of this group is competent to become induced by the anchor cell and can assume any of three fates, depending on its proximity to the anchor cell. The cell directly beneath the anchor cell divides to form the central vulval cells. The two cells flanking that central cell divide to become the lateral vulval cells, while the three cells farther away from the anchor cell generate hypodermal cells.
tf the anchor cell is destroyed, a l l six cells of the equivalence group later use. As they grow older, they develop ovaries. The eggs "roll" through the region of sperm storage, are ferti lized inside the nematode, and then pass out of the body through the vulva (see Figure 5.43). The formation of the vulva in C. el.. gans represents a case in which one inductive signal generates a variety of cell types. This organ forms during the larval stage from six cells called the vulval precursor cells (Vpes). The cell connecting the overlying gonad to the vulval precursor cells is called the anchor cell (Figure 3.34). The anchor cell secretes the LIN-3 protein, a paracrine factor (Similar to mammalian epidermal growth factor, or EGF) that activates the RTK pathway (Hill and Sternberg 1 992). If the anchor cell is (D) J" \ LrN-3 -- (Continued on next page) 8 t , ' '- ,' 3· Fig u re 3.34 C. elegans vu lval precursor cells and their descendants_ (A) locat ion of the gon.d, a nchor cell, and VPCs in the second instar larva. (B,C) Rel atiOnShip of the anchor cell to the six VPCs and their subsequent lineages. Pri mary l ineages result in the central vulval cells; 2° l ineages constitute the lateral vulval cells; 30 li neages generate hypoder mal cells. (C) Outline of the vulva in the fourth instar larva.
The circles represent the posi· tions of the nuclei (D) Model for the determination of vulval cell lineages in C. elegans. The l IN-3 SIgnal from the anchor cell causes the determ i nat ion 0/ the P6.p cell to gener ate the central vulval li neage (dark purple). lower concentrations of l I N-3 cause the P5.p and P7.p cells to form the lateral vulval lineages. The P6.p (central lineage) cell also secretes a short-range juxtacrine signal that induces the neighboring cells to activate the llN-12 (Notch) protein. This signal prevents the P5.p and P7.p cells from generating the primary, central vulval cell lineage. (After Katz and Sternberg 1 996.) 3· 4 @ 7 vul on . .p ' Im- 12 on- 2 (lateral) Signal ◆i◆t◆;◆tes Signal activates Vulval genes ,, - ., , , , I 1 SIgnal ---◆,. . activates S r..t◆◆ (lateral) ---◆ 3· 3 @ ---◆ · Hypodermis .rVulva (central) Q"◆,◆ o n _ Im-12 on V I" } } Hypodermis 1 00 CHAPTER J SIDELIGHTS & SPECULATIONS (Continued) divide once and contribute to the hypodermal tissue. If the three central VPCs are destroyed, the three outer cells, which normally form hypoder mis, generate vulval cells instead. The LIN-3 protein is received by the LET-23 receptor tyrosine kinase on the VPCs, and the signal i s transferred to the nucleus through the RTK path way.
The target of the kinase cascade is the LIN-31 protein (Tan et al. 1 99B). When this protein is phosphorylated in the nucleus, i t loses its i n hibitory protein partner and is able to function as a transcription factor, promot.ing vulval cell fates. Two mechanisms coordinate the formation of the vulva through this i nduction, as shown in Figure 3.34 (Katz and Sternberg 1 996; Felix 2007): , . The UN-3 protein forms a concen tration gradient. Here, the VPC clos est to the anchor cell (i.e., the P6.p cell) receives the highest concentra tion of LIN-3 protein and generates the central vulval cells. The two VPCs adjacent to it (PS.p and P7.p) receive a lower amount of LIN-3 and become the lateral vulval cells. The VPCs farther away from the anchor cell do not receive enough LIN-3 to have a n effect, so they become hypo dermis (Katz et al. 1 995). 2. In addition to forming the central vulval lineage, theVPC closest to the anchor cell also signals laterally to the two adjacent (P5.p and P7.p) cells and instructs them not to generate the central vulval lineages. The P5.p and P7.p cells receive the signal through the LIN-12 (Notch) proteins on their cell membranes. The N otch signal activates a microRNA, mir-6 1 , which represses the gene that would specify central vulval fate, as well as promot- ing those genes that are involved in forming the lateral vulval cells (Stern berg 1 988; Yoo et al. 2005). The later-al cells do not instruct the peripheral VPCs to do anyth i ng, so they become hypodermis (Koga and Ohshima 1 995; Simske and Kim 1 995). Cell-cell interactions and chance in the determination of cell types The development of the vulva in C. e/egans offers several examples of induction on the cellular level. We have already discussed the reception of the EGF-like LIN-3 signal by the cells of the equ ivalence group that forms the vulva. But before this induc tion occurs, there is an earlier interac tion that forms the anchor cell. The for mation of the anchor cell is mediated by lin-12, the C. efegans homologue of the Notch gene. In wild-type C. efe gans hermaphrodites, two adjacent cells, Zl .ppp and Z4.aaa, have the potential to become the anchor cell. They interact in a manner that causes one of them to become the anchor cell while the other one becomes the pre cursor of the uterine tissue. In loss◆of function Iin- 7 2 mutants, both cells become anchor cells, whereas in gai n of-function mutations, both cells become uterine precursors (Greenwald et al. 1 983). Studies using genetic mosaics and cel! ablations have shown that this decision is made in the sec ond larval stage, and that the lin-I 2 gene needs to (unction only i n that cell destined to become the uterine precur sor cell. The presumptive anchor cell does not need it. Seydoux and Green wald (1 989) speculate that these two cells originally synthesize both the sig nal for uterine differentiation (the LAG- Figure 3.35 Model for the generation of two cell types (anchor cell and ventral uterine pre cursor) from two equivalent cells (Z I . ppp and Z4.aaa) in C. e/egans. (A) The cells start off as equivalent, producing fl u ctu ating amounts of signal and receptor (inverted arrow). The lag-2 gene is thought to encode the signa l; the Iin- 1 2 gene is thoughl to encode the receptor. Reception of the signal turns down LAG-2 (Della) production and upregulate, Ll N- 1 2 (Notch). (8) A stochastic (chance) event causes one cell to produce more LAG-2 than Ihe other cell at some particular critic al time. This stimulates more UN-12 production in the neighboring cell. (C) This difference is amplified, since Ihe cell producing more LIN- 1 2 pro duces less LAG-2. Eventually, just one cell is delivering the LAG-2 signal, and the other cell is receiving it. (0) The signaling cell becomes the anchor celf; the receiving cell becomes the ventral uterine precursor. (After Greenwald and Rubin 1 992.) 2 protein, homologous to Delta) and the receptor for this molecule (the LIN12 protein, homologous to Notch; Wilki nson et al. ·1994). During a particular time in larval development, the cell that, by chance, is secreting more LAG-2 causes its neighbor to cease its production of this differentiation signal and to increase its production of UN-12 protein. The cell secreting LAG-2 becomes the gonadal anchor cell, while the cell receiving the signal through its IIN- 1 2 protein becomes the ventral uterine precursor cell (Figure 3.35). Thus, the two cells are thought to determine each other prior to their respective differentiation events. When the LIN-1 2 protein i s used again during vulva formation, it is activated by the primary vulval line age to stop the lateral vulval cells from form ing the central vulval phenotype (see Figure 3.34). Thus, the anchor cel l/ventral uterine precursor decision i l lustrates two important aspects of determination in two originally eq uiv alent cells. First, the ini ti a l difference between the two cells is created by ◆ chance. Second, this initial difference is reinforced by feedback. (')◆◆ Signal Recepto r "'◆◆) (q◆◆) ® Anchor cell Ventral uterine precursor CELL-CELL COMM U N I CATION I N DEVELOPMENT 1955; Saxen and 1966; Gurdon 1988), where ilie capacity to express a developmental potential exists only when a critical cell density of induced cells is present. In other duces a "community effect" (Grobstein Maintaining the Differentiated State Wartiovaara Development obviously means more than initiating gene expression. For a cell to become conunitted to a particular phenotype, gene e xpress ion must be maintained. Evolu tion has resulted in four major pathways for maintaining diffe rentia tion once it has been initiated (Figure 3.36): words, once a group of cells has been induced, autocrine c omplete their In Xenopus muscle development. this community effect is mediated through FGF Signaling. Standley and colleagues (2001) have shown (1) that FGF signaling can simulate the community effect in isolated muscle precursor cells; (2) that the muscle precursor cells have the recep tors for FGFs at the critical time; and factors can sustain that induction and differentiation.'" 1. The transcription factor whose gene is activated by a signal transduction cascade can bind to the enhancer of its own gene. In this way, once the transniption factor is made, its synthesis becomes independent of the sig nal tl,at induced it originally. The MyoD transcrip tion factor in muscle cells is produced in this manner. 2.
A cell can stabilize its differentiation by synthesizing proteins that act on chromatin to keep the gene accessi ble.
Such proteins include ilie Triiliorax family discussed in Chapter 2. 3 . A cell can maintain its differentiation in an autocrine fashion. If differentiation is dependent on a particular signaling molecule, the cell can make boili iliat Signal ing molecule and that molecule's receptor. This pro(A) """"r! 'I◆""", ◆: ◆◆O 1 *Conununity effect is also extremely important in bacterial devel QU I n Enha cer 1 I Enhancer 2 lJ I Promotor)) mation, invasiveness, ,md virulence. These phenotypes are expressed only i n groups of bacteria and not tions (see Zhu et al. 2002; Podbielski and Kreikemeyer 2004). that will induce the phenotype only at relatively hlgh c nce tra Receptor bi ds liga d on same cell ec reti on ◆=-,rm ◆ ___ ◆ 050 Initial activation n ne ha c y=!◆◆ o ◆ n n ◆ 0f S , 1Stimul:S 1◆ (C) .--.1 .- - E '-__ o -_ r o n individUAls. Each bacteriwn makes a small amount of a diffusible autocrine ind ucer (B) (D) _1="7 in mitting emergent phenotypes such as light production, biofilm for factor ◆◆ i opment. Here it is called "quorum sensing," and i t is cr tical in per Transcription Initial actiVatiO 1 01 ein prevents nudeosornesp from ih x rot Tr t ora forming (J Ū I Enhancer Translation c Transcription I)) I Promotor ' FIGURE 3.36 Four ways of maintaining differentiation aher the i nitial si gn al has been given . (A) The initial stimulus (1) activates enhancer 1 , which sti m ul ates the p romoter (2) to tra nscri be the gene. The gen e p rodu ct (3) is a t ransciption factor, and one of its targets is enhancer 2 of its own gene (4).'This activated enhancer can now stimulate the promoter (5) to make more of this prote i n . (8) Prote i n s such as those of the Trithorax group prevent mldeo somes from forming so that the gene remai ns accessible. (C) Autocrine stimulation of the differentiated state. The cell is stimu lated to activate those genes that enable it to synthesize and bind its own sti mul atory proteins. (D) aracrine loop between two cells such that a paracrine factor from one cell stimulates the differen ti ated state of the second cell, and that differentiated state includes the secretion of a pa ra c ri ne factor that maintains the first cell's dif ferentiated s tate. P 1 02 CHAPTER 3 (3) that the muscle precursor cells express FGFs at this time. It thus appears that part of the developmental pro gram for Xenopus muscle cells is to make an FGF pro tein to which those same cells can also respond (Le., an autocrine factor). This protein has to be present in suffi ciently high density for the continuation of the process es leading to muscle development.
group 01 a 01 cells that become the ectoderm around the this manner.
Here, a Nodal In the ectoderm of sea urchins, the identity mouth is also coordinated in paracrine factor from one cell causes the phosphoryla tion of Smad2 transcription factor in the neighboring cell receiving the signal. This activated transcription fac tor causes the neighboring cell to also make and secrete a Nodal Signal. This keeps all the cells in the field active ly secreting Nodal (Bolouri and Davidson 2009). 4. A cell rnay interact with its neighboring cells such that each one stimulates the differentiation of the other, and part of each neighbor's differentiated phenotype is the production of a paracrine factor that stimulates the other's phenotype. This typ e of I-scratch-your-back you-scratch-mine strategy is found in the neighboring cells of the developing vertebrate limb and insect seg ments. This technique can be used to generate two dif ferent types of cells next to one another (as it does in insect segments; see Chapter 6). Or this mechanism can be used to generate a single population of cells (if the signal is the same). If this is so, it will also generate a "community effect," like the sea urchin example described for mechanism 3. attached glycosaminoglycan polysaccharide side chains.
Two of the most widespread proteoglycans are heparan sulfate and chondroiton sulfate proteoglycans. Heparan sulfate proteoglycans can bind many members of the TGF p, Wnt, and FGF families, and they appear to be essential C. eiegans, and mice, muta for presenting the paracrine factor in high concentrations to their receptors.
In Drosophila, tions that prevent proteoglycan protein or carbohydrate synthesis block normal cell migration, morphogenesis, and differentiation (Garcia-Garcia and Anderson et a1. 2003; Hwang 2003; Kim-Safran et a1. 2004). The large glycoproteins are responsible for organizing the matrix and the cells into an ordered structure. Fibronectin is a very large (460 kDa) glycoprotein dimer synthesized by numerous cell types. One function 01 fibronectin is to serve as a general adhesive molecule, link ing cells to one another and to other substrates such as col lagen and proteoglycans. Fibronectin has several distinct 01 cells with 3.37A). Fibronectin also binding sites, and their interaction with the app ropriate molecules results in the proper alignment their extracellular matrix (Figu re has an important role in cell migration, since the "roads" over which certain migrating cells travel are paved with this protein. Fibronectin paths lead germ ceJ.ls to the gonads and heart cells to the midline of the embryo.
If chick embryos are injected with antibodies to fibronectin, the heart-forming cells fail to reach the midline, and two\' separate hearts develop (Heasman et a1. Lash 1981; Linask and 1988). Laminin (another large glycoprotein) and type rv col lagen are major components of a type of extracellular matrix called the basal lamina. The basal lamina is char The Extracellular Matrix as a Source of Developmental Signals acteristic of the closely knit sheets that surround epithelial tissue (Figure 3.37B). The adhesion of epithelial cells to affutity of mesenchymal cells for libronectin (to which they laminin (upon which they sit) is much greater than the 01 macromolecules secreted by cells into their immemust bind and release if they are to migrate). Like diate envirorunent. These macromolecules form a region fibronectin, laminin plays a role in assembling the extra The extracellular matrix is a critical region for much of ani changing cell shape, and permitting cell migration The extracellular matrix is an insoluble network consist ing 01 noncellular material in the interstices between the cells. all depend on the mal development. Cell adhesion, cell migration, and the formation of epithelial sheets and tubes ability of cells to form attachments to extracellular matri cellular matrix, promoting cell adhesion and growth, (Hakamori et a1. 1984; Morris et a1. 2003). See VADE MECUM Elements of the ECM ces. In some cases, as in the formation of epithelia, these attachments have to be extremely strong. In other made, broken, and made again. In some cases, the extra Integrins: Receptors for extracellular matrix molecules cellular matrix merely serves as a permissive substrate to The ability of a cell to bind to adhesive glycoproteins instances, as when cells migrate, attachments have to be wruch cells can adhere, or upon wruch they can migrate. depends on its expressing membrane receptors for the cell In other cases, it provides the directions for cell movement binding sites of these large molecules. The main libronectin or the signal for a developmental event. Extracellular receptors were identified by using antibodies that block matrices are made up of coHagen, proteoglycans, and a the attachment of cells to fibronectin (Chen et a1. variety of specialized glycoprotein molecules such as Knudsen et aI. fibronectin and laminin. found not unly to bind fibronectin on the outside uf the 1985; 1985). The fibronectin receptor complex was Proteog/yenl1s play critically important roles in the deliv ceil, but also to bind cytoskeletal proteins on the inside of ery of the paracrine factors. These large molecules consist the cell.
Thus, the fibronectin receptor complex appears to of core proteins (such as syndecan) with covelently span the cell membrane and wlitc two types of matrices. ,. D CELL-CELL COMMU N I CATI O N I N DEVELOPMENT (A) ◆'/◆\/ l " " ' ., ◆ . . ,. ' . I }' i (6) J Fibronectin FIGURE 3.37 Extracel lul a r matrices in the developing embryo. (A) Fluorescent antibodies to fibronectin show fibronectin deposi lion as a green band in the Xenopus embryo during gastrulation . The f,bronectin will orient the movements of the mesoderm cells. (B) Fibronectin links together migrating cells col l agen, hepa r i n sulfate proteoglycans, and other extracellular matrix proteins. This scanning electron mi crograph shows the extracellular matrix at the junction of the epithelial cells (above) and mesenchymal cells (below). The epithelial cells synthesize a tight, laminin-based basal lamina, while the mesenchymal cells secrete a loose reticu lar l a mina made primarily of collagen. (A courtesy of M. Marsden and D. W. DeSimone; B courtesy of R. L. Trelsted) , . On the outside of the cell, it binds to the fibronectin of the extracellular matrix; on the inside of the cell, it serves as an anchorage site for the actin microfilaments that move the cell (Figure 3. 3 8) . Horwitz and co·workers (1986; Tamkun et aJ. 1986) have called this family of receptor protems integrins because they integrate the extracellular and intracellular scaffolds, allowing them to work together. On the extracellular side, integrins bind to the sequence arginine-glycine-aspartate (RGD), found in several adhesive proteins i.n extracellular matrices, including fibronectin, vitronectin (found in the basal lamina of the eye), and laminin (Ruoslahti and Pier schbacher 1987). On the cytoplasmic side, integrins bind to talin and a.-actinin, two proteins that connect to actin Mcrofilaments. This dual binding enables the cell to move by contracting the actin microfilaments against the fi xed extracell u lar ma trix Bissell and her colleagues (1982; Ma rtins·Green and Bis sell 1995) have shown that the extracellular matrix is capa ble of inducing specific gene expression in developing tis- sues, especially those o f the liver, testis, and mammary gland. In these tissues, the induction of specific transcrip tion factors depends on cell-substrate binding (F igu re 3.39; Liu et a!. 1991; Streuli et al. 1991; Notenboom et aJ. 1996). Often, the presence of bound integrin prevents the activa tion of genes that specify apoptosis (Montgomery et aJ. 1994; Frisch and Ruoslahti 1997). The chondrocytes that produce the cartila ge of our vertebrae and limbs can sur vive and differentiate only if they are surrounded by an extra cel lul ar matrix and are joined to that matrix thrqugh their integrins (Hirsch e t a!. 1997). U chondrocytes from the developing chick sternum a re incubated with antibodies that block the binding of in tegrins to the extracellular Fibronectill . FIGURE 3.38 Simpl ified diagram of the fibronectin receptor complex. The i ntegrins of the complex are membra ne spanning receptor proteins that bind fibronectin on the outside of the cell while binding cytoskeletal proteins on the inside of the cell. (After - Llina and Hitl 1 992.) 103 m icrofilalnent 1 04 CHAPTER 3 gene for lactoferrin is expressed, as is the gene for p21, a cell division inhibitor. The c-myc and cyciinDl genes become silent. Eventually, all the genes for tl1e developmental prod ucts of the mammary gland are expressed, and tl1e cell divi sion genes remain turned off. By this time, the mammary gland cells have enveloped themselves in a basal lamina, forming a secretory epitl1elium reminiscent of ilie mamma- (AI (Bl (A) No basal lamina -ElSO\ 0\ O\O SJi. c'-mye, t FIGURE 3.39 Role of the extracellular matrix in cell differentia tion.
L igh micrographs of rat testis Sertoli cells grovm for 2 weeks on tissue culture pl astic d i shes (A) and on dishes coated with basal lamina (8). The tvvo photographs were taken at the same magn ifi cation, 1 2 00x.
(From Ha d l ey et al. 1 985, courtesy of M. Dym.) Tijuncti ght ons Tight ons juncti . ma trix, they shrivel up and die. Indeed, when the focal adhesions linking the epitl1elial cell to its extracellular matrix are broken, ilie caspase-dependent apoptosis path way is activated and the cells die. Such "death-upon detachment" is a special type of apoptosis called anoikis, and it appears to be a major weapon against cancer (Frisch and Francis 1994; Chiarugi and Giannoni 2(08). While the mechanisms by which bound integrins inhib H apoptosis remain controversial, the extracellular matrix is obviously an important source of signals that can be transduced into ilie nucleus to produce specific gene expression. Some of the genes induced by matrix attachment are being identified. When plated onto tissue culture plastic, mouse mammary gland cells will divide (F igur e 3 .40). Indeed, the genes for cell division (c-myc, cyciinDl) are expressed, while the genes for the differentiated products of ilie mammary gland (casein, lactoferrin, whey acidic pro tein) are not expressed. If the same cells are pia ted on to plastic coated with a laminin-containing basement mem brane, ilie cells stop dividing and the differentiated genes of ilie mammary gland are expressed. This happens only after the integrins of tl1e mammary gland cells bind to the laminin of ilie extracellular basement membrane. Then tl1e CD) Lactoferri B-casein, p21n, c-myc, cyclin WAI' Prol eins characteri stic ofbasall a rni n a of secretory tissue milk proteins Dl, FIGURE 3.40 Basement membrane-directed gene expression in mammary gl and tissue. (A) Mouse ma m ma ry gland tissue divides wh en placed on ti ssue culture plastic. Th e genes encoding cell division proteins are on and the genes capable of sy n thes i zi ng the differentiated products of the mammary gland-lactoferrin, casein, and whey acidic pro tei n (WAP)-are off. (B) When these cells a re pl aced on a basement membrane that contains laminin {basal lamina), the genes for cell division proteins are turned off, while the genes encodi ng inhibitors of ce l l division (such as p21) and the gene for lactoferri n are turned on. (C,D) The mammary gl a d cells wrap the basal l am i n a around them, forming a secre tory epithelium. The genes for casein and WAP a re sequentially activated. (Aher Bissell et al.
2003.) , n n " e i, tJ n e- CELL-CELL COMM U N I CATION I N DEVELOPMENT ry gland tissue. The binding of integrins to laminin is essen tial for the transcription of the casein gene, and the inte grins act in concert with prolactin (see Figure 3.23) to a cti vate that gene's expression (Roskelley et a1.
1994; Muschler et al. 1999). Several studies have shown that the binding of integrins to an extracellular matrix can stimulate the RTK pathway. When an integrin on the cell membrane of one cell binds to fibronectin Or collagen secreted by a neighbor ing cell, the integrin can activate the RTK cascade through an adaptor protein-like complex that connects the integrin to the Ras G protein (Wary et al. 1998).
Epithel ial-Mesenchymal Transition One innportant developmental phenomenon, epithelial mes enchyma l transition, or EMT, integrates all the processes we have discussed in this chapter. EMT is an orderly series of events whereby epithelial cells are trans formed into mesenchymal cells. In this transition, a polar ized stationary epithelial cell, which normally interacts with basement membrane through its basal surface, becomes a migratory mesenchymal cell that can invade tis sues and form organs in new places (Figure 3.41A). EMT is usually initiated. when paracrine factors from neighboring cells activate gene expression in the target cells, instruct ing the target cells to downregulate their cadherins, release aten m ca her inl§ ctin ' c◆ ◆ t § i1i Ullil l§:UU! ;◆ ◆ ◆ ◆0◆:.jl ◆◆ ◆l§◆ ◆ q \J (A) junction Neural rube 7 Basement membrane Notochord--o 1 . Integrm (C) their attachment to laminin and other basement membrane components, rearrange their actin cytoskeleton, and secrete new extracellular matrix molecules characteristic of mes enchymal cells. Epithelial-mesenchymal transition is critical during development (Figure 3.41 B,C). Examples of developmen tal processes in which this transition is active include (1) the formation of neural crest cells from the dorsalmost region of the neural tube; (2) the formation of mesoderm in chick embryos, wherein cells that had been part of an epithelial layer become mesodermal and migrate into the embryo; and (3) the formation of vertebrae precursor cells from the somites, wherein these cells detach from the somite and migrate around the developing spinal cord. EMT is also important in adults, in whom it is needed for wound healing. However, the most critical adult form of EMT is seen in cancer metastasis, wherein cells that have been part of a solid tumor mass leave that tumor to invade other tissues and form second◆ry tumors elsewhere in the body. It appears that in metastasis, the processes that gen erated the cellular transition in the embryo have been reac tivated, allowing cancer ceUs to migrate and become inva sive. Cadherins are downregulated, the actin cytoskeleton is reorganized, and the cells secrete mesenchymal extra cellular matrix while undergoing cell division (Acloque et a1. 2009; Kalluri and Weinberg 2009). , CelJ adhesions broken; basement membrane dissolved Signals from trT§ _]◆12:1 Lateral cells Medial cells cITlThi1ffi\\i◆ c {n;:r!-r:c } adherens junctions containing eadherin, eaten ins, and actin rings. Th ey are attached to the basement membrane through integrins. Paracrine factors can repress the expression of genes that encode hese cellular components, causing the cel! to lose polarity, lose anach m en t to the basement membrane, and lose cohesion with Cell released from basement membrane UJillil◆ lUJ -+- -+- o Epi thelial ceDs Epiblast Mesendlyme ◆-@:1 z:ib. ◆◆ Basement /◆ ◆ ◆ @ 1$1 ◆◆ ◆ forming ◆ rftr membrane - ◆ ..qp.. ◆ /-==-=_◆◆----= == mesoderrn ◆ ◆ Hypoblast FIGURE 3.41 Epithel ial - mesenchym al transition, or EMT. I A) Normal ep ithel i al cells are attached to one another through 1 05 Mesenchymal cells other epithelial cells. Cytoskeletal remodeling occurs, as well as the secretion of proteases and extracellular matrix molecules that enable the migration of the newly formed mesenchym a l cell.
(B,C) EMT is seen in vertebrate embryos during the normal forma tion of neural crest from the dorsal region of the neural tube (6), and du ri ng the formation of the mesoderm by mesenchym a l cells delaminating from the epiblast IC). 1 06 CHAPTER 3 Snapshot Summary: Cell-Cell Communication 1. The sorting out of one cell type from another results from differences in the cell membrane. 2. The membrane structures responsible for cell sorting out are often cadherin proteins that change the sur face tension properties of the cells. 3. Cadherin proteins can cause cells to sort out by both quantitative differences (different amounts of cad herin) or qualitative differences (different types of cadherin). Cadherins appear to be critical during certain morphological changes. 4. Migration occurs through changes in the actin cytoskeleton. These changes can be directed by inter nal instructions (from the nucleus) or by external instructions (from the extracellular matrix or chemoattractant molecules). 5. Inductive interactions involve inducing and responding tissues. 6. The ability to respond to inductive signals depends on the competence of the responding cells. 7. Reciprocal induction occurs when the tvvo interact ing tissues are both inducers and are competent to respond to each other's signals. 8. Cascades of inductive events are responsible for organ formation. 9. Regionally specific inductions can generate different structures from the same responding tissue. 10. The specific response to an inducer is detennined by the genome of the responding tissue. 11. Paracrine interactions occur when a cell or tissue secretes proteins that induce changes in neighboring cells. Juxtacrine interactions are inductive interac tions that take place betvveen the cell membr,ll1es of adjacent cells or between a cell membrane and an extracellular matrix secreted by an9ther cell. 12. Paracrine factors are proteins secreted by inducing cells. These factors bind to cell membrane receptors in competent responding cells. 13. Competent cells respond to paracrine factors through signal transduction pathways. Competence is the ability to bind and to respond to inducers, and it is often the result of a prior i.nduction. 14. Signal transduction pathways begin with a paracrine or juxtacrine factor causing a conformational change in its cell membrane receptor. The new shape results in enzymatic activity in the cytoplasmic domain of the receptor protein. This activity allows the receptor to phosphorylate other cytoplasmic proteins. Even tually a cascade of such reactions activates a tran scription factor (or set of factors) that activates or represses specific gene activity. 15. Programmed cell death is one possible response to inductive stimuli. Apoptosis is a critical part of life. 16. The maintenance of the differentiated state can be accomplished by positive feedback loops involving transcription factors, autocrine factors, or paracrine factors. 17.
The extracellular matrix is a source of signals for the differentiating cells and plays critical roles in cell migration. 18. Cells can convert from being epithelial to being mes enchymal and vice-versa. The epithlial-mesenchy mal transitiion is an series of transformations involved in the dispersion of neural crest cells and the creation of vertebrae from somitic cells. In adults, the EMT is involved in wound healing and cancer metastasis. For Further Reading Complete bibliographical citations for all literature cited in this chapter can be found at the free-access website www.devbio.com Ananthakrishnan, R and A. Ehrlicher. 2007. The forces behind cell move ment.
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2004. Townes and Holtfreter (1955): Directed movements and selective adhesion of embryonic amphibian cells. J. Exp. Zool. A: Camp Exp. Bioi. 301: 701-706. Go Online 3.1 FGF receptor m uta ti o ns. Mutations of human FGF receptors hav e been associated with se veral skeletal malformation syndromes, incl ud ing syndromes wherein skull cartilage, rib cartilage, or limb car tila ge fail to grow or differentiate. WEBSITE WEBSITE 3.2 The uses of apop tosis. A poptosi s is used for numerous processes throughout dev elopment . This website explores the role of apoptosis in such phenomena as Drosophila germ cell development and the eyes of blind cave fish. WEBSITE that 3.3 Notch mutations. Mutations in the genes encode Notch proteins can cause nervous system abnormalities in humans. Humans have more than one Notch gene and more than one li gand . Their interac tions may be critical in neural develop men t. Moreover, the asso ciation of Notch with the presenilin protease suggests that dis ruption of Notch functioning might lead to Alzheimer disease. a J ") Vade Mecum The differential adhesion hypothesis. These movies show the p ioneering work of Townes and Holtfreter and Mal colm Steinberg. These experiments demonstrated the phe nomenon of cell sorting and how cell su rface adhesion molecules can direct sor ting behaviors. Induced cydopia in zeb r.fish . As seen in the segment on zebrafish development, a1cohol can act as a teratogen and induce cyclopia in these embryos. Elements of the ECM. Movies on Vade Mecum review the molecula r components of the extracellular matrix, how cells are influenced bv them, and the work of Elizabeth Hay, who was among tl1e first scientists to show the impor-tance of the ECM to tissue differentiation. PART S PECI F I CATI O N Introducing Cell Commitment and Early Embryonic Developmen t ter 4 =erti l i zation: Begi n n i ng a new Jig anism er 5 :a r l y development i n selected 1vertebrates -er 6 le genetics of axis specification - Drosophila The cartoon shows the fundamental phenomenon and mystery of development: a compl

As the concentration declines below a certain th resho l d, cells become white, and where it falls below another threshold, cells become red. The resulting pattern is that of the French flag.
(8) An important feature of this model is tha t a piece of tissue tra n spla nted from one region of an embryo to another retains i ts ide ntity of origin, but differentiates according to its n ew positional instructions. This phenomenon is indicated schematically by reciprocal "grafts" between the flags of the United States and France. (After Wolpert 1978.)
1i n IS D- o ra ****** * * * * - *.*.*.*.*.*.*.*.* ..... * * * * * ..... ***** ****** develtioopns thei r final posi in "donor" flag Reciprocal transplants according to plished by threshold concentrations of the morphogen. Cells sensing the h ighest con centrations of morphogen become blue.
Then there is a threshold of morphogen con centration below which cells become white. As the decli ning concentration of mor phogen falls below another threshold, the cells become red. According to such models (see Crick 1 970), the morphogen diffuses from its site of synthesis (source) to its site of degradation (sink), its concentration dropping along the way. This drop in concentra tion can be due to s i m p le diffusion; to the cells' b i n d i ng the morphogen and thus "using it up"; or to a combination of a source synthesizing the morphogen and an environment containing an enzyme that degrades it. Later i n the book we w i l l see th e concept of morphogen gradients used to model how regions of the vertebrate body axis are established by retinoic acid, Fgf8, and Wnts (Chapters 7 and 8); how the different regions of the mesoderm are specified by bone morphogenetic protein (BMP) from the lateral plate mesoderm (Chapters 1 1 and 1 2); and how vertebrate limbs and digits are specified by Sonic hedgehog IChapter 1 3). Syncytial Specification In addition to autonomous and conditional specification, there is another strategy that uses elements of both. In early embryos of insects, nuclei divide within the egg; but the c e l l does not divide. In other words, many nuclei are formed within one common cytoplasm. A cytoplasm that contains many nuclei is called a syncytium, and the specification of presumptive cells within such a common cytoplasm is called syncytial specification.
As in the other eggs we have mentioned, the i nsect egg cyto plasm is not un iform. Nuclei in the anterior part of the cel l w i l l be exposed to cyto- the 118 PART I I Syncytial specification in Orosophila mefanogaster. Anterior-posterior specification originates from morphogen gradients in the egg cytoplasm, specifically the morphogenetic transcription fac tors Bicoid and Caudal. The concentrations and ratios of these two proteins distinguish each position along the axis from any other posi tion. When nuclear division occurs, the amounts and ratios of each morphogen differentially activate transcription of the various nuclear genes that specify the segment identities of the larval and the adult fly. (As we will see in Chapter 6, the Caudal gradient is itself constructed by interactions among constituents of the egg cytoplasm.) FIGURE 11.10 Gradient of-" I \ Bicoid in cytoplasm Posterior Anterior tI An te rio r Thorax Nuclear genes activated by morphogen gradients Posterior Abdomen plasmic transcription factors that are not present in the posterior part of the cell, and vice versa. The i nteractions of nuclei and transcription factors, which eventu a l l y result i n c e l l specification, take place i n a common cytoplasm. Each nucleus in Drosophila is given positional information (i.e., whether that nucle us is to become part of the anterior, posterior, or midsection of the body) by transcrip tion factors acting as morphogens.
These transcription factors are made in specific sites in the embryo, diffuse over long distances, and form concentration gradients where the highest concentration is at the point of synthesis and gets lower as the mor phogen diffuses away from its source and degrades over time. The concentration of specific morphogens at any particular site tells the nuclei where they are in relation to the source of the morphogens. As we will deta i l in Chapter 6, the anteriormost portion of the Drosophila embryo produces a morphagen called Bicoid with a concentration that is highest i n the anterior and declines toward the posterior. The posteriormast portion of the egg forms a posterior-to-anterior gradient of the morphogen Caudal. Thus, the long axis of the Drosophila egg is spanned by opposing morphogen gradi ents-Bicoid coming from the anterior, and Caudal from the posterior (Figure II.IO). Bicoid and Caudal are both transcription factors, and different concentrations and ratios of Bicoid and Caudal proteins activate different sets of genes i n the syncytial nuclei. Those nuclei i n regions containing high amounts of Bicoid and little Caudal are i nstructed to activate those genes necessary for producing the head. Nuclei i n regions with slightly less Bicoid a n d a s m a l l amount of Caudal are instructed to acti vate genes that generate the thorax. Nuclei in regions that have little or no Bicoid but plenty of Caudal are instructed to form the abdominal structures (Nusslei n-Volhard et a l . 1 987). Thus when the syncytial nuclei are eventually incorporated into cells, these cells will have their general fate specified. Afterward, the specific fate of each c e l l w i l l become determined both autonomously (from the transcription factors acquired by the cell's nucleus frolll the egg cytoplasm) and conditionally (by i nterac tions between the cell and its neighbors). SPECIFICATION TABLE 11.2 I. Modes of cell type specification and their characteristics Autonomous specification Predominates in most invertebrates. Specification by differential acquisi tion of certain cytoplasmic molecules present in the egg, Invariant cleavages prod llce the same lineages in each embryo of the species.
Blastomere fa tes aIe generally invariant. Cell type specification precedes any large-scale embryonic cell mjgration. Produces lost. II. "mosaic" development: cells cannot change fate if a blastomere is Conditional specification Predominates in vertebrates and a few invertebrates. Specification by interactions between cells. Relative positions are important. Variable cleavages produce no invariant fate assignments to cells. Massive cell rearrangements and migrations precede or accompany specification. Capadty for "regulative" development; allows cells to acquire different functions. m, Syncytial specification Predominates in most insect classes. Specification of body regions by interactions between cytoplasmic regions prior to ceUularization of the blastoderm.
d h ke p IC 115 m of to on on ost laL Id i LOt md ial idal ◆ in Icti but lard ells, ,ach .ors ◆c- Variable cleavage produces no rigid cell fates for particular nuclei. After cellularization, both autonomous and conditional specification are seen. Source: After Davidson 1991. Summary Each of the three major strategies of cell specification (summarized in Table II,2) offers a different way of providing an embyronic cell with a set of transcription fac tors that w i l l activate specific genes and cause the cell to differentiate into a partic ular cell type, In autonomous specification, the transcription factors are already pres ent in different regions of the egg cytoplasm. In conditional specification, the set of transcription factors is determined by paracrine and juxtacrine interactions between neighboring cells, In syncytial specification, there are interactions, not between cells, b u t between regions of the egg cytoplasm, These regional i n teractions give each nucleus a different ratio of particular transcription factors, and these ratios determ ine which genes are on and which are ofL The chapters that follow describe the early development of several organisms, I n these chapters, we shall see how the mechanisms of ferti lization, cleavage, and gas trulation use the three modes of specification to produce comm itted cell types and to organize the early embryo, 119 Fertilization Beginn ing a New Organ ism FERTILIZATION I S THE PROCESS whereby the sperm and the egg--- 1. Contact and recognition between sperm and egg. In most cases, this ensures that the sperm and egg are of the same species. 2. Regulation of sperm entry into the egg. Only one sperm nucleus can ultimate ly unite with the egg nucleus. This is usually accomplished by allOWing onl y one sperm to enter the egg and actively inhibiting any others from enter ing 3. Fusion of the genetic material of sperm and egg.
4. Activation of egg metabolism to start development .
. WALT WHITMAN (1855) The filial aim of all love in trigues, be they comic or tragic, LI really of more importance than all other ends in human life. What it turns upon is nothing less than the composicion of the next genera cion. A. SCHOPENHAUER Structure of the Gametes A complex dialogue exists between egg and sperm. The egg activates the sperm metabolism that is essential for fertilization, and the sperm reciprocates by acti ' vating the egg metabolism needed for the onset of development. But before we investigate these aspects of fertilization, we need to consider the structures of the sperm and egg-the two cell types specialized for fertilization.' Sperm Urge and urge and urge, Always the procreant lirge of the world. Out of the dimness opposite equals advance, Always subscance and increase, always sex, Always a kllit of identity, always distinction, r it is only within the past 135 years that the spe m s role in fertilization has been ' known.
Anton van Leeuwenhoek, the Dutch microscopist who co-discovered sperm in the 16705, first believed them to be parasitic animals living within the semen (hence the term spermatozoa, meaning "seed animals"). Although he orig inally assumed that they had nothing to do with reproducing the organism in which they were found, he later carne to believe that each sperm contained a pre------- ------_- ---- "\.1any courses begin with gametogenesis and meiosis. This author believes that meiosis and gillletogenesis are the culminating processes in development, and that they cannot be prop· erly appreciated without first understanding somatic organogenesis and differentiation. Also, having goni"ld formation and gametogenesis in the last lectures completes a circle. (QUOTED BY C.
DARWIN, 1871) 1 22 CHAPTER 4 iormed embryo. Leeuwenhoek (1685) wrote that sperm .....-ere seeds (both spenlla and semen mean "seed"), and that the female merely provided the nutrient soil in which the "",ds were planted.
1n this, he was returning to a notion of procreation promulgated by Aristotle 2000 years earlier. Try as he might, Leeuwenhoek was continually disap pointed in his attempts to find preformed embryos with in spermatozoa. Nicolas Hartsoeker, the other co-discov erer of sperm, drew a picture of what he hoped to find: a miniscule hwnan ("homunculus") within the spenm (Figure 4.1). This belief that the sperm contained the entire embry onic organism never gained much acceptance, as it implied an enormous waste of potential life. Most investigators regarded the sperm as unimportant.* See WEBSITE 4.1 Leeuwenhoek and images of homunculi The first evidence suggesting the importance of sperm in reproduction came from a series of experiments per formed by Lazzaro Spallanzani in the late 1700s. Spallan zani induced male toads to ejaculate into taffeta breeches and found toad semen so filtered to be devoid of sperm; such semen did not fertilize eggs. He even showed that semen had to touch the eggs in order to be functional. However, Spal1anzani (like many others) felt that the sper matic "animals" were parasites in the fluidi he thought the embryo was contained within the egg and needed sper matic fluid to activate it (see Pinto-Correia 1997). The combination of better microscopic lenses and the elucidation of the cell theory (that all life is cellular, and all cells come from preexisting cells) led to a new apprecia tion of sperm function. In 1824, J.
L Prevost and J.
B. Dumas claimed that sperm were not parasites, but rather the active agents of fertilization.
They noted the wtiversal existence of sperm in sexually mature males and their absence in immature and aged individuals. These obser vations, coupled with the known absence of sperm in the sterile mule, convinced them that "there exists an intimate relation between their presence in the organs and the fecundating capacity of the animal." They proposed that the sperm entered the egg and contributed materially to the next generation. These claims were largely disregarded until the 1840s, when A. von Kolliker described the formation of sperm from cells in the adult testes. He ridiculed the idea that the semen could be normal and yet support such an enormous number of parasites. Even so, von Kolliker denied there was any physical contact between sperm and egg. He believed that the spenm excited the egg to develop in much the same way a magnet communicates its presence to iron. It was not until 1876 that Oscar Hertwig and Herman Fol independently demonstrated sperm entry into the egg and *lndeed, sperm was discovered around 1676, while the events of fertilization were not elucidated until 1B76. Thus, for nearly 200 years, people had no idea what the spenn actually did. See Pinto Correia 1997 for details of this remarkable story. FIGURE 4. 1 Th e human infant preformed in the sperm, as depicted by Nicolas Hartsoeker (1 694).
the union of the two cells' nuclei.
Hertwig had been seeking an organism suitable for detailed microscopic obser va Hans, and he found the Mediterranean sea urchin (Paracentrotlls lividlls) to be perfect for this purpose. Not only was it common through out the region and sexually mature throughout most of the year, but its eggs were avail able in large numbers and were transparent even at high magnifications.
When he mixed suspen sions of sperm together with egg suspensions, Hertwig repeatedly observed sperm entering the eggs and saw sperm and egg nuclei unite. He also noted that only one sperm was seen to enter each egg, and that all the nuclei of the resulting embryo were derived mitot icallyfrom the nucleus created at fertilization. Fol made simi lar observations and also detailed the mechanism of spenm entry. Fertilization was at last recognized as the union of sperm and egg, and the union of sea urchin gametes remains one of the best-srudied examples of fertilization.* See WEBSITE 4.2 The origins of fertilization research Each sperm cell consists of a haploid nucleus, a propul sion system to move the nucleus, and a sac of enzymes that enable the nucleus to enter the egg. In most species, almost all of the cell's cytoplasm is eliminated during sperm mat uration, leaving only certain organelles that are modified for spermatic function (Figure 4.2). During the course of maturation, the sperm's haploid nucleus becomes very streamlined and its DNA becomes tightly compressed.
1n front of this compressed haploid nucleus lies the acroso mal vesicle, or acrosome. The acrosome is derived from *Hertvvig and Fol were actually not the first persons to report fertil ization in the sea urchin. At least three other astute .observers Adolphe Dufosse, Karl Ernst von Baer, and Alphonse Derbes observed sperm-egg contact in 1847. Briggs and Wessel (2006) suggest that the convulsions of Europe during 1848, the low opin ion German scientists had of French biology, and the tenuousness of these results (given poor microscopy and the lack of a theory in which to place them) may have confined these papers to obscurity.
FERTILIZATION (AI Nucleus ': ◆. 1- "' ◆ \]◆' ◆ Golgi apparatus Acro vesicle and granule 1 Goigi remnant. -Flagellum Centl iole Centriole Mi t o c h o n d r i a """ Acrom··somal◆ ◆ d Cell vesicle MItochondlla ◆\\-;.;.-i I,/ (I'' 'o◆◆◆ ::z ◆_ ◆ Nucleus Axoneme '---.r----''-,-----''-----y.---◆' ' v__' End Sperm Midpiece Tail ri n)f 11at -t .t ed of ry In •m ;0- til- .. s of ty. (B) head Modification of a germ cell to form a mam malian sperm. (A) The centri ole produces a long flagellum at what will be the posterior end of the sperm, and the Goigi apparatus forms the acrosomal vesicle at the future anterior end. The mito chondria collect around the flagellum near the base of the haploid nucleus and become incorporated into the mid piece ("neck") of the sperm. The remaining cytoplasm is jettisoned, and the nucleus condenses. The size of the mature sperm has been enlarged rela tive to the other stages. (S) Mature bull sperm. The DNA is stained blue with DAPI, the mitochondria are stained green, and the tubu lin of the flagellum is stained red. (C) Acroso me of mouse sperm, stained green by the fusion protein proacrosin-GFP. (A after Cler mont and Leblond 1 955; B from Sutovs ky et al. '1996, courtesy of G. Schatten; C, courtesy of K.-S. Kim and G. L Gerton.) FIGURE 4.2 the cell's Golgi apparatus and contains enzymes that digest proteins and complex suga rsi thus, the acro so me can be considered a modified se cretor y vesicle. The enzymes p a th tlu-ough the outer c o verings of the egg. In many species, a region stored in the acrosome are used to di gest a o f globular actin pro teins lies between 1 23 the sperm nucleus (e) piece Ys ◆. and the acrosomal vesicle.
These proteins are used to aerosomal p roc ess &om the sperm dur In sea urchins and sev era other species, recognition be tween sperm and egg l extend a fingerlike ing the early stages of fertilization. involves molecules on the acrosomal process.
Together, the acrosome and nucleus constitute the sperm head . The means by which sperm are propelled vary accord ing to how the species has adap ted to environmental con ditions. In most species (the major exception is, once again, the nematodes, where the sperm is formed a t the sites l travel by whipping its flagellum. where fertilization occurs), an individual sperm is able to The major motor portion of the fl age um is the axoneme, a structure formed by microtubules emanating from the centriole at the base of the sperm nucleus. The core of the axoneme consists of two 124 CHAPTER 4 central microtubules surrounded by a row of 9 doublet microtubules (Figure 4.3A,B).
Actually, only one micro tubule of each doublet is complete, having 13 protofila ments; the o ther is C-shaped and has only 11 protofila ments (Figure 4.3C). The interconnected protofiJaments are made exclusively of the dimeric protein tubulin. Although tubulin is the basis for the structure of the fla gellum, other proteins are also critical for flagellar func tion. The force for sperm propulsion is provided by dynein, a protein attached to the microtubules (see Figure 4.3B).
Dynein is an ATPase, an enzyme that hydrolyzes ATP, converting the released chemical energy into mechan ical energy to propel the sperm. This energy allows the active sliding of the outer doublet rnicrotubules, causing the flagellum to bend (Ogawa et a1. 1977; Shinyoji et al. 1998). The importance of dynein can be seen n i individu als with a genetic syndrome known as the Kartagener triad. These n i dividuals lack functional dynein in all their ciliat- (A) Sperm ed and flagellated cells, rendering these structures immotile (Afzelius 1976). Males with Kartagener triad are sterile (immotile sperm). Both men and women affected by the syndrome are susceptible to bronchial infections (immotile respiratory cilia) and have a 50% cilance of having the heart on the right side of the body (immotile cilia in the center of the embryo; see Chapter 8). The "9 + 2" microtubule arrangement with dynein arms has been conserved in axonemes throughout the eukary otic kingdoms, suggesting that this arrangement is extremely well suited for transmitting energy for move ment. The ATP needed to whip the flagellum and propel the sperm comes from rings of mitochondria located in the mid piece of the sperm. In many species (notably mam mals), a layer of dense fibers has n i terposed itsell between the mitochondrial sheath and the cell membrane. This fiber layer stiffens the sperm tail. Because the thickness of this layer decreases toward the tip, the (lbers probably prevent the sperm head from being whipped around too suddenly. Thus, the sperm cell has undergone extensive modifica tion for the transport of its nucleus to the egg. In mammals, the sperm released during ejaculation are able to move, but they do not yet have the capacity to bind to and fertilize an egg. The final stages of mammalian sperm maturation, cumulatively referred to as capacitation, do not occur until the sperm has been inside the female reproductive tract for a certain period of time. FIGURE 4.3 Motile appa ratus of the sperm. (A) Cross section of the flagellum of a mammalian spermatozoon, showing the central axoneme and the extemal (course) fibers, (B) Interpretive diagram of the ax oneme showing the 1/9 + 2" arrangement of the micro tubules and other flagellar componen ts . The dynein arms conta i n the ATPases tha t provide the energy for flagellar movem ent. (C) Association of tubulin protofi laments into a microtubule doublet.
One portion of the doubl et is a fully circular microtubule com pris ing 1 3 protofilaments. 1he second portion of the doublet contains only t 1 (occasionally 1 0) protofilaments. (A © D. M. , Phillips/Photo Researchers, tnc.) (B) (C) r s e d n FERTILIZATION The egg All the material necessary for the beginning of growth and in the egg, or ovum. Where- as the sperm eliminates most of its cytoplasm as it matures, the developing egg (called the oocyte before it reaches the development must be stored Cortical granules stage of meiosis at which it is fertilized') not only conserves Mitochondrion the material it has, but actively accumulates more. The mei otic divisions that form the oocyte conserve its cytoplasm 16.30); at the same time, the oocyte either synthesizes or absorbs pro teins such as yolk that act as food reservoirs for the devel op ing embry o Birds' eggs are enormous single cells, rather than giving half of it away (see Figure . swollen with accllln ulated yolk. Even eggs with relative ly sparse yolk are large compared with sperm . The volume 200 picoliters (2 x 10-4 mm3), 10,000 times the volume of sea urchin sperm (Figure 4.4). So, even though sperm and egg have equal i a sea urchin egg is about more than haplOid nuclear components, the egg also accumulates a remarkable cytoplasmic storehouse during its maturation. This cytoplasmic trove includes the following: • N utri t ive pro tei ns It will be a long time before the . embryo is able to feed itself or even obtain food from its mo ther, so the early embryonic cells need a supply of energy and amino acids. In many species this is accom FIGURE 4.4 Structure of the sea urchin egg at ferti l ization. The drawing shows the relative sizes of egg and sperm. (After Epel 1977.1 , e .1 1 25 plished by accumulating yolk proteins in the egg. Many of these yolk proteins are made • in other organs (e.g., liver, fat bodies) and travel through the matemal blood to the oocyte. o tRNA. The early embryo needs to make of its own structural proteins and enzymes, and in some species there is a burst of protein synthesis soon Ribosomes and ribosomes and tRNA that exist in the egg. The develop iug egg has special mechanisms for synthesizing ribo somes; certain amphibian oocytes produce as many as 1 0 12 ribosomes during their meiotic prophase. • Messenger RNAs. The oocyte not only accumulates pro teins, it also accumulates mRNp"s that encode proteins for the early stages of development. It is es timated that sea urchin eggs contain thousands of different types of mRNA that remain repressed until after fertilization (see Chapter 2). cells during cleavage (see Chapter many after fertilization. Protein synthesis is accomplished by Morphogenic factors. Molecules that direct the d iffer entiation of ceUs into certain cell types are present n i the egg. These include transcription factors and paracrine factors. In many species, they are localized in different regions of the egg and become segregated into different • 5). Protective chemicals. The embryo cannot nm away from predators or move to a safer environment, so it must come equipped to deal with threats. Many eggs contain ultraviolet filters and DNA repair enzymes that protect from s unlight .
Some eggs contain molecules that potential preda tors find distasteful, and the yolk of bird eggs even contaius antibodies them . Within this enormous volume of egg cytoplasm resides a large nucleus. In a few species (such as sea uIchins), the female pronucleus is already haploid at the time of fertil ization. In other species (including many worms and most mammals), the egg nuc1ells is still diplOid-the sperm meiotic divisions are completed (Figure 4.5). In these species, the final stages of egg meio sis will take place after the sperm's nuclear m a terial (the male pronucleus) is already inside the egg cytoplasm . enters before the egg's ◆ggs over easy: TIle terminology of eggs is confusing. In general, .m egg is a female g,lmete capable of binding sperm and being fertil lZed. An oocyte is a developing egg that cannot yet bind sperm or be -ertilized (WesseI2009). The problems in terminology come from :he iact that different species of animals have eggs in different ◆ges of meiosis (see Figure 4.5). The human egg for instance is in X(ond meiotic metaphase when it binds sperm, whereas the sea -!chin egg has finished alJ of its meiotic divisions when it binds .;yerm.
The con ten ts of the egg also vary greatly from species to """?t-""'Cies. The syn thesis and placemen t of these materials will be ◆dresscd in Chapter 16, when we discuss the di fferentiation of the .:eml cel ls , . , See WEBSITE 4.3 The egg and its environment Enclosing the cytoplasm is the egg cell membrane. This membrane must be capable of fUSing with the sperm cell membrane and must regulate the flow of certain ions dur ing fertil ization . Outside the cell membrane is an extracel lular matrix that forms a fib rous mat around the egg and is o ften involved in sperm-egg recognition (Correia and Carro11 1997). In invertebrates, this structure is usually (◆S 1 26 CHAPTER 4 Germinal __ • le Primary oocyte First metaphase Second metaphase Meiosis complete The roundworm Ascaris The mesozoan Dicyema The spo nge Gralltia The polychaete wOrm Myzosto,.na The clam wOrm Nereis The clam Spisula The echiuroid worm Urechis Dogs and foxes The nemertean worm Cerebratulus The polychaete worm Chaetopterus The mollusc Demolium The core worm Pectitloria Many insects Starfish The lancelet Bratlchiostoma Am phibians Cnid a r ans (e.g., anemones) Sea urchins 4.6). Most mammaL..
Fish i FIGURE 4.5 Stages of egg maturation at the time of sperm entry in different animal species. Note that in most spec i es, sperm entry occurs before the egg nucleus has completed meiosis. The germinal vesicle is the name given to the large diploid nucleus of the primary oocyte. The polar bodies are nonfunc tional cells produced by meiosis (see Chapter 1 6). (After Austin 1 965.1 envelope contains several different glycoproteins. It is sup The egg, then, is a cell speciaUzed for receiving sperm and plemented by extensions of membrane glycoproteins from In mammalian eggs, the extracellular envelope is a sep the cell membrane and by proteinaceous "posts# that arate and thick matrix called the zona pelluci da. The mam called the vitelline envelope (Figure The vitelline adhere the vitelline envelope to the membrane (Mozingo and Chandler 1991). The vitelline envelope is essential for the species-specific binding of spenn. initiating development. malian egg is also surrounded by a layer of cells called the cumulus (Figure 4.7), which is made up of the ovarian fol licular cells that were nurturing the egg at the time of its Many types of eggs also have a layer of egg jelly out release from the ovary. Mammalian sperm have to get past side the vitelline envelope (see Figure 4.4). This glycopro these cells to fertilize the egg. The innennost layer of cwnu tein meshwork can have numerous functions, but most Ius cells, immediately adjacent to the zona commonly it is used either to attract or to activate sperm. called the corona radiata. (A) (B) Microvilli -◆=--'>-◆ pellucida, is Cell membrane Vitelline envelope Cortical --"-'"-,; granul e Sea urchin egg cell surface. (AI Scanning electron micrograph of an egg before ferti lization. The cell membrane is exposed where the vitelline envelope has been torn.
(B) Transmission electron micrograph of an unfertil ized egg, showing microvilli and cell membrane, which are closely covered by the vitelline envelope. A cortical granule lies directly beneath the cell membrane. (From Schroeder 1 979, courtesy ofT. E. Schroeder.) FIGURE 4.6 FERTILIZATION 127 (A) Cumulus pellZonaucida FIGURE 4.7 Mammalian eggs immediately before iertilization.
(A) The ham ster egg, or ovum, is encased in the zona pellucida. This, in turn, is su rroun ded by the cells of the cumulus. A polar body cell, produced during meiosis, is also visible within the zona pellucida. (B) At lower magnification, a mouse oocyte is shown surrounded by the cumulus. Colloidal carbon particles (India ink, seen here as the b lack background) are excluded by the hyaluronidate matrix. (Courtesy of R. Ya nag imach i) . Recognition o f egg and sperm The interaction 01 sperm and egg generally proceeds according to five basic steps (Figure 4.8; Vacquier 1998): 1. 2. Lying immediately beneath the cell membrane 01 most eggs is a thin layer (about 5 ◆lm) of gel-like cytoplasm called the cortex.
The cytoplasm in this region is stiffer than the internal cytoplasm and contains high concentra tions of globular actin molecules. During fertilization, these actin molecules polymerize to form long cables of actin known as microfilaments. Microfilaments are nec essary for cell division.
They are also used to extend the egg surface into small projections called microvilli, which may aid sperm entry into the cell (see Figure 4.6B; also see Figure 4.16). Also within the cortex are the cortical granules (see Fig ures 4.4 and 4.6B). These membrane-bound, Golgi-derived 5tructures contain proteolytic enzymes and are thus homol ogous to the acrosomal vesicle of the sperm.
However, 'w hereas a sea urchin sperm contains just one acrosomal ,",,,ide, each sea urchin egg contains approximately 15,000 (artical granules. Moreover, in addition to digestive enzymes, the cortical granules contain mucopolysaccha rides, adhesive glycoproteins, and hyalin protein. As we will soon detail, the enzymes and mucopolysaccharides r:elp prevent polyspermy-they prevent additional sperm .:rom entering the egg after the first sperm has entered md the hyalin and adhesive glycuproteins surround the early embryo and provide support for the cleavage-stage =lastomeres. See VADE MECUM Gametogenesis 3. 4. 5. 111e chemoattrachon of the sperm to the egg by soluble molecules secreted by the egg The exocytosis of the sperm acrosomal vesicle to release its enzymes The binding of the sperm to the extracellular matrix (vitelline envelope or zona pellucida) 01 the egg The passage of the sperm through this extracellular matrix Fusion 01 egg and sperm cell membranes Sometimes steps 2 and 3 can be reversed (as in mammalian fertiliza han; see Figure 4.8B), and the sperm binds tu the extracellular matrix of the egg before releasing the contents of the acrosome. After these five steps are accomplished, the haploid sperm and egg nuclei can meet and the reac tions that initiate development can begin. In this chapter, we will focus on the fertilization events of sea urchins, which undergo external fertilization, and mice, which undergo internal lertilization. In subsequent chapters, the variahons of fertilization will be described as we study the development of particular organisms. External Fertilization in Sea U rchins In many species, the meeting of sperm and egg is not a simple matter. Many marine organisms release their gametes into the enviromnent.
That environment may be as small as a tide pool or as large as an ocean (Mead and Epel 1995). Moreover, this environment is shared with other species that may shed their gametes at the same time. Such organisms are faced with two problems: How can sperm and eggs meet in such a dilute concentration, and how can sperm be prevented from attempting to fertilize 1 28 CHAPTER 4 (A) SEA URCHIN Jelly layer (extracellular coat) Centriole Actin Vitelline __ ◆Q"'◆-I o Sperm contacts - jelly layer ◆ 8 Acrosome reaction _ ◆ Nucleus __ envelope (e",racellular" I :gg cellr V Egg cell (B) o Sperm activated by female reproductive tract Flagellum Nucleus Acrosome f) Sperm binds to zona pellucida e Acrosome reaction o Binding to vitelline envelope o Sperm lyses hole in zona " Fusion of acrosomal process membrane and egg membrane " Sperm and egg membranes fuse eggs of another species? In addition to simply producing enormous numbers of gametes, two major mechanisms have evolved to solve these problems: species-specific sperm attraction and species-specific sperm activation. Here we describe these events as they occur in sea urchins. Sperm attraction: Action a t a distance Species-specific sperm attraction has been documented in numerous species, including cnidarians, molluscs, echin oderms, amphibians, and urochordates (Mille r 1985; Yoshi da et at. 1993; Burnett et a!. 2008). In many species, sperm __ =:;;;¢ o Digestion of jelly layer FIGURE 4.8 Summary of events leading to the fusion of egg and sperm cell membranes in (A) the sea urchin and (8) the mouse. (A) Sea urchin fertilization is external. (1) The sperm is chemotactical ly atlracted to and activated by the egg. (2, 3) Contact with the egg jelly triggers the acrosome reaction, allowing the acrosomal process to form and release proteo lytic enzymes. (4) The sperm adheres to the vitelline envelope and lyses a hole i n it. (5) The sperm adheres to the egg cell membrane and fuses with it. The sperm pronucleus can now enter the egg cytoplasm . (8) Mam malian ferti lization is internal. (1) The contents of the female reproductive tract capacitate, attract, and activate the sperm.
(2) The acrosome-intact sperm b inds to the zon.a pellucida, which is thicker than the vitelline envelope of sea urchins. (3) The acro· some reaction occurs on the zona pellucida.
(4) The sperm digests a hole in the zona pellucid •. (5) The sperm adheres to the egg. an d their cell membranes fuse. Cumulus Zona peUucida layer (extraceUular (extracellular matrix) coat) __ ern b ane matnx) Acrosome membrane I-◆- I I MOUSE are attracted toward eggs of their species by chemotaxis that is, by following a gradient of a chemi cal secreted by the egg.
In 1978, Miller demonstrated that the eggs of the cnida rian Orthopyxis caliculata not only secrete a chemotac tic factor but also regul a te the timing of its release. Devel oping oocytes a t various stages in their maturation were fixed on microscope slides, and sperm were released at a certain distance from the eggs. Miller found that when sperm were added to oocytes that had not yet completed their second meiotic diVision, there was no attraction of sperm to eggs. However, after the second meiotic division was finished and the eggs were ready to be fertilized, the sperm migrated toward them. Thus, these oocytes control not only the type of spenn they attract, but also the time at which they attract them. The mechanisms of chemotaxis differ among species (see Metz 1978; Eisenbach 2004), and the chemotactic mol ecules are different even in closely related species. In sea urchins, sperm motility is acqUired when the sperm are spawned into seawater. As long as sperm cells are in the testes, they cannot move because their internal pH is kept low (about pH 7.2) by the high concentrations of CO2 in the gonad. However, Once spawned into seawater, sperm pH is elevated to about 7.6, resulting in the activation of the dynein ATPase. The splitting of ATP provides the ener gy for the flagella to wave, and the sperm begin swimming vigorously (Christen et aI. 1982). FERTI LIZATION (A) (D) (C) (B) FIGURE 4.9 Sperm chemotaxis in the sea urchin Arbacia punc One nanaliter of a 1 O-nM solution of Tesact is injected into a 20-microliter drop of sperm suspension. (A) A l -second photo graphic exposure showing sperm swimming in tight circles before the addition of resact.
The position of the injection pipette is shown by the white lines. (8-D) Similar 1 -second exposures showing migration of sperm to the center of the Tesac! gradient 20, 40, and 90 seconds after injection.
(From Ward et _I.
1 985, cou rtesy ofV. D. V_cq uier) 1 29 tulata. . But the ability to move does not provide the sperm with direction. 1n echinodermsr direction is provided by small chemotactic peptides such as resact. Resact is a 14-amino acid peptide that has been isolated from the egg jelly of the sea urchio Arbacia pUl1ctulata (Ward et a!. 1985). Resact dif fuses readily from the egg jelly into the seawater and has a profound effect at very low concentrations when added to a suspension of Arbacia sperm. When a drop of seawa ter containing Arbncia sperm is placed on a microscope slide, the sperm generally swim in circles about 50 Jlm in diameter. Withio seconds after a small amount of resact is injected into the drop, sperm migrate into the region of the (A) Cytoplasm (B) Resact injection and congregate there (Figure 4.9). As resact dif fuses from the area of injection, more sperm are recruited into the growing cluster. Resact is specific for A. punctula to and does not attract sperm of other species. (An analo gous compound, speract, has been isolated from the pur ple sea urchio, Strongylocentrotus purp"raIIls.) A. punclulata sperm have receptors in their cell membranes that bind resact (Rarnarao and Garbers 1985; Bentley et al. 1986). When the extracellular side of the receptor binds res act, it activates latent guanylyl cyclase activity in the cytoplas mic side of the receptor (Figure 4. 1 0). This causes the sperm cell to make more cyclic GMP (cGMP), a compound that activates a calciurn channel, allowing the inflnx of calciunl ions (Ca2+) from the seawater into the sperm, thus proVid ing a directional cue (Nishigaki et al 2000; Wood et al. 2005). Recent studies have demonstrated that the binding of a single resact molecule is able to provide direction for the sperm, which swim up a concentration gradient of this compound until they reach the egg (Kaupp et al.
2003; Kirkman-Brown et al. 2003) . . FIGURE 4. 1 0 Moce! for chemotactic peptides in sea urchin sperm, (A) Resact from Arbacia egg jelly binds to its receptor on the sperm. This activates the receptor s guanyl y l cyclase (RGe) activity, form i ng intracellular cGMP in the sperm. The cGMP opens calcium channels i n the sperm celf membrane, allowing Ca2+ to enter the sperm. The influx of Ca2+ activates sperm moti li ty, and the sperm swims up the resact gradient toward the egg. (8) Ca2+ levels in different regions of Strongylocentrotus purpuratus sperm after exposure to 1 25 nM speract (this species' analog of resact). Red indicates the highest level of Ca2+, blue the lowest. The sperm head reaches its peak (a2+ levels within 1 second. (A aher Kirkman-Brown et _I. 2003; 8 from Wood et al. 2003, cour tesy of M. Whitaker) ' GTP Swims toward egg CHAPTER 4 1 30 In addition to its function as a sperm-attracting peptide, resact also acts as a sperm◆activating peptide. One of the some reaction in sea urchins is Lnitiated by contact of the process (Dan 1952: Colwin and Colwin 1963). The acro major roles of the egg jelly is to increase the motility of sperm with the egg jelly. Contact causes the exocytosis of the sperm's acrosomal vesicle and proteolytic enzymes and sperm, and sperm-activating peptides cause dramatic and immediate increases in mitochondrial respiration and proteasomes (protein-digesting complexes) that digest a sperm motility (Hardy e t a1. 1994: Inamdar et a!. 2(07). The path through the jeUy coat to the egg surface (Dan 1967: increases in cyclic GMP and Ca2+ also activate the mito Franklin 1970). Once the sperm reaches the egg surface, chondrial ATP-generating apparatus and the dynein the acrosomal process adheres to the vitelline envelope and ATPase that stimulates flagellar movement in the sperm tethers the sperm to the egg. It is possible that proteasomes upon meeting resact, Arbacia sperm are instructed where to go and are given the motive force to get there. to digest the vitelline envelope at the point of attachment and proceed toward the egg (Yokota and Sawada 2007). The acrosome reaction interactions of the spenn cell membrane with a specific com from the acrosome coat the acrosomal process, allowing it (Shimomura et a1. 1986; Cook and Babcock 1993). Thus, A second interaction In sea wchins, the acrosome reaction is initiated by the plex sugar in the egg jelly. These sulfate-containing polysac charides bind to specific receptors located on the sperm cell between sperm and egg jelly results in the acrosome re a c ti on. In mo st marine invertebrates, memb rane direc tly above the acrosomal vesicle. The egg the acrosome reaction has two components: the fusion of jelly factors that initiate the acrosome I;eaction are often rugh exocytosis that results in the release of the contents of the one species of sea urchin fail to ac tivate the acrosome reac the acrosomal vesicle with the sperm cell membrane (an ly specific to each species, and egg jelly carbohydrates from acrosomal vesicle), and the extension of the acrosomal (A) tion even in closely related species (Figure 4.11: Hirohasru Arbaaa lixl/fa 40 oR ... .::. 30 ·E c u ◆ ◆ " 20 -,; a 0 " 0 10 " u < ""'>: .-:!" (' Sulfated polysaccharides ◆-;,.◆ -?-. ? ◆ from: 0 ◆.
Sperm fi:om: 9 .,. "' A. lixlda E. luwnrer L. IB) A. lixula HO◆O· · · 0350 °3S0 HO ' ◆ :rv tH) JfL\ HO ' ◆ CHl o so o so 0,50 ..... I CHj a so 0 50) 0) I . L. vanegatu5 O'SO I CH3 0 Species-specific induction of the acrosome reac tion by sulfated polysaccharides character i z i ng the egg jelly coats of three species of sea urchins th(lt co-inhabit the intertidal around Rio de J a nei r .
(A) The h i st gra ms compare the ability of each polysaccharide to induce the acrosome reaction i n the different species of sperm. (8) Chem i c a l structures of the acro FIGURE 4.11 variegatus ./ tHPH E. fucunter 0..... o o some reaction-inducing sul fated polysaccharides reveal t he i r species-specificity. (After Vilela Silva et al. 2008; photographs le◆ to right () Interfoto/Alamy: 131 FERTILIZATION and Vacquier 2002; Hirohashi et al. 2002; Vilela-Silva et al. 20(8). Thus, the activation of the acrosome reaction consti cutes a barrier to interspecics (and thus Wlviable) fertiliza :ions. This is important when nwnerous species inhabit the same habitat and when their spawning seasons overlap. In the sea u rchin Stongylocentrotus pZll'pUratas, the acro some reaction is initiated by a repeating polymer of fucose sulfate . When this

(0) The fertilization envel ope is complete, and excess sperm have been removed.
(From Vacquier and Payne 1973, courtes)1 ofV. D. Vacqu ier.) fibrous mat of vitelline envelope proteins. Several proteins are released by this cortical granule exocytosis.
One is a trypsin-like protease called cortical granule serine pro tease. This enzyme cleaves the protein posts that connect the vitelline envelope proteins to the cell membrane, and it clips off the bindin receptors and any sperm attached to them (Vacquier et a1.
1973; Glabe and Vacguier 1978; Haley and Wessel 1999, 2004). The components of the cortical gra nu les bind to the vitelline envelope to form a fertilization envelope (Wong and Wessel 2004, 2008). This fertilization envelope is elevat ed from the cell membrane by glycosa1llil1oglycans released by the cortical granules. These viscous compounds absorb water to expand the space between the cell membrane and the fertilization envelope, so that the envelope moves radi ally away from the egg (see Figures 4.19 and 4.20). The fer tilization envelope is then stabilized by crosslinking adja cent proteins through egg-specific peroxidase enzymes (soluble ovoperoxidase from the cortical granules and Udx1 in the former cortical gran ule membrane) and a transglutaminase released from the cortical granules (Foerder and ShapirO 1977; Wong et a1. 2004; Wong and Wessel 2009). This crosslinking allows the egg and early embryo to resist the shear forces of the intertidal waves. As shown in Figure 4.19, FERTILIZATION Cort ical granul es Time Egg cytoplasm (C) (8) Udxl , - the fertilization envelope starts to form at the site of sperm entry and continues its expansion around the egg. This process starts about 20 seconds after sperm attachment and is complete by the end of the first minute of fertilization. As this is happening, a fourth set of cortical granule proteins, :/ 137 FIGURE 4.20
Cortical granul e exocy tosi s . (A) Schematic diagram showing the events leading to the formation of the fertilization envelope and hyaline layer. As cortical granules undergo exo cylosis, th ey release cortical granule serine protease (CGSP), which cleaves the proteins linking the vitelline enve lope to die cell membrane. Mucopoly saccharides released by the cortical granules form an osmotic gradient, thereby causing water to enter and swell the space between the vitelline envelope and the cell membrane. Per oxidases (OVOP and Udx 1 1 and transg lutaminases (TG) then harden the vitelline envelope, now called the fertilization envelope. Transmis sion electron micrographs of the cortex of an unfertilized sea urchin egg IB) and the same region of a recently fertilized egg (C). The raised fertilization envelope and the points at which the corti cal granules have fused with the egg cell membrane of the egg (arrows) are visible in IC). IA a�er Wong et al. 2008; B,C from Chandler and Heuser 1 9 79, courtesy of D. E. Chandler.) including hyalin, forms a coating arouund the egg (Hylander and Summers 1982). The egg extends elongated microvilli whose tips attach to this hyaline layer. This layer provides support for the blastomeres during cleavage.
cium-activated luminescent dyes such as aequorin (like See WEBSITE 4.6 Building the egg's extracellular matrix GFP, a protein isolated from dye and then fertilized, a striking wave of calcium release Calcium as the initiator of the cortical granule reaction The mechanism of cortical granule exocytosis is similar to that of the exocytosis of the acrosome, and it may involve Dlany of the same molecules.
* Upon fertilization, the con centration of free Ca2+ in the egg cytoplasm increases great granule membranes fuse with the egg cell membrane, releasing their contents (Figu re 4.20). Once the fusion of the cortical granules begins near the point of sperm entry, a wave of cortical granule exocytosis propagates around the cortex to the opposite side of the egg . In sea urchins and mammals, the rJse in Ca2+ concen , luminescent jellyfish) or flu orescent dyes such as fura-2. These dyes emit light when they bind free Ca2+. When a sea urchin egg is injected with See VADE MECUM Sea urchin fertilization ly. In this high-calcium environment, the cortical from within the egg itself. The release of calcium from intracellular storage can be monitored visually using cal trC'ltian responsible far the cortical granule reaction is not dlle to an influx of calcium into the egg, but rather comes *Exocytotic reactions like the cortical granule reaction and the acro solfle reaction are also seen in the release of insulin from pancreatic ceUs and in the release of neurotram;mitters from synaptic terminals.
In all cases, there is Ca2+-medlatcd fusion of the secretory vesicle and the cell membrane. Indeed, the Similarity of acrosomal vesicle exocy tosis and synaptic vesicle exocytosis may be quite deep. Studies of acrosome reactions in sea urchins and mammals suggest that when the receptors for the sperm-activating ligands bind these molecules, the resulting depolarization of the membrane opens voltage-depend ent Ca2+ channels in a manner reminiscent of synaptic transmission (Gonzalez-Martinez et aJ.]992; Tulsani and Abou-HaHa 20(4). The proteins that dock the cortical granules of the egg to the cell mem brane also appear to be homologous to those used in the axon tenni nal. The synaptic granules of the neurons, the acrosomal vesicle of the sperm, and the cortical granules of the egg aU appear to use synapto tagmin to bind calcium and initiate fusion of the vesicle with the cell membran.e (Bi et aJ. 1995; Leguia et al. 2006; Roggero et ru. 200(7). 138 CHAPTER 4 FIGURE 4.21 Wave of (a2+ release across a sea urchin egg during fertilization. The egg is pre-loaded with a dye that fluoresces when it binds (a1-+, When a sperm fuses with the egg, a wave of calcium release is seen, beginning at the site of sperm entry and propagating across the egg. The wave takes 30 seconds to traverse the egg. (Courtesy of G. Schatten.) (Figure 4.21). Starting at the light traverses the cell (Steinhardt et al. 1977; Hafner et a1. 1988). The calcium ions propagates across the egg point of sperm entry, a band of do not merely diffuse across the egg from the point of Rather, the release of Ca2+ starts a t one end of the cell and proceeds actively to the other end, creating a wave of ca lciunl across the egg. The entire release of Ca2+ is complete within roughly 30 seconds, and free Ca" is re seques te red shortly after being released. If two sperm ente r the egg cytoplasm, Ca2• release can be seen starting at the two separate points of entry on the cell surface (Hafner et a1. 1988).
sperm entry. Several experiments have demonstrated that Ca2+ ions Moreover, this reaction occurs in the absence of any Ca2+ in the surrounding water. Therefore, the A23187 must be stimulating the release of Ca2-+ already sequestered in organelles within the egg (Chambers et al. 1974; Steinhardt and EpeI 1974). In sea urchins and vertebrates (but not snails and worms), the calcium ions responsible for the cortical gran ule reaction are stored in the endoplasmic reticulum of the egg (Eisen and Reynolds 1985; Terasaki and Sardet 1991). In sea urchins and frogs, this reticulum is pronounced in the cortex and surrounds the cortical granules (Figure 4.22; Gardiner and Grey 1983; Luttmer and Longo 1985). The cor tical granules are themselves tethered to the cell membrane are directly responsible for propagating the cortical granule by a series of integral membrane proteins that facilitate cal reaction, and that these ions are stored within the egg itself. cium-mediated exocytosis (Conner et al.
1997; Conner and The drug A23187 is a calcium ionophore (a compound that allows the diffusion of ions such as Ca2+ across lipid mem branes, pennitting them to traverse otherwise imperme able barriers) . Placing unfertilized sea urchin eggs into sea water containing A23187 causes the cortical granule reaction and the elevation of the fertilization envelope. Wessel 1998). Thus, as soon as Ca" is released from t he endoplasmic reticulwn, the cortical granules fuse with the cell membrane above them. Once initiated, the release of calcium is self-propagating.
Free calcium is able to release sequestered calciwn from its storage sites, thus ca usin g a wave of Ca2+ release and cortical granule exocytosis. FERTIL IZATION (Al 1 39 FIGURE 4.22 Endoplasmic reticulum surrounding cortical granules in sea urchin eggs. (A) The endoplasmic reticulum has been stained to allow visualization by transmission electron microscopy. The cortical granule is seen to be surrounded by dark·stained endoplasmic rctic�lum. (B) (B) An entire egg stained with fluorescent antibodies to calcium-dependent calcium release channels.
The antibodies s how these cha n n els in the cortical endoplasmic reticulum. (A from luttmer and Longo 1 985, courtesy of S.
LUllmer; B from Mc Phe rson el al. 1 992, co urtesy of F.
J . Longo.) Activation of Egg Metabolism in Sea Urchins Release of intracellular calcium ions Although fertilization is often depicted as merely the means to merge two haploid nuclei, it has an equally important role in initiating the processes that begin devel opment.
These events happen in the cytoplasm and occur without Ihe involvement of the parental nuclei.° In addi changing the ionic composition of the cytoplasm (Figure 4.23A). This mechanism, as we will see later, probably works in mammals. The other mechanism, proposed by Loeb's rival Frank Lillie (1913), is that the sperm acts like a big hormone, binding to receptors on the egg cell surface and changing their conformation, thus initiating reactions within the cytoplasm that activate the egg (Figure 4.23B). This is probably what happens in sea urchins. IP3 : THE RELEASER OF CALCIUM IONS If Ca2+ from the egg's tion to initiating the slow block to polyspermy (through endoplasmic reticulum is responsible for the cortical gran when the sperm enters the egg is critical for activating the releases Ca'+? lluoughout the animal kingdom, it has been egg's metabolism and initiating development. Calcium found that inosilol l,4,S- trisphosphate ions release the inhibitors from maternally stored mes mechanism for releasing Ca2+ from intracellular storage. cortical granule exocytosis), the release of Ca2+ that occurs sages, allowing these mRNAs to be translated; they also release the inhibition of nuclear division, thereby allowing cleavage to occur. Indeed, throughout the animal kingdom, calcium ions are used to activate development during fer tilization. However, the way calcium ions are released varies between species (see Farrington et al. 2007). One way, first proposed by Jacques Loeb (1899, 1 902), is that a soluble factor from the sperm is introduced into the egg at the time of cell fusion, and this substance activates the egg by ule reaction and the reactivation of development, what The IP3 pathway is shown n i Figure (0\) is the primary 4.24. The membrane phospholipid phosphatidylinositol 4,S-bisphosphate (PIP,) is split by the enzyme phospholipase C (PLC) to yield two active compounds: IP3 and diacylglycerol (DAG). IF3 is able to release Ca2+ into the cytoplasm by opening the cal cium channels of the endoplasmic reticulum. DAG acti vates protein kinase C, which in turn activates a protein that exchanges socliuru ions for hydrogen ions, raising the pH of the egg (Nishizuka 1 986; Swann and Whitaker 1986). This Na+ /H+ exchange pump also requires Ca'+ for its activity. The result of pLC activation, therefore, is the Jib eration of Ca2+ and the alkalinization of the egg, and both • • "In certain salilmandeTS, this developmental function of fertilization has been totally divorced from the genetic function. The silver sala mander (Ambystomn platineum) is a hybrid subspecies consisting solely of fetnales. Each female produces an egg with an wueduced chromosome number. nus egg, however, cannot develop on its own, so the silver salamander mates with a male Jefferson salaman der (A. jeffersoniamfm). The sperm from the male Jefferson salaman der only st.i mulates the egg':; development; it does not contribute :genetic material (Uzzell 1964). For details of this complex mecha nism of procreation, see Bogart et al. 1989. of the compounds this activation creates--1P3 and DAG-- are involved in the initiation of development. In sea urchin eggs, IF3 is formed initially at the site of sperm entry and can be detected within seconds of the eggs being fertilized. The inhibition of IF3 synthesis prevents Ca'· release (Lee and Shen 1998; Carroll et al. 2000), while injected 11'3 can release sequestered Ca'·, leading to corti cal granule exocytosis (Whitaker and Irvine al. 1 984; Busa et 1985). Moreover, these IP3-mediated effects can be CHAPTER 4 140 AFTER GAMETE (A) ACTIVATION MEMBRANE FUSION Probable mechanisms of egg activation.
In both cases, a phospholipase C (PLC) is activated and makes IP) and DAG. (A) (a + release and egg activation by acti vated PLC from the sperm, or b a substance from the sperm that activates egg PLC. This may be the mechanism in mammals. (8) The bindin receptor (perhaps acting through a G protein) adivates an Src ki nase, which acti vates PLC. This is probably the mechanism used by sea urchins. FIGURE 4.23 2 y Soluble factors from sperm activate PLC \&S ,.!pW:D1G '::':/- . '.. Iooo--....-.-- thwarted by preinjecting the egg with calcium-chelating agents (Turneret aI . 1 986) . IP3-responsive calcium channels have been found in the egg endop lasmi c reticulum. Tlle IT'3 formed a t the site of sperm entry is thought to bind to the IP3 receptors of these channels, effecting a local release of calcium (Ferris et al. 1989; Furuichi et al. 1989). Once released, Ca2+ can diffuse directly, or it can facilitate the release of more Ca2+ by binding to calcium-release receptors, also located in the cortical endoplasmic reticulum (McPherson et al. 1992). The binding of Ca2+ to these receptors releases mare Ca2+, which bind s to more receptors, and so on. The resulting wave of calcium release is propagated throughout the cell, starting at the point of sperm entry_ The cortical granules, which fuse with the cell membrane in the presence of high calcium concentrations, respond with a wave of exocyto sis that follows the calciwn wave. M�hri and colleagues (1995) have shown that IT'3-released Ca2+ is both necessary and sufficient for initiating the wave of calcium release. PHOSPHOLIPASE C, T H E GENERATOR OF I P 3 The question then becomes, What activa tes phospholipase C enzymes? This question has not been easy to address, since (1) there are numerous types of PLC that (2) ca n be activated tlu-ough different pathways, and (3) different species use different mechanisms to activate PLC Results from stud ies of sea urchin eggs suggest that the active PLC in echin oderms is a member of the y (gamma) family of PLCs (Car roll et al. 1997, 1999; Shearer et al. 1999). Inhibitors that specifically block PLCy inhibit IP3 production as well as Ca2+ release. Moreover, these inhibitors can be circwnvent ed by mi croinjecting IT'3 into the egg. Ca'+ �In Ca2+ (B) ACTIVATION PRIOR TO GAMETE FUSION Sperm receptor ? ��-�!�pW:D1G Tyrosine kinase (Src family) Ca2+ IP) recePt�;�TltD, ..., Endoplasmic reticulum Ca.1,+ KINASES: A LINK BETWEEN SPERM AND PlCy The finding that the r elass of PLCs was responsible for generating IP3 dur ing echinodenn fertilization spurred investigators to look at exacUy which proteins activated this particular class of phos pholipases. Their work soon came to focus on the Sec family of protein kinases. Src proteins are found in the cortical cyto plasm of sea urchin and starfish eggs, where they can fonn a complex with PLCy Inhibition of Src protein kinases low ered and delayed the amount of Ca2• released (Kinsey and Shen 2000; Giusti et aL 2003; Townley et al.
2009). So what activates Src kinase activity? One possibility is heterotrimeric G proteins in the cortex of the egg (F igu re 4.25). Such G proteins are known to activate Src kinases in mammalian somatic cells, so the cortical G proteins of sea u rchin eggs seem like good candidates; blocking these G proteins prevented Ca2+ release (Voronina and Wessel 2003, 2004). It is also possible that these G proteins a ctivate PLC directly. Indeed, there may be more than one pathway and more than one way to activate Ca2+ release. Thus, in sea urchins, it is thought that the binding of sperm to the egg (or possibly the fusion of sperm and egg) activates PLCy through G proteins and Sec kinases. The IT'3 thus generated opens calcium channels in the nearby cor tical endoplasmic reticulum, allOWing the initial and local outflow of Ca2+. This first efflux of ions opens calcium ga ted calciwn release channels, causing a wave of Caz+ that flows across the egg from the point of sperm entry to the opposite side of the egg. In so doing, some of the Ca2+ ini- tiates cortical granule exocytosis, fusing .the cortical gran ule with the egg cell membrane. Other Ca'+ would be bound by proteins such as calmodulin, which is activated by Ca2+ and can regulate numerous functions. 141 FERTILIZATION OR1 I OR: I 0 II 0-01-0 - I CHz-CH- CH2 HO H H OH H HO H ◆ ◆ OPO◆J H H opo2jPIP, H IP, HOPO-J C t, recePtor _ _ J __ , Endoplasmic 0.2+ retic.ulum F I G URE 4.24 Roles of inositol phos phates in releasing calcium from the endoplasmic reticulum and the in itiat ion oi development. Phosphol ipase C spl its PIP, into IP, and DAG. IP, releases calci um from the endopl asm ic reticulum, and DAG, with assistance from the released (a1+, activates the sodium-hydrogen exchange pump in the membrane, ◆r◆◆◆◆) exocytosis Stimula t.ion of cell division, DNA synthesis. '" RNA translation _ _ '- \ Egg activation C ( ) (A l 5 t Alkal ini ty P(}) IP, IP, PATHWAY (Cytoplasm H -OJPOH H Activation I Sperm contact and fusion t t t G protein aClivation I Src activation .
n PLCy activation ' :i ,f :l • , II r- , , '" Jl If' d FIGURE 4.25 G protein involvement i n (a2+ entry i nto sea urchin eggs.
(A) \lalure sea urch i n egg immunologically abeled for the cortical granule protein ""aline (red) and the G protein Gaq reen). (The overl a p of sig nals produces 'oe yellow color.) Gaq is localized to the cortex. (8) A wave of Ca" appears in the control egg (computer-enhanced to show ◆elative i ntensit ies, with red b e ing the " ghest). but not in the egg injected with an in hibitor of the Gaq protein. (C) Pass; o!e model for egg activation by the i nflux oi Ca2-+-.
(After Voronina and Wessel 2003; photographs courtesy of G. M. Wessel.) PIP, (B) I IP,. DAG ea2+ release jI jI Control Gaq inhibjtor added t t Cortical granule exocytosis Slow block to polyspermy ---- -- " I " Inactivation of MAP kinase Reinitiation of cell cycle -- -- 1 42 CHAPTER 4 TABLE 4.1 Events of sea urchin fertilization Event NAADP It is possible that diffusible compounds from the sperm also release calcium directly to the endoplasmic reticulum. There is evidence that nicotinic acid adenine din ucleotide phosphate (NAADP), a linear dinucleotide derived from NADP, serves as a sperm-borne Ca2+ releaser. NAADP frees stored Ca2+ from membrane vesicles during muscle contraction, insulin secretion, and neurotransmitter release (Lee 2001). Upon contact with egg jelly, NAADP concentration in sea uchin sperm increases tenfold, reach ing levels that appear to be more than sufficient to release stored Ca2+ within the egg (Churchill et aJ. 2003; Morgan and Galione 2007). Thus, among sea urchins, two pathways may have evolved for the release of stored calcium. EARLY RESPONSES Sperm-egg binding Approximate time postinseminationQ o seconds Fertilization potential rise (fast block to polyspermy) within 1 sec Sperm-egg membrane fnsion within 1 sec Calcium increase first detected 10 sec Cortical granule exocytosis (slow block to polyspermy) 15-60 sec LATE RESPONSES Activation of NAD kinase starts at 1 min Increase in NADr+ and NADPH starts at 1 min Effects of calcium Increase in 02 consumption Sperm entry starts at "1 min 1-2 min The flux of calcium across the egg activates a prepro grammed set of metabolic events. The responses of the egg to the sperm can be divided into "early" responses, which occur within seconds of the cortical granule reaction, and "late" responses, which take place several minutes after fertilization begins (Table 4. 1). Acid efflux 1-5 min EARLY RESPONSES As we have seen, contact or fusion betvveen sea urchin sperm and egg activates the hvo major blocks to polyspermy: the fast block, initiated by sodium influx into the cell; and the slow block, initiated by the intracellular release of Ca2+.
The same release of Ca2+ responsible for the cortical granule reaction is also responsible for the re-entry of the egg into the cell cycle and the reactivation of egg protein synthesis. Ca27 levels in the egg increase from 0.1 to 1 rru\1, and in almost all species, this occurs as a wave or succes sion of waves that sweep across the egg beginning at the site of sperm-egg fusion (Jaffe 1983; Terasaki and Sardet 1991; Stricker 1999; see Figure 4.21). Calcium release activates a series of metabolic reactions that initiate embryonic development (Figure 4.26). One of these is the activation of the enzyme NAD+ kinase, which converts NAD+ to NADP+ (Epel et aJ. 1981). This change may have important consequences for lipid metabolism, since NADP+ (but not NAD+) can be used as a coenzyme for lipid biosynthesis. Thus the conversion of NAD+ to NADP+ may be important in the construction of the many new cell membranes required during cleavage. NADP+ is also used to make NAADP (see above), which appears to boost calcium release even further. Calcium release also affects oxygen consumption. A burst of oxygen reduction (to hydrogen peroxide) is seen during fertilization, and much of this "respiratory burst" is used to crosslink the fertilization envelope. The enzyme responsible for this reduction of oxygen is also NADPH-dependent (Heinecke and Shapiro 1989; Wong et aJ. 2004). Lastly, NADPH helps regenerate glutathione and ovothiols, molecules that may be crucial scavengers of free radicals that could othervvise damage the DNA of the egg and early embryo (Mead and EpeI 1995). Increase in pH (remains high) 1-5 min Sperm chromatin decondensation 2-12 min Sperm nucleus migration to egg center 2-12 min Egg nucleus migration to sperm nucleus 5-10 min Activation of protein synthesis starts at 5-10 min Initiation of DNA synthesis 20--40 min Mitosis 60-80 min First cleavage 85-95 min Malo sources: Whitaker and Steinhardt 1985; Mohri et aJ. 1995. Approximate times based on data from S. purpuratus (15-17°C), L. pictlls (16-18cC), A. prmctulata (18-20°C), and 1. variegafus (22-24°C). The timing of events within the flrst minute is best known for LytechinU5 variegatus, so times are listed for that species. Q LATE RESPONSES: RESUMPTION OF PROTEIN AND DNA SYN THESIS The late responses of fertilization include the activa tion of a new burst of DNA and protein synthesis. The fusion of egg and sperm in sea urchins causes the intracel lular pH to increase.* This rise in intracellular pH begins with a second influx of sodium ions, which causes a 1 : 1 exchange between sodium ions from the seawater and hydrogen ions from the egg. The loss of hydrogen ions causes the pH of the egg to rise (Shen and Steinhardt 1978; Michael and Walt 1999). It is thought that pH increase and Ca2 + elevation act together to stimulate new DNA and protein synthesis *This is due to the production of diacylglycerol, as mentioned above. The sea urchin egg has a Na+ /H+ antiport'protelo that is reg ulated by the ionic and cytoskeletal changes at fertilization (Rangel Mata et al. 2007). Again, variation among species may be prevalent. In the much smaller egg of the mouse, there is no elevation of pH after fertilization, and there is no dramatic increase in protein syn thesis immediately following fertilization (Ben-Yosef et aL 1996).
r Membrane Na+ influx -+- potential ◆ change L- r -" _ _ _ Sperm binding and/or fusion to egg cell membrane L 1 43 FERTILIZATION I Activation of Conversion of NAD+ kinase ◆ NAD+ to NADP+ -..- lr--'--'------.I l :: IP3 ◆ Ca2+ release ---... Cortical granUle -i production exocyto,i, Kinase ◆ Phospholipase C stimulation adjvation L Degradation of cydin and inactivation .. .
of MAP kinase l _ .◆""' -Diacylglycerol Protein kin ' ase C production ◆ activation FIGURE 4.26 Postulated pathway of egg activation in the sea u rchin (After Epel 1 980 and L. A. Jaffe, pers. comm.) . t (Na+/H+ exchange◆ 1980; Whitaker and Steinhardt 1982; Rees et al. 1995). If one experimentally elevates the pH of an unfer tilized egg to a level similar to that of a fertilized egg, DNA synthesis and nuclear envelope breakdown ensue, j ust as if the egg were fertilized (Miller and EpeI 1999) Calcium ions are also critical to new DNA synthesis. The wave of free Ca2+ inactivates the enzyme MAP kinase, converting it from a phosphorylated (active) to an unphosphorylated (Winkler et al. . (inactive) form, thus removing an inhibition on DNA syn thesis (Carroll et a1. 2000). DNA and protein synthesis can Increase in intracellular pH Restoration of mitotic cell cycle ;::Slow == ===:;; block to Membrane biosynthesis polysnermy t' Formation of hyaline layer Stimulation of protein synthesis, DNA replication, and cytoplasmic movements of morphogenetic material maskin, an inhib itor of translation in the unfertilized amphibian oocyte. In sea urchins, a similar inhibitor binds translation initiation factor eIF4E at the 5' end of several mRNAs and prevents these mRNAs from being translated. Upon fertilization, however, this inhibitor-the eIF4E-bind ing pr otein-becomes phosphorylated and is degraded, thus allowing eIF4E to complex with other translation fac tors and permit protein synthesis from the stored sea urchin mRNAs (Cormier et a!. 2001; Oulhen et aI. 2007). One of the mRNAs "freed" by the degradation of eIF4E-binding pro- then resume. In se a urchins, a burst of pro tein synthesis usually occurs 'Within several minutes after sperm entry. This pro tein syn thesi s does not depend on the synthesis of new messenger RNA; rather, it uses mRNAs already present in the oocyte cytoplasm (Figure 4.27; also see Table 2.2). These (A) 8 .◆ during e arly a burst of protein sy n thesis can be induced by artifiCially raising the pH of the cy toplas m using arrunonium ions (Winkler et a1. 1980). One mechanism for this global rise in the translation of messages stored in the oocyte appears to be the release of inhibitors from the mRNA. In Chapter 2, we discussed and morphogenetic factors that are used development. Such , I i 5 I; 5 I· l t FIGURE 4.27 A bursl of p ro ei n synthesis al fertil izalion uses mRNAs stored in the oocyte cytoplasm. (A) Protein synthesis in embryos of the sea urchin Arbacia puncwlata fertilized in the presence or absence of actinomycin 0, an inhibitor of transcrip tion. For the first few hours, protein synthesis occurs with very lit tle new transcription from the zygote or embryo nuclei. A second burst of protein synthesis occurs during the mid◆blastula stage, at about 1 2 hours after fertilization. This burst represents translation of newly transcribed messages and therefore is not seen in embryos gr()\A.ling in actinomycin. (B) The percentage of ribosomes recruited into polysomes increases during the firsl hours of sea urchin development, espec ia lly du ring the first cell cycle. (A afler Gross et al. 1 964; B aher Humphreys 1 97 1 .) o (:◆ o,o og , mRNAs encode proteins such as histones, tubulins, actins, f Q◆ .' 0 25 • 5 " , .. 10 :◆ ..... r· ! • Development time (hours) • ◆ __ -L __'. . ◆ -.•. 20 5 . 0 ◆ ◆ ◆ __ 0 o 0 Normal seawater ActinOmyci seawater o (B) 0 _---- 15 ◆ ◆ __ 20 .- 25 1 44 CHAPTER 4 0\.i4Dnet.z.uz:-, ...-;:.,'.,"·@II....Q"7': ◆ Rules of Evidence: " Find It, lose It, Move It" B iology, like all science, does not deal with Facts; rather, it deals with evidence. Several types of evidence will be presented in this book, and they are not equivalent i n strength . As a n example, we will use the analysis of the role of Ca'+ in egg activation. Correlative evidence The first, and weakes t, type of evi- dence is correlative evidence. H ere, we find correlations between two or more events and then make the infer ence that one event causes the other. For example, upon the meeting of sea urchi n sperm and egg, a wave of free Ca2+ spreads across the egg (see Figure 4.2 1), and this wave of Ca'+ is thought to activate the egg.
This chain of events has been shown in several ways, most convincingly by aequorin fluorescence (Steinhardt et al. 1 977; Shimomura 1 995; Ste inhardt 2006). A l thou gh o ne mi ght infer that the meeting of egg and sperm caused the Ca2+ wave, and that this Ca2+ wave caused egg activation, such a correla tion of even ts wit h one a nother does not n ecessa ri ly demonstrate a causa l rel a t io nshi p . It is pos s i ble the meet in g of am etes fi rst ca used the flow of Ca + a cross the egg and then, sepa rately and by some other mechanism, activated the egg. It is a Iso conceiv a b l e that some aspect of egg activa ti on caused the Ca2+ release. The correlat ed occurrence of these events could even be coi nci d ental and have no rela ti o ns h i p to one anot her.* Correla- ◆ *In a tongue-i n-cheek letter spoofing s uch correla tive evi dence, Sies (1 988) demonslrated a remarkably good corre lation between the number of storks seen in West Germany from 1 965 to 1 980 and the number of babies born duri ng those same years. Any cause-and-effect scenario between storks and babies, however, would certai nly fly in the face of the evidence presented in this cha pter. tive evidence provi d es a starting point for many investigations, but one can not say that one event causes another based solely on correlation. Functional evidence The next type of evidence is cal led loss-of-function evidence, also known as negative inference evidence. Here, the absence of th e postul ated cause is associated with the absence of the effect. Wh i Ie stronger than correlative evidence, loss-of-function evidence still does not exclude other explana tions. For instance, when calcium chelators such as EDTA were injected into the egg prior to fertilization, released Ca2+ failed to activate the egg. T h i s would imply that Ca2+ is necessary for egg activation. However, data from such i n h ibitory studies (incl uding studies from loss-of-func tion mutations) a lways leave open the possibi lity that the i n h i b itor suppresses more than just th e process be i ng stud ied. For instance, when protease i n h i bitors caused the fai l u re of mam malian ferti lization, it was assumed that these inhibitors were blocking th e action of proteases released from the acrosome. As a result, biologists thought that the mammalian acro some releases soluble proteases that d igest the zona. Later experiments, however, demonstrated that the pro tease i n h i bitors inhibited the acro some react i on itself so that the pro teases were never released (Llanos et al. 1 993). Thus, one couldn't tell whether soluble proteases played any . role in mammalian fertilization. The strongest type of evidence is gain-of-function evidence. Here the i nitiation of th e first event causes the second event to happen even in instances where or when neither event usually occ u rs . Thus, when cal cium ionophores (which can shuttle Ca2+ across membranes) were added to unfertilized eggs, Ca2+ was released from i ntrace l l u l a r storage and the egg became activated even without ferti lization (Steinhardt and Epel 1 974). Progression of evidence and coherence Correlative ("find it"), loss-of-function ("lose it"), a n d ga i n-of-function ("move if') evidence mu st cons i stently support each other to establish and solidify a conclusion. This progression of "find it; lose it; move it" evidence is at the core of nearly a l l studies of develop m en tal mechanism (Adams 2000). Sometimes it can be fou nd in a s i n g le paper, and so meti mes, as the case above illus trates, the evidence comes from many laboratories. " Every scientist," writes Fleck (1 979), "knows just how l i ttle a single experiment can prove or con vince.1I Rather, /Ian entire system of experiments and controls is needed." Science is a communal endeavor, and it is doubtful that any great d iscovery is the achievement of a single experi ment, or of any individual. Science also accepts evidence bet ter when it fits into a system of other findings. This is often called coher· ence. For i nstance, the ability of ca lci um to ac t ivate the egg became a stan dard part of fertilization physiology when Ca2+ was shown to cause both the resumption of cell division and the i nitiation of translation-two separate tivation. Also, components of egg ac once the sperm was found to activate phopholipase C-the enzyme that synthesizes IPJ-and 1P3 was found to activate intracellular calcium release in n u merou s cells, the release of Ca2+ became understood as being the cen tral element of sea urchin egg activa tion. It fit into a much wider picture of phys io logica l calcium release, and the mechanisms for its syntheSiS an d its effects a l l fit together.
, FERTILIZATI O N tein is the message encoding cyclin B (Salaun et aL 2003, 2004). The cyclin B protein combines with Cdk1 cyclin to create mitosis-promoting factor (MPF), which is required to initiate cell division. Thus fertilization activates pathways that target the translational inhibitory protein for degradation, and the newly accessible 5' end of the mRNA can interact with those proteins that allow the message to be translated.
One of these mRNAs encodes a protein critical for cell division.
in such a manner, fertilization can initiate mitosis and the sea urchin can begin to form c1 multicellular organism. Fusion of genetic material sea urchins, the sperm nucleus enters the egg perpen dicular to the egg surface. After the sperm and egg cell membranes fuse, the sperm nucleus and its centriole sep arate from the mitochondria and flagellum. The sperm's mitochondria and the flagellum disintegrate n i side the egg, so very few, if any, sperm-derived mitochondria are found in developing or adult organisms. Thus, although each gamete contributes a haploid genome to the zygote, the mitochondrial genome is transmitted primarily by the maternal parent. Con◆ersely, in almost all animals studied (the mouse being the major exception), the centrosome needed to produce the mitotic spindle of the subsequ ent divisions is derived from the sperm centriole (see Fi gure -l.17; Sluder et al. 1989, 1993). Fertilization in sea urchin eggs occurs after the second meiotic diVision, so there is a haploid female pronucleus in the cytoplasm of the egg when the sperm cnters. Once inside the egg, the sperm nucleus undergoes a dramatic transformation as it decondenses to form the haploid male pronucleus. First, the nuclear envelope vesiculates into small packets, expDsing the compact sperm chromatin to the egg cytoplasm (Longo and Kunkle 1978; Poccia and Collas 1997). Then proteins holding the sperm chromatin in its condensed inactive state are exchanged for other pro teins derived from the egg cytoplasm. TIus exchange per mits the decondensation of the sperm chromatin. Once econdensed, the DNA adheres to the nuclear envelope, where DNA polymerase can initiate replication (Infante et al. 1973). In sea urchins, sperm chromosome decondensation appears to be initiated by the phosphorylation of the nuclear envelope lamin protein and the phosphorylation of two sperm-specific his tones that b ind tightly to the DNA. The process begins when sperm comes into contact with a certain glycoprotein in the egg jelly that elevates the level of cAMP-dependent protein kinase activity. These protein kinases phosphorylate several of the basic residues of the sperm-specific histones and thereby interfere with - eil' binding to DNA (Garbers ot aL 1980; Por ter and Vac 'luier 1986; Stephens et al. 2002). This loosening is thought : facilitate the replacement of the sperm-specific histones In , with 145 other histones that have been stored in the oocyte cytoplasm (Green and Poccia 1985). After the sea urchin sperm enters the egg cytoplasm, the male pronucleus separates from the tail and rota tes 180 degrees so that the sperm centriole is between the spe rm pronucleus and the egg pronucleus. The sperm centriole then acts as a microtubule organizing center, extending its own microtubules and integrating them with egg micro tubules to form an aster.' Microtubules extend throughout the egg and contact the female pronucleus, at which point the two pronuclei migrate toward each other. Their fusion forms the diplOid zygote nucleus (Figure 4.28). DNA syn thesis can begin either in the pronuclear stage (during migration) or after the formation of the zygote nucleus, and depends on the level of Ca2+ released earlier in fertil ization Gaffe et al. 2001). Internal Fertilization in Mammals It is very difficult to study any interactions between the mammalian sperm and egg that might take place prior to these gametes making contact. One obvious reason for this is that mammalian fertilization occurs inside the oviducts of the female: while it is relatively easy to mimic the con ditions surrounding sea urchin fertilization using either natural or artificial seawater, we do not yet know the com ponents of the various natural environments that mam malian sperm encowlter as they travel to the egg. A second reason why it is difficult to study mammalian fertilization is that the sperm population ejaculated into the fema le is probably v ery heterogeneous, containing spermatozoa at different stages of matu ration. Out of the 280 x l(? human sperm normally ejaculated during coitus, only about 200 reach the vicinity of the egg (Ra l t et al. 1991). Thus, since fewer than 1 in 10,000 sperm even gets close to the egg, it is difficult to assay those molecules that might enable the sperm to swim toward the egg and become activated. Getting the gametes into the oviduct: Translocation and capacitation reproductive tract is not a passive conduit set of tissues that actively regulates the transport and maturity of both gametes. 130th the male and female gametes use a The female through which sperm race, but a highly specialized *Vo1hen Oscar Hertwig observed this radial array of sperm asters forming in his newly fertilized sea urchin eggs, he called it "the sun in the egg C'lnd thought it was the happy indicC'ltion of a successful fertilization (Hertwig 1877).
More recently, Simerly and co-workers (1999) f01.U1d that certC'lin types of human male infertility are due to defects in the centriole's ability to form these microtubular asters. This deficiency results in the failure of pronuclear migration and the cessation of further development. " 146 CHAPTER 4 ◆) (e) (◆ o 100 200 400 Time (sec) 600 800 FIGURE 4.28 Nuclear events in the fertilization of the sea urchin. (A) Sequent ia l photographs showi ng the migration of the egg pronuc leus and the sperm pronucl eus toward each other in an egg of Clypeasler japonicus. The sperm pronuc leus is sur rounded by its aster of microtubules. (B) The two pronucl ei migrate toward each other on these microtubular processes. (The is stained blue by Hoechst dye.) The micro tubules (stained green with fluorescent antibodies to tubulin) radi ate from centrosome associated with the (smaller) male pronu cleus and reach toward the female pronucleus. (C) Fusion of pronuclei in the sea u rc i n egg. (A from H a maguc h i and Hiramo to 1 980, courtesy of the au thors; B from Holy and Schallen 1 99 1 , cou rtesy of J .
Holy; C courtesy of F. J. Longo.) the pronuc lear DNA combination of small-scale biochemical interactions and large-scale physical propulsion to get to the ampulla, the region of the oviduct where fertilization takes place. A mammalian oocyte just released from the ovary is surrounded by a matrix containing cumulus ceUs. (Cumulus ceUs are the cells of the ovarian follicle to which the developing oocyte was attached; see Figures 4.7 and 16.31). U this matrix is experimentally removed or sig nificantly altered, the fimbriae of the oviduct will not "pick up" the oocyte-aunulus complex (see Figure 8.15), nor will the complex be able to enter the oviduct (Talbot et al. 1999). Once it is picked up, a combination of ciliary beating and muscle contractions transport the oocyte-cumulus com plex to the appropriate position for its fertilization in the oviduct. The translocation of sperm from the vagina to the oviduct involves many processes that work at different times and places. Sperm motility (i.e., flagellar action) is probably a minor factor in getting the sperm into the oviduct, although motility is re quired for mouse sperm to travel through the cervical mucus, and for sperm to TRANSLOCATION h encounter the egg once they are in the oviduct.
Sperm are found in the oviducts of mice, hamsters, guinea pigs, cows, and humans within 30 minutes of sperm deposition in the vagina-a time "too short to have been attained by even the most Olympian sperm relying on their own flagellar power" (Storey 1995). Rather, sperm appear to be trans ported to the oviduct by the muscular activity of the uterus. Recent studies of sperm motility have led to several con clusions, including the following: 1. Uterine muscle contractions are critical in getting the sperm into the oviduct. 2. The region of the oviduct before the ampulla may slow down sperm and release them slowly. 3. Sperm (flag

candidates for sperm fusion proteins; indeed, there may be several sperm-egg binding systems operat ing, and each of them may be necessary but not sufficient binding and fusion. It is not yet known whether the Izumo on the sperm and the crn on to insure proper gamete the egg bind one another. FERTILIZATION (A) (B) 1 53 (C) , " d or 0> n s, '" eel an FIGURE 4.35 P ronu c l ear movements during human fertilization. The microtubules have been stained green, while the DNA is dyed blue. The arrows point to the sperm tail. (A) The mature unfertilized oocyte completes the first meiotic division, budding off a polar body. 18) As the sperm enters the oocyte (left side), mlcrotubules condense around it as the oocyte compl etes its second meiotic division at the periphery. (C) By '1 5 hours after fertili zation, the two pronuclei have come together, and the centrosome splits to organize a bipolar microtubule array. The sperm tail is still seen (arrow), (0) At prometaphase, chromosomes from the sperm and egg intermix on the metaphase equator and a mitotic spindle initiates the first mitotic division. The sperm tail can still be seen.
(From Simerly et al. 1995, courtesy of C.
Schatten.) Polyspermy is a problem for the developing mammal as well as for the sea urchin (see Figure 4.17). In mammals, an electrical "fast" block to polyspermy has not yet been detected; and it may not even be needed/ given the limit ed number of sperm that reach the ovulated egg (Gardner and Evans 2006). However, a slow block to polyspermy in mammals occurs by enzymes from the cortical granules modifying the zona pellucida proteins. Released enzymes modify the zona pellucid a sperm receptors such that they can no longer bind spenn (Bleil and Wassarman 1980). Cor tical granules of mouse eggs have been found to contain -'!-acetylglucosaminidase enzymes capable of cleaving N- .acetylglucosamine from ZP3 carbohydrate chains. N3cetylglucosamine is one of the carbohydrate groups to ·,·;hich sperm can bind. Miller and co-workers (1992, 1993) have demonstrated that when the N-acetylglucosamine ⊕sidues are removed at fertilization/ ZP3 will no longer serve as a substrate for the binding qf other sperm. ZP2 is dipped by another cortical granule protease and loses its ⊕bility to bind sperm as well (Moller and Wassarman 1989). Thus/ once one sperm has entered the egg/ other sperm can no longer initiate or maintain their binding to the zona pel .ucida and are rapidly shed. Fusion of genetic material .-\5 in sea urchins, the mammalian sperm that finally enters :he egg carries its genetic contribution in a haploid pronu ...!eus. In mammals, the process of pronuclear migration -=> 'es about 12 hours/ compared with less than 1 hour in me sea urchin. The mammalian sperm enters almost tan- (D) gentially to the surface of the egg rather than approaching it perpendicularly, and it fuses with numerous microvilli (see Figure 4.34A). The DNA of the sperm nucleus is bound by basic proteins called protamines, which are tightly com pacted through disulfide bonds. Glutathione in the egg cytoplasm reduces these disulfide bonds and allows the uncoiling of the sperm chromatin (Calvin and Bedford 1971; Kvist et a1. 1980; Perreault et a1. 1988). The mammalian sperm enters the oocyte while the oocyte nucleus is "arrested" in metaphase of its second meiotic divi sion (Figure 4.35A,B; see also Figure 4.5). The calcium oscil lations brought about by sperm entry inactivate MAP kinase and allow DNA synthesis.
But unlike the sea urchin egg, which is already in a haploid state, the mammalian oocyte still has chromosomes in the middle of meiotic metaphase. Oscillations in the level of Ca2+ activate another kinase that leads to the proteolysis of cyctin (thus allowing the cell cycle to continue, eventually resulting in a haploid female pronu cleus) and securin (the protein holding the metaphase chro mosomes together) (Watanabe et a1. 1991; Johnson et a1. 1998).
Mammals appear to undergo several waves of Ca2+ release; it is possible that events initiated by the first wave might not go to completion without additional calcium waves (Ducibella et a1. 2002) . DNA synthesis occurs separately in the male and female pronuclei. The centrosome (new centriole) accompanying the male pronucleus produces its asters (largely from pro teins stored in the oocyte). The microtubules join the two CHAPTER 4 154 toward one anoth er other. Upon meeting, the two nuclear envelopes break down (Figu re 4.35C). How e ve r, instead of pro ducing a common zygote nucleus (as in sea urchins), the chromatin condenses into chromosomes tha t orient themselves on a common mitotic spindl e (F igure 4.35C, D). Thus, a true diploid nucleus in mammals is fust seen not in the zygote, but at the 2- of cytoplasm.
The sperm mitochondria and their DNA are p ronuclei and enable them to migrate degraded in the egg cy toplasm, so that all of the new indi vidual's mitochondria are derived from its mother.
The egg and embryo appear to get rid of U,e paternal mitochondria both by dilution and by actively targeting them for destruc tion (Cummins et a!. 1998; Shitara et al. 1998; Schwartz and Vissing 2(02). However, in mos t mammals, the sperm cen triole not only survives but serves as the organizing agen t for making the new mito tic spindle. SIDEL I G H TS SPECULATIONS t The Nonequivalence of Mammalian Pronuclei I i s generally assumed that males and females carry equivalent hap loid genomes. Indeed, one of the fundamental tenets of Mendelian genetics is that genes derived from the sperm are functionally equivalent to those derived from the egg. However, as we saw i n Ch apter 2, genomic imprinting can occur in mammals such that the spe rm de rived genome and the egg-derived genome may be functionally different and play comple mentary roles during certain stages of development. This imprinting is tho ught to be caused by the different patterns of cytosi ne methylation on the genome. The first evidence for nonequiva- lence came from studies of a h u ma n tumor called a hydatidiform mole, which resembles placental tissue. A majority of such moles have been shown to arise when a haploid sperm fertil izes an egg in which the female pronucleus is absent. After enteri ng the egg, the sperm chromosomes dupl icate themselves, thereby restor ing the diploid chromosome number. However, the entire genome is derived from the sperm Uacobs et a!.
1 980; Ohama et al. 1 98 1) . The cells survive, divide, and have a normal chromo some number, but development is abnormal . Instead offarming a n emb ryo, the egg becomes a mass of - placenta-like cells. Normal develop ment does not occur when the entire genome comes from the male parent. Normal development also does not occur when the genome is derived totally from the egg. The ability to develop an embryo without spermatic c ontributio n is called parthenogenesis (Greek, "virgin birth").
The eggs of many invertebrates and some verte brates are capable of deve lo ping nor mally in the absence of sperm (see Chapter 1 6). Mammals, however, do not exhibit parthenogenesis. Placi ng mouse oocytes in a culture medium that artikially activates the oocyte while suppressing the formation of the second polar body produ ces diploid mouse eggs whose genes are derived TABLE 4.2 Class of reconstructed zygotes Bimatemal Bipaternal Control exclusively from the oocyte (Kaufman et al. 1 977). These eggs divide to form embryos with spinal cords, muscles/ skeletons, and organs, including beat ing hearts. However, development does not continue, and by day 1 0 or 1 1 (halfway through th e mouse's gesta tion), these parthenogenetic embryos deteriorate. Neither human nor mouse development can be completed solely with egg-derived chromosomes. That male and female pronuclei are both needed for normal development was also shown by pronuclear trans plantation experiments (Surani and Barton 1 983; McGrath and Salter 1 984; Surani et a!.
1 986). Either male or female pronuclei can be removed from recently ferti lized mouse eggs Pronuclear transplantation experiments --@{9 Operation ⊕ 0_ --0Q) Source: McGrath and Solter 1984. Number of successful transplants Number of pros.e⊕y su rvrvmg 339 0 328 0 348 18 FERTI LIZATION 155 SIDELIGHTS & SPECULATIONS (Con t i n u ed) and added to other rec e nt l y fertilized eggs.
(The two pronuclei can be dis tinguished at this stage because the female pronucleus i s th e one beneath the po l a r bodies.) Thus, zygotes with two male or two female pro nuc l e i can be constructed. Alth ough these eggs w i l l form diploid cells that undergo normal cleavage, eggs whose genes are derived s o lel y from sperm nudei or solely from oocyte nuclei do not develop to birth. Control eggs under- going such tra nspl a ntat io n (i .e., eggs co n tai n i ng one male and o ne fem a l e pro nu c l eu s taken from different zygotes) can d eve l op normally (Table 4.2). Thus, for mammalian develop ment to occur, both the sperm-derived and the egg d e r ived pro nu c l e i a re cr i ti ca l . The importance o f DNA methyla tion in this block to parthenogenesis was demonstrated when Ko no a nd co l l ea gues (2004) generated a fe m a l e - Activation of the mammalian egg As in every other animal studied, a transient rise in cyto 2 plasmic Ca + is necessary for egg activation in mammals. The sperm induces a series of Ca2+ waves that can last for mouse whose genes came exc l u sive ly from two oocytes. To accomplish this feat, they had to mutate the DNA met hy l at io n system i n one of the oocyte genomes to make it more l i ke that of a male mouse, and then they had to perform two rounds of nuclear tra nsfer. liMen," as one reviewer remarked, "do not need to fear becoming redundant any time soon" (Vogel 2004). ' tle or no functional PLCS. In fact, normal human tiperm 2 can activate C a + oscillations when injected into mouse eggs, but sperm lacking PLC⊕ do not (Yoon et a1. 2008). of meiosis, cortical granule exocytosis, and the release of Whereas sea urchin eggs usually arc activated as a sin 2 gle wave of Ca + crosses from the point of sperm entry, the mammalian egg is traversed by numerous waves of Ca2+ the lnhlbition on maternal mRNAs) and the formation of (Miyazaki et a1. the male and female pronuclei. And, again as in sea 2 urchins, fertilization triggers intracellular Ca + release Fissore hours, terminating in egg activation (i.e., the resumption through the production of IP3 by the enzyme phospholi pase C (Swann et a1. 2006; Igarashi et a1. 2007). 2008). 1992; Ajduk et al.
2008; Ducibella and In mammals, the calcium ions released by IP3 bind to a series of proteinti including calmodulin-acti vated protein kinase (which will be important in eliminat ing the inhibitors of mRNA translation), MAP kinase However, the mammalian PLC responsible for egg acti (which allows the resumption of meiosis), and synaptotag vation and pronucleus formation may in fact corne from the min (which helps initiate cortical granule fusion). Calcium sperm rather than from the egg. Some of the first observa ions that are not used are pumped back into the endoplas tions for a sperm-derived PLC carne from studies of intra mic reticulum, and additional calcium ions are acquired cytoplasmic sperm injection (ICSI), an experimental treat ment for curing infertility. Here, sperm are directly injected from outside the cell. If mammalian oocytes are cultured 2 in media without Ca +, the nwnber and amplitude of oscil into oocyte cytoplasm, bypassing any interaction with the lations decrease (Igusa and Miyazake 1983; Kline and Kline egg plasma membrane. To the surprise of many biologists 1992). The extent (i.e., amplitude, duration, and number) 2 of C a + oscillations appears to regulate the timing of egg (who had asswned that sperm binding to some egg receptor 2005; Toth et al. 2006). 111OS, cortical protein was critical for egg activation), this treatment activation (Ozil et al. worked. The human egg was activated and pronuclei granule exocytosis occurs before the reswnption of meiosis formed. Injecting mouse sperm into mouse eggs will also z induce fertilization-like Ca + oscillations in the egg and lead and the translation of maternal mRNAti. to complete development (Kimura and Yanagimachi 1995). 2 It appeared that an activator of C.a + release was stored Coda in the sperm head. This activator turned out to be a solu Fertilization is not a moment or an event but a process of PLCE, (zeta), that is delivered to carefully orchestrated and coordinated events including the egg by gamete fusion. In mice, expression of PLC⊕ mRNA in the egg produces Ca 2+ oscillations, and remov the contact and fusion of gametes, the fusion of nuclei, and ble sperm PLC enzyme, the activation of development. It is a process whereby two ing PLC⊕ from mouse sperm (by antibioclies or RNAi) abol cells, each at the verge of death, unite to create a new ishes the sperm's calciwn-inducing activity (Saunders et orgamsm that will have numerous cell types and organs. 2002; Yoda et al. 2004; Knott et a1. 2005). Human sperm It is just the beginning of a series of cell-cell interactions al. that are unsuccessful in lCSl have been shown to have lit- that characterize animal development.
1 56 CHAPTER 4 Snapshot Summary: Fertilization 1. Fertilization accomplishes two separate activities: sex (the combining of genes derived from two parents), and reproduction (the creation of a new organism).
2. The events of fertilization usually include (1) contact and recognition between sperm and egg; (2) regulation of sperm entry into the egg; (3) fusion of genetic material from the two gametes; and (4) activation of egg metabolism to start development. 3. The sperm head consists of a haploid nucleus and an acrosome. The acrosome is derived from the Golgi apparatus and contains enzymes needed to digest extracellular coats surrounding the egg. The midpiece and neck of the sperm contain mitochondria and the centriole that generates the microtubules of the flagellum. Energy for flagellar motion comes from mitochondrial ATP and a dynein ATPase in the flagellum. 4. The female gamete can be an egg (with a haploid nucleus, as in sea urchins) or an oocyte (in an earlier stage of development, as in mammals). The egg (or oocyte) has a large mass of cytoplasm storing ribosomes and nutritive proteins. Some mRNAs and proteins that will be used as morphogenetic factors are also stored in the egg. Many eggs also contain protective agents needed for survival in their particular environment. 5. Surrounding the egg cell membrane is an extracellular layer often used in sperm recognition. In most animals, this extracellular layer is the vitelline envelope. In mammals, it is the much thicker zona peUucida. Cortical granules lie beneath the egg's cell membrane. 6. Neither the egg nor the sperm is the "active" or "passive" partner. The sperm is activated by the egg, and the egg is activated by the sperm. Both activations involve calcium ions and membrane fusions. 7. In many organisms, eggs secrete diffusible molecules that attract and activate the sperm. 8. Species-specific chemotactic molecule⊕ secreted by the egg can attract sperm that are capable of fertilizing it. In sea urchins, the chemotactic peptides resact and speract have been shown to increase sperm motility and provide direction toward an egg of the correct species. 9. The acrosome reaction releases enzymes exocytotically. These proteolytic enzymes digest the egg's protective coating, allowing the sperm to reach and fuse with the egg cell membrane.
In sea urchins, this reaction in the sperm is initiated by compounds in the egg jelly. Globular actin polymerizes to extend the acrosomal process. Bindin on the acrosomal process is recognized by a protein complex on the sea urchin egg surface. 10. Fusion between sperm and egg is probably mediated by protein molecules whose hydrophobic groups can merge the sperm and egg cell membranes. In sea urchins, bindin may mediate gamete fusion. 11. Polyspermy results when two or more sperm fertilize an egg. It is usually lethal, since it results in blastorneres with different numbers and types of chromosomes. 12. Many species have two blocks to polyspermy. The fast block is immediate and causes the egg membrane resting potential to rise. Sperm can no longer fuse with the egg. In sea urchins this is mediated by the influx of sodium ions. The Slbw block, or cortical granule reaction, is physical and is mediated by calcium ions. A wave of Ca2+ propagates from the point of sperm entry, causing the cortical granules to fuse with the egg cell membrane. The released contents of these granules cause the vitelline envelope to rise and harden into the fertilization envelope. 13. The fusion of sperm and egg results in the activation of crucial metabolic reactions in the egg. These reactions include reinitiation of the egg's cell cycle and subsequent mitotic division, and the resumption of DNA and protein synthesis. 14. Genetic material is carried in a male and a female pronucleus, which migrate toward each other.
In sea urchins, the male and female pronuclei merge and a diploid zygote nucleus is formed.
DNA replication occurs after pronuclear fusion.
- 15. In all species studied, free Ca2+, supported by the alkalization of the egg, activates egg metabolism, protein synthesis, and DNA synthesis. Inositol trisphosphate (IF3) is responsible for releasing Ca2+ from storage in the endoplasmic reticulum.
DAG (diacylglycerol) is thought to initiate the rise in egg pH. 16.
IP3 is generated by phospholipases. Different species may use different mechanisms to activate the phospholipases. 17.
Mammalian fertilization takes place internally, within the female reproductive tract. The cells and tissues of the female reproductive tract actively regulate the transport and maturity of both the male and female gametes. 18.
The translocation of sperm from the vagina to the egg is regulated by the muscular activity of the uterus, by the binding of sperm in the isthmus of the oviduct, and by directional cues from the oocyte (immature egg) and/ or the cumulus cells surrounding it. (::c FERTILIZATION 19. Mammalian sperm must be capacitated in the female reproductive tract before they are capable of fertilizing the egg. Capacitation is the result of bio chemical changes in the sperm cell membrane. 20. Capacitated mammahan sperm must penetrate the cumulus and bind to the zona pellucida before lmdergoing the acrosome reaction. In the mouse, this binding is mediated by ZP3 (zona protein 3) and several sperm proteins that recognize it. 21. ZP3 initiates the mammahan acrosome reaction on the zona pellucida, and the acrosomal enzymes are concentrated there. 22. In mammals, blocks to polyspermy include the mod ification of the zona proteins by the contents of the cortical granules so that sperm can no longer bind to the zona. 157 23.
The rise in intracellular free Ca2+ at fertilization in amphibians and mammals causes the degradation of cyclin and the inactivation of MAP kinase, allowing the second meiotic metaphase to be completed and the formation of the haploid female pronucleus. 24. In mammals, DNA replication takes place as the pronuclei are traveling toward each other. The pronuclear membranes disintegrate as the pronuclei approach each other, and their chromosomes gather arow1d a common metaphase plate.
25. The male and female pronuclei of mammals are not equivalent. If the zygote's genetic material is derived solely from one parent or the other, normal develop ment will not take place. This difference in the male and female genomes is thought to be the result of different methylation patterns on the genes. For Further Reading Complete bibliographical citations for all literature cited in this chapter can be fOW1d at the free-access website www.devbio.com Bleil, j. D. and P. M. Wassarman. 1980.
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Vacquier, V. D. and G. W. Moy. 1977. Isolation of bindin: The protein respon sible for adhesion of sperm to sea urchin eggs. Fmc. Natl. Acad. Sci. USA 74: 2456-2460. WEBSITE 4.3 The egg and its environment The labora WEBSITE 4.1 Leeuwenhoek and images of homunculi.
Scholars in the 1600s thought that either the sperm or the egg carried the rudiments of the adult body. Moreover, :hese views became distorted by contemporary commenta :Drs and later historians. tory is not where most eggs are found. Eggs have evolved remarkable ways to protect themselves in particular envi⊕ ronments. W"EBSITE 4.2 The origins of fertilization research. Stud binding. In the early 1900s, fertilization research was e; by Her twig, Fol, Boveri, and Auerbach investigated fer ;::il ization by integrating cytology with genetics. The ⊕bates over meiosis and nuclear structure were critical in -..ese investigations of fertilization. framed by a dispute between F.
R. Lillie and jacques Loeb, who disagreed over whether the sperm recognized the egg through soluble factors or through cell-cell interactions. . WEBSITE 4.4 The Lillie-Loeb dispute over sperm-egg 1 58 CHAPTER 4 WEBSITE 4.5 Blocks to polyspermy. Theodore Boveri's analysis of polyspermy is a classic of experimental and descriptive biology. E. E. Just's delineation of the fast and slow blocks was a critical paper in embryology. Both papers are reprinted here, along with commentaries. WEBSITE 4.6 Building the egg's extracellular matrix. In sea urchins, the cortical granules secrete not only hyalin but a number of proteins that construct the extracellular matrix of the embryo. This highly coordinated process results in sequential layers. Vade Mecum Gametogenesis. Stained sections of testis and ovary illus trate the process of gametogenesis, the streamlining of developing sperm, and the remarkable growth of the egg as it stores nutrients for its long journey. You can see this in movies and labeled photographs that take you at each step deeper into the mammalian gonad. E.
E. Just. Blocks to polyspermy were discovered in the early 1900s by the African American embryologist Ernest Just, who became one of the few embryologists ever to be honored on a postage stamp. The Sea DIehm segment con tains videos of Just's work on sea urchin fertilization.
Sea urchin fertilization. The remarkable reactions that pre vent polyspermy in a fertilized sea urchin egg can be seen in the raising of the fertilization envelope. The Sea Urchin segment contains movies of this event shown in real time. Early Development i n Selected I nvertebrates THE GENERAL POLARITY, SYMMETRY, A D PATIERN of the embryo are egg char which were determined before fertilization," wro te Edwin Grant Conklin in 1920. Indeed, as we will see, the egg cyto plasm plays a major role in deter mining patterns of cleavage, gas trulation, and cell specification. I t does so by mteracting with the nuclear genome established at fertilization. Fertilization gave the organism a new genome and rearranged its cyt oplasm. :\ow the zygote begins the production of a multicellular organism During cleav ⊕ e, rapid ceU divisions divide the cytoplasm of the fertilized egg into numer ous cells. These cells undergo dramatic disp lacements during gastrulation, a rocess whereby they move to different parts of the embryo and acquire new neighbors During cleava ge and ga s trulation the major axes of the embryo are determined and the embryOnic cells begin to acquire their respective fates. While cleavage always precedes gastrulation, axis formation in some species begin as early as oocyte forma tion. It can be comp leted during cleavage (as Drosophila) or extend all the way through gas trulation (as in Xenopus). Three y axes must be specified: the anteri or-posterior (head-tail) axis; the dorsal . entral (back-belly) axis; and the left -ri ght axis. Di fferen t species specify these .;xes at different times, using different mechanisms. This chapter will look at .' e different ways these universal processes take place in some invertebrate embryos. • ers . . / , EARLY DEVELOPMENTAL PROCESSES: 4.N OVERVIEW Cleavage ce fertilization is complete, the development of a multicellular organism proby a process called cleavage, a series of mi totic divisions whereby the enor ;no us volume of egg cytoplasm is divided into numerous smaller, nucleated cells. -::nese cleavage-stage cells are call e d blastomeres. In most species (mammals ""ing the chief exception), both the initial rate of cell di vision and the placement ' the blastomeres with respect to one another are under the control of the pro 5 and mRNAs stored in the oocyte. Only later do the rates of cell division nd the placement of cells come under the control of the newly formed genome. During the ini tial pha se of development, when cleavage rhythms are con ⊕ lied by maternal fa ct ors, t he cytop lasmiC volume does not increase. Rather, he zygote cytoplasm is divided into increasingl y smaller ceUs. First the zygote . Hence, studying the period of cleav age, we approacli rhe source whence emerge tlie progressively branched streams of differelliar.ion that end finally in almost quiet pools, rhe indi vidual cells organism. of the complex adult E. E. /UST (1 939) It i.l not birth, marriage, or death, but gastrulation, which is truly the most imporrant time in your life. LEWIS WOLPERT (1 986) 1 60 CHAPTER 5 qUickly and to restore the somatic ratio of nuclear volwne MPF is degrad S phase (Gerhart et aJ. 1984; Newport and Kirschner 1984). What causes this cyclic al activity of MPF?
Mitosis-pro to cytoplasmic volume. The embryo often accomplishes moting factor consists of two subunits. The larger subunit, is divided in half, then quarters, then eighths, and so forth. denses into chromosomes. After an hour, Cleavage occurs very rapidly in most invertebrates, prob ed and the c1u-omosomes return to ably as an adaptation to generate a large number of cells this by abolishing the gap periods of the cell cycle (the G [a nd G2 phases), when growth can occur. A frog egg, for example, can divide into 37,000 cells in just 43 hours. Mito sis in cleavage-stage Drosophila embryos occurs every 10 minutes for over 2 hours, and some 50,000 cells form in just 12 hours. From fertilization to cleavage B, displays the cyclical behavior that is key to mitot S and being degraded after the cells have reached M (Evans et al. 1983; Swenson et al. 1986). Cyelin B is often encoded by mRNAs stored in the oocyte cytoplasm, and if the translation 01 this message is specifically inhibited, the cell will not enter mitosis (Min shull et a1. 1989). Cyclin B regulates the small subunit 01 MPP, the cyel in dep en dent kinase. This kinase activates mitosis by phos cyelin ic regulation, accumulating during As we saw in Chapter 4, fertilization activates protein syn phorylating several target proteins, including his tones, the thesis, DNA synthesis, and tlle cell cycle. One of the most nuclear envelope lamin proteins, and the regulatory sub important events in this transition from fertilization to unit of cytoplasmiC myosin. It is the actions of this small cleavage is the activation of mitosis-promoting factor, or kinase subunit that bring about chromatin condensation, MPF. MPF was first discovered as the major factor respon nuclear envelope depolymerization, and the organization sible for the reswnption of meiotic cell divisions in the ovu of the mitotic spindle . Without the cycIin B subunit, how lated frog egg.
It continues to play a role after fertiliza tion, ever, the cyclin-dependent kinase subunit regula ting the cell cycle of early blastomeres. function. Blastomeres generally progress through a biphasic cell cycle consisting of just two steps: M (mitosis) and 5 (DNA synthesis) (Figure 5 . 1). The MI'F activity of early blas S. S phases in blastomeres is driven solely by the gain and loss of MPF activity.
When MPF is microinjected into these cells, they enter M.
Their 01 MPF will not In most The presence of cyclin B is controlled by several proteins that ensure its periodic synthesis and degradation. species studied, fue regula tors of cyclin B (and thus of MPF) tomeres is highest during M and undetectable during are stored in the egg cytoplasm. Therefore, the cell cycle The shift between the M and remains independent of the nuclear genome for a num ber nuclear envelope breaks down and their chromatin con- (A) Mitosis of cell divisions. These early divisions tend to be rapid and In several synchronous. However, as the cytoplaSmiC components are used up, tl,e nucleus begins to synthesize them . (B) Mitosis k se (MPF) Active cdc2 protein ina M s Inactive protein kinase Cyclin degradation Synthesis FIGURE 5.1 Cell cycles of somatic cells and early blastomeres. (A) The bi phasic cell cycle of early amphibian blastomeres has only two states, S and M. Cycl i n B syn thesis allows progression to M (mitosis), while degradation of cyclin B al lows cells to pass into 5 (synthesis) phase. (B) The complete cell cycle of a typical somat ic cell. Mitosis (M) is fol lowed by an i n terphase stage. Interphase Interphase is subdivided intn Gr. S (synthesis), and G, phases . Cel l s th at are differentiating are usually taken "out" of the cell cyd e and are in an extended G1 phase cal led G o ' The cyc lins responsible for the progression through the cell cycle and their respective kinases are shown at their point of cell cycle regulation. (8 after Nigg 1 995.) EARLY DEVELOPMENT IN SELECTED I NV ERTEBRATES (A) Microfilaments (contractile ring) ⊕r.⊕=lh ⊕! (B) :⊕'r Chromosome C) ·,,⊕, ⊕ .'.' ⊕.'⊕.•⊕ species, the embryo now enters a mid-blastula transition, in which several new properties are added to the biphasic cell divisions of the embryo. First, the "gap" stages (Gj and G,) are added to the cell cycle (see Figure 5.18). Xenopus embryos add Gj and G, phases to the cell cycle shortly after the twelfth cleavage.
Drosophila adds G, dw-ing cycle 14 and Gl during cyde 17 (Newport and Kirschner 1982; Edgar et al. 1986). Second, the syncluonicity of cell division is lost, ;:;ecause different cells synthesize different regulators of \IPF. After numerous synchronous rounds of mitosis, the cells begin to "go their own way." Third, new mRNAs are transcribed, Many of these messages encode proteins that "{ill become necessary for gastrulation. In several species, ⊕. transcription is blocked cell division will still occur at nor mal rates and times, but the embryo will not be able to ini tiate gastrulation. Many of these new messenger RNAs are also used for cell specification. As we will see in sea urchin embryos, the new mRNA expression patterns of the mid ⊕Iastula transition map Qut territories where specific types of cells will later differentiate. The cytoskeletal mechanisms of mitosis Cleavage is the result of two coordinated processes. The :-jrst of these is karyokinesis, the mitotic division of the .:ell's nucleus. The mechanical agent of karyokinesis is the mitotic spindle, with its microtubules composed of tubu- . . :⊕ . ' :" . . . . 1 61 FIGURE 5.2 Role of microtubules and microfilaments in cell division.
(A) Diagram of first-cleavage te lop hase in a sea urchin egg. The chromosomes are being drawn to the cen trioles by microtubules, while the cytoplasm is being pinched in by the co ntracti on of micro filaments.
IBI Confocal fl uorescent image of an ech inoderm embryo undergoing first cleav age (early anaphase). The mic rotubu les arc stained green, actin microfil⊕ments are stained red. (C) Confocal fluorescent image of sea urchin embryo at the very end of first cleav age.
M i cro tub u les are ora nge; actin proteins (both unpolymerized and in microfilaments) are blue. (B,C courtesy of G.
von Dassow and the Center for Cell Dynamics.) lin (the same type of protein that makes up the sperm fla gellum).
The second process is cytokinesis: the division of the celL The mechanical agent of cytokinesis is a con tractile ring of microfiJaments made of actin (the same type of protein that extends the egg microvilli and the sperm acrosomal process). Table 5.1 presents a compari son of these agents of ceJl division. The relationship and coordination between these two systems during deavage are depicted in Figure 5.2A, in which a sea urchin egg is shown undergoing first deavage. The mitotic spindle and contractile ring are perpendicular to each other, and the spindle is internal to the contractile ring. The contractile ring creates a cleavage furrow, which eventually bisects the plane of mitOSiS, thereby creating two genetically equivalent blastomeres.
The actin rnicrofilaments are found in the cortex (outer cytoplasm) of the egg rather than in the central cytoplasm. Under the electron microscope, the ring of mkrofilaments can be seen forming a distinct cortical band 0.1 11m wide (Figure 5.2B, C). This contractile ring exists only during cleavage and extends 8-10 11m into the center of the egg. It is responsible for exerting the force that splits the zygote into blastomeres; if the rijJg is disrupted, cytokinesis stops. Schroeder (1973) likened the contractile ring to an "intra cellular purse-string," tightening about the egg as cleav age continues. 11us tightening of the microfilamentous ring creates the cleavage furrow. Microtubules are also seen near the cleavage furrow (in addition to their role in creat ing the mitotic spindle), since they are needed to bring membrane material to the site of membrane additiun (Danilchik et aJ. 1998). Although karyokinesis and cytokinesis are usually coor dinated, they are sometimes modified by natural or exper imental conditions. The placement of the centrioles is crit ical n i orienting the mitotic spindle, and thus the division plane of the blastomeres. Depending on the placement of the centrioles, the blastomeres can separate either into dor sal and ventral daughter cells, anterior and posterior daughter cells, or left and right daughter cells. The spin- 1 62 CHAPTER 5 TABLE 5.1 Karyokinesis and cyloldnesis Process Mechanical agent Major protein composition locatio n Major disruptive drug Karyokinesis Mitotic spindle Tubulin microtubules Central cytoplasm Colchicine, nocodazolEf Cytokinesis Contractile ring Actin micro filaments Cortical cytoplasm Cytochalasin B tlBecause colchicine has been found to independently inhibit several membrane functions, including osmoregulation and the transport of ions and nucleoside..,>, nocodazole has become the major drug used to inhibit microtubule-mediated processes (see Hardin 1987). die can even be at an angle such that one daughter cell is clockwise or counterclockwise to the other. As we will ieam Chapter 6, cleavage in insect eggs consists of karyokinesis several times before cytokinesis takes place. In this man ner, numerous nuclei exist within the same cell. (The outer membrane of that one big cell will eventually indent to sep arate the nuclei and form individual cells.) Patterns of embryonic cleavage Tn 1923, embryologist E.
B. Wilson reflected on how little we knew about cleavage: "To our limited intelligence, it would seem a simple task to divide a nucleus in to equal parts. The cell, manifestly, entertains a very different opin ion." Indeed, different organisms W\dergo cleavage in dis tinctly different ways. The pattern of embryoniC cleavage peculiar to a species is determined by two major parame ters: (1) the amount and distribution of yolk protein with in the cytoplasm, and (2) factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation. The amount and distribution of yolk determine where cleavage can occur and the relative size of the blastomeres. In general, yolk inhibits cleavage. When one pole of the egg is relatively yolk-free, cellular divisions occur there at a faster rate than a t the opposite pole.
The yolk-rich pole is referred to as the vegetal pole; the yolk concentration in the animal pole is relatively low. The zygote nucleus is fre quently displaced toward the animal pole. Figure 5.3 pro vides a cla$ification of cleavage types and shows the influ ence of yolk on cleavage symmetry and pattern. At one extreme are the eggs of sea urchins, mamma1s, and snails. These eggs have sparse, equally distrib uted yolk and are thus isolecithal (Greek, "equal yolk"). In these species, cleavage is holoblastic (Greek holos, "complete"), meaning that the cleavage furrow extends through the entire egg. With little yolk, these embryos must have some other way of obtaining food. Most will generate a vora cious larval form, while mammals will obtain their nutri tion from the maternal placenta. At the other extreme are the eggs of insects, fish, rep tiles, and birds. Most of their cell volumes are made up of yolk. The yolk must be slifficient to nourish these animals throughout embryonic development. Zygotes containing large accumulations of yolk undergo meroblastic cleavage (Greek meros, "part"), wherein only a portion of the cyto· plasm is cleaved. The cleavage furrow does not penetrate the yolky portion of the cytoplasm because the yolk platelets impede membrane formation there. Tnsect eggs have yolk in the center (i.e., they are centrolecithal), and the divisions of the cytoplasm occur only in the rim of cyto· plasm, around the periphery of the cell (i.e., superficial cleavage). The eggs of birds and fish have only one small area of the egg that is free of yolk (telolecithal eggs), and therefore the ceU divisions occur only in this small disc of cytoplasm, giving rise to the discoidal pattern of cleavage. These are general rules, however, and even closely related species have evolved different patterns of cleavage in dif ferent environments. Yolk is just one factor influenCing a species' pattern of cleavage. There are also, as Conklin had intuited, inherited patterns of ceU djvision superimposed on the constraints of the yolk. The importance of this inheritance can readily be seen in isolecithal eggs. In the absence of a large can· centration of yolk, holoblastic cleavage takes place, and four major patterns of this cleavage type can be observed: radial, spiral, bilateral, and rotational holoblastic cleavage. We will see examples of all of these cleavage patterns as this chapter takes a more detailed look at four different invertebrate groups.
Gastrulation The blastula consists of numerous cells, the positions of which were established during cleavage. During gastrula· tion, these cells are given new positions and new neigh bors, and the multilayered body plan of the organism is established. The cells that will form the endodermal and mesodermal organs are brought to the inside of the embryo, while the cells that will form the skin and nerv ous system are spread over its outside surface. Thus, the three germ layers-outer ectodenn, inner endoderm, and interstitial mesoderm-are first rroduced during gastru lation. In addition, the stage is set for the interactions of these newly positioned tissues. Gastrulation usuaUy involves some combination of sev eral types of movements (Figure 5.4) . These movements involve the entire embryo, and cell migrations in one part of the gastrulating embryo must be intimately coordinat ed with other movements that are taking place simultane- EARLY DEVELOPMENT I N SELECTED I NVERTEBRATES I. HOLOBLASTIC (COMPLETE) CLEAVAGE A.
rsolecithal (Sparce, evenly distributed yolk) 1. 2. Radial cleavage Echinodenns. amphioxus Spiral cleavage Annelids, molluscs, flatworms 3. Bil ateral cleavage Tunicates 4. Rotational cleavage Mammals, nematodes ea B. Mesolecithal (Moderatl! veg t l yolk disposition) Displaced radial cleavage Amphibians II. MEROBLASTIC (INCOMPLETE) CLEAVAGE A. (Dense yolk throughout most of cell) Telolecithal I. 2. Bilateral cleavage Cephalopod molluscs Discoidal cleavage Fish, reptiles.
birds (Yolk in center of egg) 13. Ce ntrolecithal Superficial cleavage Most insects CURE 5.3 .. ..
'C::> .... ⊕ .. . ⊕ .. c=J C) . Summary of the main patlerns of deavage. ⊕ .. .
..
:. : :.: :⊕ . . . .. ⊕ 1 63 1 64 CHAPTER 5 cell sheet ae Invagination: Involution: Infolding of cell lnturning of sheet into embryo .0 ·· · · · over the basal su rface of an outer l y r . . . .
· . . .
.
. . .. " , . ,⊕, " . ,' .. · ' Sea urchin Example: Amphibian mesoderm Example: endoderm n • • • • • Cell Specification and Axis Formation CeU fates are specified by ceU-ceU signaling or by the asym metric distribu han of patterning molecules into particular cells, These unevenly distributed molecules are usually transcription

(A) Micromeres are transplanted from the vegetal pole of a 1 6-cell embryo into the animal pole of a host 1 6-cell em b ryo . (8) The transpl anted m i c romeres i n vaginate into the bl astocoel to create a new set of skeletoge ni.c mesenchyme ce lls, and they induce the animal cells next to them to become vegetal pl ate endoderm cells. (e) The tran sp l anted micromeres dif ferentiate into skeletal cables, while the induced animal cap cells form a secondary archenteron. Meanwhile, gastrulation p roceeds norm ally from the original vegetal plate of the host. (After Ransick and Davidsnn 1 993.) FIGURE 5 . 1 0 (D) EARLY DEVELOPMENT I N S E L ECTED INVERTEBRATES The initial regulatory inputs are the transcription factors Disheveled and p-catenin, both of which are found in the cytoplasm and are inherited by the micromeres as Soon as Illey are formed at the fourth cleavage. During oogenesis, Disheveled becomes located in the vegetal cortex of the egg (Figure 5.1 1 A; Weitzel et al. 2004; Leonard and Etten sohn 2007), where it prevents the degrada tion of p-catenin in the micromere and macromere cells. The ⬝-catenin is therefore allowed to enter into the nucleus, where it com bines with the TCF transcription factor to activate gene expression. Several pieces of evidence suggest that p-catenin speci fies the micromeres. First. during normal sea u.rchin devel- 1 69 opment, p-catenin accumulates in the nuclei of those cells fated to become endoderm and mesoderm (Figure 5.1 1 B). This accwnulation is autonomous and can occur even if the micromere precursors are separated from the rest of the embryo. Second, this nuclear accumulation appears to be resporu;ible for specifying the vegetal half of ti1e embryo. It is possible that the levels of nuclear p-catenin accumu lation help determine the mesodermal and endodermal (A) FIGURE 5.11 Role of Disheveled and p-catenin proteins in specifying the vegeta l cells of the sea urchin embryo. (A) Local ization of Disheveled (arrows) i n the vegetal cortex of the sea urchin oocyte before fertilization (left) and in the region of a 1 6⬝ cell embryo about to becom e the micromeres (right). (B) During normal development, p-catenin accumulates predominantly in the micromeres a nd somewhat less in the veg2 tier cells. (C) In embryos treated with l ith i um ch lori de, p catenin accumulates in the nuclei of all blastula cel ls (probably by liCi blocking the GSK3 enzyme of the Wnt pathway), and the animal cells become specified as endoderm and mesoderm. (D) When p eatenin is prevented from entering the nuclei (i.e., it remains in the cytoplasm), the vegetal cell fJtes are not specified and the entire embryo deve l ops as a ciliated ectodermal ball. (A,B from Weitzel et a1. 2004, courtesy of C. Ettensohn; B-O from Logan ot al. 1 998, courtesy of D. MCClay.1 - Mesomeres (B) \-"':T'3i:='ri--- Large micromeres Small micromeres (C) (D) micl'omeres Cf" -v'TER 5 1 70 FIGURE 5.12 Simplified, double-negative gated "circuir' for micromere specification. (A) In situ hybridization reveals the accumulation of Pmarl mRNA (dark pUlpl e) in the micromeres. (B) Otx and ⬝-catenin from the maternal cytoplasm are concentrated i n at the vegetal pole of the egg. These transcriptional regulators are inherited by the m icromeres and activate the Pmarl gene. Pmarl encodes a repressor of HesC.
which in turn encodes a repressor (hence the "double-negative") of several genes involved in micromere specificat i on (e.g., A/x 1, Tbr, and Ets). Genes encoding signaling protei ns (e. g., Della) are also under the co nt rol of HesC. In the micromeres, where ac tivated Pmarl represses the HesC represso r, the rn icrom ere specification and signaling genes are active. I n the veg1 ceU s, Pmar 7 is not activated and the HesC gene product shuts down the skel etogeni c genes; however, those cell s co ntain in g Notch can respond to the Delta si gn al from the skeletogenic mesenchyme. U, ubiquitous activating tran scription factors. (After Ol iveri et al. 2008; photograph courtesy of P. O l iveri .) (A) B) MATERNAL MICROMERE FACfORS Otx ⬝-catenin Pm.rl t t rr----..l⬝ ⬝ i i HesC i ⬝⬝⬝ v v v v t Del ta ! Transcription factors act.ivating skeleton-forming genes VEG, CELL Pmarl I'---⬝-_J fates of the v egetal cells.
Treating sea urchin embryos with lithium chloride causes the accumulation of ⬝-catenin in every cell and transforms the presumptive ectoderm into endoderm. Conversely, experimental procedures that inhibit ⬝-catenin accumulation in the vegetal cell nuclei prevent the formation of endoderm and mesoderm (F ig ure 5.1 1 C,D; Logan et a!. 1998; Wikramanayake et al. 1998). The next regulatory input comes from the Otx transcrip tion factor that is enriched in the micromere cytoplasm. the Pmarl gen es to activate their transcription in the t 1 Thr I ⬝ Notc h a11 u t t ---+- v t Ets Non-skeletogenic mesenchyme genes I v + Delta 1 No transcription factors n act ivati g skeleton of that repressor (in other words, when activation occurs by the repression of a repressor), is called a double-nega same genes in every other cell type (Oliveri et a!. 2008). micromere specification and differentia tion: Alxl, Etsl, Tbr, The genes repressed b y HesC are those involved in Tel, and Soxc. Each of these genes can be activated by ubiq tion factors cannot w ork while HesC repressor protein tine sP'" evol men gate ry fE ties I havE geni regu placl becaJ represse s HesC, and all these genes become active (Revilla-i-Domingo et a1. 2007). The newly Spel vate skeletal determinants. These transcription factors also The tein is present, it activated genes synthesize transcription factors that acti embryo except those of the micromeres.* In the micromeres, fication and these genes can be tmIocked by the repressor genes. This stabilizes the regulatory state of the skeleto activate each other's genes, so that once one factor is acti vated, it maintains the activity of the other skeletogeruc genic mesenchyme cells.
In contrast to the double-negative gate, the control of *This is an oversimplification of a very complex process. Other transcription factors, such as Blimp, are also needed, and the mater nal transcription factor SoxBl has to be eliminated from the vegetal pole or else it will inhibit the activation of Pmorl. In addi tion, the cytoskeletal processes partitiOlUng the cells and anchoring certain factors are not being considered here.
For complete details of the model, see the continually updated website endomesl . \", the undl vate another set of genes, mos t of which are genes that acti where Pntarl is activated, the Hese gene is repressed. 1ltis mechanism, whereby a repressor locks the genes of speci is of these genes where the input occurs, and it represses the regulation of fate specification: it promotes the expression binds to their respec tive enhancers. When the Pmarl pro HesC is expressed in every nucleus of the sea urchin the lllal genl micromeres shortly after their formation (Figure S.12A; repressor of HesC, a gene that encodes another repressive ske tive gate (Figure S.1 2B, 5.13A) . Such a gate allows for tight uitous transcription factors, but these positive transcrip transcription factor.
When the Pmarl gene is repressed, to t der de,· The ⬝-catenin/TCF and Otx interact at the enhancer of O l iveri et a1. 2008). Pmar1 protein is a transcriptional Ie v⬝w u Alxl FIC bl. ba: pro HesC u G the differentiation genes that make the sea urchin skeleton operates on a feedforward process (Figure 5. 13B) .
Here, reg ulatory gene A produces a transcription factor that is need ed for differentiation gene C and also activates regulatory gene B, which produces a transcription factor also needed for differentiation gene C. The skeletogenic portion of the micromere genetic regulatory nenvork appears to be due c: the al prodl The t (AnS' As so Otxa, tion , Wnt8.
it (aut genes sets ul that eo EARLY DEVELOPMENT I N S ELECTED INVERTEBRATES (A) Gene A GeneC 1 1 GeneB GeneD I IfB notactivae:t ve C. D. If B actinotvactie ve: GeneE C, D, E. inactive I A C. active c i A FIGURE 5.13 "Logic circuits" for gene expressi on. (A) In a dou ble·neg⬝tive gate, a si ngle gene encodes a repressor of an entire batte y of genes. When this repressor gene i s repressed, the bat tery of genes is ex p essed . (B). In this feedforward circuit, gene product A activates both gene B and gene C, and gene B also acti vates gene C. Feedforward circuits p ovi de an efficient way of amplifying a signal in ope direction. r ,. i· i o ic 1'i· r r to the recruibnent of a "subroutine" that in most echino derms (including sea urchins) is used for making the adult skeleton (Gao and Davidson 2008). The cooption of subroutines by a new lineage is one of the ways evolution OCCliS. It happens that the genetic reg ulatory network of the rnicromeres in sea urchin embryos is very different from that in other echinoderms. Only in the micromeres of sea urchins has the skeletogeruc subrou tine (which in aU other ech;noderms is activated late in development) come under the control of the genes that specify ceUs to the m;cromere lineage. The most important evolutionary events were those placing the skeletogenic genes AlxI and EtsJ (necessary for adult skeletal develop ment) and Tbr (used in later larval skeleton formation) under the regulation of the Pmar1-HesC double-negative gate. This occurred through mutations in the cis-regulato ry regions of these genes. Thus, the skeletogenic proper ties that distinguish the sea urchin m;cromeres appear to have arisen through the co-option of a preexisting skeleto genic regulatory system by the micromere lineage gene regulatory system. When skeletal gene expression was placed under the control of the dl"uble-negative gate, it became a property specifically of the micromeres. Specification of the vegetal cells The abilities of the primary rnicromeres go well beyond the autonomous development into skeletal cells. They also produce Signals that can induce changes in other tissues. The first of these signals is the paracrine factor Wnt8 (Angerer and Angerer 2000; Wikramanayake et al. 2004).
As soon as the micromeres form, maternal ⬝-catenin and Otx activate the Blimpl gene, whose product (in conjunc tion with more p-catenin) activates the gene encoding WntB.
WntB is then received by the rnicromeres that made it (autocrine regulation), activating the micromeres' own genes for p-catenin. Since p-catenin activates BlimpI, this sets up a positive feedback loop between Blimp1 and Wnt8 that establishes a source of p-catenin for the nuclei of the (B) Regul Gene atory 171 Regulatory Gene B A DiGeneC fferentic ation micromeres.
The experiments described in Figure 5.10 demonstrated that the micromeres are able to induce a sec ond embryoniC axis when transplanted to the animal hemi sphere. However, rnicromeres from embryos in which p catenin is prevented from entering the nucleus are unable to induce the animal cells to form endoderm, and no sec ond axis forms (Logan et al. 1998).
D,e second inducing signal is an as yet unidentified "early signaL" llis early signal (ES) gene is under the con trol of the double-negative gate of Pmar1 and HesC (see Figure 5.12B). The early signal instructs the adjacent cells (i.e., cells above the micromeres) to have an endomesoder mal fate. It is also responsible for establishing a second axis when micromeres are transplanted to the animal region of the embryo.' If Pmn,.I mRNA is injected into an animal ceU, that an;mal cell will develop into a skeletogeruc mes enchyme ceU, and the ceU adjacent to it w;U start develop ing like a macromere endomesodermal cell (Oliveri et al. 2003). It appears that both Wnt8 and the unidentified "early signal" are needed, and in this particular order. If either one is not present, the vegz cells fail to make non skeletogenic mesenchyme or endoderm (Ransick and Davidson 1995; Sherwood and McClay 1999; Sweet et al. 1999). The early signal may create competence in the veg, cells to respond to the Delta signal. The thi.rd signal is the juxtacrine protein Delta, which is also controlled by the double-negative gate.
It functions later in development by activating the Notch protein on the adja cent ceUs. Notch protein on these cells tells the cells below them to become the non-skeletogeruc mesenchyme cells. Finally, Wnt8 makes another appearance, a product of the micromeres and endoderm cells. Wnt8 appears to act in an autocrine manner to boost the specification of both the veg, endoderm cells and the rnicromeres and to facilitate their separation into two distinct lineages. Wnt8 also acts as a paracrine factor on the endoderm precursor cells to Initiate the invagination of the vegetal plate at the start of gastrula tion (Davidson et al. 2002; Wlkramanayake et al. 2004). micromeresthe ""The experiments described in Figure 5.10 demonstrated that the are able to induce a second embryonic axis when trans planted to ond animal hemisphere. However, micromeres from embryos in which ⬝-catenin is prevented from entering the nucleus are unable to induce the animal cells to form endoderm, and no sec axis fomls (Logan et al. 1998). 1 72 CHAPTER 5 rn sum, the genes of the sea urchin mlcromeres specify their cell fates autonomously and also specify the fates of their neighbors conditionally. The original inputs come from the maternal cytoplaslll, and activate genes that unlock repressors of a specific cell fate. Once the cytoplasmic factors accomplish their functions, the nuclear genome takes over. Axis specification In the sea urchin blastula, the general cell fates (ectoderm, endoderm, skeleton, etc.) line up along the animal-vegetal axis established in the egg cytoplasm prior to fertilization. The animal-vegetal axis also appears to structuJ:e the futuJ:e anterior-posterior axis, with the vegetal region sequester ing those maternal components necessary for posterior development (Boveri 1901; Mantyama et aJ. 1985).]n most sea urchins, the dorsal-ventral and left-right axes are specified after fertilization, but the marmer of their specification is not well understood. Lineage tracer dye injected into one blastomere at the 2-ceIJ stage demonstrat ed that, in nearly all cases, the oral pole of the future oral aboral (mouth-anus) axis lay 45 degrees clockwise from the first cleavage plane as viewed from the animal pole (Cameron et al. 1989). This oral-aboral axis appears to form through the activation of the Nodal gene in the oral (but not in the aboral) ectoderm (Duboc et al. 2004; Flowers et a!. 2004).
As we will see, Nodal (a member of the TGF-⬝ paracrine family; see Chapter 3) is also used by vertebrates to establish the right-left body axis. The role of Nodal was discovered through the classic "find it, lose it, move it" mode of experimentation that we described in Chapter 4 (see p. 144). First, researchers cloned a sea urchin Nodal gene and, using in situ hybridization, demonstrated that Nodal protein becomes expressed in the presLunptive oral ectodenn at about the 6O-cell stage.
Nodal then becomes prominent on one side of the blastula and on the presumptive oral side of the gastrula. When the researchers prevented translation of the Nodal message, development was normal until the mesenchyme blastula stage-but the larvae never obtained bilateral symmetry, the archenteron did not bend to one side to form the mouth, and the skeletogenic mesenchyme did not separate into the two sets of spicule-forming skeleton cells.
Moreover, genes usually expressed in the oral ectoderm were not expressed. Conversely, when researchers induced ectopic expression of the Nodal gene tIuoughout the ectoderm, all of the ecto derm appeared to become oral. Thus, Nodal appears to be crucial in establishing the oral ectoderm. See WEBSITE 5.1 Sea urchin cell specification Sea U rchin Gastrulation The late sea urchin blastula consists of a single layer of about 1,000 cells that form a hollow ball, somewhat flat tened at the vegetal end. The blastomeres are derived from different regions of the zygote and have dillerent sizes and properties. Figu res 5.14 and 5.1 5 ill llstrate development of the blastula through gastrulation to the pluteus larva stage that is characteristic of sea urchins. The drawings show the fate of each cell layer dLIling gastrulation. Ingression o f the skeletogenic mesenchyme Shortly after the blastula hatches from its fertilization enve lope, the descendants of the large micromeres change their shape, lose their adhesions to their neighboring cells, and then break away from the apical layer to enter the blasto coel as skeletogenic mesenchyme cells (see Figure 5.15, 9-10 hours). The skeletogenic mesenchyme cells then begin extending and contracting long, thin (250 nm in diameter and 25 f1ITI long) processes called filopodia. At first the cells appear to move randomly along the inner blastocoel sur face, actively making and breaking filopodiaI cormections Invaginating endodel m (gu,) (D) Skeletogenic mesenchyme cells Blastopore Normal sea urchi n devel opment, following th e fate of the cellular layers of the blastu la. (A) Fate map of the zygote (B) Late blastula with c ili ary tun and flattened vegetal pl ate (C) B l astu la with skeletogenic mesenchyme. (0) G as tru la with non-skeletogcnic mesenchyme. (E) Prism-stage larva. (F) Pluteus larva. Fates or the zygote cyto plasm can be followed through the color pattern (see Fi gure 5.9). (Courtesy of D. McClay.) FIGURE 5.1 4 . . Skeletal rods EARLY DEVELOPMENT I N SELECTED INVERTEBRATES 1 73 to the wall of the blastocoel. Eventually, however, they tomere adhesion to the hya line layer, to the basal lamina become localized within the prospective ventrolateral lining the blastocoel, and to other bl astomeres region of the blastocoel. Here they fuse into syncytial . Originally, all the cells of the blastula are cormected on cables, which will form the axis of the calcium carbonate their ou te r surface to the hyaline layer, and on their inl1er spicules of the larval skeletal rods (see Figure 5.14D-F). surface to a basal lamina secreted by the cells. On their lat eral surfaces, each cell has another cell for a neighbor. Fink THE IMPORTANCE OF EXTRACELLULAR LAMINA INSIDE THE and McClay found that the prospective ectoderm and BLASTOCOEL The ingression of the large micromere descen endoderm cells (descendants of dants into the blastocoel is a result of their losing their macromeres, respectively) bind tightly to one another and affinity for their neighbors and for the hyaline membrane; lamina (Ta b le instead they acquire a strong affinity for a group of pro-teins that line the blastocoel. This model of mesenchymal the mesomeres and to the hyaline layer, but adhere only loosely to the basal 5.2). The micromeres originally display a similar pattern of binding. However, the micromere pat Gustafson and Wolpert (1967) and was confirmed in 1985, when Rachel Fink and their tight binding to the hyaline layer and to their neigh David McClay measured the strength of sea urchin blas- bors, the skeletogenic mesenchyme precursors lose their migration was first proposed by tern changes at gastrulation. Whereas the other cells retain affinities for these structures (which drop to about 2% of their original value), while their affinity for com ponents of the basal lam⬝a and extracellular matrix (such as fibronectin) increases a hundredfold. These changes have been correlated with changes in cell surface molecules that occur during this time (Wes sel and McClay 1985), and proteins such as Hbronectin, integrin, laminin, and cad her ins have These changes in affinity cauSe the skel etogenic been shown to be invulved in cellular ingression. mesenchyme precursors to release their attachments to the extemal hyaline layer and to their neighboring into the blastocoel (Figure S.16A). There is a heavy con cells and, drawn in by the basal lami.na, to migra te up centration of extracellular material around the ingress ing mesenchyme cells (Figure 5.1 6B,C). Once inside the blastocoel, these cells appear to migrate along the extracellular matrix of the blastocoel wall, extending their filopodia in front of them (Galileo and Morrill 1985; Karp and Solursh 1985; Cherr et al. 1992). Sev eral proteins (including a fibronectin-like protein and a particular sulfated glycoprotein) are necessary to ini- FIGURE 5.15 Entire sequence of gastrulation in Lytechinus variegatus.
Ti m es show the length of development at 25°(, (Courtesy of J. Morrill; pluteus larva courtesy of C . Watch maker.) 1 74 t CHAPTER 5 ⬝) (8) Ex r acellula r Skeleto genic mesenchyme cell 10 (C) FIGURE 5.1 6 Ingression of skeletogenic mesenchyme cells.
(A) I⬝terpretative depiction of changes in the adhesive affinities of the presumptive skeletogenic mesenchyme cells (pink). These cells lose their affinities for hyalin and for their neighboring blastomeres while gaining an affinity for the proteins of the basal lamina. Nonmesenchymal blas tomeres retain their original high affinities for the hyaline layer and neighboring cells. (8) Scanning electron micrograph of skeletogenic mesenchyme cells enmeshed in the extra cellular matrix of an early Strongylocentrotus gastrula. (C) Gastrula-stage mesenchyme cell migration. The extracellular matrix fibrils of the blastocoel lie parallel to the animal vegetal axis and are intimately associated with the skeletogenic mesenchyme cells. (B,C from (herr et al. 1 992; courtesy of the authors.) hate and maintain this migration (Wessel et a1. 1984; Lane and Solursh 1991; Berg et a1. 1996). At two sites near the future ventral side of the larva, many skeletogeruc mesenchyme cells cluster together, fuse with one another, and initiate spicule formation (Figure 5 . 1 7; Hodor and Ettensohn 1998). If a labeled micromere from another embryo is injected into the blastocoel of a gastrulating sea urchin embryo, it migrates to the correct location and contributes to the formation of the embryon ic spicules (Ettensohn 1990; Peterson and McClay 2003). It is thought that the necessary positional information is pro-e.t fa (R il"r ar TABLE 5.2 Affinities of mesenchymal and nonmesenchymal cells to cellular and extracellular components' Dislodgment force (in dynes) Cell type Hyaline Gastrula cell monolayers Basal lamina 16-celI-stage micromeres 5.8 X 10-5 6.8 X 10-' 4.8 X 10-7 Migratory-stage mesenchyme cells 1 . 2 X 10-7 1.2 x 10-7 1.5 X 10-5 Gastrula ectoderm and endoderm 5.0 X 10-5 5.0 X 10-5 5.0 X 10-7 1985. Source: After Fink and McClay GTested cells were allowed to adhere to plates containing hyaline, extracellular basal lamina, or cell monolayers. The plates were inverted and centrifuged at various strengths to dislodge the cells. The dislodgement force is calculated from the centrifugal force needed to remove the test cells from the substrate. Fie the der is. ⬝ reg· gra. cell In\', lul2 1'-" iII EARLY DEVELOPMENT I N S E L ECTED I NVERTEBRATES (A) " ided by the prospective ectodermal cells and their basal laminae (Figure 5.18A; Harkey and Whiteley 1980; Arm strong et al. 1993; Malinda and Ettensohn 1994). Only the skeletogeruc mesenchyme cells (and not other cell types or 1 75 (B) FIGURE 5.17 Forma ti on of syncytial S cables by skelelogenic mes enchyme cells of Ihe sea urchin. (AJ kelelogenic mesenchyme cells in the early gastrul a align and fuse to lay down the matrix of the calcium carbonate sp ic ule (arrows). (B) Sca n ni n g electron micrograph of spicules formed by the fusing of skel etogeni c mesenchyme cells into syncytial cables. (A from Ettensohn ' 990; B from Morrill and Santos ' 985.1 latex beads) are capable of responding to these patterning cues (Ettensohn and McClay 1986). Miller and colleagues (1995) reported that the extremely fine filopodia on the skeletogenic mesenchyme cells appear to explore and sense the blastocoel wall and may be responsible for picking up (A) dorsa!·ventral and animal-vegetal patterning cues from the ectoderm (Figure 5.186; Malinda et al.
1995). Two signals secreted by the blastula wall appear to be aitical for this migration. VEGF paraaine factors are emit ted from two small regions of the ectoderm where the skeletogenic mesenchyme cells will congregate (Duloquin et 01. 2007), and a fibroblast growth factor (FGF) paracrine factor is made in the equatorial belt between endoderm and ectoderm and then becomes defined into the lateral domains where the skeletogenic mesenchyme cells collect Rottinger et al. 2008). The skeletogenic mesenchyme cells migrate to these points of VEGF aI)d FGF synthesis and arrange themselves in a ring along the animal-vegetal axis. INTEGRATION OF THE GENETIC REGULATORY NETWORK AND \10RPHOGENESt S The skeletogenic mesenchyme cell recep tor for VEGF is under the control of the micromere genet- (B) Localization of skeletogenic mesenchyme cells. (Al loealization of -he mic romeres to form the calc i um carbonate skeleton is determined by the ecto dermal cells. The skel etogenic mesenchyme cells are stained green, while p-caten in s stained red. The skelelogen ic mesenc hyme cells appear to accumulate in those 'egions ch a racte ri zed by high p-catenin concentrations. (8) N o ma rsk i videomicro ⬝raph showing a)on& thin filopodium extendi ng from a skeletogenic mesen chyme cel l to the ectodermal wall of the gastrula, as well as a shorter filopodium extending nward from the ectoderm. The mesenchymal filopodia extend through the extracel tJJar matrix and directly contact the cell membrane of the ectodermal ce l ls (8 from .Iiller et a l . , 995; photographs courtesy of J. R. Miller and D. McClay.1 FIGURE 5.18 . ⬝,.,., . - 1 76 (A) CHAPTER 5 (B) Micromeres (skeletogenic mesenchyme) ic regulatory network and thereby COImects morphogene sis to cell specification. As we have seen, skeletogenic mes enchyme cells ingress into the blastocoel at a particular time. Moreover, this ingression is a " cell⬝autonomous" property (i.e., not controlled by neighboring cells), and skeletogenic mesenchyme cells will ingress into the blas tocoels even il transplanted into younger or older embryos (Peterson and McClay 2003). The p repara tion lor cell ingression involves the changing of the micromere cell membranes by endocytosing the original membrane and replaCing it with a new one (Wu et al. 2007). This new membrane lacks the cadherin necessary lor cell-cell adhe sion (and presumably altered receptors lor hyalin and basal lamina proteins as well). Endocytosis and the downregula lion 01 cadherins are controlled by the transcription lactor Snail. And the gene encoding the Snail protein is activat ed by the Alxl transcription factor that is regulated by the double-negative gate of the gene regulatory network. The use of Snail protein to downregulate cadherins and pre pare the cell for an epithelial-to-mesenchymal transition is a morphogenetic subroutine that exists throughout the ani mal kingdom. Here, it has been cormected to the specifica tion program of the micromere lineage.. In this manner, the micromere cells alter their cell membranes and leave the blastula epithelium by ingressing into the blastocoel. Invagination of the archenteron FIRST STAGE OF ARCHENTERON INVAGINATION As the skele togenic mesenchyme cells leave the vegetal region of the spherical embryo, important changes are occurring in the cells that remain there. These cells thicken and flatten to form a vegetal plate, changing the shape of the blastula (see Figure 5.15, 9 hours).
The vegetal plate cells remain bound to one another and to the hyaline layer of the egg, and they move to fill the gaps caused by the ingreSSion of the skele togenic mesenchyme. Moreover, the vegetal plate involutes inward by altering its cell shape, and then invaginates about one-fourth to one-half the way into the blastocoel (Figure 5.19A; see also Figure 5.15, 10.5-11.5 hours). Then inv agi- FIGURE 5.19 I nvagi nati on of the vegetal plate.
{AI Vegetal plate invagination in Lytechinus variegacus, seen by scanning electron microscopy of the external surface of the early gastrula. The blastopore is clearly visible. (B) Fate map of the vegetat plate of the sea urchin embryo, looking lup ard at the ve etal surface. The central portion becomes the non-skeletogenic mesen chyme cells, while the concentric layers around midgut, and hindgut, respectively. The t e endo derm meets the ectoderm the anus. mesenchyme and foregu t from the veg2 l aye l the midgut comes from and veg, celis, a d i dgut (and the ecto derm in co tac with it) comes from veg, layer. (A from and Sa tos 1 985, cou rtesy of j. B. Mo il l; B aher Loga a d McC l y 1 999.1 g it become the foregut, boundary where h marks The non-skeletogenic come r n the h n the Morrill rr n n I a n veg, n t w " nation suddenly ceases. The invaginated region is called the archenteron (primitive gut), and the opening of the archenteron at the vegetal pole is called .the blastopore. The movement of the vegetal plate into the blastocoel appears to be initiated by shape changes in the vegetal plate cells and in the extracellular matrix underlying them (see Kominami and Tak a ta 2004 for a review). Actin micro filaments collect in the apical ends 01 the vegetal celis, caus ing these ends to constrict, forming bottle-shaped vegetal cells that pucker inward (Kimberly and Hardin 1998; Beane et al. 2006). Destroying these ceUs with lasers retards gas trulation. In addition, the hyaline layer at the vegetal plate buckles inward due to changes in its composition (Lane et al. 1993). At the stage when the skeletogenic mesenchyme cells begin ingressing into the blastocoel, the fates of the vege tal plate cells have already been specified (Ruffins and Ettensohn 1996). The non-skeletogenic mesenchyme is the first group of cells to invagi na te, fo rm ing the tip of the archenteron and leading the way into the blastocoel. The non-skeletogenic mesenchyme will form the pigment cells, the musculature around the gut, and contribute to the coelomic pouches. The endodermal cells adjacent to the micromere-derived mesenchyme become foregut, migrat ing the farthest distance into the blastocoel. The next layer of endodermal cells becomes midgutl and the last circwn ferential row to invaginate forms the hindgut and anus (Fig ure 5.19B). • (EARLY DEVELOPMENT IN SELECTED INVERTEBRATES Early gastrulation Later Archenteron fiGURE 5.20 Cell rearrangement during extension of the archenteron in sea urchin embryos. In this species, the early archenteron has 20 to 30 cells around its ci rc umference. Later in gastrulation, the archenteron has a circumference made by only 6 to B cells.
(Aher Ha rdi n 1 990.) gastrulation ⬝ : , , 0 0 0 o : 0 o SECOND AND THIRD STAGES OF ARCHENTERON INVAGINATION : ' o After a brief pause following the initial invagination, the second phase of archenteron forIrultion begins. The archen teron extends dramatically, sometimes tripling its length. In this process of extension, the wide, short gut rudiment is transfomned into a long, thin tube (Figure 5.20; see also Figure 5.15, 12 hours). To accomplish this extension, the cells change their shape and start migrating. First, the thick ened cells of the vegetal plate become thinner; then these cells rearrange themselves, intercalating between one anoth er, like lanes of traffic merging (Ettensohn 1985; Hardin and Cheng 1986). This phenomenon, where cells intercalate to narrow the tissue and at the same time move it forward, is called convergent extension (Martins et al. 1998). In this marmer, the archenteron is elongated. The final phase of archenteron elongation is initiated by the tension provided by non-skeletogenic mesenchyme cells, which form at the tip of the archenteron and remain there. These cells extend filopodia through the blastocoel fluid to contact the inner surface of the blastocoel wall (Dan and Okazaki 1956; Schroeder 1981). The filopodia attach to the wall at the junctions between the blastomeres and then shorten, pulling up the archenteron (Figure 5.21 ; see also Figure 5.15, 12 and 13 hours). Hardin (1988) ablated non-skeletogeruc mesenchyme cells of Lytechinlls pictlls gas trulae with a laser, with the result that the archenteron could elongate only to about two-thirds of the normal length. If a few non-skeletogenic mesenchyme cells were left, elongation continued, al though at a slower rate. Thus, in this species, tile non-skeletogenic mesenchyme cells play an essential role in pulling the archenteron upward to the blastocoel wall during the last phase of invagination.
1 77 0 But can the non-skeletogenic mesenchyme filopodia attach to any part of the blastocoel wall, or is there a specif ic target in the aninnal hemisphere that must be present for attachment to occur? Is there a region of the blastocoel wall that is already committed to becoming the ventral side of the larva? Studies by Hardin and McClay (1990) show that fiGURE 5.21 Mid-gastrula stage of Lytechinus pictus, showing filopodial exten sians of non-skeletogenic mesenchyme. (Al Non-skel etogeni c mesenchyme cells extend filopodia (arrows) from the tip of the arch enteron . (8) Filopodial cables con nect the blastocoel wall ta the archenteron tip.
The ten sion o(the cables can be seen as they pull on the blastocoel wall at the point of attachment (arrows). (Courtesy of C Ettensohn.) (A) (B) 1 7B CHAPTER 5 there is a specific target site for the filopodia that differs from other regions of the animal hemisphere. The filopodia extend, touch the blastocoel wall at random sites, and then retract. However, when the filopodia contact a particular region of the wall, they remain attached there, flatten out against this region, and pull the archenteron toward i t. When Hardin and McClay poked in the other side of the blasto coel wall so that contacts were made most readily with that region, the filopodia continued to extend and retract after touching it. Only when ti,e filopodia found their target tissue did they cease these movements. U the gastrula was con stricted so that the filopodia never reached the target area, the non-skeletogenic mesenchyme cells continued to explore until they eventually moved off ti,e archenteron and found the target as freely migrating cells. There appears, then, to be a target region on what is to become the ventral side of the larva that is recognized by the non-skeletogenic mes enchyme cells, and which positions the archenteron near the region where the mouth will form. As the top of the archenteron meets the blastocoel wall in the target region, many of the non-skeletogenic mes enchyme cells disperse into U,e blastocoel, where they pro liferate to form the mesodermal organs (see Figure 5.15, 13.5 hours). Where the archenteron contacts the wall, a mouth eventually forms. The mouth fuses with the archen teron to create a continuous digestive tube. Thus, as is char acteristic of deuterostornes, the blastopore marks the posi tion of the anus. As the pI uteus larva elongates, the coelomic cavities form from non-skeletogenic mesenchyme and veg2 cells. Under the influence of Nodal protein, the right coelomic sac remains rudimentary. However, the left coelorrtic sac undergoes extensive development to form many of the structures of the adult sea urchln. The left sac splits into three smaller sacs. An invagination from the ectoderm fuses with the middle sac to form the imaginal ru dimen t. This rudiment develops a fivefold symmetry (Figure 5.22), and skeletogenic ruesenchyme cells enter the rudiment to synthesize the first skeletal plates of the shell. The left side of the pluteus becomes, in effect, the future oral surface of the adult sea urchin (Bury 1895; Aihara and Aruemiya 2001). During metamorphosis, the in1aginal rudin1ent sep arates from the larva, which then degenerates. While the in1aginal rudin1ent (now called a juvenile) is re-forming its digestive tract and settling on the ocean floor, it is depend ent on the nutrition it received from the jettisoned larva. The echinoderm pattern of gastrulation provides the evolutionary prototype for deuterostome development. In deuterostomes (echinoderms, turucates, cephalochordates, and vertebrates), the first opening (i.e., the blastopore) becomes the anus while the second openi.ng becomes the mouth (hence, deutero stoma, "mouth second "). Moreover, in sea urchins, we see the phenomena of convergent exten sion and the use of Nodal gene expression for the establish ment of axes. We will return to the subject of deuterostome development later in this and in succeeding chapters, but Imaginal rudiment FIGURE 5.22 The imaginal rudiment growing in the l eh side of the pilitelis larva of a sea urchin. The rudiment will become the adult sea urchin, wh il e the larval stage will be j ettison ed . The five fold symmetry of the rudiment is obvious. (Courtesy of G. Wray.) we now turn to development in a protostome group, the molluscs. See VADE MECUM Sea urchin development EARLY DEVELOPMENT IN SNAILS C leavage in Snail Embryos Spiral holoblastic cleavage is characteristic of several ani mal groups, including annelid worms, some flahvorms, and most molluscs (see Lambert 2010). It differs from radi al cleavage in numerous ways. First the cleavage planes are not parallel or perpendicular to the animal-vegetal axis of the egg; rather, cleavage is at oblique angles, forming a "spiral" arrangement of daughter blastomeres. Second, the cells touch one another at more places thm do those of radially cleaving embryos. In fact, they assume the most thermodynamically stable packing orientation, much like adjacent soap b ubbles. Third, spirally cleaving embryos usually undergo fewer divisions before they begin gastru lation, making it possible to follow the fate of each cell of the blastula. When the fates of the individual blastomeres from annelid, flatworm, and mollusc embryos were com pared, many of the same cells were seen in the same places, and their general fates were identical (Wilson 1898). Blas tulae produced by spiral cleavage have no blastocoel and are called stereoblastulae.
Figures 5.23 depicts the deavage pattern typical of many molluscan embryos. The first two cleavages are nearly meridional, producing fow' large macromeres (labeled A, B, C, and D).
in many species, these four blastomeres are dif ferent sizes (D being the largest), a characteristic that allows them to be individually identified.. In each successive cleav age, each macromere buds off a small micromere at its ani mal pole. Each successive quartet of micromeres is dis- .- ,. EARLY DEVELOPMENT I N SELECTED I NVERTEBRATES 1 79 A) View from animal pole 3D B) Side view Polar body ⬝⬝ '⬝. B⬝) /70;> / FIGURE 5.23 B Spiral cleavage of the mol lusc Trochus v iewed from the animal pol e (AI and from one side (BI, The cells deri ved 'i-om the A blastomere are shown in color.
The mitotic spindles, 5 'e tc hed in the ear ly stages, divide the cells unequally and at an angle to the vertical and horizontal axes. Each successive quartet of rnicromeres (indicated with lowercase letters) is d i sp l aced to the iight or to the left of its sister macromere (uppercase letters), creat ..ng the cha racteristic sp iral pattern. placed to the right or to the left of its sister macromere, cre ating the characteristic spiral pattern.
Looking down on the embryo from the animal pole, the upper ends of the mitotic spindles appear to alternate clockwise and coun :erclockwise (Figure 5.24) . This arrangement causes alter nate micromeres to form obliqu⬝ly to the left and to the right of their macromeres. At the third cleavage, the A macromere gives rise to hvo daughter cells, macromere 1A and micromere 1a. The B, C, Mid D cells behave sirnilarly, producing the first quartet of micro meres. In most species, these micromeres are to the right of their macromeres (looking down on the animal pole). At the fourth cleavage, macromere 1A divides to :orm macromere 2A and micromere 2a, and micromere 1a divides to form tvvo more micromeres, 1a1 and 1a2 (see fig ure 5.23).
The micromeres of this second quartet are to the left of the macromeres. Further cleavage yields blastomeres 3_-'\ and 3a from macromere 2A, and micromere 1a2 divides :0 produce cells 1a21 and 1a22. In normal development, the first-quartet micromeres form the head structures, while ilie second-quartet micromeres form the statocyst (balance organ) and shell.
These fates are specified both by cyto- plasmic localization and by induction (Cather 1967; Clement 1967; Render 1991; Sweet 1998).
The orientation of the cleavage plane to the left or to the right is controlled by cytoplasmic factors ir1 the oocyte. This was discovered by analyzing mutations of snail coiling. Some snails have their coils opening to the right of their shells (dextral coiling), whereas the coils of other snails open to the left (sinistral coiling). Usually the direction of coiling is the same for all members of a given species, but occasional mutants are found (i.e., in a population of right coiling snails, a few individuals will be found with coils that open on the left). Crampton (1894) analyzed the embryos of such aberrant snails and found that their early cleavage differed from the norm. The orientation of the cells after the second cleavage was different in the sinis trally coiling snails as a result of a different orientation of the mitotic apparatus (Figure 5.25). In some species (such as the pond snail Physa, an entirely sir1istral species), the sinistrally coiling cleavage patterns are mirror images of the dextrally coiling pattern of the right-handed species. In other instances (such as Lynmaea, where about 2% of the snails are lefties), sinistrality is the result of a two-step process: at each division, the initial cleavage is radial; how ever, as the cleavage furrow forms, the blastomeres shift to the left-hand spiral position (Shibazaki et a1. 2004). In Figure 5.25, one can see that the position of the 4

Ascidians are characterized by bilateral holoblastic cleavage, a pattern found primarily in tunicates (Figure 5.34). The most striking feature of this type of cleavage is that the first cleavage plane establishes the earliest axis of symmetry in the embryo, separating the embryo into its future right and left sides.
Each successive division orients itself to this plane of symmetry, and the half-embryo i-armed on one side of the first cleavage plane is the mir wr image of the half-embryo on the other side. The second cleavage is meridional, like the first, but unlike the first division, it does not pass through the center of the egg.
Rather, it creates mo large anterior cells (the A and a blas :omeres) and two smaller posterior cells (blastomeres B and b). Each side now has a large and a small blastomere. Indeed, from the 8- through the 64-cell stages of tuni (ate development, every cell division is asymmetricat such ::hat the posterior blastomeres are always smaller than the anterior cells (Nishida 2005; Sardet et a1. 2007). Prior to each The tunicate fate map The fate map and cell lineages of the tunicate Styela partita are shown in Figure 1 .12. Most of the early tumcate blas tomeres are specified au tono:r.nously, each cell acquiring a specific type of cytoplasm that will determine its fate.
In many tunicates, the different regions of cytoplasm have distinct pigmentation, and the cell fates can easily be seen to correspond to the type of cytoplasm taken up by each cell. These cytoplasmic regions are apportioned to the egg during fertilization. In the unfertilized egg of Styela partita, a central gray cytoplasm is enveloped by a cortical layer containing yellow lipid inclusions (Figure 5.35A). During meiosis, the breakdown of the nucleus releases a clear sub stance that accumulates in the animal hemisphere of the egg. Within 5 minutes of sperm entry, the inner clear and cortical yellow cytoplasms contract into the vegetal (lower) hemisphere of the egg (Prodon et a1. 2005, 2008; Sardet et a1. 2005). As the male pronucleus migrates from the vege tal pole to the equator of the cell along the future posterior side of the embryo, the yellow lipid inclusions migrate with it. This migration forms the yellow crescent, extend ing from the vegetal pole to the equator (Figure 5.35B-0); (e) (B) ectoderm Vegetal pole Anterior Vegetal pole CICURE 5.34 Bilateral symmetry in th e egg of the ascidian tuni ate Stye!a partita. (The cell l ineages of Stye!a are shown in Figure 1 2C.) (A) Uncleaved egg. The regions of cytoplasm destined to "iorrn particular organs are labeled here and coded by color 'nroughout the diagrams. (B) 8-cell embryo, showing the blas•. Posterior View from vegetal pole tomeres and the fates of various cells. The embryo can be viewed as two 4-cell balves; from here on, each division on the right side of the embryo has a mirror-image division on the left. (C,O) Views of later embryos from the vegetal pole. The dashed line shows the plane of bilateral symmetry. (A after Balinsky 1981 .) 1 88 (A) CHAPTER 5 Animal pole (B) (D) (C) Nuclear Clear { Yellow Cho ion material Vegetal po le Styela partita. (A) Before Cytoplasmic rearrangement in the fertilized egg of fertilization, yellow cortical cytoplasm surrounds the gray yolky inner cytoplasm. (8) Aher sperm entry (in the vegetal hemisphere of the oocyte), the yellow cortical cyto plasm and the clear cytoplasm derived from the breakdown of the oocyte nucleus contract vegetally toward the sperm. (e) As the sperm pronucleus migrates animally toward the newly formed egg pronucleus, the yel low and clear cytoplasms move with it. (D) The final position of the yellow cytoplasm marks the location where cells give rise to tail muscles. (Aher Conklin 1 905.) FIGURE 5.35 this region will produce most of the tail muscles of the tuni cate larva. The movement of these cytoplasmic regions depends on microtubules that are generated by the sperm centriole and on a wave of calcium ions that contracts the animal pole cytoplasm (Sawada and Schatten 1989; Speksnijder et al. 1990; Roegiers et al. 1995). Edwin Conklin (1905) took advantage of the differing coloration of these regions of cytoplasm to follow each of the cells of the tunicate embryo to its fate in the larva (see Figure 1.l2C). He lound that cells receiving clear cytoplasm become ectoderm; those containing yellow cytoplasm give rise to mesodermal cells; those that incorporate slate gray inclusions become endoderm; and Light gray cells become the neural tube and notochord. The cytoplasmic regions are localized bilaterally around the plane of symmetry, so they are bisected by the first cleavage furrow into the right and left halves of the embryo. The second cleavage caus es the prospective mesoderm to lie in the two posterior cells, while the prospective neural ectoderm and chor damesoderm (notochord) will be formed from the two anterior cells (see Figure 5.34). The third division lurther partitions these cytoplasmic regions such that the meso derm-forming cells are confined to the two vegetal poste rior blastomeres, while the chordamesoderm cells are restricted to the two vegetal anterior cells. See WEBSITE 5.4 The experimental analysis of tunicate cell specification crescent Autonomous and conditional specification of tunicate blastomeres The autonomous specification of tunicate blastorneres was one of the first observations in the field 01 experimental embryology (Chabry 1888). Cohen and Berrill (1936) con firmed Chaby's and Conklin's results, and by counting the number of notochord and muscle cells, they demonstrat ed that larvae derived from only one of the first two blas tomeres had half the expected number of cells. Reverberi and Minganti (1946) extended this analysis in a series of isolation experiments, and they, too, observed the self-dif ferentiation of each isolated blastomere and of the remain ing embryo. (The results of one of these experiments are shown in Figure 11.2.) When the 8-cell embryo is separat ed into its four doublets (the right and left sides being equivalent), both mosaic and conditional specification are seen. The animal posterior pair of blastomeres gives rise to the ectoderm, and the vegetal posterior pair produces endoderm, mesenchyme, and muscle tissue-just as expected from the fate map. Autonomous specification is seen in the tunicate gut endoderm, muscle mesodenn, skin ectoderm, and neural cord. However, organisms are formed by both autonomous and conditional specification. Some organs are not formed by the localization of cytoplasmic determinants. Rather, they are formed by the interactions of cells. Conditional specification (by induction) is seen in the formation of the brain, notochord, heart, and mesenchyme cells. AUTONOMOUS SPECIFICATION OF THE MYOPLASM: THE YEL LOW CRESCENT From the cell lineage studies of Conklin and others, it was known that only one pair of blastomeres (posterior vegetal; B4.1) in the 8-cell embryo is capable of producing tail muscle tissue (Whittaker 1982). These cells contain the yellow crescent cytoplasm. When yellow cres cent cytoplasm is translerred from the B4.1 (muscle-form ing) blastomere to the b4.2 (ectoderm-forming) blastomere of an tunicate embryo, the ectoderm-forming blas tomere generates muscle cells as well as its normal ecto- S-cell EARLY DEVELOPMENT IN SELECTED INVERTEB RAT ES 1 89 the egg of 8011enia villosa. The yell ow cres cent, originally seen in the vegeta l pol e, becomes segregated i n to the B4.1 bl as tomere pair and thence into the muscle cells. (From Swalla 2004, courtesy of B.
Swal la, K.
Zigl er, and M. Bal tzley.) FIGURE 5.36 Cytoplasmic segregation in (D) dermal progeny (Figure 5.36; see also Figure 11.3). More (E) was found to be concentrated (f) in the vegetal hemisphere of over, cytoplasm from the yellow crescent area of the fertil the unfertilized egg and remains present during early fer ized egg can cause the a4.2 blastomere to express muscle tilization. It appears to migrate with the yellow crescent specific proteins (Nishida 1992a). Conversely, when larval cytoplasm into the posterior vegetal region of the egg dur cell nuclei are transplanted into enucleated tunicate egg ing the second half of the first cell cycle. By the 8-cell stage, fragments, the newly formed cells show the structures typ ical of the egg regions providing the cytoplasm, not of maclw-l mRNA is found only in the B4.1 blastomeres. At the 16- and 32-cell stages, it is seen in those blastomeres the muscle cells' (Figure 5.37). those cells providing the nuclei (Tung et al. 1977). We can that give rise to egg cytoplasm cause the fonnation of certain tissues. These mRNA were injected into unfertilized eggs, the resulting conclude, then, that certain determinants present in the morphogenetic determinants appear to work by selective ly activating or inactivating specific genes. The determina tion of the blastomeres and the activation of certain genes are controlled by the spatial localization of the morphoenetic determinants within the egg cytoplasm. Using RNA hybridization techniques, Nishida and When antisense oligonucleotides to deplete macho-l larvae Jacked all the muscles usually fom1ed by the descen dants of the B4.1 blastomere. (They did have the second A4.1 and b4.2 blastomeres.) The tails of these maclw-l ary muscles that are generated through the interactions of depleted larvae were severely shortened, but the other regions of the tadpoles appeared structurally and biomem- Sawada (2001) found particular mRNAs to be highly Halo l.."Ynthia roretzi.
One of these RNA ﬠessages encodes a zinc finger rranscription factor called Macho-I. Macho-l mRNA enriched in the vegetal hemisphere of the tunicate fiGURE 5.37 Autonomous spec fication by a morphogenetic fac· lOr. Th e macho- ! mRNA message 3 localized to the muscle-forming amicate c op lasm . In situ bridization shows the macho· 7 .... essage found nrst the vegetal ;>ole cytop l sm (A), then m igr ti ng _p the presumptive posterior sur· ce of egg (B) ocalize in t e 84."1 l astomere C. (rom yt a in a the and becoming d h b F Nishida and Sawada _001 , courtesy of H. Nishida and . Satoh.) (A) enchyme. However, FCF Signals from the endoderm prevent these mesenchyme precursors from developing into muscle celis, as we will see later. "'Macho-l mRNA is also localized in the cells that become the mes (B) (e) 1 90 CHAPTER 5 (A) (B) (C) staining of - a e n protein ho nd derm FIGURE 5.38 A ntib po e ,1 Ciona em b r . formation. (A) No p ca e n is seen in the nuclei i n he endoderm pre (6) the " O-cell stage. (e) not c or precursor bec m e endoderm and e p e s alkaline normal endoderm; the black show notochordal e pres in g endodermal enzymes. (From Imai et al. 2000, ourtesy H . ody p c t ni s ws its involvement with e o - t ni in the animal l nuclei of a O-cell yo In contrast, nuclear p-catenin is readily seen t vegetal cursors at When p-catenin is expressed in o h dal cells, those cells will o x r s enclodermal markers such as phosphatase. The white arrows show arrows cells that are x s c of Nishi da and N. Satoh.) ically normal.
Moreover, B4.1 blastomeres isolated from 11U1cho-I-depleted embryos failed to produce muscle tissue.
Nishida and Sawada then injected Inacho-l mRNA into cells that would not normally fonn muscle, and found that these ectoderm or endoderm precursors did generate muscle cells when given macho-I mRNA. Macho-l turns out to be a transcription factor that is required for the activation of several mesodermal genes, including muscle acHl1, myosin. tbx6, an d snail (Sawada et aJ. 2005; Yagi et al. 200Sa). Of these gene products, only the Tbx6 protein produced muscle differentiation (as Macho-l did) when expressed in cells ectopically.
Macho-1 thus appears to directly activate a set of tbx6 genes, and Tbx6 proteins activate the rest of muscle development (Yagi et al. 200Sb). Thus, the macho-I message is found at the right place and at the right time, and these experiments suggest that Macho-I protein is both necessary and sufficient to promote muscle differentiation in certaiﬠ ascidian cells.
The Macho-1 and Tbx6 proteins also appear to activate the muscle-specific gene snail. Snail protein is important in preventing 8rachyury (T) exp ressio n in presumptive muscle cells, and is therefore needed to prevent the mus cle precu rsors from becoming notochord cells.
It appears, then, that the Macho-l transcription factor is a critical com ponent of the tunicate yellow crescent, muscle-forming cytoplasm. Macho-l activates a transcription factor cas cade that promotes muscle differentiation while at the same time inhibiting notochord specification. AUTONOMOUS SPECI FICATION OF T H E E N DODERM: p-CATENIN Presumptive endoderm originates from the veg etal A4.1 and B4.1 blastomeres.
The specificati on of these cells coincides with the scription factor localization of p-catenin, a tran discussed earlier in regard to sea urchin endoderm specification. Inhibition of p-catenm results in the loss of endoderm and its replacement by ectoderm in the ascidian embryo (Figure 5.38; Imai et aL 2000). Con versely, Increasing p-catenin synthesis causes an increase in the endoderm at the expense of the ectoderm (just as in sea urchins). The p-catenin transcription factor appears to function b y activating the synthesis of the homeobox tran scription factor Lhx-3. Inhlbition of the lhx-3 message pro hibits the differentiation of endoderm (Satou et al. 2001). CONDITIONAL SPECIFICATION OF T H E .MESENCHYME AND NOTOCHORD BY THE ENDODERM While most of the mus cles are specified autonomously from the yellow crescent cytoplasm, the most posterior muscle cells form through conditional specification by ceB interactions with the descendants of the A4.1 and b4.2 blastomeres (Nishida 1987, 1992a,b). Moreover, the notochord, brain, heart, and mesenchyme also form through inductive Interactions. In fact, the notochord and mesenchyme appear to be induced by the fibroblast growth factor that is secreted by the endo derm cells (Nakatani et aL 1996; Kim et aL 2000; Imai et al. 2002). The posterior cells that will become mesenchyme respond differently to the FGF signal due to the presence of Macho-1 in the posterior vegetal cytoplasm (Figure 5.39; Kobayashi et aL 2003). Macho-1 prevents notochord induc tion in the mesenchymal cell precursors by ac tiva ting the snnil gene (which will in turn suppress the activation of 8rachyllry). Thus, Macho-l is not only a muscle-activating determinant, it is also a factor that distinguishes cell response to the FGF Signal. These FGF-responding cells do not become muscle, because FGF also activates cascades that block muscle formation-another role that is con served in vertebrates. As can be seen in Figure 5.40, the : EARLY DEVELOPMENT I N SELECTED I N V E RTEB RATES FIGURE 5.39 Th e two-step process for specifying the marginal cells of the tuni cate embryo. The first step involves th e acquis ition (or nonacquisition) by the cells of the Macho-1 transcripti on factor. The secon d step involves the reception or non reception) of the FGF signal from :he endoderm. (After Ko bayash i et al.
1003.) Anterior ,---,---,_-, en ﬠ Macho-1 RNA"- Posterior presence 01 Macho-1 changes the responses to endodennal FGFs, causing the anterior cells to form notochord willIe the posterior cells become mesenchyme. Specification of the embryonic axes The axes of the tunicate larva are among its earliest com mitments. Indeed, aU of its embryonic axes are determined by the cytoplasm of the zygote prior to first cleavage (see Sardet et a1. 2007). The first axis to be determined is the orsal-ventral axis, which is defined by the cap of cyto plasm at the vegetal pole. This vegetal cap is eruiched for mitochondria, endoplasnuc reticum components, and spe cific maternal mRNAs (such as l17acho-1) that will be - volved in cell specification. Tills vegetal cap prefigures the future dorsal side of the larva and the site where gas Intlation is initiated (Bates and Jeffery 1988). When small regions of vegetal pole cytoplasm were removed from zygotes (between the first and second waves of zygote cytoplasmic movement), the zygotes neither gastrulated nor formed a dorsal-ventral axis. The anterior-posterior axis is the second axis to appear and is determined during the migration of the oocyte cyto plnsm during fertilization. Microtubules originating from the sperm centrosome, followed by cortical actin microfil aments, cause the vegetal cap to become repositioned to -,,'hat will be the posterior region of the embryo. This can be followed readily, since the yellow crescent forms in the region of the egg that will become the posterior side of the rva (see Figures 5,35 and 5.36). When roughly 10% of the c-vtoplasm from this posterior vegetal region 01 the egg was removed after the second wave of cytoplasmic movement, most of the embryos failed to form an anterior-posterior axis.
Rather, these embryos developed into radially sym metrical larvae with anterior fates. This posterior vegetal cVloplasm (PVC) is "dominant" to other cytoplasms in that when it was transplanted into the anterior vegetal region or zygotes that had had their own PVC removed, the ante :ior of the cell became the new posterior, and the axis was reversed (Nisillda 1994). The specification of the left-right axis in tunicates is poorly understood. The first cleavage divides the embryo . to its future right and lelt sides, bu t we do not yet know how these sides are specified.
As in sea urchins and verte brates, the lelt-right asymmetry is predicated on the expre... Macho-l abs t o ten 1 91 Nerve cord No Macho-I; No FGF Notochord No Macho-I; FGF End}derm ﬠ+Mesenchyme Macho-l, FGF Macho-I, No FGF Muscle """" ﬠ " stage ""-' 64-ceU sion of the Nodal gene. In tunicates, Nodal becomes expressed specifically in the lelt-side epidermis of the tail bud-stage embryo (Morokuma et a1. 2002; Yoshida and Sauga 2008). But how it becomes expressed there is still not known. Gastrulation in Tunicates Tunicates, like sea urchins and vertebrates, are deuteros tomes and follow a pattern of gastrulation in which the blastopore becomes the anus. Tunicate gastrulation is char acterized by the invagination of the endoderm, the invo lution of the mesoderm, and the epiboly of the ectoderm. About 4-5 hours after fertilization, the vegetal (endoderm) cells assume a wedge shape, expanding their apical mar gins and contracting near their vegetal margins (Figure 5.40), The A8.1 and B8.1 blastomere pairs appear to lead this invagination into the center of the embryo. The n i vagi nation forms a blastopore whose lips will become the mesodermal cells, The presumptive notochord cells are now on the anterior portion of the blastopore lip, while the presumptive tail muscle cells (from the yellow crescent) are on the posterior lip. The lateral lips comprise those ceUs that will become mesenchyme. The second step of gastrulation involves the involution of the mesoderm. The presumptive mesoderm cells invo lute over the lips of the blastopore and, by migrating over the basal surfaces of the ectodermal cells, move inside the embryo. The ectodermal cells then flatten and epibolize over the mesoderm and endoderm" eventually covering the em bryo. After gastrulation is complete, the embryo elongates along its anterior-posterior axis. The dorsal ecto dermal cells that are the precursors of the neural tube invaginate into the embryo and are enclosed by neural folds. Tills process forms the neural tube, willch will form a brain anteriorly and a spinal chord posteriorly. Mean while, the presumptive notochord cells on the right and lelt sides of the embryo migrate to the midline and inter digitate to form the notochord. The 40 cells of the noto chord rearrange themselves from a 4-by-10 sheet of cells into a single row of 40 cells (Jiang et al. 2005). This interca lation and migration of notochord cells is called convergent extension (Figure 5.41 ; we also saw this phenomenon in OUI discussion of the sea urchin archenteron), and it extends the body axis along the anterior-posterior dimension. 192 CHAPTER 5 IA) FIGURE 5.40 Gastrulation in the tunicatc. Cross sections (A-C) and scanning electIon micrographs viewed from the vegetal pole (O-F) i l 1ustr'ate the invagination of the endoderm (A,D), the invo lution of the mesoderm (B,E), and the epibol y of the ectoderm (C,F). Cell fates are color-coded as in Figure 5.34. (From Satoh '1978 and Jeffery and Swall" 1 997, courtesy of Satoh.1 Polar body N. endoderm IB) Notochord the genes responsible for heart differentiation. These two cells migrate to form tvvo regions of cardiac mesoderm on the left and right ventral sides IE) of the tadpole, just anterior to the tail. Like the heart precursor cells of vertebrate embryos, these two cell clusters migrate to meet at the ven tral midline of the larva (Davidson and Levine 2003; Satou et a!. 2004; Christiaen et aI. 2008). After metamorphosis, they will form the functional heart of the adult. During this metamorphosis, the tail and brain degenerate and the tuni cate no longer moves.· (C) THE NEMATODE C. ELEGANS Mesenchyme IF) Our ability to analyze development requires appropriate model organisms. Sea urchins have long been a favorite of embryologists because their gametes are readily obtain able in large nwnbers, their eggs and embryos are trans parent, and fertilization and development can occur under laboratory conditions. But sea urchins are difficult to rear in the laboratory for more than one generation, making their genetics difficult to study. Drosophila. The Geneticists (at least those who have worked with mul ticellular eukaryotes) have always favored fruit fly's rapid life cycle, its readiness to breed, and the polytene Indeed, the convergent extension of notochordal precur sor cells is characteristic across all the chordate phyla. (jeffery and Swalla 1997). The muscle cells of the tail differentiate on either side of the neural tube and notochord chromosomes 67.5 blastomere pairs express the conserved heart transcription factor Mesp. The anterior daughters of these B7.S blastomeres respond to FGF signals to activate the cytoskeletal genes responsible for migra tion as well as the larva (which allow gene *Such a process, according to neurobiologist Rudolfo L1inas (1987" is "par This forms the tadpole-like body of the larva. At the 110cell stage, the of FIGURE S.41 Convergenl extension of the t unicate notochord. The notochord is visualized by a green fluorescent protein (GFP) probe fused to a promoter of the ilrachyury gene, which is usually expressed in the notochord. The notochordal precursor cells con verge and extend the notochord down the length of the animal's tail. (From Deschct et EARLY DEVELOPMENT I N SELECTED INVERTEB RAT ES localization) make it superbly suited for hereditary analy 1 93 etc.-although there is no skeleton), and it exhibits a n sis. But Drosophila development, as we will detail in Chap aging phenotype before it dies. In addition, C . elegans is ter 6, is complex. particularly friendly to molecula r biologists. DNA inject A research program spearheaded by Sydney Brenner (1 974) was established to identify an organism wherein it might be possible to identify each gene involved in devel opment as well as to trace the lineage of each and every cell. Nematode roundworms seemed like a good group to start with, since embryologists such as Goldschmidt and Baveri had shown that several nematode species have a relatively small number of chromosomes and a small nwn ber of cells with invariant ceU lineages. ed into C. elegans cells is readily incorporated into their nuclei, and C elegans can take up antisense RNA from its culture medium. Cleavage and Axis Formation in C. e/egans Rotational cleavage of the C. elegans egg The zygote of Caenorhabditis exhibits rotational holoblastic Eventually, Brenner and his colleagues settled on cleavage (Figure 5.42C,D) . During early cleavage, each Caenorllabditis elegans, a small (1 mrn long), free-living (i.e., asymmetrical division produces one founder cell (denot-nonparasitic) soil nematode (Figure 5.42A) . C. elegans has ed AB, E, MS, C, and D) that produces differentiated a rapid period of embryogenesis (about 1 6 hours), which descendants; and one stem cell (the Pl-P4 lineage). In the it can accomplish in a petri dish, and relatively few cell first cell division, the cleavage furrow is located asymmet types. Moreover, its predominant adult form is hermaph rically along the anterior-posterior axis of the egg, closer roditic, with each individual produCing both eggs and to what will be the posterior pole. It forms an anterior sperm. These roundworms can reproduce either b y self founder cell (AB) and a posterior stem cell (Pl). During the fertilization or by cross-fertilization with the infrequently second division, the founder cell (AB) divides equatorial occurring maJes. ly (longitudinally; 90 degrees to the anterior-posterior axis), The body of an adult C. eiegans hermaphrodite contains exactly 959 somatic cells, and the entire cell lineage has been traced through its transparent cuticle (Figure 5.42B; ulston and Horvitz 1977; Kimble and Hirsh 1979). Fur thermore, lmlike vertebrate cell lineages, the C. elegans lin while the PI cell divides meridionally (transversely) to pro duce another founder cell (EMS) and a posterior stem cell (P2). The stem cell lineage always undergoes meridional division to produce (1) an anterior founder cell and (2) a posterior cell that will continue the stem cell lineage. eage is almost entirely invariant from one individual to the The descendants o f each founder cell can be observed next. There is little room for randomness (Sulston et al. through the transparent cuticle and divide at specific times \983). It also has a very compact genome. Although it has in ways that are nearly identica1 from individual to indi about the same number of genes as hcunan beings (c. ele gal1s has 18,000-20,000 genes, whereas H. sapiens has 20,000-25,000), the nematode has only about 3% the num ber of nucleotides in its genome (Hodgkin 1998, 2001).* The C. elegans genome was the first complete sequence ever obtained for a multicellular organism ing Consortium 1999).
(c. elegans Sequenc C.
elegans has the rudiments of nearly all the major .vpes of bodily systems (feeding, nervous, reproductive, vidual. In this way, the exactly 558 cells of the newly hatched Jarva are generated. Descendant cells are named according to their positions reJative to their sister ceUs. For instance, ABal is the "left-hand" daughter cell of the Aba cell, and ABa is the "anterior" daughter cell of the AB cell. Anterior-posterior axis formation The elongated axis of the C. elegans egg defines the future anterior-posterior axis of the nematode's body.
The deci sion as to which end will becOlne the anterior and which this Similarity in gene number was rather surprising, to say the .east.
" What does a worm want with 20,000 genes?" wrote Jonathan Hodgkin. curator of the C. elega715 gene map. Humans have trillions of cells, a four-chambered hea rt. an incredibly regionalized brain, m trica le limbs, and remarkable vascular networks. C. eiegan.5, on the ()ther hand. has no hands. Nor does the nematode have chambers in its heart, or any head to speak of. Thousands of these organisms would fit under our fingernails (which C. elegans also lacks). the posterior seems to reside with the position of the sperm pronucleus. When it enters the oocyte cytoplasm, the cen triole associated with the sperm pronucleus initiates cyto plasmic movements that push the male pronucleus to the nearest end of the oblong oocyte.
That end becomes the posterior pole (Goldstein and Hird 1996). The integration of cell division, cell specification, and morphogenesis is coordinated by the sperm and several PAR ("partitioning") :,e more LOultifunctional than their nematode counterparts. Where- proteins. The sperm provides a protein, CYK-4, that acti "'piked RNAs as human genes do. In addition, C. e1egall5 may have plasm activates the egg actin microfilaments to repOSition --ble Jor the huge physical dWerences between worms and human females are mated with males whose CYK-4 has been Hodgkin (2001) notes that human genes and their proteins tend to C. elegnns protein appears to have inst a single ftmction. Nematode hUlnan developmenttll proteins often have many fwlCHons, each ;:enes do not have nearly the capacity for producing alternatively duplicated many of its genes, thus inflating its gene number. What ever the casc, the mere number of genes docs not seem to be respOll .oeings. vates GTFases ter 3, GTPases in the egg cytoplasm. As discussed in Chap (G proteins) are often involved in altering cytoskeletal proteins. Here, CYK-4 from the sperm cyto PAR proteins, pushing them anteriorly (Figure 5.43) . If 1 94 CHAPTER 5 (A) ﬠl size Actu (-lmm) (8) ,' .' . ' Spermatheca (sperm storage region) Anterior ! (D) PO e Zygot ﬠ' p ma h c S er t e a Posterior ﬠ ----Chori --ﬠ o/ ------AS PI AB.a AB.p EMS AB.a] AB.ar AB.pl AB.pr MS E P2 C P3 Pharynx, hypodennis, Pharynx, hypodermi ne r s s, 389 cells Hypodcnnis, neurons Hypodermis, pharynx, ganads (80 cefts) (20 cells) hypodermis, 2 (47 cells) e (20 cells) D P4 Z2 Germ line Z3 '----neurons u on neurons Muscle, Intestine Muscle, neurons Muscl ﬠ 1 95 EARLY DEVELOPMENT I N SELECTED INVERTEB RATES .. FIGURE 5,42 The nematode Caenorhabditis elegam, (A) Side view of adult hermaphro dite. Sperm are stored such that a mature egg must pass through the sperm on its way to ",e vulva. (B) The gonads. Near the distal end, the germ cells undergo mitosis. As they "'love farther from the distal tip, they enter meiosis. Early meioses form sperm, which are ;tored in the spermatheca. Later meioses form eggs, which are fertilized as they roll "mough the spermatheca. (C) Early development, as the egg is fertilized and moves toward n :.
e vulva. The P lineage consists of stem cells that will eventually form the germ cells. (0) ﬠbreviated cell lineage chart. The germ line segregates into the posterior portion of the -,ost posterior (P) cell. The first three cell divisions produce the AS, C, MS, and E lineages. The number of derived cells (in parentheses) refers to the 558 cells present in the newly :uitched larva.
Some of these continue to divide to produce the 959 somatic cells of the adult. (Aher Pines '1992, based on Sulston and Horvitz ' 977 and Sulston et al. ' 983,) PAR-3 (in cortex) A) Oocyte chromatin Sperm chromatin Anterior Posterior MEX-5 PAR proteins and the establishment of polarity, (A-E) PAR protein distribution. (A) When sperm enters the egg, the egg nucleus is undergoing meiosis (left). The cortical cytoplasm contains PAR-3 (orange), and the internal cytoplasm contains MEX-5 (shaded gray), (B) Cytoplasm begins flowing toward the sperm pronucleus, which contains CYK-4. Near the newly formed sperm asters, PAR-2 (purple) replaces PAR-3 in the corti cal cytoplasm. (C) The domain of PAR-2 expression expands as the sperm nucleus migrates toward the center of the cell. (0) At the first diviSion, about half the PAR-2, PAR-3, and MEX-5 polarity remains. (EI At the end of the first division, the AB blastomere has MEX-5 and PAR-3, while most of the Pl blastomere has very little MEX-5 and has a cortex of PAR-2 (except where the two blastomeres meet). (F) In this dividing C. e/egans zygote, PAR-2 protein is stained green and ONA is stained blue. In second division, the AS cell and the Pl cell divide 90 degrees differently. (A-E aher Nance 2005; F, courtesy of j, Ahrenger; courtesy of j, White,) FIGURE 5,43 B) PAR-2 t Sperm _1---'_ pronucleus (CYK 4) Oocyte pronudeu As ers Cytoplasmic=ﬠ ", now _ _ _ I C) (G) G, (F) (D) (G) (E) PAR-2 AB PI 1 96 CHAPTER 5 knocked down with RNAi, many of the result ing embryos lack polarity and die (Jenkins et al. 2006). Some of the most important entities localized by tlie PAR proteins are the P-granules, ribonu cleoprotein complexes that specify the germ cells. The P-granules appear to be a collection of trans lation regulators.
The proteins of these granules include RNA helicases, polyA polymerases, and translation initiation factors (Amiri et al. 2001; Smith et al. 2002; Wang et al. 2002). Using fluo rescent antibodies to a component of the P-gran ules, Strome and Wood (1983) discovered that shortly after fertilization, the randomly scattered P-granules move toward the posterior end of the zygote, so that they enter only the blastomere (Pl) formed from the posterior cytoplasm (Fig ure 5.44). The P-granules of the Pl cell remain in the posterior of the Pl cell when it divides and are thereby passed to the P2 cell. Dming the divi sion of P2 and P3, however, the P-granules become associated with the nucleus that enters the P3 cytoplasm. Eventually, the P-gramues will reside in the P4 cell, whose progeny become the sperm and eggs of the ad Lilt. The localization of the P-granules requires microfilaments but can occur in the absence of micro tubules. Treating the zygote with cytochalasin D (a microfilament inhibitor) prevents the segregation of these gran ules to the postenor of the cell, whereas deOlecol cine (a colchicine-like microtubule inhibitor) fails to stop this movement. = See WEBSITE 5,5 P-granule migration Formation of the dorsal-ventral and right-left axes The dorsal-ventral axis of the nematode is seen in the division of the AB cell.
As the AB cell divides, it becomes longer than the eggshe ll is wide. This causes the ceHs to slide, resuJting in one A B daLighter cell being anterior and one being posterior (hence their respective nmes, ABa and ABp; see Figme 5.43). This squeeZing also caLises the ASp cell to take a position above the EMS cell that results from the division of the PI blastomere. The ABp cell defines the futme dorsal side of the embryo, while the EMS cell the precursor of tile muscle and gut cells-marks the future ventral surface of the embryo.
The left right axis is specified later, at the 12-cell stage, when the MS blastomere (from the division of tile EMS cell) contacts half tile "granddaughters" of the ABa cell, distinguishing the right side of the body from the left side (Evans et a1. 1994). FIGURE 5.44 Segregation of the P-granules into the germ line l ineage of the C. elegans emhryo. The len column shows the cell nu c l ei (Ihe DNA is siained blue by Haeschl dye); Ihe right co lumn shows the same embryos stained for P granules. AI eac h successive division, {he P-granules enter the P-lincage bl as tomere/ whose progeny will become the germ cells.
(Courtesy of S. Strome) . EARLY DEVELOPMENT IN SELECTED INVERTEBRATES nus asymmetric signaling (by Delta in the MS blastomere activating Notch in the cell it contacts) sets the stage for several other inductive events that make the right side of the larva differ from the left (Hutter and Schnabel 1995). Indeed, even the different neuronal fates seen on the left and right sides of the C elegans brain can be traced back to that single change at the 12-cell stage (Poole and Hobert 2006). Control of blastomere identity C. elegans de monstrates both the conditional and autonomous modes of cell specification. Both modes can be seen if the first two blastomeres are experimentally sep arated (Priess and Thomson 1987). The PI cell develops autonomously without the presence of AB, generating all the cells it would normally make, and the result is the pos terior half of an embryo. Howe e r the AB cell in isolation makes only a small fraction of the ce11 types it would nor mally make.
For instance, the resulting ABa blastomere fails to make the anterior pharyngeal muscles that it would have made in an intact embryo.
Therefore, the specifica tion of the AS blastomere is conditionat and it needs to interact with the descendants of the PI cell in order to evelop normally. v , UTONOMOUS SPECIFICATION The detennination of the PI lineages appears to be autonomous, with the cell fates determined by internal cytoplasmic factors rather than by in teractions with neighboring cells. The P-granules are localized in a way consistent with a role as a morpho- genetic determinant, and they act through translational regulation (the exact mechanism is not well understood) to specify germ cells. Meanwhile, the SKN-l, PAL-I, and PIE-I proteins are thought to encode transcription factors that act intrinsically to determine the fates of cells derived from the four PI-derived somatic founder cells (MS, E, C, and D).
The SKN-l protein is a maternally expressed polypep tide that may control the fate of the EMS blastomere, which is the cell that generates the posterior pharynx. After first cleavage, only the posterior blastomere-PI has the ability to produce pharyngeal cells when isolated.
After PI divides, only EMS is able to generate pharyngeal muscle cells in isolation (Priess and Thomson 1987). Sim ilarly, when the EMS cell divides, only one of its progeny, MS, has the intrinsic ability to generate pharyngeal tissue.
These findings suggest that pharyngeal cell fate may be determined autonomously, by maternal factors residing in the cytoplasm that are parceled out to these particular cells. Bowerman and co-workers (1992a,b, 1993) found matemal effect mutants lacking pharyngeal cells and were able to isolate a mutation in the skn-l gene. Embryos from homozygous skn-l-deficient mothers lack both pharyn geal mesoderm and endoderm derivatives of EMS (Figu e 5.45) . Instead of making the normal intestinal and pha ryngeal structures, these embryos seem to make e t ra hypodermal (skin) and body wall tissue where their intes tine and pharynx should be. In other words, the EMS blas tomere appears to be respecified as C. Only those cells des tined to form pharynx or i ntest ine are affected by this r x Wild-type (A) FIGURE 5.45 Defic ienc ie of ntestine and pharynx in skn- l mutants of C. e1egam. Embryos derived from wild-type females -I.C) and from fem al e homo y 'Ous :. for m utant skn- 7 (B,O) were . led for the presence of pharyn al mLlscles (A,S) and gut-specific ; anul es (CD). A pharyngeal mus de-specific antibody labels the pharynx musculature of those embryos derived from wi Id-type ,ales (A), but does not bind to any st u ctu e i n the embryos from n-l muta t females (B). Simi larly, "le birefringent gut granules char acteristic of em b y ic intestines o are absent (rom embryos -ierived from the skn-/ mutant ..
males (D). (From Bowerman el a l . 492a, courtesy o f B .
Bowerman.) (B) s s z Pharynx _muscle antigen · r r n r on (C) Gut specific granules 197 (D) skn-J mutant 1 98 CHAPTER 5 Zygote 2  POP- 1 - SKN-l PAL- l EMS . / MED-l,2 MS . TBX- 35 Blastomere specification 1 -   . - - n doderm) E fate (e • HLH - l --"'- fate (ectoderm) (muscle, hypodermis) fates Pharynx ..M " SCle _____... C Tissue identity FIGURE 5.46 Mode l for specification of the MS blastomere. Maternal SKN- 1 activates GATA transcription factors MEO-l and MED-2 in the EMS ce l l. The POP-l signal prevents these proteins from activating the endodermal transcription factors (such as END-l) and instead activates the tbx-35 gene. The TBX-35 I ran scription factor activates mesodermal genes in the MS cell, including pha-4 in Ihe pharynx lineage and hlh- / (which encodes a myogenic transcription factor) in muscles. TBX-35 also in h ibits pal- 1 gene express ion , t h ereby preventing the MS cell from acquiring the C-blastomere fates. (After Broitman-Madura et al.
2006) mutation. Moreover, the protein encoded by the skn-l gene has a DNA-binding site motif similar to that seen in the bZip family 01 transcription factors (Blackwell et al. 1994). SKN-l is a maternal protein, and it activates the tran scription of at least two genes, med-l and med-2, whose products are also transcription factors. The MED transcrip-tion factor.; appear to specify the entire fate of the EMS cell, since expression of the med genes in other cells can cause non-EMS cells to become EMS even if SKN-l is absent (Maduro et al. 2001). A second putative transcription factr, PAL-l, is also required for the differentiation of the PI lineage. PAL-l activity is needed for the normal development of the somatic descendants of the P2 blastomere. Embryos lacking PAL-1 have no somatic cell types derived fro.m the C and D stem cells (Hunter and Kenyon 1996). PAL-1 is regulated by the MEX-3 protein (see Figure 5.44), an RNA-binding protein that appears to inhibit the translation of pal-l mRNA. Wherever MEX-3 is expressed, PAL-1 is absent. Thus in mex-3-deficient mutants, PAL-l is seen in every blastomere. SKN-1 also inhibits PAL-l (thereby prevent ing it from becoming active in the EMS cell). A third putative transcription factor, PIE-I, is necessary for germ line fate.
PIE-1 is placed into he P blastomeres through the action of the PAR-1 protein, and it appears to inhibit both SKN-1 and PAL-1 lunction in the P2 and sub sequent germ line cells (Hunter and Kenyon 1996). Muta tions of the maternal pie-l gene result in germ line blas- t MS ' tomeres adopting somatic fates, with the P2 cell behaving similarly to a Wild-type EMS blastomere. The localization and the genetic properties of PIE-1 suggest that it repress es the establishment of somatic cell late and preserves the totipotency of the germ cell lineage (Mello et al. 1996; Sey doux et al. 1996). CONDITIONAL SPECIFICATION c As mentioned earlier, the C.
elegans embryo uses

was a difficult organism on which to study embryology. ambushes. " ,. , ot Drosophila to its development, the Although Jack Schultz (originally in Morgan's laboratory) and others follow ing him attempted to relate the genetics of fly embryos proved too complex and intractable to study, being neither large enough to manipulate experimentally nor transparent enough to observe micro scopically. It was not until the techniques of molecular biology allowed researchers to identify and manipulate the genes and RNAs of the insect that its genetics could be related to its development. And when that happened, a revolution occurred in the field of biology. This revolution is continuing, in large part because of the ability to generate transgertic flies at high frequency (Pfeif fer et a!. 2(09). This enables researchers to identify developmental interactions taking place in very small regions of the embryo, and it identifies enhancers that control developmental processes taking place rapidly and in small areas. The merging of our knowledge of the molecular aspects of Drosophila genetics with our knowledge of the fly's development built the foundations on which the current sciences of developmental genetics and evolutionary developmen tal biology are based.
Those of us who are at war" on Drosophi la find a particular point to the question. For the genetic material available is all l.hat could be desired, and even embryological experiments can be done. . . I t is for us to ma/Ie use of these opportunities. We have a COIll plete story to unravel, because we can work l.hings from both ends al once. JACK SCHULTZ (1 935) The chief advantage of Drosophila initially was one that historians have overlooked: it was an excellent organ ism for student projects. ROBEIIT E. KOH LER (1994) 204 CHAPTER 6 EARLY DROSOPH I LA DEVELOPMENT In Chapter 5 we discussed the specification of early embry onic cells by cytoplasmic detenninants stored in tl1e oocyte. The cell membranes that form during cleavage establish the region of cytoplasm incorporated into each new blas tomere, and the incorporated morphogenetic determinants then direct differential gene expression in each cell. Dur ing Drosophila development, however, cellular membranes do not form until after the thirteenth nuclear division. Prior to this time, all the dividing nuclei share a common cyto plasm, and material can diffuse throughout the whole embryo. In these embryos, the specification of cell types along the anterior-posterior and dorsal-ventral axes is accomplished by the interactions of cytoplasmic materials within the single multinucleated cell. Moreover, the initia tion of the anterior-posterior and dorsal-ventral differences is controlled by the position of the egg within the moth er's ovary, Whereas the sperm entry site may fix the axes in asddians and nematodes, the fly's anterior-posterior and dorsal-ventral axes are specified by interactions between the egg and its surrounding follicle cells. is only one site-the micropyle, at the future dorsal anteri or region of the embryo-where the sperm can enter the egg. The micropyle is a tunnel in the chorion (eggshell) that allows sperm to pass through it one at a time. The micropy le probably prevents polyspermy in Drosophila. There are no cortical granules to block polyspermy, although corti cal changes are seen. Third, by the time the sperm enters the egg, the egg already has begun to specify its axes; thus the sperm enters an egg that is already organizing itself as an embryo. Fourth, there is competition between sperm. A sperm tail can be many times longer than the adult fly, and this huge tail is thought to block other sperm from entering the egg. In Drosophila melanagaster, the sperm tail is 1.8 mm-about as long as the adult fly, and some 300 times longer than a hwnan sperm. The entire sperm (huge tail and all) gets incorporated into the oocyte cytoplasm, and the sperm cell membrane does not break down until after it is fully inside the oocyte (Snook and Karr 1998; Clark et aI. 1999). See WEBSITE 6.1 Drosophila fertilization C leavage Fertilization Drosophila fertilization is not your standard sperm-meets egg story. First, the sperm enters an egg that is already acti vated. Egg activation in Drosophila is accomplished at ovu lation, a few minutes before fertilization begins. As the Drosophila oocyte squeezes through a narrOw orifice, ion channels open, allowing calcium ions to flow into it. The oocyte nucleus then resumes its meiotic divisions and the cytoplasmic mRNAs become translated, even without fer tilization (Mahowald et al. 1983; Fitch and Wakimoto 1998; Heifetz et aI. 2001; Homer and Wolfner 2008). Second, there Most insect eggs undergo superficial cl eav age, wherein a large mass of centrally located yolk confines cleavage to the cytoplasmic rim of the egg. One of the fascinating fea tures of this cleavage pattern is that cells do not form until after the nuclei have divided several times. KaryokineSiS (nuclear d ivision) occurs without cytokineSiS (cell divi sion), and the rapid rate of division is accomplished (as it is in sea urchin embryos) by eliminating the gap (G) stages of the cell cycle. Cleavage in the Drosophila egg creates a syncytium, a single cell with many nuclei residing in a common cytoplasm (Figure 6.1). The zygote nucleus under- .:'" -\I: Laser confocal micrographs of stained chromatin showing superficial cleavage in a Drosophila embryo. The future anterior is positioned upward, and the numbers refer to the cell division cyc le. The early n ucl ea r divisions occu r centra l ly. Later, the nuclei and thei r cytoplasmic islands (energids) migrate to the periphery of the cell. This creates the syncytial blastoderm. A�er cycle 1 3 , the oocyte membranes ingress between the nuclei to form the cellular blastoderm. The pole cells (germ cell precursors) form in the posterior. (Courtesy of Daily and FIGURE 6.1 W. Sullivan.) D.
CT: --, , -. - -co "-= Iat - , >e - THE GENETICS O F AXIS SPECIFICATION I N DROSOPHILA A) N uc lea r and cell division in Drosophila. FIGURE 6.2 (B) 205 (A) Nu clea r division (but not cell division) can be seen in th e s ingle cel! of the Orosophila embryo us i ng a dye th a t stains DNA. The firsl region 10 cell u lar ize, th e po l e region, ca n be see n fo rm i ng the cells that will evenlually become Ihe germ cells (sperm or eggs) of the fly.
(B) Confocal nuo rescence photomicrographs of nuclei divid ing during cellularization of the blastoderm. While there arc no cell boundaries, actin (green can be seen forming regions within which each nucleus divides. The micro tubules of the mitotic re stained red wilh antibodies to l b l in .
(C,D) Cross section of a part of the stage 1 0 Orosophila embryo shOW i n g nu cl ei (green) in th e cortex of the s n c tia l cel l, of actin m icro fi ia men ls red). (C) I nterphase nuclei. (D) Nuc lei in anaphase, dividing parallel 10 the cortex and enabling the nuclei to stay in Ihe cell per i hery. Bo n n efoy el a I . 2007; B from S ll ivan elal. 1 993, cou rlesy ofW. Theurkauf and W. S u l l iv n ; CD from Foe 2000, courtesy of V. Foe.)) apparatus a uu (D) y y p goes several mitotic divisions within the central portion of the egg; 256 nuclei are produced by series of eight nuclear divisions averaging 8 .minutes each. During the ninth divi sion cycle, about five nuclei reach the surface of the poste rior pole of the embryo. These nuclei become enclosed by cell membranes and generate the pol e cells that give rise to the gametes of the adult. At cycle 10, the other nuclei migrate to the cortex (periphery) of the egg, where the mitoses continue, albeit at a progressively slower rate (Fig ure 6.2; Foe et al. 2000). During these stages of nuclear divi sion, the embryo is called a syncytial blastoderm, since no (ell membranes exist other than that of the egg itself. The nuclei divide within a common cytoplasm, but this joes not mean the cytoplaSfll is itself uniform. Karr and _Alberts (1986) have shown that each nucleus within the syncytial blastoderm is contained within its own little ter itory of cytoskeletal proteins. When the nuclei reach the • eriphery of the egg during the tenth cleavage cycle, each nucleus becomes surrOLUlded by microtubules and micro filaments. The nuclei and their associated cytoplasmic tslands are called energids. Following division cycle 13, �he oocyte plasma membrane folds inward between the nuclei, eventually partitioning off each somatic nucleus mto a single cell. This process creates the cellular blasto derm, in which all the cells are arranged in a single-lay ered jacket around the yolky core of the egg (Turner and '.Iahowald 1977; Foe and Alberts 1983). Like any other cell formation, the formation of the cellu lar blastoderm involves a delicate interplay between micro tubules and micro filaments.
The membrane movementsl me nuclear elongation, and the actin polymerization each a (u near a layer (A from a appear to be coordinated by the microtubules (Riparbe\li et a1. 2007).
The first phase of blastoderm ceJlularization is characterized by the invagination of cell membranes between the nuclei to form furrow canals (Figure 6.3).
This process can be inhibited by drugs that block microtubules. After the furrow canals have passed the level of the nuclei, the second phase of cellularization occurs. The rate of invagination increases, and the actin-membrane complex begins to constrict at what will be the basal end of the cell (Foe et a1. 1993; Schejter and Wieschaus 1993; Mazumdar and Mazumdar 2002). In Drosophila, the cellular blastoderm consists of approximately 6000 cells and is formed within 4 hours of fertilization. The mid-blastula transition After Ihe nuclei reach the periphery, the time required to complete each of the next four divisions becomes progres sively longer. While cycles 1-10 average 8 minutes each, cycle 13-the l st cycle in the syncytial blastoderm-takes 25 minutes to complete. C ycle 14, in which the Drosophila embryo forms cells (i.e., after 13 divisions), is asynchro nous. Some groups of cells complete this cycle in 75 min utes, other groups take 175 minutes (Foe 1989). Zygotic gene transcription (which begins around cycle 11) is great ly enhanced at this stage. This slowdown of nuclear divi sion, cellularizahon, and concomitant increase in new RNA tran&ription is often refeued to as the mid-blashlla lra.n sition (see Chapter 5). It is at this stage that the maternal ly provided mRNAs are degraded and hand over control of development to the zygotic genome (Brandt et al. 2006; a 206 CHAPTER 6 W (B)) if'�fijr'il (C) Centrosomes f? ;Nucleus . . ..
·v uu / J'� M i cro t b les . 10 min I ::.� : ActlI1 __ , '�: . • •• Ccllularization I 1;\ 55 min De Renzis et a1. 2007; Benoit et a1. 2009). Such a matemal to-zygotic transition is seen in the embryos of numerous vertebrate and invertebrate phyla. In Drosophila, the coordination of the mid-blastula tran sition and the maternal-to-zygotic transition is controlled by several factors, including (1) the ratio of chromatin to cytoplasm; (2) Smaug protein; and (3) cell cycle regulators. The ratio of chromatin to cytoplasm is a consequence of the increasing amount of DNA while the cytoplasm remains constant (Newport and Kirschner 1982; Edgar et a1. 1986a). Edgar and his colleagues compared the early development of wild-type Drosophila embryos with that of haploid mutants. The haploid Drosophila embryos had half the wild type quantity of chromatin at each cell division. Hence, a haploid embryo at cell division cycle 8 had the same amount of chromatin that a wild-type embryo had at cycle 7. The investigators found that, whereas wild-type embryos formed a cellular blastoderm immediately after the thir teenth division, haploid embryos underwent an extra, four teenth, division before cellularization. Moreover, the lengths of cycles 11-14 in wild-type embryos corresponded to those of cycles 12-15 in the haploid embryos. Thus, the haplOid embryos followed a pattern similar to that of the wild-type embryos-but they lagged by one cell division.
Smaug (yes, it's named after the dragon in Lord of the Rings) is an RNA-binding protein often involved in repress ing translation. During the mid-blashila transition, howev er, it targets the maternal mRNAs for destruction (Tadros et al. 2007; Benoit et al. 2009). Maternal Smaug mutants dis rupt the slowing down of nuclear division, prevent cellu- FIGURE 6.3 Formation of the cellular blastoderm in Drosophila . Nuclear shape change and cellularization are coordinated through the cytoskeleton. (A) Cellularization and nuclear shape change shown by staining the embryo for microtubles (green), microfilaments (blue), and nuclei (red.) The red stain in the nuclei is due to the presence of the Kugelkern protein, one of the earliest proteins made from the zygotic nuclei. It is essential for nuclear elongation. (B) This embryo was treated with nocadozole to dis rupt microtubules. The nuclei fail to elongate, and cellularization is prevented. (C) Diagrammatic representation of cell formation and nuclear elongation. (After Brandt et a l. 2006; photographs courtesy of J . Grosshans and A. BrandL) larization, and thwart the increase in zygotic genome tran scription. Smaug is encoded by a maternal mRNA, and Smaug protein levels increase during the early cleavage divisions. These levels peak when the zygotic genome begins efficient transcription. Moreover, if Smaug is artifi cially added to the anterior of an early Drosophila embryo, there results a concomitant gradient-in the timing of mater nal transcript destruction, cleavage cell cycle delays, zygot ic gene transcription, cellularization, and gastrulation. Thus, Smaug accumulation appears to regulate the progression from maternal to nuclear control of development and coor dinates this progression with the mid-blastula transition. Cell cycle regulators are critical for introducing the gap stages into the cell cycle and slowing it down.
As the maternal replication factors are depleted, the zygotically encoded replication factors take over and regulate the accu mulation of cyclinE in the cell (Sibon et aL 1997; Royou et a1. 2008). See VADE MECUM Drosophila development See WEBSITE 6.2 The ear ly development of other insects Gastrulation Gastrulation begins shortly after the mid-blastula transi tion. The first movements of Drosophila gastrulation segre gate the presumptive mesodenn, endoderm, and ectoderm. The prospective mesoderm-about 1000 cells constituting the ventral midline of the embryo-folds inward to pro duce-the ventral furrow (Figure 6.4A). This furrow eventu ally pinches off from the surface to become a ventral tube within the embryo. The prospective endoderm invaginates THE GENETICS OF AXIS SPECIFICATION I N DROSOPHILA A) 207 (8) m idgU l invagination Cephalic furrow Ventral furrow Ventral furrow Pole cells in posterior m idg ut inV"dginat·ion ,.,;;:o:::::-_-- Pole cells DJ Clypeo labrum (E) Procephalic regio n { Ma Optic ridge Dorsal ridge Thorax (Tl-D) Head Cephalic ..trrow Future Lb l,-':�: {.. Abdomen { AI A2 Germ band AS At A7 A6 AS A4 AS A3 IGURE 6.4 G ast ru lation in Dro50phila. The anterior po ints u pward in each figure. .; Ventral furrow beginning to form as cells flanking the ventral midline invaginate. (B) ing of ventral furrow, with mesodermal cells placed i nternal ly and surface eeto ;>erm fla nki ng Ihe venlral midline. (C) Dorsal view of a slightly older embryo, showing pole cells and posterior endoderm sinking into the embryo. (0) Dorsolateral view an embryo at fullest germ band extension, just prior to segmentation. The cephalic _trOW separates the future head region (procephalon) from Ihe germ band, which will m the thorax and abdomen. (E) lateral view, showing fu llest extension of the germ :.and and the beginnings of segmen tation . Subtle indentations mark the incipient seg· .....nts along the germ band. Ma, Mx, and Lb correspond to Ihe mandibular, max i l l ary, :-.l labial head segments; Tl-T3 are the thor.cic segment'; and A l-A8 are the axlominal segments. (F) Germ band reversing direction. The true segments are now .s.ible, as well as the other territories of the dorsal head, such as the c1ypeolabrum, ",ephalic region. optic ridge, and dorsal ridge. (G) Newly halched firsl-instar larva. otographs courtesy of F. R. Turner. D aher Campos-Ortega and Hartenstein t 98S.) . (G) 208 CHAPTER 6 FIGURE 6.5 Schematic representation of gastrulation in Drosophila. Anterio r is to the left; dorsal is facing upward. (A,B) Surfac e and cutaway views showing the fates of the tissues immediately prior to gastrulation. (C) The begi n ni ng of gastrul atio n as the ven tra l mesoderm i nvaginates into the embryo.
(0) This view corresponds to Figure 6.4A, while (E) correspon ds to Figure 6.4B,C. I n (E), the neuroectoderm is largely differentiated into the nervous system and the epidermis. (After Campos� Ortega and Ha rtenstein 1 985.) the anterior and posterior ends of furrow. The p ole cells are internalized a long with the endoderm (Figure 6.4B,C) . At this time , the embryo b ends to form the cephalic furrow. The ectodermal cells on the surface and the mesoderm tmdergo convergence and extension, migrating toward the ventral midline to form the germ band, a collection of cells along the ventral midline that includes all the cells that will form the trunk of the emb ryo. The germ band extends pos- teriorly and, p erha p s because of the egg case, w ra ps around the top (dorsal) surface of the embryo (Figure 6.40) . Thus, at the end of germ band formation, the cells destined to form the most posterior larval structures are located immediately behind the future head region (Figure 6.4E) . At this time, the b ody segments begin t o appear, dividing the ectoderm and mesoderm. The germ band then retracts, placing the presumptive posterior segments at the poste rior tip of the embryo (Fi gure 6.4F) . At the dorsal su rface, the two sides of the epidermis are brought together in a process called dorsal closure. The amnioserosa, which had Internal ectoderm Amnioserosal covering of (A) to form two p ocke ts at the ventral (B) (C) (D) been the most dorsal structure, interacts with the epider I t cells to encourage their migration (reviewed in Pan filio 2007; Heisenbe rg 2009). While the germ band is in its extended position, sever al key morphogenetic processes occur: organogenesis, seg mentation, and the segregation of the imaginal discs.' In addition, the nervous system forms from mo regions of mal ��· ---'� ventral ectoderm.
Neuroblasts differentiate from this neu rogenic ectoderm ",ithin each segment (and also from the nonsegmented region of the head ectoderm). Therefore in insects like Drosophila, the nervous system is located ven� tra lly, rather than be ing derived from a dorsal neural tube as in verteb rates (Figure 6.S) .
The general body plan of Drosophila is the same in the embryo, the larva, and the a dul t each of which has a dis tinct head end and a distinct tail end, between which are repeating segmental units (Figure 6.6).
Three of these seg ments form the thorax, while another eight segments form the abdomen. Each segment of the adult fly has its own identity. The first thoracic segment, for exa mple, has only Epidermis , , *lmaginal discs are those cells set aside to produce the adult struc in Chapter 1 5 . For more information on Drosophila developmental tures. The details of imaginal disc differenti(ltion will be discussed (lnatomy, see Bate and Martinez�Arias 1993; Tyler and Schetzer 1996: and Schwatm 1997. Nervous system legs; the second thoracic segment has legs and wings; and the third thoracic segment has legs and halteres (balancing organs). Thoracic and abdominal segments can also be dis- tinguished from each other by differences i.n the cu tiele of the newly hatched first-instar larvae. GENES THAT PATTERN THE D ROSOPH I LA BODY PLAN Most of the genes involved in shaping the· larval and adult 1990s u sing a powerful "forward genetics" approach. The basic strat egy was to randomly mutagenize flies and then screen for mutations that disrupted the normal formation of the body forms of Drosophila were identiiied in the early THE G E N ETICS OF AXIS SPECIFICATION IN A) DROSOPHILA 20 9 (B) Head Metatho rax Abdominal gments FIGURE 6.6 Comparison of larval l eft) and adult (right) segmen tation in Drosophila. In th e adult, the three thoracic segments can be distinguished by their appendages: Tl (protherax) has legs o n l y; T2 m esoth e ra x) has wings and legs; T3 (metatherax has nalteres (not visible) and legs. (BI Segmen ts i n ad u lt tra nsgen ic Drosophila in which the gen e fo r green fl uorescen t protei n has n fused to the cis-regulatory region of the engrai/ed gene. Thus, GFP is produced in the areas of engraifed transcription, ... hich is active at the border of each segment and in the posterior l e es . compartment of the wing. (B courtesy of (A) (( ) A. K b 1 Primary Axis Formation during Oogenesis The processes of embryogenesis may "offiCially" begin at fertilization, but many of the molecular events critical for Drosophila embryogenesis actually occur during oogene sis. Each oocyte is descended from a single female germ cell-the oogonium-which is surrounded by an epithe lium of follicle cells. Before oogenesis begins, the oogonium divides four times with incomplete cytokinesis, giving rise to 16 interconnected cells: 15 nurse cells and the single oocyte precursor. These 16 cells constitute the egg cham ber (ovary) in which the oocyte will develop, and the oocyte will be the cell at the posterior end of the egg cham Ian. Some of these mutations were quite fantastic, and ber (see Figure 16.4). As the oocyte precursor develops, included embryos and adult flies in which specific body numerous mRNAs made in the nurse cells are transport structures were either missing or in the wrong place. These :JIutant collechons were distributed to many different lab ed on microtubules through the cellular interconnections into the enlarging oocyte. oratories. The genes involved in the mutant phenotypes were cloned and then characterized with respect to their expression patterns and their functions, This combined Anterior-posterior polarity in the oocyte effort has led to a molecular understanding of body plan The follicular epithelium surrounding the developing Drosophila that is unparaUeled in aU of biol oocyte is initially uniform with respect to cell fate, but this y, and in 1995 the work resulted in a Nobel Prize for uniformi ty is broken by two signals organized by the evelopment in E ward Lewis, Christiane Niisslein-Volhard, and Eric .. 'jeschaus, The rest of this chapter details the genetics of Drosophi the same gene, gllrken. The gllrken message appears to be oocyte nucleus. Intereshngly, both of these signals involve synthesized in the nurse cells, but it becomes transported development as we have come to upderstand it over the specifically to the oocyte nucleus. Here it is localized past two decades. First we will examine how the dorsal between the nucleus and the cell membrane and is trans 'ental and anterior-posterior axes of the embryo are estab lated into Gurken protein (Caceres and Nilson 2005). At . hed by interactions between the developing oocyte and this time the oocyte nucleus is very near the posterior tip .- surrounding follicle cells. Next we will see how dorsal of the egg chamber, and the Gurken signal is received by -ental patterning gradients are formed within the embryo, the follicle cells at that position through a receptor protein d how these gradients specify cli!ferent tissue types. The encoded by the torpedo gene< (Figure 6.7A). This signal rmed along the anterior-posterior axis, and how the dif results in the "posteriorization" of these follicle cells (Fig- =w-d part of the discussion will examine how segments are :erent segments become specialized. Finally, we will briefly !low how the positioning of embryOniC tissues along the ..'o primary axes specifies these tissues to become partic �, ":ar organs.
*Molecular analysis has established that gurken encodes a homo logue of the vertebrate epidermal growth factor (EGF), while torpe do encodes a homologue of the vertebrate EGF recep tor (Price et a1. 1989; Neuman-Silberberg and Sch upbach 1993). CHAPTER 6 210 (A) Posterior Anteri or Nurse cells Nucleus o T rpedo (Curken receptor) e T r min al follicle c lls e Uncommitted polar fol l icle ce lls o j o o (8) Anterior follicle cells Curken protein Microtubules o Posterior follicle (D) bicoid mRNA Anterior border cells oskar mRNA in associa tion with ki nesin I Nucleus (E) Dorsal I) o o Curken 0 Oskar protein (F) ��- An terior FIGURE 6.7 The anterior- posteri o r axis is specified during ooge nesi s. (A) The oocyte moves into the posterior region of the egg chamber, while nurse cells fill the anterior portion. The oocyte nucleus moves toward the terminal follicle cells and synthesizes Gurken protein (green). The terminal follicle cells express Torpe do, the receptor for Gurken. (8) When Gurken binds to Torpedo, the terminal fol licle cells differentiate into posterior follicle cells and synthesize a molecule that activates protein kinase A in the egg. Protein kinase A orients the microtubules such that the grow ing end is at the posterior. (C) The Par-l protein (green) localizes to the cortical cytoplasm of nurse cells and to the posterior pole of the oocyte. (The Stauffen protein marking the posterior pole is labeled rcd; the red and green signals combine to Ouoresce yel low.) (D) The bicoid message binds to dynein, a motor protein associated with the non -growing end of microtubules. Dynein moves the bicoid message to the anterior end of the egg. The oskar message becomes complexed to kinesin I, a motor protein that moves it toward the growing end of the microtubules at the posterior region, where Oskar can bind the nanos message. (E) The nucleus (with its G u rken protein) migrates along the micro tubules, inducing the adjacent follicle cells to become the dorsal follicles. (F) Photomicrograph of bicoid mRNA (s tai ned black) passing from the nurse cells and localizing to the anterior end of the oocyte during oogenesis. Ie co u rtesy of H. Docrflinger; F from 5tephanscn et 01. 1 988, courtesy of the authors.) ure 6.7B). TI,e posterior follicle cells send a signal back into O,e oocyte. The identity of this signal is not yet known, but it recruits the par-l protein to t he po s ter i or edge of the Posterior oocyte cytoplasm (Figure 6.7C; Doerflinger et a1. 2006). Par I protein org aniz es microtubules specifically with their T H E G E N ETICS OF AXIS SPECI FICATION I N DROSOPHILA (8) 211 (C) D) nllilus (cap) and plus (growing) ends at the anterior and posterior ends of the oocyte, respectively (Gonzalez-Reyes 1995; Roth et a1. 1995; Januschke et a1. 2006). The orientation of the m.icrotubules is critical, because different microtubule motor proteins will transport their et a1. FIGURE 6.8 Expression of the gurken message and protein between the oocyte nucleus and the dorsal anterior cell mem brane. (A) The gurken mRNA is l ocal i zed between the oocyte nucleus and the dorsal follicle cells of the ovary. Anterior is to the left; do rsal faces upward. (8) The Gurken protein is si m ila rly locat ed (sh ow n here in a younger stage oocyte than A). (C) Cross sec tion of the egg through the reg ion of Gurken protein expressi on. (D) A more mature oocyte, showing Gurken protein (yellow) across the dorsal region. The actin is stained red, showi ng cell boundaries. As the oocyte grows, follicle cells migrate across the top of the oocyte, becomi n g exposed to Gurken. (A from Ray and Schupbach 1 996, courtesy of T.
Schupbach; B,C from Peri et al. 1 999, courtesy of S. Roth; D courtesy of C. van Bu ski rk and T. Schupbach.) A or protein cargoes in different directions. The motor protein kinesin, for instance, is an ATPase that will use the energy of ATP to transport material to the plus end of the motor protein that will transport its cargo the opposite way. ing event takes place. Here the gurken message becomes localized in a crescent between the oocyte nucleu::i and the One of the messages transported by oocyte cell membrane, and its protein product forms an microtubule. Dynein, however, is a "minus-directed" kinesin along the microtubules to the posterior end o f the oocyte is oskar A (Zimyanin et a1. 2008). The oskar mRNA is not able :. be translated until it reaches the posterior cortex, at which time it generates the Oskar protein. Oskar protein ruits more par-l protein, thereby stabilizing the micro :ubule orientation and allowing more material to b e rECruited to the posterior pole o f the oocyte (Doerflinger 2006; Zimyanin et a1. 2007). The posterior pole will :hereby have its own distinctive cytoplasm, called pol e et .1. ?lasm, which contains the determinants for producing the bdomen and the germ cells. This cytoskeletal rearrangement in the oocyte is accom :-anied by an increase in oocyte volllIT!e, owing to transfer i cytoplasmic components from . icoid and rponents the nature cells. These include maternal messengers such as the Hllnos mRNAs. These mRNAs are carried by =totor proteins along the micro tubules to the anterior and ""sterior ends of the oocyte, respectively (Fi gure 6.7D-F) . .\s we shall soon see, the protein products encoded by -oid and 1IatJos are critical for establishing the anterior :'OSterior polarity of the eU1bryo. (Fi gure 6.8; Neuman-Silberberg and Schupbach 1993). Since it can diffuse only a short distance, Gurken protein reaches only those follicle cells closest to the oocyte nucleus, and it signals those cells to become the more columnar dorsal follicle cells (Montell et al. 1 991; SchUp bach et a1. 1991; see Figure 6.7E). This establishes the dor anterior-posterior gradient along the dorsal surface of the oocyte sal-ventral polarity in the fullicle cell layer that surrounds the growing oocyte. Maternal deficiencies of either the gurkell or the torpedo gene cause ventralization of the embryo. However, gurken is active only in the oocyte, whereas torpedo is active only in the somatic follicle ce lls. This fact was revealed by experi ments with germline/somatic chimeras. Tn one such exper iment, SchUpbach (1987) transplanted genm cell precursors from wild-type embryos into embryos whose mothers car ried the torpedo mutahon. Cunversely, she transplanted the germ cell precursors from embryos (Figure torpedo mutants into wild-type 6.9). The wild-type eggs produced mutant, ventralized embryos when they developed in a torpedo mutant mother's egg chamber. The torpedo mlltant eggs were able to produce normal embryos if they developed • :Jorsal-ventral patterning in the oocyte oocyte volume increases, the oocyte nucleus moves to m anterior dorsal position where a second major signal- in a wild-type ovary. Thus, unlike Gurken, the Torpedo protein is needed in the follicle celis, not in the egg itself.
TI,e GlUken-Torpedo signal that specifies dorsalized fol licle cells initiates a cascade of gene activities that create 212 CHAPTER 6 n /-'" ; Embryo from wild-type ! ! mother torpedo.deficient germ cells in a wild-type female \ \ , , \ ) n·- -.' V Embryo from / mother i deficient in :. torpedo gene (\. \ -+ torpedo-deficient oocyte in wild-type follicle pOle cells ,--(germ ceU precursors) .... . ----1.� , Wild-type germ ceJls ill a torpedo deficient female \ - :;; E'-- i V -' .. . ' '-;:---- tollOid decapcntaplegic IZl ':' { wist FIGURE 6.1 4 Subdivision of the Drosophila dorsal-ventral axis by the gradient of Dorsal protein in the nLlclei. (A) Dorsal protein activates the zygotic genes rhomboid, twist, {gfB, fgfB receplOr, and snail, depending on its nuclear concentration. The mesoderm forms whe e Twist and Snail are present, and the glial cells form where Twist and Rhomboid interact. Those cells with RhombOid, but no Snail or TWist, the neu ogenic ectoderm. The Fgf receptor is expressed in the mesoderm, and the Fgf8 iga nds for this receptor are expressed in the mesectoderm (glia and midline central nervous system), adjacent to the mesoderm. The binding o(Fgf8 to its receptor triggers the cell movements required for the i ngression of the mesoderm. (8) Interactions in the specification o(t e ven tra l porti on of the Drosophila embryo. Dorsal protein inhibits those genes that would give rise to dorsal structu res (to/ laid, decapenlaplegic, and ze,knOlll) while activating the three ventral genes. Snail protein, formed most ventrally, nhibi ts the transcription of rhomboid and prevents ectoderm formation. Twist activa es dMet2 and bagpipe (which activate muscle differentia tion) as wel l as tinman (heart muscle development).
(A afte Stew ard and Govind 1 993; B after Furlong ot al. 200 1 an d Leptin and Affolter 2004.) l i r o snail E:;J (B) derm invagina tes and brings these v entro lateral regions toge ther. This mesectoderm gives rise to glial cells and to the midline system. Unlike the neurogenic ectoderm adj acent to it, the mesectoderru cells never form typical neuroblasts, never fonn epidermis, and are not a stem cell population (see Figure 6.14).
The high concentration of Twist pro tein in the nuclei of the ventralmost cells ac ti zerkniil1t, structures of the central nervous tolloid, decape/1taplegic bagpipe fgfS receptor fin man dMet2 vates the gene for the Fgf8 receptor (the produ c t of the heartless gene) in the pre sunlpti ve mesoderm Giang and Lev ine 1993; Gryzik and Miiller 2004; Strathopou los et aI. 2004). The expression and secretion of Fgf8 by the presumptive neural ectoderm is received by its receptor on the mesoderm cells, causing these mesoderm cells to invaginate into the embryo and flatten since their enhancers do not bind Dorsal with a very high affinity (Thisse et a1. 1988, 1991; Jiang et al. 1 99 1 ; Pan et a1. 1991). Both Snail and Twist are also needed for the com plete mesodenmal phenotype and proper gastrulation (Lep tin et a!. 1991b). The Twist protein activates mesodermal genes, while the Snail protein represses partic u lar non mesodermal genes that might othe rwise be active. The rhomboid and fg[S genes a re interesting because they are acti va ted by Dorsal but repressed by Snail . Thus, rhomboid and fg[8 are not expressed in the most ventral cells (i.e., the mesodermal precursors) but are expressed in the cells adja cent to the mesoderm. These rhomboid- and fg[S-expressing against the ectoderm (see Figure 6.13). Meanwhile, intermediate levels of nuclear Dorsal acti vate transcription of the Slmt gastrulation (Sog) gene in two lateral str ipes that flank the ventral twist expression domain, each 12-14 cells wide (Fran�ois et a!.
1994; Srini vasan et a!. 2(02). Sog encodes a protein that prevents the r ectoderm in this region from becoming epidermis and begins the processes of neural differentiation (Figure 6.15). otein also determines the mesoderm indirect ly. In addition to ac tiva ting the mesod e rm - stimu la ting genes (twist and sl1ail), i t directly inhibits the dorsalizing genes zerkniillt (zen) and decapentaplegic (dpp). Thus, in the same cells, Dorsal can act as an activator of some genes and Dorsal p cells will become the mesectoderm. The mesectoderm tis a repressor of others. Whether Dorsal activates or repress sue is fated to become the ventral midline, once the meso- es a given gene depends on the structure of the gene's T H E G E N ETICS OF AXIS SPECIFICATION I N DROSOPHILA 217 Dorsal-ventral pattern ing in Drosophila. The readout of the Dorsal gradi e nt can be seen in the anteri or region of a whole-moun t s ta i ned embryo. The expression of the most ventral gene, ventra/ nervous system defective (blue), is from the n eurogen ic ectoderm. The intermediate neuroblast defective gene (green) is expressed in lat eral ectoderm. Red represents the muscle-specific homeobox gene, ex pressed in the mesoderm above the intermediate neuroblasts.
The dorsalmost tissu e expresses decapenlaplegic (yellow). (From Kosman et al. 2004, co urtesy of D. Kosman Bier.) FIGURE 6. 15 and E. enhancers. The zen enhancer has a silencer region that con lains a binding site for Dorsa l as well as a second binding sile for two other DNA-binding pro teins. These two o ther pro teins enable Dorsal to bind a transcrip tiona l repressor pro tein (Groucho) and bring it to the DNA (Valentine et aJ. \ 998) . Mutants of Dorsal express dpp and zel! genes throughout the embryo (Rushlow et aI. 1987), and embryos deficient in dpp and zel! fail 10 form dorsal structures (Irish and Gelbart 1987). Thus, in wild-type embryos, the meso ermal precursors express twist and snail (bu t not zen or dpp); precursors of the dorsal epidermis and amnioserosa express zen and dpp (but not twist or snail). Glial (mesecto derm) precursors express twist and rhomboid, while the lal eral neural ectodermal precursors do not express any of five genes (Kosman et aI. 1991; Ray and Schupb ach 1996). By the cellular responses to the Dorsal protein gradi ent, the embryo becomes subdivided from the ventral to dorsa l regions into mesoderm, neurogenic ectoderm, epi dermis (from the lateral and dorsal e c tode r m), and these amnioserosa (see Figure 6.12A). SIDEL IGH TS SPECULATIONS The left- Right Axis V e ry little is known aboul lhe for mation of the l eft-right axis in Drosophila. Al though the fiy may oak b ilatera lly symmetric, there are asymmetries in the em b ryoni c h indgut wh i ch l oops to the left) and the adult ,indgul a nd gona ds.
This asymmetry appears to be regu la ted by microfi la "ents (Hozumi et a l . 2006; Speder et . al. 2006). The mechanism lhat prouces this asymmetry is different from 'nat known to prod uce left- ri gh t asym etry in vertebrates, which a ppea rs to "" regu l ated by m i c rotu bu l e s . Ii t h e actin microfilaments are dis -Jpted in the Drosophila e m b ryo, "'lany defec ts occur, and Ihe left-r ight oattern is randomized. Loss-of-function ...,utations of certa in genes for myosinproteins (which interact with micro aments) can reverse the insect's left ght asymmetry (Figure 6.16). Wild type Myosin mutant Wild type Myosin mutant Ventral view Dorsal view Figure 6.16 l.eh-right axis formation in Drosophila involves the microfilament cytoskeleton.
Mutations in the myosin gene Myo3 10F can reverse the inseces left-right asymmetry. Here the embryonic gut is seen in dorsa l and ventral perspectives, showi ng that in the larva with myosin mutant, the asymmetry of the gut is reversed. HG, hindgut; MG, midgut; FG, foregut. (From Hozum! et a l 2006, courtesy of K. Matsuno.) . 21 8 CHAPTER 6 Segmentation and the Anterior-Posterior Body Plan The gene tic screens pioneered by Nusslein-Volhard and Wieschaus identified a hierarchy o f genes that establish a n terior-poste ri or polarity, and divide the embryo into a speci fi c number of segments with different identities (Fig ure 6.1 7). This hierarchy is ini ti a ted by maternal effect genes that produce messenger RNAs that are placed in dif- FIGURE 6.1 7 Generalized model of Drosophila anterior-posteri or pattern formation. Anterior is to the left; the dorsal surface faces upward. (A) The pattern is establ ished by maternal effect genes that form gradients and regions of morphogenetic proteins. These proteins are transcription factors that activate the gap genes, which define broad territories of the embryo. The gap genes enable the expression of the pair-rule genes, each of which divides the embryo into regions about two segments wide. The segment polarity genes then divide the embryo into segment-sized units along the anterior-posterior axis. Together, the actions of these genes define the spatial domai ns of the homeotic genes that define the identities of each of the segments.
In this way, periodic� ily is generated from nonperiodicity, and each segment is given a unique identity, (B) Maternal effect genes. The anterior axis is s peci fied by the gradient o f Bicoid protein (yellow through red).
(0 Gap gene protein expression and overlap.
The domain of Hunchback protein (orange) and the domain of Kruppel protein (green) overlap to form a region containing both transcription fac� tors (yellow). (D) Products of the lushi laraw pair-rule gene form seven bands across the blastoderm of the embryo. (E) Produc� of the segment polarity gene engrailed, seen here at the extended germ band stage (8 courtesy of C. Nlisslein-Volhard; C courtesy of C. Rush l ow and M. Levine; D courtesy of D. W. Knowles; cou rtesy of S. Carroll and S. Paddock.) ferent regions of the egg. These messages encode transcrip tional and translational regulatory proteins that diffuse through the syncytial blastoderm and activate or repress the expression of certain zygotic genes.
The first such zygotic genes to be expressed are called gap genes (because mutations in them cause gaps in the segmentation pattern). These genes are expressed in cer tain broad (about three segments wide) partia lly overlap ping domains. These gap genes encode transcription fac, (B) (C) . E (A) (D) Cytoplasmic polari ty (maternal effect) Gap genes Pair-rnle genes (E) Segment polarity Renes Homeotic eenes T H E GENETICS OF AXIS SPECIFICATION IN DROSOPHILA tors, and differing combinations and concentrations o f the gap gene proteins regulate the transcription of pair-rule genes, which divide the embryo into periodic units. The transcription of the different pair-rule genes results in a striped pattern of seven transverse bands perpendicular to the anterior-posterior ax

localization. But having Exuperantia in the oocyte is not sufficient to bring the bicoid message into the oocyte (Cha- et a1. 2001; Reichmann a.nd Ephrussi 2005).
The bicoid-protein complex is transported out of the nurse cells and into the oocyte via microtubules. 111e complex seems to ride on a kinesin ATPase that is crit- Posterior (B) Proteiu Bicoid mRNA and protein gradi ents shown by in situ hybridization and confocal ent acros s the anterior portion of the oocyte . llicroscopy. (A) Bicoid mRNA shows a steep gradi 31 When the mRNA is translated, the Bicoid ')fntein gradient can be seen in the anterior nuclei. ""'Jlterior is to the left; the dorsal surface is upward. \iter Spirov et aI., courtesy of S. Baumgartner.) Anterior Posterior CHAPTER 6 222 ical for taking U,e bicoid message into the oocyte (Am et al. 2003). Once inside the oocyte, the bicoid-mRNA complex attaches to dynein proteins that are maintained at the microtubule organizi.ng center (the "minus end") at the anterior of the oocyte (see Figure 6.7; Cha et a!. 2001). NANOS MRNA LOCALIZATION IN THE POSTERIOR POLE OF THE OOCYTE The posterior organizing center is defined by the activities 01 U,e llallOS gene (Lehmalm and Nusslein-Vol hard 1991; Wang and Lehmann 1991; Wharton and Struhl 1991). While bicoid message is bound to the anchored end 01 the microtubules by active transport along micro tubules, the nmlOS message appears to get "trapped" in the posteri or end of the oocyte by passive diffusion. The nanos mes sage becomes bound to the cytoskeleton in the posterior region 01 the egg through its 3' VIR and its association with the products 01 several other genes (osm/', valois, vasa, staufell, and tudor).' If nOllOS (or any other of these maternal effect genes) is absent in the mother, no abdomen forms in the embryo (Lehmann and Nusslein-Volhard 1986; Schup bach and Wieschaus 1986). Bul before the can get "trapped" nanos message in the posterior cortex, a nanas mRNA specific trap has to be made; this trap is the Oskar protein FIGURE 6.22 Caudal protein gradient in the syn cyti al blasto derm 01 a wild·type Drosophila embryo. Anterior is to the left. The protein (stainee darkly) en ters the nuclei and helps specify poste·rior fates. Compare with the complementary gradient of Bicoid protein in Figure 6.2 1 . (from Macdonald and Struh1 1 986, cour tesy of G. Struhl.) they diffuse toward the middle regions 01 the early embryo, creating the two opposing gradients that estab lish the anterior-posterior polarity 01 the embryo. (1I1mchback, lib; caudal, cad) are critical for patterning the anterior and Two other maternally provided mRNAs and (Ephrussi et a!. 1991). The oska/' message and the Staulen posterior regions of the body plan, respectively (Lehmalm protein are transported to the posterior end 01 the oocyte et al. 1987; Wu and Lengyel 1998). These two mRNAs are by the kinesin motor protein (see Figure 6.7). There they synthesized by the nurse cells 01 the ovary and transport become bound to the actin microfilaments 01 the cortex. ed to the oocyte, where they are distributed ubiquitously Staulen allows the translation 01 the oskar message, and the throughout the syncytial blastoderm. But if they are not resulting Oskar protein is capable 01 binding the localized, how do they mediate their localized patterning nnllos mes sage (Brendza et a!. 2000; Hatchet and Ephrussi 2004). Most llanos mRNA, however, is not trapped. Rather, it is bound in the cytoplasm by the translation inhibitors Smaug and CUP.
Smaug binds to the 3' UTR 01 nanos mRNA and recruits the CUP protein that prevents the asso ciation of the message with the ribosome. If the nanos activities? It turns out that translation of the hb and cad mRNAs is repressed by the diffusion gradients 01 Nanos and Bicoid proteins, respectively. In anterior regions, caudal's Bicoid binds to a specific region 01 3' UTR. Here, it binds d4HEP, a protein that inhibits translation by binding to the 5' cap of the message, Smaug-CUP complex reaches the posterior pole, howev thereby preventing its binding to the ribosome. By recruit er, Oskar can dissociate CUP from Smaug, allowing the ing this translational inhibitor, Bicoid thus prevents trans-mRNA to be bound at the posterior and ready for transla lation of Caudal in the anterior section of the embryo (Fig ure 6,22; Rivera-Pomar et a!. 1996; Chan and Strul,1 1997; hon (Forrest et aJ. 2004; Nelson et a!. 2004). bicoid mes Cho et a!. 2006).
This suppression is necessary because if nunDS message is tethered to the posterior end (Frigerio et and thorax do not form properly.
Caudal is critical in spec a!. 1986; Berleth et al. 1 988; Gavis and Lehmann 1992). ifying the posterior domains 01 the embryo, activating the These mRNAs are dormanl until ovulation and fertiliza genes responsible lor the invagination 01 the hindgut. Therefore, at the completion of oogenesis, the sage is anchored at the anterior end of the oocyte, and the tion, at which time they are translated.
Since the Bicoid and Nanos protein products are not bound to the cytoskeleton, Caudal protein is made in the embryo'S anterior, the head At the other end 01 the embryo, Nanos functions by pre venting hunchback translation (Ta utz 1988). Nanos protein in the posterior 01 the embryo lorms a complex with sev eral other ubiquitous proteins, induding Pumilio and Brat. message is determined by its 3' UTR.
if the bicoid 3' UTR is experi "'Like the placement of the bicoid message, the location of the nanos mentally placed on the protein-encoding region of nanDS mRNA, the llanos message gets placed in the anterior of the egg.
When the RNA is translated, the Nanos protein inhibits the h'anslation of hunchback and bicoid mRNAs, and the embryo forms two abdomens-one in the anterior of the embryo and one in the poste rior (Gavis and Lehmmn 1992). We will see these proteins again in Chapter 16, since they are critical in forming the germ cells of Drosophila. Pumilio appears to direct the complex to the 3' UTR 01 the hunchback message, and Brat appears to recruit d4HEP, which will inhibit the translation 01 the hunchback message (Cho et al. 2006). The result 01 these interactions is the cre • An anterior-ta-posterior gradient of Bicoid protein • An anterior-to-posterior gradient 01 Hunchback protein ation 01 lour maternal protein gradients in the early embryo (Figure 6.23) : (.A) Oocyte THE GENETICS OF AXIS SPECIFICATION IN DROSOPHILA mRNAs hunchback mRNA / "calldal mRNA (e) ANTERIOR bicoid mRNA Cortex, Grauzone, Staufen c p r o t e i n AnEarltyecriloearvage proteins POSTERIOR - bicoid mRNA rIa nos _ mRNA Post ri or e B) 223 e m bryo I Bi I oi d Oskar I proteinI Nanos . Anterior p5 f---------i . Pumilio r-�-:--:-l Posterior fiGURE 6.23 Model of anterior-posterior pattern generation by Orosophila maternal effect ",nes. (Al The bicoid, nanos, hunchback, and caudal mRNAs are placed in the oocyte by the (J\arian nurse cells. The bieoid message is sequestered anteriorly; the nanos message is sent to "!e posterior pole. (8) Upon translation, the Bicoid protein gradient extends from anterior to "JOsterior, while the Nanos protein gradient extends from posterior to anterior. Nanos inhibits -..e translation of the hunchback message (in the posterior), while Bicoid prevents the trans la iOn of the caudal message (in the anterior). This inhibition results in opposing Caudal and "iunchback gradients. The Hunchback gradi�nt is secondarily strengthened by transcription of ;e hun chba ck gene in the anterior nuclei (since Bicoid acts as a transcription factor to acti a;e hunchback transcription). (C) Para ll e l interactions whereby translational gene regulation es • A posterior-to-anterior gradient of Nanos protein • .-\ posterior-to-anterior gradient of Caudal protein needed for the formation of anterior structures. Moreover, one could hypothesize that the substance these mutants lack is the one postulated by Sander and Kalthoff to turn ne Bicoid Hunchback, and Caudal proteins are transcrip ::-.on fac tors whose relative concentrations can activate or on genes for the anterior struchlres and turn off genes for , ress particular zygotic genes. The stage is now set for =e ac t iva tion of zygotic genes in those nuclei that were :-T; Y dividing while these four protein gradients were Several studies support the view that the product of the , ::-eing established. 'he anterior organizing center: T�e Bicoid and Hunchback gradients - Drosophila, the phenotype of the bicoid mutant provides a!Uable information about the function of morphogenet gra dients (Figure 6.24A-C).
Instead of having anterior -"ctures (acron, head, and thorax) followed by abdominal bicoid mutant -;Jctures and a telson, the structure of the :elson·abdomen-abdomen-telson (Figure 6.240). It would ear that these embryos lack whatever substances a re the telson structures (compare Figures wild-type bicoid gene is 6.18 and 6.240). the morphogen that controls ante rior developmen t. The firs t type of e vi dence came from gradient. As we have seen, the exuperantia and swallow experiments that altered the shape of the Bicoid protein genes are responsible for keeping the bicoid message a t the anterior pole of the egg. In their absence, the bicoid mes sage diffuses farther into the posterior of the egg, and the protein gradient is less steep (Oriever and Niisslein-Vol hard 1988a). The phenotype produced by exuperantia and swallow mutants is similar to that of bicoid-deficient embryos but is less severe; these embryos lack their most anterior structures and have an extended mouth and tho racic region. Furthermore, by adding extra copies of the bicoid gene, the Bicoid protein gradient can be extended (D) (A) (E) bicoid-deficient mutant (C) 150 Bicoid protein gradient in the early Drosophila embryo. (A) Localization of bicoid mRNA to the anterior tip of the embryo in a steep gradient.
(B) Bicoid protein gradi en t shortly after ferti lization. Note that the concentration is greatest anteriorly and trails off posteriorly. Notice also that Bicoid is concentrated in the nuclei. (el Densitometric scan of the Bicoid protein gradient. The up per c rve represents the Bicoid gradient in wild type embryos. The l ower cu rve represents Bicoid in embryos of bicoid-mutant mothers. (0) Phenolype of cuticle from a strongly affected embryo from a female fly deficient in the bicoid gene compared with the wild-type cuticle pattern. The head and thorax of the bicoid mutant have been replaced by a second set of posterior telsan structures, abbreviated fk (fi lzk6rper neurons) and ap (anal plates). (A from Kaufman et al. 1 990; B,C from Dri ever and Nusslein-Volhard 1 988b; D from Driever et al. 1 990, courtesy of the authors.) FIGURE 6.24 \ \.
. Wild type . •/ , ... bicoid mutant .", -/ ....!..•.• A._·.·.·.···.·.·";!e;;;

of the embryo (Skeath and Carroll 1992). The cells in each clus ter interact (via the Notch pathway discussed in Chapter 3) to generate a single neural ceU from each cluster.
Skeath and colleagues (1993) have shown that the pattern of neu ral gene transcription is imposed by a coordinate system. Their expression is repressed by the Decapentaplegic and Snail proteins along the dorsal-ventral axis, while positive enhancement by pair-rule genes along the anterior-poste (B) inhibits very llkely, then, that the positions of organ primordia in tem based on the intersection of the anterior-posterior and dorsal-ventral axes. Coda Genetic studies on the Drosophila embryo have uncovered numerous genes that are responsible for specification of the anterior-posterior and dorsal-ventral axes. We are far from a complete understanding of Drosophii11 pattern for mation, but we are much more aware of its complexity than we were a decade ago. Mutations of Drosophila genes have given us our first glimpses of the multiple levels of pattern regulation in a complex organism and have enabled us to isolate these genes and their products. Most importantly, as we will see in forthcoming chapters, the Drosophila genes provide clues to a general mechanism of pattern fonnation that is used thrQughout the animal king dom. activates dpp 1 rior axis causes their repetition in each half-segment. It is the fly are specified via a two-dimensional coordinate sys scr inhibits t dorsal {.
a al/ Iiv 1 , t FIGURE 6. 39 Cartesian coordinate system mapped out by gene expression patterns. (A) A grid (ventral view, looking "up" at the embryo) formed by the expression of short-gastrulation (red), intermediate neuroblast defective (green), and muscle seg ment homeobox (magenta) along the dorsal-ventral axis, and by the expression of wingless (yellow), and engrai/ed (gree n) tran scripts along the anterior-posterior ax is. (B) Coordinates for the expression of genes giving rise to Drosophifa salivary glands. These genes are activated by the protein produ ct of the sex combs reduced (ser) homeolic gene in a narrow band along the anterior-posterior axis, and they are inhibited in the regions marked by decapentaplegic (dpp) and dorsal gene prod ucts along the dorsal-ventral axis. This pattern allows sal ivary glands to form in the midline of the embryo i n the second parasegment. (A cou rtesy of D. Kosman; B aher Panzer et al. ' 992.) Snapshot Summary: Drosophila Development and Axis Specifica tion 1 . Drosophila cleavage is superficial. The nuclei divide 13 times before forming cells. Before cell formation, the nuclei reside in a syncytial blastoderm. Each nucleus is surrounded by actin-filled cytoplasm. 2. When the cells form, the Drosophila embryo under goes a mid-blastula transition, wherein the cleavages become asynchronous and new mRNA is made. At this time, there is a transfer from maternal to zygotic control of development. 3.
Gastrulation begins with the invagination of the most ventral region (the presumptive mesoderm), which causes the formation of a ventral furrow. The germ band expands such that the luture posterior segments curl just behind the presumptive head. CHAPTER 6 238 4. The genes regulating pattern lormation in Drosophila the ventral cell fates and represses those genes operate according to certain principles: • whose proteins would specify dorsal cell fates. There are morphogens-such as Bicoid and Dorsal 10. Dorsal protein forms a gradien t as it enters the whose gradients determine the specification of di f various nuclei. Those nuclei at the most ventral su r ferent cell types. These morphogens can be tran temporal order wherein different classes of face incorpora te the most Dorsal protein and become scription factors . • There is a genes are transcribed, and the products of one gene often regulate the expression of another gene. • mesoderm; those more lateral become neurogenic ectoderm. 11. BOllndaries of gene expression can be created by the Caudal activates genes responsible for posterior interaction between transcription factors and their gene targets. Here, the transcription factors tran • Translational control is extremely important in the scribed earner regula te the expression of the next development. 12. given field is divided and subdivided, eventua lly maternal effect gene proteins. Their protein products tein defines specific regions of the embryo. 14. moters such that they become activated in seven Maternal ellect genes are responsible for the initia tion of anterior-posterior polarity.
"stripes . " Their boundaries of transcription are Bicoid mRNA is defined by the gap genes. The pair-rule genes form bound by its 3' UTR to the cytoskeleton in the future nanas mRNA is sequestered by its 3' UTR in the luture poster ior pole. Hunchback and cau dal messages are seen throughout the embryo. seven bands of transcription a long the anterior-pos anterior pole; 6. Dorsal-ventral polarity is regulated by the en try 01 Dorsal protein into the nucleus. Dorsal-ven tral polarity is initiated when the nucleus moves to the dorsal-anterior of the oocyte and transcribes the gurken message, which is then transported to U)e region above the nucleus and adjacent to the follicle cells. 7. Gurken protein is secreted lrom the oocyte and binds to its receptor (Torpedo) on the follicle cells. This binding dorsalizes the follicle cells, preventing them from synthesizing Pipe. 8. Pipe protein in the ventral follicle cells modifies an as yet unknown factor that modifies the Nudel p ro tein. This modification allows Nudel to activate a cascade of proteolysis in the space between the ven As a result 01 this cascade, the Sp atzle protein is acti tral follicle cells and the ventral cells of the embryo. vated and binds to the Toll protein on the ventral embryonic cells. 9. The activated Toll protein initiates a cascade U)at phosphorylates the Cactus protein, which has been bound to Dorsal. Phosphorylated Cactus is deg rad ed, allowing Dorsal to enter the nucleus. Once in the nucleus, Dorsal activates the genes responsible for The gap gene proteins activate and repress the pair rule genes. The pa ir-ru le genes have modular pro regulating individual ceil lates. 5. ' interact with each other such tht each gap gene pro Individual cell fa tes are not delined immediately.
Rather, there is a stepwise specification wherein a The unsegmented anterior a nd posterior extremities 13. The gap genes respond to concentrations of the patterning the embryo. • are regula ted by the activation of the Torso protein at the anterior and posterior p oles 01 the egg.
set of genes.
early embryo, and localized mRNAs are criti cal in genes responsible lor the anterior portion 01 the fly; The Bicoid and Hunchback proteins activate the terior axis, each one comprising two parasegments. 15. The pai r-rule gene products activate engrailed and willgless expression in adjacent cells. The ellgrailed expressing cells form the anterior boundary of each parasegment. These cells lorm a signaling center that organi zes the cuticle formation and segmental struc ture of the embryo. 16. The homeotic selector genes are found in two com plexes On chromosome 3 of Drosophila.
Toge ther, these regions are called Hom-C, the homeotic gene complex. The genes are arranged in the same order as their transcriptional expression . The Hom-C genes s pecify the individual segments, and muta tions in these genes are capable of transforming one segment into another.
17. The exp ression 01 each homeotic selector gene is reg ulated by the gap and pair-rule genes. Their expres sion is refined and maintained by interactions 01 neighboring Hom-C genes. whereby their protein products prevent the tran scription lB. The targets o f the Hom-C pro tein s are the realisator genes, These realisator genes are responsible for con structing the specific structure. 19. Organs lorm at the intersection 01 dorsal-ventral and anterior-pos terior regions of gene expression.
T H E GENETICS OF AXIS SPEC I F I CATION IN DROSOPHILA 239 For Further Reading bibliogra phical citations for all literature cited in this chapter can be found at the free-access website www.devbio.com Complete Driever, W. and C. Ni.isslein-Volhard. 1988a. The Bicoid protein determines position in the Drosophila embryo in a concen.tration-dependent marmer. Cell 54: 95-104. Emi-Sarker, G. L.
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Tools for neuroanatomy and neurogenehcs in Drosophila. Proc. Nat!. Acad. Sci. USA 105: 9715-9720. Go Online WEBSITE 6,1 Drosophila fe rti lization. Fertilization of Drosophila can only occur in the region of the oocyte that will become the anterior of the e mbryo. Moreover, the sperm tail appears to stay in this region. WEBSITE 6.2 The early d evelopmen t of other insects. Drosophila is a highly deriv ed species. There are other insect species that dev elo p in ways very different from the "standard " fruit fly. WEBSITE 6.3 Evidence for gradients in insect develop ment. The origina l· evidence for gradients in insect devel opment came from studies provjding evidence for two "o rganiza tion centers]J in the egg, one located anteriorly and one loca ted poste riorly. WEBSITE 6.4 Christiane Niisslein-Volhard and the molecular approach to development. The research that rev ol utioni zed deve lop ment al biology had to wait for someone to synthesi ze molecular biology, embryology, and Drosophila gene tics. WEBSITE 6,5 Asymmetrical spread of morphogens. I t is unlikely tha t morphogens such a s Wingless spread by free diffusion. The asymmetry of Wingless diffusion sug gests that neig hboring cells p lay a crucial role in moving this protein . WEBSITE 6.6 G e tting a head i n the fl y. The segment pola rity genes may act differently in the head than in the trunk. Indeed, the formation of the Drosophila head may differ signific antl y from the way the rest of the bo dy is formed. Vade Mecum Drosophila development . The Vade Mecum sites have remarkab le time-lap se sequences of Drosophila develop ment, includ ing cleavage and gast rulati on. This segment also provides access to the fly life cyeie . The color coding superimposed on the germ lay ers allows you to readily understand tissue movements. Outside Sites "The Interactive Fly," compiled by Thomas Brody, provides an index to the maj or Drosophila websites worldwide. It is hosted by the SOCiety for Developmental Biology (SDB) a t . Two notable entries accessible through the si te are II A tlas of Fly De v el op me n t" by Voker Har tenstein (. sdbonline.orgifly/atias/OOatlas.htm) and "S tages in Fly Development: The M ovies" (fly/aimainl2stages.hlm).
Am phibians and Fish Early Development and Axis Formation AMPHIBIAN EMBRYOS ONCE DOMINATED the field of experimental embryolo gy.
With their large cells and rapid development, salamander and frog embryos were excellently suited for transplantation experiments. However, amphibian embryos feU out of favor during the early days of developmental genetics. Frogs and salamanders undergo a long period of growth before they become fertile, and their chromosomes are often found in several copies, precluding easy muta genesis.* But new molecular techniques such as in situ hybridization, antisense oligonucleotides, chromatin immunoprecipitatiolI, and dominant negative pro teins have allowed researchers to return to the study of amphibian embryos, and to integrate recent molecular analyses of development with earlier experimen tal findings.
The results have been spectacular, revealing new vistas of how ver Dania retia, commonly known as the tebrate bodies are patterned and structured. Also in recent years, the teleost fish zebra fish, has become a model organism for those who study vertebrate devel opment. Zebrafish have large broods, breed all year, are easily maintained, have transparent embryos that develop outside the mother In addition, these fish develop rapidly. At 24 hours after fertilization, the embryo has already formed most of its organ primordia and displays a characteristic tadpole-like form. In addition, the ability to rnicroin ject fJuorescent dyes into individual cells has allowed scientists to follow indi vidual Hving cells as an organ develops. Therefore, much of the description of fish development later in this chapter is based on studies of Dania.
The analysis of zebra fish rnutants has confirrned many of the principles of development originally elucidated in amphibian embryos, and has shown that many of the same molecules are 1:lsed.
Together, the Xenopus and zebrafish research communities have established the general rules of vertebrate develop ment, which (as we will see in Chapter 8) are maintained among the amniote vertebrates (reptiles, birds, and mammals).
We start here with the amphibians, because it was these organisms that provided us with the "backbone" of ques tions and tentative conclusions that have enabled vertebrate developmental biol ogy to flourish. "' In the 19605, Xell0pus {acvis replaced Rana frogs and the salamanders because it could be to mate throughout the year. Unfortunately, Xenopus 'aevis has four copies of each chromosome rather than the more usual two, and it takes 1-2 years to reach sexual maturity. Another Xenopus species, X. tropicalis, is often studied now, since i t has all the advantages of X. lnevis, plus it is diploid and reaches sexual maturit y in a mere 6 months (Gurdon and Hopwood 2000; Hirsch et a1. 2(02). induced We are standing and walking with parts of our body which could hafle been used for thin.hing had they devel oped in another part of the embryo. HANS SPEMANN (1 943) Theories come and theo" ies go. The frog remains. JEAN ROS1AND (1 960) 242 CHAPTER 7 EARLY AMPHIBIAN DEVELOPMENT Fertilization, Cortical Rotation, and Cleavage Fertilization can occur anywhere in the animal hemisphere of the amphibian embryo. The point of sperm entry is important because it determines the orientation of the dor sal-ventral axis of the larva (tadpole). The point of sperm entry will mark the ventral side of the embryo, while the site 180 degrees opposite the point of sperm entry will mark the dorsal side.' *The axis between the point of sperm entry and the dorsal side approximates, but does not exactly correspond to, the actual ven tral-dorsal axis of the amphibian larva (Lane and Smith 1999; Lane and Sheets 2002). However. as the literature in the field has tradi tionally equated these two axes, we will use the c1assica1 terminolo gy here. (A) The sperm centriole organizes the microtubules of the egg and causes them to arrange themselves in a parallel array in the vegetal cytoplasm, separating the cortical cyto plasm from the yolky internal cytoplasm (Figure 7.1A,B). These microtubular tracks allow the cortical cytoplasm to rotate with respect to the inner cytoplasm. Indeed, the arrays are first seen immediately before rotation starts, and they disappear when rotation ceases (Elinson and Rown ing 1988; Houliston and EUnson 1991). In the I-cell embryo, the cortical cytoplasm rotates 30 degrees with respect to the internal cytoplasm (F igure 7.1 C). In some eggs, this exposes a band of inner gray cytoplasm in the marginal region of the I-cell embryo, directly opposite the sperm entry point (Figure 7.1 D; Raux 1887; Ancel and Vintenberg er 1948). This region, the gray crescent, is where gastrula tion will begin. Even n i Xenopus eggs, which do not expose a gray crescent, corneal rotation occurs and cytoplasmic movements can be seen (Manes and Elinson 1980; Vincent et al. 1986). As we will see later, the site opposite the point 0.50 (8) 0.70 FIGURE 7.1 Reorganization of the cyto plasm and cortical rotation produ ce the gray crescent in frog eggs.
(A,B) Parallel arrays of microtubules (visualized here using fluorescent antibodies to tubulin) form i n the vegetal hemisphere of the egg, a long the future dorsal-ventral axis. (A) With 50% of the first cell cyc le complete. microtubufes are present, but they lack polarity. (B) By 70% completion of the cycle, the vegetal shear zone is character· ized by a pa ra l lel array of micro tubules; cortical rotation begins al this time. At the end of rotation, the microtubules will depolymerize. (C) Schematic cross section of cortical rotation. At left, the egg is shown about midway through the first cell It has radial symmetry around the animal-vegeta l axis. The sperm nucleus has entered at one side and is migrating inward. At right, 80% into first cleavage, the cortical cytoplasm has rotated 30 degrees relative to the internal cytoplasm. Gastrulation will begin in the gray cres· cent-the region opposite the point of sperm entry, where the greatest displace ment of cytoplasm occurs. (D) Gray cres cent of Rana pipiens. Immediately after cortical rotation (left), lighter gray pigmen tation is exposed beneath the heavily p ig mented cortica l cytoplasm . The first cleav· age furrow (right) bisects this gray crescent. (A,B from Cha and Gard 1 999, courtesy of the authors; C after Gerhart et al.
1 989; 0 courtesy of R. P. El i n son) cycle. · · . (C) (" g r a y ") p a g e sperm ..... A; ro Clear Panigmaelnrtegdion Vegetal Diffuse bl ack i m nt Pomt of entry Sperm centriole Pigmented cortic l cytoplasm R ! Mlcro tubules Inner . cytoplasm Shear zone cortical cytoplasm (D) region AMPHIBIANS AND FISH (A) 1 I1 (E) II (H) (e) (D) (F) (G) (H) l a V V of sperm entry will be the place where gastrulation begins, and the microtubular array will become extremely impor tant in initiating the dorsal-ventral and anterior-posterior axes of the larva. Unequal radial holoblastic cleavage Cleavage in most frog and salamander embryos is radial ly symmettical and holoblastic, just like echinoderm cleav age. The amphibian egg, however, contains much more yolk. This yolk, which is concentrated in the vegetal hemi sphere, is an impediment to cleavage. Thus, the first divi sion begins at the animal pole and slowly extends down into the vegetal region (Figure 7.2; see also Figure 1.2E). In the axolotl salamander, the cleavage furrow extends through the animal hemisphere at a rate close to 1 mm pe" minute, slowing down to a mere 0.02-0.03 mm per minute as it approaches the vegetal pole (Hara 1977). In many species (especially salamanders and frogs of the genus Rnna), the first cleavage division bisects the gray crescent. At the frog egg's first cleavage, one can see the differ ence in the furrow between the animal and the vegetal 243 FIGURE 7.2 Cleavage of a frog egg.
Cleavage furrows, designated by Roman numerals, are numbered in order of appearance.
(A,B) Because the vegetal yolk impedes cleavage, the second division begins in the animal region of I he egg before Ihe firsl divi sion has divided Ihe vegelal cyto plasm. (Cnhe Ihird division is dis placed loward Ihe animal pol e. (D-H) The vegelal hemisphere ullimalely contains larger and fewer blastomeres Ihan Ihe animal half. (H) Cross section Ih rough a mid-blaslula stage embryo. (Afler Carlson 1 98 1 .) hemispheres (Figure 7.3A). While the cleavage furrow is still cleaving the yolky cytoplasm of the vegetal hemi sphere, the second cleavage nas already started near the animal pole. This cleavage is at right angles to the first one and is also meridional (Figure 7.3B). The third cleavage is equatorial. However, because of the vegetally placed yolk, the tlUrd cleavage furrow is not actually at the equator but is displaced toward the animal pole (Valles et al. 2002). It divides the amphibian embryo into four small animal blas tomeres (micromeres) and four large blastomere, (macromeres) in the vegetal region (Figure 7.3C). Despite their unequal sizes, the blastomeres contin ue to divide at the same rate until the tweUth cell cycle (with only a small delay of the vegetal cleavages). As cleavage progresses, the animal region becomes packed with numerous small ceils, while the vegetal region contains a relatively small num ber of large, yolk-laden macromeres (see Figures 7.2 and 1.21). Au amphibian embryo containing 16 to 64 cells is commonly called a morula (plural morulae; Latin, "mul berry," whose shape it vaguely resembles). At the 128-cell stage, the blastocoel becomes apparent, and the embryo is considered a blastula. FIGURE 7.3 Scanning electron micrographs of frog egg c l eavage. (A) Firsl c l eavage. (B) Second cleavage (4 cells) . (C) Fourth cleavage (1 6 ce lls), showing the size discrepancy between the CHAPTER 7 244 (A) (B) FIGURE 7.4 Depletion of EP-cadherin mRNA in the Xenopus oocyte results in the loss of adhesion between blstomeres and the obliteration of the blastocoel. (A) Control embryo. (8) EP-cadherin-depleted embryo. (From Heasman et al. 1 994b, courtesy of J. Heasman.) The amphibian blastocoel serves two major functions. First, it permits cell migration during gastrula tion; and sec ond, i t prevents the cells beneath it from interacting pre Drosopllila) the time of this transition can be changed exper imentally by altering the ratio of chromatin to cytoplasm in the cell (Newport and Kirschner 1982a,b). maturely with the cells above it. When Nieuwkoop (1973) Some of the events that trigger the mid-blastula transi in the animal hemisphere (a region often caJ!ed the animal tion involve chromatin modification. First, certain promot took embryOniC newt cells from the roof of the blastocoel ers are demethylated, allOWing transcription of these genes. Xenopus (unlike mammals) cap) and placed them next to the yolky vegetal cells from In the base of the blastocoel the animal cap cells differentiat DNA are ed into mesodermal tissue instead of ectoderm. Thus, the derived chromosomes. However, during the late blastula . , high levels of methylated seen in both the paternal ly and mate rnal ly blastocoel prevents the contact of the vegetal cells destined stages, there is a loss of methylation on the promoters of to become endoderm with those cells in the ectoderm fated genes that are activated at MBT. This demethylation is not to give rise to the skin and nerves. seen on promoters that are not activated at MBT, nor is it Numerous ceJ! adhesion molecules keep the cleaving observed in the coding regions of MBT-activated genes . blastomeres together One of the most important of these The methyla tion of lysine-4 on histone H3 (forming a is EP-cadherhl The mRNA for this protein is supplied in trimethylated lysine that is associated with active transcrip . . the oocyte cytoplasm: If this message is destroyed by anti tion) is also seen on the sense oligonucleotides so that no EP-cadherin is made, the MBT.
It appears, then, that modificatiOn of certain promot 5' ends of many genes during the adhesion between blastomeres is dramatically reduced, ers and their associated nucleosomes may play a pivotal 7.4; role in regulating the tinling of gene expression at the mid resulting in the obliteratiQn of the blastocoel (Figure Heasman et aJ. 1994a,b). blastula transi tion (Stancheva et a J . 2002; Akkers et aJ. 2009). It is thought that once the chromatin at the promoters The mid-blastula transition: Preparing has been remodeled, various transcription factors (such as for gastrulation the VegT protein, forme d in the vegetal cytoplasm from An impo rtant precondition for gastrulation is the activa localized maternal mRNA) bind to the promoters and ini tion of the genome. In the model organism tiate new transcription. For instance, the vege tal cells Xenopus lnwis be tran (under the direction of the VegT protein) become the endo sc ribed during ea rly cleavage. For the most part, nuclear derm and begin secre ting factors that induce the cells genes are not activated until late in the twelfth cell cycle above them to become the mesoderm (see Figure 7.23). (African clawed frog), only a few genes appear to (Newport and Kirschner 1982a,b; Yang et al.
2002). At that time, the embryo experiences a (MBT; see Chapters 5 and mi d b las tula transition See WEBSITE 7.1 Amphibian development movies - 6); different genes begin to be transcribed in different cells, the cell cycle acquires gap phases and the bJastomeres acquire the capacity to become , Amphibian Gastrulation motile. It is thought that some factor in the egg is being The study of amphibian gastrulation is both one of the old absorbed by the newly made chromatin becalJse (as in est and one of the newest areas of experimental embryolo- AMP H I B IANS AND FISH EEpiderxmteirsi_-,,or endoderm endoderm (B) I n t e r i o r Neural msd m (A) 245 plate Su pra blastoporal Lateral plate e o er \- ;! onnit." and notochord Blastopore Subblastoporal r m FIGURE 7.5 Fate maps of the Xenopus /aevis blastu la exterior (A) and i n terior (B). Most of lhe mesodermal derivatives are formed from the i n terior cells. The p lacement of ven t al mesoderm remains controversial (see Kumano and Smith 2002; Lane and Sheets 2006). (Aher lane and Smith New a n and Kreig 1 999.) 1 999; gy (see Beetschen 2001; Braukmann and GiJbert 2005).
Even though amphibian gastrulation has been studied extensive ly for the past century, most of our theories conceming its mechanisms have been revised over the past decade. The study of these developmental movements has been com plica ted by the fact that there is no single way amphibians ga strula te; d ifferen t sp eci es employ different means to achieve the same goaL In recent years, the most intensive inves tiga tions have focused on Xenopus laevis, so we will concentrate on the mechanisms of g astrulation in tha t species. Vegetal rotation and the invagina tion of the bottle cells Amphibian blastulae are faced with the same tasks as the invertebrate blastulae we followed in C hapters 5 and 6- namely, to bring inside the embryo til0se areas destined to form the endodermal organs; to surround tile embryo with capable of forming the ectoderm; and to place the mesodermal cells in the proper positions between them. Fa te mapping by Levtrup (1975; Landstrom and Levtrup 1979) and by Keller (1975, 1976) ha s shown that cells of Xenopus blastulae have different fates, depending on whether they are located in the deep or the superficial lay ers of the embryo (Figure 7.5) . In Xenopus, the mesodermal precursors exist mostly in the deep laye r of cells, while the cells ectoderm and endoderm arise from the superficial layer on the surface of the embryo. Most of the p recursor s for the notochord and other mesodermal tissues are located beneath the surfa ce in the equatorial (marginal) region of the embryo. ' Gastrulation movements in frog embryos act to pOSition the mesoderm between the outer ectoderm and the in:ner endoderm. These movements are initiated on the future dorsal side of the emb ryo, just below the equator, in the region of the gray crescent (Figure 7.6)-the region opposite the paint of sperm entry. Here the cells invaginate to form a slitli ke blastopore (Figure 7.7) . These cells change their The main body of each cell is displaced the i nsid e of th e embryo while maintaining COn tact w i th the outside surface by way of a slender neck. These bottle cells line the archenteron (prin1iti ve gut) as it forms, and as in the gastrulating sea urchin, an invagina tion of cells initiates formation of the archenteron. How ever, unlike sea urchins, gastrulation in the frog begins not in the most vegetal region but in the margi nal zone-the region surrounding the equator of the blastula, where the animal a nd vege tal hem ispheres meet (see Figu re 7.6B). Here the endodermal cells are not as large or as yolky as the most vegeta l blastomeres . Working with Xenopus, Ray Keller and his students showed that the peculiar shape cha nge of the bottle cells is needed to initiate gastrulation; it is the constriction of these cells that forms the bla stopore . However, once the subsurface marginal cells are brought into contact with the basal region of the surface blastomeres, they begin to invo l ute on the extracellular matrix secreted onto the basal region of these cells. \'Vhen such involution movements are underway, bottle cells are no longer essential. When bot tle cells are removed after their formation, involution and blastopore formation and closure continue (Keller 1981; Ha rd in and Keller 1988). Thus, in Xenopus, the maj or fac tor in the movement of cells into the embryo appears to be the involution of the subsurface cells rather than the super ficial margi nal cells_ But cell involution is not a passive event. At least 2 hours shape dramatically. toward r before the bottle cells form, internal cell rearrangements p opel the cells of the dorsal floor of the b lastocoel toward od in r *In urodeles (salamanders such as Triturus and Ambystoma) and in some frog species, many more of the notochord and mes erm pre cursor cells are found among the surface cells (Purcell and Keller 1993i et al. 2002).
Here, the superficial mesoderm cells move into the deeper layers by forming a primitive streak simila to that of amniote embryos (see Chapter For ways which the pattern ing of the mesoderm differs between anurans and urodeles, see Hurtado and De Robertis 2007. Sho k 8). CHAPTER 7 246 (A) Animal pole (AP) (B) (C) Dorsal mesoderm Dorsal blastopore lip Mesoderm Blastocoel displaced Endoderm Vegetal pole (E) (F) Mesenchyme blastopore lip E blaSiopore l ip blaSiopore tip ctoderm plug endomesoderm blastopore l ip . ;;(!:;,;,\ ▪ '. . . .
. " --'¡¡¡. . . . ;'::'. '." i'. '" - . , ', ' ". '. ;: FIGURE 7.6 Cell movements during frog gastrulation. The drawings show meridional sections are cut through the middle of the embryo a nd posit ioned so that the vegetal pole is tilted toward the observer and slightly to the left. The major cell movements are indicat· ed by arrows, and the superficial animal hemisphere cells are colored so that their move ments can be followed. Below the drawings are corresponding micrographs imaged with a surface imaging microscope (see Ewald et a t. 2002). (A,B) Early gastrulation. The bottle cells or the margin move inward 10 form the dorsal lip of the blastopore, and the mesoder mal precursors involute under the roof of the blastocoel. AP marks the position of the ani mal pole, which will change as gastrulation continues. (C,D) Mid-gastrulation. The archen· teron forms and displaces the blastocoel, and cells migrate from the lateral and ventral lips of the blastopore into the embryo. The cells of the animal hemisphere migrate down toward the vegetal region, moving the b lastopore to the region near the vegetal pole. (E,F) Toward the end of gastrulation, the blastocoel is obliterated, the embryo becomes sur· rounded by ectoderm, the endoderm has been internalized, and the mesodermal cells have been positioned be,een the ectoderm and endoderm. (Drawings aher Keller 1 9B6; micrographs courtesy of Andrew Ewald and Scott Fraser.) mesoderm AMPHIBIANS AND FISH 247 INVOLUTION AT THE BLASTOPORE LIP The next phase of gas trulation involves the involution of the marginal zone cells the whlle the animal cells undergo epiboly and converge at FIGURE 7.7 Su r face view of an early dorsal bl astopore lip of Xenopus. The size difference between the animal and vegetal blastomeres is readily apparent. (Courtesy of C. Phillips.) blastopore (see Figure 7.6C,D). When the migrating mar ginal ceUs reach (and become) the dorsal lip of the blasto pore, they tum inward and travel along the inner surface of the outer animal hemisphere cells (i. e., the blastocoel roof). 11,US, the cells constituting the lip of the blastopore are con stantly changing. The order of the march into the embryo was determined by the vegetal rotation that abutted the prospective pharyngeal endoderm against the inside of the anin,.1 cap tissue. The first cells to compose the dorsal blastopore lip and enter into the embryo are the prospec tive pharyngeal endoderm of the foregut (including the bottle ceUs). As these first cells pass into the interior of the embryo, the dorsal blastopore lip becomes composed of cells that involute into the embryo to become the pre chordal pl ate (the precursor of the head mesoderm) . The next cells involuting into the ·embryo through the dorsal the animal cap. This vegetal rotation plac es the prospec adj acent to the blastocoel and inunediately above the involut ing mesoderm. 111€se tive pharyngeal endoderm cells cells then migrate along the basal surface of the blastocoel roof, travel ing toward the future anterior of the embryo (F igure 7.8; Nieuw koop and Florschiitz 1950; Winklbauer and Schillfeld 1999; Ibrahim and Wmklbauer 2001). The superficial layer 01 marginal cells is pulled inward to form the endodermal Uning of the archenteron, merely because it is attached to the actively migrating deep cells. Although experimentally removing the bottle cells does not affect the involution of the deep or superficial marginal zone cells into the embryo, removal of the deep invol u tin g margin al zone (IMZ) cells stops archenteron formation. See VADE MECUM Amphibian development (A) Penhdaorydnegrmeal rm / (cerberus)   Chmeosrdaerm· meHeasod erm blastopore formation (Xbra) blastopore lip are the chordamesoderm cells. These cells will form the notochord, a transient mesodermal rod that plays an important role in inducing and patterning the nervous system.
Thus the cells constituting the dorsal blastopore lip are constantly changing as the original cells cells mi grat ing down, inward, and upward. As the new ceUs enter the embryo, the blastocoel i s dis placed to the side opposite the dorsal lip of the blastopore. migrate into the embryo and are replaced by Meanwhlle, the lip expands laterally and ventrally as the processes of bottle cell formation and involution continue arowld tile blastopore. The widening blastopore "crescen' develops lateral lips and finally a ventral lip over which additional mesodermal and endoderinal precursor cells pass.
With the formation of the ventral lip, the b las topore has formed a ring around the large endodermal cells that (C) (S) IMZ (goosecoid) FIGURE 7.8 Early movements of Xenopus gastrulation. Orange represents the prospecti ve pharyngeal endoderm (as described by cerberus expression). Dark orange represents the prospective heacl mesoderm (goosecoid expression), and the chordameso derm (Xbra expression) is red. (A) At the beginning of gastrulation, (he involuting marginal zone (IMZ) forms. (B) Vegetal rotation (D) c e l s Blastopore (arrows) pushes the prospective pharyngal endoderm to the side of the blastocoel. (CD) The vegeta l endoderm movements push the ph aryngeal endoderm forward, driving the mesoderm pass i ve ly into the embryo and toward the animal po e. The ectoderm (bl u e) begins epiboly. (After Winklbauer and Schurleld 1 999.) l 248 CHAPTER 7 (B) Dorsal lip Ventral lip FIGURE 7.9 Epiboly of the ectoderm . (A) Changes in the region around the blastopore as the dorsal, lateral, and ventral lips are formed i n succession. When the ventral lip completes the circle, the endcxlerm becomes progressively i nternalized. Numbers ii-v correspond to Figure 7.6 B-E, respect ively. (B) Summary of ep i boly of the ectoderm and involution of the mesodermal cells migrating into the blastopore and then under the surface. The endoderm beneath the blastopore lip (the yolk plug) is not mobile and is enclosed by these movements. (A from Balinsky 1 975, cou rtesy of B.
I. Balinsky.) remain exposed on the vegetal surface (Figure 7.9).
This remaining patch of endoderm is called the yol k plug; it, too, is eventually internalized. At that point, all the endo dermal precursors have been brought into the interior of the embryo, the ectoderm has encircled the surface, and the mesoderm has been brought between them . CONVERGENT EXTENSION OF THE DORSAL MESODERM invo-lution begins dorsally, led by the pharyngeal endoderm and the head mesoderm. These tissues will migrate most anteriorly beneath the surface ectoderrn. * Meanwhile, as the lip of the blastopore expands to have dorsolateral, lat eral, and ventral sides, the prospective heart, kidney, and . ventral mesodermal precursor ceUs enter iI1to the embryo. Figure 7.10 depicts the behavior of the involuting mar ginal zone cells at successive stages of Xenopus gastrula tion (Keller and SchoenwoU 1977; Hardin and Keller 1988). The IMZ is originally several layers thick. Shortl y before their involution through the blastopore lip, the several lay ers of deep IMZ cells intercalate radially to form one thin, broad layer.
This intercalation further extends tile IMZ veg- *The pharyngeal endoderm and hcad mesoderm cannot be sepa ra t ed experimentally at this stage, so they are somehmes referred to collectively as the head endomesoderm. The notochord is the basic unit of the dorsal mesoderm, but it is thought that the dorsal por tion of the somites may have similar properties to the notochord. Blastopore lip etally (Figure 7.10A). At the same time, the superficial cells spread out by dividing and flattening. When the deep cells reach the blastopore lip, they involute into the embryo and initiate a second type of intercalation. This intercalation causes a convergent extension along the mediolateral axis that integrates several mesodermal streams to form a long, narrow band (Figure 7.1 OB). This movement is reminiscent of traffic on a highway when several lanes must merge to form a shngle lane (or for that matter, the cell movements of the sea urchin archenteron). The anterior part of this band migrates toward the animal cap. Thus, the mesoder mal streanl continues to migrate toward the animal pole, and the overlying layer of superficial cells (including the bottle cells) is p a ssively pulled toward the animal pole, thereby forming the endoderma] roof of tile archenteron (see Figures 7.6 and 7.lDA). The radial and mediolateral intercalations of the deep layer of cells appear to be respon sible for the continued movement of mesoderm into the embryo. Several forces appear to drive convergent extension. The first force is a polarized cell cohesion, wherein the involu t ed mesodermal cells send out protrusions to contact one another. These "reachings out" are not random, but occur toward the midline of the embryo and require an extracel lular fibronectin matrix (Goto et al.
2005; Davidson et a1. 2006). The second force is differential cell cohesion. During gastrulation, the genes encoding adhesion proteins parax ial p rotoc adh erin and axial p ro toca dh erin become expressed specifically in the paraxial (somite-forming) mesoderm and the notochord, respectively (Figure 7. 1 1). An

Their prospective fate was determined, and the cells developed independently of their new embryonic location.
Specifical ly, prospective neural cells now developed into brain tis sue even when placed in the region of prospective epider mis (Figure 7.16B), and prospective epidermis formed skin even in the region of the prospective neural tube. Within the time separating early and late gastrulation, bhe poten cies of these groups of cells had become restricted to their eventual paths of differentiation. Something was causing Results of tissue transplantation during early- and late-gastrula stages in t e newt Host region Differentiation of donor tissue Conclusion Prospective neurons Prospective epidermis P ospective epidermis Prospective neurons Epidermis Neurons Conditional development Conditional development LATE GASTRULA Prospective neurons Prospective epidermis Neurons Prospective epidermis Prospective neurons Epidermis Autonomous development (detenmined) Autonomous development (dete mined) Donor region EARLY GASTRULA r r AMP H I BIANS AND F I S H tissue was transplanted into the presumptive belly skin (A) Transplantation in early gastrula Presumptive neural ectoderm region of another gastrula, it not only continued to be dor sal blastopore lip but also initiated gastrulati on and Presumptive embryogenesis in the surrounding tissue! Neural plate E p idermis forms (B) Transplantation in late gastrula Presumptive neural ectoderm 255 In these experiments, Spemann and Mangold once Triturus taeniatus and T.
cristatus so they could identify host and again used the differently pigmented embryos of donor tissues on the basis of color. When the dorsal lip of an early T. taeniatus gastrula was removed and impl;mted into the region of an early T. cristatus gastrula fated to become ventral epidemus (belly skin), the dorsal lip tissue invaginated just as it would normally have done (show ing self-determination) and disappeared beneath the veg etal cells (Figure 7.1 7A). The pigmented donor tissue then continued to self-differentiate into the chordamesoderm (notochord) and other mesodermal structUres that nomlal Presumptive Iy form from the dorsal lip (Figure 7.1 7B). As the donor to participate in the production of a new embryo, becoming derived mesodermal cells moved fOlOWard, host cells began organs that normally they never would have formed. In this secondary embryo, a somite could be seen containing ��l!§- both pigmented (donor) and unpigmented (host) tissue. Even more spectacularly, the dorsal lip cells were able to plate tissue forms FIGURE 7.16 Determination of ectoderm d ur ing newt gastrula tion. P resum ptive neural ectoderm from on e n ewt embryo is t ran spl anted into a region in another embryo that normally becomes epiderm i s. (A) When the tissues are transferred between early gastrul ae, the presumptive neural tissue develops into epi dermis, and only one neural plate is seen. (8) When the same experiment is performed using late-gastrula tissues, the presump tive neural cells form neural tissue, thereby causing two neural p lates to form on the host. (After Saxen and Toivonen 1 962.) them to become committed to epidermal and neUIaI fa tes. What was happening? interact with the host tissues to form a complete neural plate from host ectoderm. Eventually, a secondary embryo formed, conjoined face to face with its host (Figure 7.17C). The results of these technically difficult experiments have species, including Xenopus (Figure been confirmed many times and in many amphibian 7.1 70; Capuron 1968; 1985). Smith and Slack 1983; Recanzone and Harris See WEBSITE 7.3 Spernann, Mangold, and the organizer SpemarUl referred to the dorsal lip cells and their deriv atives (notochord and head endome50derm) as the organ izer because (1) they induced the host's ventral tissues to change their fates to form a neural tube and dorsal meso dermal tissue (such as somites), and (2) they organized host and donor tissues into a secondary embryo with clear ante during normal development, these cells " organi ze " the rior-posterior and dorsal-ventral axes. He proposed that Hans Spemann and Hilde Mangold: Primary embryonic induction dorsal ectoderm into a neural tube and transform the flank The most spectacular transplantation experiments were mann published by Spemann and Hilde Mangold in 1924.* They and his students) that the interaction of the chordameso showed that, of all the tissues in the early gastrula, only derm and ectoderm is not sufficient to organize the entire one has its fate autonomously determined. This self-deter events. Because there are numerous inductions during mining tissue is the dorsal lip of the blastopore-the tis sue derived from the gray crescent cytoplasm. When this ing mesoderm into the anterior-posterior· body axis (Spe 1938). It is now known (thanks largely to Spemann embryo. Rather, it initiates a series of sequential inductive embryonk development, this key induction-in which the progeny of dorsal lip cells induce the dorsal axis and the Hilde Proescholdt Mangold died in a tragic accident in 1924, when her kitchen's gasoline heater exploded. She was 26 years old, and ner paper was just being published.
Hers is one of the very few doc· toral theses in biology that have directly resulted in the awarding of .3 Nobel Prize. For more information about Hilde Mangold, her times, and the experiments that identified the organizer, see Ham burger 1984, 1988, and Fassler and Sander 1996. neural tube-is traditionally called the primary embryon ic induction.t tnus classical term has been a source of confusion because the induction of the neural tube by the notochord is no longer consid ered the first inductive process in the embryo. We vvill soon discuss inductive events that precede this "primary" induction. 256 CHAPTER 7 i (A) J noeo• umop e .. ---I., Pr s t iv t ch rd Presumptive Dobiplartsoapl ore ePnredsoondmeirptmeisve SNeomuriatel tube Notochord invagination Pinrvimagainryation (8) f invagination Induced secondary structures Primary structures FIGURE 7.17 Organization of a secondary axis by dorsal blastopore lip tissue.
Spe mann and Mangold's 1 924 experiments visual zed the process by usi ng differently pigmented newt em bryos. (A) Dorsal lip tissue from an early T. taenialUs gastrula is transplanted into a T. crista/us gastrula in the regi on that normally becomes ventral epidermis. (B) The donor tissue invaginates and forms a second archenteron and then a second embryonic axis. 80th donor and host ti ssues are seen in the new neural tube, notochord, and so i tcs. Ie) Eventually, a second embryo forms joined to the hosl. (D) Live twinned Xenopus larvae generated by transplanting a dorsal blastopore lip into the ventral region of an early-gastrula host embryo. (E) Similar twinned larvae are seen from below and stained for notochord; the original and sec ondary notochords can be seen. (A--C aker Hamburger 1 988; D,E p hotographs by Wills, courtesy of R. Harland.) (A-Ci , m A. Lumen of gut (C) (D) ,....-----. tube (E) Molecular Mechanisms of Amphibian Axis Formation The experiments of Spemann and Mangold showed that the dorsal lip of the blastopore, along with the dorsal meso derm and pharyngeal endoderm that form from it, consti tuted an "organizer" able to instruct the formation of embryonic axes. But the mechanisms by which the organ izer itself was constructed and through which it operated remained a mystery. Indeed, it is said that Spemann and Mangold's landmark paper posed more questions than it answered. Among those questions were: • How did the organizer get i ts prope rties? What caused the dorsal blastopore lip to differ from any other region of the embryo? • What factors were being secreted from the organizer to • How did the different parts of the neural tube become cause the formation of the neural tube and to create the anterior-posterior, dorsal-ventral, and left-right axes? established, with the most anterior becoming the senso ry organs and forebrain, and the most posterior becom ing spinal cord? Spemann and Mangold's description of the organizer was the starting point of one of the first truly internation al scientific research programs: the search for the organiz er molecules. Researchers from Britain, Germany, France, the United States, Belgium, Finland, Japan, aJ1d the former Soviet Union all tried to find these remarkable substances (see Gilbert and Saxen 1993). R. G. Harrison referred to the amphibian gastrula as the "new Yukon to which eager min ers were now rushing to dig for gold around the blasto- AMPH I B IANS A N D FISH pore" (see Twitty 1966, p. 39). Unfortunately, their early p i cks and shovels proved too blunt to uncover the mole cules involved. The proteins responsible for induction were present in concentrations too small for biochemical analy ses, and the large quantity of yolk and lipids in the amphibian egg further interfered with protein purification (Grunz 1997). The analysis of organizer molecules had to wait until recombinant DNA technologies enabled owes tigators to make cDNA clones from blastopore lip mRNA and to see which of these clones encoded factors that could dorsalize the embryo. Today, however, we are able to take up each of the above four questions in turn. How does the organizer form?
Why are the dozen or so initial cells of the organizer posi tioned opposite the point of sperm entry, and what deter mines their fate so early? Recent evidence provides an unexpected answer: these cells are in the right place at the right time, at a point where two Signals converge. The first signal tells the cells that they are dorsal. The second signal says that they are mesoderm. See WEBSITE 7.4 The molecular biology of organizer formation THE DORSAL SIGNAL: -CATENIN It turns out that one of the reasons the organizer cells are spedal is that the&:! meso dermal cells reside above a special group of vegetal cells. One of the major clues in determining how the dorsal blastopore lip obtained its properties came from the exper hnents of Pieter Nieuwkoop (1969, 1973, 1977) and Osamu Nakamura. These studies showed that the organizer receives its properties from the ectoderm beneath it. Nakamura and Takasaki (1970) showed that the meso derm arises from the marginal (equatorial) cells at Ule bor der between the animal and vegetal poles.
The Nakamura and Nieuwkoop laboratories then demonstrated that the properties of this newly formed mesoderm were induced by the vegetal (presumptive endoderm) cells underlying them. Nieuwkoop removed the equatorial cells (i.e., pre sumptive mesoderm) from a blastula and showed that nei ther the animal cap (presumptive ectoderm) nor the vege tal cap (prestunptive endoderm) produced any mesodermal tissue.
However, when the two caps were recombined, the animal cap cells were induced to form mesodermal struc tures such as notochord, muscles, kidney cells, and blood cells (F i g u re 7.1 8). The polarity of this induction (i.e., "...,hether the animal cells formed dorsal mesoderm or ven tral mesoderm) depended on whether the endodermal (vegetal) fragment was taken from the dorsal or the ventral side: ventral and lateral vegetal cells (those closer to the side of sperm entry) induced ventral (mesenchyme, blood) and intennediate (kidney) mesoderm, while the dorsalinost veg etal cells specified dorsal mesoderm components (somites, notochord)-mcluding those having the properties of the organizer. These dorsalmost vegetal cells of the blastula, 257 which are capable of ind ucing the organizer, have been called the Nieuwkoop center (Gerhart et a!. 1989). The Nieuwkoop center was demonstrated in the Xeno pus embryo by transplantation and recombination exper iments. First, Gimlich and Gerhart (Gimlich and Gerhart 1984; Ginuicl1 1985, 1986) performed an experhnent anal ogous to the Spemann and Mangold studies, except that U,ey used early Xenopus blastulae rather than newt gastru lae. When they transplanted the dorsalinost vegetal blas- (A) ----:- - Animal od m Mac(ceaqlpsurcgaeitnoasrlial) Mesoderm Vecelgsetal Anectomadermca)pis(pcroensvuemrtpedtivteo byfactors fromvegetal cel s .. 1 Ect , -- __ --- ---..., (n) i er I 1 1 1 Endoderm1 l 1Mesoderm1 released (e) Dosignrsasl mesoderm-inducing Mesoderm-inducing signals i NieubywkNo p center r u r n li m t n n rl i l ll t l t . . . :.: . ' . - : Summary of exper men ts ieuwkoop an d by Nakamura and Takasaki, showing mes0dermal i nduction by vege tal end ode m . (A) I sol ated animal cap cells become a mass of cili ated ectoderm, isolated eq ato ia l (margi a l zone) cells become mesoderm, and isolated vegetal cells generate gut ke tissue. IB) If animal cap cells are combined with vegetal cap cells, many of the animal cells generate mesodermal tissue. IC) Simplified odel for mesoderm induction i n Xenopus. A ventral signal (probably a com p lex set of signals from aClivin·like TGF-p fac ors and FGFs) is released throughout Ihe vegetal region of the embryo. This si g a l i nd uces the marginal cells to become mesoderm. On the dorsal si de (away from the point of sperm entry), a signal is released by Ihe vegetal cells of the Nieuwkoop center.
This dorsal signal induces the formation of the Spema organizer in the ove y ng marg ina zone cells. The possible i denti ty of this signal will be dis cussed later in this chapter. Ie after De Robertis et al. '1992.) FIGURE 7.18 CHAPTER 7 258 (Al NORMAL DONOR \ (E l NORMAL RECIPIENT Point of sperm en tly j (j)CD t ':i':;" lJ-+" Vegetal pole New gastrulation site and body axis form 2 FIGURE 7.19 Transplantation and recombination experiments on Xcnopu-, embryos demonstrate that the vegetal cells underly ing the prospective dorsal blastopore lip region are responsible for initiating gastrul atio n .
(0) p-Catenin dorsal localization persists through the gastrula stage.
(A,D courtesy of R. T. Moon; B,C from Schneider ct al. 1 996, courtesy of P. Hausen.) (D) ing and straightening the microtubular array and in ma in t aining the direction of transport when the kinesin com plexes occasionally jump the track (Weaver and Kimelman 2004). Once at the site opposite the point of sperm entry, GBP and Dsh are released from the microtubles. Here, on the future dorsal side of the embryo, they inactivate GSK3, allowing p-catenin to accumulate on the dorsal side while ventral Jl-catenin is degraded (Figure 7.210, E). site sperm entry? The answer appears to reside in the But the mere translocation of these proteins to the dor translocation of Wntll and the Disheveled (Dsh) protein sal side of the embryo does not seem to be sufficient for pra from the vege tal pole to the dorsal side of the egg during tecting p-catenin. It appears that a Wnt paracrine factor has From research done on the Wnt pathway, we to be secreted there to activate the Jl-catenin protection path fertilization. have learned that p-catenin is targeted for destruction by way; this is a ccomplished by Wnt11 (see Figure 7.21). If glycogen synthase kinase 3 (GSK3; see Chapter 3). Indeed, activated GSK3 destroys p-catenin and b locks axis forma tion when added to the egg, and if endogenous GSK3 is Wntll synthesis is suppressed (by the injection of antisense ventral ceUs of the early embryo, a .second axis forms (see -igure 7.21F; He et al. 1995; P ierce and Kimelman 1995; vegetal cortex during oogene sis and is tho ught to be translocated to the future dorsal portion of the embryo by Yo st et al. lated into a protein that becomes cOncentrated knocked out by a dominant nega tive form of GSK3 in the 1996). GSK3-binding p ro tein CBP) and Disheveled. These two proteins release GSK3 ITom the degradation complex and prevent it from bindg p-catenin and target ing it for destruction. During the iirst cell cyde, when the microtubules form p arallel tracts In the vegetal portion of the egg, GBP travels alon g the GSK3 can be inactivated by the :nicrotubules by binding to kinesin, an ATPase motor pro :?in that travels on microtubules. Kinesin always migrates Wntll oligonucleotides into the oocyte s), the organizer fails to form (Tao et al. 2005).
Wntll mRNA is localized to the the cortical rotation of the egg cytoplasm. Here it is trans ed on the dorsal side of the embryo (Ku and Mel ton in and secret 1 993; Schroeder et al. 1999; White and Heasman 2008). Thus, during first cleavage, GBP, Dsh, and Wntll are brought into the future dorsal section of the embryo. Here, GBP and Dsh can initiate the inactivation of GSK3 and the consequent protection of p-catenin. The signal from Wntll stabilizes GBP and Dsh and organizes them to protect catenin. p The p-catenin tra nscrip tion factor can associate ; ward the growing end of the microtubules, and in this with other transcription factors to give them new proper ;-ase, that means moving to the point opposite sperm entry, _e., the future dorsal side (Figure 7.21A-C) . Disheveled, ties. 1t is known that Xenopus p-catenin can combine with a -hich is originally found in the vegetal pole cortex, grabs onto the CPB, and it too becomes translocated a l ong the ;n icrotubular monorail (Miller et al. 1999; Wea ver et a1.)J()3) .
The cortical rota tion is probably important in orient- ubiq uitous transcription factor known as Td3, converting the Tcf3 repressor into an activator of transcrip tion. Expres sion of a mutant form of Tcf3 (one that lacks U,e p-catenin binding doma in) results in embryos without dorsal stmc tures (Molenaar et a1. 1996). CHAPTER 7 260 (A) (F) Fertil zation Foansmit rcarnostpuobrutles Sclorwtictrlarnostpaotriotn Icnytoeprlasm i\ j GBP\Sh Wn\ t by DisheveUed protein (Dsh) o (8) C rtica1 rotation v ...
11 mRNA (C) Doof Dsrsahl eanrdicGBhmPent v FIGURE 7.21 Model of the mechanism by which the Dish eveled protein sta bilizes p-catenin in the dorsal portion of the amphibian egg. (A) Disheveled (Dsh) and GBP associate with kinesin at the vegetal pole of the unfertilized egg. Wnt1 1 is also in vesicles at the vegetal portion of the egg. (B) After fertilization hese vegetal vesicles are translocated dorsally along subcortical microtubule tracks. Cortical rotation adds a "slow" form of transportation to the fast-track microtubu le ride. (e) Wnt1 1 , Osh, and GBP a e then released from the micro tubules an are distributed in the future dorsal third of the 1 00eil embryo. (D) Dsh and GBP bind to and block the action of GSK3, thereby preventing the degradation p-catenin on the dorsal side of the embryo. Wnt1 1 probably is needed to stabilize this eaction keeping an active source of Dsh. (E) The nuclei of the blastomeres in the dorsal region of the embryo receive j3 caten in while the nuclei of those in the ventral region do not. (F) Formation of a second dorsal axis caused by the injection of both blastomeres of a 2-cell Xenopus embryo with dominant inactive GSK3. Dorsal fate is actively suppressed by wild-type CSK3_ (A-E aher Weaver and Kimelman 2004; F from Pierce and Kimelman 1 995, courtesy of D. Kimelman.) D t (D) oDof GSrsaKl3inhibit on D - /')(st-acbaltenin dpecgartedneidn "-"- (E) Doo(prs-acl etnrinchment D in vepn-ctratleniundei in dorsal nuclei v v No d of r r , , - . The -catenin/Td3 complex appears to bind to the pro moters of several genes whose activity is critical for axis formation. Two of these genes twin and siartlois, encode homeodomain transcription factors and are expressed in the organizer region immediately following the mid-blas tula transition. If these genes are ectopically expressed in the ventral cells, a secondary axis emerges on the former ventral side of the embryo; and if cortical microtubular polymerization is prevented, siamais expression is elimi nated (Lemaire et al. 1995; Brannon and Kimelm an 1996). The Tcf3 protein is thought to inhibit siamais and twin tran scription when it binds to those genes' promoters in the absence of -catenin.
However, when p-catenin binds to Tcf3, the repressor is converted into an activator, and twin and siamais are activated (Figure 7.22). AMP H I B IANS AND FISH 261 FIGURE 7.22 Tcprof3f\: siamois, twin Summary of events hypothesized to bring about induction of the organizer in the dorsal mesoderm. Microtubules a llow the translocation of Disheveled and Wntl 1 proteins to the dorsal side of the embryo. Dsh (from the vegetal cortex and newly made by Wntl l) binds GSK3, thereby al lowing D-catenin to accu mulate in the future dorsal portion of the embryo. During c leav age, -catenin enters the nuclei and bi nds with Tef3 to form a transcription factor that activates genes en cod i ng protei n s such as Siamois and Twin. Siamois and Twin interact in the organizer with Smad 2/4 transcription factors activated by vegetal TGF-D family members (Nodal-related proteins, Vgl , activin, etc.). Together, these two sets of trans cri ption factors adivate the "organizer" genes such as chordin, noggin, and goosecoid The presence of theVegT transcription factor in the endoderm prevents the organ izer genes from being expressed outside the organizer area. (After Moon and Kimelman 1 998.) -catenin . Siamols and twin proteins Transcription Organizer gene /Or g a n i z e r p r o t e i n Transcription I Smad2/4 from TGF-D family paracrine factors (chordin, noggin, goosecoid, etc.) tIC? b \ (chordin, noggin, goosecoid, etc.) 51amois and Twin bind to the enhancers of several genes involved in organizer function (Fan and Sokol 1997; Kessler transcription fac for the ability of the vegetal cells to induce the cells above them to be mesodermal. One of Goosecoid and Xlim1 (which appear to be critical in these encodes Vg1, a member of the TGF-D superfamily 1997). These include genes encoding the tors Two maternal RNAs tethered to the vegetal cortex appear to be crucial 7.23A). specilying the dorsal mesoderm) and the paracrine factor (Figu re antagonists Noggin, Chorclin, Frzb, and Cerberus (Laurent whose Vg1 has been depleted lack et a!. 1997; Engleka and Kessler 2001). VegT appears to Vg1 is critically important, since embryos organizer gene expres sion and also lack notochords (Birsoy et al. 2006). The other inhib i t the expression of these genes in the vegetal cells vegetally tethered mRNA encodes VegT, a transcription (Brannon et a!. 1997; Ishibashi et a!. 2008). Thus one could factor that instructs the endoderm to synthesize and secrete expect that if the dorsal side of the embryo contained D TGF- family members activin, Derriere, and several Nodal eatenin, then -catenin would allow this region to express proteins 01 the organizer. (Latinkic et al. 1997; Smith 2001).
These proteins Twin and Siamois proteins, and these proteins would ini have overlapping functions. Each of them can activate the liate formation Xbra (Brachyury) gene encoding a transcription factor that THE VEGETAL TGF-p-liKE SIGNAL Yet another transcription instructs the cells to become mesoderm. Derriere can induce animal cap cells to become mesoderm over the This other factor, Smad2/4, long-range distances predicted by Nieuwkoop's experi is induced in the dorsal mesoderm cells by TGF- family types of mesoderm at different concentrations.
At moder paracrine lactors secreted by the vegetal cells beneath them ate concentrations, activin activates the Xbra gene, while factor also appears to be critical in activating the genes that characterize the organizer cells. ments (White et a!. 2002), and activin can induce different (Brannon and Kimelman 1996; Engleka and Kessler 2001). TGF- proteins in the Nieuwkoop center induce the cells in the dorsal marginal zone above them to express Smad2/4 traunscription lactors, which then bind to the pro moter of the organizer genes and cooperate with Twin and 2000) . Siamois to activate them (see Figure 7.22; Germain et al. at higher concentrations it induces organiz·er genes to become expressed (Green and Smith 1990; Moriya and Asashima 1992; Piepenburg et a!. 2004). Thus the TGF-ll-like signal from the endoderm is known type 01 mesoderm induced. to be critical for mesoderm induction; moreover, the amount of this signal may control the Since activin, Vg1, Derrier, and Nodal proteins all act 262 CHAPTER 7 (A) (B) Stage 8 -ca{enin VegT, Vgl < FIGURE 7.23 Vegetal induction of mesoderm. (AI The maternal RNA encoding Vgl (brighl white crescent) is tethered to the vege tal cortex of a Xenopus oocyte. The message (along with the maternal VegT message) will be translated at fertilization. Both proteins appear to be cruc ial for the abil ity of vegetal cells to induce cells above them to become mesodermal. (8-0) Model for mesoderm induction and organizer formation by the interaction of -caten in and TGF- proteins. (BI At late blastula stages, Vg1 and VegT are found i n the vegetal hemisphere; -catenin is locat ed in the dorsal region. (C) p-Cateni n acts synergistica l l y with Vgl (D) Stage 10 (C) Stage 9 Gradient of BMP4, Xwnt8 (ventral and lateral mesoderm) related high ., Nodal --------I. Organizer Nodal related low Ventral --I _ _ _ _ _ _ mesoderm and VegT to activate the Nodal-related (Xnr) genes. This creates a gradien t of Xnr proteins across the e ndoderm, highest in the dor sal region. (D) The mesoderm is specified by the Xnr grad ien t. Mesodermal regions with li ttle or no Xnr have high levels of BMP4 and Xwnt8; they becomc ventral mesoderm.
Those having intermediate concentrations of Xnr becomc lateral mesod erm. Where there is a high concentration of Xnr, goosecoid and other dorsal mesodermal genes are activated and the mesodermal lissue becomes the organi ze r. (A courtesy of D. Melton; B-D after Agius et al. 2000.1 throu gh the same pathway (activating the Smad2/4 tran- specification, particularly when combined with dorsal scription factor; see Figure 7.22), the amowlt of each of catenin signaL these is expected to be additive (Agius et aJ. 2000).
Indeed, this appears to be the case. In summary, then, the fonnation of the dorsal mesodenn and the organizer originates through the activation of crit proteins (including Xnrl-6) are expressed in a gradient ical transcription factors by intersecting pathways. The first pathway is the Wnt/- throughout the endoderm with a low concentration ven- encoding the Siamois and Twin transcription factors. The trally and a high concentration dorsaUy (Onuma et al. 2002; second pathway is the maternal VegT pathway that acti by the activation of Xenopus Nodal-related gene expres vates the expression of Vgl and other Nodal-related paracrine factors,.which in turn activate the Smad2/4 tran and his colleagues presented a model, shown in F ig u re high levels of Smad2/4 and Siamois/Twin transcription 7.23B-D, in which the dorsally located j)- factor proteins work within the dorsal mesoderm cells and During the late blastula stage, several Nodal-related Rex et aJ. 2002; Wright et at. 2005). This gradient is formed sion by the synergistic action of VegT with j)-catenin. Agius scription factor in the mesodermal cells above them. The vegetally located Vgl signals interact to create a gradient activate the genes that give these cells their "organizer" of the Nodal-related proteins across the endoderm. Those regions with little Nodal-related protein induce the cells properties (review Figures 7.20-7.23). above them to become ventral mesoderm; regions above vegetal cells with some Nodal-related protein become lat Functions of the organizer era! mesoderm; and regions above vegetal cells containing While U1e Nieuwkoop center cells remain endodermal, the large amounts of these proteins (plus Vgl) become the cells of the organizer become the dorsal mesoderm and organizer. Achvin, Vgl, and Derriere gradients may behave migrate underneath the dorsal ectoderm. There, the dor in the same way. Thus, the initial specification of the meso sal mesodeml induces the central nervous system to form. derm along the dorsal-ventral axis appears to be accom The properties of the organizer tissue can be divided inlo plished by Nodal-like TGF- paracrine factors. 111e region four major functions: with the highest concentration of these factors may pro vide the vegetal signal for dorsal mesoderm (organizer) 1. The ability to self-differentiate dorsal mesoderm (pre chordal plate, chordamesoderm, etc.) AMP H I BIANS AND FISH 2. The ability to dorsalize the surrounding mesodenn n i to paraxial (somite-forming) mesoderm when i t would otherwise form ventral mesoderm 3. The ability to dorsalize the ectoderm and induce forma tion of the neural tube 4. The ability to initiate the movements of gastrulation It is now thought that the cells of the organizer ulti mately contribute to four cell types: pharyngeal endo 263 TABLE 7.2 Proteins expressed solely or almost exclusively in the organizer (partial list) Nuclear proteins Secreted proteins Twin Chordin Siamois Dickkopf Xliml ADMP Xnot Frzb Otx2 Noggin XFDl Follistatin derm and prechordal plate lead the migration of the XANF1 Sonic hedgehog organizer tissue and induce the forebrain and midbrain. Goosecoid Cerberus The dorsal mesoderm induces the hindbrain and trunk.
HNF3 Nodal-related proteins (several) derm, head mesoderm (prechorda l plate), dorsal meso dem1 (prinlarily the notochord), and the dorsal blastopore lip (Keller 1976; Gont et al. 1993). The pharyngeal endo The dorsal blastopore lip remaining at the end of gastrula tion eventually becomes the chordaneural hinge that induces the tip of the tail. As we have just seen, the Smad2/4 and Il-catenin tran scription factors cooperate to activate several genes (Table of the ectoderm that is protected from epidermal induction 7.2).
Many of these genes encode secreted proteins that will by BMP-inhibiting molecules (Hemmati-Brivanlou and act to organize the embryo. Melton 1994, 1997). In other words, (1) the "default fate" See WEBSITE 7.5 Early attempts 10 locale Ihe organizer molecules Induction of neural ectoderm and dorsal mesoderm: BMP inhibitors Evidence from experinlental embryology showed that one of the ectoderm is to become neural tissue; (2) certain parts of the embryo induce the ectoderm to become epidermal tissue by secreting BMPs; and (3) the organizer tissue acts by secreting molecules that block BMPs, thereby allowing the ectoderm "protected" by these BMP inhibitors to become neural tissue. See WEBSITE 7.6 Competence and bias of the most critical properties of the organizer was its pro Three of the major BMP inhibitors secreted by the duction of soluble factors. The evidence for such dilfusible organizer are Noggin, chordin, and follistatin. The noggin, signals from the orgarrizer carne &om several sources, First, chordin, and follistatin genes are some of the most critical Hans Holtlreter (1933) showed that if the notochord fails genes activated by Smad2/4 and Siamois/Twin (Camac et to migrate beneath the ectoderm, the ectoderm will not al. 1996; Fan and· Sokol 1997; Kessler 1997). become neural tissue (and will become epidermis). More definitive evidence for the importance of soluble factors came later from the transfilter studies of Finnish investi gators (Saxen 1961; Toivonen et al. 1975; Toivonen and Wartiovaara 1976). Here, newt dorsal lip tissue was placed on one side of a filter fine enough so that no processes could fit through the pores, and competent gastrula ecto derm was placed on the other side. After several hours, neural structures were observed in the ectodermal tissue (Figure 7.24). The identities of the factors diffusing from the organizer, however, took another quarter of a century to find. It turned out that scientists were looking for the wrong mechanism. They were searching for a molecule secreted by the organizer and received b y the ectoderm that then converted the ectoderm into neural tissue. However, molecular studies led to a remarkable and non-obvious conclusion: it is the epidermis that is induced to form, not the morp hogene ti c proteins ! BMPs), while the nervous system forms from that region neural tissue. The ectoderm is induced to become epider mal tissue by binding bone FIGURE 7.24 N eu ra l structures induced in presumptive ecto derm by newt dorsal lip tissue, separaled from the ectoderm by a nucleopore fi lter with an average pore diameter of 0.05 mm. Anterior neu ral tissues are eviden t, including some induced eyes. (From Toivonen 1 979, courtesy of L. Saxen.) 264 (A) CHAPTER 7 (B) (i) (ii) (iii) (iv) NOGGIN In 1992, Smith and Harland constructed a eDNA plasmid library from dorsalized (lithium chloride-treated) gastrulae. Messenger RNAs synthesized from sets of these plasmids were injected into ventralized embryos (having no neural tube) produced by irradiating early embryos with ultraviolet light. Those plasmid sets whose mRNAs rescued dorsal structures in these embryos were split into smaUer sets, and so on, W1til single-plasmid clones were isolated whose mRNAs were able to restore the dorsal tis sue in such embryos. One of these clones contained the gene for the protein Noggin (Figure 7.25A). Injection of 1I0g gin mRNA into I-cell, UV-irradiated embryos completely rescued dorsal development and allowed the formation of a complete embryo.
Noggin is a secreted protein that is able to accomplish two of the major functions of the organizer: it induces dor sal ectoderm to form neural tissue, and it dorsalizes meso derm cells that would otherwise contribute to the ventral mesoderm (Smith et aJ. 1993). Smith and Harland showed that newly transcribed noggin mRNA is first localized in the dorsal blastopore lip region and then becomes expressed in the notochord (Figure 7.25B) . Noggin binds to BMP4 and BMP2 and inhibits their binding to receptors (Zimmerman et aJ. 1996). FIGURE 7.25 The soluble protein Noggin dorsalizes the amphib ian embryo. (A) Rescue of dorsal structure5 by Noggin protein. When Xenopus eggs are exposed to ultraviolet radiation, cortical rotation fails to occur, and the embryos lack dorsal structures (top). If such an em bryo is i njected with noggin mRNA, it devel· ops dorsal structures in a dosage-related fashion (top to bottom). If too mu ch n.oggin message is injected, the embryo produces dorsal anterior tissue at the expense of ventral and posterior tissue, becoming linle more than a head (bonom). (B) Localization of noggin mRNA in the organizer tissue, shown by in silu hybridiza· tion. At gastrulation (i), noggin mRNA (dark areas) accumulates in the dorsal marginal zone. When cells involute (ii), noggin mRNA is seen in the dorsal blastopore lip. During con vergent extension (iii), noggin is expressed in the precursors of the notochord, pre· chordal plate, an d ph aryngea l endoderm, which (iv) extend beneath the ectoderm in the center of the embryo. (Courtesy of R. M. Harland.) CHORD IN The second organizer protein found was chord in.
It was isolated from clones of cDNA whose mRNAs were present in dOlsalized, but not in ventralized, embryos (Sasai et al. 1994). These clones were tested by injecting them into ventral blastomeres and seeing whether they induced secondary axes. One of the clones capable of inducing a secondary neural tube contained the chardin gene. Chordin mRNA was found to be localized in the dor sal blastopore lip and later in the notochord (Figure 7.26) . Of all organizer genes observed, chardin is the one most acutely activated by - AMPHIBIANS AND FISH Local izati on of chordin mRNA. (A) Whole-mount in situ hybridization shows that just prior to gastrulation, chardin mRNA (dark area) is expressed in the region that will become the dorsal blastopore lip. (B) As gastrulation begins, chordin is expressed at the dorsal blastopore lip. (e) In later stages of gastru lation, the chardin message is seen in the organizer tissues (From Sasai et al. 1 994, courtesy of E. De Robertis.) 265 FIGURE 7.26 . 2003). Like Noggin, chordin binds directly to BMP4 and BMP2 and prevents thele complexing with their receptors (Piccolo et a1. 1996). FOLLISTATIN The mRNA for a third organizer-secreted pro tein, follistatin, is also transcribed in the dorsal blastopore lip and notochord. Follistatin was found in the organizer through an unexpected result of an experiment that was looking for something else. Ali Hemmati-Brivanlou and Douglas Melton (1992, 1994) wanted to see whether the protem activin was required for mesoderm induction. In searching for the mesoderm inducer, they found that fol listatin, an inhibitor of both activin and BMPs, caused ecto derm to become neural tissue. They then proposed that under normal conditions, ectoderm becomes neural unless induced to become epidermal by th BMPs. This model \vas supported by, and explai.ned, certain cell dissociation experiments that had also produced odd results. Three 1989 studies-by Grunz and Tacke, Sato and Sargent, and Godsave and Slack-had shown that when whole embryos or their animal caps were dissociated, they formed neural tissue. This result would be explainable if the "default state" of the ectoderm was not epidermal, but neural, and tissue had to be induced to have an epidermal phenotype. Thus we conclude that the organizer blocks this epidermalizing induction by inn.ctivatil1g EMPs. See WEBSITE 7.7 Specification of the endoderm Epidermal inducers: The BMPs In Xenopus, the epidermal inducers are the bone morpho genetic protein BMP4 and its close relatives BMI'2, BMP7, and ADMP (anti-dorsalizing morphogenetic protein, a BMP-like paracrine factor).
There is an antagonistic rela tionship between these BMPs and the organizer. If the mRNA for BMP4 is injected into Xenopus eggs, all the mesoderm in the embryo becomes ventrolateral meso derm. Involution is delayed and, when it does occur, has a ventral rather than a dorsal character (Dale et a1. 1992; Jones et a1. 1992). Conversely, overexpression of a domi nant negative BMP4 receptor results in the formation of twumed axes (Graff et a1.
1994; Suzuki et a1. 1994). In 1995, Wilson and Hemmati-Brivanlou demonstrated that BMP4 induces ectodermal cells to become epidermal. By 1996, several laboratories had demonstrated that Noggin, chordin, and follistatin.are all secreted by the organizer, and that each of them prevents BMP from binding to and inducing the ectoderm and mesodenm cells near the organ izer (Piccolo et a1. 1996; Zimmerman et al. 1996; Iemura et a1. 1998). lJMP4 is expressed initially throughout the ectodenmal and mesodermal regions of the late blastula.
However, dur ing gastrulation, bmp4 transcripts become restricted to the ventrolateral marginal zone. This is because the Goosec oid protein (and some other transcription factors) are induced by the Siamois/Twin and Smad2/4 interactions in the dorsal (organizer) mesoderm starting at the begin ning of gastrulation (Blitz and eho 1995; Yao and KeSsler 2001). These transcription factors repress bmp4 and wntB transcription (Hemmati-Brivanlou and Thomsen 1995; Northrop et a1. 1995; Steinbeisser et a1. 1995; Glavic et a1. 2001). In the ectoderm, BMPs repress the genes (SUcll as neurogenin) involved in forming neu.ral tissue, while acti vating other genes involved in epidermal specification (Lee et a1. 1995). In the mesoderm, it appears that graded lev els of BMP4 activate different sets of mesodermal genes, thereby specifying the dorsal, intermediate, and lateral mesodermal tissues (F ig u re 7.27; Gawantka et aI. 1995; Hemmati-Brivanlou and Thomsen 1995; Dosch et a1. 1997). In 2005, tvv'o important sets of experiments confirmed the default model and the importance of blocking BMI's to specify the nervous system. Flrst, Khokha and colleagues 266 CHAPTER 7 (A) Animal I ectoderm (A) (B) Ventral ORGANIZER MOLECULES: Chordin, Noggin, Follistatin. Xnr3 endoderm Vegetal (B) (C) / BMP4 c .g g u Noggin ___ 8 '------'--'"-.5 Ventrolateral pla te, Notochord 2- kidney (Xvent2) (Chordin) c of; , OJ ..3 , / Yentral, blood mesenchyme (Xventl) Dorsolateral. muscle (mylS) Vent raj Dorsal FIGURE 7.27 Model for the action of the organizer.
(A) BM?4 (along with certain other molecules) is a powerful ventralizing factor.
Organizer proteins such as chordin, Noggin, and follistatin block the action of BMP4; their inhibitory effects can be seen in all three germ layers. (8) BMP4 may elicit the expression of differ ent genes in a concentration-dependent fashion, Thus, in the regions of noggin and chordin expression, BMP4 is totally pre vented from binding, and these tissues become notochord (organ. izer) tissue. Slightly farther away from the organizer, the myf5 gene is activated, producing a marker for the dorsolateral mus cles. As more and more BMP4 molecules afe allowed to bind to the cells, the Xventl (ventrolateral) and Xventl (ventral) genes become expressed. (Aher Dosch et al. 1 997; De Robertis et al. FIGURE 7.28 Control of neural specification by the levels of BM?s. (A,B) Lack of dorsal structures in Xenopus embryos whose BMP-inhihitor genes (chordin, noggin, and (ollistatin) were elimi nated by antisense morphQlino oligonucle

left and the gut coils in a clockwise manner.
(From Ryan et al. 1 998, courtesy of J. C. I zpis ua-B elmonte.) AMPHIBIANS AND FISH (iii) (ii) Yolk Yolk syncytial layer cell Animal pole (v) (vi) Vegetal pole (ix) (x) 273 Otic vesicle (ear) (vii) (viii) (xi) (xii) Notochord FIGURE 7.37 Zebrafish development occurs very rapidly. In th e first 24 hours of em bryogen es is shown here, the l -cell zygote becom es a vertebrate embryo with a tadpole-like form. (From Langeland and Kimmel 1 997; drawings by N . Haver.l , EARLY ZEBRA FISH DEVELOPMENT Xenopus eggs are holoblastic, dividing the entire egg, whereas the yolky zebra fish egg is meroblastic; despite this difference, however, Xenopus and zebra fish fonn their axes and specify their cells in very similar ways. The rapid development, transparency, and breeding ability 01 zebrafish parental fish are treated with a chemical mutagen that will cause random mutations in their germ cells (Figure 7.38). Each mutagenized male is then crossed to (i.e., mated with) a Wild-type female fish to generate Fj fish. individuals in the F, generation carry the mutations inherited from their (Danio rerio) have allowed them to confirm many lather. If the mutation is dominant, it will be expressed in of the principles gleaned from studying Xenopus. Zebrafish the Fl generation. If these mutations are recessive, the FI 24 hours alter lertilization, the fish will not show a mutant phenotype, since the wild-type develop so rapidly that by embryo has lormed most of its organ primordia and dis pl ays the characteristic tadpole-like lorm (Figure 7.37; see Cranato and Nusslein-Volhard me 1996; Langeland and Kim l 1997). dominant allele will mask the mutation. The Fj fish are that includes both males and lemales who carry the mutant then crossed to wild-type fish to produce an F, generation allele. When tvvo F2 parents carry the same recessive muta The zebrafish is the first vertebrate for which intensive ",utagenesis has been attempted. By treating parents with mutagens and selectively breeding their progeny, scientists 25% chance that their offspring will show the mutant phenotype. Since zebralish development occurs tion, there is a in the open (as opposed to within an opaque shell or inside .. ve fOWld thousands of mutations whose normally func the mother's body), abnormal developmental stages can :ioning genes are critical for zebrafish development. The be readily observed, and the defects in development can :;aditional method of genetic screening (modeled after rge-scale screens in Drosophila) begins when the male often be traced to changes in a particular group 01 cells (Driever et aJ. 1996; Haffter et al. 1996). 274 CHAPTER 7 ◆◆ B I Parents M:tagenized d' x F I generation d' Wild type +1+ Mutagen B◆ B◆ +Im x +1+ x +1+ 100% wild type (Figure 7.39). vertebrates and the above-mentioned ability of this fish to be genetically manipulated has given the zebrafish an important role in investigating the genes that operate dur ing human development. When developmental biologists screened zebrafish mutants for cysti,c kidney disease,. they found 12 different genes. Two of these genes were known 10 to cause human cystic kidney disease, and the other were as-yet unknown genes that were found to interact with the first tvvo in a common pathway. Moreover, that pathway,. which involves the synthesis of cilia,. was not closed an important and previously unknown pathway to explore human birth defects (Sun et a1. 2004). Zebrafish embryos are also permeable to small molecules placed in +1+ X +Im +Im x +Im ◆ ◆ 'J) ◆ \) \) ◆ ◆ ◆ ◆ ◆ ◆ ◆ ◆ 3◆ 100% wild type (+1+ or +Im) and places as they express what had been expected. Thus, the zebrafish studies dis +1+ +1+ CFP at the same times The similarity of developmental programs among all d' x fused with specific zebrafish promoters and enhancers and inserted into the fish embryos. The resulting transgenic fish amazing thing is that one can observe the reporter protein -@ F3 gen eration and lar gene is required for a particular ftmction. Furthermore,. · the green fluorescent protem (CPP) reporter gene can be in living transparent embryos' . _-- - - B◆ B◆ +Im 2001), researchers can use this method to test whether a particu the proteins controlled by these regulatory sequences. The F2 generation B◆ pholino antisense molecules (Zhong et a1. express x +11'11 " Zebrafish genes arc extremely amenable to study.
Like Xenopus embryos,. zebrafish embryos are susceptible to mor 100% wild type *GFP-transgenic zebrafish are the first commercially available transgenic pets, and arc marketed as "Glo-Fish." (A) (B) wild type, 1 mutant FIGURE 7.38 Screen ing protocol for identifying mutations of zebrafish development. The male parent is mutagellized and mated with a wi ld-type (+1+) fe mal e. If some of the male's sperm carry a recessive mutant allele (m), then some of the Fl progeny of the mating will inherit that allele. F1 individuals (here shown as a male carrying the mutant allele m) are then mated with wild-type partners. This creates an F2 generation wherein some males and some females carry the recessive mutant allele. When the F2 fish are mated, some of their progeny will show the mutant pheno type. (After Haffter et al. 1 996.) FIGURE 7.39 The gene for green fluorescent protein (GFP) was fused to the regulatory region of a zebrafish sonic hedgehog gene. As a result, GFP was synthesized wherever the Hedgehog protein is normally expressed in the fish embryo. (A) In the head of a zebrafish embryo, GFP is seen in the developing retina and nasal placodes. (B) Because GFP is expressed by individual cells, scien tists can see precisely which cells make GFP, and thus which cells normally transcribe the gene of interest (in this case, sonic hedge hog in the retina). (Photographs courtesy of Strahle and C. Neumann.) U. AM PHIB IANS A N D FISH the water-a property that allows us to test drugs that may be deleterious to vertebrate development. For instance, zebrafish development can be altered by the addition 01 ethanol or retinoic acid, both of which produce malforma tions in the fish that resemble human developmental syn dromes known to be caused by these molecules (Blader and Strahle 1998). As one zebrafish researcher joked, "Fish really are just little people with lins" (Bradbury 2004). Cleavage The eggs of most bony fish are lelolecithal, meaning that most of the egg cell is occupied by yolk. Cleavage can take place only in the blastodisc, a thin region of yolk-free cyto plasm at the animal pole of the egg. The cell divisions do not completely divide the egg, so this type of deavage is called meroblastic (Greek m.eros, "part"). Since only the blastodisc becomes the embryo, this type of meroblastic cleavage is referred to as discoidal. Scanning electron micrographs show beautifully the incomplete nature of discoidal meroblastic cleavage in fish eggs (Figure 7.40). The calcium waves initiated at fertiliza tion stimulate the contraction of the actin cytoskeleton to squeeze non-yolky cytoplasm into the animal pole of the egg. This process converts the spherical egg into a pear- (A) 275 shaped structure with an apical blastodisc (Leung et aJ. 1998, 2000). In fish, there are many waves of calcium release, and they orchestrate the processes of cell division. The calcium ions coordinate the mitotic apparatus with the actin cytoskeleton; they help propagate cell division across the cell surface; they are needed to deepen the deavage furrow; and they heal the membrane after the separation of the blastomeres (Lee et al. 2003). The fust cell divisions follow a highly reproducible pat tern of meridional and equatorial cleavages. These divi sions are rapid, taking about 15 minutes each. The first 12 divisions occur synchronously, forming a mound of ceHs that sits at the animal pole of a large yolk cell. This mound of cells constitutes the blastoderm. Initially, all the cells maintain some open connection with one another and with the underlying yolk cell, so that moderately sized (17-kOa) molecules can pass freely from one blastomere to the next (Kimmel and Law 1985). Maternal effect mutations have shown the importance of oocyte proteins and mRNAs in embryonic polarity, cell FIGURE 7.40 D i scoi da l meroblastic cleavage in a zebrarish egg. (A) 1 -cell embryo. The mound atop the cytoplasm is the blas tod isc (8) 2-cell embryo.
(C) 4-cell embryo. (D) 8-cell embryo wherein two rows or four cells are formed. (E) 32-cell embryo. (F) 64-cell embryo, where i n the blastodisc can be seen atop the yolk cell. (From Beams and Kessel 1 976, cou rtesy of the authors.) .
, (8) (C) (E) (F) 276 (HAPTER 7 (A) Blastoderm { Internal yolk syncytial Yolk (8) layer Deep --Yolk cell ,L-fLj ri (e) Animal pole FIGURE 7.41 Fish blastula. (A) Prior to gastrulation, the deep cells are surrounded by the envel oping layer The animal surface of the yolk cell is flat and contairis the nuclei of the yolk syncytial layer (YSL). Microtubules extend through the yolky cyto . plasm and the external YSL. (B) Late blastula-stage embryo of the minnow Fundulus, showi ng the external YSl. The nuclei of these cell s were derived from cells at the margi n of the blastoderm, which released their nuclei into the yolky cytop lasm . (C) Fate map of the deep cells aher cell mixing has stopped.
This is a lateral view; for the sake of clarity, not all organ fates are label ed. (A,C after Langeland and Kimmel B from Trinkaus 1 993, courtesy of J. P. Trinkaus.) (EVL). 1 997; Ventral Dorsal toderm to form the in ter n a l YSL, and others will move vegetally, staying ahead of the blastoderm margin, to form the extern al YSL (Fi gu re 7.41 B). .The YSL will be important for directing some of the cell movements of gastrula hon.
The second cell population distinguished at the mid Vegetal pole blastula transition is the envelopin g layer (EVL). [t is made up of the most superfioal cells from the blastoderm, wrucl, form an epithelial sheet a single cell layer thick. The EVL division, and ceU cleavage (Dosch et al. 2004). As in frogs, the microtubules are important roads upon which mor is an extraembryonic protective covering that is Sloughed off during later development. Between th e EVL and the phogenetic determinants travet and maternal mutants YSL are the deep cells. These are the cells that give rise to affecting the formation of the rrticrotubal cytoskeleton pre the embryo proper. vent the normal positioning of the cleavage furrow and of mRNAs in the early embryo (Kishimoto et al.
2004).
As in Xenopus and many other embryos, there is a mid The fates of the early blastoderm cells are not deter mined, and cell lineage studies (in wruch a nondiffusible when zygotic gene transcription begins, cell divisions slow, fluorescent dye is injected into a cell so that its descendants can be followed) show that there is much cell mixing dur ing cleavage. Moreover, any one of these early blastomeres and cell movement becomes evident (Kane and Kimmel can give rise to an W1predictable variety of tissue descen blastula transition, seen around the tenth cell division, 1993). At trus time, three distinct cell populations can be distinguished (Figure 7.41A). The first of these is the yolk syncytial layer, or YSL. The YSL is formed at the ninth or tenth cell cycle, when the cells at the vegetal edge of the blastoderm fuse with the underlying yolk cell. T1Us fusion produces a ring of nuclei in the part of the yolk cell cyto plasm that sits just beneath the blastoderm. Later, as the blastoderm expands vegetally to surround the yolk cell, some of the yolk syncytial nuclei will move under the blas- dants (Kimmel and Warga 1987; Heide et a1. 1994). A fate map of the blastoderm cells can be made shortly before gastrulation beginS. At this time, cells in specific regions of the embryo give rise to certain tissues in a highly pre dictable manner, although they remain plastic, and cell fates can change if tissue is grafted to a new site (Figure 7.41C; see also Figure 1.11). See VADE MECUM Zebrafish development AMPHIBIANS AND FISH (A) layer Cells ingress (C) (8) Animal pole 277 Animal pole Yolk Deep Involuting cells Yolk Vegetal pole (D) syncytial nuclei inducing sign al Animal pole Vegetal pole n Mesoderm • Ectoderm neuroectoderm 0 Pluripotential epiblast , _ Ventral EO\'elope layer Vegetal pole Envelope layer / Yolk granule cells layer syncytial • Mescndoderm: precursors to both mesoderm and endoderm 0 Endoderm • Envelope layer (EVL) Dorsal Ectoderm I E) Anterior dorsal inducing signals ,::◆◆◆;◆◆◆Head region Animal pole .. . FIGURE 7.42 Cell movements during zebrafish gastrulation. (A) The blastoderm at 30% c ompl etion of epiboly (abou t 4.7 hours).
(8) Formation of the hypoblast, either by involution of cells at the margin of the epibolizing blastoderm or by delamination and ingression of cells from the epiblast 16 hours). IC) Close up of the ma rgi na l region. (0) AI 90% epi boly (9 hours), mesoderm can be seen s urrounding the yolk between the endoderm and ectoderm . (E) Com p l etio n of gastrulation 110.3 hours).
(After Driever 1 995; Langeland and Kimmel l 997.) - , Somite # l Ventral Dorsal deep cells spring back to the top of the yolk, while U,e YSL the YSL and the EVL. When this is done, the EVL and the continues its expansion around the yolk cell (Trinkaus 1984, 1992). E-cadherin is critical for these morphogenetic move ments and for the adhesion of the EVL to the deep cells Tailbud (Shimizu et a!.
Posterior -- Vegetal pole 2005). The expansion of the YSL depends partially on a net work of microtubules within it, and radiation or drugs that block the polymerization of tubulin slow epiboly (Strahle and Jesuthasan 1993; Solnica-Krezel and Driever 1994) . During epiboly, one side of the blastodenn becomes notice Gastrulation and Formation of the Germ layers ably thicker than the other.
Cell labeling experiments indi ca te that the thicker side marks the site of the future dor sal surface of the embryo (Schmitz and Campos-Ortega The first cell movement of fish gastrulation is Ule epiboly of 1994). Ulis movement, the deep cells of the blastoderm move out ward to intercalate with the more superficial cells (Warga zebrafish yolk cell (this occurs earlier in fish species whose the blastoderm cells over the yolk. In the initial phase of and Kimmel 1990). Later, this concatenation of ceUs moves ,-egetally over the surface of the yolk cell and envelops it completely (Figure 7.42).
This downward movement t ward the vegetal pole is a result of radial intercalation of After the blastoderm cells have covered about half the eggs have larger yolks), a thickening occurs throughout the margin of the epibolizing blastoderm. TIus thickening, epiblast; and an inner layer, the hypoblast. The hypoblast called the germ ring, is composed of a superficial layer, the in fish (but not in birds and marumals) contains the pre the deep cells into the superficial layer. The enveloping cursors of the endoderm and mesoderm. The hypoblast Ll yer is tightly joined to the yolk syncytial layer and is dragged along with it. That the vegetal migration of the forms in a synchronous "wave" of internalization (Keller et a!. 20OS) that has some characteristics of ingression (espe ':>lastoderm margin is dependent on the epiboly of the YSL cially in the dorsal region; see Cannany-Rampey and Schi can be demonstrated by severing the attacJunents between er 2001) and some elements of involution (especially in the 278 CHAPTER 7 Endodermal behavior simulation Animal pole Ventral (◆,J ::_:◆.::◆'.:'"◆',',.;◆.:::::.:::.:,:::.◆::.:.::-:::◆◆. Dorsal Vegetal pole Onset of gastrulation I (/:::,; :◆ Mesoderm: AP-directed movement AP , V : . . ;:..◆:\ ... . . .;;:. .. ..:: . .
. ' \ . " .. . . . . " . . . . ' VP Mid-gastrulation :, D V \ Endoderm: Random walk . AP . " " ,. " ", ' . . " " . : .' . . . .' . ' . . \ : :. : : - . : . . '" . ' . " 'i D . • • .
' . ' . • • • t VP Mid-gastrulation FIGURE 7.43 Cel l migration of endodermal and mesodermal precursors. At the beginning of gastrulation, endoderma! and mesodermal cells are localized in the hypoblast. Once the hypoblast is internalized, the mesodermal cells migrate vegetally and then toward the animal pole. In contrast, endodermal cells spread th rough the internal regions of the embryo by a "random walk" over the yolk. (After Pezeron et af. 2008.) future ventral regions; see Figure 7A2C). Thus, as the cells of the blastoderm undergo epiboly arpund the yolk, they are also internalizing cells at the blastoderm margin to form the hypoblast. As the hypoblast cells internalize, the future mesoderm cells (the majority of the hypoblast cells) initially migrate vegetally while proliferating to make new mesoderm cells. Later, they alter their direction and pro cede toward the animal pole. The endodermal precursors, however, appear to move randomly, walking over the yolk (Figure 7-43; Pezeron et aL 2008). Once the hypoblast has formed, cells of the epiblast and hypoblast intercalate on the future dorsal side of the embryo to form a localized thickening, the embryonic shield (Figu re 7-44A). Here, the cells converge and extend anteriorly, eventually narrowing along the dorsal midline (Fig u re 7-44B). This convergent extension in the hypoblast forms the chordamesoderm, the precursor of the notochord (Trinkaus 1992; Figure 7A4C). As we will see, the embry- onic shield is functionally equivalent to the dorsal blasto pore lip of amphibians, since it can organize a secondary embryonic axis when transplanted to a host embryo (Oppenheimer 1936; Ho 1992). The cells adjacent to the chordamesoderm-the paraxial mesoderm cells-are the precursors of the mesodermal somites. Concomitant con vergence and extension in the epiblast bring presumptive neural cells from all over the epiblast into the dorsal mid line, where they form the neural keel. The neural keel is usually quite a broad band of neural precursors, and it extends over the axial and paraxial mesoderm. Those cells remaining in the epiblast become the epidermis. On the ventral side (see Figure 7.44B), the hypoblast ring moves toward the vegetal pole, migrating directly beneath the epiblast that is epibolizing itself over the yolk celL Eventually the ring closes at the vegetal pole, completing the internalization of those cells that are going to become the mesoderm and endoderm (Keller et aL 2008). The zebrafish fate map, then, is not much different from tha t of the frog. If you can visualize opening a Xenopus blastula at the vegetal pole and then stretching that open ing into a marginal ring, the resulting fate map closely resembles that of the zebra fish embryo at the stage when half of the yolk has been covered by the blastoderm (see Figure 1.11; Langeland and KirnmeI 1997). Meanwhile, the endoderm arises from the most marginal blastomeres of the late blastula-stage embryo. See WEBSITE 7.9 GFP zebrafish movies and photographs Axis Formation in Zebrafish By different mechanisms, the Xenopus egg and zebrafish egg have reached the same basic state. They have become multicellular, they have undergone gastrulation, and they have positioned their germ layers such that the ectoderm is on the ou tside, the endoderm is on the inside, and the mesoderm is between them.
As might be expected, zebrafish also form their axes in ways very similar to those of Xenopus, and using very similar molecules. Dorsal-ventral axis formation The embryoniC shield of fish is, as mentioned above, homologous to the dorsal blastopore lip of amphibians, and it is critical in establishing the dorsal-ventral axis.
Shield tissue can convert lateral and ventral mesoderm (blood and connective tissue precursors) into dorsal meso derm (notochord and s◆mites), and it can cause the ecto derm to become neural rather than epidermaL This trans formative capacity was shown by transplantation experiments in which the embryonic shield of an early-gas trula embryo was transplanted to the ventral side of anoth er (Figure 7-45; Oppenheimer 1936; Koshida et aL 1998). Two axes formed, sharing a cornmon yolk celL Although the prechordal plate and notochord were derived from the AMPH I B IANS AND FISH (A) Animal pole 279 Dorsal view (D) .JJr-:'Cfo--.hl◆¥.I'! "b◆W' :/◆◆s-,'--- - Epiboly :/--- Yolk cell (C) 12.5 hours FIGURE 7.44 Convergence and extension in the zebrafish gas trula. (A) Dorsal view of convergence and extension movements duri ng zebrafish gastrulation. Epiboly spreads the blastoderm over the yolk; involution and ingression generate the hypoblast; con◆ vergence and extension bring the hypoblast and epiblast cells to the dorsal side to form the embryonic shield. Within the shie ld, intercalation extends the chordamesoderm toward the animal pole. (B) Model of mesendoderm (hypoblast) formation. Numbers indicate hours a fter fertilization, On the future dorsal side, the internalized cells undergo convergent extension to form the chor damesooerm (notochord) and the paraxial (sami!ic) mesoderm adjacent to it. On the ventral side, the hypoblast cells migrate with the ep ibolizing epiblast toward the vegetal pole, eventually converging lhere. (C) Convergent extension of the chordameso derm of the hypoblast cells. These cells are marked by th eir expression of the no-tail gene (dark areas) encoding a T-box tran scription factor. (Ale from Langeland and Kimmel 1 997, courtesy of the authors; B aher Keller et al. 2008.) . donor embryonic shield, the other organs of the second ary axis came from host tissues that would normally form ventral structures. The new axis had been ind uced by the donor cells . Like the amphibian blastopore lip, the embryonic shield fonns the prechordal plate and the notochord of the devel oping embryo. The precursors of these two regions are responsible for inducing ectodenn to become neural ecto- derm. Moreover, the p res umptive notochord and pre chordal plate appear to do this in a manner very much like the homologous structures in amphibians.' Like amphib ians, fish specify the epidermis by using bone morpho genetic proteins (BMPs) and certain Wnt proteins to induce the ectoderm (see Tuc.ker e t al. 2008). These BMPs and Wnts are made in the ventral and lateral regions of the e mbryo. The notochords of bo th fish and amphibians secrete factors that block this induction, thereby allowing the ectoderm to become neural. In fish, BMP2B induces embryonic cells to acqui re ventral and lateral fates, and •Another similarity between the amphibian and fish organi7.ers is that they can be duplicated by rotating the egg and changing the orientation of the microtubules (Fluck et al. 1998). One difference in the axial development of these groups is that in amphibians, the prechordal plate is necessary for inducing the anterior brain to form. In zebrafish, although the prechordal plate appears to be nec essary for forming ventra] neural s tructu res, the anterior regions of the brain can fOffil in its absence (Schier et al. 1997; Schier and Tal bot \998).
280 CHAPTER 7 Animal (A) Ventral \"-◆--"'-r Dorsal Vegetal g ◆◆ Embryo n ic hield Embryonic shield Host t WntB ventralizes, lateralizes, and posteriorizes the embry onic tissues (see Schier 2001). The protein secreted by the chordamesoderm that binds with and inactivates BMP2B is the chordin homologue called Chordino (F igu re 7.46; Kishimoto et a1. 1997; Schulte-Me rk er et al. 1997). If the chordino gene is mutated, the neural tube fails to form prop erly; and if chordino, >1oggiIl, andfollistatin homologues are all inactivated, the neural tube fails to form at all, just like in Xenopus (Dal-Pra et al. 2006). Extracellular BMP antagonists (such as Chordino and Noggin) are not the only inlportant factors for specifying the neural plate. FGFs made in the dorsal side of the embryo also inhibit BMP gene expresson (Furthauer et al. 2004; Tsang et al. 2004; Little and Mullins 2006). In the cau dal region of the embryo, FGF signaling is the predomi nant neural specifier (Kudoh et al. 2004). And as in Xeno pus, insulin-like growth factors (lGFs) also play a role in the production of the anterior neural plate. Zebrafish IGFs appear to upregulate chordin.o and goosecoid while restrict ing the expression of bmp2b. Although fGFs appear to be made throughout the embryo, during gastrulation the IGF receptors are found predominantly in the anterior portion of the embryo (Eivers et a1. 2004). Also, Wnt inhibitors appear to play roles in head formation, as antisense mor- (B) (A) (B) .BMP2B ",---.l' """ Mesoderm Dorsal mesoderm in ducin g sign al FIGURE 7.45 The embryoni c shield as orga ni zer in the fish embryo. (AI A donor embryonic shield (about 1 00 cells from a staine◆ embryo) is transplanted into a host embryo at the same ear ly-gastrula stage.
The result is t"vo embryonic axes joined to the hos◆s yolk cell. In the photograph, both axes have been stained for sonic hedgehog mRNA, which is expressed in the ventral mid line. (The embryo to the ri ght is the secondary axis.) (B) The same effect can be achieved by activating nucl ear catenin in embryos at sites opposite where the embryonic shield will fo rm . (A after Shinya et al . 1 999, photograph courtesy of Ihe authors; B courtesy of j . C.
Izpisua-Belmonte.) Dorsal m esoderm i n ducing signal Mesoden FIGURE 7.46 Ax is formation in the zebra fish embryo. (A) Prior to gastrula tion, the zebrafish blastoderm is a rra nged with the presumptive ecto derm near the animal pole, the pre sumptive mesoderm beneath it, and the presumptive endoderm sitting atop the yolk cell. The yolk syn cyti al layer (and possib ly the endoderm) sends two si gnals to the presumptive mesoderm. One si gnal (l ighter arrows) indu ces the mesoderm, while a second signal (heavy arrow) specifically induces an area of mesoderm to become the dorsal mesoderm (embryonic shield). (B) Formation of th e dorsal-ventral axis.
Durin g gastrulation, the ventral mesoderm secretes BMP2B (arrows) to in duce the ventral and I J tera l mesodermal and epider mal differentiation.
The dOfsJI mesoderm secretes factors (such as Chordino) that hlock BMP2B and dorsalize the mesoderm and ectoderm (converting the latter into neural tissue). (Aher Sch ier and Talbot 1 998.) AMPH I B IANS AND FISH pholinos that downregulate Wnt3a and Wnt8 lead to the 281 anteriorization of the neural plate at the expense of trunk Inducing p-catenin accumulation on the ventral side of the ' egg results in dorsalization and a second embryonic axis structures (Shimizu et a l. (Kelly et a1. 1995). p-C a tenin in the embryonic shield combines with the 2005). zebrafish homologue of Tcf3 to form a transcription factor The fish Nieuwkoop center that activates genes encoding several mesoderm-pattern It thus appears that zebrafish have an "organizer" region ing proteins. One of them is an FCF that represses BMP that dorsalizes the region through BMP and Wnt inhibitors gene expression on the dorsal side o f the embryo related to similar proteins seen in Xenopus.
It also turns out (Furthauer et a1. that both zebrafish and XenopllS use !}-catenin- and Nodal proteins induced by p-catenin include Squint and Bozo related proteins to form the dorsal mesoderm and enable zok, which are very similar to the proteins that pattem the 2004; Tsing et a1.
2004). Other patterning this mesoderm to express the organizer genes. Both species amphibian mesoderm. Squint is a Nodal-like paracrine fac use BMPs and Wnts to lateralize and posteriorize the tor, while Bozozok* is embryo, and in both groups the orgaruzer genes encode the amphibian Nieuwkoop center protein Siamois. a homeodomam protein similar to proteins such as chordin, Noggin, and Dickkop! that inhib Bozokok protein works in several ways. First, acting it BMPs and Wnts.
Furthermore, later in development both alone, it can repress BMP and Wnt genes that would pro zebrafish and mote ventral functions (Soinica-Krezel and Driever 2001). Xenopus use a particular Wnt protein to pos teriorize the ectoderm, forming the trunk neural tube.
In different ways, but in both groups the result is a structure some instances, fish and amphibians use these proteins in that is definitely recognizable as a vertebrate embryo. The zebrafish embryonic shield appears to acquire its orgaruzing ability in much the same way as its amphibian counterparts. In amphibians, the endodermal cells beneath Second, it suppresses a transcriptional inhibitor (the vegal gene), allowing the organizer genes to function (Kawahara 2000). Third, Bozozok and Squint act individually to chordino gene, and they act synergistically to activate other organjzer genes such as goosecoid, noggin, and dickkopf (Figure 7.47B; Sampath et aL 1998; Critsman et al. 2000; Schier and Talbot 2001). These genes encode the et a1. activate the the dorsal blastopore lip (i.e., the Nieuwkoop center) accu proteins that block BMPs and Wnts and allow the specifi mulate p-catenin synthesized from maternal rnessages◆ cation of the dorsal mesoderm and neural ectoderm. Thus, enabling the amphibian endoderm to induce the cells the embryonic shield is considered equivalent to the above it to become the dorsal blastopore lip. amphibian organizer, and the dorsal part of the yolk cell, In zebralish, the nuclei in that part of the yolk syncytial layer lying beneath the cells that will become the embryonic shield similarly accumulate p-catenin . Indeed, the presence of P catenin distinguishes dorsal YSL from the lateral and ven tral YSL regions' (Fi gure 7.47A; Schneider et a1. 1996). ◆me of the endodermal cells that accumulate p-catenin will become the precursors of the ciliated ceUs of Kupffer's vesicle Cooper and 0'Amico 1996).
As we will discuss in the last section of dle chapter, these cells are critical in detennining the left-right axis of the embryo. *Bozozok is Japanese slang for an arrogant youth on a motorcycle. The gene's name was deJ.ived from the severe loss-of-function phe notype wherein CI single-eyed embryo CUl-VCS ventrally over the yolk cell (i.e., resembling a rider on a fast motorcycle), However, this gene is also known as Dharma (after a famous Buddhist priest) because embryos with gain-of-function-too much of this protein, the result of experimentally injecting its mRNA into the embryo develop huge eyes and head, but no trunk or tail; they thus resem◆ ble Japanese Dharma dolls (Yamanaka et al. l998; Fekany et al. 1999). (B) ,◆.. squjnt p-catenin ◆ -◆[--" bozozok I c11Ordino noggin, goosecoid, dickkopf) chordino '---. Stat3 ----+- -+-- CeU movement FIGURE 7.47 p-Catenin aelivates organ izer genes in the zebrafish. (A) N uclear localization of p-catenin marks the dorsal side of the Xenopus blastula (larger i m age) and helps form its Nieuwkoop center beneath the o rganizer. In the zebra fish late blastula (smaller image), n uclear l oca l izatio n of ◆.catenin is seen in the yolk syncytial layer nuclei beneath the future embryonic shield. (B) p-Catenin activates squint and bozozok, whose proteins activate organizer-specific genes as well as the Star3 gene whose product is necessary for gaslrulation movements. (A courtesy of S. Schneider; B aher Schier and Talbot 2001.) 282 CHAPTER 7 (A) Late blastula ,,,. 1.
, Early gastrula Otx2 cyp26 1. •.c;◆;◆. yp26 Late g ., 1. -"f , "r--◆:..=c.:.:..:"-----,J . . FGF, Wnt ◆ ◆",.."...1.
Collve◆ ·gent extenSions move margin posteriorly • 1. 1. RA--llOxbJ F, Wn t '-___G-F:.. • ◆◆◆◆, • • • _-.,p' } I) k.:::;◆"" Anterior neural ectoderm • FIGURE 7.48 Moeel for specification of zebrafish neural ecto derm by Wnt, and retinoic acid (RA) signaling. The cyp26 gene encodes retinoic acidA·hydroxylase, a cytochrome family enzyme that degrades RA. (A) Temporal sequence of the posterior ization process. At the late blastula stage,. the cyp26 gene is con fined to the anterior region by FGFs and Wnts from the margin. After the start of gastrulation, convergent extension takes the mar· gin farther from the anterior. RA accumulates in this region acti· vating genes associated with the posterior neural ectoderm.
(B) Pathway through which a boundary can form between anterior (cyp26-, Otx2-expressing) and posterior (haxb 7-, mids3-express ing) neural ectoderm. In the po◆terior region, FGFs and/or Wnt suppress anterior genes such as Otx2. The FGFMlnt signal in the posterior also suppresses expression of cyp26 (which encodes the enzyme that degrades RA) and enhances the expression of aldh 7a2 (which encodes the enzyme that synthesizes RA), enabl i ng RA to accumulate and activate the posterior genes.
(A◆er Kudoh et aJ. 2002; White et aJ. 2007.) FGF, , together with the dorsal marginal biastomeres (the precur sors of the Kupffer cells; see below) can be thought of as the Nieuwkoop center of the teleost fish embryo. Anterior-posterior axis formation The patterning of the neural ectoderm along the anterior posterior axis in the zebrafish appears to be the result of the interplay of FGFs, Wnts, and retinoic acid, similar to that seen in Xenopus. In fish embryos, there seem to be two separate processes. First a Wnt signal represses the expres sion of anterior genes; then Wnts, RA, and FCFs are required to activate the posterior genes. This regulation of anterior◆posterior identity appears to be coordinated by retinoic acid-4-hydroxylase, an enzyme that degr ades RA (Kudoh et al.
2002; Dobbs-McAuliffe et al. 2004). The gene encoding this enzyme, cyp26, is expressed specifica lly in the region of the embryo destined to become the anterior end. Indeed, this gene's expression is first seen during the late blastula stage, and by the time of gastrulation it defines the presumptive anterior neural plate. Retinoic acid-4-hydroxylase preven ts the accumula tion of RA at the embryo'S anterior end, blocking the expression of the posterior genes there. This inhibition is reciprocated, since the posteriorly expr essed FGFs and Wnts inhibit the expression of the cyp26 gene, as well as inhibiting the expression of the head-specifying gene Olx2. This mutual inhibition creates a border between the zone of posterior gene expression and the zone of anterior gene expression. As epiboly continues, more and more of the body axis is specified to become posterior (Figure 7ABA). Retinoic acid acts as a morphogen, regulating cell prop erties depending on its concentration. Cells receiving very little RA express anter ior genes; ceUs receiving high levels of RA express posterior genes; and those cells at intermedi· ate Jevels of RA express genes characteristic of cells bcnveen the anterior and posterior regions. This morpho geneSis is extremely important in the hindbrain, where dif ferent levels of RA specify different types of cells along the anterior-posterior axis (White et al. 2007).
FGFs positively regulate RA accumulation both by inhibiting the expres sion of cyp26 (which encodes the enzyme that degrades RA) and by enhanCing the synthesis of the enzyme that synthesizes RA from vitamin A (Figure 7A88). The result is a gradient of Cyp26, which in turn generates an RA gra dient that is stabl e and can be sustained throughout the time the embryo is elongating. See VADE MECUM: Retinoic acid as a teratogen Left-right axis formation In all vertebrates studied, the right and left sides differ both anatomicall y and developmenta l l y. [n fish, the heart is on (A) ◆ ca' / Notch, BMP, Pitx2 AMPH I B IANS A N D FISH ◆, _" Nodal cilia 283 (B) PGF signaling Kupffer's vesicle (node) Left Left-right asymmetlY in the zebrafish embryo. (AI Model for asymmetric gene expression . As in other vertebrates, Nodal-related genes are expressed solely on the embryo's left side: Nodal cilia in Kupffer's ves icl e create a current that releases Ca'·, st imulati ng the Notch and B MP4 pathways and activating the Pitx2 transcription factor in the left-hand mesoderm (blue).
On the other hand, FGF expression is secn predominantly on the right-hand side (red).
(6) Brain asymmetry in zebra fish, Antibody stai n i ng of the Leftover (red) and Right-on (gree n) proteins in neu rons of the habenular nucleus (a behavior-controlling region of the zebrafish forebra in) and the axonal projections to their mid brain target (the interpedu n cular nucleus) reveals marked asym metry. Most Leftover-positive axons emerge from the left haben ula to innervate the targeL (A after Okada et al. 2005; B ◆01l1 Gamse et al.
2005, courtesy of M. Halpern.) Righ t FIGURE 7,49 the left side and there are different structures in the left and right regions of the brain (Figure 7.49). Moreover, as in other vertebrates, the cells on the left side of the body are given that information by Notch and Nodal signaling and by the Pitx2 transcription factor, while the cells on the right side of the body are exposed to FGF Signaling, The ways the different vertebrate classes accomplish this asymme try differ, but recent evidence suggests that the currents produced by cilia in the node may be responsible for left right axis formation in all the vertebrate classes (Okada et a l. 2005); when the gene for a dynein subunit of cilia was cloned, it was found to be expressed in the ventral portion of the node (or organizer) in mouse, chick, Xenopus, and zebra fish embryos (Essner et aI, 2002, 200s), Using extreme ly rapid (500 frames! sec) photography, Okada and col leagues (2005) showed that the clockwise rotational motion of cilia and the leftward flow of particles were conserved in all these vertebrate groups, despite the different types and shapes of their nodal structures, In zebrafish, the nodal structure housing the cilia that ;:ontrol left-right asymmetry is a transient fluid-filled organ called Kup ffer's v esi cl e , As mentioned earlier, Kupffer's \·esicle arises from a group of dorsal cells near the embry >ruc shield shortly after gastrulation, Essner and colleagues 2002, 2005) were able to inject small beads into Kupffer's eside and see their translocation from one side of the vesi- expression nucleus de to the other, Blocking ciliary function by preventing the synthesis of dynein or by ablating the precursors of the cil iated cells prevented normal left-right axis formation, The cilia activate the Nodal-related genes on the lelt side of the embryo. These genes are critically important in initiating the cascades of paracrine factors that are specific to the left side of the body (Rebagliati et al. 1998; Long et al. 2003). Coda Researchers analyzing the development of Xenopus ia!!uis and Oauin rerio are finally putting names to the "agents" and "soluble ractors" postulated by the early experimen tal embryologists, We are also delineating the intercellular pathways of paracrine and transcription factors that con stitute the first steps in organogenesis. The international research program initiated by Spemann's laboratory in the 1920s is reaching fruition, and this research has revealed layers of complexity beyond anything Spemann could have conceived.
Just as Spemann's experiments told us how ruuch we didn't know, the answers we have found to these older questions have generated a whole new set of ques tions, The ability to perform live imaging of cells, the abil ity to delete and add new genes to particular cells, and the ability to produce mutant lines of zebrafish have all helped us to understand the mechanisms by which order is gen erated in the embryo. The remarkable conservation of mechanisms and molecules between model amphibian and fish species, despite the initial differences in their cleav age, shows very strong evolutionary constraints on how tills order comes .into being. 284 CHAPTER 7 Snapshot Summary: Early Development of Amphibians and Fish 1. Amphibian cleavage is holob lastic, but it is unequal due to the presence of yolk in the vegetal hemisphere. 2. A mphibian gastrulation begins with the invagina tion of the bottle cells, followed by the coordinated involution of the mesoderm and the epiboly of the ectoderm. Vegeta l rotation plays a significant role in di recting the involution. 3. The driving forces for ectodermal epiboly and the convergent extension of the mesoderm are the inter calation events in which several tissue layers merge. Fibronectin plays a critical role in enabling the meso dermal cells to migrate into the embryo. 4. The dorsal lip of the blastopore forms the organizer tissue of the amphibian gastr ula. This tissue dorsal izes the ectoderm, transforming it into neural tissue, and it transforms ventral mesoderm into lateral and dorsal mesoderm. 5. The organizer consists of pharyngeal endoderm, head mesoderm, notochord, and dorsal blastopore lip tissues. The organizer functions by secreting pro teins (Noggin, chordin, and follistatin) that block the BMP signal that would otherwise ventralize the mesoderm and activate the epiderma l genes in the ectoderm. 6. The organizer is itself induced by the Nieuwkoop center, located in the dorsalmost vegetal cells.
This center is formed by the translocation of the Dishev eled protein and Wntll to the dorsal side of the egg to stabilize p-catenin in the dorsal cells of the embryo. 7. The Nieuwkoop center is formed by the accumula tion of p-catenin, which can complex with Tcf3 to form a transc

*Frank M. Balfour proposed the homology of the amphib plate Primitive B I RDS AND MAMMALS 291 FIGURE 8.4 Migration of endoclermal and mesodermal s ea k. (A) Stereogram a gastrulating c i ck showing the relationship of th e migrating cells, and hypoblast and epi b l as t of blastoderm.
The lower layer becomes a mosaic of hypoblast and endodermal cells; the hypoblast cells eventually sort out form a layer beneath the endoderm and contribute to the yolk sac. Above each region of the ste eog am are micrographs showing the tracks of GFP-Iabeled cells at that positi n in the pri m i ti e streak. Cells migrating rough Hensen's node travel anteriorly to form the prech rda l plate and notochord; cells through the primitive tr of h embryo, primitive streak, the the the ◆:◆◆◆n◆o:d=e (A) ◆r◆:'◆::◆s:tr:ea:k=_ E◆: j as:t i.Blastocoel f J Pib:◆ =" _ _ _ to r r th v o o those migrating through the next anterior region of the streak travel laterally, but con verge near the midline make notochord and somites; those from the mi dd l e the streak form intermediate m esode rm and la eral plate mesode m (see the fate map in Figure 8.3). Farther posterior, t e cells m igrating through e p i iti e streak make the mesoderm (not shown), (8) This scanning electron m i c og raph shows epiblast cells passing into the blasto coel and extending their a pi cal ends to become bottle cells. (A after Balinsky 1 975, p h tograp s from Yang et al. 2002; Solursh and Re el 1 978, c u rtesy of M. Solu s and C. j. Weij er.) to r Migrating cells (mesoderm) th r m v t extraembryonic o v B from rh B) r h of h o l ELONGATION OF THE PRIMİTIVE STREAK As ce ls enter the primitive streak, they und ergo an epithelial-to-mesenchymal transformation, and the basal lamina beneath them breaks down. The streak elongates toward the future head region as more anterior ells migrate toward the center of the embryo. Cell division adds to the length pro duced by convergent extension, and some of the ells the anterior portion of the epiblast con tribute to the formation of Hensen's node (Streit et al. 2000; Lawson and Schoenwolf 2001 b). At the same time, the secondary hypoblast (endoblast) cells continue to migrate anteriorly from the posterior margin of the blastoderm (see Fig ure 8.2E). The elongation of the prim i tive streak appears to be coextensive with the anteri or migration of these secondary hypoblast cells, and the hypoblast directs the movement of the primitive streak (Waddington 1933; Foley et al. 2000). The streak eventual ly extends to 60-75% of the length of the area pellucida . c c from . the prechordal mesoderm, notochord, and medial part the somites. Cells that ingress through the middle of the -.eart and kidneys .
Cells in the posterior p ortion of the -:reak give rise to the lateral part of the somites and to the (Psychoyos and Stern 1996). Afte r the ing ression of eak make the lateral plate and extraembryonic meso ::erm :ae mesoderm cells, epiblast cells remaining outside, but to, the streak will form medial (dorsal) structures such the neUJal plate, while those epiblast cells farther from FORMATION OF ENDODERM AND MESODERM As soon as the primitive streak has formed, epiblast cells begin to migrate through it and into the blastocoel (Figure 8.4). The streak thus has a continually changing cell populatio n. Cells = streak will become epidermis (see Figure 8.3, right migrating through the anterior end pass down into the -.and panels). blastocoel and migrate anteriorly, forming the endoderm, 292 CHAPTER 8 head mesoderm, and notochord; ceUs passing through the more posterlor portions of the primitive streak give rise to the majority of mesodermal tissues (Rosenquist et al. Schoenwolf et al. 1966; 1992). The first cells to migrate through Hensen's node are those destined to become the pharyngeal endoderm of the foregut. Once deep within the embryo, these endodermal cells migrate anteriorly and eventually disp lace the hypoblast cells, causing the hypoblast cells to be confined to a region in the anterior portion of the area pellucida. This anterior region, the germin al crescent, does not form any embryonic structures, but it does contain the precur sors of the germ ceJJs, which later migrate through the blood vessels to the gonads (see Chapter 16). entering through Hensen's node also move anteriorly, but they do not travel as far ventrally as The next ceJJs the presumptive foregut endodermal cells. Rather, they remain between the endoderm and the epiblast to form the prechordal plate mesoderm (Psychoyos and Stern 1996). Thus, the head of the avian embryo forms anterior (rostral) to Hensen's node. The next cells passing through Hensen's node become the chordamesoderm. The chordamesoderm has two com ponents: the head process and the notochord. The most anterior part, the head process, is formed by central meso derm ceUs migrating anteriorly, behind the prechordal plate mesoderm and toward the rostral tip of the embryo (see Figures 8.3 and 8.4). The head process will underlie those cells that will form the forebrain and midbrain. As the prim itive streak regresses (see below), the ceUs deposited FIGURE 8.5 Time Medi ol ateral intercalation in the formation of the primitive streak. Chick embryos at (A) stage XII (immediately p rior to primitive streak formation) an d (B) stage 2 He wrote pathway in turn appears to be activated by fibroblast growth factors (FCFs) produced by the hypoblast. If the hypoblast is rotated, the orientation of tlie primitive streak foHows it. Moreover, if FGF signaling is activated in the margin of U,e epiblast, Wnt signaling will occur there, and the orientation of the primitive streak will change, as if tile of labeled cells under the microscope in hypoblast had been placed there. The cell migrations that cour tly dance wherein men and women move form the primitive streak thus appear to be regulated by that these movements reminded him of the Polonaise, a in para llel rows along the sides of the room, and then a man and a FGFs coming from the hypoblast, wruch activates the Wnt planar cell polarity pathway in the epiblast. woman at the "posterior end" leave their respective lines to dance forward through the center. The mechanism for ' this "dance" was revealed by Voiculescu and colleagues (2007), who used a modern version of cinemicrography PREPARING TO MIGRATE The basic rule of amniote ceU spec ification is that germ layer identity (ectoderm, mesoderm, or endoderm) is established before gastrulation, but the (specifically, multi photon time-lapse microscopy) that specific type of cell is controlled by ind uctive influences could identify individual moving cells. They found that during and after migration through the primitive streak.
cells came down the sides of the epiblast to undergo a Chapman and colleagues mediaUy directed .intercalation of cells in the posterior mar cells from one area of quail embryos to a different area in gin where the primitive streak was forming (Figure 8.5). (2007) transplanted groups of chick embryos and showed that presumptive mesoderm And wrule the movement may look like a dance from far cells remain mesodermal and w ill away, "at high power, it looks like a rush hour" (Stern layer, even if they enter the primitive streak from a new 2007). This rush to the center is mediated by the activation of the Wnt planar cell polarity pathway (see Chapter 3) in (usually endodermal) si te Similarly, presumptive endo dermal cells transplanted to a new position (where prc the epiblast next to Koller's sickle, a t the posterior edge of sumptive mesodermal cells usually enter the streak) the embryo. If this paU,way is blocked, the mesoderm and migr..te to the middle . ingress into the lower layer and become epithelial. BI RDS A N D MAMMALS (A) Hoxb4 (B) Hoxb9 While they are still in the epiblast, but close to the prim itive streak, the mesoderm cells appear to receive instruc tions that tell them exactly where they are along the ante rior-posterior axis (Iimura and Pourquie 2006). At this point, they are induced to activate specific Hox genes. As we will detail later in the chapter, Hox genes are the ver tebrate homologues of the homeotic (Hom-C) genes of Drosophila.
And just like in Drosophila embryos, vertebrate Hox genes specify the identity of cells along the anterior posterior axis of the embryo.
In the case of vertebrates, however, there are four gene clusters (HoxA, HoxB, HoxC, and HoxD) instead of just one, and rather than individual Hox genes appearing at particular segmental levels, there is a nested set of Hox gene expression. For example, the mesodermal precursor cells are patte◆ed along the ante rior posterior axis by the HoxB genes, which appear to inform the cells when to leave the epiblast and ingress into the primitive streak.
"Anterior" Hox genes (which are iden tified with lower numbers, e.g., Hoxb4) are expressed early and extend farther anterior than genes such as Hoxb9, which is expressed later and does not extend as far into the embryo's anterior (F ig u re 8.6). Thus, the more posterior cells have more Hox genes expressed than the more ante rior cells. 293 8.6 Hox gene activation begins when the mesodermal precursor cells are stil l in the epiblast. These genes are activated in an anterior-to◆posterior fashion. Migration into the primitive streak is reguM lated by the Hox gene expression pattern (the most posterior Hox gene having preference). (A) Hoxb4 expression in stage 4, 5 , and 6 chi ck embryos. (8) Hoxb9 expression in stage 7, 8, and 8+ chi ck embryos. Note that the anterior border of expression is more posterior than the anterior border of Hoxb4 expression. (From limura and Pou rqu ie 2006, cour tesy of o. Pourquie.) FIGURE through the primitive streak (see Figure 8.4).
They were able to deflect these normal trajecto ries by using beads that released Fgf8. Once inside the embryo, the 'lateral movement of the mesoderm cells appears to be regulated in part by platelet-derived growth factor (PDGF), a paracrine lactor made by the epiblast (Yang et al. 2008). Meanwhile, cells continue migrating inward through the primitive streak. As they enter the embryo, the cells separate into two layers. The deep layer joins the hypoblast along its midline, displac ing the hypoblast cells to the sides. These deep moving cells give rise to all the endodemlal organs of the embryo, as well as to most of the extraembryonic membranes (the hypoblast and peripheral cells 01 the area opaca lorm the rest). The second migrating layer spreads to form a loose layer of cells between this endoderm and the epiblast. This middle layer of cells generates the mesodermal portions of the embryo and the mesoderm lining the extraembry onic membranes.
Movement away lrom tihe streak appears to be guided by Fgf8-mediated chemorepulsion, and the further move ment of the mesodermal precursors appears to be regu lated by Wnt proteins. In the more posterior regions, WntSa is unopposed and directs the ceUs to migrate broad ly to become the lateral plate mesoderm. In the more ante rior regions of the streak, however, WntSa is opposed by Wnt3a, which inhibits migration and causes the cells to lorm paraxial mesoderm. Indeed, the addition of Wnt3a secreting pellets to the posterior primitive streak suppress es lateral migration and prevents the formation of lateral plate mesoderm (Sweetman et al. 2008). By 22 hours of incubation, most of the presumptive endodermal cells are in the interior of the embryo, although presumptive meso dermal cells continue to migrate inward lor a longer tirile. MIGRATION THROUGH THE PRIMITIVE STREAK The migration of the mesodermal cells through the anterior primitive streak and their condensation to form the chordamesoderm appear to be controlled by FGF signaling. Fgf8 protein is expressed in the primitive streak and repels migrating cells away from the streak. Yang and colleagues (2002) were able to follow the trajectories 01 these ceIls as they migrated Regression of the primitive streak and epiboly of the ectoderm Now a new phase of development begins. As mesodermal ingression continues, the primitive streak starts to regress, moving Hensen's node from near the center of tihe area pel- ---------- 294 CHAPTER 8 (A) (e) (8) Blood islands Head fold streak (D) IE) d ----ir----ir----◆----t_ '0 '" o c d' 1] t b ◆ ◆ c b' c ◆ '2 c' Posterior border of pellucida area 0.0 10.5 20.5 Hours lucida to a more posterior position (Figure 8.7). The regress ing streak leaves in its wake the dorsal axis of the embryo, including the notochord.
The notochord is laid down in a head-to-tail direction, starting at the level where the ears and hindbrain form and extending caudally to the tail bud. As in the frog, the pharyngeal endoderm and head mesoendoderm will induce the anterior parts of the brain, while the notochord will induce the hindbrain and spu,al cord. By this time, all the presumptive endodermal and mesodermal cells have entered the embryo and the epi blast is composed entirely of presumptive ectodermal cells. While the pres umptive mesodermal and endodermal cells are moving inward, the ectodermal precursors prolif erate and migrate to surround the yolk by epiboly. The of the epiboly of the amphibian ectoderm) enclosure of the yolk by the ectoderm (again, reminiscent is a Herculean task that takes the greater part of 4 days to comple te. 11 involves the continuous production of new cellular mate- FIGURE 8.7 Chick gastrulation 24-28 hours after fertilization.
(A) The primitive streak at full extension (24 hours). The head process (anterior notochord) can be seen extending from Hensen's node.
(6) Two-somite stage (25 hours). Pharyngeal endoderm is seen anteriorly, while the anterior notochord pushes up the head process beneath it. The primitive streak is regressing, (q Four somite stage (27 hours). (0) At 28 hours, the primitive streak has regressed to the caudal portion of the em bryo. (E) Regression of the primitive streak, leaving the notochord in its wake. Various points of the streak (represented by letlers) were followed after it achieved its maximum length. The x ax is (time) represents hours after achiev ing maximum length (the reference line is about 1 8 hours of incu bation). IA-O courtesy of K. l i nask; E after Spratl l 947.l rial and the migration of the presumptive ectodermal cells along the underside of the vitelline envelope (New 1959; Sp ratt 1963).
Interestingly, only the cells of the outer mar gin of the area opaca attach firmly to the vitelline envelope. These cells are inherently different from the other blasto derm cells, as the y can extend enormous (500)lm) cyto plasmi c processes onto the vitelline envelope. These elon- B I RDS A N D MAMMALS gated filopodia are believed to be the locomotor appara tus of the marginal cells, by which the marginal cells pull other ectodermal cells around the yolk (Schlesinger 1958). The filopodia bind to fibronectin, a laminar protein that is a component of the chick vitelline envelope. If the contact between the marginal cells and the fibronectin is experi mentally broken by adding a soluble polypeptide similar to fibronectin, the filopodia retract and ectodermal migra tion ceaSes (Lash et a1. 1990). Thus, as avian gastrulation draws to a dose, the ecto derm has surrounded the yolk, the endoderm has replaced the hypoblast, and the mesoderm has positioned itself between these two regions. Although we have identified many of the processes involved in avian gastrulation, we are only beginning to understand the molecular mecha nisms by which some of these processes are carried out. See WEBSITE 8.1 Epiblast cell heterogeneity See VADE MECUM Chick development Axis Specification and the Avian "Organizer" As a consequence of the sequence in which the head endomesoderm and notochord are established, avian (and mammalian, reptilian, and teleost fish) embryos exhibit a distinct anterior-to-posterior gradient of developmental maturity. While cells of the posterior portions of the embryo are still part of a primitive streak and colonizing the meso derm, cells at the anterior end are already starting to form organs (see Darnell et a!. 1999).
For the next several days, the anterior end o f the embryo is more advanced in its development (having had a "head start," if you will) than the posterior end. Although the formation of the chick body aXes is accomplished during gastrulation, axis specification begins earlier, during the cleavage stage. The role of gravity and the PMZ The conversion of the radially synuneiricaJ blastoderm into a bilaterally symmetrical structure appears to be determined b y gravity. As the ovum passes through the hen's reproduc tive tract, it is rotated for about 20 hours in the shell gland.
(A) Surface view of egg (B) Cross section through egg 295 This spinning, at a rate of 10-12 revolutions per hour, shifts the yolk such that its lighter components (probably contain ing s tored maternal determinants for development) lie beneath one side of the blastoderm. This imbalance tips up one end of the blastoderm, and that end becomes the posterior marginal zone (PMZ) of the embry (C) Surface view of yolk FIGURE 8.8 Specification of the chick anterior-posterior axis by gravi ty.
(A) Rotation in the shell gland results in (8) the lighter components rotates in shell of the yolk pushing up one side of the blastoderm. (C) This m ore elevat ed region becomes the posterior of the embryo. (Aher Wolpert et a1. 1 998.) 296 CHAPTER 8 I Anterior (A) Koller's r- ji (B) FIGURE 8.9 Formation of Hensen's node from Kollel◆s sickle. (A) Diagram of posterior end of an early (pre-streak) embryo, showing the cells labeled wilh nuorescent dyes i n the photographs. (B) Just hefore gastrulation, cells in the anteri or end of Koller's sickle (the epiblast and middle layer) were labeled with green dye. Cells of the posterior portion of Koller's sickle were labeled with red dye As the cells migrated, the anterior cells formed Hensen's node and its notochord derivatives. The posterior cells formed the posterior region of the primitive streak.
The time after dye injection is labeled on each photograph. (A after Bachvarova et 01. 1 998; B courtesy of R. F. Bachvarova.) Epiblast sickle . Marginal zone Posterior marginal zone (PMZ) Area opaca Poster ior primitive streak in the posterior epiblast. Once formed, however, the streak secretes its own Nodal antagonist (the Lefty protein), thereby preventing any further primitive streaks from forming. Eventua lly, the Cerberus-secreting hypoblast cells are pushed to the future anterior of the embryo, where they contribute to ensuring that neural cells in this region become forebrain rather than more posteri or structures of the nervous system. The chick "organizer" The "organizer" of the chick embryo forms from cells ini tially located just anterior to the posterior marginal zone.
(A) (B) Transplantation of duck Hensen':) node The epiblast and middle layer cells in the anterior portion of Koller's sickle become Hensen's node, as described ear lier. The posterior portions of Kolier's sickle contribute to the posterior portion of the primitive streak (Figure 8.9). Hensen's node has long been known to be the avian equiv alent of the amphibian dorsal blastopore lip, since (1) it is the region whose cells become the prechordal plate and chordamesoderm, (2) it is the region whose cells can both induce and pattern a second embryonic axis when trans planted into other locations of the gastrula (Figure 8.10; Waddington 1933; 1934; Gallera 1966; Nicolet 1970), and (3) it expresses the same marker genes as Spemann's organ izer in amphibians and the embryonic shield 01 teleost fish es, such as the transcription factor Goosecoid (Izpisua-Bel monte et al. 1993). Moreover, Hensen's node can induce Induced neural tube Host embryo 2° neural tube 2° head forming Host head forming Duck embryo FIGURE 8.10 I nduction of a new embryo by Iransplantation of Hensen's node. (A) A Hensen's node from a duck embryo is transplanted into the epiblast of a chick embryo. (8) A secondary embryo is i nduced (as is evident by the neural tube) from host tissues at the graft site.
(C) C raft of Hensen's node from one embryo into the periphery of a host embryo. After further incubation, the host embryo has a neural tube whose regionaliza- tion can be seen by i n situ hybridization. Probes to OLX2 (red) recognize the head region, while probes to Hoxb 1 (blue) recognize the trunk neural tube. The donor node has induced the formation of a secondary axis, complete with head and trunk regions. (A,B aher Waddington 1 93 3 ; C from Boettger et al. 2001.) Host neural tube BIRDS AND MAMMALS (B) ----- , (A) 297 (el __"; _ , Hensen's node _f, _Hensen's_ _ node Nogg in BMP?
FIGURE 8.11 Possible contribution to chick neural induction by the inhibition of BMP signaling. IA) In a neurulating embryo, Nog gin protein (purple) is expressed in the notochord and the pharyn geal endoderm. (B) Bmp?
expression (dark purple), which had encompassed the entire epiblast, becomes restricted to the non neural regions of Ihe ectoderm. (e) Similarly, the product of BMP signaling, the phosphorylated form of Smadl Irecognized hy anti bodies to the phosphorylated form of the protein, dark brown) is not seen in the neural plate. (From Faure et al. 2002, cou rtesy of the authors.) neural tissue when grafted into fish, amphibian, or mam malian embryos (Waddington 1936; Kintner and Dodd 1991; Hatta and Takahashi 1996). As is the case in all vertebrates, the dorsal mesoderm is able to induce the formation of the central nervous system '1 the ectoderm overlying it. The cells of Hensen's node and its derivatives act like the amphibian organizer, and they secrete bone morphogenetic protein (BMP) antago nist proteins such as chordin, Noggin, and Nodal. These proteins repress BMP signaling and dorsalize the ectadenm dnd mesoderm (Figure 8.1 1). However, repression of the B.v1 P sig!lal by these antagonists does not appear to be suf Cicient for neural induction (see St◆rn 2005). Fibroblast ◆rowth factors synthesized in the hypoblast and in Hensen's node precursor cells just prior to gastrulation ◆ppear to be critical lor preparing the epiblast to generate 'lemonal phenotypes. Although FCFs can block BMP sig -aling, this does not account for the ability of FCFs to duce a transient expression of pre-neural genes in the iblast (Streit et al. 1998, 2000). These neural genes do not -!ay active unless they are supported by BMP antagonists 5treit et aJ. 1998, 2000, Albazercru and Stern 2006).
Thus, "CF signaling inhibits BMPs from inducing the genes that -pecify ectoderm to become epidermis, and they activate -;..e genes that specify to ectoderm to become neural. Indeed, fibroblast growth factors play four fundamental - ◆es in cell specification during gastrulation. • • First, as in all vertebrates, FGFs are responsible for spec iiying the mesoderm. FCFs lrom the hypoblast (in col- Smadl laboration with Nadal from the. posterior marginal zone) accomplish this specification by activating the BrachyuMj and Tbx6 genes in the cells passing through the primi tive streak (Figure 8_12; Sheng et al. 2003). • Second, FGFs separate mesoderm formation from neu rulation_ The mechanism by which FCFs help end meso derm ingression and stabilize the epiblast appears to be due to a gene called Churchill.
While FCFs are rapidly indUCing the mesoderm, they are also slowly inducing activation of Churchill in the ectoderm. The Churchill protein (so named because the protein's two zinc fingers extend like the British prime minister's famous "V for Victory symbol) can activate the Smad-interacting pro tein SIPl. SIPI controls the genes whose expression is required for ingression 01 cells through the primiti ve streak. Thus, once activated, SIP1 helps prospective neu ral plate cells remain in the epiblast. " • Third, FCFs help bring about neurulation in the central ectodermal cells. SIP1, probably through its interaction with Smadl, may make tile prospective neural plate cells less sensitive to BMP. • Fourth, FCFs induce ERN! and 5m3, two pre-neural genes that initiate the Signaling cascade leading to the production of neural tissue.
Thus, FCFs appear to be critically important regulators of cell fate in the early chick embryo (Streit et al. 2000; Sheng et al. 2003; Albazerchi and Stern 2006). Anterior-posterior patterning The patterning of the definitive anterior-posterior axis occurs differently for the mesoderm and neural ectoderm, but in both cases the process involves timing (the sequen tial generation of cells from a zone of undifferentiated pro liferating cells) and the influence 01 caudalizing molecules. In the ectoderm, most of the initial neural plate corre sponds to the future head region (from lorebrain to the level of the fu ture ear vesicle, which lies adjacent to Hensen's node at full primitive streak stage). A small 298 CHAPTER 8 ◆ ... ◆ C (A) ..3◆ _____Uncommitted cells ERNl, Sox3 ◆ t Hypoblast FgfB , (in epiblast) Nodal T , Cerberus; t FGF8 - Endoblast cells FIGUREJl.12 Model by which FGFs regulate mesoderm formation and neurulation. (A) Stage XI, where the hypoblast (green) secretes Fgf8, which induces pre-neural genes ERNI and 50x2 (blue) in the epi blast. The cells in this domain, however, remain uncommitted. Nodal, expressed in the posterior epiblast, cannot functioni it is inhibited by the Cerberus protein secreted by the hypoblast. (B) At around stage 1 , the hypoblast i s displaced from the posterior edge by the endoblast (secondary hypoblast; gold), allowing Nodal to function. Nodal plus Fgf8 induces Brachyury and Tbx6 expression to specify the mesoderm and initiate the ingression of mesoderm cells through the primitive streak (red). (C) At stage 4/ continued Fgf8 expression activates Churchill in the epi b last. (0) By end of stage 4, Churchill protein induces SIP1, which blocks Brachyury and Tbx6, preventing further ingression of epiblast cells through the streak. The remaining epiblast cells can now become sensitized to neural inducers from Hensen's node (purple). (Aher Sheng et al. 2003.) "young" and undifferentiated as it regresses, and that this is antagonized by retinoic acid (RA) ac tivity as cells leave this zone (Figure 8.13; Diez del Corral et aJ. 2003). In the mesodenn, the anterior-posterior axis is also relat ed to time. The entire length of the notochord at the mid line is derived from cells that are present in Hensen's node by the fulJ prin1itive streak stage. A population of progen itor cells remains in the node; their descendants gradual (C) ly leave as the node regresses, laying down the chordame plate of the spinal cord) (Selleck and Stern 1991; Psychoyos and Stem 1996; Tzo uanaco u et al. 2009). Therefore anterior-posterior identities along the axis from soderm and the ventral midline of the neural tube (the future floo r ◆ TFgf8t c= ◆ : ◆ Th 'cerberus the hindbrain to the tail are specified as a function of the time of emergence from the primitive streak and Hensen's node. [t has been proposed that the length of time cells are Endoblast resident in this region determines which Hox genes are can also be under the influence of the FGF and retinoic acid expressed by the cells. This pattern of Hox gene expression gradients (Gaunt 1992; Wilson et al. 2009). Left-right axis formation The vertebrate body has distinct right and left sides. The heart and spleen, for instance, are generally on the left side .
of the body, while the liver is usually on the righ t The dis Neural ◆: i induction Tb x6 - ... -;;;;;;" "' ...1 '\T tinction between the sides is primarily regulated by two proteins: the paracrine factor Nodal and the transcription factor Pitx2. However, the mechanism by which nodal gene expression is activated in the left side of the body differs among the vertebrate classes. The ease with which chick -the pathways of left-right axis determination in birds more readily than in other vertebrates. embryos can be manipula ted has allowed scientists to elu region of neural ectoderm just lateral and posterior to the give rise to the rest of the nervous system, includ ing the node (sometimes called the caudal lateral epib l as t) will and all of the spinal cord. As the prim itive streak regresses, this latter region regresses with the posterior hindbrain node and adds cells to the caudal end of the elongating neural plate. [t appears that FGF signaling in the streak and paraxial (future somite) mesoderm keeps this region cidate As the primitive streak reaches its maximum length, sonic hedgehog (shll) e mbryo, controlled b y activin and its receptor (F i gure 8.14A). Activin signaling, along with BMP4, appears to block the expression of Sonic hedgehog and to activate expression of Fgf8 protein on the transcription of the restricted to the left side of the gene becomes BIRDS A N D MAMMALS ROSTRAL o o ◆ Neurons 1 ,5 , _ I ◆ RA , ◆ _◆ c-, 3,4 CAUDAL I 299 FIGURE 8.
1-3 Signals that regulate axis extension in chick embryos.
In the stage 1-0 chick embryo, FgfB inhibits expression of the retinoic acid (RA) synthesizing e nzyme Raldh2 in the presomilic mesoderm (1) and the expression of the retinoic acid receptor RAR◆ in the neural ectoderm (4), thu s preventing RA from triggering differentiation in the caudal-later al epiblast cells (those cells adjacent to the node/streak border and which give rise to lateral and dorsal neural tube) and the caudalmost paraxial mesoderm (1,5). In add itio n , FgfB inhibits Sonic hedgehog (Shh) expression in the neural tube noorplate, controlling the onset of ventral patterning genes (1). FGF signaling is also required for expression of Delta1 in the medial portion of the caudal lateral epi blast cells (2) and promotes expression of Wnt8c (4). As Fgf8 decays in the caudal paraxial mesoderm, Wnt signal i ng, most likely provided by WntBc, now acts to promote Raldh2 in the adjacent paraxial mesoderm (4). RA produced by Raldh2 activity represses FgfB (1) and WntBc (3,4). (After Wilson et al. ----------, 2009.) , o Raldh2/RARP 0 Fgf8 0 Shh right side of the embryo. FgfB blocks e xpression of the paracrine factor Cerberus on th e right-hand side; it may also activate a Signaling cascade that instructs the mesoderm to have right-sided capacities (Schlueter and Brand 2009). Meanwhile, on the left side of the body, 5hh protein ac tiv a tes Cerberus (F igu re B.14B), w h ich in this case acts with BMP to stimulate the synthesis of Nodal pro tein (Yu et a1. 2008). Nodal activates the pitx2 gene while repressing snail.
In a ddition, Leftyl in the ven tral midline prevents the Cerberus signal from pass ing to the right side of the embryo (Figure 8.14(,0) As in Xenopus, Pitx2 is crucial in directing the asymmetry of the embryonic structures. Experimental ly induced expression of either Nodal or Pitx2 on the right side (A) (B) LEFT RIGHT Hensen's node "'- I ◆MP,J . . . . t Cerberus \ .".
Nodal . ... J. Snail \ Shh /-- Activin FgfS --- BMP Shh ◆ Cerberus Pitx2 Midline FIGURE 8.14 Model for generating l eft-right asymmetry in the chick embryo.
(A) On the left side of Hensen's node_ Sonic hedgehog (Shh) activates Cerberus, which sti m u late BMPs to induce the expression of Nodal. In the presence of Nodal, th e pitx2 gene is activated. Pitx2 pro tein is active in the various organ primordia and spec ifies which side will be the left. On the right side of the em bryo, activin is expressed, along with activin receptor IIa. This activates Fgf8, a protein that blocks exp res sion of the gene for Cerberus. I n the absence of Cerberus, Nodal is not activated and thus Pitx2 is not expressed. (B) Whole-mount in situ hybridization of cerberus mRNA. This view is from the ventral surface ("from below", so the expression seems to be on th e right. Dorsally, the expression pattern would be on the leh. (C) Who le-mo u nt i n si tu hybridization using probes for the chick nodal message (stained purple) shows its expression in the lateral pl a te mesoderm only on the left side of the embryo.
This view is from the dorsal side. {DJ Similar in situ hybridization, using the probe for pitx2 at a later stage of development. The embryo i s seen from its ventral surface. At this stage, the heart is forming, and pitx2 expression can be seen 0!1 the left side of the heart tube (as well as symmetrically in more anterior tissues. (A after Raya and Izpisua-Belmonte 2004; B from Rodriguez-Esteban el al. 1 999, courtesy of) . Izpisua-Belmonte; (courtesy of C. Stern; 0 from Logan et al. 1 99B, courtesy of C. Tabin.) 300 CHAPTER 8 of the chick reverSes the asyrrunetry or cause randomiza tion of asymmetry on the right or left sides' (Levin et al. 1995; Logan et al. 1998; Ryan et al. 1998). The real mystery is, What processes create the originaJ asymmetry of Shh and Fgf8? One important observation is that the first asymmetry seen during the formation 01 Hensen's node in chicks involves Fgf8- and Shh-express ing cells rearranging themselves to converge on the eight hand side of the node (Cui et al. 2009; Gros et al. 2009). Therefore, the differences in gene expression can be traced back to differences in cell migration to the right and left sides of the embryo. What establishes this initial asymme try is still unknown. EARLY MAMMALIAN DEVELOPMENT Cleavage It is not surprising that early mammalian development has been incredibly difficult to study.
Mammalian eggs are among the smallest in the animal kingdom, making them hard to manipulate experimentally. The human zygote, for instance, is only 100 ◆ in diameter-barely visible to the eye and less than one-thousandth the volume of a Xenopus {nevis egg. Also, mammalian zygotes are not produced in numbers comparable to sea urchin or frog zygotes; a female mammal usually ovulates fewer than 10 eggs at a given time, 50 it is difficult to obtain enough material for biochemical studies. As a final hurdle, the development of mammalian embryos is accomplished inside another *tn humans, homozygous loss of PITX2 causes Rieger's syndrome, a condition characterized by asymmetry anomalies. A similar con- dition is caused by knocking out the Pitx2 gene in mice (Fu et at. 1999; Lin et aJ. 1999). organism rather than in the external environment. Only recently has it been possible to duplicate some of these internal conditions and observe mammalian development in vih"o. The unique nature of mammalian cleavage It took time to surmount the difficulties, but our knowl edge of mammalian cleavage has turned out to be worth waiting for.
Mammalian cleavage is strikingly different from most other patterns of embrYOnic cell division. Prior to fertilization, the mammalian oocyte, wrapped in cumulus cells, is released from the ovary and swept by the fimbriae into the oviduct (Figure 8.1 5). Fertilization occurs in the ampulla of the oviduct, a region close to the ovary. Meiosis is completed aft◆r sperm entry, and the first cleavage begins about a day later (see Figure 4.35). Cleav ages in mammalian eggs are among the slowest in the ani mal kingdom, taking place some 12-24 hours apart. Mean while, the cilia in the oviduct push the embryo toward the uterus; the first cleavages occur along this journey. In addition to the slowness of cell division, several other features distinguish mammalian cleavage, including the unique orientation of mammalian blastomeres with rela tion to one another. The first cleavage is a normal merid ional division; however, in the second cleavage, one of the two blastomeres divides meridionally and the other divides equatorially (Figure 8.1 6). This is called rotational cleavage (Gulyas 1975). Another major difference between mammalian cleav age and that of most other embryos is the marked asyn chrony of early ceU division. Mammalian blastomeres do not all divide at the same time. Thus, mammalian embryos do not increase exponentially from 2- to 4- to 8-cell stages, but frequently contain odd numbers of cells.
And the mam- Zona pellucida Early stage of implantation FIGURE 8.15 Developm en t al a human embryo from ferti l izat ion to implantation. Compaction of th e human em bryo occurs on day 4, at the 1 O cel l stage. The embryo "hatches" from the zona pell ucida upon reach ing the uterus. During its migration to the uterus, the zona prevents the em bryo from prematurely adh ering to the oviduct rather than trave l ing to the uterus. - B I R DS A N D MAMMALS (A) Echinoderm and amphibian \ Cleavage plane II Cleavage plane 1 I (B) Mammal Cleavage plane IIA \ 301 Compaction Cleavage plane I I One of the most crucial differences between mammalian cleavage and all other types involves the phenomenon of compacti on Mouse blastomeres through the 8-cell stage form a loose arrangement with plenty of space betw een them (Figure 8.1 7A,B). Following the third cl eavage, how ever, the blastomeres Wldergo a spectacular change in their behavior. C el l adhes ion proteins such as E-cadherin become expressed, and the blastomeres suddenly huddle together and form a compact ball of cells (Figure 8.17C,D; Peyrieras et a!. 1983; Fleming et a!. 2001). This t igh tl y packed arrangement is stabilized by tight j unctions that form betwe en the outside cells of the ba l l, sealing off the inside of the sphere.
The cells within the sphere form gap j unctions, thereby enab ling small molecules and ions to pass between them .
I/T==◆J \ Cleavage plane IIB FIGURE 8.16 Comparison of early cl eavage in (A) echinoderms and amphibians (radial c l eavage) and (B) mammals (rotational c leavage) Nematodes also have a rotational form of c leavage, but they do not form the blastocyst structure characteristic of mam mals. (After Gulyas 1 975.) . See WEBSITE 8.2 Mechanism's of compaction and formation of the inner cell mass . See WEBSITE 8.3 Human cleavage and compaction. The cells of the compacted 8-cell embryo divide to pro duce a 1 6-cell morula (Figure 8.17E). The momla consists of a smal l group of internal cells surrounded by a larger group of externa l cells (Barlow et a!. 1972). The internal cells a re called the inner cell mass ([CM). They will give rise to the embryo. Most of the descendants of the exter nal cells become the tro ph ob l ast (trophectoderm) cells. This g roup of cells produces no embryonic structures . Rather, it forms the tissu e of the chorion, the emb ryonic malian genome, unlike the genomes of rapidly develop mg animal s, is activated du ring early cleavage, and it is ti,e newly formed nuclei (rather than the oocyte cytoplasm) th at produce the pro teins necessary for cleavage and devel opment. In the mouse and goat, the switch from maternal to zygotic control OCCliIS at the 2- 1989). Most research on mammalian development has focused on the mouse since mice are relatively easy to breed, have large litters, and can be housed easily in laboratories 11ms, most of the s tudies discllssed here will concern murine developmen t , FIGURE 8.17 Cleavage of a single mouse embryo i n vitro. (A) 2cell stage. (8) 4-cell stage.
(C) Early 8-cell stage. (D) Compacted 8cell stage. (E) Morula. (F) B lastocyst. (G) Electron micrograph . through the center of a mouse blastocyst. (A-F from Mulnard -1967, courtesy of J. G.
Mulnard; G from Ducibella et " I. 1 9 75, courtesy ofT. Ducibella.) .
A) (B) (C) D) (E) (F) (G) 302 CHAPTER B portion of the placenta. The chorion enables the fetus to asymmetrically localized in the fertilized egg, and there get oxygen and nourjshment from the mother. It also does not seem to be any predetermined polarity. secretes hOImones that cause the mother's uterus to retain The mouse embryo proper is derived from the inl1er ceU of the 16-cell stage, supplemented by cells dividing to the 32-ceU stage (Pedersen et a1. 1986; Fleming 1987; Bischoff et al. 2008). The cells of the rCM-w ill give rise to the embryo and its associa ted yolk sac, allantois, and the fetus and produces regulators of the inunw1e response mass will not reject the embryo (as she would an organ gra ft). [t is important to remember that the cru from the outer ceUs of the morula during the transition so that the mother cial outcome of these first divisions is to generate ceUs that will stick to the uterus. Thus, formation of the trophecto derm is the first differentiation event in mamma lian devel amnion. By the 64-cell stage, the irmer cell mass (approxi opment. These external cells first adhere to the uterine lin mately 13 ceUs) and the trophoblast cells have become sep that allow s the embryo to lodge wall. to the other a1. 1987; Fleming 1987). The inl1er cell mass ing, then digest a path arate cell layers, with neither contributing cells itself in the uterine group (Dyce et l! is a matter of chance which cells become trophoblast actively supports the trophoblast, secreting proteins such as and which become inl1er cell mass (see Dard et a1. 2009) . Fgf4 tha t stimulate the trophoblast cells to divide (Tanaka Indeed, tin1e-lapse photomicroscopy appears to show that et a!. 1998). the plane of the first division is totally irrelevant to the allo The earliest blastomeres (such as each blastomere of an embryo) can form b oth trophoblast cells and the cation of blastomeres along the embryonic/ abembryonic 8-cell the position of the inl1er ceU mass within the trophoblast to the area of the trophoblast d irect ly opposite the reM). The descendants of both of the first embryo precursor ceUs. These very early ceUs are said be totipotent (Latin, "capable of everything"). The inl1er two blastomeres were seen to contribute to the trophoblast things"). That is, each cell of the rCM can generate any ceU and to the inl1er cell mass (Hfuagi and Solter 2004; Motosu type in the body, but because the distinction between reM axis (the axis defined by gi et a1. cell mass is said 2005; Kurotaki et a1. 2(07). The mammalian embryo to be pluripotent (Latin, "capable of many and trophoblast has been established, it is thought that rCM cells are not able to form the trophoblast. has no known proteins or other subcellular components SIDEL IGHTS SPECULATIONS Trophoblast or I CM? T decision to become either tro phob la st or inner cell mass b las tomere is the fjrst binary decision in OUf l ives. Pr io r to bl astocyst forma tion

red blood cells are seen in the maternal blood circulation. apposed See WEBSITE 8.4 Placental functions *The.re are numerous types of placentas, and the extraembryonic membranes form differently in different orders of marrunals (see Cruz and Pooersen 1991). Although mice and humans gastrulate and implanl in a similar fashion, their extraerubryonic structures are distinctive. It is very risky to extrapolate developmental phe nomena from one group of mammals to another. Even Leonardo da Vinci gol caught (Renfree 1982). His remarkable drawing of the human felus inside the placenta is stunning art, but poor science: the placenta is that of a cow. a l r BIRDS AND MAMMALS 309 SIDEL IGHTS SPECULATIONS Twins and Chimeras T he early cells of the mammalian embryo can replace each other and compensate for a missing cell. This regulative ability was first demonstrated in 1 952, when Seidel destroyed one cell of a 2-cell rabbit embryo and the remaining cell pro duced an entire embryo. Once the inner cell mass (ICM) has become sep arate from the trophoblast, the ICM cells constitute an equivalence group. In other words, each ICM cell has the same potency (in this case, each cell can give rise to all the cell types of the embryo, but not to the trophoblast), and their fates will be detenmined by interactions among their descendants. Gardner and Rossant (1 9 76) also showed that if cells of the ICM (but not trophoblast cells) are injected into blas- tocysts, they contribute to the new embryo. Since the ICM blastomeres can generate any cell type in the body, the cells of the blastocyst are referred to as pluripotent. The regulative capacity of the ICM blastomeres is also seen in humans. Human tvvins are classified into two major groups: monozygotic (one egg, or identical) twins and dizygotic (two egg, or fraternal) twins. Fraternal twins are the resu lt of two separate fertiliza tion events, whereas identical twins See WEBSITE 8.5 Nonidentical monozygotic twins See WEBSITE 8.6 Conjoined twins 2-cell embryo Figure 8.25 Diagram showing the timing of human monozygotic twi n ning with relation to extra.- embryonic membranes. (C) (A) Splitting occurs before formation of the t roph o blast, so each twi n has its own chorion and amnion. (B) Splitting occurs after trophoblast formation but before amnion for- ation, r su l ti ng in twi ns having individual amnionic sacs bu t shari ng one chorion. (C) Splitting alter ammon formation leads to twi ns in one amnionic sac and a si n gl e chorion. (After Langman t981.) are formed from a single embryo whose cells somchow become dissoci ated from one another, Identical tvvi ns may be produced by the separation of early blastomeres, or even by the sepa ration of the inner cell mass into two regions within the same blastocyst. Identical twins occur in roughly 0.25% of human births. About 33% of identical twins have two complete and separate chorions, indicating that separation occ,urred before the forma tion of the trophoblast tissue at day 5 (Figure 8.25A). The remaining identical twins share a common chorion, sug gesting that the split occurred within (Continued on next page) 2 Chorions 1 Chorion 310 CHAPTER 8 SIDELIGHTS & SPECULATIONS (Cont i n u ed) the inner cel! mass after the tro ph ob l a st formed. By day 9, the human emb ryo has co m p l eted the co ns tr uc tion of an other extraemb ryonic l ayer the l ining of the amnion. This tissue, derived from the ec toclerm and meso , derm, forms the amnionic sac, which surrounds the embryo with amnionic fluid and protects it from desiccation and ab rupt movement. If th e separa tion of the embryo were to come after the formation of the chorion on day 5 b u t before the formation of the amnion on day 9, then the res u l tin g Figure 8.26 Producti on of chimeric mice. (A) Experimental procedu res used to produce chimeric mice Ea rly 8-cell embryos of gen eti ca l l y disti n c t . mice (here, with (Oelt color diiferences) are isolated from mouse oviducts and brought together after their zonae are removed by proteo lytic enzymes.
The cells form a com posi te blastocyst, which is implanted into the uterus of a foster mother. The photograph shows one of the actual chimeric mice produced i n this manner. (B) An adult female chimeric mouse (bottom) produced from the fus ion of th rec 4-cell embryos: one from two white-furred parents, one from two black-furred par ents, and one from two brown-furred parents. The resulting mouse has coal colors from a l l three embryos. More over, each embryo contributed germline cells, as is ev idenced by the three colors of offspring (above) pro duced when this chimeric female was mated with recessive (white-furred) mal es. (A courtesy of B. M intz; B from Markert and Petters 1 978, counesy of C. Markert.) embryos should have one chorion and two a mnions (Figure 8.2SB).
This hap pens in about two-thirds of human identical tw i ns A sma l l percentage of identical twins a re born within a s in gle chorion and amnion (F igu re 8.2SC).
T h is means the division of the embryo came after day 9. The abil ity to prod uce an en t i re embryo from cells that no rma l l y would have con tri b uted to on l y a portion of the embryo called regulation was d i scussed in the l ntroduction to Part II. Regulation is a l so seen in t h e abi l ity of , , ,

The mammalian hematopoietic niche is found i n the hollow cavities of trabecu , . lar bones (such as the sternum) where the bone marrow resides (see Figure 1 11.2). Here , Dentin • • Labia) (lips) FIGURE 1 1 1.5 The cervical loop of the mouse i ncisor is a stem cell niche for the enamel secreting ameloblast cells. These cel15 migrate from the base of the stellate reti cu l u m into the enClmel layer, allowing the teeth to keep grow ing. (Aher Wang et al. 2007.J Lingual (tongue) ,t> :.

.. \'. . .. : Enamel ·: · :. Ameloblast progenitor cells , AmeIo blast s (making enamel) stem cell T H E STEM CELL CONCEPT (A) (C) (8) GermJine stem cell Hub ccUs Cadherins " '"" Centrosome Hub cells Germline stem cell Gonialblast FIGURE 11 1.6 Stem cell niche in Drosophila tes tes.
(A) The apical hub consists of about 1 2 somatic cells, to which are attached 5 9 germ stem cells. The germ stem cells divide asym m etrica l ly to form another germ stem cell (which remains attached to the somatic hub cells) Jnd a gonialblast that will divide to form the sperm precursors (the spermatogonia and the spermatocyte cysts where meiosis is i n i ti ated) . (B) Reporter ga lac tosidase inserted into the gene for Unpaired reveals that this prote i n is transcribed in the somatic hub cells. (C) Cell division pattern of the germ l i ne stem cells, wherein one of the centrosomes remains in the cortical cytoplasm near the site of hub cell adhesion, while the other centrosome migrates to the opposite pole of the germl i ne stem cell. This results in one cell remaining attached to the hub and the other cell detaching from it and differentiating. (Arter Tul ina and Matunis 2001; - B courtesy of E. Matunis.) the stem cells are in close proximity to the bone cells (osteocytes) and the endothelial cells that l i ne the blood vessels. A complex cocktai l of paracrine factors, including Wnts, angiopoietin, and stem cel l factor, combines with cell s u rface signals from Notch and integrin to regulate stem cell prol iferation and differentiation. Hormonal signals and pressure from the blood vessels, as well as neurotransmitters from adja cent axons, also help regulate hematopoiesis (see Spiegel et a l . 2008; Malhotra and K i ncade 2009) . In many instances, germ cells are continuously p rod uced in stem cell niches. In h Drosophila tes tes, the stem cells for sperm reside in a regulatory microenvironment called the hub (Figure 1 11.6). The ub consists of about a dozen somatic testes cells and is surrounded by 5-9 germ stem cells. The division of the sperm stem cell is asym metric, always producing one cel l that remains attached to the hub and o n e unat tached cell. The daughter cell attached to the hub is maintained as a stem cell, while the cell that is not touching the hub becomes a gonialblast a committed cell that will divide to become the precursors of the sperm cells. The somatic cells of the hub create this a symmetric proliferation by secreting the paracrine factor Unpaired onto the cells attached to them. Unpaired protein activates the JAK-STAT pathway in the adja cent germ stem cells to specify their self-renewal. Those cells that are distant from the paracrine factor cannot receive this signal, so th ey begin their differentiation into the s perm cell lineage (Kiger et al. 2001; Tul ina a n d Matunis 2001). Physically, this asymmetric division involves the i nteractions between the sperm stem cells and the somatic ce l l s I n the division of the stem cel l, one centrosome remains attached to the cortex at the contact site between the stem cell and the somat ic cells. The other centrosom e moves to the opposite side, thus establishing a mitot ic spindle that w i l l produce one daughter cell attached to the hub and one daughter . 329 330 PART I I I cell away from it (Yamashita et a l . 2003). (We w i l l see a similar inheritance of cen trosomes in the division of mammalian neural stem cells.nhe cell adhesion mole cules linking the hub and stem cells together are probably i nvolved in retai n i ng one of the centrosomes in the region where the two cells touch. The stem cell niche is a criti ca l component of our phenotype, and it does nothing less than regulate the ratio of cell division to cell differentiation.
This means that main tenance of such niches is critical for our health. Too much stem cell d ifferentiation depletes the stem cells and promotes the phenotypes of agi ng or decay.
Too much stem cel l division can cause cancers to arise. The "graying" of mammalian hair (in mice as well as humans) can involve a breakdown in the maintenance of the stem cell niche such that both daughters of the divi di ng stem cells d ifferentiate, thereby leaving a smaller population of stem cel ls that cannot continue to make pigment forming melanocytes (Nishimura et a l . 2005; Steingrimson et a l . 2005).
Conversely, myeloproliferative disease-a cancer of blood stem cells and their (non-lymphocytic) derivatives-results when the stem cell niche is unable to provide the signals need ed for proper blood cell differentiation (Walkley et a l . 2007a,b) Thus, stem cell niches provide microenvironments that regulate stem cell renew al, survival, and differentiation. Their paracrine factors, cell adhesion molecules, and architecture al low asymmetric cell divisions such that a stem cell divides i n a man ner that a l lows one of its daughter cells to have a high probability of leaving the niche and beginning to differentiate according to the new signals it encounters. . Mesenchymal Stem Cells: Multipotent Adult Stem Cells Adult stem cells are used in the adult body to replace worn-out somatic cells on a regu lar basis. Our epidermis, our intestinal epithelium, a n d our blood cells are con ti nual ly being replaced with cells generated by d i vidi ng a d u l t stem cells. Most (if not a l l) adult stem cells are restricted to forming only a few cell types (Wagers et al. 2002). When hematopoietic stem cells marked with green fluorescent protein were placed in mice, their labeled descendants were found throughout the animals' blood but not i n any other tissue. Some adult stem cells, however, appear to have a sur prisingly large degree of plasticity. These mul ti potent cells are called mesenchymal stem cells, or MSCs (sometimes called bone marrow-derived stem cells, or BMDCs), and their potency remains a controversial subject. Originally found in bone marrow (Friedenstein et a l. 1 968; Caplan 1 99 1), m ultipo tent MSCs have also been found in adult tissue such as fat, muscle, thymus, and den tal pulp, as well as i n the umbilical cord (see Kuhn and Tuan 201 0). Indeed, the find ing that human umbilical cords and deciduous ("baby") teeth contain MSCs (Gronthos , et a l . 2000; H i rata et aL 2004; Traggiai et a l . 2004; Perry et a l . 2008) has led some physicians to propose that parents freeze cells from their chi ld's umbil ical cord or teeth so that these cells w i l l be available for transplantation later in life. However, the crucial test for pluripotency-the ability of a mo u se stem cell to generate cells of a l l germ layers when inserted into a blastocyst-has not yet been achieved. Mesenchymal stem cells are able to give rise to numerous bone, cartilage, mus cle, and fat l ineages !Pittenger et al. 1 999; Dezawa et al. 2005). The differentiation of MSCs is predicated on both paracrine factors and cel l matrix molecules in the stem cell niche. Certai n cell matrix components, especially lam in in, have been implicated in keeping MSCs in a state of undifferentiated "stemness" (Kuhn and Tua n 201 0). Cer tain paracrine factors appear to direct development into specific lineages. In one study (Ng et a l . 2008), platelet-derived growth factor was critical for chondrogenesis and fat formation, TGF signaling was important for chondrogenesis, and FGF Signaling was crucial for the d ifferentiation of MSCs into bone cel ls I n addition to paracrine factors, the repertoire of cell types from mesenchymal stem cells may also be enhanced by the surfaces on which the stem cells reside. Human . THE STEM CELL CONCEPT - p 3 tubulin (neural) - CBra! (bone) .....
MyoD (muscular) ..... + Dlebbistatin (no cytoskeleton) • • • Subslrare elasticity (kPa) P3 Tubulin (neurogenic) MyoD (myogenic) CBFal (osteogonic) MSCs can differentiate according to the elasticity of the surface on which they are placed. If p laced on soft matrices of collagen-coated polyacrilamide, these stem cells differentiate into neurons. A moderately elastic malrix of the same materials causes the same stem cells to become muscle cells, while harder matrices cause the MSCs to produce bone cells (Figure 111.7; Engler et al. 2006).
It is not yet known whether this range of potency is found normally in the body. Mesenchymal stem cells have recently been linked to normal growth and repair conditions in the human body. Indeed, one premature aging syndrome, Hutchinson Gilford progeria, appears to be caused by the inability of MSCs to differentiate into certain cell types, such as fat cells (Scaffidi and Misteli 2008). These fi ndi n gs lead to speculation that the loss of MSC ab i l i ty to differentiate may be a component of the normal aging syndrome. Moreover, MSCs may work in ways other than differentiating into needed cel l types. They may produce paracrine factors that aid olher, more spe cific stem cells to divide and repair tissues (Gnecchi et al. 2009).' A New Perspective on Organogenesis The ability to create, isolate, and manipu late stem cells offers a vision of regenera tive medicine, wherein a patient can h ave his Or her d iseased organs regrown and replaced by one's own stem cells. We wi l l deta i l the medical possibilities of stem cell therapy in Chapter 1 7 . But beyond their pOlential medical uses, stem cells tell us a great many facts about how the body is constructed and how it maintains its struc ture.
Organs often form by the regulation of stem cells, and we will see that the skin, hair, blood, and parts of the nervous system rout i nely use stem cells in their construc tion. Slem cells certainly give credence to the view that "development never ends," and offer fascinating (if not frightening) potential ways to modify development.
331 FIGURE 111.7 Mesenchymal slem cell dirrerentialion is influenced by the elasticity of the matrices upon which the cells sit. {On collage-coated gels having elasticity similar to that of the brain (aboul 01-1 kPa), Human MS(s differentiated into cells containing neu ral markers (such as p3 lubulin) bul not into cells containing muscle cell mark ers (MyoD) Or bone cel l markers «(BFal). As the gel s became stiiier, the MSCs generated cells exhibiting mus cle-specific proteins, and even stiffer matrices elicited the differentiation of cells with bone markers. Differentia tion of the MSC on Clny matrix could be abolished wilh blebbi statin. which inhibits micro filamenl assembly al the cell 'membrane. (A er Engler et al. 2006; photographs courtesy of).
Shields.) The Emergence of the Ectoderm Central Nervous System and Epidermis "WHAT IS PERHAPS THE MOST INTRIGUING question of all is whether the brain is powerful enough to solve the problem of its own creation." So Gregor Eichele (1992) ended a review of research on mammalian brain development. The con struction of an organ that perceives, trunks, loves, hates, remembers, changes, deceives itsel(and coordinates all of our conscious and unconscious bodily processes is undoubtedly the most challenging of all developmental enigmas. A combination of genetic, cellular, and organismal approaches is now giving us a very preliminary understanding of how the basic anatomy of the brain becomes ordered. The fates of the vertebrate ectoderm are shown in Figure 9.1 . In the past two chapters, we have seen how the ectoderm is instructed to form the vertebrate nervous system and epidermis. A portion of the dorsal ectoderm is specified to become neural ectoderm, and its cells become distinguishable by their columnar appearance. This region of the embryo is called the neural plate. The process by which the neural plate tissue forms a neural tube-the rudiment of the cen tral nervous system-is called neurulation, and an embryo undergoing such changes is called a neurula (Figure 9.2). The neural tube forms the brain ante riorly and the spinal cord posteriorly. This chapter will look at the processes by which the neural tube and the epidermis arise and acquire their distinctive patterns. Establishing the Neural Cells This chapter follows the story of the committed neural and epidermal precur sor cells. Neural cells become specified through their interactions with other cells. There are at least four stages through which the pluripotent cells of the epi blast or blastula become neural precursor cells, or neuroblasts (see Figure 111.4; Wllson and Edlund 2001): • Competence, wherein multipotent cells can become neuroblasts if they are exposed to the appropriate combination of Signals. • Specification, wherein cells have received the appropriate signals to become neuroblasts, but progression along the neural c1ifferentiation pathway can still be repressed by other Signals. o Commihnent (determination), wherein neuroblasts enter the neural differen tiation pathway and will become neu rons even in the presence of i.nhibitory Signals. • Differentiation, wherein the neuroblasts leave the mitotic cycle and express those genes cl1aracteristic of neurons. For the real a mazement, if you wish to be amazed, is this process. You start out as a single cell derived from the coupling of a sperm and an egg; this divides in two, then four, then eight, and so on, and at a certain stage there emerges a single cell which has as all its progen y the human brain. The mere existence of such a cell should be one of the great astonishments of the earth. People ought to be walking around all do)" all through rheir wak ing hours calling to each other in end less wonderment, talking of nothing except that cell. LEWtS THOMAS (1 979) CHAPTER 9 334 Epidern1is Hair Nails Sebaceous glands Olfactory epithelium Mouch epithelium Lens. cornea Ectoderm t Anterior pituitary Tooth enamel Surface ectoderm Cheek ium (epidermis) epithe l Schwaun Peri p heral cells nervous system Neuroglial cells Adrenal Neural medulla Sympathetic crest ----1 Melanocytes nervous system ParasymFacial pathetic cartilage nervous Dentine system of teeth Brain Neural pituitary Neural Spinal tube ---t- cord Motor neurons Retina <> Q r ,. Neural p la te 2 , Primitive streak <> o Qo 1il) 0 0 0 " " FIGURE 9.1 Major derivatives of the ectoderm germ layer.
The ectoderm is divided into three major domairw the surface ecto derm (primarily epidermis), the neural crest (peripheral neurons, p i gm ent fac i a l cartil age), and the neural tube (brain and spinal cord). , Neural folds (E) FIGURE 9.2 Gastrulation an d neurulation in a ch ick embryo (dorsal view). (A) Flat neural plate. (B) Flat neural plate with under lying notochord (head process). (e) Neural groove. (D) Neura l folds begin closing at the dorsalmost region, forming the inc ipie nt neural tube. (E) Neura l tube, showing the three brain regions and the spinal cord. The neural tube rema i ns ope n at the a nterior end and the optic bulges (which become the retinas) have extended to the lateral margins of the head. (F) 24-hour chick embryo, as n (D). The cephal i c (head) region has undergone neurulation, w le the caudal (tail) regions are still undergoing gastrulation. (A E cou tesy of G. C.
Schoenwolf; F aher PaNen 1 971 .) , i hi r CONSTRUCTING THE CENTRAL NERVOUS SYSTEM Formation of the N eural Tube There are two major ways of converting the neural plate into a neural tube. In primary neurulation, the cells sur roundi.ng the neural plate direct the neural plate cells to proliferate, invaginate, clnd pinch off from the surface to form a hoUow tube. In secondary neurulation, the neural tube arises from the coalescence of mesenchyme cells into a solid cord that subsequently forms cavities that coalesce to create a hollow tube. In general, the anterior portion of the neural tube is made by primary neurulation, while the posterior portion of the neural tube is made by secondary neurulation. The comp ete neural tube forms by joining these two separately formed tubes together (Ha rringt on et aJ. 2009). l T H E EMERGENCE O F T H E ECTODE RM (F) Anterior Ectoderm mesoderm ;;;;;O =,\NeuraJ region of coelom Yolk adherent [0 endoderm groove of blastoderm r== \- Cnmial mesenchyme Ecoxtelroaembryonic ,, Lateral body fold Ect \ Nellral fold l N"u"a] groove Notochord Subcephalic space fold ===:. ' =" An'terior intestinal por tal + jii m somite = (= E J f\ Neural fold 335 mesoderm Neu ral plate Endoderm olf idgut = Neural folds diverging . Blood island Primitive streak == _ = _ . , ;;;;;" mesoderm Hensen's node . pit Pnmltlve .I, Mesoderm .....? .iil . ve = = = = =:;; primitive g roo \ 1 I Posterior In birds, the neural tube anterior io the twenty-eighth somite pai r (i.e., everything anterio r to the hindlimbs) is made by primary neurulation (pasteels 1937; Catala et a1. 1996). In mammals, secondary neurulation begins at the level of the sacral vertebrae of the tail (Schoenwolf Nievelstein et a1. 1993). In amphibians such as 1984; Xenopus, Primary neurulation The events of primary neurulation divide the original ecto derm into three sets of cells: (1) the internally positioned neural tube, which will form the brain and spinal cord; (2) the externally positioned epidermis of the skin; and (3) the only the tail neural tube is derived from secondary neuru neural crest cells (see Figure lation (Gont et a l . in the region that connects the neural tube and epidermis, 1993); the same pattern occurs in fish 9.1). The neural crest cells form (whose neural hlbes were formerly thought to be formed but they migrate to new locations where they solely by secondary neurulation) (Lowery and Sive 2(04). ate the peripheral neurons and glia, the pigment cells of See WEBSITE 9.1 Homologous specification of the neural tissue will gener the skin, and several other cell types (see Chapter 10). The process of primary neurulation appears to be simi lar in all vertebrates; F i gu re 9.3 illustrates the process in 336 CHAPTER 9 EARLY NEURULA Neural plate (AI DORSAL SECTION "" VIEW LATE NEURULA MID-NEURULA Anterior Neural fold Neural tube Transverse section Midsagittal section Posterior tube plate (8) MIDSAGITTAL SECTION Cavity of gut Cavity of gut Remnant blastocoel Endoderm Neural plate (C) TRANSVERSE SECTION c Epidermis Neural plate .... Neural fold \ Mesoderm Notochord .. Endoderm amphibians (Gallera 1971). Shortly after the neural plate hils formed, its edges thicken and move upward to form the neural folds, while a U-shaped neural groove appears in the center of the plate, dividing the future right and left sides of the embryo. The neural folds migrate toward the midline of the embryo, eventuaUy fUSing to form the neural tube beneath the overlying ectoderm. The cells at the dorsalmost portion of the neural tube become the neural crest cells. Prin1ary neurulation can be divided into four distinct but spatially and temporally overlapping stages: (l)forma liO/1 andfolding of the neural plate; (2) shaping and elevatio/1 of the neural plate; (3) cOllvE'1ge1lce of tlhe neural folds, cre a ting a neural groove, and (4) closure of the neural groove to form the neural tube (Smith and Schoenwolf 1997; Colas Neural tube Mesoderm CaVlty of gut Epidermis FIGURE 9.3 Th ree views of neurulation in an amphibian embryo, showing early (Ieh), middle (center), and late (right) neu rulae in each case. (A) Looking down on the dorsal surface of the whole embryo. (B) Midsagittal section through th e m edia l plane of the embryo. (C) Transverse section through the center of the embryo . (Aher Balinsky 1 975.) N otoehord . Cavity of gut and Schoenwolf 2(01). Figu re 9.4 illustrates these stages in the chick embryo. See VADE MECUM Chick neurulation FORMATION AND SHAPING OF THE NEURAL PLATE The pro cess of neurulation begins when the underlying dorsal mesoderm (and the pharyngeal endoderm in the head region) signals the ectodermal cells above it to elongate into columnar neural plate cells (Smith and Schoenwolf 1989; KeUer et af. 1992). Their elongated shape distinguish es the cells of the prospective neural plate from the flatter pre epidermal cells surrounding them. As much as half of the ectoderm is included in the neural plate. The neural plate is shaped by tlhe movements of tlhe epi dermal and neural plate regions. The neural plate length ens along the anterior-posterior axis and narrows by con vergent extension, intercalating severa] layers of cells into a few layers. These convergence and extension movements are critical for the shaping the neural plate, and mutations disturbing these movements can block neural tube closure (Ueno and Greene 2003). THE EMERGENCE OF T H E ECTODERM 41> Formation (A) 33 7 ,.N:.e i u:.r:al.:: .l:.p :.a:t e Neural ,,;;;.... crest Folding (B) e Elevation of neural crest (C) T Notochord • Convergence (0) o Closur. FIGURE 9.4 Pr i ma ry neurulation: neural tube formation In the chick embryo. (A, 1 1 Cells of the neural plate can be distinguished as elongated cells In the dorsal region of the ectoderm. Folding begins as the medial hinge point (MHP) cells anchor to the notochord and change their shape, while the presumptive epidermal ce l s move toward the dorsal midline. (B,2) The neu ral folds are elevated as the presumptive epidermis continues to move toward the dorsal midline. (C,3) Convergence of the neural folds occurs as the dorsolateral hinge point (DLHP) cells become wedge-shaped and the epidermal cells push toward the center.
(0,4) The neural folds are brought into contact with one another, and the neural crest cells link the neural tube with the epidermis. The neural crest cells then disperse, leaving the neural tube separate from the epidermis. (photographs courtesy of K. Tosney and G. Schoenwolf; drawings after Smith and Schoenwolf 1 997.) l e cr st Epidermis Neura ceUs l crest 338 CHAPTER 9 Divisions of the neural plate cells are preferentially in the anterior-posterior direction (in chick, often referred to as the rostral-caudal, or beak-tail, direction). These events occur even if the involved tissues are separated. If the neu ral plate is isolated, its cells converge and extend to make a thinner plate, but fail to roll up into a neural tube. Howev er, if the "border region" containing both presumptive epi dermis and neural plate tissue is isolated, it will form small neural folds in culture Oacobson and Moury 1995, Moury and Schoenwolf 1995, Sausedo et al. 1997). Hence the epi dermis is an important factor in shaping the neural plate. BENDING AND CONVERGENCE OF THE NEURAL PLATE The bending of the neural plate involves the fonnation of hinge regions where the neural plate contacts surrounding tis sues. In birds and mammals, the cells at the midllne of the neural plate form the medial hinge point (MHP). The cells of the MHP are derived from the portion of the neural plate just anterior to Hensen's node and from the anterior mid line of Hensen's node (Schoenwolf 1991a,b; Catala et al. 1996). MHP cells become anchored to the notochord beneath them and form a hinge, which forms a furrow at the dorsal midllne. The notochord induces the MHP cells to decrease in height and to become wedge-shaped (van Straaten et al. 1988; 5mith and Schoenwolf 1989); cells lat eral to the MHP do not undergo such a change (see Figure 9.4B,C). Shortly thereafter, two other hinge regions form furrows near the connection of the neural plate with' the remainder of the ectoderm. These regions are the dorso lateral hinge points (DLHPs), and they are anchored to the surface ectoderm. The DUll' cells also increase their height and become wedge-shaped. Cell wedging is intimately linked to changes in cell shape. In the DLHPs, microtubules and microfilaments are both involved in these changes. Colchicine, an inhibitor of microtubule polymerization, inhibits the elongation of these cells, while cytochalasin B, an inhibitor of microfiJa ment formation, prevents the apical constriction of these cells, thereby inhibiting wedge formation (KarfunkeI 1972, Burnside 1973; Nagele and Lee 1987). As the neuroepithe lial cells enlarge (by means of the microtUbules), the micro filaments and their associated regulatory proteins accumu late at the apical ends of the cells (Zolessi and Arruti 2001). Constriction of these microfilaments allows the cell shape to change. After the initial furrowing of the neural plate, the plate bends around these hinge regions.
Each hinge acts as a pivot that directs the rotation of the cells around it (Smith and 5choenwolf 1991). In Xenopus, the actin-bind ing protein Shroom is critical in initiating this apical con striction to bend the neural plate (Haigo et al. 2003). Meanwhile, extrinsic forces are also at work. The sur face ectoderm of the chick embryo pushes toward the mid line of the embryo, providing another motive force for bending the neural plate (see Figure 9.4C; Alvarez and 5choenwolf 1992; Lawson et aJ. 2001).
This movement of the presumptive epidermiS and the anchoring of the neu- ral plate to the underlying mesoderm may also be impor tant for ensuring that the neural tube invaginal"es inward, or into the embryo and not outward. If small pieces of neu ral plate are isolated from the rest of the embryo (includ ing the mesoderm), they tend to roll inside out (5choen woIf 1991a). The pushing of the presumptive epidermis toward the center and the furrowing of the neural tube ere- ate the neura l folds. CIOSURE OF THE NEURAL TUBE The neural tube closes as the paired neural folds are brought together at the dorsal midline. The folds adhere to each other, and the cells from the two folds merge. In some species, the cells at this junc tion form the neural crest cells. In birds, the neural crest cells do not migrate from the dorsal region of the neural tube until after the neural tube has closed at that site. In mammals, however, the cranial neural crest cells (which form facial and neck structures; see Chapter 10) migrate while the neural folds are still being elevated (i.e., prior to neural tube closure), whereas in the spinal cord region, the neural crest cells do not migrate until closure has occurred (Nichols 1981; Erickson and Weston 1983). The closure of the neural tube does not occur simulta neously throughout the ectoderm. This phenomenon is best seen in those vertebrates (such as birds and mammals) whose body axis is elongated prior to neurulation. In arnniotes, induction occurs in an anterior-to-posterior fash ion; so in the 24-hour chick embryo, neurulation in the cephalic (head) region is well advanced, while the caudal (tail) region of the embryo is still undergoing gastrulation (see Figure 9.2D-F). The two open ends of the neural tube are called the anterior neuropore and the posterior neu ropore.
In chicks, neural tube closure is initiated at the level of the future midbrain and "zips up" in both directions. By contrast, n i manunals neural tube closure is initiated at sev eral places along the anterior-posterior axis. In hwnans, there are probably three sites of neural tube closure (fig ure 9.5A-C; Nakatsu et al. 2000; O'Rahilly and Muller 2002). Failure of closure in different regions of the neural tube causes different neural tube defects. Failure to close the posterior neuropore around day 27 of development results in a condition called spina bifid a, the severity of which depends on how much of the spinal cord remains exposed. Failure to close sites 2 and 3 in the rostral neural tube keeps the anterior neuropore open, resulting in a lethal condition called anencephaly in which the forebrain remains in contact with the amnionic fluid and subsequent ly degenerates (Figure 9.5D). The fetal forebrain ceases development, and the vault of the skull fails to form. The failure of the entire neural tube to close over the entire body axis is called craniorachischisis. Collectively, neural tube defects are quite common in humans, as they occur in about 1 in every 1000 live births. Neural tube closure defects can often be detected during pregnancy by various physical and chemical tests. T H E EMERGENCE O F T H E ECTODERM (H) (A) (e) Anterior neuropore 339 (D) Cutedge- of am nion 22 days FIGURE 9.5 N eurulation in the human embryo. (A) Dorsal view of a 22-day (8-somite) huma n embryo initiating neurulation. Both anterior and posterior neuropores are open (0 the amn ionic fluid. (B) 1 O-somite human embryo show i ng the three major sites of neural tube closure (arrows). (C) Dorsal view of a 23-day neuru lating human embryo with only its neuropores open. (0) Photo graph of a stillborn infant with an encephaly. (8 fro m Nakatsu et al. 2000; 0 courtesy of National March of Dimes.) The neural tube eventually forms a closed cylinder that separates from the surface ectoderm.
This separation appears to be mediated by the expression of different cell adhesion molecules. Although the cells that will become the neural tube originally express E-cadherin, they stop producing this protein as the neural tube forms, and instead synthesize N-cadherin and N-CAM (Fig ure 9.6A). As a result, the surface ectoderm and neural tube tissues no longer adhere to each other. If the surface ectoderm is experimentally made to express N-cadherin (by injecting N-cadherin mRNA into one cell of a 2-cell Xellopus embryo), the separation of the neural tube from the pre- (A) Normal development E-cadherin sumptive epidermis is dramatically impeded (F ig ure 9.6B; Detrick et a1. 1990; Fujimori et aJ. 1990). Human neural tube dosure 'is the result of a complex interplay between genetic and environmental factors (Cabrera et aJ. 2005; Fournier-Thibault et al. 2009). The Pax3, Sonic hedgehog, and openbrai.n genes are essential for the formation of the mammalian neural tube, but dietary factors, such as cholesterol and folate (also known as folic acid or vitamin B9), also appear to be critical. It has been estimated that more than half of all human neural tube birth defects could be prevented by a pregnant woman's taking supplemental folate. Therefore, the U.s. Public Health Service recommends that women of childbearing age take 0.4 milligrams of folate daily (Miiunsky et al. 1989; Centers for Disease Control 1992, Czeizel and Dudas 1992). While the mechanism by whidl folate facilitates neural tube closure is not understood, recent studies have demon strated expression of a folate receptor protein on the dor salmost regions of the mouse neural tube immediately prior to fusion (Figure 9.7; Saitsu et al. 2003). Rothenberg and colleagues (2004) showed that most women who deliv- (B) Neural plate Experimental N-cadherin and N-cadherin N-cadherin FIGURE 9.6 Expression of N- and E-cadherin adhesion proteins during neurulation in Xenopus. (A) Normal development. In the neural plate stage, N-cadherin is seen in the n eura l p la te while E-cadherin is seen on the presumptive epidermis . Eventually, the N-cadherin-bearing neural cells separate from the E-cadherin, containing epidermal cells.
(The neural crest cells express neither N- nor E-cadherin, and they disperse.) (B) No separation of the neural tube occurs when one side of the frog em bryo is injected with N-cadherin mRNA, so that N-cadherin is expressed in the ep idermal cells as well as in the presumptive neu ral tube . 340 CHAPTER 9 (A) (B) (e) (B) _ (C) – FIGURE 9.7 Folate-binding protein in the neural folds as neural tube closure occu rs (A) 1 O-somite mouse embryo stained for folate-binding protein mRNA.
(B,C) Sections t hrough embryo (A) at t he two arrows, showing the folate-binding protei n (dark blue) at the poi nts of neura l tube closure. (From Sa i tsu et al.
2003; pho tographs courtesy of K. Shiota.) . ered babies with neural tube defects had antibodies against this receptor protein, whereas such antibodies were sel dom found in women whose babies did not have open neural tubes. Mice with mutations in the gene encoding the folate receptor were born with a high incidence of neu ral tube abnormalities. The incidence of neural tube defects decreased if pregnant mice were fed folate supplements (Piedrahita et al. 1999; Spiegelstein et al. 2004). Folate deficiency appears to be only one risk factor, however. Women in low socioeconomic groups appear to have a higher incidence of babies with nelUai tube defects, even when vitamin use is taken into account (Little and Elwood 1992a; Wasserman et a l. 1998). Moreover, there appears to be a seasonal variation in the incidence of neuCondensing (A) (B) Medullary cord ccUs Transitional (C) ral tube defects (Little and Elwood 1 992b). Although this phenomenon remains mysterious, one possible explana tion may be contaminated crops. Marasas and colleagues (2004) documented that a fungal contaminant of com pro duces a teratogen (fumonisin) that causes neural tube fail ure by perturbing the function of several lipids and pro teins including the folate receptor protein. This fungus has been found in regions where neural tube defects are prevalent. In mice the teratogenic effects of fumonisin can be reduced by the administration of folate supplements during pregnancy. , Secondary neurulation Secondary neurulation involves the production of mes enchyme cells from the prospective ectoderm and endo derm, followed by the condensanon of these cells into a medullary cord beneath the surface ectoderm (Figure 9.8A,B). After this mesenchymal-ta-epithelial transition, the central portion of this cord undergoes cavitation to form tube Secondary neuru lation in the caudal region of a 25-somite chick e mbryo . (AI Mesenchymal cells condens e to form the medu l lary cord at a slightly more anterior position in the tail bud. (C) The neural tube is cavita ting and Ihe noto chord forming; note the pres ence of sep a ra te lumens. (D) The lumens coal esce to form the central canal of the neural tube. (From Catala et a l . ' 995; photo graphs courtesy of N. M. le FIGURE 9.8 (D) Douarin.) T H E EMERGENCE OF T H E ECTODERM several hollow spaces, or lumens 341 (Figure 9.8C); the lumens (Figure 9.80; 1980). We have seen that after wall of the neural tube rearrange themselves to form the Hensen's node has migrated to the posterior end of the selves dilierentiate into the numerous types of nerve cells then coalesce into a si ngle central cavity Schoenwolf and De longo gives rise embryo, the caudal region of the epiblast contains a precur sor cell population that to both neural ectoderm and mesoderm as the embryo's trunk elongates (Tzouana cou et aJ. 2009). The ectodermal cells that invaginate (and which will form the secondary neural tube) express the Pax2 gene, while the mesodermal cells adjacent to them (which will form somites) do not express and Takahashi 2010). Presumably, Pax2 (Shimokita different functional regions of the brain and spinal cord. Finally, on the cellular level, the new-oepithelial cells them (ne uron s) and supportive cells (glia) present in the body The early development of most vertebrate brains is sim ijar, but because the human brain may be the most organ ized piece of matter in the solar system and is arguably the most in teresting organ in the animal kingdom, we will con centrate on the develo pmen t that is supposed to make Homo sapient. these precursor cells pro vide the material for both the neural tube and mesoderm.
In human and chick embryos, there appears to be a tran The anterior-posterior axis sitional region at the junction of the anterior ("primary") The early mammalian neural tube is a straight structure. and posterior ("secondary") neural tubes. In human However, even before the posterior portion of the tube has embryos, coalescing cavities are seen in the transitional formed, the most anterior portion of the tube is undergo region, but the neural tube also forms by the bending of ing drastic changes.
In the anterior region, the neural tube neural plate cells.
Some posterior neural tube anomalies balloons into the three primary vesicles: the forebrain result when the two regions of the neural tube fail to coa (prosenc ephalon), midbrain (mesencephalon), and hind lesce (Saitsu et al. 2007). Given the prevalence of human brain posterior spinaJ cord terior end of the neural tube closes, secondary bulges--the malformations, fwther understanding of the mechanisms of secondary neurulation may have inlportant clinical implications . BUILDING THE BRAIN Differentiation of the neural rube into the various regions of the central nervous system (Le., the brain and spinal cord) occurs Simultaneously in three different ways. On the gross anatomical level, the neural tube and its lumen bulge and constrict to form the chambers of the brain and spinal cord. At the tissue level, the cell popula tions in the Wall Cavity 11 / Telencephalon L0","""""_,_,,,,," (Mesencephalon) Metencephalon - Hindbrain ,,"---","," , erally from each side of the developing forebrain. The prosencephalon becomes subdivided into the ante telencephalon and the more caudal diencephalon (see 9.9). The telencephalon will eventually form the cerebral hemispheres. The diencephalon will form the rior FIGURE 9.9 Early human brain development. The three primary brain vesicles are subdivided as development conti nues. At the right is a list of the adult derivatives formed by the walls and cavi t ies of the brain. (After Moore and Persaud 1 993.) 5 Secondary vesicles -» I)::,,"' (","""' " \j ,.", " optic vesicles, which will become retina have extended lat Figure Differentiation of the Neural Tube 3 Primary vesicles (rhombencephalon) (Figure 9.9). By the time the pos Spinal cord y Olfactor lobes Hippocampus Cerebrum Optic vesicle Ep it hal am us Thalamus Hypothalamus L- Midbrain Cerebellum Pons Medulla Adult d erivatives - Smell - Memory storage -Association ("intelligence") - Vision (retina) - Pineal gland - Relay center for optic and auditory neurons - Temperature, sleep, and breathing regulation - Fiber tracts between anterior and posterior brain, optic lobes, and tectum - Coordination of complex muscular movements - Fiber tracts between cerebrum and cerebeUum (mammals only) - Reflex ceuter of involuntary activities 342 (B) (A) CHAPTER 9 F I GURE 9.1 0 Rhombomeres of the chick hindbrain. (A) H i n d brain of a 3 day chick embryo. The roof plate has been removed so t hat the segm ented mo rphology of the neural epithelium c an be seen. The r1 /r2 boundary is at the upper arrow, and the r6/r7 bou nd ary is at the lower arrow.
(8) A ch i ck hindbrain at iI similar stage stained with antibody to a neurofilament subunit. The rhom bomere boundnries are em p hasi zed because they serve as c han nels for neurons crossing from one side of the brain to the other. (From Lumsden 2004, courtesy of A. Lumsden.) optic vesicles the future retina-as well as the tha1amic and hypothalamic brain regions, which receive neural input from the retina. Indeed, the retina itself is a deriva tive of the diencephalon. The mesencephalon does not become subdivided, and its lumen eventually becomes the different fate. The neural crest cells above each rhom cerebral aqueduct. The rhombencephalon becomes subdi vided into a posterior myelencephalon and a more ante riar metencephalon. The myelencephalon eventually becomes the medulla Oblongata, whose neurons generate bomere will form ganglia dusters of neuronal cell bod the nerve centers responsible for pain relay to the head and in the even-numbered rhombomeres, r2, r4, and r6 (Figu re neck, auditory connections, tongue movement, and heart 9.1 0; Lumsden and Keynes 1989). Neurons originating beat, as well as respiratory and gastrointestinal move from r2 ganglia form the fifth (trigeminal) cranial nerve; ments. The metencephalon gives rise to the cerebellum, the part of the brain responsible for coordinating move those from r4 fonm the seventh (facial) and eighth (vestibu loacaustic) cranial nerves; and those from r6 form the ninth ments, posture, and balance. (glossopharyngeal) cranial nerve. The rhombencephalon develops a segmental pattern that specifies the places where certain nerves originate. Periodic swellings called rhomborneres divide the rhombencephalon into smaller compartments. The rhorn bomeres represent separate "territories" in that the cells within each rhombomere mix freely within it, but not with cells from adjacent rhombomeres (Guthrie and Lumsden 1991; Lumsden 2004).
Moreover, each rhombomere has a ies whose axons form a nerve. The generation of the cra nial nerves from the rhombomeres has been snldied most extenSively in the chick, in which the first neurons appear See WEBSITE 9_2 Specifying the brain boundaries The zebrafish neural tube follows the same basic differ entiation pattern as the mammalian neural tube (although the eyes form earlier). When red fluorescent dye is injected into the zebrafish hindbrain as that vesicle is forming, the dye soon diffuses into the midbrain and forebrain as well (Figure 9.1 1). This diffusion is dependent on the inflation (8) (A) Inject red dye into bindbrain ventricle (I8 h postfertilization) ::= =,,= 1 8h 20 h = ... 22 h 24 h S/takehead mutant FIGURE 9.11 Brain ventri c l e for mation in zebrafish. (A) Wh en a fluorescent red dye is injected into t h e i nc i p ien t h in dbra in ven tr ic le 30 h of 1 R-hour z ebrafi s h emb ryos, the dye diffuses to the midbrain and forebrai n regi ons . As developm en t proceeds, the brain ventricles are s haped by regional differences in cell proliferation and adhe sion. (B) In the snakehead mutant, the Na+/K+ ATPase i s deficient. The brain ventricles fail to form, and dye injec ted into the pre sumptive hindbrain does not diffuse. (After lowery and Sive 2005, p hotograp hs courtesy of H. Sive.) T H E EMERGENCE O F T H E ECTODERM the lumen by the Na+ /K+ ATPase, which sets u p an fill the ventricles* (Low " and Sive 2005). (A similar mechanism is used to fill the ma uunalian blastocoel.) Further shaping of the ventricles "pends on the secretion of the cereb rosp inal fluid and on :egional cell proliferation and cell adhesion. Both the mid Drain and hindbrain venlTicles are shaped by lateral hinge regions where the c pithelium bends shar

differentiates FIGURE 9.20 (E) into the ventricular (ependymal), mantle, and marginal zones, as we ll as the roof and floor plates.
IE) A segment of the sp i nal cord with its sensory (dorsal) and motor (ventral) roots. (After larsen 1 993.) Spinal nerve 9.21B). A typical Purkinje neuron may form as many as 100,000 synapses with other neurons more connections than any other type of neuron studied. Each Purkinje neu root layer Jayer layer ron also sends out a slender axon, which connects to neu rons in the deep cerebellar nuclei.
Purkinje nenrons are critical in the electrical pathway of the cerebellum. All electric impulses eventually regulate the activity of these neurons, which are the only output Cerebellar organization In the brain, cell migration, differential neuronal prolifera tion, and selective cell death produce modifications of the neurons of the cerebellar cortex. For this to happen, the proper cells must differentiate at the appropriate place and time. How is this accomplished? three-zone pattern seen in Figure 9.19. In the cerebellum, One mechanism thought to be important for position some neuronal precursors enter the marginal zone to fqrm ing young neurons in the developing mammalian brain is unit serving as a relay nuclei (clusters of neurons; see second footnote, p. 348). glial guidance (Rakic 1972; Hatten 1990). Throughout the Each nucleus works as a functional cortex, neurons are seen to ride a "glial monorail" to their station between the outer layers of the cerebellum and respective destinations. other parts of the brain. Other nenronal precursors nUgrate precursors travel on the long processes of the Bergmann In the cerebellum, the granule cell away from the germinal neuroepithelium. These cerebel• glia (see Figure 9.218; Rakic and Sidman 1973; Rakic 1975). lar neuroblasts migrate to the ou tee surface of the devel As oping cerebellwn and form a new germinal zone, the exter complex and fascinating series of events, involving recip nal granular layer (see Figure 9.19.), near the outer rocal recognition between glia and neuroblasts (Hatten boundary of the neural tube. Figure 9.22 illustrates, this neuron gUa interaction is a 1990; Komuro and Rakic 1992). The neuron maintains its At the outer boundary of the external granular layer, adhesion to the glial cell through a number of proteins, one which is 1-2 ceUs thick, neuroblasts proliferate and come of them an adhesion protein called astrotaclin. If the astro into contact with cells that secrete BMP factors. The BMPs tactin on a neuron is masked by antibodies to that protein, specify the postntitotic products of these neuroblasts to the neuron will fail to adhere to the glial processes become a type of neuron called the granule cells (Alder et (Edmondson et a!. 1988; Fishell and Hatten 1991). Mice a1.
1999). Granule cells nUgrate back toward the ventricu deficient in astrotactin have slow neuronal migration rates, lar (ependymal) zone, where they prod lice a region called the internal granular layer (see Figure 9.19). Meanwhile, abnormal Purkinie cell development, and problems coordi nating their balance (Adams et aJ. 2002). The direction of the original ventricular zone of the cerebellum generates this nUgration appears to be regulated by a complex series a wide variety of neurons and glial cells, including the dis of events orchestrated by brain-derived neurophic factor tinctive and large Purkinje neurons, the major cell type of the cerebellum (Figure 9.21).
Purkinje neurons secrete Sortic (BONF), a paracrine factor that is made by the internal granular layer (Zhou et al.
2007). hedgehog, which sustains the division of granule cell pre Much insight into the mechanisms of spatial ordering cursors in the external granular layer (Wallace 1999). Each in the brain has come from the analysis of neurological Purkinje neuron has an enormous dendritic arbor that mutations in mice.
More than 30 mutations are known to spreads like a tree above a bulblike cell body (see Figure affect the arrangement of cerebellar neurons. Many of these T H E EMERGENCE OF T H E ECTODERM A) 351 FIGURE 9.21 Cerebellar organization. (AI Sagittal sec tion of a fluorescentJy labeled rat cerebel lum pho tographed "sing dual-photon confocal microscopy. (B) A vast enlargement of one area of (A). Purkinje neurons are light blue with bright green processes, Bergmann glia are red. ancl granule cells are dark blue. This dose-up illus trates the highly structured organization of n eu rons and glial cells.
(Courtesy ofT. Deerinck and M. Ellisman, University of Cal i fornia, San Diego.) distinct ways. First, like the cerebellum, it is organ ized vertically into layers that interact with one another. Certain neuroblasts from the matin zone migrate on glial processes through the white matter to generate a second zone of neurons at the outer surface of the brain. This new layer of gray matter will become the neocortex. The specification of the neocortex is accompHshed largely through the Lhx2 transcription factor, which activates numerous other cerebral genes. In Lhx2-deficient mice, the cerebral cortex faUs to fonn (Mangale et al.
2008; Chou et al. 2009). The neocortex eventually stratifies into six lay (B) ers of neuronal cell bodies; the adult forms of these layers are not completed until the middle of child hood. Each layer of the neocortex differs from the others in its functional properties, the types of neu rons found there, and the sets of connections they make. For instance, neurons in layer 4 receive their major input from the thalamus (a region that forms from the diencephalon), whereas neurons in layer 6 send their major oulput back to the thalamus. In addition to the six vertical layers, the cerebral cortex is organized horizontally into more than 40 regions that regulate anatomically and functional ly distinct processes. For instance, neurons in layer 6 of the visual cortex project axons to the lateral geniculate nucleus of the thalamus, which is involved in vision (see Chapter 10), while Jayer 6 neurons neurons of the auditory cortex (located more ante riorly than the visuaJ cortex) project axons to the medial geniculate nucleus of the thalamus, which functions in hearing. One of the major questions n i cerebellar mutants have been found because the pheno developmental neurobiology is whether the different func type of such mutants-namely, the inabUity to maintain balance while walking- in the ventricular region or if specification is accomplished much later by the synaptic connections between the ous reasons, these mutations are given names such as weav er, reeler, and staggerer. See WEBSITE 9.4 Cerebellar mutations of the mouse tional regions of the cerebral cortex are already specified , regions. Evidence that specification is early (and that there might be some "proto-map" of the cerebral cortex) is sug gested by certain human mutations that destroy the layer ing and functional abilities in only one part of the cortex, leaving the other regions intact (Piao et al. 2004). Cerebral organization Indeed, most of the neuroblasts generated in the ven The three-zone arrangement of the neural tube is also mod tricular zone migrate outward along radial glial processes to form the cortical plate at the outer surface of the brain. ified in the cerebrum. The cerebrum is organized in two As in the rest of the brain, those neurons with the ea rliest 352 CHAPTER 9 FIGURE 9.22 Neuron-glia i n terac tion in the m ou se. (A) Diagram of a cortica l neuron migrating On a glial cell process.
(B) Sequential ph oto- graphs of a neu ron migrating on a cerebellar glial process. The l eadi ng process h as several filopodia I exten sions. The neuron can reach speeds around 40 JlOl per hour as i t travels . (A a fter Rakic 1 975; B from Hatten 1 990, photograph courtesy of M. H atten.) (A) •------ (B) Leading process of ne u ron Migrating neuron • , I • birthdays form the layer closest to the ventricle. Subsequent neurons travel greater distances to fann the more superficial layers of the cortex. This process forms an "inside-out" gradient of development (Rakic 1974). McConnell and Kaznowski (1991) have shown that the determination of laminar iden tity (i.e., which layer a cell migrates to) is made during the final cell division.
Newly generated neuronal precursors transplanted after this last division from young brains (where they would form layer 6) into older brains whose migra tory neurons are forming layer 2 are committed to their fate, and migrate only to layer 6. However, if these cells are transplanted prior to their final division (during mid-S phase), they are uncommitted and can migrate to layer 2 (Figure 9.23). The fates of neuronal precursors from older brains are mOre fixed. While the neuronal precursor cells formed early in development have the potential to become any neuron (at layers 2 or 6, for instance), later pre cursor cells give rise only to upper-level (layer 2) neurons (Frantz and McConnell 1996). Once the cells arrive at their final destination, it is thought that they produce particu lar adhesion molecules that organize them together as brain nuclei (Matsunami and Takeichi 1995). According to Gaiano (2008), "the construction of the mammalian neocortex is perhaps the most complex bio logical process that Occurs in nature. A pool of seemingly homogeneous stem cells first undergoes proliferative expar'l.sion and diversification and then initiates the pro duction of successive waves of neurons. As these neurons are generated, they take up residence in the nascent corti cal plate where they integrate into the developing neocor tical circuitry.
The spatial and temporal coordination of neuronal generation, m.lgration, and cliffcrentiation is tight ly regulated and of paramount importance to the creation of a mature brain capable of processing and reacting to sen- sory input from the environment and of conscious thought." And as if this weren't enough after neuroge nesis subsides, the neuronal stem cells start making glia! See WEBSITE 9.5 Constructing the cerebral cortex STEM CELLS AND PRECURSOR CEllS The stem cells of the neocortex are originally generated by symmetrical divi sion of the neural tube epithelium before neurogenesis (neuron formation) actually occurs. Initially, the prolifera tive layer of the mouse cortex forms the ventricular zone (VZ). Shortly thereafter (arow1d day 13), it divides to give rise to a subventricular zone (SVB) directly outside it. Together, these zones form the germinal strata that gener ate the neuroblasts (neuronal precursor cells) that migrate into the cortical plate and form the layers of neurons.
The VZ will form the lower (deeper) layers of neurons, while the SVB will give rise to those cells that form the upper layer of neurons (Frantz et a1. 1994). There are three major progen..itor cells in th e germinal strata: radial glial cells (RGCs), short neural precursors (SNPs), and intermediate progenitor cells (IPCs; Figure 9.24). RGCs (which aren't really glia but tend to look like them) are thought to be stem cells, and they are found in the VZ. At each division, they generate another VZ cell and a more committed cell type. Interestingly, at each division, the cell receiving the "old" centriole (which contains dif ferent proteins than the newly made centriole) stays in the VZ, while the cell receiving the "young" centriole leaves to differentiate (Wang et a1. 2009). The more committed cells can be either neuroblasts (which divide to generate neurons) or [PCs, which migrate to the SVZ, where they generate neuroblasts. The SNPs also appear to generate neuroblasts, but from the VZ. A single stem cell In the ven tricular layer can give rise to neurons (and glial cells) in any of the cortical layers (Walsh and Cepko 1988). T H E EMERGENCE O F T H E ECTODERM (A) 100 [3HJ FIGURE 9.23 Determination of cortical laminar identity in the ferret cere brum. (A) "Early" neuronal precursors (birthdays on embryonic day 29) migrate to layer 6. (B) "Late" neuronal precursors (birthdays on postnatal day 1) migrate farther, into layers 2 and 3. (0 When early neuronal precursors (dark blue) are transplanted into older ventricular zones after their last mitotic 5 phase, the neurons they form migrate to layer 6. (D) If these precursors are transplanted before or during their last 5 phase, however, they migrate (with the host neu rons) to layer 2. (Aher McConnell and Kaznowski 1 99 1 .) -thymidine administered on embryonic day 29 80 f 2 60 40 " • • ::? (e) 20 0 • 1 , 2/3 4 5 Cortical laye rs 6 , Whmat er it (B) [3H J •thymidine administered ,on postnatal day 1 0 oI •r n H st neu o s • ..2 100 ';, .E 80 .j • • >-- " .. • 3 2/3 4 5 6 • ••• Subventricular zone (white matter) 60 40 20 • .,,---- ' Whma iter 2/3 5 4 6) Cortical layer s Homigsrtanteiounral • tO t Vewntericular Cel -autonomousfate ..h.. ena e transplanted after last S ph s thre FIGURE 9.24 Cortical neurons are generated from types of neural precursor cells: radial glia cells, short neural precursors, and intermediate pro genitor cells. RGCs and SNPs divide al the apical (luminal) surface of the ven- tricular layer. SNPs are committed neu ral precursors. IPes divide away from the luminal surface in the ventricular ?nd subventricular zones. Most IPes undergo neurogenic divisions, with a small fraction undergoing symmetrical proliferative divisions (dotted circular arrow).
Through asymmetrical divisions, RGCs give rise to IPes that migrate to the subventricular layer. The ventricular zone generates lower-layer neurons; the subventricular zone generates upper layer neurons. (After De ay and Kennedy 2007.) h 353 t Radial glial cell (RGC) • Short neuronal precursor (D) Homi••gsrtanteiounral cel n e phfatse wh n _ Glial Host (conditional) t ra spJam d in S e 354 CHAPTER 9 SIDEL IGH TS SPECULATIONS Adult Neural Stem Cells O (B) CA) ntil recently, i t was generally believed that once the mam malian nelVQUS system was mature, no new neurons were "born" -in other words, the neurons formed in utero and during the first few years of life were all we could ever expect to have. The good news from recent studies, however, is that the adult brain is capable of producing new neurons, and environmental stim ulntion can increase the n umber of these new neurons. In these experiments, researchers injected adult mice, rats, or mar mosets with bromodeoxyuridine (BrdU), a n ucleoside that resemb les thymidine. SrdU is incorporated into a ce l l 's DNA only if the cell is undergo i n g DNA replication; therefore, any cell labeled with BrdU must have been undergoing DNA synthesis dur ing the time it was exposed to BrdU. Figure 9.25 Evidence of adult neural stem This l abe l ing technique revealed that cells. The green s tai n i ng, which indicates newly divi ded cells, is from a fluo rescent thousands of new neurons are being antibody agai nst BrdU (a thym idi ne ana made each day in adult mice. More l ogue that is taken up only duri ng the 5 over, these new brain cells integrated phase of the cell cycle). (A) Newly gen erated with other cells of the brain, had ner adult mouse neurons (green cells) have a mal neuronal morphology, and exhib no rma l mo rpho logy and receive synaptic ited action potentials (Fi g u re 9.25A; inputs. The red spots are synaptophysin, a van Praag et a l . 2002). pro tei n found on the dendrites at the synaps- Injecting h u mans with SrdU is usu• es of axons from other neurons. (8) Newly ally unethical, since l a rge doses of generated neuron (arrow) in the adul t BrdU are often lethal. However, i n human brain.
This cell is located in the den certain cancer patients, the progress of tate gyrus of the hippocampus. The red fluo chemotherapy is monitored by trans rescence is from an anti body that stains only fusing the patient with a small amount neural cells.
Yellow indicates the overlap of of BrdU. Gage and colleagues (see red and green. Clial cells are stained purple. Erikkson et al. 1 998) took postmortem (A from van Praag et al. 2002; B from Erikks samples from the brains of five such son et al. 1 998, photograph courtesy of F. H. patients who died between 1 6 and Gage.) 781 days after the BrdU infusion. tn all five subjects, they saw labeled (new) neurons i n the granular cell layer of The existence of neural stem cells the h i ppocampal dentate gyrus (a part in adults is now well established for of the brain where memories may be the olfactory epithelium and the hip formed). The BrdU-labeled cells also pocampus {Kempermann et al. stained for neuron-specific markers 1 997a,b; Kornack and Rakic 1 999; (Figure 9.256).
Thus, although the rate van Praag et al. 1 999; Kato et a l . of new neuron formation in adulthood 2001). These cells respond to Sonic may be relatively low, the human hedgehog and can proliferate to brain is not an a natomical fait accom become multiple ce l l types for at least pli at birth, or even after childhood. the first year of a mouse's l ife (Ahn and Joyner 2005). I t appears that the stem cells prodUCing these neurons are located in the ependyma (the for mer ventricular zone, i n which the embryonic neural stem cells once resided) or in the subventricular zone (SVZ) adjacent to it (Doetsch et al.
1 999, Johansson et al. 1 999; Cassidy and Frisen 2001). These adult neural stem cells represent only about 0.3% of the ventricle wall cell population, but they can be distinguished from more differentiated cells by their cell surface proteins* (Rietze et a l . 2001). In the adult mouse, thousands of new neuroblasts are generated each day, migrating from the lateral SVZ to the olfactory bulb, where they differenti ate into several different types of neu rons. Recent evidence suggests that *These neural stem cells may have partic• u l ar physiological roles as wel l. During pregnancy, prolactin stimulates the pro• duction of neuronal progenitor cells in the subventricular zone of the adult mouse forebrain. These progenitor cells migrate to produce olfactory neurons that may be important for maternal behavior of rearing offspring (Shingo e t a l . 2003). T H E EMERGENCE O F T H E ECTODERM 355 SIDELIGHTS & SPECULATIONS (Conti n u ed) ste m ce l ls are n ot mult i poten t beco ming spec i fi ed only when th ey reach the o lfacto ry bulb) but i nstead are a pop u latio n of h eterogenous neu robl asts that a re already committed to becoming certain neuronal types ,V\e rk l e e et al. 2007). These adult neu ral stem cells proliferate in respo nse to exercise, learni ng, and stress (Zhang et th ese al.
2008). The existence of adult neural stem cells in the cortex is more controver sial (see Gould 2007). Some i nvestiga- tors (Gould et al. 1 999a,b; Magavi et al. 2000) c laim to have identified them; other scie ntists (see Rakic 2002) q uestion the existence of these corti cal neural stem cells. The mechanisms by which neural stem cells are kept i n a state of ready qu iescence well into adulthood are c urrently being explo red .
Before they become neu rons, neural stem cells are character ized by the expression of the NRSE translational inhibi tor that prevents neuronal differentiation by binding to a si lencer region of DNA (see Chapter 2). When n eu ra l stem cells begin to differe nt i a te, they synthesize a small, double-stranded RNA that has the same sequence as the si lencer and which might bind NRSE and thereby perm it neuronal differentiation (Kuwabara et al. 2004). The use of c u l tured neuronal stem cells to regener ate or repair parts of the brain wi ll be considered in Chapter 1 7 . CORTICAL CELL MIGRATION The first cortical neurons to be times. One of these switches is the gene encoding the tran- generated migrate out of the germinal zone to form the When the mouse neuronal progen to form the first layer of cortical neurons, Foxgl is not exp ressed in the progenitor cells or in the first formed neurons. However, later, when the progenitor cells generate those neurons destined for layers 4 and 5, they express tlus gene.
If tl.e FoxgI gene is conditionally knocked out of this In.eage, the neural precursor cells con tinually give rise to layer 1 neurons. Therefore, it seems .ransient prep l ate (Kawauch.i and Hoshino 2008). Subse quently generated neurons migrate into the preplate and "'parate it into two layers: the Cajal-Retzius layer and the 5ubplate.
The Cajal-Retzius layer becomes and remains the most superficial layer of the neocortex, and its cells express the cell surface glycoprotein Reelin. The subplate remains .he deepest layer through which the successive waves of neuroblasts travel to form the cortical plate. The Reelin producing cells of the Cajal-Retzius layer are critical in the separation of the preplate. In Reelin-deficient mice, the pre plate fails to sp lit, and the neurons produced by the ger minal layers pile up behind the previously generated neu scription factor Foxg 1 . itor cells divide that the Foxgl transcription factor is required to suppress the "layer 1" neural fate. Neither the vertical nor the horizontal organization of the cerebral cortex is clonally specified-that is, none of the functional units form from the progeny of a single cell. (instead of migrating through them). By activating the Rather, the developing cortex forms from the mixing of \latch pathway, Reelin on the surface of the Cajal-Retzius cells derived from numerous stem cells. The early region rons cells allows the neuronal stem cell to produce a long fiber alization of the neocortex is that extends through the cortical plate (Hashimoto-Torii et paracrine factorS secreted by the epidermiS and neural crest 2008; Nomura et al. 2008).
This (and the fact that it pro- cells a t the margins of the developing brain (Rakic et al.
2009). The paracrine factors induce the expression of tran scription factors in the specific brain regi ons, which then mediate the survival, differentiation, proliferation, and migration of the newly generated neurons. For instance, Fgf8 protein is secreted by the anterior neu ral ridge and is important for specifying the telencephalon (Figure 9.26). If Fgf8 is overexpressed n. the ridge, specifi cation of the telencephalon is extended caudally, whereas if Fgf8 is ectopically added to the caudal region of the cortex, part of that caudal region will become anterior (Fukuchi Shimogori and Grove 2001, 2005). Sonic hedgehog is secret ed by the medial ganglionic€ eminence and helps form the ventral neurons of the cortex, including those of the sub al. uced some proteins thought to be glial-specific) caused the neural stem cell to be called the radial glial cell. The process from this cell becomes cr iti.cal for the migra tion of the neural cells produced by the germinal zones. We also know that there are mutations that specifically affect the microtubular cytoskeleton of the migrating neu geJ:>e prevent neuronal with microtubule assembly; humans with such mutations have been seen to suffer from mental dysfunctions, among them autism, bipolar migration in the cortex by interfering *DISCl mutations are not the cause of most cases of these diseases, but they are seen in the rMe cases where these behavioral diseases are transmitted as Mendelian traits (see Marx 2(07). thought to be organized by stantia nigra (whose absence causes Parkinson disease). NEURONS TO GliA The stem cells of the vertebrate cortex make neurons first and then make glial cells. In mice, neu rons are formed from embryonic day 12 through embry onic day 18. Then, at embryonic day 18, the same precursor cells generate glia. When cortical progenitor cells (cortical 356 CHAPTER 9 13 FIGURE 9.26 Building the proto-map of the cerebral cortex. (A) Day mouse embryo at the point of the formation of the wo telencephali c vesicles. Fgf8 is secreted from the anterior neural ridge and the com missural plate; Wnts and BMPs are secreted from the cOlinical hem; and sonic hedgehog (Shh) is secreted from the medial ganglionic eminence, These result in a graded expres sion of transcription factors that translates inlo a molecular regionali z ti on of the ger minal zone. (B) Fgf signal i ng affects the identity of cortical regions. EctopiC expres sion of Fgf8 in the caudal pole of the neo cortex generales a partial dupli cation of the S 1 barrel-field (the receptive field for whisker responses) usually seen in the anlerior. (Aher Dehay and Kennedy 2007.) a :: ;; ;; ;; ; I :: :: :: • (7 - : : - • whisker (B) (A) :: ---- 51 cau Paracrine factors , • Fgf8 Shh D Wnts, BMPs a (• ••• Intrinsic Pgf8 Rostral l ••_r a /\- r- stem cells such as RGCs and their more coounitted descen dants) are cultured on embryonic day 12, tl1ey differenli a te into neurons for the firs l few d ys. After mulliple days of culture, Ihe progenilors swilch and slart making glia (GOtz and Barde 2005). Retroviral tracers and time-lapse microcinematography have shown that individual precur sor cells make both neurons and gha (Reid et al. 1995; Qian et al. 2000; Shen el al. 2006).
_ No m l •X\ Sl \••) (s whisker neurons Ectopic PgfS Both internal and external faclors regulate this transi tion. Cortica l precursor cells form neurons when cultured on embryonic cortical slices, but they become glia when placed on older cortical hces (Morrow el aJ. 2001).
One ot the main factors involved in this environmental regula tion is CT l, a pa racrin e factor that aclivates the jAK-STAT pathway. The STAT transcription factors activ a te glial spe cifi genes. Moreover, this transition must be done in the absence of neurotrophic factors, a s these activate the RTK-MAPK pathway, resulting in neural speci fic gene expres sion (see Miller and Ga u thier 2007).
- SIDEL I G H TS SPECULATIONS The Unique Development of the H uman Brain T a . There are many differences betvveen humans and chim- pa nzees These include our hair less, sweaty skin and striding bipedal posture. However, the most striking nd sign ifi c a nt differences occur i n brain deve l op ment The e no rmou s growth and asymmetry of our neoc or tex and ou r ability to reason, remem ber, plan for the future, and learn lan- guage and cultural skills make h u ma ns unique in the animal k i ngdo m (Varki el al. 2008). The deve l opment of the h u ma n neocortex is st ri k i ngly plastic and i s a n a l most cons tan t wo rk i n progress . Se ve ra l developme nta l phe nomena have been identified that dis tingu ish the devel opmen t of the human brain from that of other species, including other primates: . - 1 . The retention of the fetal growth rate after birth. neu ronal 2 . Th e activity of human-specific RNA genes. 3. Th e high activity of transcription.
4.
Human-s pecific alleles of develop mental reg u l atory genes. 5. The conti nu atio n of brain matura tion into adulthood. Fetal neuronal growth rate after birth If there is one important develop menta l trait that d isti ngu ish es humans from the rest of the animal k i n gdom, it is our retention of the fetal neuronal growth rate. Both human and ape brains have a high growth rate before birlh. After b i rth, however, this rate slows greatly in the apes, whereas human brai n growt h continues at a rapid rate for about 2 years (Figu re 9.27A; Ma rl i n 1 990). Dur i ng early postnatal development, we add ap proxi mate l y 250,000 neurons per minute (Purves and Lichtman 1 985). The ratio of brain weight 10 body weight at birth is similar for great apes and h u ma ns, bu t by adulthood the ratio for h u ma ns is l iterally "off the chart"- when compared with that of othe r pri mates (Figu re 9.27B; Bogi n 1 997). At the cel l u lar level, we fi nd that no fewer than 30,000 synapses per second per cm2 of cortex are formed during the f i rst few years of human l i fe . It is th oug h t that this increase in neuron number may (1) generate new modules (addressable sites) thai can acquire new functions; (2) s to re n ew THE EMERGENCE O F THE ECTODERM 357 SIDELIGHTS & SPECULATIO NS (Continued) (A) • • B• '" ,g -g, .• • .S - • co NeWbor•O Adult h um an " h u man \• I Ad ult 0 chimpanzee Body weigh' (log scale) Figure 9.27 (B) E• o 1400 Human 1200 - .!'!9 1000 .• 00 800 " 600 .• - .D "3 ." < 400 200 0 ..!. -:o: C h i m p au zee o .,. , ,.... 20 Reten ti on of fetal neuronal growth rate in humans. (A) Whereas other pri mates, such as chimpanzees, comp lete neuronal proliferation around the time of birth, the neurons of newborn humans continue to proliferate at the same rate as the fetal brain neu rons.
(B) The human brain!body weight ratio (encepha l i zation index) is about 3.5 t imes high er than that of apes. (After Bogin 1 997.) memories for use in thinking and fore genome. In 1 975, King and Wilson casting, and (3) enable learning by concluded from their studies of human interconnecti ng among themselves and chimpanzee proteins that "The and with prenatally generated neurons organismal differences between chim (Rose 1 998; Bari naga 2003). it is in panzees and humans would then this early postnatal stage that i n terven result chiefly from genetic changes i n a tion is thought to be able to raise IQ few regu latory systems, while amino (reviewed in Wickelgren 1 999). acid substitutions i n general would The prodigious rate of continued rarely be a key factor in major adaptive neuron production has important con shifts." This was one of the first sugges sequences. Portmann (1 94 1), Montagu tions that evolution can occur through (1 962), and Gould (1 977) have each changes in developmental regulatory made the claim that we are essential l y genes. When the total genomes are Ifextrauterine fetuseslf for the first year compared, humans and chimpanzees of life. If one follows the charts of ape differ a t about 4% of their sequences, maturity, actual human gestation is 2 1 and most of these are in the noncoding months. Our "premature" birth" is an regions (see Varki et aL 2008).
evolutionary compromise between Although there are some brain maternal pelvic width, the fetal head growth genes (e.g., ASPM and micro circumference, and lung maturity. The cephalin) whose sequences differ mechanism for retaining the fetal neu between humans and apes, these dif ronal growth rate has been called ferences have not been cor•lated with hypermorphosis-the phyletic exten the huge growth of human brains. sion of development beyond its ances- .
Rather, the critical differences may tral state (Vrba 1 996; Vinicius and reside in the DNA sequences that Lahr 2003). encode noncoding RNAs. These RNAs are highly expressed in the developing See WEBSITE 9.6 Neuronal brain and may regulate the transcrip growth and the invention of tion or translation of neuronal tran childhood scription factors. Computer analysis comparing different mammalian Genes for neuronal growth genomes may have found such genes (Pollard et aL 2006a,b; Prabhakar et aL Yet humans and chimpanzees have 2006). First, these studies identified a remarkably similar genomes. When relatively small group of noncoding the protein-encoding DNAs are com- DNA regions that were conserved pared, we are around 99% identical. among non-human mammals. This rep However, the protein-coding regions resents around 2 % of the genome, and comprise only around 2% of the Corina - 0 __ Orangutan 60 100 Adult body weight (kg) 140 it was assumed that if these regions were conserved throughout mam- malian evolution, they were important. Second, they compared these sequences to their human homologues to see if any of these regions have changed between humans and other mammals. This study identified 49 such regions where the sequence is highly conserved among mammals but has diverged rapidly between humans and chimps. The most rapid divergence is seen in the sequence HAR I (human accelerated region-1), where 1 8 sequence changes were seen between chimps and humans when one would expect, based on mammalian evolu tion, to see only one or no substitu tions. HAR 1 is expressed in the devel oping brains of humans and apes, especially in the Reelin-expressing Cajal- Retzius neurons that are known to be responsible for directing neuronal migration during the formation of the Six-layered neocortex. Research is ongoing to discover the function of HAR I and the other HAR genes that are in the conserved noncoding region of the genome. High transcriptional activity In the 1 970s, A. C. Wilson suggested that the difference between humans and chimpanzees might reside in the amount of proteins made from their genes (see Gibbons 1 998), and there is now evidence supporting this hypothe sis. Using rnicroarrays to study global patterns of gene expression, several (Continued on next page) SIDELIGHTS & SPECULATIONS (recent investigations have found
that, although the quantities and types of genes expressed in human and chimp l ivers and blood are i ndeed extremely simi lar, human brains produce more than five times as much mRNA as chimpanzee brains (Enard et a l . 2002a; Preuss et al. 2004). In humans, tran scription of some genes (such as SPTLC1 , a gene whose defect causes sensory nerve damage) was elevated l 8-fold over the same genes' expres sion in the chimp cortex, while other genes (such as DDX 1 7, whose product is i nvolved in RNA processing) are expressed 1 0 times less in human than in chimp cortices. Speech, language, and the FOXP2 gene Spoken language is a un iquely human trait and is presumed to be the prereq uisite for the evolution of cultures. Speech entails the fine-scale control of the larynx (voice box) and mouth. tndi- viduals who are heterozygous for mutations at the FOXP2 locus have severe problems with language articu lation and with forming sentences (Vargha-Khadem et al. 1 995; Lai et a l . 2001). This observation has provided genetic anthropologists with an inter esting gene to study. Enard and col- leagues (2002b) have shown that, although the FOXP2 gene is conserved throughout most of mammalian evolu tion, it has a unique form i n humans, having accumulated at least two amino acid-changing mutations just since our divergence from the com- man ancestor of humans and chim panzees. These differences are signifi cant, since human and chimpanzee forms of the Foxp2 protein differential ly regu late more than l OO genes (Konopka et al. 2009). In the mouse, the Foxp2 gene is expressed i n the developing brain, but its major site of expression is the lung (Shu et al. 2001). In humans, FOXP2 is predominantly expressed in those brain regions that coordinate speech (i.e., the caudate nucleus and i nferior olive nuclei); these sites are abnormal i n patients with FOXP2 deficiency (Lai et 3 1 . 2003). In birds, the Foxp2 protein is associated with song learning, and the experimental downregulation of Foxp2 expression i n certain areas of the brain prevents young male birds from imitating their species-specific song (Teramitsu and White 2006; Haesler et a l . 2007). Although it is not certain that FOXP2 is the most critical gene for human language acquisition, it seems to be very important for allowing the orofacial movements and grammar characteristic of human speech. Teenage brains: Wired and unchained Until recently, mm,t scientists thought that after the initial growth of neurons during fetal development and early childhood, rapid neural proliferation ceased.
However, magnetic resonance imaging (MRI) studies have shown that the brain keeps developing until around puberty, and that not a l l areas of the brain mature simultaneously . . . (Giedd et a l . 1 999; Sowell et a l . 1 999). Soon after puberty, neuronal growth ceases and pruning occurs. The time of this "pruning" correlates with the time when language acquisition becomes difficult (which may be why children learn language more readily than adults). There is also a wave of myelin production ("white matter" from the glial cells that surround neuronal axons) at this time. Myelination is criti cal for proper neural functioning, and the greatest differences between brains in early puberty and those in early adulthood i nvolve the frontal cortex (Figure 9.28; Sowel l et al. 1 999; Cogtay et a l .
2004). These differences in brain develop ment may explain the extreme responses teenagers have to certain stimuli, as well as their abil ity to learn certa i n tasks. In tests using functional MRI to scan subjects' brains while emotion-charged pictures flashed on a computer screen, the brains of young teens showed activity in the amyg dala, which mediates fear and strong emotions. When older teens were shown the same pictures, most of thei r brain activity was centered in the frontal lobe, an area involved in more reasoned perceptions (Baird et a ! . 1 999; Luna e t a l . 2001). The teenage brain is a compl icated and dynamic entity, and (as any parent knows) one that is not easily understood. But if one survives these years, the resulting adult brain is usually capable of mak- ing reasoned decisions, even in the onslaught of emotional situations. Anterior 5 yrs. 8 yrs. 16 yrs. 12 yrs. Figure 9.28 Dorsal view of the human brain showing the pro gression of myeli nati on ("white matter") over the cortical surface during adolescence. (Images courtesy of N. Cogtay.) Low Myelination (percent "white matter") 20 yrs. High THE EMERGENCE OF THE ECTODERM DEVELOPMENT OF THE 359 lens pla code, which then invaginates to form the lens. This invagi the head ectoderm, it induces the formation of a VERTEBRATE EYE nation is accomplished by the cells of the lens placode An individual gains knowledge of its environment through As the optic vesicle bends to form the optic cup, the pre evelop from interactions of the neural tube with a series sumptive lens cells are brought inside the embryo of epidermal thickenings called the cranial ectodermal pla (Chauhan et aJ.
2009). As the optic vesicle becomes the optic its sensory organs. The major sensory organs of the head codes (discussed in more detail in Chapter 10). Most of these placodes form neurons and sensory epithelia. The two olfactory placodes form the nasal epithelium (smell receptors) as well as the ganglia for the olfactory nerves. Similarly, the two oti c placodes extending adhesive filopodia to contact the optic vesicle. cup, its two layers differentiate in different ways. The cells than the neural crest cells tl,at can form of the outer layer produce melanin pigment (being one of the few tissues other this pigment) and ultimately become the pigmented reti invaginate to form the na.
The cells of the inner layer proliferate rapidly and gen inner ear labyrinth, whose neurons fann the acoustic gan erate a variety of glia, ganglion ceUs, intemeurons, and gl ia that enable us to hear. Tn this section, we will focus on light-sensitive photoreceptor neurons. Collectively, these the development of the eye from the lens placode.
The lens placode does not form neurons; rather, it forms ceUs constitute the neural retina. The retinal ganglion cells are neurons whose axons send electric impulses to the the transparent lens that allows light to impinge on the reti brain. Their axons meet at the base of the eye and travel na. The interactions between the cells of the lens placode down tile optic stalk, which is then called the and the presumptive retina structure the eye via a cascade that it will become the optic vesicle? It appears that a group of reciprocal changes that enable the construction of an of intricately complex organ.
optic nerve.
How is a specific region of neural ectoderm informed transcription factors-Six3, Pax6, and Rxl-are expressed together in the most anterior tip of the neural The Dynamics of Optic Development plate.
This single domain will later split into the bilateral regions that form the optic vesicles (Adelmann 1929; Math \'Ve first discussed the interactive induction of the verte ers et a!. 1995; Chiang et aJ. 1996). Again, we see the simi endoderm and mesoderm interact with the adjacent tems, for these three proteins are also necessary for the prospective head ectoderm to give the head ectoderm a formation of the brate eye in Chapter 3. At gastrulation, the involu ting larities between the Drosophila and vertebrate nervous sys Drosophila eye. lens-farming bias (Saha et aJ. 1989). But not all parts of the head ectoderm eventually form lenses, and the lens must Formation of the Eye Field have a precise spatial relationship with the retina. The acti vation of the head ectoderm's latent lens-forming ability Eye formation has been studied in several organisms, but and the positioning of the lens in relation to the retina are historically the amphibian eye has received U>e most atten accomplished by the optic vesicle (Fi gu re 9.29). The optic vesicle extends from the diencephalon, and where it meets (A) 4-mm embryo tion. (Indeed, this was this organ that Spemann began studying (B) 4.S-mm embryo Lens placode in fin de siecle Germany; see Saha (C) S-mm embryo Lens vesicle (D) 1991.) Using 7-mm embryo Retina Lens Optic vesicle FIGURE 9.29 Development of the verteb ra te eye (see also Figure 3.1 5). (A) The opti c ves i c l e evaginates from the brain and contacts the overly ing ectoderm, inducing a lens placode. (B,C) The overlying ectoderm dif ferentiates i nto lens cells as the opti c vesicle folds in on itself, and the lens placode bec omes the lens vesicle. (C) The opti c vesicle becomes the neural and pigmented retina as the lens is internalized. (D) The lens vesi cle induces the overlying ectoderm to become the cornea. (A-C from Hilfer and Yang 1 980, courtesy of 5. R. Hiller; 0 courtesy of K. Tosney.) Optic cup Cornea) 360 CHAPTER 9 @ @ E T , R x t , Ot x 2 , 3-1'• Eye IA) 0 o pa:.•ru. _: _• X6_', _:-,i Op t x 2 iSntNeadguect1i0o.n5 FsoprSec/taimifgiecda1btiroain spSeEtcayigeefcia1e2tldi.o5n IB) •Six3• -!! ET ___Rx-..,1 t in---=or1f-Nogr•' j 1LOP •hx2/ tx2 Stage Stage 12.5 Dynamic formation of the eye field in the anterior neural plate. Formation of the eye field. Light blue rep resents the neural plate; moderJ.te blue indicates the area of Otx2 expression FIGURE 9.30 INoA) g in ET, Rxl, • • u ral lforebrain), and dark blue indicates the region of the eye field as it forms in the • forebrain. (8) Dynamic expression of tran- scription factors leading to specification of the eye field.
Prior to stage '10, Noggin inhibits £T expression but promotes expression of Otx2.
Otx2 protein then represses the inhibition of ET by Noggin signaling. The ET transcription factor acti o vates Rx1, w h i ch encodes a transcription factor that blocks 01x2 and promotes Pax6 expression. Pax6 protein i nitiates the cascade of gene expression constituting the eye field lat right). Ie) Location of the transcription factors in the nascent eye field of stage 1 2 .5 learly neurula) and stage 1 5 Imid-neurula) Xenopus embryos, showing a concent ric organization of ---l.• _. 15 (e) transcription factors having domains of decreasing size: Six) > Pax6 > Rx l > Lhx2 > ET. IAfter Zuber et al. 2003.) i . ill Rxl Sru Lhx2 • yPax6\\ • 2)) Zi --1:/ ET ___in situ hy b ridizati on and injec tion of tra nse ription factor mRNA into developing Xenopus embryos, Z

above them, activating the keratinocyte differentiation corneum). Throughout life, the dead keratinocytes of the pathway and preventing further Cell divisions (see Mack cornified layer are shed humans lose about 1.5 grams of et a1. 2005; Blanpain and Fuchs 2009). If these cells every day* and are replaced by new cells. In *Most of this skin becomes "house dust" on furniture and floors. you doubt this, bum some dust, i t will smell like singed skin. The Cutaneous Appendages The epidermis and dermis interact at specific sites to cre ate the sweat glands and the cutaneous appendages: hairs, scales, or feathers (depending on the species). The forma Thickened Keratin granules ❷2SS❷;;} ❷❷.❷ ❷❷❷❷❷❷❷❷ :::: tion of these appendages requires a series of reciprocal inductive interactions between the dermal mesenchyme and the ectodermal epithelium, resulting in the formation of epidermal thickenings called pIa codes that are the base ❷;"" precursors of hair follicles. Epidermal cells in the regions capable of forming these placodes secrete Wnt protein Transitional } cell Granular layer tiation of follicle development (see Reddy et al. 2001; Andl among themselves, and Wnt signaling is critical for the ini In mammals, the first indication that a hair follicle pla et al. 2002). code will form at a particular place is an aggregation of cells in the basal layer of the epidermis (Figure 9.38A,B) . This aggregation is directed by the underlying dermal fibroblast cells and occurs at different times and different places in the embryo.

The basal epidermal cells elongate, divide, and sink into the dermis. The dermal fibroblasts respond to this ingression of epidermal cells by forming a small Spinous node (the dermal papilla) beneath the hair germ layer (Figure 9.38C). The dermal papilla then pushes up on the basal stem cells and stimulates them Malpighian layer Basal to divide more rapidly. The basal cells respond by prodUCing postmitotic cells that will differ entiate into the keratinized hair shaft (Figure layer Layers of the human epidermis. The basal cells are mitotically active, whereas the fully keratinized cells characteristic of external skin are dead and are continually shed.

The kerati n ocytes obtain their pigment through the transfer of melanosomes from Ihe processes of mela nocytes that reside in the basal layer. (Aner Montagna and Parakkal I 974.) FIGURE 9.37 Basal lamina T H E EMERGENCE OF T H E ECTODERM (A) Basal keratinocytes of epidermis ❷❷ Dermal fibroblasts (C) (B) Inner Placode Dermal papilla fibroblasts Epidermis Melanin in early hair shaft (D) 367 Epidermis root sheath Dermal papilla (E) (F) -Hair shaft Sebaceous gland Melanocytes See WEBSITE 9.8 Developmental genetics of hair formation 9.38D,E; see Hardy 1992; Miller et .1. 1993; Duverger and 'vlorasso 2009). Wnt Signaling is critical in placode formation. Forced expression of the Wnt inhibitor Dickkopf, or deletion of epithelial ❷-catenin, preclude placode formation, while mutation of epithelial ❷-caterun to a constitutively active form results in adoption of hairlike fate by the entire sur face ectoderm.

This indicates that ectodermal p-caterun sig naling determines hair follicle versus epidermal fate (Gat et at. 1998; Andl et al. 2002; Narhi et aJ. 2008; Zhang et aJ. 2008, 2009). Patterning of placode fate within the surface ectodenm is thought to rely on a reaction-diffusion mecha nism based on competition between. placode promoting factors (such as Wnts) and secreted inhibitors (including !he Dickkopf family of Wnt inhibitors and several BMP iarnily members; Jiang et aL 2004; Mou et aL 2006; Sick et al. 2006; Bazzi et al. 2007). In one such regulatory loop, Wnt activates expression of its own inhibitor, Dkk4, in placodes. As Dkk4 is thought to be more diffusible than Wnt ligands, it may suppress pla code fate in cells adjacent to the placode, tl1ereby contribut ing to patterning (Figure 9.39, Sick et aL 2006; Bazzi et aJ. 2007). Expression of Dkk4 is additionally enhanced by another Signaling pathway that is crucial for placode forma tion: the ectodysplasin (EOA) pathway (FJinjaux et at. 2008; Zhang et al. 2009; see Sidelights & Speculations, p. 369). FIGURE 9.38 Early development of the hair follicle and hair shah. (A) Signals initiate local proliferation of the basal keratinocytes in the epidermis. (B) Proliferation of epiderma l cells results in formation of the hair follicle placode, which signals the dermal mesenchymal cells to aggregate beneath it into a dermal papilla. (C) The papilla signals the prolifera tion of the hair germ, making it into a primitive hair shaft (or "hair peg"). (D) The hair shaft engulfs the dermal papi l la and forms the i nner hair root di rectl y above the papilla . Seba ceous cells (sebocytes) and the bulge appear as melanin granules enter into the cortex. (E) Sebaceous glands form, and the hair canal is made. The hair shaft differentiates the inner root sheath of epidermal cells. (F) The sebaceous gland is localized on the lateral wall of the follicle, while the hair shaft extends into the hair canal and out past the skin. (A-F a her Phif pott and Paus 1 998.) Interestingly, signaling interactions similar to those that take place in the embryo can occur during healing of large wounds in adult mouse skin, resulting in regeneration of follicles within the wound. The epidermis appears to revert back to an embryonic state and uses the same Wnt signal ing pathway to establish new hair follicles (Ito et al. 2007). This regenerative process is inhibited by forced expression of Dkkl and enhanced by overexpression of Wnt7a (indicating that it requires Wnt activity). Once placodes are established, Signals from each pla code induce clumping of underlying dermal fibroblasts, forming a dermal condensate (see Figure 9.38B). In response to a " second dermal message" from the dermal condensate, the epithelial placode cells proliferate and invade the dermis, eventually surrounding the dermal con densate, which develops into the hair follicle dermal papil la, an important signaling center in the mature hair folli cle. Further proliferation and differentiation of the ep ithelial cells result in the formation of the inner root sheath and hair shaft of the mature follicle, processes that are likely to reguire lateral communication between epithe lial cells (see Hardy 1992; Andl et aL 2002). As the hair fol licle matures, an epithelial swelling begins to form on the periphery of the hair germ and eventually develops into the sebaceous gland (see Figure 9.38E,F). Sebaceolls glands produce an oily secretion known as sebum that functions to lubricate the hair follicles and skin. 368 CHAPTER 9 (A) I" . . . . . ❷ (0) Epidermis '❷ DermiS ---' '- _ _ _

and co-work found that premigratory neural crest cells from ��[ ]{}[ ]n[mOO OO]m drome wherein the intestine cannot properly void its solid of the foregut and midgut, thus cells and explained the genetic deficiencies that cause Hirschsprung disease. In this model, the vagal crest cells are committed soon after their formation, the differentiation of the ventrally migrating neural crest cells depends on the Meanwhile, pathway they follow and their final location. or not the colonization is complete depends on the initial there is good evidence that, although some neural crest cells are]{[[[[[r in the anterior portion of the gu t. Rather, they prolifer ate until all the niches in that region of the intestine are sat synthesizing both acetylcholine and norepinephrine. Thus urated, after which the migrating front ruoves posteriorly. the gut itself continues to elongate. Whether 382 CHAPTER 1 0 (A) (B) m FIGURE 10.9 Neural crest cell m igration in the dorsolateral pathway through the skin. (A) Whole ou nt in situ hybridization of day 1 1 mouse embryo staining neural crest-derived melanoblasts (purple). (8) Stage 1 8 chic k embryo seen in cross-section through the trunk. Melanoblasts (arrows) can be seen moving through the dermis, from the neu ral crest region toward the periphery. (A from Baxter and Pavan 2003; 8 from Santiago and Erickson 2002, cou rtesy of C. Erickson.) number of vagal crest cells entering the anterior gut and the ratio of cell motility to gut growth. These results were not obvious, and they show the power of combining exper imental and mathematical approaches to development. THE ADRENAL GLAND Chemotactic factors are also impor tant for fOrm!Dg the adrenal gland, an organ generated from two distinct cell types. The outer portion, or cortex, of the adrenal gland secretes hormones such as cortisol, while the inner portion the medulla-secretes hormones such as epinephrine. The cells of the medulla are derived from the trunk neural crest, while those of the adrenal cortex are derived from intermediate mesoderm (see Chapter 11). TI,e neural crest cells are directed into the medulla-forming region by chemotactic factors from those tissues of the inter mediate mesoderm that form the adrenal cortex. If other regions of the intermediate mesoderm are converted into adrenal cortex (by electroporating the SFl transcription fac tor into these cells), the crest cells will migrate to those ectopic places (Saito and Takahashi 2005; Huber et al. 2008). This migration appears to be directed by a graclient of BMP coming from the adrenal cortical precursors, If Noggin is overexpressed near the adrenal-forming region, trunk neu ral crest cells are not induced to migrate there. Thus, both extracellular mabices and soluble chemotactic factors influ ence the migration of these neUIal crest cells. The dorsolateral migration pathway It appears that the cells that take the dorsoiateral pathway have already become specified as melanoblasts-pigment cell progenitors-and they are led a long the dorsolateral route by chemotactic factors and cell rnatrix glycoproteins (Figure 1 0.9). In the chick (but not in the mouse), the first neural crest cells to migrate enter the ventral pathway, while cells that migrate later enter the dorsolateral pathway (see Harris and Erickson 2(07), These late-migrating cells remain above the neural tube in what is often called the "staging area," and it is these cells that become specified as melanoblasts (Weston and Butler 1966; Tosney 2004). The switch between glial/neural precursor and melanoblast pre cursor seems to b e controlled by the FoxD3 transcription factor. If FoxD3 is present, it represses expression of MITF, a transcription factor necessary for melanoblast specifica tion and pigment production. If FoxD3 gene expression is downregulated, MITF is expressed 'and the cells become melanoblasts. MITF is involved in three signaling cascades. The first cascade activates those genes responsible for pig ment production; the second allows these neural crest cells to travel along the dorsolateral pathway into the skin; and the third prevents apoptosis in the migrating cells (Kos et al. 2001; McGill et al. 2002; Thomas and Erickson 2009). Once specified, the melanoblasts in the staging area upregulate the ephrin receptor (Eph B2) and the endothe lin receptor (EDNRB2). This allows them to migrate along extracellular matrices containing ephrin and endothelin-3 (Harris et al. 2008). Indeed, the melanocyte lineage migrates on exactly those same molecules that repelled the gliallneural lineage of crest cells. Ephrin expressed along the dorsolateral migration pathway stimulates the migra tion of melanocyte precursor cells. Ephrin activates its receptor, Eph 82, on the neural crest cell membrane, and this Eph signaling appears to be critical for promoting this migration.

Disruption of Eph signaling in late-migrating neural crest cells prevents their dorsolateral migration (Santiago and Erickson 2002). In mice (but not in chicks), the Kit receptor protein is crit ical in causing the committed melanoblast precurors to migrate on the dorsolateral pathway.

This protein is found on those mouse neural crest cells that also express MITF that is, the presumptive neuroblasts. Kit protein binds stem cell factor (SCF), which is made by the dermal cells.

When bound to SCF, Kit prevents apoptosis and stimulates cell division among the melanoblast precursors. SCF is critical for dorsolateral migration. If SCF is experimentally secret ed from tissues (such as cheek epithelium or the footpads) that do not usually synthesize this protein (and do not usu ally have melanocytes), neural crest cells will enter those regions and become melanocytes (Kunisada et al. 1998; Wil son et al. 2004). In vertebrates, all of the pigment cells (except those of the pigmented retina) are derived from the neural crest. As they travel through the dermis, they multiply. In certain mutations, the neural crest cells do not form enough pig- N E U RAL CREST CELLS AnD AXONAL SPECIFICITY 31ent ceU precursors to totally encircle the body (see Figure 1.22A).

Eventually, the melanoblasts enter the epidermis, where they rapidly enter the developing hair follicles or ieether prirnorrUa and take up residence at the base of the follicle bulge. In mice, all of the melanoblasts go into the hair follicle (the mouse epidermis is transparent), whereas in birds and humans, both the epidermis and its cutaneous appendage (hair, feather) become pigmented. In the feath er or hair follicle, the melanoblasts become melanocyte stem ceUs (Mayer 1973; Nishimura et a1. 2002). In the hair shaft, they reside in the bulge, along with the hair stem cells. A portion of these cells migrate outside the bulge at the beginning of each hair development cycle to differentiate into mature melanocytes and proVide pigmentation to the hair shaft. Here, the epithelial cells secrete Fgf2, which stim ulates the melanocytes to transfer packets of pigment into the epithelial cells (Weiner et a1. 2007). Nishimura and col leagues (2005) have documented that the reason the hair of mice and humans grays with age is that melanocyte stem ceUs become depleted from the bulge. Thus, the differentiation of the trunk neural crest is accomplished by autonomous factors (such as the Hox TABLE 1 0.2 Pharyngeal arch 383 genes distinguishing trunk and cranial neural crest cells, or MITF committing ceUs to a melanocyte lineage), by spe cific conditions of the environment (such as the adrenal cortex inducing adjacent neural crest cells into adreno medullary cells), or by a combination of the two (as when cells migrating through the sclerotome respond to Wnt sig nals depending on their types of receptors.) The fate of an inrUvidual neural crest ceU is determined both by its start ing position (anterior-posterior along the neural tube) and by its migratory path. Cranial Neural Crest The head, comprising the face and the skull, is the most anatomically sophisticated portion of the vertebrate body. It is the evolutionary novelty that separates the vertebrates from the other deuterostomes-echinoderms, tunicates, and lancelets (Northcutt and Gans 1983; Wilkie and Mor riss-Kay 2001). The head is largely the product of the cra nial neural crest, and the evolution of jaws, teeth, and facial cartilage occurs through changes in the placement of these ceUs (see Chapter 19). Some derivatives of the pharyngeal arches Skeletal elements (neural crest plus mesoderm) Arches, arteries (mesoderm) Muscles (mesoderm) Cranial nerves (neural tube) 1 Incus and malleus (from neural crest); mandible, maxilla, and temporal bone regions (from neural crest) Maxillary branch of the carotid artery (to the ear, nose, and jaw) Jaw muscles; floor of mouth; muscles of the ear and soft palate Maxillary and mandibular divisions of trigeminal nerve (V) 2 Stapes bone of the middle ear; styloid process of temporal bone; part of hyoid bone of neck (all from neural crest cartilage) Arteries to the ear region: corticotympanic artery (adult); stapedial artery (embryo) Muscles of facial expression; jaw and upper neck muscles Facial nerve (VII) 3 lower rim and greater horns of hyoid bone (from neural crest) Common carotid artery; root of internal carotid Stylopharyngeus (to elevate the pharynx) Glossopharyngeal nerYe (IX) 4 Laryngeal cartilages (from lateral plate mesoderm) Arch of aorta, right subclavian artery; original spouts of pulmonary arteries Constrictors of Superior laryngeal branch of vagus nerve (X) 6* Laryngeal cartilages (from lateral plate mesoderm) Ductus arteriosus; roots of definitive pulmonary arteries Intrinsic muscles of larynx Recurrent laryngeal branch of vagus nerve (X) Source: Based on Larsen 1993. "'The fifth arch degenerates in humans pharynx and vocal cords 384 CHAPTER 1 0 (C) (A) (/ I I \, \ Hindbrain "m[{ Frontonasal \[j Maxillomandibular prominence ([\ Lateral nasal ommence ', / \ [\ The cranial neural crest is a mixed population of cells in different stages of commitment, and about 10% of the population comprises multipotent progenitor cells that can differentiate to become neurons, glial rnelanocytes, mus cle cells, cartilage, and bone (Calloni et al. 2009). The migra tion of these cells is directed by an underlying segmenta tion of the hindbrain. As mentioned' in Chapter 9, the hindbrain is segmented along the anterior-posterior axis into compartments called rhomba meres. The cranial neu ral crest cells migrate ventrally from those regions anterior to rhombomere 8 into the pharyngeal arches and the fron tonasal process; the final destination of these crest cells will determine their eventual fate (Figure 1 0 . 1 0A; Table 1 0.2). The cranial crest cells follow one of three major streams: 1. Neural crest cells from the midbrain and rhombomeres 1 and 2 of the hindbrain migrate to the first pharyngeal arch (the mandibular arch), farming the jawbones as well as the incus and malleus bones of the middle ear. These cells will also differentiate into the neurons of the trigem inal ganglion the cranial nerve that innervates the teeth and jaw-and will contribute to the ciliary ganglion that innervates the ciliary muscle of the eye. These neural crest [. . ___]" \ \ \[:,[[\, Incus / I process ['ii- \ [[= [Pharyngeal arell' ([Malleus [} and S Factal bones (from arch 1 neural crest) ,) """ \c . T [( ) _ ___ . , Meckel s cartilage 'i[cricoid cartilage t!! \;=---' I (B) / _.-- Hyoid bone Thyroid cartilage Tracheal rings I FIGURE 10.10 Cranial neural crest cell migration in the mam malian head. (A) Migrational pathways from the cranial n eural crest into the pharyngeal arches and frontonasal process . (8) Con tinued migration of the cranial neural crest to produce the human face. The frontonasa l prominence contributes to the forehead, nose, ph i l tru m of the upper lip (the area between the lip and nose), and primary palate . The lateral nasal prominen ce generates the sides of th e nose. The maxillomandibular prom i nences give rise to the lower jaw, much of the upper jaw, and the sides of the middle and lower regions of the face. (C) Structures formed in the human face by the m esenchymal cells of the neural crest. The cra ter ia liginous elements of the pharyngeal arches are indicated by col ors, and the darker pink region indicates the facial skel eton pro duced by anterior regions of the cranial n eura l crest. (A after Le Douarin 2004; B a her Helms et al. 2005; C a her Carlson 1 999.) cells are also pulled by the expanding epidermis to form the frontanasal process, which forms the forehead, the middle of the nose, and the primary palate.

Thus, the cra nial neural crest cells generate the facial skeleton (Figure 10.10B,C; Le Douarin and Kalcheim 1999).

2. Neural crest cells from rhombomere 4 populate the sec ond pharyngeal arch, forming the hyoid cartilage of the neck as well as the stapes bone of the middle ear. These cells will also form the neurons of the facial nerve. N E U RA L CREST CELLS AND AXONAL SPECIFICITY 3. Neural crest cells from rhombomeres 6-8 migrate into the third and fourth pharyngeal arches and pouches to form the hyoid carillages and the thymus, parathyroid, and thyroid glands (Serbedzija et aJ. 1992; ereuzet et aJ. 2005). They also go to the region of the developing heart, where they help construct the outflow tracts (i.e., the aorta and pulmonary artery). If the neural crest is removed from those regions, these structures fail to form (Bockman and Kirby 1984). Some of these cells migrate caudally to the clavicle (collarbone), where they settle at the sites that will be used for the attachment of certain neck muscles (McGonnell et aJ. 2001). Observations of labeled neural crest cells from chick hind brain, wherein individually marked cells were followed by cameras fOCUSing through a Teflon membrane window in the egg, found that the migrating cells were "kept in line" by interactions with their environment (Kulesa and Fraser 2000). In frog embryos, there is evidence that the separate streams are kept apart by ephrins. Blocking the activity of the Eph receptors causes cells from the differ ent streams to mix together (Smith et aJ. 1997; Helbling et al. 1998). Neural crest cells from rhombomeres 3 and 5 do not migrate; rather, they undergo apoptosis. If this cell death is prevented, the r3 and r5 neural crest cells enter the first and third pharyngeal arches, respectively, and create muscle attachment sites that are not normally present (Ellies et aJ. 2002).

385 Intramembranous ossification: Neural crest-derived head skeleton Bones fonn in two major ways. The bones of the trunk and some of the bones in the back of the skull are made from the mesodenn. This type of bone formation, where meso dermal mesenchyme becomes cartilage and the cartilage is replaced by bone, is called endochondral ossification; we will talk about it in detail in Chapter 11. The other type of bone formation, where mesenchyme forms bones directly, is called intramembranous ossification (Figure 1 0.1 1). There are three main types of intramembranous bone: sesamoid bone (such as the patella), which fonns as a result of mechanical stress; periostal bone, which adds thickness to long bones; and dermal bone, which forms in the der mis of the skin. The first two types of intramembranous ossification are characteristic of mesodermal cells. Many of the dermal bones of the skull and face, however, originate from cranial neural crest-derived mesenchymal cells. The pathway from neural crest to intramembranous bone begins when cranial neural crest cells, under the influ ence of BMPs from the head epidermis, proliferate and con dense into compact nodules (Figure 1 0.1 2). High levels of 8MFs induce these nodules to become cartilage, while lower levels of BMPs induce them to become pre-osteoblast progenitor cells that express the Runx2 transcription fac tor and the mRNA for collagens II and IX. Later, these ceUs downregulate Runx2 and begin expressing the osteopolltin. gene, giving them a phenotype similar to a developing chondrocyte (cartilage cell); thus, this stage is called a chon- Osteocytes (A) fiGURE 10.1 1 Intramembranous ossification. (A) Mouse mes enchyme cells condense and change shape to produce osteoblasts, which deposit osteoid matrix. Osteoblasts then become arrayed along the calcified region of the matrix. Osteoblasts that are embedded within the calcified matrix become osteocytes. (8) Bone formation in chick embryo heads (days 9-1 3) as revealed with Alizarin red (which stains bone matrix). (A from Komori et aJ. 1 997; B from Abzhanov et aJ. 2007, courtesy of P. Abzhanov.) Fronetal b Premaxilal (B) ry .+ Day 9 10 tt 12 n bone De tary 13 bone CHAPTER 1 0 386 I 9 Somite stage II Embryonic day 2 3 II Embryon ic day 6 7 II Embryonic day 8+ Cranial neural crest cells Cranial crest Pre-osteoblast FIGURE 10.12 During craniofacial development, mesencephalic cranial neural crest cel l s m igrate to become (he mesenchyme of the future face and much of the skull. Cells of the early cranial skeletogenic condensations depend on BMP2/4/7 activities to form pre-osteoblastic progenitors (which eventually become bone), whereas high levels of BMP2 andlor BMP4 alone induce a chondrogenic (cartilage) fate. Differentiation into the chondro cyte-like osteoblasts is negatively regulated by both Indian hedge hog (IHH) and parathyroid hormone-related protein (PTHrP) activ ities that are probably autocrine. (After Abzhanov et al. 2007.) drocyte-like osteoblast. Under the influence of Indian hedgehog (which it secretes and receives in an autocrine fashion), the chondrocyte-like osteoblast becomes a mature osteoblast-a committed bone precursor cell (Abzhanov et a!. 2007). The osteoblasts secrete a collagen-proteogly can osteoid matrix that is able to bind calcium. Osteoblasts that become embedded in the calcified matrix become osteocytes (bone cells). As calcification proceeds, bony spicules radiate out from the region where ossification began. Furthermore, the entire region of calcified spicules becomes surrounded by compact mesenchymal cells that fonn the periosteum (a membrane that surrounds bone).

The cells on the inner surface of the Periosteum also become osteoblasts and deposit matrix parallel to ti1e exist ing spicules. In this manner, many layers of bone are formed. The vertebrate skull, or cranium, is composed of the neurocranium (skuU vault and base) and the viscerocra nium (jaws and other pharyngeal arch derivatives). Skull bones are derived from both the neural crest and the head mesoderm (Le Lievre 1978; Noden 1978; Evans and Noden 2006). While the neural crest origin of the viscerocranium has been well documented, the contributions of cranial neural crest cells to the skull vault are more controversial. In 2002, Jiang and coileagues constructed transgenic mice that expressed p-galactosidase only in their cranial neural Chondrocyte-like Mature osteoblast (CLO) osteoblast [HH [crest cells.* When the embryoniC mice were stained for p galactosidase, the cells forming most of the skull-the nasal, frontal, alisphenoid, and squamosal bones-turned blue; the parietal bone cUd not (Figure 10.13). Although the specifics may vary among the vertebrate groups, in gener al the front of the head is derived from the neural crest, while the back of the skull is derived from a combination of neural crest-derived and mesodermal bones. The neural crest contribution to facial muscle mixes with the cells of the cranial mesoderm, such that facial muscles probably also have dual origins (Grenier et al. 2009). Given that the neural crest forms our facial skeleton, it follows that even small variations in the rate and direction of cranial neural crest cell divisions will determine what we look like. Moreover, since we look more like our bio logical parents than our friends do (at least, we hope this is true), such small variations must be hereditary. The reg ulation of our facial features is probably coordinated in large part by numerous paracrine growth factors. Wnt sig naling causes the protrusion of the frontonasaJ and maxil lary prominences, giving shape to the face (Brugmann et a!. 2006). FGFs from the pharyngeal endodenn are respon sible for the attraction of the cranial neural crest cells into the arches as well as patterning the skeletal elements wiID in the arches. FgfB is both a survival factor for the cranial crest cells and is critical for the proliferation cells forming the facial skeleton (Trocovic et a!. 2003, 2005; ereuzet et al.

*These experiments were done using the ere-Lox technique. The mice were heterozygous for both (1) a 1}-galactosidase allele that could be expressed only when Cre-recombinasewas activated in that cell, and (2) a Cre-recombinase al1ele fused to a Wnt1 gene pro moter. Thus, the gene for [galactosidase was activated (blue stain) i.n those ceJls expressing Wntl-a protein that is activated in the cranial neural crest and in certa in brain cells. only N EURAL CREST CELLS AND AXONAL SPECIFI CITY (A) (B) 387 (C) FN, frontal nasal process MB, midbrain HB, hindbrain Mx, maxilla Md, mandible Sq, squamosal bone F, frontal bone P. parietal B. mineralized bone {non-neural crest} IP, interparietal bone V, trigeminal ganglion FIGURE 1 0.13 Cranial neural crest cells in embryonic mice, stainec for [gal ac tosidase expression. (A-C) Cranial neural crest migration from day 6 to day 9.5, shown by the dark blue staining neural crest cells. (D) Dorsal view of 1 7 5 day embryoni c mouse showing lack of staining in the pari eta l bone. (From Jiang et al. 2002, courtesy of G.

Morriss-Kay.) . 2004, 2005). (Lee The FGFs work in concert with BMPs, some times activating them and sometimes repressing them 2001; Holleville et al. 2003). in concert with Sonic hedgehog (Shh; Haworth et al. 2008). We saw in Chapter 9 that Shh is crit et al. FGFs also work -- in Le Douarin and her colleagues (2007). FirSt they between facial form and brain growth was highlighted studies by found that the region of cranial neural crest that forms the facial skeleton is also critical for the growth of the anteri or brain. When that region of chick neural crest was ical for the proper growth of the facial midline (see Figure reruoved, not only did the bird's face fail to form, but the 9.31), and Shh is also telencephalon failed to grow as well (Figure crucial for shaping the neural crest 10.14A,B). Next derivatives of the head. The epithelia (both neural and epi they found that the forebrain development could be res dennal) of the dOrSal part of the frontonasal process secrete cued by adding Fgf8-containing beads to the anterior neu Fgf8, while the ventral epithelia of the frontonasal process ral ridge (the neural folds of the anterior neuropore; Fig secrete Shh (see Figure 9.26). The crest-derived mesen ure 10.1 4C). But this was strange. Cranial neural crest cells chyme between the epithelia receives both signals. Where do not make or secrete Fgf8; the anterior neural ridge usu these signals meet is where a bird's beak cartilage grows ally does (Figure out. If the region of frontnasal process containing the Fgf/Shh boundary is inverted in the chick, the beak forms 10.14D; see Figure 9.26). rior neural ridge from making the Fgf8 necessary for fore in reverse direction (Hu et al. 2003; Abzhanov and Tabin brain proliferation. factorS to pattern the face and skull have become an impor anterior neural ridge region, 2004).

The interactions among these (and other) paracrine tant area of research in developmental biology. It seemed that removing the cranial neural crest cells prevented the ante Le Douarm and colleagues Looking at the effects of activated genes added to the hypotheSized that the BMP4 from the surface ectoderm was capable of blocking Fgf8. The cranial neural crest cells Coordination of face and brain growth in clinical genetics that "the face secreted Noggin and Gremlin, two extracellular proteins that bind to and inactivate BMP4. This allows the synthe reflects the brain." While this is not always the case, physi ment of the forebrain structures. Thus, the cranial neural cians are aware that children with facial anomalies may crest cells not only provide the cells that build the facial have brain malformations as well. The coordination skeleton and connective tissues, they also regulate the pro- It is a generalization sis of Fgf8 in the anterior neural ridge and the develop 388 CHAPTER 1 0 (A) Control Facial skeletogenic neural crest ablation C]\ Ablation + FgfS Fgf8 [bead " " , , , , , , I , rl . . I , " , , , , " ' , ' I " . , I I , " \ . No anterior brain (B) , , , , , , .r,' r2 Con trol , . ' . Normal anterior brain FIGURE 10.1 4 The cranial neural crest that forms the facial skeleton is also critical for the growth of the anterior region of the brain. (AI Removal of the facial skeleton forming neural crest cells from a 6-somite stage chick embryo stops the telencephalon from forming, as well as inhibiting formation of the facial skeleton.

However, telencepha lon development could be rescued by adding Fgf8-containing beads to the anterior neural ridge. (8) Embryo stained with HNK-1 (which labe l s neural crest cells green).

FgfB appears pink i n this micrograph. (After Creuzet et al. 2006, 2009; photographs courtesy of N. le Douarin.) duction of Fgf8 in the anterior neural ridge, thereby allow ing development of the midbrain and forebrain. Tooth formation The cranial neural crest cells of the first pharyngeal arch also form the interior, dentin-secreting odontoblasts of the teeth.

The jaw epithelium becomes the outer, enamel secreting ameloblasts. The mouse tooth 'is specified by either Fgf8 or BMP4. Fgf8 induces the expression of the Barxl transcription factor and molds the tooth bud to fonm molars, whereas BMP4 induces the expression of the Msxl and Msx2 transcription factors that mold the tooth to form incisors (Mina and Kollar 1987; Lumsden 1988b; Tucker et al. 1998).

The Signaling center of the tooth is the enamel knot, a group of cells induced in the epithelium by the neural crest-derived mesenchyme (jemvaU et al. 1994; Vaahtokari et al. 1996a,b; Tummers and Thesleff 2009). The enamel knot secretes a cocktail of paracrine factors, including Shh, BMPs 2, 4, and 7, and Fgf4. Shh and Fgf4 induce the pro liferation of cells to form a cusp, while the BMPs inhibit the formation of new enamel knots. As will be shown in Chapter 19, these proteins act through a reaction-diffusion mechanism to pattern the cusps of the tooth. Cardiac Neural Crest The heart originally forms in the neck region, directly beneath the pharyngeal arches, so it should not be surpris ing that it acquires cells from the neural crest. The caudal region of the cranial neural crest is sometimes called the careliac neural crest, since its ceUs (and only these particu lar neural crest cells) generate the endothelium of the aor tic arch arteries and the septum between the aorta and the pulmonary artery (Figure 10.1 5; Kirby 1989; Waldo et al. 1998). Cardiac crest cells also enter pharyngeal arches 3, 4, and 6 to become portions of other neck structures such as the thyrOid, parathyroid, and thymus glands. These cells are often referred to as the circumpharyngeal crest (Kuratani and Kirby 1991, 1992).

It is also likely that the carotid body, which morutors oxygen in the blood and reg- N EU RA L CREST CELLS A N D AXONAL SPECIFICITY .\) [eural be ru Cardiac neural crest r · : [:: [. : :: [:::::::: : ::::: : ". . . [[[[/', [.:: pulmonary /[",.. ------A [[[. : . :: . . re[.

. Aortic- se Ptum Pulmonary artery (8) • • •

first proposed by Santiago Ram6n y Cajal (1892). He sug growth of commissural axons.

Serafini and his co-workers tested them to see if any of the proteins therein mimicked and secreted from the floor plate cells, whereas netrin-2 is syntheSized in the lower region of the spinal cord, but not in the floor plate (Figure 1 0.258).

It is possible that the com missural neurons first encounter a gradient of netrin-2 and Shh, which brings them into the domain of the steeper netrin-1 gradient. The netrins are recognized by receptors, DCC and DSCAM, found in the axon growth cones (Uu et al. 2009). gested that the commissural neurons of the spinal cord Although they are soluble molecules, both netrins might be told by diffusible factors to send axons from their become associated with the extracellular matrix.* Such dorsal positions to the ventral floor plate. Commissural effect of the nelrin hom attractive to' repulsive. The growth neurons are intemeurons that cross the ventral midline to coordinate right and left motor activities. Thus, they some how must migrate to (and through) the ventral midline. The axons of these neurons begin growing ventrally down the side of the neural tube. However, about two-thirds of the way down, their direction changes, and they project through the ventrolateral (motor) neuron area of the neu (Figure 1 0.2SA). in attracting There appear to be two systems in volved ral tube toward the floor plate cells the commissural neurons to the ventral midline. The first is the Sonic hedgehog protein that is made in the floor plate (see Figure 9.14; Charron et a!. 2003). If Shh is inhibited by cyclopamine or conditionally knocked out of the floor plate cells, the commissural axons have difficulty getting to the ventral midline. However, a gradient of Shh does not pro yjde a full explanation of the migration. Some other factor is also involved. In 1994, Serafini and colleagues developed an assay that allowed them to screen for the presence of a presumptive diffusible molecule that might be guiding the commissural neu rons. When dorsal spinal cord explants FIGURE 10.25 Trajeclory of commissural axons in the rat spinal cord. (A) Schematic drawing of a model wherein commissural neurons first experience a gradi ent of Sonic hedgehog and netrin-2, and then a steeper gradient of netrin- 1 . The commissural axons are chemotacti cal l y guided ventrally down the lateral margin of the spinal cord toward the floor plate. Upon reaching the floor plale, contact guidance from the floor plate cells causes the axons to change direction. (B) Autoradiographic localization of netrin-' mRNA by in situ hybridization of an ti sense RNA to the hindbrain of a young rat embryo. Netrin- 1 mRNA (dark area) is concentrated in the floor plate neurons.

(B from Kenn edy el al. 1 994, cou€esy of M. Tessier-Lavigne.) associations can play important roles and may change the cones of Xenopus retinal neurons, for example, are attract ed to netrin-1 and are guided to the head of the optic nerve by this diffusible factor. Once there, however, the combi nation of netrin-1 and laminin prevents the axons hom leav ing the optic nerve. It appea rs that the laminin of the extra cellular matrix surrounding the optic nerve converts the netrin from an attractive molecule to a repulsive one (Hop ker et al. 1999). The structures of the netrin proteins have numerous regions of homology with UNC-6, a p rotein implicated in *The binding of a soluble factor to the extracellular matrix milkes for an interesting ambiguity between du€motaxis and migrating on Nature doesn't necessarily con the ca tegories we create. There is also some confusion between the terms neurotropic and nettrotropllic. Neurotropic (Latin, tropicus, "a turning movement") means that something attracts the neu ron. Neurotrophic (Greek, trophikos, "n u rsing") refers to a fac tor's ability to keep the neuron alive, usually by supplying growth factors. Since many agents have both properties, they are alterna tively called neurotropins and neurotrophins. In the recent literature, neurotrophin appears to be more widely used. preferred substrates (Imptotaxis). fonn to (A) (B) Neural tube neuron Netrin l gradient plate Floor plat.€ N E U RA L CREST CELLS AND AXONAL SPECIFICITY C. elegam 399 FIGURE 10.26 UNC expression and function in axonal gU i d ance. (A) In the body of the wild-type C. elegans embryo, sensory neurons project ventrally and motor neurons project dorsally. The ventral body wall epidermoblast5 expressing UNC-6 are darkly s haded . (6) In the unc-6 mutant embryo, neither of these m igra tions occurs.

(C) The unc-5 loss-of function mutation affects on ly the dorsal movements of the motor neurons. (D) The unc-40 loss-of function mutation affects only the ventral migration of the sen sory growth cones. (After Goodman 1 994.) A) growth cones of Drosophila neurons express a Roundabout (Robo) protein, which is the receptor for Slit. In this way, most Motor neuron Drosophila neurons are prevented from migrating across the midline. However, the commissural neurons that traverse the embryo from side to side avoid this repulsion by downregulating Robo protein as they approach the mid C) line. Once the growth cone is across the middle of the embryo, the neurons re-express Robo and become sensi tive again to the midline inhibitory actions of Slit (F igure 1 0.27A; 2004). Brose et al. 1999; Kidd et al. 1999; Orgogozo et al. In vertebrates, the Slit/Robo system cooperates with the netrin/DCC system to permit the commissural neurons to cross the midline; there are several vertebrate Robo and Slit proteins, and they do different tasks (Mambetisaeva et 2005). As the axon extends toward its target in the devel directing the circumferential migration of axons around al. the body wall of Caenorhabditis eIegans. In the wild-type oping brain, the neuron is kept from crossing the midline (Figure 1 0.27B). nematode, UNC-6 induces axons from certain centrally by Slit, which binds to Robo1 located sensory neurons to move ventrally while inducing Robo1 protein prevents DCC from binding to the netrin In Imc-6 Ioss-of function mutations, neither of these axon al movements OCCUrS (Hedgecock et aJ. 1990; Ishii et a!. 1992; Hamelin et a!. 1993). Mutations of the I1nc-40 gene proteins. When the axon gets near the midline, however, disrupt ventral (but not dorsal) axonal migration, where line. The axon grows through the midline, but after cross as mutations of the ul1c-5 gene prevent only dorsal migra ing it, Rob03 is downregulated while Robol is upregulat €tentrally placed motor neurons to extend axons dorsally. tion (Figure 1 0.26) .

Genetic and biochemical evidence sug gests that UNC-S and UNC-40 are portions of the UNC-6 receptor complex, and that UNC-5 can convert a UNC-40mediated attraction into a repulsion (Leonardo et aJ. 1997; Hong et a!. 1999; Chang 2004). There is reciprocity in science, and just as research on vertebrate netrin genes led to the discovery of their C. ele gans homologues, research on the nematode unc-5 gene led to the discovery of the gene encoding the human netrin receptor. This turns out to be a gene whose mutation in Expressed Rob03 is expressed and blocks RoboL Robo1 is no longer able to bind Slit or to block DCC. This enables netrin to bind DCC and turns the axon growth cone toward the mid ed. This allows Slit to act as a chemorepellent, forCing the growth cone away from the midllne. DCC is once again blocked, preventing the axon from going back (Woods 2004).

Mutations in the human ROB03 gene disrupt the normal crossing of axons from one side of the brain's medulla to the other Gen et al. 2004). Among other prob lems, people with this mutation are unable to coordinate their eye movements. See WEBSITE 1 0.6 The early evidence for chemotaxis mice causes a disease called rostral cerebellar malforma- tion (Ackerman et a!. 1997; Leonardo et a!. 1997). See WEBSITE 1 0.5 Genetic control of neuroblast migration in C.

elegans SLIT AND ROBO Target selection In some cases, nerves in the same ganglion may have sev eral different targets. How do the different neurons know where to go? It seems they use the same strategy as the Diffusible proteins can also provide guid neural crest cells. In some cases, different neurons in the ance by repulsion. One important chemorepulsive mole same ganglion have dilierent receptors and can therefore cule is the Slit protein. In Drosophila, Slit is secreted by the respond to certain cues and not others. For instance, some neural ceUs in the midline, and it acts to prevent most neu neurons in the superior cervical ganglia (the biggest gan rons from crossing the midline from either side. The glia in the neck) go toward the carotid artery, while other 400 (A) CHAPTER 1 0 S t protein (B) li —, Robo protein Axon I 1.1 -\ € (E) 1 Robo Dec (e) Wild type Nervous system midline I €0 Slit 0 chemorepellant 0 1€ + fJI Netrin Q blo cked I:l € btOSliCkt€ o (D) Slit ! 0 chemoattractant Q chemorepellanc € Slit 0 Raba3 FIGURE 10.27 RobolSlit regu lation of midline crossing by neu rons. (A-D) Roba and Slit in the Drosophila central nervous sys tem. (A) Antibody staining reveals Slit protein in the midline neu rons. (B) Roba protein appears along the neurons of the longitudinal tracts of the CNS axon scaffold. (C) Wi ld type CNS axon scaffold shows the ladderlike arrangement of neurons cross ing the midline. (D) Staining of the CNS axon scaffold with anti bodies to all eNS neurons in a slil loss-of function mutant shows axons enter ing but failing to leave the midline (instead of running a longsid e it). (E) Regulation by Slit and Robo in vertebrates. Neu rons are prevented from crossing the midline by Slit, which acti vates the Robo 1 gene. Robot then blocks DCC from inding to the netrin proteins. When the axon gets near the midline, Roba3 is exp ressed and blocks these functions of Rob0 1 , thus enab l i ng netrin to bind to DeC and turning the axon growth cone toward the midline.

Aiter the cone crosses the midline, Robol is upregu lated and Rob03 is down regu lated This allows Slit to act as a chemorepellent, forcing the growth cone away from the midline. (A-D from Kid d et al. 1 999, courtesy of C. S.

Goodman; E after Woods 2004.) - b o o 0. € blocket d /' Q Ne rin 0 Rabal Dec — . neurons from these ganglia do not. It appears that those axons extending to the carotid artery follow the blood ves sels that lead there. These blood vessels secrete small pep tides called endothelins. In addition to their adult roles constricting blood vessels, endothelins appear to have an embryonic role as well, as they are able to direct the exten sion of certain sympathetic axons that have endothelin receptors (Makita et a!. 2008). Similarly, the trigeminal gan glion has three peripheral axon bundles that innervate the eye regions, the upper jaw, and the lower jaw (Figure 1 0.28); these include the neurons dentists "put to sleep" with novocaine while filling cavities or extracting teeth. BMP4 from the target organs causes the differential growth and differentiation of these neurons, but intrinsic differ ences in transcription factors enable them to respond differ ently to this Signal and allow their axons to migrate in their particular ways (Hodge et al. 2007). Once a neuron reaches a group of ceUs in which lic its potential targets, it is responsive to various proteins pro duced by the target cells. In addition to the proteins already mentioned, some target cells produce a set of chemotactic N E U RAL CREST CEllS A N D AXONAL SPECIFICITY nerve (nose) Cinerves liary , ...“' FIGURE 10.28 The trigeminal ganglion has three main branches: the ophthalmic (to the eyes), the max i l lary (to the upper jaw), and the man d i bu lar (to the lower jaw). The growth of these nerves is regulated by BMP4 from the target lissues combined with differ- ential receptors for the BMPs on the neuron s.

() €, € b =I € €€€.rr.;,; al " .€ r VI €€ _ _ I. ' m ;J € €€€€ibular R €€ .:' ' ' ' I (cornea) / " \ (€ .. _ €" (€€€lary € interneuron axons in the developing cerebrum and to ini tiate the elongation and fasciculation of long-range axons from the cerebral cortex \ €: € Inferi or alveolar \:.... — € .. 401 ... nerve (teeth) \ \ 2(08). The attachment (Harkany el al. 2008; Mulder et al. of an axon to its target can be either Udigital'" or "analogue. '€ In "analogue" mode, different axons recognize the same molecule on the target, but the amount of the molecule on the target appears to be critical fish brain 2008). In other cases, there may be ex tre mely molecule specific ("digita l") binding such that certain con nections are neuron-specific. This may be the case for reti to the connections that form. This may be the case in the attachment of retinal neurons to the tectum in the (Gosse et al. - nal neurons in Drosophila . Given the complexity of neural connections, it is probable that both qualitative and quan factors collectively called neurotrophins. These proteins titative cures are used. Growth cones do not rely on a sin include nerve growth factor (N G F), brain-derived neu rotrophic factor (BDNF), and neu rotrophins 3 and 4/5 gle type of molecule to recognize their target, but integrate These proteins are released from potential selecting their targets based on the combined input of these (NT3, NT4/5). target tissues and work at short ranges as either chemotac the sinnultaneously presented attractive and repulSive cues, multiple signals (Wmberg et al. 1998). tic factors or chemorepulsive factors (Paves and Saarma 1997). Each can promote and attract the growth of some axons to its source while inhibiting other axons. For are attracted to sources of NT3 instance, sensory neurons from the rat dorsal foot ganglia (Figure 1 0.29) but are inhib ited by BONE These factors are probably transported from the axonal growth cone to the soma of the neuron. For instance, NGF derived from the hippocampus of the brain binds to receptors on the axons of basal forebrain neurons and is endocytosed into these neurons.

It is then transport ed back to the nerve cell body, where it stimulates gene expression. Increased amounts of the gene App (a gene on chromosome 21 encoding ameloid precursor protein) are seen in people with Down syndrome and Alzheinner's dis ease, and increased App locks the retrograde transport of NGF from the axon to the cell soma (Salehi et o 2 6 min lOmin m in aJ. 2006). Another group of soluble molecules that appear to steer receptors during developme nt developing axons are endocannabinoids, which normal ly bind to cannibinol '-Endocannabinoids appear to regulate the migration of cans not toco d d i t s c nnabi n oi d a the en led to concern ng tastiAnswad g alter-et o2009). d fetalg brain (Campololongosubtle et al.and 2009;lofutras*Exogenous cannabinoids, such as those in marijuana, these receptors. While marijuana and nsi ere classic teratogens, brain developm importance of endocannaboids in t has smoking urin pregnancy can cause ali n s in the .1. also bind a are over whether marijuana FIGURE 10.29 Embryonic axon from a rat dorsal root ganglion turning in response to a source of NT3. The photographs docu ment the growth cone's turn over a 1 O minute period. The same growth cone was insensitive to other neurotrophins. (From Paves and Saarma 1 997, cou rtesy of M. Saarma .) 402 CHAPTER 1 0 (A) Growth cone contacts myotube (D) (B) receptors Neural receptorsagritonclinuduces ster ACh Sheathi Schwannngcelbyls (E) In maturity Muscl fiber e (e) Synaptic basal lamina forms (F) Neuromuscul juncti on ar Schwann cells FIGURE 1 0.30 Differentiation of a motor neuron synapse with a muscle in mammals. (A) A growth cone approaches a developing muscle cell. (B) The axon stops and forms an unspecialized con tact on the muscle surface. Agrin, a protein released by the neu ron, causes acetylcholine (ACh) receptors to.cluster near the axon. (C) Neurotransmitter vesicles enter the axon terminal, and an extracellular matrix connects the axon terminal to the muscle cell as the synapse widens. This matrix contains a nerve-specific laminin. (0) Other axons converge on the sam€ synaptic site. The Forming the synapse: Activity-dependent development When an axon contacts its target (usually either a muscle or another neuron), it forms a specialized junction called a synapse. Neurotransmitters from the axon terminal are released at these synapses to depolarize or hyperpolarize the membrane of the target cell across the synaptic cleft. The construction of a synapse involves several steps (Burden 1998). When motor neurons in the spinal cord extend axons to muscles, growth cones that contact newly wider view (below) shows muscle innervation by several axons (seen in mammals at birth). (E) All but one of the axons are elimi- nated. The remaining axon can branch to form a complex neuro muscular junction with the muscle fiber. Each axon terminal is sheathed by a Schwann cell process, and folds form in the muscle cell membrane. The overview shows muscle innervation several weeks after birth.

(F) Whole-mount view of mature neuromuscular junction in a mouse.

(A-E after Hall and Sanes 1 993; Purves 1 994; Hall 1 995; F courtesy of M. A. Ruegg.) formed muscle cells migrate over their surfaces. When a growth cone first adheres to the cell membrane of a mus cle fiber, no specializations can be seen in either membrane.

However, the axon terminal soon begins to accumulate neurotransmitter-containing synaptic vesicles, the mem branes of both cells thicken at the region of contact, and the synaptic cleft between the cells fills with an extracellu lar matrix that includes a specific form of laminin (Figure 1 0.30A-C). This muscle-derived lamirun specifically binds the growth cones of motor neurons and may act as a "stop signal" for axonal growth (Martin et a!. 1995; Noakes et a!. Dorsalro t (C) Nodose (taste) N EURAL CREST CELLS AND AXONAL SPECIFICITY NGF BDNF (A) Sympathetic .. . . (B) . FIGURE 10.31 Effects of NCF (top row) and BDNF (bottom row) on axonal outgrowths from (A) sympa thetic ganglia, (B) dorsal root ganglia, and (e) nodose (taste perception) ganglia. While both NGF and BDNF h ad a mild stimulatory effect on dor sal root gangl ia axonal outgrowth, the sympathetic ganglia responded dramatically to NCF and hardly at a l l to BDNF; th e converse was true o f the nodose ganglia. (From Ibanez et a!. 1 99 1 .) . • . ' ., _ € . I , ' • 403 - 1995). In at least some neuron-to-neuron synapses, the synapse is stabilized by N-cadherin. The activity of the synapse releases N-cadherin from storage vesicles in the growth cone (Tanaka et al. 2000). In muscles, after the first contact is made, growth cones from other axons converge at the site to form additional synapses. During mammalian development, all muscles that have been studied are innervated by at least two axons. However, this polynecttronal intlervation is transient, soon after birth, all but one of the axon branches are retract ed (Figure 1 0.30D-F). This "address selection" is based on competition between the axons (Purves and Lichtman 1980; Thompson 1983; Colman et aJ. 1997). When one of the motor neurons is active, it suppresses the synapses of the other neurons, possibly through a nitric oxide-depend ent mechanism (Dan and Poo 1992; Wang et al.

1995). Eventually, the less active synapses are eliminated. The remaining axon terminal expands and is ensheathed by a Schwann cell (see Figure 10.30E). Differential survival after innervation: Neurotrophic factors One of the most p uzzling phenomena in the development of the nervous system is neuronal cell dea th. l.n many parts of the vertebrate centraJ and peripheral nervous systems, more than half the neurons die during the normal course of development (see Chapter 3).

Moreover, there do not seem to be great siJnilarities in apoptosis patterns across species. For example, about 80% of a cat s retinal ganglion cells die, while in the chick retina, this figure is only 40%. l.n fish and amphibian retinas, no ganglion cells appear to die (patterson 1992). The apoptotic death of a neuron is not caused by any obvious defect in the neuron itself. Indeed, these neurons have differentiated and have success fully extended axons to their targets. Rather, it appears that the target tissue reg ulates the number of axons innervating it by limiting the supply of a neurotrophin. In addition to their roles as chemotrophic factors described in the previolls section, neu rotrophins regulate the survival of different subsets of neu rons (Figure 1 0.31). NGF, for example, is necessary for the survival of sympathetic and sensory neurons. Treating ' mouse embryos with anti-NGF antibodies reduces the num ber of trigeminal sympathetic and dorsal root ganglion neu rons to 20% of their control numbers (Levi-Montalcini and Booker 1960; Pearson et al. 1983). Furthermore, removal of these neurons' target tissues causes the death of the neurons that would have innervated them, and there is a good corre lation between the amount of NGF secreted and the survival of the neurons that innervate these tissues (Korsching and Thoenen 1983; Harper and Davies 1990). BDNF does not affect sympathetic or sensory neurons, but it can rescue fetal motor neurons in vivo from nonnal ly occurring cell death, and from induced cell death fol lowing the removal of their target tissue. The results of these in vitro studies have been corroborated by gene knockout experiments, wherein the deletion of particular neurotrophic factors causes the loss of only certain subsets of neurons (Crowley et al. 1994; Jones et a1. 1994). Neurotrophic factors are produced continuously in adults, and loss of these factors may produce debilitating diseases. BDNF is required for the survival of a particular subset of neurons in the striatum (a region of the brain involved in modulating the intensity of coordinated mus cle activity such as movement, balance, and walking) and enables these neurons to differentiate and synthesize the receptor for dopamine . BDNF in this region of the brain is upregulated by Huntingtin, a protein that is mutated in Huntington disease. Patients with Huntington disease have decreased production of BDNF, which leads to the death of striatal neurons (Guillin et a l. 2001; Zuccato et al. 2001). The result is a series of cognitive abnormalities, involun tary muscle movements, and eventual death.

Two other neurotrophins-glial-derived ne urotrophic factor (GDNF, which we discussed earlier in terms of neural crest migra tion) and conserved dop a mine neurotrophic factor (CDNF)-enhance the survival of another group of neu rons, the midbrain dopaminergic neurons, whose destruc tion characterizes Parkinson disease (Lin et al. 1993; Lind holm et al.

2007). These neurons send axons to the striatum, whose abilit y to respond to their dopamine signals is dependent on BDNF. GDNF and CDNF can prevent the death of these neu rons in adult brains (see Lindsay 1995) and are being considere d as possible therapies for Parkin son disease (Zurn et al. 2001).

404 CHAPTER 1 0 SIDEL IG H TS . SPECULATIONS The Brainbow O one of the difficulties encoun tered by developmental biolo gists is trying to follow any one of the thousands ofaxons from its source to its specific destination. (Think about how difficult it is to trace wires from a computer to a surge pro tector; then multiply by several hun dred thousand.) But now there i s a way that scientists can color-code axons. Livet and colleagues (2007) made a transgene for genes encoding four fluo rescent proteins, each fluorescing a t a different wavelength (orange, yel low, magenta, and blue). After each of these genes is a 3' UTR, and in front of each gene is a variant cite encoding a spe cific lox recombination site (Figure 1 0.32A). At the beginning of the trans gene is a constitutively active promoter and three variant lox recombi nation sites. When ere is i ntroduced i nto the cell, there is random recombination that can cause the promoter to be brought next to any of the fluorescent protein genes.

Moreover, if more than one transgene enters the neuron, com bi natorial expression can yiel d nearly 1 00 d iffe rent colors (Figure 10.32B). Th is ability to uniquely label and trace Promotor J€ , = €€€ : ••€' • ___ Construct loxN Outcomes lox2272 Orange fluorescent protein lox? € _ €l 0' • u, Magenta fluorescent protein Yellow fluorescent protei n r i Blue flllorescent p ote n (B) individual axons through the embryo should greatly facil itate studyi ng the devel opmen t of com plex neural Circuitry. FIGURE 10.32 Maki ng a "brai nbow." (Al Exp eri mental ly man i pu lated mice contain a reg io n or DNA constructed such that lox sites at the pro moter random ly iind homologous lox sites adjacent to one of four genes, each encoding a different fluorescent protein. Depend i ng on the rearrangement induced by the ere protein! different nuorescent proteins are expressed in different neurons. (B) The result is that adjacent neurons fluoresce diffe rent colors, all owing researchers to trace axonal routes and connections throug h the embryo. (A after Livet et al. 2007; B courtesy of J. Lichtman.) Paths to glory: The travels of the retinal ganglion axons Nearly all the mechanisms for neuronal specification and specificity mentioned in this chapter can b e seen in the way s individual retinal neurons send axons to the axon area s of the brain. Even when retinal ave are transplanted far away from the eye, they are able to find these brain areas (Harris 1986). The ability of the brain to gU de the axons of translocated neu ro ns to their appropriate large t sites implies that the guidance cues are not distributed solely along the normal pathway but vision-processing neurons i (A) N E U RAL CREST CELLS AND AXONAL SPECIFICITY Ephrin-B EphB 405 (B) Retinal Retina ganglion cell I Optic nerve Chiasm FIGURE 10.33 Multiple guidance cues direct the movement of retinal gangl io n cell (RGC) axons to the optic tectum. Guid ance molecules belonging to the netri n, sl i t, semapho ri n, a nd eph ri n families are expressed in discrete regions at several sites along the pathway to d i rect the RGC growth cones. RGC axons are repelled from the retinal periphery by chondroitin sulfate. At the optic disc, the axons exit the retina and enter the optic nerve, gui ded by netrin/DCG-mediated attraction. Once in the optic nerve, the axons are kept within the pathway by inhibitory inter actions. Slit protei ns in the optic chiasm create zo nes of inhibi tion. Zi c2-expressing gangl ia in the ventro temporal retina project EphB 1 -expressing axons, which are repe l l ed at the chiasm by ephrin-B2, thus termi nati ng at ipsi lateral (same-side) targets. \!eurons from the medial portions of the retina do not express EphB 1 and proceed to the oppos i te (contral ateral) s ide. A) Cross secti on . (B) Dorsal view. Not all cues are shawn. A aher van Horck et al. 2004; B after Harada et al. 2007.) • Chondroitin sulfate • DCC, Rob02 • Zic2, EphB I • Netrin • Semaphorins • Ephrin-B2 nerve Sli t 1/2 der 1992; Ohta et a1. 1999). Moreover, N-CAM may also be especiaily important here, since the directional migration of the retinal ganglion growth cones depends on the N-CAM-expressing glial endfeet at the inner retinal surface (Stier and Schlosshauer 1995). The secretion of netrin-1 by the cells of the optic disc (where the axons are assembled to form the optic nerve) plays a role well. Mice lacking in this migration as netrin-l genes (or the genes for the netrin receptor found in the retinal gangUon axons) have poorly formed optic nerves, as many of the axons fail to leave the eye and grow randomly aroun'd the disc (Deiner et a1. netrin-l is co surface of the retina. 1997). The role of netrin may change in different parts of exist throughout the embryonic brain. Guiding an axon the eye. At the entrance to the optic nerve, from a nerve ceU body to its destination across the embryo expressed with laminin on the is a complex process, and several different types of cues may be used Simultaneously to ensure that the correct con Laminin converts netrin from having an attractive signa] nections are estabUshed.

Although we are looking here at the growth cone away from the retinal surface and into the non-mammalian vertebrates, the processes we describe apply to mammals as weU. GROWTH OF THE RETINAL GANGLION AXON TO THE OPTIC "ERVE The first steps in getting retinal ganglion ceU (RGC) to having a repulSive signal. This repulsion might "push" head of the optic nerve, where netrin is expressed without larninin (Mann et al. 2004). Upon their arrival a t the optic nerve, the migrating axons fasciculate (bundle) with axons already present there. N-CAM and L1 cell adhesion molecules are critical to in a disorderly axons to their specific regions of the. optic tectum take place this fasciculation, and antibodies against L1 or N-CAM within the retina. As the RGCs differentiate, their position cause the axons to enter the optic nerve in the inner margin of the retina is determined by cadherin fashion, which in turn causes them to emerge at the wrong molecules (N-cadherin and retina-specific R-cadherin) on positions in the tectum (Thanos et a!. 1984; Brittis et al. al. 2004). The RGC axons grow along the inner surface of 1995; Ym et al. 1995). heir ceU membranes (Matsunaga et al. 1988; van Horck et the retina toward the optic disc (the head of the optic nerve). The mature human optic nerve will contain over a million re t inal ganglion a xons . The adhesion and growth of the retinal ganglion axons along the inner surface of the retina may be governed by its laminin-containing basal lamina. However, Simple adhe GROWTH OF THE RETINAL GANGLION AXON THROUGH THE OPTIC CHIASM When the axons enter the optic nerve, they grow on glial cells toward the midbrain. In non-mammalian vertebrates, the axons will go to a portion of the brain cailed the optic tectum. (Mammalian axons go to the lateral genic ulate nuclei).

After leaVing the eye, the retinal axons appear sion to laminin cannot explain the directionality of this to grow on surfaces of netrin, surrounded on all sides by growth. Other factors also play a role. The embryonic lens semaphorins keeping them on track and the periphery of the retina secrete inhibitory factors cues (see Harada et a1. 2007). Upon entering the brain, probably chondroitin sulfate proteoglycans) that repel the by proViding repulsive if they are to continue straight mammaUan retinal ganglion axons reach the optic chiasm, ganglial cell axons, thereby preventing them from travel where they have to "decide" .ng in the wrong direction (Figure or if they are to turn 90 degrees and enter the other side of 10.33; Hynes and Lan- 406 CHAPTER 1 0 the brain. In the optic chasm area, semaphorins are no longer present, but Slit proteins take over their function in establishing corridors through which the axons can travel. It appears that those axons not destined to cross to the opposite side of the brain are repulsed from doing so when they enter the optic chiasm (Godement et al. 1990). The basis of this repulsion appears to be the synthesis of ephrin on the neurons in the chiasm and ephrin receptors on the retinal axons (Cheng et al. 1995; Marcus et al. 2000). In the mouse eye, the Eph Bl receptor is expressed on those temporal axons that are repelled by the optic chi asm's ephrin B2, and those axons project to the side of the tectum on the same side as their eye (the ipsilateral projec tions); Eph Bl is nearly absent on axons that are allowed to cross over. Mice lacking the EphBI gene show hardly any ipsilateral projections. This pattern of EphBI expres sion appears to be regulated by the Zic2 transcription fac tor found on those retinal axons that do forni ipsilateral projections (Herrera et al. 2003; Wtlliams et al. 2003; Pak et al. 2004).

Ephrin appears to play a similar role in the retinotectal mapping in the frog. In the developing frog, the ventral regions express the Eph B receptor, while the dorsal axons do not. Before metamorphosis, both axons cross the optic chiasm. However, when the frog nervous system is being remodeled during metamorphosis, the chiasm expresses ephrin B, which causes a subpopulation of ventral cells to be repulsed and project to the same side rather than cross the chiasm (Mann et al. 2002). (This allows the frog to have binocular vision, which is very good if one is trying to catch flies with one's tongue.) Laminin appears to promote crossing of the optic chi asm. On their way to the optic tectum, the axons of nOI1mammalian vertebrates travel on a pathway (the optic tract) over glial cells whose surfaces are coated with laminin. Very few areas of the brain contain laminin, and the laminin in this pathway exists only when the optic nerve fibers are growing on it (Cohen et al. 1987). TARGET SELECTION When the axons come to the end of the laminin-lined optic tract, they spread out and find their specific targets in the optic tectum. Studies on frogs and fish (in which retinal neurons from each eye project to the opposite side of the brain) have indicated that each retinal ganglion axon sends its impulse to one specific site (a cell or small group of cells) within the optic tectum (Figure 1 0.34A; Sperry 1951).

There are two optic tecta in the frog brain. The axons from the right eye form synapses with the left optic tectum, and those from the left eye form synaps es in the right optic tectum. The map of retinal connections to the frog optic tectum (the retinotedal projection) was detailed by Marcus Jacob son (1967). Jacobson created this map by shining a narrow beam of light on a small, limited region of the retina and noting, by means of a recording electrode in the tectum, (A) Caudal (posterior) (B) Rostral Optic tract -€_ _ _ _ Optic entasrn Optic neIve- €'' _!. _ _ _ _ _ _ _ _ _ _ _ _ _ _ FIGURE 1 0.34 Retinotectal proj ect ions . IA) Confoca l micro graph of axons entering th e tectum of a s-day zebrafish embryo. Fluorescent dyes were injected into the eyes of zebrafish embryos mounted in agarose. The dyes diffused down the axons and into the tectum, showing the retinal axons from the right eye going to the left tectum and vice versa. (S) Map of the normal retinotectal projection in ad u l t Xenopus. The right eye innervates the left tec tum, and the left eye innervates the right tectum. The dorsal por tion of the retina (D) innervates Ihe lateral IL) regions of the tec tum. The nasal (anterior) region of the retina projects to the caudal Ie) region of the tectu m . (A courtesy of M. Wi lson; B after Holt 2002, cou rtesy of C. Hall.) (anterio r) Visual image N E U RA L CREST CELLS AND AXONAL SPECIFICITY Tectal membranes Anterior Posterior Anterior Posterior 407 FIGURE 10.35 Differential repulsion of temporal retinal ganglion axons on tectal membranes. Alternating stripes of anterior and posterior tecta I membranes were absorbed onto filter paper. When axons from temporal (posterior) retinal ganglion cells were grown on such alternating car pets, they preferentially extended axons on the an teri o r tecta I memb ranes. (From Wal ter et al. 1 987.) Anterior Posterior Anterior which tectal cells were being stimulated. The retinotectal projection of Xenopus faevis is shown in Figure 1 0.34B. Light illuminating the ventral part of the retina stimulates cells on the lateral surface of the tectum. Similarly, light focused on the temporal (posterior) part of the retina stimulates cells in the caudal portion of the tectum. These studies demonstrate a point for point correspondence between the cells of the retina and the cells of the tectum. When a group of retinal cells is activated, a very small and specific group of tectal cells is stimulated. Furthermore, the points form a continuum; in other words, adjacent points on the retina project onto adjacent points on the tectum. This arrange ment enables the frog to see an unbroken image. This intri cate specificity caused Sperry (1965) to put forward the chemoaffinity hypothesis: The complicated nerve fiber circuits of the brain grow, assemble, and organize themselves through the use of intricate chemical codes under genetic control. Early in development, the nerve cells, numbering in the millions, acquire and retain thereafter, individual identification tags, chemical in nature, by which they can be distin guished and recognized from one a.nother. Current theories do not propose a point-for-point speci -icity betw"een each axon and the neuron that it contacts. Rather, evidence now demonstrates that gradients of adhe sivity (especially those involving repulsion) play a role in defining the territories that the axons enter, and that activ ity-driven competition between these neurons determines the final connection of each axon. €DHESIVE SPECIFICITIES IN DIFFERENT REGIONS OF THE OPTIC TECTUM TI,ere is good evidence that retinal ganglion cells can distinguish between regions of the optic tectum. Cells taken from the ventral half of the chick neural retina pref erentially adhere to dorsal (medial) halves of the tectum, and vice versa (Gottlieb et aJ. 1976; Roth and Marchase 1976; Halfter et al. 1981). Retinal ganglion cells are speci fied along the dorsal-ventral axis by a gradient of transcrip- lion factors. Dorsal retinal cells are characterized by high concentrations of ThxS transcription factor, while ventral cells have high levels of Pax2. These transcription factors are ind uced by paracrine factors (BMP4 and retinoic acid, respectively) from nearby tissues (Koshiba-Takeuchi et al. 2000). Misexpression of nx5 in the early chick retina results in marked abnormalities of the retinotectal projec tion. Therefore, the retinal ganglion cells are specified according to their location. One gradient that has been identified functionally is a gradient of repulsion that is highest in the posterior tec tum and weakest in the anterior tectum. Bonhoeffer and colleagues (Walter et al. 1987; Baier and Bonhoeffer 1992) prepared a "carpet" of tectal membranes with alternating "stripes" of membrane derived from the posterior and the anterior tecta. They then let cells from the nasal (anterior) or temporal (posterior) regions of the retina extend axons into the carpet. The nasal ganglion cells extended axons equally well on both the anterior and posterior tectal mem branes. The neurons from the temporal side of the retina, however, extended axons only on the anterior tectal mem branes (Figure 10.35). When the growth cone of a tempo ral retinal ganglion axon contacted a posterior tectal cell membrane, the growth cone's filopodia withdrew, and the cone collapsed and retracted (Cox et al. 1990). The basis for this specificity appears to be two sets of gradients along the tectum and retina.

The first gradient set consists of ephrin proteins and their receptors.

In the optic tectum, ephrin proteins (especially ephrins A2 and AS) are fowld in gradients that are highest in the posterior (caudal) tectum and decline anteriorly (rostrally) (Figure -1 O.36A) . Moreover, cloned ephrin proteins have the abili ty to repulse axons, and ectopically expressed ephrin will prohibit axons from the temporal (but not from the nasal) regions of the retina from projecting to where it is expressed (Drescher et al. 1995; Nakamoto et al. 1996). The complementary Eph receptors have been found on chick retinal ganglion cells, expressed in a temporal-to-nasal gra dient along the retinal ganglion axons (Cheng et al. 1995). CHAPTER 1 0 408 the medial region and lowest laterally (like the ephrin gra This gradient appears to be due to a spatially and tempo rally regulated expression of retinoic acid (Sen et a1. 2005). Ep hrin appears to be a rem a rkab l y pliable molecule Concentration differences in ephrin A in the tectum can account for the smooth topographic map (wherein the posi tion of neurons in the retina maps continuously onto the targe ts Hansen and collea gues (2004) h a ve shown that ephrin A can be an attractive as well as a rep ulSive signal for retinal axons. Moreover€ their quantitative assay for axon growth showed tha t the origin of the axon deter mined whether it was attracted or repulsed by ephrins Axon growth is promoted by low ephrin A concentrations that a re anterior to the proper target and inhibited by high er concentrations posterior to the corre ct target (F i gure 1 0.36B). Each axon is thus led to the appropriate place and then told to go no further. At that equilibrium point, there would be no growth and no inhibition, and the synapses dient). In the retina, a gradient of Wnt receptor is highest ventrally (like the Eph proteins). The two sets of gradients .) are both required to specify the posi tion of the axon in the tectum (Schmitt et a!. 2006).

The Development of Behaviors: Constancy and Plasticity robiology is the correlation of certain neuronal COIUlectiOns One of the most fascinating aspects of develop menta l neu . with certain behaviors. Although this could be the subj ect of another (large) textbook, two remarkable aspects of this phenomenon need to be mentioned here. First, there are ca ses in which complex behavioral patterns are inherent beat of a 19-day chick embryo quickens when it hears a ly present in the " circuitry" of the brain at birth The heart .. The second set of gradients parallels the ephrins and with the target tectal neurons could be made. distress call, and no other call will evoke this response (Gottlieb 1965). Furthermore, a newly hatched chick will Ephs. The tectum has a gradient of Wnt3 that is highest at immediately seek shelter if presented with the shadow of a hawk An actual hawk is not needed; the shadow cast by a paper silhouette will suffice The shadow of a different bird will not elicit the sam e response (Tmbergen 1951). There appear, then, to be certain neuronal connections th t lead to "hard-wired" behaviors in vertebrates. Synapses also can fonn as the result of neural actiVity, and activity dependent synapse formation appears to be involved in the final stages of retinal projection to the brain. In frog, bird, and rodent embryos treated with tetrodotox in, axons will grow normally to their respective territories and will make synapses with the tectal neurons. Howev er, the retinote etal map is a coarse one, lacking fine resolu . a - Ey e tase) E p h r i n s S (l i g a d) . . €_• . . . .:: : : : € n :;;. "'€.€Or rAn;in;te;hrio;r. :::;. €..€. ..... €€... :. "'::::;. Posterior :: :: € :; ;: €:: Nasal Telnporal . Tectwn Temporal retina . . Attractiuonl Re lsi n Attraction Equi(targleibt)rium ph (re c .. .. € "€;;;:€1 (B) . €... . r€€€:::::.) Eq ili rium b (target) . ... "'..€'' "_'. .. "_ . . . . p or .. • •• . . ..

and ventral body wall; and the dermamy otome, which contains skeletal muscle progenitor cells, (including those that migrate into the limbs) and the cells that generate the dermis of the back. In addition, other, smaller compartments are formed from these three. The syndetome arising from the most dorsal sclerotome cells generates the tendons, while the most internal cells of the sclerotome (sometimes called the arthratome) become the vertebral joints, the intervertebral discs, and the proximal l PARAXIAL A N D I NTERMED IATE MESODERM (A) P&#65533; rimit ve strea ;&#65533;Epibi)last EndoEpdeirdmer"'' mis - - .

Mlgratmg mesodermalceUs Endoderm Notochord Lateralmesoderm IntermediatSeclerotome ofsomite of son"t" to coelom _'li p (B) (C) CD) . Dermatome . L _&#65533;lt : . .

&#65533; k ,# / . . ;AO$&#65533;y. 415 FIGURE 1 1 .2 Gastrulation and neu rulation in the chick embryo, focusing on the mesoderma l component. (A) Primitive streak region, showing migrating mesodermal and endoder mal precursors. (8) Formation of the notochord and paraxial mesoderm. (C, D) Differentialion of the somites, coelom, and the two aortae (which will eventually fuse). A-C, 24-hour embryos; D, 48-hour embryo. som i ti t portion of the ribs (Mi t apall el a1. 2005; Chris et a1. 2007). Moreover, an as-yet lmnamed group of cells in the posteri or sclerotome generates vascular cells of the dorsal aorta and intervertebral blood vessels (Table 1 1 .1; Pardanaud et a1. 1996; Sato et a1. 2008). See VADE MECUM Mesoderm in the vertebrate embryo Formation of the Somites The important components of somitogenesis (somite for mation) are (1) periodicity, (2) fissure formation (separa tion of the somites), (3) epithelialization, (4) specification and (5) differentiation. The first somites appear in the ante rior portion of the trunkf and new somites "bud affn from the rostral end of the presomitic mesoderm at regular inter vals (F i gu re 1 1 .4). Somite formation begins as paraxial mesoderm cells become organized into whorls of cens, sometinles called somitomeres (Meier 1979). , n FIGURE 11.3 Specification of somites. Placing Noggi n-secreti g cells into a prospective region of chick lateral plate mesoderm will respeci fy that mesoderm into somite forming paraxial mesoderm. I nduced somites (bracketed) were detected by in situ hybridization with Pax3. (From Tonegawa and Takahashi 1 998, courtesy of Y. Takahashi.) 416 CHAPTER 1 1 t The somitomeres become compacted and split apart as m esenchymal cells making up the immature somite now c hange, with the outer cells joining into an epi thelium while the inner cells remain mesenchymal. Because indi vidual embryos in any species can develop a t slightly dif ferent rates (as when chick embryos are incubated a sligh ly different emperatures), the number of so mi es presen is usually the best indicator of h o w far devel opmen has proceeded . Traditional t t t tt t view TABLE 1 1 .1 fissures separate them into discre e, immature somites. The view t it t t tli Derivatives of the somite Current DERMAMYOTOME -Myo tome forms skeletal muscles Lateral edges generate primary myo tome that forms muscle Dermatome forms Central region forms muscle, muscle stem cells, dermis, brown fa cells back dermis SCLEROTOME Forms vertebral and rib cartilage Forms vertebral and rib cart lage Dorsal region forms endons (synde ome) Medial region forms blood vessels and meninges Cen ral mesenchyma reg on forms joints (ar hro tome) Forms smooth muscle cells of dorsal aorta FIGURE 1 1 .4 Formation of new somites. (A) Neural tube and samites seen by scanning electron microscopy. When the surface ectoderm is peeled away, well-formed samites are revealed, as well as paraxial mesoderm (bottom right) that has not yet separated into distinct somites. A rounding of the paraxial mesoderm into a somitomere is seen at the lower left, and neural crest cells can be seen migrating ventrally from the roof of the neu ral tube. (B) Sequential molecular and cel l u l a r events in somitogenesis. (i) At the bou n d ary (determined by Notch s gn a l i n g), a ventral-la-dorsal signal aligns the posterior border cells (i.e., those cells i m medi ately posterior to the presumptive border. (ii, iii) Mesp2!c Mesol induces Ephs in the posterior border cells, and the Ephs induce ephri n in the cells across the border. This creates the fissure. (iv) As the fissure forms, a separate sign al aligns the cel ls that will form the posterior boundary of the somite. (v) Ectoderma l signals act on GTPases to coordinate the transition from m esenchyma l o epithelial cell, complet ng the somite. (A courtesy of K. W. Tosney, B after Takahashi and Sato 2008.) i (ll) Anterior J "'1 (i) i (i i) (i) (iv) t (v) 0000 -2 --*0000 5 8.....-. 00 0v00 0(\)00 ° -i)00 o e iVoerntrVale&#65533;ntr'"al-toDo droIsraslal ibtnhoderudpceotrsiocenrlitosralign P st r I Patonomasterkioerai-ntfidosucutrieon signals tarlig er transition Ectodermal mcsenchymal ep i theli PARAX IAL AND INTERMEDIATE MESODERM 417 1981). The total number o f somites fomled is charac 50 in chicks, 65 in mice, and as many as 500 in some snakes) . (Tam Periodicity of somite formation teristic of a species (about Somite formation depends on a "clock and wave" mecha nism, in which an oscillating signal (the "clock") is provid ed by the Notch and Wnt pathways, and a rostral-to-caudal Where somites form: The Notch pathway gradient provides a moving "wave" of an FGF that sets the somite boundaries. In the chick embryo, a new somite is Although we do not completely understand the mecha formed about every nisms controlling the temporal periodicity of somite for 90 minutes.

In mouse embryos, this time frame is more variable (Tam 1981). However, somites somites form is the Notch signaling pathway (see Aulchla mation, one of the key agents in determining where appear at exactly the same time on both sides of the and Pourquie cells first enter the presomitic mesoderm. If the presomitic embryo, and the clock for somite formation is set when the 2008). When a small group of cells from a region constituting the posterior border at the presump and the rostral end faces the tail, somite fomlation will start mesoderm is inverted such that the caudal end is rostral tive somite boundary is transplanted into a region of unsegmented mesoderm that would not ordinarily be part from the caudal end and proceed rostrally. Even if isolat of the boundary area, a new boundary is created. The ed from the rest of the body, the presomitic mesoderm will transplanted boundary cells instruct the cells anterior to stages of presomihc mesoderm formation. When Xenopus border area. However, these nonboundary cells can acquire segment at the appropriate time and (Palmeirim et aJ. 1997). in the right direction them to epithelialize and separate. Nonboundary cells will not induce border formation when transplanted to a non Moreover, the number of somites is set at the initial boundary-forming ability or mouse embryos are experimentally or genetically reduced 2002). in size, the number of somites remains the same (A) Quail Chic.ken Chic.ken o II Cl Cl 11 · · · · · · __ ····· · New border formed fiGURE 1 1 .5 · · ·

[1 cells &#65533; Quail Q&#65533;(47%) (B) Quail 1 -2 Chicken Chicken III 0 OJ B ln :+&#65533; &#65533;. Notch signaling and somite formation. (AI Transplanta tion of a prospective somite boundary region into a nonboundary region creates a new boundary and a new somite. The transplanted quail cells can be identified by staining for a quail-specific protein. (8) Transplanta tion of non boundary cells into a nonboundary region does not create a new boundary or a new somite. (C) Transplantation of a nonboundary region that has had Notch activated will cause a new somite boundary to occur. (D) Dorsal views of a control mouse and its littermate (E) with t he Delta-like3 gene (the gene encoding a N otch ligand) knocked out. The 0113 mutant has several ossification centers (white squares) in rows instead of in a column, and its ribs are malformed. (A-C after Sato et al. 2002, photographs courtesy ofY. Takahashi; D from Dunwoodie et aJ. 2002, courtesy of S. Dunwoodie.) 1 1 .5A-C; Sato et a1. (2005) have been able to Morimoto and colleagues t ControL no new border formed [if an activated Notch protein is electroporated into those cells (Figure (D) Wild type 1&#65533; Electroporate activated Notch Chicken Chicken (C) Quail 1 .-- 2· . . t&#65533; r,;) 1[H t &#65533; &#65533; &#65533;.. New border formed ----.n (E) 0113 mutant 418 CHAPTER 1 1 visualize the endogenous level of Notch activity in mouse embryos, and have shown that it oscillates in a segmental ly defined pattern. The somite boundaries were formed at the interface between the Notch-expressing and Notch nonexpressing areas. Notch has also been implicated in somite fissioning by mutations. Segmentation defects have been found in mice that are mutant for important components of the Notch pathway. These include the Notcll protein itseil, as well as its ligands Delta-likel and Delta-like3 (Dlll and 0113). Mutations affecting Notch signaling have been shown to be responsible for aberrant vertebral formation in mice and humans. In humans, individuals with spondylocostal dys plaSia have numerous vertebral and rib defects that have been linked to mutations of the Delta-like3 gene. Mice with knockouts of Dll3 have a phenotype similar to that of the human syndrome (Figure 1 1 .5D; Bulman et aJ. 2000; Dun woodie et aJ. 2002). Moreover, Notch Signaling follows a remarkable wave like pattern wherein the Notch gene becomes highly expressed in the posterior region of the forming somite, just anterior to the fissure. Notch genes are transcribed in a cyclic fashion and function as an autonomous segmenta tion "clock" (Palmeirim et aI. 1997; Jouve et aJ. 2000, 2002). If Notch signaling determines the placement of somite for mation, then Notch protein must control a cascade of gene expression that ultimately separates the tissues. This wave can be illustrated by following the expres sion pattern of hairyl, a segmentation gene regulated by FIGURE 1 1.6 Som i te formation correlates with the wavel ike expression of the hairy 1 gene in the chick. (A) tn the posterior por tion of a chick embryo somite, 51 has just budded off the presomitic mesoderm. Exp ression of the hairy 1 gene (purple) is seen in the caudal half of this somite, as well as in the posterior portion of the pre somitic mesoderm and in C1 thin band that somite (Sol. (B) A caudal fissure (small arrow) begins to separate the new somite from the pre somitic mesoderm. The posterior region of hairy! expres ion extends anteri orly. (c) The newly formed somite is now referred to as 5 1 ; it retains the expression of hairy! i n its caudal half, as the posterior domain of hairy 1 exp ress ion moves farther anteriorly will form the caudal half of the n ext (A) Som ite ___ S, Region So Notch activity. I t i s expressed in the presomHic segmen tal plate in a cyclic, wavelike manner, cresting every 90 minutes in the chick embryo (Figure 1 1 .6). The caudal domain of the hairyl expression pattern rises anteriorly and then recedes like &#65533; wave, leaving a band of expres sion at what will become the posterior half of the somite. The caudal boundary of this domain is exactly where the transplantation experiments showed Notch expression to be important. One of the most critical genes in somite formation i s Mesp2/c-Mesol (the first name refers to the mouse homo logue, the second to the chicken homologue). This gene is activated by Notch, and its protein product, a transcrip tion factor, initiates the reactions that suppress Notch activ ity. This activation-suppression cycle causes Mesp2/c-Mesol expression to oscillate in tune and space. Wherever it is expressed, that site is the most anterior group of cells in the next somite, and the boundary forms immediately anterior to those cells (see Figure i1.4B). Mesp2/c-Mesol induces Eph A4 (one of the compounds whose repulsive interaction separates the somites) in the rostral half of the somite (Saga et aI. 1997; Watanabe et al. 2005).

In the caudal (posterior) half of the somite, Mesp2/ c-Mesol induces the expression of the transcription factor Uncx4.l (Takahashi et al. 2000; Saga 2007).

In this way, the somite boundary is determined, and the somite is given anterior/posterior polarity at the same time. This, as we saw in Chapter 10, is critical for the migration patterns of neural crest cells and neurons. (;:) (;:) of S So (;:) 5, S, So (;:) (;:) &#65533; (;:) ---+ hairyl _ expression r------ S,, (;:) Rostral (D) (C) (8) so forms) 90 minutes (one mite and shortens.

The former 51 somite, now called 52! undergoes differentiation. (D) The formation of somite 51 is complete, and the anterior region of what had been the posterior hairy 1 expression pattern is now the anterior expression pattern. It will become the caudal domain of the next somi te. The entire process takes 90 minutes. --------0--11 Caudal PARAXIAL AND INTERMEDIATE MESODERM 419 SIDEL IGH TS SPECULATIONS Coordinating Waves and Clocks i n Som ite Formation A CYci ie activation of Notch appears to be critical for forming the s&#65533;mites, but what controls Notch activation? The predominant model of somite formation is the clock-and-wavefront model, first pro posed by Cooke and Zeeman (1 976). In zebrafish, this been found to be rel atively simple: the clock involves a negative feedback loop of the Notch signaling pathway. One of the proteins activated by the Notch protein is also able to inhibit Notch, which would become active again. Such a cycle would create a " clock" whereby the Notch gene would be turned on and off by a protein it itself induces.

These off-and-on oscillations could provide the molecular basis for the periodicity of somite segmentation (Holley and NUsslein-Volhard 2000; l iang et al. 2000; Dale et al. 2003). More recent reports indicate that the Mesp2/c-Mesol protein may be such a regulator of Notch (Morimoto et al. 2005). The output from this protein controls the ephrins that mediate the separation of the block of cells that form the somite, The wave appears to be the gradi ent of Fgf8, which moves caudally as more cells are added to the posterior. Fgf8 --- (Axin Wavefront Bridge (As long as the unsegmented paraxial mesenchyme is in a region of relative ly high Fgf8, the clock will not func tion.

This appears to be due to the repression of Delta, the protein that is the major ligand of Notch. The bind ing of Fgf8 to its receptor enables the expression of the Her13.2 protein, which is necessary to inhibit the tran scription of Delta (see Dequeant and Pourquie 2008). Interestingly, the Fgf8 signal can be perturbed by the right left laterality signals being given at the same time, and the presence of retinoic acid is needed to insu late the somite wavefront from the laterality signals of Nodal proteins. If retinoic acid is not made, somitogenesis becomes asymmetric (Kawakami et al. 2005; Brend and Holley 2009). In chicks and mice, the situation appears to be far more complicated.

The Notch pathway sti l l provides the clock, but the clock appears to be sen sitive not only to Fgf but also to the Wnt signaling pathway, which also exhibits a posterior-to-anterior gradi ent and the retinoic add gradient that extends in the opposite direction. The details of how the clock and wave front are coordinated in amniotes have not been elucidated and are an extremely active area of research. It may be that the FgfB wavefront is Lfringe -+- Mesp2 Clock Effector Figure 1 1 .7 Hypothetical pathway for regulation of the clock through which an FgfS gradient regulates a Wnt oscillating clock, which in turn controls a Notch clock that can inhibit its own activity i n a negative feedback loop. Different species might use different molecules in such a scheme. merely providing the needed amount of fl-catenin that can be localized into the nucleus by Wnt signaling. Alterna tively, Fgf signaling may provide another clock whose oscillations form a segment when they coincide with both a Wnt clock and a Notch clock (Figure 1 1 .7). This latter model has been proposed by Goldbeter and Pourquie (2008), who show that this might have the best fit mathematically. One way to unravel the complex interactions of signaling molecules i n amn iotes may b e to look a t embryos that have extreme numbers of somites. Snake embryos, for instance, can have hundreds of somites. The pattern of gene expression in corn snake embryos indicates that they have the same FgfB and Wnt waves and Notch clockwork as chicks and mice; but the snake somite clock is four times faster relative to the embryo'S growth rate, rapidly segregating off blocks of pre somitic mesenchyme into more numerous, albeit smaller, somites (Gomez et al. 2008). If we can eluci date the alterations that are involved in regulating the speed of this clock, we might be able to figure out how the Signaling pathways become i nte grated to give a single output at specif ic temporal and spatial intervals. 420 CHAPTER 1 1 seen in Figure 1l.4A, the cells of the newly formed somite a re randomly organized as a m esenchymal mass. These cells have to be compacted into an outer epithelium and an internal me>enchyme (Figure 1 1 .9). Ectodermal Signals appear to cause the peripheral somitic cells to undergo mesenchymal-to epithelial transition by lowering the Cdc42 levels in these cells. Low Cdc42 levels alter the cytoskeleton, allowing epithelial cells to form a box around the remaining mesenchymal cells, which have a higher level of Cdc42. Another small GTPase, Rac1, must be at a certain level that allows it to activate Paraxi&#65533;, another tran scription factor involved in epithelialization (Burgess et a1. 1995; Barnes et aJ. 1997; Nakaya et aJ. 2004).

The epithelialization of each somite is stabilized by syn thesis of the extracellular matrix protein fibronectin and the adhesion protein N-cadherin (Lash and Yamada 1986; Hatta et aJ. 1987; Saga et al. 1997; Linask et al. 1998). N-cad herin links the adjoining cells into an epithelium while the fibronectin matrix acts alongside the Ephrin and Eph to promote the separation of the somites from each other (Martins et a1. 2009). Separation of somites from the unsegmented mesoderm Two proteins whose roles appear to be critical for fissure formation and somite sep aration are the Eph tyrosin e kinases and their ligands, the ephrin proteins.

We saw in Chapter 10 that the Eph tyrosine kinase receptors and their ephrin ligands are able to elicit cell-cell repulsion between the posterior somite and migrating neural crest cells. The separation of the somite from the presomitic mesoderm occurs at the ephrin B2/Eph A4 border. In the zebra fish, the boundary between the most recently separated somite and the presomitic mesoderm forms between ephrin B2 in the posterior of the somite and Eph A4 in the most anteri or portion of the presomitic mesoderm (Figure 1 1 .8; Durbin et a1. 1998). Eph A4 is restricted to the boundary area in chick embryos as well. Interfering with this signallng (by injecting embryos with mRNA encoding dominant nega tive Ephs) leads to the formation of abnormal somite bOllllldaries. In addition to the posterior-ta-anterior induction of the fissure (from Eph proteins to ephrin proteins on their neighboring cells), a second signal originates from the ven tral posterior cells of the somite, putting all the ceUs in reg ister so that the cut is clean from the ventral to the doral aspects of the somite (Sato and Takahashi 200S) . , Somite specification along the anterior-posterior axis Although all somites look identical, they will form differ ent structures.

For instance, the somites that form the cer vical vertebrae of the neck and lumbar vertebrae of the abdomen are not capable of forming ribs; ribs are generat ed only by the somites forming the thoracic vertebrae. Moreover, spedfication of the thoracic vertebrae occurs Epithelialization of the somites Several studies in the chick have shown that epithelializa tion occurs immediately after somitic fission occurs. As (A) (B) Somites: Anterior Posterior 2 3 Somite number 5 4 7 -l ephriu 82 --+- "" EphA4 --+ &#65533; Unsegmented paraxial mesoderm FIGURE 1 1 .8 Ephrin and its receplor constitute a poss i ble fissure site for somite formation.

(A) Expression pattern of the receptor tyrosine kinase Eph A4 (blue) and its ligand, ephrin 62 (red), as somites deve lop. The somite boundary forms at the junction between the region of ephrin expression on the posterior of the last somite formed and the region of Eph A4 expression on the anterior of the next somi te to form. In the presomitic mesoderm, the pattern is created anew as each somite buds off. The posteriormosl (egion ofthe next somite to form does not express ephrin until that somite is ready to separale. (6) In situ hybridization showing Eph A4 (dark blue) expression as new somites form in the chick embryo. (A after Durbin el al. 1 998; 6 courtesy of J . Kaslner.) 421 PARAXIAL AND INTERMEDIATE MESODERM (A) Epithelial cel s &#65533;&#65533;t> 1 r&#65533;&#65533;&#65533;&#65533;&#65533;&#65533;&#65533;,&#65533;&#65533;,! &#65533;e /&#65533;"':]1&#65533;&#65533;&#65533;&#65533;&#65533;.vlkQ Formed &#65533;,...i •-.. • •&#65533;C::!:C&#65533;_ I t . Mes nchymal ce)]s ""-'.,..".. &#65533;&#65533; FIGURE 1 1 .9 Epithelialization and de-epithelial ization i n somites of a chick embryo. (A) Changes in cell shape from mes&#65533; enchymal (pink) to epithelial (gray) cells when a somite forms from presomitic mesenchyme. A formed somite is surrounded by epithelial cells, with mesenchymtd cells rem aini ng inside. I n chickens, epit e ia i zati on occurs first at the pos terior edge of the somite, with the anterior edge becoming epithelial l ater. (8) Changes in cell po a rity as somites form are revealed by staining thtlt visualizes F&#65533;actin accumLdation (red), (After Nakaya et a . 2004; B courtesy ofY. Takahashi.) h l ll l ized into somites (Carapuc;o et al. 2005). Mice that are homozygous for a loss-of function mutation of Hoxe8 con vert a lumbar vertebra into an extra thoracic vertebra, com plete with ribs (see Figure 8.32). The Hox genes are acti vated concomitantly with somite formation, and the (B) If Fgf8 levels are manip embryo appears to "count somites" in setting the expres wil sion boundaries of the Hox genes. ulated to create extra (albeit smaller) somites, the appro priate Hox gene expression be activated in the appropriately numbered somite, even if it is in a different position along the anterior posterior axis. Moreover, when mutations affect the activation of the appropriate Hox genes (Dubrulle et al. 2001; Zakany et al. 2001). Once established, very early in development. The segmental plate mesoderm each somite retains its pattern of Hox gene expression, even is determined by its position along the anterior-posterior if that somite is transplanted into another region of the axis before somitogenesis. lf one isolates the region of chick embryo (Nowicki and Burke 2000). The regulation of the segmental plate that will give rise to a thoracic somite and Hox genes b y the segmentation clock should allow cOor 01 a younger embryo, the host embryo will develop ribs in the new segments.

transplants this mesoderm into the cervical (neck) region donn has been transplanted (Figure 1 1 .10; Kieny et aI. 1972; dination between the formation and the specification of its neck but only on the side where the thoracic meso Nowicki and Burke 2000). Differentiation of the somites The somites are specifie d according to the Hox genes In contrast to the early commitment of the presomitic seg they express (see Chapter 8). These Hox genes are active mental plate mesoderm along the anterior posterior axis, in the segmental pla te mesoderm before it becomes organ- the commitment of the cells within a somite occurs relative- Cervicral V-crt"b",developing from donor tis ue ve teb ae tDois nuoer ,/\ vertebrae • • l FIGURE 1 1 .10 When seg menta l plate mesoderm that would ordinarily form i.horacic somites is transp anted into a region in a younger embryo (caudal to the iirst somite) that would ordinarily give rise to cervical (neck) somites, the grafted mesoderm differentiates according to its origina p05ition and forms ribs in the eck. IAher Kieny et al.

1972.) l 422 CHAPTER 1 1 (E) (A) 2-day embryo Epidermis Neural tube &#65533; Notochord ---- &#65533; Somite (B) 3-day embryo (C) 4-day embryo Proliferating myoblasts P rimax.ial lip Primaxial ---i--- ir- &#65533;f]tl myotome (F) Abaxial myotome Sclerotome cells r&#65533;&#65533;&#65533;j&#65533;&#65533;:re&#65533;g&#65533;ion (D) Late 4-day embryo Primaxial Abaxial dermamyotome lip Central myotome lip Dorsal root --!-, -1 ganglia s Myobl a t, Sclerotome FIGURE 1 1.1 1 Transverse section through the trunk of a chick e mbryo on days 2 4. (A) In the 2-day somite, the sclerotome cells can be distinguished from the rest of the somite. (S) On day 3, the sclero tome cells lose their adhesion to one another and migrate toward the neural tube. (C) On day 4, the remaining cells divide. The medial cells form a primaxial myotome beneath the dermamyotome, while the lat- eral cells form an abaxial myotome. (0) A layer of muscle cell precur sors (the myotome) forms beneath the epithelial dermamyotome. (U) Scanning electron micrographs correspond to (A) and (D), respective ly; 1 , neural tube; 2, notochord, 3, dorsal aorta; 4, surface ectoderm; 5, intermediate mesoderm; 6, dorsal half of somite; 7, ventral half of somite; 8, somitocoellarthrotome; 9, central sclerotome; 1 0, ventral sclerotome; 1 1 , lateral sclerotome; 1 2, dorsal sclerotome;. 1 3 , der mamyotome.

(A,S after Langman 1 98 1 ; C,D aher Ordahl 1 993; E,F from Christ et al. 2007, courtesy of H. J. Jacob and S. Christ.) Abaxial somitic bud PARAXIAL A N D INTERMEDIATE MESODERM of its cells can become any of the somite-derived structures. These structures include: • • formed in the region farthest from the neural tube produce aorta and the intervertebral blood vessels The cellular sheaths, or meninges, of the spinal cord that tionally been called the dermatome, since its major prod- The muscles of the rib cage, limbs, abdominal wall, back, • The tendons that connect the muscles to the bones • The dermis of the dorsal skin • ribs and the deep muscles of the back; those myoblasts the ab axial muscles of the body wall, limbs, and tongue (F igure 1 1 .1 2). The bounda ry between the primaxial and abaxial muscles and between the somite-derived and lat eral plate-derived dermis is called the l ateral sontitic f,on tier (Christ and Ordah1 1995; Burke and Nowicki 2003; Nowicki et aJ. 2003.) Various transcription factors distin gui sh the primaxial and abaxial muscles. The central portion of the dermamyotome has tradi The cartilage of the vertebrae and ribs and tongue • 423 Vascular cells that contribute to the formation of the protect the central nervous system Thus, the sontite contains a population of multipotent cells , . hose specification depends on their location within the sontite. Paracrine factors from the surrounding tissues (neu ral tube, notochord, epidermis, and intermediate mesoderm) will have profound influences on the regions of the somite *The terms prirnaxial and abaxial are used here to designate the mus cles from the medial and lateral portions of the somite, respectively. The terms epaxial and hypaxial are commonly used, but these terms are derived from secondary modifications of the adult anatomy (the hypaxial muscles being innervated by the ventral regions of the spinal cord) rather than from the somitic myo tome lineages. adjacent to them. As the somite matures, its various regions become committed to forming only certain (those cells closest to the neural tube and notochord) cell types. The ventral-medial cells of the somite undergo ntitosis, lose their round epithelial charac (A) teristics, and become mesenchymal cells again. The portion of the somite that gives rise to these cells is tube the sclerotome, and these me se nchymal cells ulti mately become the cartilage cells (cllondrocytes) of the vertebrae and a major part of each rib (Figure . Primaxial o Abaxiaj 1 1 .1 1 A,B; see also Figure 11.2). The remaining epithelial portion of the sontite Dorsal aorta is the dermarnyotome. Fate mapping with chick quail chimeras (Ordahl and Le Douarin 1992; Brand-Saberi et aL 1996; Kato and Aoyama 1998) has reveaJed that the dermamyotome is arranged (B) into three regions (Figure 1 1 .1 1 C,D). The cells in the two lateral portions of this epithelium (i.e., the dor somedial and ventrolateral lips closest to and far thest from the neural tube, respectively) are the myotomes and will form muscle cells. The central region, the dermatome, will form hack dermis and several other derivatives. In the lateral myotomes, muscle precursor cells will migrate beneath the der mamyotome to produce a lower layer of muscle precursor cells, the myoblasts. Those.myoblasts in (C) the myotome closest to the neural tube form the centrally located primaxial muscles/' which include the intercostal musculature between the FIGURE 1 1 .12 Primaxial and abaxial domains of vertebrate mesoderm.

(A-C) Color-coded diagrams of chick d evelopment where the darker color represents somite-derived mesoderm and the lighter represents lateral platederived mesoderm. (A) Early-stage chi ck embryo as mesoderm forms. (B/C) Two view of a day 9 chick embryo, showing regionalization. (D) Day 1 3 chick in which Prox I gene expres sion is shown by dark stain. In the trunk, this gene is expressed in the abaxial region. The boundary between the stained and unstained regions is the lateral somatic frontier. (After Winslow et al. 2007; D courtesy of A. C. Burke.) (D) 424 CHAPTER 1 1 . Ileratmg ProI"c Prim axia l "'&#65533;&#65533;,,&#65533;:::::::\" / (body wall and limb) muscles tube Lateral plate mesoderm FIGURE 1 1 .13 Model of major postulated interactions in the p attern ing of the somite. A c om bi n ati on of Wnts (p robably Wntl and WntJa) is induced by BMP4 in the dorsal neural tube. These Wnt proteins, in combination with low concentrations of Soni c hedgehog from the notochord and floor pl ate, induce the primaxi al myotome, which synthesizes the myogenic transcription factor Myf5.

High concentrations of Shh ind uce Paxl expression in those cel l s fated to become the sclerotome.

Certain co n cen tra tions of neurotrophin-3 (NT3) from t he dorsal neural tube appear to specify the derm atome while Wnt proteins from the epidermis, in conjunction with BMP4 and Fgf5 from the latera l p late meso derm/ are thought to induce the primaxial myotome. (After Cossu et al. 1 996b.1 , uct is the precursors of the dermis of the back. (The der undifferentiated, and they prolifera te mis of the ventral portion of the body is derived from the for most of the myoblast cells. While most of these progen rapidly to account lateral plate, and the dermis of the head and neck comes, itor cells differentiate to form muscles, some remain undif in part from the cranial neural crest.) However, ferentiated and surround the mature muscle cells. These at least recent studies have shown that this central region of the 2005; Relaix et a1. 2005). Therefore, Ordah1 1995; Christ et a!. derm a myo tome also gives rise to a third population of muscle ceUs (Gros et a!. some researchers (Christ and 2007) prefer to retain the term dermamyotome (or central undifferentiated cells become the satellite cells responsible for postnatal muscle growth and muscle repair. Determination of the sclerotome dermamyotorne) for this epithelial region. Soon, however, Like this part of the somite also undergoes an epithelial-to-mes somitic region depends on three things: enchymal and location. As shown in Figure 1 1.13, the locations of the transi tion (EMT). FGF signals from the Snail the proverbial piece of real estate, the destiny of a location, location, gene, somitic regions place them close to different signaling cen a well-known regulator of EMT (see Chap ters 3 and 10; Delfini et a1. 2009). During this EMI, the mitotic spindles of these epithelial cells are realigned so that ceU division takes place along the dorsal-ventral axis. One daughter cell enters the ventral region and becomes part of the myotome, wIllie the other daughter cell loca tes dorsally to become a precursor of the dermis. ters such as the notochord (source of Sonic hedgehog and myotome activate the transcription of the whose product is The N-cadherin holding the cells together is downregu lated and the cells go their separate ways, with the remain ing N-cadherin found only on the cells entering the myotome (Ben-Yair and Kalcheim 2005). These muscle precursor cells thC'lt delaminate from the epithelial plate to join the primary myotome cells remain Noggin), neural tube (source of Wnts and BMPs), and sur face epithelium (also a source of Wnts and BMPs). The specification of the somite is accomplished by the interaction of several tissues. The ventromedial portion of the somite is induced to become the sclerotome by paracrine factors (especiaUy Noggin and Sonic hedgehog) 1994; Johnson et al. 1994). If portions of the notochord are trans secreted from the notochord (Fan and TeSSier-Lavigne planted next to other regions of the somite, those regions will also become sclerotome cells. Paracrine factors induce the presumptive sclerotome cells to express the transcrip tion factor Paxl, which is required for their epithelial to- PARAXIAL A N D INTERME DIATE MESODERM mesenchymal transition and subsequent differentiation into cartilage (Smith and Tuan 1996). In this EMT, the epithelial cells lose N-cadherin expression and become motile (Sosic et al. 1997). Sclerotome cells also express 1mf, an inhibitor of the myogenic (muscle-forming) family of transcription factors (Chen et al. 1996). The sclerotome contains several regions, each of which becomes specified according to its location. While most sclerotome cells become the prescursors of the vertebral and rib cartilage, the medial sclerotome cells closest to the neural tube generate the meninges (coverings) of the spinal cord as well as giving rise to blood vessels that will pro vide the spu,al cord with nutrients and oxygen (Halata et al. 1990; Nimmagadda et al. 2007). The cells in the center of the somite (which remain mesenchymal) also contribute to the sclerotome, becoming the vertebral joints, the mter vertebral discs, and the portions of the ribs closest to the vertebrae (Mittapalli et al. 2005; Christ et al. 2007). This region of the somite has been called the arthrotome. The notochord, with its secretion of Sonic hedgehog, is critical for sclerotome formation. Moreover, we will also see that it produces compounds that direct the migration of sclerotome cells to the center of the embryo to form the vertebrae. But what happens to the notochord, that central mesodermal structure that induced the nervous system and caused the sclerotome to form? After it has provided the axial integrity of the early embryo and has u,duced the formation of the dorsal neural tube, most of it degenerates by apoptosis. This apoptosis is probably signaled by mechanical forces_Wherever the sclerotome cells have formed a vertebral body, the notochordal cells die. How ever, between the vertebrae, the notochordal cells form part of the intervertebral discs, the nuclei pulposi (Asz6di et al. 1998; Guehring et al. 2009). These are the spinal discs that "slip" in certain back injuries. Determination of the central derma myotome The central dermamyotome generates muscle precursors as well as the dermal cells that COFtstitute the connective tissue layer of the dorsal skin. The dermis of the ventral and lateral sides of the body is derived from the lateral plate mesoderm that forms the body wall. There is a sharp demarcation between the somite- and lateral plate-derived dermis. This corresponds to the lateral somitic frontier (see Figure 1 1 .12; Nowicki et al. 2003; Shearman and Burke 2009), a boundary that may have medical importance for the spread of skin diseases (such as viruses that cause rash es only in the chest and belly but not in the back).

The maintenance of the central dermamyotome de pends on Wnt6 coming from the epidermis (Christ et al. 2007), and its epithelial-ta-mesenchymal transition appears to be regulated by two factors secreted by the neural tube: neurotrophin-3 (NT3) and Wntl. Antibodies that block the activities of NT3 prevent the conversion of epithelial der matome into the loose dermaJ mesenchyme that migrates 425 beneath the epidermis (Brill et al. 1995). Remov:ing or rotat ing the neural tube prevents this dermis from forming (Takahashi et al. 1992; Olivera-Martinez et al. 2002). The Wnt signals from the epidermis promote the differentia tion of the dorsally migrating central dermamyotome cells into dermis (Atit et a1. 2006). But muscle precursor cells and dermal cells are not the only derivatives of the central dermamyotome. Atit and her colleagues (2006) have shown that brown adipose cells ("brown fat") are also somite-derived and appear to come from the central derma myotome. Brown fat plays active roles in energy utilization by burning fat (unlike the bet ter known adipose tissue, or "white fat," which stores fat). Tseng and colleagues (2008) have found that skeletal mus cle and brown fat cells share the same somitic precursor that originally expresses bHLH proteil1s. In brown fat pre cursor cells, the transcription factor PRDM16 is induced (probably by BMP7); PRDM16 appears to be critical for the conversion of myoblasts to brown fat cells, as it activates a battery of genes that are specific for the fat-burning metabolism of brown adipocytes (Kajimura et al. 2009).

Determination of the myotome All the skeletal musculature in the vertebrate body (with the exception of the head muscles) comes from the der mamyotome of the somite. In similar ways, the myotome is induced in two different places by at least two distinct sig nals.

Studies using transplantation and knockout mice indi cate that the primaxial myoblasts coming from the medi al portion of the somite are induced by factors from the neural tube probably Wntl and Wnt3a from the dorsal region and low levels of Sonic hedgehog from the ventral region (Mlinsterberg et al. 1995; Stem et al. 1995; Borycki et al. 2000). The abaxial myoblasts coming from the lateral edge of the somite are probably specified by a combina tion of Wnt proteins from the epidermis and signals from the lateral plate mesoderm (see Figure 11.13; Cossu et a1. 1996a; Pourquie et al. 1996; Dietrim et al. 1998). These pro teins, which probably include Scatter factor (a protein that induces epithelial-to-mesenchymal transition), cause the myoblasts to migrate away from the dorsal region and delay their differentiation until they are in a more ventral position. These myoblasts are also the cells that form the muscles of the limb (Chapter 13). In addition to positive signals, there are inhibitory sig nals preventing the positive signals from affecting an inap propriate group of cells. For example, Sonic hedgehog and Noggin both inhibit BMP4 (Marcelle et al. 1997). Indeed, one model of myotome determination holds that the two conditions needed to produce muscle precursors in the somite are (1) the presence of Wnt signals and (2) the absence of BMPs (Reshef et al. 1998). Recent studies (Ger hart et al. 2006) have traced the development of these BMP-inhibiting centers to cells that arise prior to somite forma tion. They found a small population of surface epiblast celis 426 CHAPTER 1 1 (A) Control &#65533;__ll'f' (B) Ablated FIGURE 1 1 ,14 Ablating Noggin-secreting epiblast cells results in severe muscle defects.

Noggin-secreting epiblast cells were ablat ed in Stage 2 chi ck embryos using antibodies against G8. (A) The control embryo has normal morphology and abundant staining of myosin (lower photograph) in the muscles. (B) Embryos whose Noggin-secreting epiblast cells are ablated have severe eye defects, severely reduced somatic musculature, and the herniation of abdominal organs through the thin abdominal wall. Severely reduced musculature (sparse myosin in lower photograph) is char acteristic of these embryos. (From Gerhart et al. 2006, courtesy of J.

Gerhart and M. George-Weinstein.) that express the mRNA for MyoD but do not translate this message into protein. These particular cells migrate to become paraxial mesoderm and specifically sort out to the dorsomedial and ventrolateral lips of the dermanlyotome. There, they synthesize and secrete the BMP inhibitor Nog gin to promote the differentiation of myoblasts. If these particular cells are removed from the epiblast, there is a decrease in the skeletal musculature i&#65533;oughout the body, and the ventral body wall is so weak that the heart and abdominal organs often are herniated through it (Figure 1 1 .14), (This defect can be prevented by implanting Nog gin-releasing beads into the somites lacking these cells.) See WEBSITE 1 1 ,1 Calling the competence of the somite into question See WEBSITE 1 1 ,2 Cranial paraxial mesoderm Myogenesis: The Generation of Muscle Myogenic bHLH proteins What do these Wnt signals activate in the absence of BMPs? As we have seen, muscle cells corne from two cell lineages in the somite, the primaxial and the abaxial. In both lineages, paracrine factors instruct cells to become muscles by inducing them to synthesize the MyfS and MyoD proteins (see Figure 11.13; Maroto et aL 1997; Tajbakhsh et aL 1997; Pownall et aL 2002). MyoD and MyfS belong to a family of transcription factors called the bHLH (basic helix-loop-helix) proteins (sometimes also referred to as myogenic regulat ory factors, or MRFs), The proteins of this family all bind to similar sites on the DNA and acti vate muscle-specific genes. The mechanism of induction of b

Eventually, the endothelial cells from the sclerotome replace the cells of the primary dorsal aorta (which will become part of the blood stem ceU pop Ulation) (Figure 1 1 .20; Pouget et a1. 2008; Sato et aJ. 2008). aorta. (C) The sclerotome cells take up residence in the dorsal region of the vessel . (D) These cel ls then spread along both t he anterior posterior and dorsal-ventral axes, ultimately occupyi ng the entire region of the aorta. The pri mary aortic endothelial cells become blood cel l precursors. (Afier Sato et al . 2008.) Tendon Formation: The Syndetome The most dorsal part of the sclerotome will become the fourth compartment of the somite, the syndetome (Greek syn, "connected "). Since the tendons connect muscles to bones, it is not surprising that the syndetome is derived from the most dorsal portion of the sclerotome that is, from sclerotome cells adjacent to the muscle-forming myotome.

The tendon-forming cells of the syndetome can be visualized by their expression of the scieraxis gene (Fig ure 1 1 .2 1; Schweitzer et a1. 2001; Brent et a1.

2003). Because there is no obvious morphological distinction between the sclerotome and syndetome cells (O,ey are both mesenchy mal), Our knowledge of this somitic compartment had to wait until we had molecular markers (Pax] for the sclero tome, scleraxis for the syndetome) that could distinguish them and allow one to follow their ceUs' fates. The syndetome is made from the myotome's secretion of FgfB onto the immediately subjacent roW of sclerotome ceUs (Figure 1 1 .22A; Brent et a1. 2003; Brent and Tabin 2004). Other transcription factors limit the expression of sclerax is to the anterior and posterior portions of the syndetome, causing two stripes of scleraxis expression, Meanwhile, the developing cartilage cells, under the influence of Sonic hedgeljog from the notochord and floorplate, syntljesize Sox5 and Sox6 transcription factors that block scleraxis transcription (Figure 1 1 .22B). In this way, the cartilage pro tects itself from any spread of the FgfB S igna l . The tendons then associate with the muscles directly above them and with the skeleton (including the ribs) on either side of them (Figure 1 1 .22C; Brent et al. 2005). 433 PARAXIAL AND I NTERMEDIATE MESODERM (A) In (B) FIGURE 1 1 .21 Scleraxis is expressed in the progenitors of the tendons. (A) situ hybridization showing scleraxis pattern in the developing chick embryo. (BI Areas of scleraxis expression in the tendons of a newborn mouse forelimb (ventral view).

The GFP gene had been fused on the scle- raxis enhancer. (C) Wrist and digit of a newborn mouse, showing scleraxis in the tendons (green) connecting muscles (red) to the digit and wrist. (A from Schweitzer et al. 2001, all photographs courtesy of R. Schweitzer.) (e) (B) (A) CD-o Sclerotome My(secorteoemseFgf8) Sclerotome Syndetome Ne u r a l t u b eVertebralprogenitors(cailge)progenitors Anterior=\''1=/:\ ::::.

(C) Posterior Muprogselneitors Tendon ◆F F G I Sclerotome ! ◆ P e a 3 . -i S h (t r n (I◆naotorcp!aotred), factosrc)ription Sox9 1 Sox6 Erm sots, t Cartilage sderaxis Tendon Intercostal muscle Vertebrae FIGURE 1 1.22 tnduction of scleraxis in the chick sclerotome by Fgf8 from the myotome. (AI The dermatome, myotome, and sclero- tome are established before the tendon precursors are specified.

Tendon precursors (syndetome) are specified in the dorsalmost tier of sclerotome cells by FgfS received from the myotome. (B) Path- way by which FgfB signals from the muscle precursor cells induce the subjacent sclerotome cells to become tendons. (C) Synde(ome cells migrate (arrows) along the developing vertebrae. They diffel" entiate into tendons that connect the ribs to the intercostal muscles beloved by "spare-ribs" devotees. (A,C after Brent et al. 2003.) 434 CHAPTER 1 1 INTERMEDIATE MESODERM: development (day THE UROGENITA L SYSTEM pronephric duct arises in the intermediate mesoderm just 22 in humans; day 8 in mice), the ventral to the anterior s◆mites.

The cells of this duct The intermeruate mesoderm generates the urogenita l sys migrate caudally, and the anterior region of the duct tem-the kidneys, the gonads, and their respective duct induces the adjacent mesenchyme to form the pronephros, systems. Reserving the gonads for our discussion of sex o r tubules of the initial kidney (Figure determination in Chapter pronephric tubules form functioning kidneys in fish and 14, we will concentrate here on the development of the mammalian kidney. 1 1 .23A).

The in amphibian larvae, but they are not believed to be active in amniotes. In mammals, the pronephric tubules and the anterior portion of the pronephric duct degenerate, but the The Progression of Kidney Types Homer Smith noted in more caudal portions of the pronephric duct persist and 1953 that "our kidneys constitute 1945; Saxen 1987).

This serve as the central component of the excretory system the major foundation of our philosophical freedom. Only throughout development (Toivonen because they work the way they do has it become possible remaining duct is often referred to as the nephric, or Woli for uS to have bone, muscles, glands, and brains." While fian, duct. this statement may smack of hyperbole, the human kidney As the pronephric tubules degenerate, the middle por is an incredibly intricate organ whose importance cannot tion of the nephric duct induces a new set of kidney be overestimated. Its functional unit, the nephron, contains tubules in the adjacent mesenchyme. This set of tubules more than 10,000 cells and at least 12 different cell types, constitutes the mesonephros, or mesonephric kidney (Fig each cell type having a speCific function and being locat ure ed in a particular place in relation to the others along the mammalian species, the mesonephros functions briefly in 1 1 .23B; Sainio and Raatikainen-Ahokas 1999). ln some urine filtration, but in mice and rats it does not function as length of the nephron. Mammalian kidney development progresses through a working kidney. In humans, about 30 mesonephric three major stages.

The first two stages are transient; only tubules form, beginning around day 25. As more tubules the third and last persists as a functional kidney. Early in are induced caudally, the anterior mesonephric tubules (A) ◆ Nephrogenic :. . - : , '--- (B) (C) cord Gonad cord UrNeoptherriicc Nephric duct --c◆'-fl ,.◆"◆ --'o; burt -"",, fl FIGURE 11 .23 General scheme of development in the vertebrate kidney. (AI The original tubules, constituti ng th e pronephros, are i nduced from the neph rogen ic mesenchyme by the pronephric duct as it migrates caudally.

(Bl As the pronephros degenerates, the m esoneph ri c tubules form. {OThe final mammalian kidney, the metanephros, is induced by the ureteric bud, which branches Metubsuolensephric Gonad Ne(Wpohlffrican) duct MemesteancehpyhmroUrgeenteicr h (D) from the nep ric duct. (D) Intermediate mesoderm of a 1 3 -day mouse embryo showing initiation of the metanephric kidney (bottom) while the mesonephros is stil l apparent. The duct tissue is stained with a fluorescent antibody to a cytokeratin found in the pronephric duct and its derivatives. (A-C after Saxen 1 987; D courtesy of S. Vainio.1 PARAXIAL AND INTERMEDIATE MESODERM (A) ◆rfl◆ :::◆::◆ ◆ ◆◆W !I◆U Vn ◆ Paraxial / \0 (8) 435 (e) Lateral plate ◆ d ate mesoder mesoderm : Cut separating paraxial mesoderm from intermediate mesoderm ! Pronephric Uninduced intermediate kidney mesoderm FIGURE 1 1 .24 Signals from the parax i a l mesoderm induce pronephros formation in the intermediate mesoderm of the chick embryo . (A) The paraxial mesoderm was surgically sepa rated from the intermediate mesoderm on the right side of the body (B) As a result, a proneph r i c kidney (Pax2-staining duct) developed only on the left side. (C) L i m l expression in an 8-day mouse embryo, showing the prospective i ntermediate mesoderm. (A,B after Mauch et a l. 2000; B courtesy ofT. J. Mauch and G. C. Schoenwolf; C courtesy of K. Sainio and M. Hytonen.) . begin to regress through apoptosis (although in mice, the Specification of the I ntermediate Mesoderm: Pax2/8 and Liml The intemlediate mesoderm of the chick embryo acquires its ability to form kidneys through its interactions with the paraxial mesoderm. While its bias to become interme diate mesoderm is probably established through the BMP gradient mentioned earlier, specification appears to become stabilized through signals from the paraxial meso anterior tubules remain while the posterior ones regress; derm. Mauch and her colleagues (2000) showed that sig Figure 1 1 .23C,D). While it remains unknown whether the nals from the paraxial mesoderm induced primitive kid human mesonephros actually filters blood and makes urine, it does provide important developmental functions ney formation in the intermediate mesoderm of the chick embryo. They cut developing embryos such that the inter Dzierzak 1996; Wmtour et al. 1996). Second, in male mam mediate mesoderm could not contact the paraxial meso derm on one side of the body. That side of the body (where contact with the pa rax ial mesoderm was abolished) did not form kidneys, but the undisturbed side was able to form kidneys (Figure 1 1 .24A, B).

The paraxial mesoderm urethra (the epididymis and vas deferens; see Chapter 14). The permanent kidney of amniotes, the metanephros, since co-culturing lateral plate mesoderm with paraXial during its brief existence. First, as we will see in Chapter 12, it is one of the main sources of the hematopoietic stem ceUs necessary for blood ceU development (Medvinsky and mals, some of the mesonephric t ubules persist to become the tubes that transport the sperm from the testes to the is generated by some of the same components as the ear lier, transient kidney types (see Figure 11.23C). It is thought to originate through a complex set of interactions between epithelial and mesenchymal components of the interme appears to be both necessary and sufficient for inducing kidney-forming ability in the intermediate mesoderm, mesoderm causes pronephric tubules to fonm in the later al plate mesoderm, and no other cell type can accomplish this. These interactions induce the expression of a set of diate mesoderm. In the first steps, the me tan ep hrogen ic es enchym e is committed and forms in the posterior homeodomain transcription factors including Liml, Pax2, the formation of a branch from each of the paired nephric Kobayashi et al. 2005). In Xenopus, the PaxB and Liml pro m regions of the intermediate mesoderm, where it induces ducts. These epithelial branches are called the ureteric buds. These buds eventually separate from the nephriC duct to become the collecting ducts and ureters that take the urine to the blad der When the ureteric buds emerge .

from the nephriC duct, they enter the meta ne phrogenic mesenchyme. The ureteric buds induce this mesenchymal tissue to condense around them and differentiate into the nephrons of the mammalian kidney. As this mesenchyme differentiates, it tells the ureteric bud to branch and grow.

and Pax8 that cause the intenmediate mesodenm to form the kidney (Figure 1 1 .24C; Karavanov et aJ. 199B; teins have overlapping boundaries, and kidney develop ment originates from cells expressing both. EctopiC co expression of the PaxB and Liml genes will produce kidneys in other tissues as weU (Carroll and Vize 1999). In the chick emb ryo, Pax2 and Lim 1 are expressed in the intenmediate mesodenm, starting at the level of the sixth somite (i.e., only in the trunk, not in the head); if Pax2 is experimentally expressed in the presomitic mesoderm, it converts that paraxial mesoderm into intermediate meso- 436 CHAPTER 1 1 (A) FIGURE 1 1 .25 Reciprocal induc lion in the developmen t of the mam malian kidney. (A) As the ureteric bud enters the metanephrogenic mesenchyme, the mesenchyme induces the bud to branch. (B-G) At the tips of the branches, the epitheli um induces the mesenchyme to aggregate and cavitate to form the renal tubules and glomerul i (where the blood from the arteriole is fil tered), When the mesenchyme has condensed into an epithelium, it digests the basal lamina of the ureteric bud cells that induced it and connects to the ureteric bud epithel ium. A portion of the aggre gated mesenchyme (the pretubu la r condensate) becomes the nephron (renal tubules and Bowman's cap sule), while the ureteri c bud becomes the co ll ecting duct for the urine. (After Saxen 1 987; Sa riol a ....◆ ... (Metanephrogenic mesenchyme ac T "' Ureteric condensate Glomerulus (G) t OlleC'ing duct capsule Distal --i' .# tubule Proximal tubule ej'l ·:t◆ -: 2002.) derm, expresses Lim1, and forms kidneys (Mauch et a!. 2000; Suetsugu et at. 2005). Similarly, in mouse embryos with knockouts of both the Pax2 and Pax8 genes, the meS enchyme-ta epithelial transition necessary to form the kid ney duct fails, the cells undergo apoptosis, and no nephriC structures form (Bouchard et at. 2002). Moreover, in the mouse, Liml and Pax2 proteins appear to induce one another. Liml plays several roles in the formation of the mouse kidney. First it is needed for converting the intemlediate mesenchyme into the kidney duct (Tsang et at. 2000), and later it is required for the formation of the ureteric bud and the tubular structure both in mesonephric and metanephric mesenchyme (Shawlot and Behringer 1995; Karavanov et a1. 1998; Kobayashi et at.

2005). The anterior border of the Lim1- ana Pax2-expressing cells appears to be established by the cells above a certain region losing their competence to respond to the activin secreted by the neural tube. This competence is established by the transcription factor Hoxb4, which is not expressed in the intermediate mesoderm. The anterior boundary of Hoxb4 is established by a retinoic acid gradient, and adding activin locally will allow the kid ney to extend anteriorly (Barak et aJ. 2005; Preger-Ben Noon et at.

2009). The metanephrogeniC mesenchyme causes the ureteric bud to elongate and branch. The tips of these branches induce the loose mesenchyme cells to form epithelial aggregates. Each aggregated nodule of about 20 cells proliferates and differentiates into the intricate struc ture of a renal nephron. Each nodule first elongates n i to a "corruna" shape, then forms a characteristic S-shaped tube. Soon afterward, the cells of this epithelial structure begin to differentiate into regionally specific cell types, including the capsule cells, the podocytes, and the proximal and dis tal tubule cells. While this transformation is happening, the epithelializing nodules break down the basal lamina of the ureteric bud ducts and fuse with them.' This fusion creates a connection between the ureteric bud and the newly formed tubule, allowing material to pass from one into the other (Bard et a1. 2(01). These tubules derived from the mesenchyme form the secretory nephrons of the func tioning kidney, and the branched ureteric bud gives rise to the renal collecting ducts and to the ureter, wlicll drains the urine from the kidney. Clifford Grobstein (1955, 1956) documented this reciprocal interaction in vitro. He separated the ureteric bud from the metanephrogenic mesenchyme and cultured them either indiVidually or together.

In the absence of mes enchyme, the ureteric bud does not branch. In the absence Reciprocal Interactions of Developing Kidney Tissues capiJlaries that the nephuons filter is accomplished by the secretion The two intermediate mesodermal tissues-the ureteric bud and the metanephrogeniC mesenchyme-interact and reciprocally induce each other to form the kidney (Figure 1 1 .25) . *The intricate coordination of nephron development with the blood of VEGF from the podocytes. VEGF, as we will see in Chap ter 12, is a powerful inducer of blood vessels, causing endothelial cells from the dorsal aorta to fonn the capillary loops of the glomerular filh'a Han apparatus (Aitkenhead et al. 1998; Klanke et al. 2(02). PARAXIAL A N D I NTERMEDIATE MESODERM of the ureteric bud, the mesenchyme soon dies. But when they are placed together, the ureteric bud grows and branches, and nephrons form throughout the mesenchyme. This has been confirmed by experiments using GFP-labeled proteins to monitor cell division and branching (Figure 1 1 .26; Srinvas et a1.

1999.) Mechanisms of reciprocal induction The induction of the metanephros can be viewed as a dia logue between the ureteric bud and the metanephrogenic mesenchyme. As the dialogue continues, both tissues are altered. We will eavesdrop on this dialogue more intently than we have done for other organs. This is because the kidney has become a model for organogenesis. There are many reasons for this.

FiIst, early kidney development has only two major components. Second, the identity and roles of many of the paracrine and transcription factors pro duced during this dialogue have been discovered from studies of knockout mice. Third, the absence of many of these transcription factors is associated with serious pathologies characterized by absent or rudimentary kid neys. While there are several simultaneous dialogues between different groups of kidney cells, there appear to be at least nine critical sets of signals operating in the recip rocal induction of the metanephros. STEP 1 : FORMATION OF THE METANEPHROGENIC MESEN CHYME: FOXES, HOXES, AND WTl Only the metanephrogenic mesenchyme has the competence to respond to the ureteric bud and form kidney tubules; indeed, this mes enchyme cannot become any tissue other than nephrons. If induced by non-nephric tissues (such as embryonic sali vary gland or neural tube tissue), metanephric mes enchyme responds by forming kidney hlbules and no other structures (Saxen 1970; Sariola et a1. 1982). The positional specification of the metanephrogenic mesenchyme is positively regulated by Hoxb4 and nega tively regulated by the transcription factors Foxc1 and Foxc2. Foxc1-/2- double-mutant m◆ce have an expanded metanephric area that induces extra ureters and kidneys (Kume et a1. 2000). Next, the permanent kidney-forming metanephrogenic mesenchyme is specified by genes of the Hoxll paralogue group. When Hoxll.genes are knocked out of mouse embryos, mesenchymal differentiation is arrested and the mesenchyme cannot induce the ureteric bud to form (Patterson et a1. 2001; Wellik et a1. 2002). The competence to respond to ureteric bud inducers is regu lated by a tumor suppressor gene, WTl . Without WTl, the metanephric mesenchyme cells remain uninduced and die (Kreidberg et a1. 1993). In situ hybridization shows that, normally, WTl is first expressed in the intermediate meso derm prior to kidney formation and is then expressed in the developing kidney, gonad, and mesothelium (Pritchard-Janes et a1. 1990; van Heyningen et a1. 1990; Armstrong et a1. 1992).

Although the metanephrogenic mesenchyme appears homogeneous, it may contain both mesodermally derived tissue and some cells of neural crest origin (Le Douarin and Tiellet 1974; Sariola et a1. 1989; Sainio et a1. 1994).

STEP 2: THE METANEPHROGENIC MESENCHYME SECRETES i GDNF TO INDUCE AND DIRECT n-E URETERIC BUD SO what are all these factors doing in the metanephrogenic mes enchyme? It seems that they are setting the stage for the secretion of a paracrine factor that can induce the ureteric buds ta emerge.

This second signal in kidney development is gli al-derived neurotrophic factor (GDNF) ' GDNF is expressed through a complex network initiated by Pax2 and Hoxll (Xu et a1. 1999; Wellik et a1.

2002). Pax2 and Hoxll (in concert with other transcription factors that per mit this interaction) activate GDNF expression in the metanephrogenic mesenchyme (see Brodbeck and Englert 2(04). If GDNF were secreted throughout the metanephro genic mesenchyme, numerous epithelial buds would sprout from the nephric duct, thus GDNF expression must be limited to the posterior region of this mesenchymal tis sue. This restriction is accomplished by the proteins Sprouty1 and Rab02 (Roba2 being the same protein that helps deflect axon growth cones).

If the Robo2 protein is mutated, both the anterior and posterior metanephrogenic *This is the same compound that we saw in Chapter 10 as critical for the induction of dopaminergic neurons in the mammalian brain and for the entry of neural crest cells into the gut. GDNF is one busy protein; we will meet it again when we discuss sperm cell production in Chapter 16. O.5 mm o hrs 24 hrs 60 hrs 437 84 hrs FIGURE 1 1.26 Kidney induction observed in vitro. (A) A kidney rudiment from an 1 1 .5-day mouse embryo was pla ced into culture. This transgenic mouse had a CFP gene fu sed 10-a Hoxb7 promoter, so it expressed green fluorescent protein in the nephric (Wolf fian) duct and in the ureteric buds. Since GFP can be photogra phed in living tis sues, the kidney could be fol lowed as it developed. (Srinivas et al. 1 999, cour tesy of F. Costa nt ini .) 438 CHAPTER 1 1 STEP 3: THE URETERIC BUD SECRETES FGF2 AND BMP7 TO PREVENT MESENCHYMAL APOPTOSIS The third signal in kid ney development is sent from the ureteric bud to the metanephrogenic mesenchyme, and it alters the fate of the mesenchyme cells. If left uninduced by the ureteric bud, the mesenchyme cells undergo apoptosis (Grobstein 1955; Koseki et al. 1992). However, if ind uced by the ureteric bud, the mesenchyme ceUs are rescued from the precipice of death and are converted into proliferating stem ceUs (Bard and Ross 1991; Bard et al. 1996). The factors secret ed from the ureteric bud include Fgf2 and BMP? Fgf2 has three modes of action in tha t it inhibits apoptosis, promotes the condensation of mesenchyme cells, and maintains the syntheSiS of WTl (Perantorri et al. 1995). BMP?

has similar effects (Dudley et al. 1995; Luo et al. 1995). mesenchyme express GDNF, and the nephric duct sends out an anterior and a posterior ureteric bud (Grieshammer et aJ. 2004). Sprouty1 is an inhibitor of FGF Signaling. In mice whose Sprolltyl gene is knocked out, GDNF is pro duced throughout the metanephrogenic mesenchyme, ini tiating numerous ureteric buds (Basson et al. 2005). The receptors for GDNF (the Ret tyrosine kinase recep tor and the GFRa1 co receptor) are synthesized in the nephric ducts and later become concentrated in the grow ing ureteric buds (Schuchardt et al.

1996). Indeed, during formation of the ureter, those cells expressing the Ret recep tor migrate to positions closest to the source of GDNF (Fig ure 1 1 .27A; Chi et al. 2009). Whether this is a sorting-out phenomenon or whether the cells are attracted to the GDNF is not yet known. Mice whose grin! or GDNF recep tor genes are knocked out die soon after birth from renal ageneSis-lack of kidneys (Figure 1 1 .27B-D; Moore et al. 1996; Pichel et al. 1996; Sanchez et al. 1996). The ability of nephric duct cells to prolilerate appears to be su ppressed by activin, and the major mechanism of GDNF action may be to locally suppress the activities of inhibitory activin. When activin was experimentally inhibited, GDNF induced numerous ureteric buds (Maeshima et al. 2006). -STEP 4: WNT9B AND WNT6 FROM THE URETERIC BUD tNDUCE MESENCHYME CELLS TO AGGREGATE The ureteric bud caus es drama tic changes in the behavior of the metanephro genic mesenchyme cells, converting them into an epithe lium. The newly induced mesenchyme synthesizes E-cadherin, which causes the mesenchyme cells to dump together. These aggregated nodes of mesenchyme now (A) -- (B) -- (C) rNt ePhric (D) . , , . ' duct - . FIGURE 1 1 .27 reteric bud growth is depend ent on GDNF and its receptors (A) When mi ce are con structed from Ret defic i ent cel ls (green) and Ret-expressing cells (blue), the cells express ing Ret migrate to form the tips of the ureteric bud.

(B) The ureteric bud from an , , S day wild-type mouse kidney cultured for 72 hours has a charac teristi c bran ching pattern. (C) In embryonic mice heterozygous for a mutation of the gene encoding GDNF, both the size of the ureteri c bud and the number and length of il5 branches are redu ced . (D) I n mouse embryos missing both cop ies of the gdnf gene, the ureteric bud does n ot form. (A from Chi et al. 2009, courtesy of F. Costantini; B-O from Pichel et al. ' 996, courtesy of J . G. Pichel and H.

5oriol a) U . - . · (A) Wn t9b Wntll Wolffian (nephric) duct PARAXIAL AND I NTERM E D I ATE MESODERM 439 STEP 5: WNT4 CONVERTS AGGREGATED MESENCHYME CELLS INTO A NEPHRON Once induced, and after it has started to condense, the mesenchyme begins to secrete Wnt4, which acts in an autocrine fashion to complete the transition from mesenchymal mass to epithelium (Stark et a!. 1994; Kispert et a!. 1998). Wnt4 expression is found in the condensing bud mesenchyme cells, in the resulting S-shaped tubules, and in the region where the newly epithelialized cells fuse with the ureteric bud tips. In mice lacking the Wnt4 gene, the mesenchyme becomes condensed but does not form epithelia.

Therefore, the ureteric bud induces the changes (8) in the metanephrogenic mesenchyme by secreting FGFs, Wnt9, and Wnt6; but these changes are mediated by the effects of the mesenchyme'S secretion of Wnt4 on itself. One molecule that may be involved in the transition from aggregated mesenchyme to nephrons is the Lim1 homeodomain transcription factor (Karavanov et aJ. 1998; Kobayashi et a!. 2005). This prptein is found in the mes Wild-type FIGURE 1 1 .28 Wnts are crilical for kidney developm en t. (A) In the l1-day mouse kidney, Wnt9b is found on the stalk of th e ureteric bud, while Wnt1 1 is found at the tips. Wnt9b i nduces the metanephrogenic mesen chyme to condense; Wnt1 1 will partition the metanep hrogeni c mesoderm to induce branching of the ureteric bud. Borders of th e bud are indicated by a dashed line. (B) A wild-type l 8.S-day male mouse (leh) has normal kidneys, adrenal glands, and ureters. I n a mouse deficient for Wnt9b [righl), the kidneys are absent. (From Carroll et al. 2005.) synthesize an epithelial basal lamina containing type IV ench yme cells after they have condensed around the ureteric bud, and its expression persists in the developing nephron (Figure 1 1 .29). Two other proteins that may be crit ical for the conversion of the aggregated cells into a nephron are polycystins 1 and 2. These proteins are the *Wnt9b appears to be critical for inducing mesenchyme-to-epitheli transitions throughout the intermediate mesoderm. This includes the formation of the nephric (Wolffian) and MUllerian ducts, as weU as the kidney tubules. ln addition to having no kid neys, Wnt9b-deficient mice have no uterus (if they are female) and no vas deferens (if male). In rats, leukemia inhibitory factor (LIF) appears to substitute for Wnt6 and Wnt9 (Barasch et al. 1999; Car roU et al. 2005). um collagen and laminin. At the same time, the mesenchyme cells syntheSize receptors for laminin, allowing the aggre gated cells to become an epithelium (Ekblom el aJ. 1994; Miller et aJ. 1997).

11,e cytoskeleton also changes from one characteristic of mesenchyme cells to one typical of epithe lial cells (Ekblom et al. 1983; Lehtonen et aJ. 1985). The transition from mesend'ym◆1 to epithelial organiza tion may be medjated by several molecules, including the expression of Pax2 in the newly induced mesenchyme cells. When antisense RNA to Pax2 prevenls the translation of the Pax2 mRNA that is transcribed as a response to induction, the mesenchyme cells of cultured kidney rudiments fail to condense (Rothenpieler and Dressler 1993). Thus, Pax2 may play several roles during Iddney formation. in the mouse, Wnl6 and Wnt9b from the lateral sides of the ureteric bud (but not from the tip) are critical for trans forming the metanephrogenic mesenchyme cells into tubu lar epithelium. The mesenchyme has receptors for these enchyme in an FGF-independent way (Itaranta et aJ. 2002), Wnts. Wnt6 appears to promote the condensation of mes and Wnt9b induces Wnt4 in the mesenchyme. As we will see, Wnt4 is very important for the formation of the nephron, and mice deficient in Wnt9b lack Iddneys' (Fig ure 1 1 .28; see also Figu re 3 .27) .

FIGURE 1 1 .29 liml expression (dark stain) in a 1 9-day embry onic mouse kidney. In situ hybridization shows high levels of Lim1 protein in the newly epithelialized comma-shaped and S-shaped bodies that will become nephrons. Compare with earlier Liml expression shown in Figure 1 1 .24C (From Karavanov et al. 1 998, courtesy of A. A. Karavanov.) 440 CHAPTER 1 1 FIGURE 11.30 Effect of GDNF on branching of the ureteric epithelium. The ureteric bud and its bra nches are stained orange (with antibodies to cytokeratin 1 8), while the nephrons are stained green (with antibodies to nephron brush border antigens).

(AI 1 3 day embryonic mouse kidney cultured 2 days with a control bead (circle) has a normal branching pattern. (B) A similar kidney cul tured 2 days with a GDNF-soaked bead shows a distorted pattern, as new branches are induced in the vicinity of the bead. (From Sainio et al. 1 997, courtesy of K. Sainio.) TGF-◆l is added to cultured kidneys, it prevents the epithe lium from branching (Figure 1 1 .31A,B; Ritvos et al. 1995). TGF-◆l is known to promote the synthesis of extracellular matrix proteins and to inhibit the metalloproteinases that can digest these matrices (Penttinen et al. 1988; Nakamu ra et al. 1990). Thus, it is possible that TGF-Ill stabilizes branches once they form. A third molecule that may be important in epithelial branching is BMP4 (Miyazaki et al. 2000). BMP4 is found in the mesenchymal cells surrounding the nephric duel, products of the genes whose loss-of function alleles give and BMP4 receptors are found in the epithelial tissue of these genes have abnormal, swollen nephrons (Ward et aJ. BMP4 restricts the branching of the duct to the appropri rise to human polycystic kidney disease. Mice deficient in the duct. Because BMPs antagonize branching signals, 1996; van Adelsberg et aJ. 1997). ate sites. When the BMP4 signaling cascade is activated STEP 6: SIGNALS FROM THE MESENCHYME I N D UCE THE BRANCHING OF THE URETERIC BUD Recent evidence has severely distorts the normal branching pattern (Figure ureteric bud, and these factors probably work as pushes XVIll, which is part of the extracellular matrix induced by matrix surrounding the epithelium, thereby preventing branching pattern implicated several paracrine factors in the branching of the and pulls. Some factors may preserve the extracellular branching from taking place (the "push"), while other fac tors may cause the digestion of this extracellular matrix, ectopically in embryonic mouse kidney rudiments, it 1 1 .31C). The fourth molecule involved in branching is collagen the mesenchyme. It may provide the specificity for the (Lin et aJ. 2001). Collagen XVIII is found on the branches of the kidney epithelium b u t not at the tips; in the developing lung, the reaprocal pattern is seen.

permitting branching to OCCur (the "pull"). This pattern is generated in part by GDNF, which down GDNF (Sainio et aJ. 1997). GDNF from the mesenchyme ureteric bud branches. When ureteric duct epithelium is The lirst protein regula ting ureteric bud branching is not only induces the initial ureteric bud from the nephric duct, but it can also induce secondary buds from the ureteric bud once the bud has entered the mesenchyme (Figure 1 1.30). GDNF from the mesenchyme promotes cell division in the Ret-expressing cells at the tip of the ureteric regulates collagen XVIll expression in the tips of the incubated in 1ung mesenchyme, the collagen XVIll expres sion pattern seen is typical of that of the lung, and the branching pattern resembles that of the lung epithelium (Figure 11.310). Wntll synthesis in these responsive cells at the tip of the STEPS 7 AND 8: DIFFERENTIATION OF THE NEPHRON AND GROWTH OF THE URETERIC BUD The interactions w e have ing GDNF levels. Wntll may also be important in inducing metanephrogenic mesenchyme cells that covers the tips of bud (Shakya et aJ. 2005). GDNF also appears to induce bud (see Figure 11.28A), and Wntll reciprocates by regulat the differentiation of the metanephrogenic mesenchyme (Kuure et al.

2007). The cooperation between the described to this point create a cap condensate of the ureteric bud branches. The cap condensate maintains GDNF production, ensuring the outward growth of the GDNF/Ret pathway and the Wnt pathway appears to ureteric branches. Moreover, the mesenchyme of this cap nephrogenic cell division such that continued metanephric can make all the different cell types of the nephron (Mug The second candidate branch-regulating molecule i s which also condense into a population of self-reneWing coordinate the balance between branching and meta development is ensured (Majumdar et al. 2003). transforming growth factor-◆l (TGF-◆l). When exogenous condensate is a multipotent, self-renewing population that ford et aI. 2009). As such, i t is not unlike the somite cells, multipotent progenitor cells. PARAXIAL A N D INTERMEDIATE MESODERM (A) (B) 441 FIGURE 1 1 .31 Signaling molecules and h ranching of the ureteric epitheli um . (A) An 1 1 ◆day embryonic mouse kidney cul tured for 4 days in control medium has a normal bra nching pat tern. (8) An 1 1 .day mouse kidney cultured in TGF-◆l shows no branching until reach ing the periphery of the mesenchyme. (B-G) At the tips of the branches, the epitheli um induces the mesenchyme to aggregate and cavitate to form the renal tubules and glomerul i (where the blood from the arteriole is fil tered), When the mesenchyme has condensed into an epithelium, it digests the basal lamina of the ureteric bud cells that induced it and connects to the ureteric bud epithel ium. A portion of the aggre gated mesenchyme (the pretubu la r condensate) becomes the nephron (renal tubules and Bowman's cap sule), while the ureteri c bud becomes the co ll ecting duct for the urine. (After Saxen 1 987; Sa riol a ....◆ ... (Metanephrogenic mesenchyme ac T "' Ureteric condensate Glomerulus (G) t OlleC'ing duct capsule Distal --i' .# tubule Proximal tubule ej'l ·:t◆ -: 2002.) derm, expresses Lim1, and forms kidneys (Mauch et a!. 2000; Suetsugu et at. 2005). Similarly, in mouse embryos with knockouts of both the Pax2 and Pax8 genes, the meS enchyme-ta epithelial transition necessary to form the kid ney duct fails, the cells undergo apoptosis, and no nephriC structures form (Bouchard et at. 2002). Moreover, in the mouse, Liml and Pax2 proteins appear to induce one another. Liml plays several roles in the formation of the mouse kidney. First it is needed for converting the intemlediate mesenchyme into the kidney duct (Tsang et at. 2000), and later it is required for the formation of the ureteric bud and the tubular structure both in mesonephric and metanephric mesenchyme (Shawlot and Behringer 1995; Karavanov et a1. 1998; Kobayashi et at.

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j., G. Yang, M. Wright, D. Smith and G. C. Schoenwolf. 2000. Sig nals from trunk paraxial mesoderm induce pronephros formation in chick intermediate mesoderm. Dev. BioI. 220: Paimeirim, I., D. Henrique, D. lsh Horowicz and O. Pourquie. 1997. Avian hairy gene expression identifies a molecular dock linked to vertebrate segmentation and somitogenesis. Cell Nowicki, j. L., R. Takimoto, and A. C. Burke. 2003. The lateral somitic frontier: Dorsa-ventral aspects of anterior-poste rior regionalization in the embryo. Piche!, j. C. and 11 others. 1996. Defects 62-75. Mec/!. Dev. 120: 227-240. Ordahl, C. P.

and N. Le Douarin. 1992. Two myogenk lineages within the developing somite. Development 114: 339-353. 91: 639-648. in enteric innervation and kid ney devel opment in mice lacking GDNE Nature 382: 73-76. Sato, Y, K. Yasuda and Y Takahashi. 2002. Morphological boundary forms by a novel inductive event mediated by Lunatic-Fringe and Notch during somitic segmentation. Developmenl 129: 3633-3644. Go Online WEBSITE 11.1 Calling the competence of the somite into question. When the tbx6 gene was knocked out from mice, the resulting emb ryos had three neural tubes in the posterior of their bodies. Without the tbx6 gene, the somitic tissue responded to the notochord and epidermal signals as if it were neural ectoderm. WEBSITE 11.2 Crania! paraxial mesodenn.

Most of the head-musculature does not come from s◆mites. Rather, it comes from the cranial paraxial (prechordal plate) meso the brain, and they migrate to their respective destinations. ate by the fusion of cells, while heart muscle becomes mult inucleate by nuclear divisions withjn a cell. WEBSITE 11.4 Paracrine factors, their receptors, and human bone growth. Mutations in the genes encoding paracrine factors and their receptors cause numerous skele tal anomalies in humans and mice. The FGF and Hedge hog pathways are especially important. Vade Mecum derm. These cells originate adjacent to the sides of Mesodenn in the vertebrate embryo. The organization of the mesoderm in the neurula stage is similar for aU verte WEBSITE 11.3 Muscle fonnation. Research on chimeric brates. You can see this organization by viewing serial sec mice has shown that skeletal muscle becomes multinuc1e- tions of the chick embryo in the Chick-Mid segment. Lateral Plate Mesoderm and the Endoderm I N THE CHAOS OF THE E GliSH CIVIL WARS, William Harvey, physician to the King end discoverer of the blastoderm, was comforted by viewing the heart as the undisputed ruler of the body, through whose divinely ordained powers the lawful growth of the organism was assured. Later embryologists saw the heart as more of a servant than a ruler, the chamberlain of the household who assured that n utr ien ts reached the apically located brain and the peripherally located muscles.

In ei ther metaphor, the heart, circulation, and digestive system were known to be absolutely critical for development.

As H arvey persuaSively argued in 165 1 , the chick embryo must form its own blood without any help from the hen, and this blood is crucial in embryonic growth. How this happened was a mystery to him. "What artificer," he wrote, could create blood "when there is yet no liver in being?" The nutrition provided by the egg was also paramount to Hervey. ! lis conclusion about the nutritive value of the yolk and albumen was "The egge is, as it were, a n exposed womb; wherein there is a substance con cluded as the Representative and Substitute, or Vicar of the breasts." This chapter outlines the mechanisms by which the Circulatory system, the respiratory system, and the digestive system emerge in the amniote embryo.

We will first discuss the formation of the heart and Ihen proceed to the mechanisms whe reb y the blood vessels and blood cells develop. At the end of the chap ter, we will bricOy follow the development of the gut tube and its associated organs. As we will see, the lateral plate mesoderm and the endoder m interact to create the Circulatory and the digestive organs. ' LATERAL PLATE MESODERM On the peripheral sides of the two bands of intermediate mesoderm resides the lateral pl. tc mesoderm (see Figures ILl and 1 1.2). Each plate splits horizontal ly into two layers. The dorsal layer is the sont. tic (parietal) mesodernt, which The ventr. l layer is tile splanclmic (viscorel) mesoderm, which overlies the endo derm and, toge ther with the endoderm, forms the s pl en ch nop leure (Figure underlies the ectoderm and, together with the ectoderm, forms the somatopleure. -Some scientists think that the mesoderm and endoderm were originally a single germ layer, in vertebrates is a broad the "mesendoderOl," that accomplished all these functions. RecaU from Chapter 5 that \",hat territory of embryonk cells is actuflly the progeny of a Single and the "mes

cardiac precursor specification and migration.

The faust gene, which encodes the GATA5 protein, is expressed in the endoderm and is required for the migra tion of cardiac precursor cells to the midline and also for their division and specification. It appears to be important in the pathway leading to activation of the Nkx2-5 gene in the cardiac precursor cells (Reiter et a1. 1999). Another particularly interesting zebrafish mutation is miles apart. Its phenotype is limited to cardiac precursor migration to the midline and resembles the cardia bifida seen in experimen tally manipulated chick embryos (Figure 12.5B,C). The miles npart gene encodes a receptor for a cell surface sphyn golipid molecule, and it is expressed in the endoderm on either side of the midline (Kupperman et a1. 2000). In mice, cardia bifida can also be produced by muta tions of genes that are expressed in the endoderm. One of these genes, Foxp4, encodes a transcription factor expressed in the early foregut cells along the pathway the cardiac pre cursors travel toward the midline.

In these mutants, each heart primordia develops separately, and the embryonic mouse contains two hearts, one on each side of the body (Figure 12.SD,E; Li et a1. 2004). 450 (B) CHAPTER 1 2 FIGURE 12.5 Migration of hearl primordia. (A) Cardia bifida (tw'O hearts) in a chick em bryo induced by surgically cutting the ventral mid line, thereby preventing the two heart prim ord ia irom fusing.

(B) Wild-type zebrafish and (C) miles apart mutant, stained with probes for the cardiac myosin light chain.

There is a lack of migration in the miles apart mutant. (D) Mouse heart stained with antisense RNA probe to ven tricular myosin shows fusion of the heart primor dia in a wi ld-type 1 2.5-day embryo. (E) Cardia bifida in a Foxp4 deficient mouse embryo. I nter esti ngly each of these hearts has ventricles and atria, and they bOlh loop and form all four chambers wilh normal leright asy mmet ry.

(A courlesy of R. L. DeHaan; B,C from Kupperman et al. 2000, courtesy ofY. R. Didier; D,E from L i el a l. 2004, courlesy of E. E. Mo rrisey.) , (e) , (D) (E) Determination of anterior and posterior cardiac domains As the cardiac precursor cells migrate, the posterior region becomes exposed to increas ingly higher concentrations of retinoic acid (RA) produced by the poslerior mesoderm (see Figune 8.13). RA is critical in specifying the posterior cardiac precursor cells into becoming the inflow, or "venous," portions of the heart-the sinus venosus and atria. Originally, these fates are not fixed, as trans plantation or rotation experiments show that tn accordance with a new environ- these precursor cells can regulate and differ entiate ment. But once the posterior cardiac precursors enter the rior tail cells do not migrate, but they express retinalde realm of active RA synthesis, they express the gene for reti hyde dehydrogenase and initiate a retinoid acid gradient naldehyde dehydrogenase; they then can produce their own (Figure RA, and their posterior fate becomes committed (Figure 1 2.6A,B; Simoes-Costa el al. 2005). This ability of retinoic add to specify and commit heart precursor to become atria 12.6C). Heart cell differentiation explains its teratogenic effects on heart development, where One of the most important new discoveries of cardiac devel n i exposure of vertebrate embryos to RA can cause expan opment has been the demonstration that all the different sion of atrial tissues at the expense of ventricular tissues cells of the heart-the ventricular myocytes, the atrial (Stainier and Fishman 1992; Hochgreb et a1. 2003). myocytes, the smooth muscles that generate the venous and Interestingly, this relationship between heart develop arterial vasculature, the endothelial lining of the heart and ment and retinoic acid appears to be conserved, even in valves, and the epicardium that forms an envelope for the embryos that form their hearts in very different ways. In hearl-are derived from the same progenitor cell type aI. 2006; Moretti et al. 2006; Wu et a!. 2006). The tunica les, the heart is a Single-layered U-shaped tube. At (Kattman et the 110-cell stage, the heart lineage is represented by a pair cardiac fields contain multipotent progenitor cells. Indeed, of mesodenmal cells near Ihe vegetal pole, the 87.5 blas there appears to be an early progenitor cell population that tomeres. These cells give rise to the anteriormost tail cells bears Ihe responSibility for forming the entire circulatory and Ihe heart precursor cells (trunk venlral cells).

When system. Under one set of influences, its descendents become the B7.5 blastomeres split into the heart lineage and the hemangioblasts, those cells that form blood vessels and anterior tail lineage, the heart precursor cells express the blood cells; under other conditions (i.e., the conditions in tunkate homologue of the the cardiac fields), they form Ihe multipotent cardiac pre Nkx2-5 gene and migrate ven 12.7; Anton et a1. 2007). Several inves trally into the "head" of Ihe developing tadpole (David cursor cells (Figure son and Levine 2003; Simoes-Costa et a!. 2005). The ante- tigators have proposed slightly different pathways for gen- L\) Chick,stage d a y s (B) Mo u s e , Ventricular 8 (outflow) (C) a a stage Ciona, l rv p to pro-l erating these cells, but the diiferences may be caused by differences in assaying and culturing procedures, and it would not be surprising were we to find that each of these pathways is actually used in differ ent situa tions. CEll DIFFERENT IATION Se v eral genes are expressed very early during heart development (see Figure 12.2C). The GATA4 transcri ption factor is first seen in the precardiac cells of chicks and mice as these cells emerge from the primitive streak. GATA4 expression is retained in all the cells making lip the heart fields on both sides FIGURE 12.6 Double in situ hybridiza tion for the expression of r?AOH2 (orange), which encodes the retinoic acid-synthesizing enzyme rctioaldehyde dehydrogenase-2; and Tbx5 (pu r l es), a marker for the early heart fields. In the devel opm ental stages seen here, the heart precursor cells are exposed gressively increasing amounts of retinoic acid. (A) Chick, stage B (26-29 hours). (B) Mouse, B days. (0 Ciona (a lun ic a te) at the larval stage. (From SimOes-Costa et a l . 200S, courtesy of J. Xavier-Neto.) • Earlyprogenitorcel Cardiac precursor cel Hemangiablast t Bry+ Is1+1- Bry+ Hcceml sa(tbopl oiedt)ic An(vgeisobelas)ts sl c-KiIcsl/+scaI c-Kil"t I [diEnf edrcornthicaltiaoI n I 1CadiIfrderioerr===t mntyiaotcioyntI ISmoothdiiferen1tmusc1iationeI Hc a r t VEGF   I N ITIAL 451 LATERAL PLATE MESODERM A N D THE E N ODERM 8 of the embryo. This transcrip tjon factor is necessary for the activation of numerouS heart-specific genes/ and i t also activates expression of the gene for N cadherin, a protein tha t is critical for the fusion of the two heart rudiments into one tube (H. Zhang et al. 2003). The BMP pathway is critical in induc ing the syn thesis of the Nkx2-5 transcrip tion factor in the m igrating cardiogenic   I t Flkl+ I + t Nkx25+1Flkt+l . ." . .. . ' .. ' -, , . t "t NW5 + NW5 I I t 1 , , FIGURE 1 2.7 Model for early cardiovascular l ineages. The splanchnic mesoderm gives rise to two lineages Ihat have Flk1 (a receptor) on their cell membranes. The earlier population gives rise to the hemangiablasts (precursors to blood cells and blCX>d vessels), while the later population gives rise to the cardiac (heart) progenitor cel ls. This latter population in turn gives rise to a variety of cell types whose relationships are still obscure. However, all the cell types of the heart can be traced back to the cardiac progenitor cells. (Mer Anton 2007.) 452 CHAPTER 1 2 mesoderm (Komuro and Jzwno 1993; Lints et aJ. 1993; Sugi and Lough 1994; Schultheiss et al. 1995; Andree et al. 1998). Nkx2-S is crucial in instructing the mesoderm to become heart tissue, and it also activates the synthesis of nwnec ous cardiac transcription factors' (especially members of the T-box, GATA, and Mcf2 families). GATA4 can also induce Nkx2-S, so activating one will activate the other and convert mesoderm into cardiac precursor cells. Working together, these transcription factors activate the expression of genes encoding cardiac muscle-specific proteins (such as cardiac actin.. atrial natriuretic factor, and the a-myosin heavy chains) (Sepulveda et aI. 1998; Kawamura et al. 200S) . The Nkx2-S transcription factor can also work as a repressor, and if the Nkx2-5 gene is specifically knocked out in those cells destined to become ventricles, these chambers express Btv1P10, causing massive overgrowth of the ventricles such that the ventricular chambers fill with muscle cells (Pashmforoush et al. 2004). Nkx2-S also can downregulate BMPs, and in early heart cell development, it limits the nwnber of heart cell precursors there can be in the cardiac fields (Prall et al. 2007). The Tbx transcription factors are also critical in heart development (Plageman and Yutzey 2004). The Tbx5 gene is expressed in the cardiac fields on both sides of the chick and mouse embryos (see Figure 12.6). In these early cells, TbxS acts wW, GATA4 and Nkx2-S to activate numerous genes involved in heart specification t Later, TbxS protein expression becomes restricted to the atria and left ventri cle. The ventricular septum (the wall separating the left and right ventricles) is formed at the boundary between those cells expr essing Tbx5 and those that do not. TbxS works antagonistically to Tbx20, which becomes expressed in the r ight ventricle. When the TbxS express ion domain is ectopically expanded, the location of the ventricular sep tum sh.ifts to this new location. Moreover, a conditional knockout of the mouse Tbx5 gene specifically inactivat ing it during ventricular development leads to the for mation of a lizard-like ventrical that lacks any septation (Takeuchi et al. 2003; Koshiba-Takeuch.i et al. 2009). Thus, TbxS is extremely important in the separation of the left and right ventricles. Mutations in human TBX5 cause Holt- *The cells of the anterior heart field express the Foxhl gene, which commits these heart precursor ceUs to become the "arterial" portion of the heart-the ventricles and outflow tracts. In combination with the Nkx2-S transcriptio n factor, Foxhl activates those genes (such as MeJ2c) that begin the differentiation o f the ventricles (von Both et at. 2004). t The Nkx2-S homeodomain transcription fctor is homologous to the Tl.ll.man transcription factor active in specifying the heart tube of Drosophf/n, Moreover, neither Tmman nor Nkx2-5 alone is sUEfi cient to complete heart development in their respective organisms.

Mice lacking the Nkx2-5 gene start heart tube formation, but the tube fails to thicken or to loop (Lyons et an. 1995). Humans with a mutation in one of their NKX2-5 genes have congenital heart mal formations (Schotl et elt. 1998). Oram syndrome (Bruneau et al. 1999), characterized by abnormalities of the heart and upper limbs. Fusion of the heart rudiments and initial heartbeats Cell differentiation occurs independently in U,e two heart forming primordia. As they m.igrate toward each other, the ventral splanchnic mesoderm cells of the primordia begin to express N-cadherin on their apices, sort out from the somatic mesoderm cells, and join together to form an epithe lial layer. This joining of the somatic mesoderm will lead to the formation of the pericardial cavity, the sac in which the heart is fonned (Linask 1992).

A small population of splanch nic mesoderm then downregulates N-cadherin and delam inates from the epitheliwn to form the endocardium, the lin ing of the heart that is continuous with the blood vessels (see Figure 12.4C,D). The epithelial layer of splanclmic meso derm forms the myocardium (Manasek 1968; Linask et al. 1997), which will give rise to the heart muscles that will pump for the lifetime of the organism. The endocardial cells produce many of the heart valves, secrete the proteins that regulate myocardial growth, and regulate the placement of nervous tissue in the heart. The fusion of the two heart primordia occurs at about 29 hours in chick developmen t (see Figure 12.4C,D) and at 3 weeks in hwnan gestation. The myocardia unite to form a single tube. The two endocardia lie within this common tube for a short while, but eventually they also fuse. The unlused posterior portions of the endocardiwn become the openings of the vitelline veins into the heart (see Figure 12.8A). These veins will carry nutrients from the yolk sac into the sinus venosus, the posterior region where the two major veins fuse. The blood then passes through a valve like flap into the atrial region of the heart. Contractions 01 the truncus arteriosus speed the blood into the aorta. In addition to the heart cells derived from the paired heart fields, cells from the rostral paraxial mesoderm also appear to form portions of the truncus (de la Cruz and Markwald 1998; Abu-Jssa et aI. 2004; Harel et al. 2009). Pulsations of the chick heart begin while the paired pri mordia are sliO fusing.

Heart muscle cells develop an ini1er ent ability to contract, and isolated heart cells from 7-day rat or chick embryos will continue to beat when placed in petri dishes (Harary and Farley 1963; DeHaan 1967; lmana ka-Yoshida et aI. 1998).

The pulsations are made possible by the appearance of the sodiwn-calcium exchange pwnp in the muscle cell membrane; inhibiting this channel's func tion prevents the heartbeat from starting (Wakimoto et al. 2000; Linask et al. 2001). Eventually, the rhythmiCity of the heartbeat becomes coordinated by the sinus venosus. The electric impulses generated here initiate waves of muscle contraction through the tubular heart. In this way, the heart can pump blood even before its intricate system of valves has been completed. Studies of mutations of cardiac cell calcium channels have implicated these channels in the LATERAL PLATE MESODERM A N D THE ENDODERM pacemaker function (Rottbauer et a1. 2001; Zhang et a1.

2002). 453 original anterior-posterior polarity o f the heart tube into i anterior the right-left polarity seen in the adult organism. When this looping is completed, the portion of the heart tube des Looping and formation of heart chambers tined to become the atria l es to the portion that In 3-day chick embryos and 4-week human embryos, the will become the ventricles (Figure 12.8) . blood and one ventricle to pump blood. In the chick Chapter embryo, the unaided eye can see the remarkable cycle o f the Hand 1 and Hand2 transcription factors.

Both Hand blood entering the lower chamber and being pumped out proteins appear to be synthesized throughout the early through the aorta.

Looping of the heart thus converts the heart tube, but as looping commences, Hand! becomes heart is a two-chambered tube, with one atriwn to receive (A) AoRigrhtitc seac c e Righvteantrriuicmle Atrioventricular o Left • • Day 21 The direction of heart looping is dependent on the lelt right patterning proteins (Nodal and Pitx2) discussed in 8. Within the heart primordium, Nkx2-S regulates Newborn Day 28 v n tri l Left atrium valve I e) (6) Cardiac looping and chamber formation. (A) Schematic diagram of cardiac morphogenesis in humans. On day 2 1 , the heart is a single-chambered tube. Regional specification of the tube is shown by the different colors. By day 28, cardiac loop ing has occurred, placing the presumptive atria anterior to the presumptive ventricles. (B/C) Xin expression in the fusion of left and right heart primordia of a chick. The cells fated to form the myocardium are shown by staining for the Xin message, whose protein product is essential for the looping of the heart tube. (B) Stage-9 chick neuru-la, in which expression of Xin (purple) is seen in the two symmetrical heart-forming fields (arrows). (C) Stage , 0 chick embryo, showing fusion of the two heart-forming regions prior to looping. (D,E) Specification of the atria and ventricles occurs even before heart loopi ng The atria and ventricles of the FIGURE 12.8 . (D) .. . . . (E) .. mouse embryo have separate types of myosin protei ns, which allows them to be differentially stained. In these photographs, atri al myosi n is stained blue and ventricular myosin is stained orange. (0) In the tubular heart (prior to looping), the two myosins (and their respective stains) overlap at the atrioventricular channel join- ing the future regions of the heart. Aer looping, the blue stain is seen in the definitive atria and inflow tract, while the orange stain is seen in the ventricles. The unstained region above the Yen- trides is the truncus arteriosus. Derived primarily from the neural crest, the truncus arteriosus becomes separated into the aorta and pulmonary arteries. (A after Srivastava and Olson 2(){x); B,C from Wang et al. ' 999, courtesy of J. J.-c. Lin; D,E from Xavier-Neto et al. t 999, courtesy of N. Rosenthal) (E) . 454 CHAPTER 1 2 FIGURE 12.9 Form at ion of and valves of the heart. (A) Diagrammatic cross section of the human hea rt at 4.5 weeks. The atrial (A) lhe cham bers Day ei um vena cava month Formamen atr Valveof vFcauenseatrdlicular vinefnaricarva cInntservcoeanntricular ovaleInferior m vena cava 33 S (8) Third p tum pr m pri and ventricular septa are growing toward the endocar dial cushions. (8) Cross sec tion of the human heart dur ing the third month of gestation. B lood ca n cross from the righ t side of the heart to the left side through openings in the primary and secondary atrial septa.

(After Larsen 1 993.) e Atrial sept.um Superior um d o rdi al u hi Atpserprimtiualm i um Foramen s sep u future left ventricle and Hand2 to the right. restricted to the Without these proteins, looping is abnormal, since the ven tricles fail to form properly' (Srivastava et al. 1995; Biben and Harvey 1997). interfering with these chamber-specific factors (Cai et aJ.

2005; SteJUlard et al.

2005). The formation of the heart valves-four leaflike flaps that must open and shut without failure once each second-Cytoskeletal proteins and their adhesion to the extra for the duration o f our life is just starting to be under cellular matrices are very important in these turning stood. In mammals, endocardial cushions form from the events. First, the myosin-like matrix protein flectin regu endocardium and divide the tube into right and left atri lates the physical tension of the heart tissues differently on oventricular channels. Meanwhile, the primitive atrium is different sides of the heart (Tsuda et al. 1996; Lu et al. 2008). partitioned by two septa that grow ventrally toward the Second, transcription factors Nkx2-5 and Mef2C activate endocardial cushions (Figure the Xin gene (Chinese for "heart"), whose product binds ions serve as a valve during early heart development 12.9A). The endocardial cush to actin microfilaments and medi.ates cytoskeletal changes (Lamers et al. 1988), but as the heart enlarges, specialized essential for heart looping (Wang et aJ. 1999; Grosskurth et valves develop to prevent the return of blood into the atTia al. 2008). Third, metalloproteinases-especially metallo and to prevent the mixing of bloods from the two sides of proteinase-2 (MMP2)-are critical for remodeling the cytoskeleton. 11 MMP2 gene expression the heart (Figure 12.9B) .

These valves begin to form when is blocked by anti cells from the myocardium produce a factor that causes bodies or pharmaceutical inhibitors, the extracellular cells from the adjacent endocardium to detach and enter matrix fails to change, the asymmetric cell divisions (which the hyaluronate-rich "cardiac jelly" extracellular matrix cause the left side to grow faster than the right) fail to between the two layers (Markwald et al. 1977; Potts et aJ. occur, and heart looping stops (Linask et aJ. 2005). 1991). In mammals, BMP2 appears to be necessary for Differential cell division is also important in structur inducing this epithelial-mesenchymal transition and for ing the right and left sides of the heart, and the regulation forming the endocardial cushions from cardiac myocytes or differential growth remains a major question in heart (Ma et aJ. 2005; Rivera-Feliciano and Tabin 2006). BMP2 development.

It is thought that many of the genes found induces TGF-, which promotes · the EMT, as well as the Tbx5 is expressed in the Wrzt11 is expressed in the atrioventricular canal that separates i n one particular area of the heart may be responsible for differential groWtll. For instance, the cells and become a major part of the cardiac jelly (Shi left ventricle and atrium, while rai et al. 2009). right side of the heart.

A series of regulators (including The septa between the atria, however, have openings in Tbx2 and Tbx20) probably control growth by activating or them, so blood can still cross from one side of the heart into r2000). r(Hove o nl "Zebrafish, with o y one ventricle, have onlyonetypeofHand p otein . When the gene encoding this protein is mutated, the entire ventricular portion of the heart fails to form (Srivastava and Olson fo Nongenetic agents are also critical in normal zebrafish heart mation. In the absence of high-pressure blood Oow, heart loop 2(03). ing, chamber f rma tion , and valve development eITe impaired et al. the other. This crossing of blood is needed for the survival of the fetus before circulation to iunctional lungs has been established. Upon the first breath, the septal openings close and the left and right Circulatory loops are established. With the formation of the septa (which usually occurs in the seventh week of human development), the heart is a four-chambered structure with the pulmonary artery con nected to the right ventricle and the aorta connected to the left ventricle. LATERAL PLATE MESODERM AND THE ENDODERM 455 SIDEL IGH TS SPECULATIONS Redirecting Blood Flow in the Newborn Mammal A lthough the developing mam malian fetus shares with the adult the need to get oxygen and nutrients to its tissues, the physiology of the fetus differs dramatically from that of the adult. Chief among the dif ferences is the fetus's lack of functional lungs and intestines. A l l of its oxygen and nutrients must come from the pla centa. This observation raises two questions. First, how does the fetus obtain oxygen and nutrients from maternal blood? And second, how is blood circulation redirected to the lungs once the umbilical cord is cut and breathing becomes necessary? Human embryonic circulation The human embryonic circulatory sys tem is a modification of that used i n other amn iotes, such a s birds and rep tiles. The circulatory system to and from the chick embryo and yolk sac is shown in Figure 12.1 0A. B lood pumped through the dorsal aorta pass es over the aortic arches and down into the embryo. Some of this blood leaves the embryo through the vitel line arteries and enters the yolk sac. Nutri ents and oxygen are absorbed from the yolk, and the blood returns through the vitelline veins to fe enter the heart through the sinus venosus. Mammalian embryos obtain food and oxygen from the placenta. Thus, although the embryo has vessels homologous to the vite l l ine veins, the main supply of food and oxygen comes from the umbilical vein, which unites the embryo with the p lacenta Figure 12.10 Embryonic circu l a tory sys tem . (A) Ci rcu l a tory system of a 2-day chick embryo The sinus term i na lis is the outer limit of the circulatory system and the site of blood ce l l ge erat i on . (B) Circulatory system of a 4-week human embryo. Although at this stage all the major blood vessels are pa i red len an d right, only Ihe r ight vessels are shown here. In both views, arteries are shown in red, veins in blue. (A adapted from Popoff 1 894; B after Carlson 1981.) s (Figure 12.108). This vein, which brings oxygenated, food-laden blood into the embryo, is derived from what would be the right vitelline vein in birds. The umbilical artery, carrying wastes to the placenta, is derived from what would have become the al lanto ic artery of the chick. It extends from the caudal portion of the aorta and proceeds along the allantois and then out to the placenta. Fetal hemoglobin The solution to the fetus's problem of getting oxygen from its mother's blood (Continued 011 next page) (Al arches Heart arteries and veins DorsaJ aorta Posteriorvein vitelline (B) Chorionic Posterior cardinal vein CO.L11lI10n cardinal vein Lung bud Pharyngeal IV Aortic arch III pouch . n artery Internal carotid artery sac 456 CHAPTER 1 2 SIDEL IGHTS & SPECULATIONS (Conti n u ed) Figure 12.11 Adult and fetal hemoglob i n molecules differ in their globin subunits. The fetal y-chain bi nds diphosphoglycerate less 100 Adult avidly than does the adult p-chain. Conse quently, fetal hemoglobin can bind oxygen more effi cien tly than can adult hemoglobin. In the placen ta, there is a net flow (arrow) of oxygen from the mother's blood (which gives up oxygen to the tissues at lower oxygen pressures) to the fetal blood (which at the same pressure is still taking up oxygen). hemoglobin O L ·-02 pressw'e involves the development of a special ized fetal hemoglobin n,e hemogl obin in fetal red blood cells differs slightly from that in adult corpuscles. Two of the four peptides the a lph a (a) chai ns that make up fetal and adult hemoglobin chains are identical, but adult hemoglobin has two beta (P) chains, while the fetus has two gamma (y) chains (Figu re 12.1 1). The p chains bind the natural regulator diphospho glycerate, which assists in the unload- ing of oxygen. The y-chain proteins do not bind diphosphoglycerate as well, and therefore have a higher affinity for oxygen. In the low-oxygen environ ment of the placen ta, oxygen is released from adult hemoglobin. In this same environment, fetal hemoglobin does not release oxygen, but bi nds it. This small difference in oxygen affinity mediates the transfer of oxygen from the mother to the fetus. In the fetus, the .

myoglobin of the fetal muscles has an even higher affinity f or oxygen, so oxy gen molecules pass from fetal hemo globin to the fetal muscles. Fetal hemo globin is not deleterious to the newborn, arid in humans, the replace ment of fetal hemoglobin-containing blood cells with adult hemoglobin-containing blood cells is not complete until about 6 months after birth. (fhe molecular basis for this switch i n glo bins is discussed in Chapter 2 .) From fetal to newborn circulation Once the fetus is no longer obtaining its oxygen from its mother, how does it restructure its circulation to get oxygen from its own lungs? D uri ng fetal devel opment, an open ing- the ductus arte ri osus- Formation of Blood Vessels Although the heart is the first fimctioual organ of the bod y, blood into the left ventricle to be pumped. This is accomplished by the foramen ovaIe, an ope ning in the sep tum separating the right and left atria. Blood can enter the right atrium, pass through the foramen into the left atri um, and then enter the left ventricle (Figure 1 2.12) . Whe n the first breath is drawn, blood pressure in the left side of the heart increases. This pressure closes the septa over the foramen ova Ie, thereby separating the pul monary and systemic circulations. Moreover, the decrease in prosta glandins experi enced by the newborn cause the muscles s urro u nding the ductus arteriosus to close that opening as well (Nguyen et a l . 1 997). Th us, when brea th ing begins, the respiratory c i rculation is shunted from the placen ta to the lungs. tn some infants, the septa fai l to close and the foramen ovale is left open. Indeed, atrial and ventricular septum defects are among the most common congenital anomalies. Usu ally the atrial ope ning is so small that there are no physical symptoms, and the foramen eventually closes. If it does not close completely, however, and the secondary septum fails to form, the atrial sep tal opening may cause enlargement of the right side of the heart, which can lead to heart fail ure in early adulthood. This fine-tun ing of septal g rowt h is controlled by m i R-1 2, a microRNA that regulates translation of several proteins involved in cardiac muscle growth and electri cal conduction (Zhao et a l . 2007).

species look very much alike, beca use the development of the circulatory system is severely constrained by physio logical, physical, and evolutionary pa rameters . it does no t even begin to pump until the vascular system of the embryo has established its first circul atory loops. Rather than sp routin g from the heart, the blood vessels form independently, linking up to the heart soon afterward. Eve ryone s c ircula tory system is different, since the genom e cannot encode the intricate series of connections between the arteries and veins. In deed .. chance plays a major role in es tablishing the microanatomy of the dreu la tory system. However, all circula tory systems in a given ' Constraints on the construction of blood vessels The first constraint on vascular development t is physwlogi cal. Unli ke new Inachines.. which d o not need to function until they have left the assembly line, new organisms have to function even as they develop . The embryOniC cells must obtain nourishment before there is an intestine, use oxy gen before there are lungs, and excrete wastes before there LATERAL PLATE MESODERM A N D T H E ENDODERM FETUS Tofromanadrm and from head Aovenarta==;Wlti aiPDusrutolecmprtieuoonsnuasry ;cava .. avretienry ropvaelnems ba rtiwae n Ivnefhearicoarva AoaDurterctrtaiuosus DeaDuorsetceat.eunsding clos s venos arteries vein vena cava Fo a SIDEL IGHTS & SPECULATIONS (Continued) To C .:\ en i r + ---- il ---- f.'fr-iT ,fi!]J are kidneys. All these functions are mediated through the embryonic circulatory system.

Therefore, the circulatory physiology of the developing embryo must cliffer from that of the adult organism .

Food is absorbed not through the intestine, but from either the yolk or the placenta, and res piration is conducted not through the gills or lungs, but u Figure 1 2.12 Red i rec ti o n of human blood flow at birth. The expansion of air into the lungs ca ses pressure changes that redirect the flow of blood in the newborn infant.

The ductus arteriosus squeezes shut, breaking off the connection between the aorta and the pulmonary artery, and the foramen Ie, a passageway between the left and ri gh t atria, also closes. In this way, pulmonary cir culation is separated from system ic circulation. ova i;;$[. ::J[;;;;;;;;; ; :: =l ----;;t 457 NEWBORN six pairs of aortic arches are formed in mammalian and avian embryos before the system eventually becomes sim plified into a single aortic arch.

Thus, even though our physiology does not require such a structure, our embry onic condition reflects our evolutionary history. The third set of constraints is physical. According to the through the chorionic or allantoic membranes. The ruajor laws of fluid moveruent, the most effective transport of flu extraembryonic structures . The second constraint is evolutionary. The mammalian vessel gets smaller, resistance to flow increases as } . 4 there is no yolk inside (see Figure 12.10). Moreover, the slowly and has access to cell membranes. 50 here is a para embryonic blood vessels must be constructed to serve U,ese embryo extends blood vessels to the yolk sac even though blood leavi.ng the heart via the truncus arteriosus passe!:> through vessels that loop over the foregut to reach the dor ids is performed by large tubes. As the radius of a blood (Poiseuille's law). A blood vessel that is half as wide as another has a resistance to flow 16 times greater. However, cliffusion of nutrients can take place only when blood flows sal aorta. dox: the constraints of diffusion mandate that vessels be small, while the laws of hydraulics mandate that vessels be and enable the gills to oxygenate the blood through the culatory systems with a hiera rchy of vessel sizes (LaBar genate the blood, such a system makes little sense-but all (aorta and vena cava) flows over 100 times faster than it Six pairs of these aortic arches loop over the phar vnx (Figure 1 2.13). In primitive fish, these arches persist gills.

In adult birds and mammals, in which lungs oxy large. This paradox has been solved by the evolution of cir bera 1990). In dogs, for example, blood in the large vessels 458 CHAPTER 1 2 Day Ecaxtrteoerrtinedal Arch of R g t ceaternal Left internal carotid the a rta caro id a y caro d te), L e f t c o m mo n Ri g h t c a r o i d a r t e r y s u b c l a v i a n arte Y V Lsuebftclavian arches . a r t e r y L i g a m e n t Trartuenricous S e v e n t h a r t e r i o s u s Raoigrhat dorsal Laoefrtadorsal ianrtelr)s,egmental Aorta t (A) Day Aortic 29 (B) Internal 49 II III IVI (C) r i s / D y 56 i h \ ar t;J!i "gj. Aortic arches of the human embryo. (A) Original ly, the truncus arteriosus pumps blood into the aorta, which branches on either side of the foregut. The six aortic arches take blood from the truncus arteriosus and allow it to flow into the dor sal aorta. (8) As deve lopme n proceeds, arches begin to disinte grate or become modified (the dotted lines indicate degenerating structures). The first two pairs of arches completely disappear. The third arches become the internal carotid arteries.

The right fourth arch becomes the right subclavian artery, and the left fourth arch becomes the maj or arch of the aorta. The fifth arches disintegrate. The right sixth arch disappears, but the left sixth arch gives rise to the pulmonary arteries and the ductus arteriosus (which will start closing on the first breath). (C) Eventually, the remaining arches are modified and the adult arterial system is formed. However, numer ous variations of this scheme are found in the human population. FIGURE 12.13 does in the capillaries. With a system of large vessels spe cialized for transport and small vessels specialized for dif fusion (where the blood spends most of its time), nutrients and oxygen can reach the individual cells of the growing organism. This hierarchy is seen very early in development; it is already well established in the 3-ciay chick embryo. But this is not the entire story. If fluid under constant pressure moves directly from a large-diameter tube into a small-diameter tube (as in a hose nozzle), the fluid veloci ty increases. The evolutionary solution to. this prOblem was the emergence of many smaller vessels branching out from a larger one, making the collective cross-sectional area of all the smaller vessels greater than that of the larger ves sel. Circulatory systems show a relationship (known as Murray's law) in which the cube of the radius of the par ent vessel approximates the sum of the cubes of the radii of the smaller vessels. Computer models of blood vessel formation must take into account not only gene expression patterns but also the fluid dynamics of blood flow, if they are to show the branching and anastomosing of the arter ies and veins (Gbdde and Kurz 2001). The construction of any circulatory system negotiates among all of these phys ical, physiological, and evolutionary constraints. Vasculogenesis: The initial formation of blood vessels The development of blood vessels occurs by two tempo rally separate processes: vasculogenesis and angiogene sis (Figure 1 2 . 1 4) . During vasculogenesis, a network of blood vessels is created de novo from the lateral plate mesoderm. During angiogenesis, this primary network is remodeled and pruned into a distinct capillary bed, arter ies, and veins. In the first phase of vasculogenesis, cells leaving the primitive streak in the posterior of the embryo become hemangioblasts,* the precursors of both blood cells and blood vessels. Labeling zebrafish embryos with fluores- cent probes to make Single-cell fate maps confirms that hemangioblasts are the common progenitor for both the hematopoietic (blood) and endothelial (vascular) lineages in zebrafish. This population of bipotential progenitor cells is found only in the ventral mesoderm, the region that had been known to produce these two cell types. The pathway whereby ventral mesoderm cells differentiate into heman gioblasts appears to be induced by the Cdx4 gene, while the determination of whether the hemangioblast becomes a blood-cell precursor or a blood vessel precursor is regu lated by the Notch signaling pathway.

Notch signaling increases the conversion of hemangioblasts into blood cell precursors, while reduced amounts of Notch cause heman gioblasts to become endothelial (Vogeli et aJ. 2006; Hart et aJ. 2007; Lee et aJ. 2009). ""The prefixes hem- and heltJato- refer to blood (as in hemoglobin). Similarly, the prefix al1gio- refers to blood vessels. The suffix -blf/st denotes a rapidly dividing cell, usually a stem cell. The suffixes -poesis and -poietic refer to generation or formation (poeisis is also the root of the word poetnj). Thus, hematopOietic stem cells are those cells that generate the different types of blood cells. The s -genesis (as in angiogenesis) means the same as the Greek -poiesis. Latin uf ix LATERAL PLATE MESODERM A N D THE ENDODERM Vasculogenesis ------l. VEGF-R2 (FLk l) VEGF-Rl (FLt!) t Hemangioblasts (birth. migra tion, and proliferation) Tube for mation. endothelial cell-cell interactions 459 Mature vessels in a ---". Angiogenesis -------' primary capillary plexus VEGF M"u VEGFRI,2 'T Recruit and interact with pericyte support cells.

Maintain vessel integrity and quiescence FIGURE 1 2.14 Vascu l oge nesis and angiogenesis. Vasculogenesis involves the formation of blood islands and the construction of capi llary networks from them. Angiogenesis i nvolves the forma tion of new blood vessels by remode li ng and building on older ones. Angiogenesis finishes the c irculatory connections begu n by \ J sc u l ogenes i s .

The maj or paracrine factors involved in each step are shown at the top or the diagram, and their receptors (on the , esseL-forming cells) are shown beneath them, (After Honahan 1 997 a nd Risau 1 997,) \ " Pruning and remodeling produce a "juvenile" vascular system Maturation and remodeling produce a mature vascular system which form the Lining of the blood vessels. In the third phase, the endothelial cells form tubes and connect to form the primary capillary plexus, a network of capillaries. SITES OF VASCULOGENESLS In amniotes, formation of the primary vascular networks occurs in two distinct and inde pendent regions. First, extraembryonic vasculogenesis occurs in the blood islands of the yolk sac. These are the In amruotes, however, there is much more controversy blood islands formed by the hemangioblasts, and they give rise to the early vasculatwe needed to feed the embryo and about hemangioblasts, and there may be more than one also to a red blood cell population that functions in the pathway by which to generate blood vessels and blood cells early embryo (Figure (Veno and Weissman 2006; Weng et aL. 2007), Heman gioblasts in the spLanchnic mesoderm condense into aggre gations that are often called blood is l ands' (ShaLaby et ai, 1997; Huber et aJ. 2004). It is generally thought that the inner culogenesis forms the dorsal aorta, and vessels fron1 this 12.15A), Second, intraembryonic vas large vessel cormect with capillary networks that form from mesodermal cells within each organ , The aggregation of endothelial-forming cells in the yolk cells of these blood islands become blood progenitor cells, sac is a critical step in amniote development, for the blood while the outer cells become angioblasts, the progenitors of islands that line the yolk sac produce the veins that bring blood vessels. In the second phase of vasculogenesis, the nutrients to the embryo and transport gases to and from angioblasts multipLy and differentiate into endothelial cells, "Again, the endoderm pLays a major role in lateral plate mesoderm specification. Here, the visceral endoderm of the splanchnopleure interacts with the yolk sac mesoderm to induce the blood islands. The endoderm is probabl)' secreting Indian hedgehog, a paracr.i.ne factor that al..'t1vates BMP4 expression in Ule mesoderm. BMP4 expression feeds b the sites of respiratory exchange (Figure 12,15B), In birds, these vessels are called the vitelline veins; in mammals, they are often called the omphalomesenteric (umbilical) veins, In the chick, blood islands are first seen in the area opaca, when the primitive streak is at its fullest extent (Par danaud et aJ. 1987), They form cords of hemangioblasts, which soon become hollowed out and become the flat endothelial cells lirring the vessels (while the central cells give rise to blood cells

existence of such hemangioblasts in amniote ulated by Prox1 is embryos. VEGFR-3, which encodes the receptor for the paracrine factor VEGF-C As inlportant as VEGF-A is for blood vessel development, VEGF-C is equally neces The original sites of hematopoiesis are associated with the blood islands in the ventral mesoderm surrounding the sary for proper lymphatic development (Figu re 1 2.22; yolk sac that is, the splanchnopleure. Karkkainen et al. 2004; Alitalo et a1. 2005). VEGF-C pro the first blood cells aTe seen duced in the area of the jugular vein attracts Proxl-posi tive endo thelial ceUs out from the vein and then promotes their proliferation and development into the lymphatic sacs (see Adams and Alitalo 2007; Hosking and Makinen 2007). Each day we lose and replace about 10" red blood cells. As red blood cells are killed in the spleen, their replace ments come from populations of stem cells. As mentioned in the Part III introduction, a stem ceU is capable of exten- In chick embryos, those blood islands that form in the posterior marginal zone near the site of hypoblast initiation.

Although the hematopoietic stem cells of these blood islands seem capable of generating all blood cell (but not lymphocyte) lineages, they usuaUy produce red blood cells (Moore and Hematopoiesis: The Stem Cell Concept in Metcalf 1970; Rampon and Huber 2003).

In non-amniote vertebrates, this splanchno pleure is also the source of the hematopoietic stem cells, and BMPs are crucial in inducing the blood-fonning cells n i all vertebrates studied. [n Xenopus, the venITal mesoderm forms a large blood island that is the lirst site of hema topoiesis. Ectopic BMP2 and BMP4 can induce blood and LATERAL PLATE MESODERM A N D THE E N DODERM 467 in Xenopus, and interference with of the yolk sac may generate the definitive hematopoiet BMP signaling prevents blood formation (Maeno et al. ic stem cells of the bone marrow and liver. However, there blood vessel form ation 1994; Hemmati-Brivanlou and Thomsen zebra fish, the 1995). swirl muta tion, which prevents In the is still a great deal of controversy and discussion about BMP2 sig this. 2009, several laboratories proposed a new mechanism This new hypothesis was based on the discovery of a new cell type, the hemogenic endothelial cell. Recall that in our discussion of somites, naling, also abolishes ventral mesoderm and blood cell pro- duction (Mullins et al. 1996). ill As mentioned above, BMP4 is critical in the formation of the blood islands for blood cell production. in the mam malian extraembryonic mesoderm. that in ammotes, hematopoi we noted that the sclerotume produces angioblasts that etic stem cells originated in the yolk sac and then subse Early studies suggested migrate to the dorsal aorta and replace most of the primary quently colonized the bone marrow and fetal liver to gen dorsal aorta cells. These primary endothelial cells of the 1974; Azar and Eyal-Giladi 1979; Kubai and Auerbach 1983). These latter rise to blood-forming stem cells (Figure 12.23A). Th.is blood sites wuuld be the places where the definitive hemato vesseJ-derived hematopoietic stem cell may be an impor erate the adult HSC p opula tion (Wilt dorsal aorta, especially those in the ventral a.rea, then give poiesis would occur throughout the lifetime of the animal tant source of adult blood stem cells. By analyzing the (after the yolk sac disappeared). However, later transplan typ es of cells made by the blood vessel endothelium, tation studies seemed to show that there are two distinct researchers were able to isola te the h rnogeni e c endothelial populations of hematopoietic stem cells: a yolk sac cell5 and showed that they produce the hematopoietic stem derived stem cell that produces the blood for the embryos, cells that migrate to and a definitive hematopoietic stem cell, originating in the 2009; Lancrin et al. 2009). Furthermore, the transition from endothelial cell to hematopoietic stem cell was seen to be mediated by the activation of the Runxl transcrip al. lateral plate splanchnopleure near the aorta, and whose progeny serve the fetus and adult (Dieterlen-Lievre and Martin 1981; Medvinsky et al. tion factor.

In mice lacking this factor, the blood stem cells 1993). This mesenchymal region, area is often called the aorta-gonad-mesonephros or AGM. failed to form in the yolk sac, umbilical arteries, dorsal 2009). nus set of observations dovetails and interacts with a sec and set of studies (Adamo et al. 2009; North et al. 2009) ,how- Recent studies, using less invasive techruques aorta, and placental vessels (Chen et al. uf genetic marking, have returned scientific attention to the hypothesis that the original hematopoietic stem cells (A) the liver and bone marrow (Eilken et (B) AGM Bone marrow FIGURE 12.23 Sources of hlood cells to adult bone marrow. (Al Sec tion through the aorta of a 3-day chick embryo, showing the cells (arrows) thaI give rise to hematopoietic stem cells in the AGM (aorta- gonad-mesonephros region) of the chick. (8) In mammals, the yolk sac, the AGM, and the placenta each probably contribute stem cells to the fetal liver.

Stem cells (rom the fetal liver then populate the bone marrow as the hematopoietic niche in the bone marrow is constructed. ll1ere is controversy JS to whether the stem cells from the yolk sac populate the AGM or the l iver, or if a new set of stem cells is formed. (C) Schematic diagram of an interaction in the stem cell niche by which bone marrow (endosteal) oSleoblasts maintain HSCs by activating the Wnt, Notch, and receptor tyrosine kinase (RTK) pathways (red dashed arrows). (A from Dieterlen-Lievre and Martin 1981, photograph courtesy of F. Dicterlen Lievre; B aher Ottersbach and Dzierak 200,S.) 468 CHAPTER 1 2 ing that blood flow causes shear forces that activ ate the Runxl gene in the ventral endothelium of the dorsal aorta.' 1 2.23C). A second signal comes from angiop oietin 1 on the osteoblasts, which activates the receptor tyrosine kinase TIe2 The shear forces appear to elevate levels of nitrous oxide on the surface of the HSC (Arai et al. 2(04). A third signal is (NO) in the endothelium, and NO in turn activates RIInxI from the Wnt pathway, localizing p-catenln into the nucle and other genes known to be critical for blood cell formation. Chen and colleagues (2009) claim that the hematopoiet made by differentiated osteocytes as well. This Wnt path ie stem cells arise from hemogenic endothelial cells that way seems critical for the self-renewal of the HSC (Reya et originate separately in the blood islands, the dorsal aorta, al. 2(03). us. Wnt proteins are made by osteoblasts, and are prObably the placental arteries and veins, and the umbilical vessels; HSCs give rise to lineage-restricted stem cells that pro and it is possible that they each contribute hematopoietic duce blood cells and lymphocytes. While there are disputes stem cells to the liver and bone marrow (Figure 12.23B). about the exact lineage and time of commitment to certain Indeed, Samokhvalov and collea gues (2007) have shown cell fates (see Adolffson et al. 2005; Hock and Orkin 2005), that the same hematopoietic stem ceUs that had formed red Figure 1 2.24 is a Simplified depiction of one plausible blood cells in the mouse yolk sac later generated stem cells model. An HSC can give rise to the blood cell precursor tha t colonized the fetal umbilical cord and AGM. This (common myeloid precursor cell, or CMFj sometimes multi-origin model of hematopoietic stem cells is a testable called the CFU-S) or to the lymphocyte stem cell (ClP). hypothesis, and the extent to which these various hemo These cells may also be stem cells, although this is not cer genic endothelial populations colonize the adult stem cell tain. The CMFs produce the megakaryocyte/erythroid pre niches in the liver and bone marrow should soon be known. cursor cell (MEP), which can generate either the red blood cell (erythrocyte) lineage or the platelet lineage. The CMF Committed stem cells and their fates can also give rise to the granulocyte/monocyte precursor pro cell (GMP), which generates the basophils, eosinophils, The bone marrow HSC is a remarkable cell, in that it is the neutrophils, and monocytes. Eventually, these cells common p recursor of red blood cells (erythrocytes), white duce progenitor cells that can divide but produce only o/le blood cells (granulocytes, neutrophils, and platelets), and lymphocytes. When transplanted into inbred, irradiated mice type of cell in addition to renewing themselves. For instance, the erythroid progeni tor cell (BFU-E) is a com (who are genetically identical to the donor cells and whose mit ted stem cell that can form only red blood cells. Its own stem ceUs have been eliminated by radiation), HSCs can mone erythropoietin to produce the first reCOgnizable d if repopulate the mouse with all the blood and lymphoid cell types. It is estimated that only about 1 in every 10,000 blood cells is a plw'ipotential HSC (Berardi et al. 1995). The HSC (sometimes called the CFU-M,L) appears to be dependent on the transcription factor SCL. Mice lacking SCL die from the absence of all blood and lymphocyte lineages. immediate progeny is capable of responding to the hor ferentiated member of the erythrocyte lineage, the proery throblast, a red blood cell precursor. This cell begins to make globin (Krantz and Goldwasser 1965). As the proery throblast matures, it becomes an erythroblast, synthesiz 1996; Robb et al. 1996).

The pluripotent HSC is also depend ing enormous amounts of hemoglobin. The mammalian erythroblast eventually expels its nucleus and becomes a reticulocyte. Although reticulocytes, lacking a nucleus, can no longer synthesize globin mRNA, they can translate exist ent on osteoblasts in the bone marrow. Osteoblasts that have ing messages into g]obins. finished making bone can still have a function: endosteal osteoblasts that line the bone marrow are responsible for age is the erythrocyte, or mature red blood cell. In this ceU. SCL is thought to speciiy blood cell fate in mesoderm cells, and it continues to be expressed in the HSCs (porcher et al. The final stage of differentiation of the erythrOid line providing the niche that attracts HSCs; prevents apoptosis, no division, RNA synthesis, or protein synthesis takes and keeps the HSCs in a state of plasticity. These osteoblasts place. The DNA of the erythrocyte condenses and trans bind HSCs (p robably through N-cadherin) and provide sev eral other signals (Calvi et aI. 2003; j. Zhang et a1. 2003; Chan et al. 2009). One Signal is proVided by tlie jagged protein, lates no further messages. Amphibians, fish, and birds retain the functionless nucleus; mammals extrude it from the cell.' The erythrocyte then leaves the bone marrow and which activates Notch protein on the HSC surface (Figure -Shear stress from blood flow is a major player in development. Recall tha t it is also required for normal heart development (Mironov et al. 2005) and for the correct patterning of blood vessels (Lucitti et at. 2007; Yashiro et at. 2(07). it is also needed for the frag mentation of the platelet precursor ceU-the megakaryocyte into plate1ets. The megakaryocyte in the bone marrow inserts small processes into the blood vessels surrounding the stem cell niche, and the shear force there fragments these processes into platelets OW'll el al. 2(07).

'"In 1846, the young Joseph Leidy (then an assistant coroner, later the most famous American biologist of his day) was probably the first person to use a microscope to solve a murder mystery.

A man accused of killing a Philadelphia farmer had blood on his clothes and hatchet. The suspect claimed irmocence, saying that the blood was from chickens he had just slaughtered. Examining the blood cells under his microscope, Leidy found no nuclei in these erythro cytes. Moreover, he found that even if he let chick erythrocytes remain outside the body for hours, they did not lose their nuclei. Thus, he concluded that the blood stains could not have been chick en blood. The suspect subsequently confessed (Warren 1998). LAT E RAL PLATE MESODERM AND THE ENDODERM BFU-E 469 (lymphocyte;" t GMP CFU-E (leukocyte precursor) (eryth roid t precursor) progenito r) Pro-NK cells Pro-T cells Pro-8 cells Pro erythrobJasls + " + Erythroblasis t Promydocytes ReliculoCYles (nucleus expelled) t ' y J Red blood cells Erythrocytes @J @   @ :* @ Basophils Eosinophils Neutrophils \ Monocytes , White blood cells (leLLkocytes) DWOD FIGURE 12.24 A model (or the origin o(mammalian blood and lymphoid cells. Other models are consistent with the data; this one summari2es reatures from several models. PI uri potential hematopoietic stem cells (HSCs) can generate two lineage.restricl ed multi potent slem cells, the common myeloid precursor (CMP) of all blood cells, and the common lymphoid precursor (CLP) of the immune system. Whether or not these are actually stem cells is still uncertain.

The CMP can generate (he precursors of red blood cells and whi te blood cells. (Afler Kluger et 01. 2004.) NK cells Dendritic cells l'_____J I I J.

Te @ n T helper lymphocytes lym phocytes B cells - , , T H I cells Lymphocytes IMMUNE SYSTEM TH2 cells Plasma cell 470 CHAPTER 1 2 (C) (B) (A) Hecelms atopoietic S i n u s o i d m Sinusoid FIGURE 1 2.25 Development of hu m a n subendothelial cells i p l an te d into 4 weeks, small mononuclear cells (stained brown with antibodies to the subendothelial cell marker CD1 46) associate with vessels (arrow). (B) 7 weeks, the el ongated CD146 + cells are found over and around sinusoids in the bone marrow (arrows). (C) At 8 wee ks, processes from (D1 46-1' cells establish contacts with hematopoietic cells (as in the human bone marrow). The red arrows show hematopoietic cells between endothelial and (D146+ cells. (Aher Sacchetti et al. 2007, courtesy of Bianco.) mice.(Al At blood At P. enters the circulation, where it delivers oxygen to the body tissues. Hematopoietic inductive microenvironments Different paracrine factors are important in causing hematopoietic stem cells to differentiate along the partic ular pathways illustrated in Figure 12.24. The paracrine factors involved in blood cell and lymphocyte formation are caJJed cytokines.

Cytokines can be made by several cell types, but they are collected and concentrated by the extra reside in the s p leen formed colonies that were predomi nantly erythroid, whereas those that came to reside in the bone marrow formed colonies that were pred ominantly granulocytic.

In fact, colonies that straddled the borders of the two tissue types were predominantly erythroid in the spleen and granulocytic in the marrow. Such regions of determination are referred to as hematopoietic inductive microenvironments (HIMs). As expected, the HIMs induce different sets of transcription factors in these cells, and these transcription factors specify the fate of the partic ular cells (see Kluger et aJ. 2(04).

cellular matrix of the stromal (mesenchymal) cells at the 1987; Whitlock et al. 1987).

For instance, granulocyte-macrophage colony-stim sites of hematopuiesis (Hunt et al. ulating factor (GM-CSF) and the multilineage growth fac tor interleukin 3 (IL3) both bind to the heparan sulfate gly cosaminoglycan of the bone marrow stroma (Gordon et al. 1987; Roberts et al. 1988). The extracellular matrix is then able to present these factors to the stem cells in concentra tions high enough to bind to their receptors. At different stages of maturation, the stem cells becoe competent to respond to different factors. Early stem cells are able to respond to stem cell factor (SCF), whereas more mature Stem cell niche construction Ian Wilmut, who directed the cloning l program that pro that

"Although the story is complicated, it is bio ogy not physics: that is to say, it is not weird" (2001, p . 17). This is an important distinction to make when one is trying to understand the complexities of blood cell formation. Because here s another layer: the hematopoietic stem cell niche is possibly derived from its own stem cell. Sacchetti and colleagues (2007) have shown that the subendothelial cells of the human hematopoiet duced Dolly, famously remarked , ' stem cells luse the ability tu respund to SCF but acquire the ic microenvironment, a relatively minor cell population in ability to respond to the bone marrow and having no IL3. The developmental path taken by the descendant of a known function , are actu ally hematopoietic niche stem cells. When such cells are pluripotential HSC depends on which growth factors it implanted into the skin of imm unosupp ressed mice, these meets, and cells divide and differentiate into small hematopoietic is therefore determined by the stromal cells. Wolf and Trentin (1968) demonstrated that short-range microenvirorunents, complete with miniature bones and interactions between stromal cells and stem cells determine more subendothelial cells (Figure 1 2.25). Mouse hematopOi the developmental fates of the stern cells' progeny. These investigators placed plugs of bone marrow in a spleen and then injected stem cells into it. Thuse CMPs that eame tu etic stem cells migrate into these regions and start forming blood. Moreover, when those new subendothelial cells are transferred to yet other mice, they too develop a new LATERAL P LATE MESODERM A N D T H E ENDODERM 471 hematopoietic microenvironment. One of the proteins the view that the embryo constructed itself "from scratch" secreted by these cells is angiopoietin-l, a paracrine factor and that no small, profonned individual resided within the 1).

known to be involved in vascular remodeling and the for sperm or egg (see Chapter mation of the stem hematopoietic microenvironment (Suri this, modem work has shown that gut tube development 1996; Arai et aJ. 2004). Thus, this hematopoietic niche begins at two sites that migrate toward each other and fuse et aJ. In addition to confirming 1986, stem cell not only differentiates into the osteoblasts that in the center (Lawson et aJ. create the hematopoietic microenvironment; it also pro the foregut, cells from the lateral portions of the anterior Franklin et aJ. 2008).

In duces a new population of bone marrow stem cells that endodenn move ventrally to form the tube of the anterior Ciln produce the paracrine factors necessary for remodel intestinal portal (AlP); the caudal i ntestinal portal ing the vasculature to integrate the blood vessels with the forms from the posterior endoderm. The AlP and CIP microenvironment. (CIP) migrate toward each other and come together to form the We ask a lot of our circulatory system. We require a ntidgut (Figure 12.26) . Dawless flow of blood through the valves each second of There is an ectodermal entrance at either end of the gut our lives; we demand fine-tuned coordination between tube.

At first, the oral end is blocked by a region of ecto our brain, heart, bone marrow, and hormones such that derm called the oral plate, or stomodeum. Eventually (at the cardiac muscle contractions adapt to our physiologi about 22 days in human embryos), the stomodeum breaks, cal needs; and we demand that the production of our creating the oral opening of the digestive tube. The open blood cells--- ing itself is lined by ectodermal cells. This arrangement in our embryo---be so precise that we get neither cancer nor ane creates an interesting situation, because the oral plate ecto mia. Given aU this, it is not surprising that blood cell dif derm is in contact with the brain ectoderm, which has forentiation, heart development, and vessel formation are curved around toward the ventraJ portion of the embryo. now among the most important fields of study in medical These two ectodermal regions interact with each other, 17, controll ing blood with the roof of the oral region forming Rathke's pouch cell differentiation and stem cell proliferation is at the root and becoming the glandular portion of the pituitary gland.

of leukemia research, and regulating angiogenesis holds The neural tissue on the floor of the diencephalon gives promise for preventing tumor formation.

Congenital heart rise to the iniund ibulum, which becomes the neural por science. As we will see in Chapter defects are among the most prevalent types of birth tion of the pituitary. Thus, the pituitary gland has a dual defects, and cardiovascular disease is the most common origin, which is reflected cause of death in industrialized nations. The questions of similar meeting of endoderm and ectoderm at the anus; cardiogenesis, angiogenesis, and hematopoiesis that this is called the anorectal junction. in its adult functions. There is a engaged Aristotle and Harvey still excite major research programs today. The Pharynx The anterior endodermal portion of the digestive and res ENDODERM The first of the erubryonic endoderm's two major functions piratory tubes begins in the pharynx. Here, the mammalian embryo produces four pairs of pharyngeal pouches. Between these pouches are the four pharyngeal arches is to induce the formation of several mesodermal organs. (Figure 12.27). The first pair of pharyngeal pouches become As we have seen iIL this and earlier chapters, the endodenn eustachjan tubes. The second pair of pouches give rise to the heart, the blood vessels, and even the mesodermal the walls of the tonsils. The thymus is derived from the germ layer. The second function is to construct the linings third pair of pharyngeal pouches; it wiJ1 direct the differ of two tubes within the vertebrate body. The digestive tube entiation of T lymphocytes during later stages of develop extends the length of the body. Buds from the digestive ment. One pair of parathyroid glands is also derived from is critical for instructing the formation of the notochord, the auditory cavities of the middle ear and the associated tube form U,e liver, gallbladder, and pancreas. The respi the third pair of pharyngeal pouches, while the other pair ratory tube forms as an outgrowth of the digestive tube is derived from the fourth pharyngeal pair. and eventually bifurcates into the two lungs. The region these paired pouches, a small, central diverticulum is In addition to of the digestive tube anterior to the point where the respi formed between the second pha ryngeal pouches on the ratory tube branches off is the pharynx. Epithelial outpock floor of the pharynx. This pocket of endoderm and mes ets of the pharynx give rise to the tonsils, to the thyroid, enchyme will bud off from the pharynx and migrate down thymus, and parathyroid glands, and eventually to the res the neck to become the thyroid gland.

The respiratory tube pu atory tube itsel!. In 1769, Caspar Friedrich Wolff, an embryologist in St. Petersburg, demonstrated that the gut tube fomled by the curving of an initiaUy flat sheet.

His De Formatione Intesti norlln1 was the first microscopic evidence for epigenesis- sprouts from the pharyngeal floor (between the fourth pair of pharyngeal pouches) to form the lungs, as we will see below. The pharynx is where the endoderm meets the ecto derm, and the endoderm plays a critical role in determin- 472 CHAPTER 1 2 (B) Sagittal section (midline) (A) Cross section (k.idneyformjng level) - 2 WEEKS mesoderm mesoderm cavities in lateral plate aorta lntermediate Allantoic j Cardiac primordia Blood island Neural folds beginning Foregut Hindgut Midgut to fuse Body Neural plate stalk Heart intestinnl portal (CIP) Intrabody cavity - 3 WEEKS coelom cavity J";.;s. 100'1.. ""aU of yolk sac Dorsal Pancreas Visceral mesoderm layer Parietal - 4 WEEKS Allantois D rsal aorta Stomach Lung bud Stomodeum (mouth precursor) layer cavity af gut membrane (peritoneum) FIGURE 12.26 Endodermal fo lding d uri ng early human development. (A) Cross sections through the kidney forming region . (B) Sagittal sections through the embryo's midline. (After Sad l e r 2009.) Brain LATERAL PLATE MESODERM A N D T H E E N DODERM ing which pouches develop. Sonic hedgehog from the endoderm appears to act as a survival factof, preventing apoptosis of the neural crest cells (Moore-Scott and Man ley 2005). In zebra fish, genetic analysis combined with transplantation studies has shown that FCFs (mainly Fgf3 and FgfS) from the ectoderm and mesoderm also are important not only for the migration and survival of neu ral crest cells, but also for the formation of the pouches themselves. Mice deficient in both FgfB and Fgf3 genes lack aU the pharyngeal pouches, even when endoderm is pres ent. Instead of migrating laterally and ventrally to form pouches, the endoderm remains in the anterior pharynx and does not spread out (Crump et al. 2004). The Digestive Tube and Its Derivatives Posterior to the pharynx, the digestive tube constricts to form the esophagus, whidl is followed in sequence by the stomach, small intestine, and large intestine. The endoder mal ceUs generate only the lining of the digestive tube and its glands; mesenchyme ceUs from the splanchnic portion of the lateral plate mesoderm will surround the tube to provide the muscles for peristalsis. As Figure 12.28A shows, the stomach develops as a dilated region of the gut close to the pharynx. The intes tines develop more caudally, and the connection between the intestine and yolk sac is eventually severed. The intes tine originally ends in the endodermal cloaca, but after the cloaca separates into the bladder and rectal regions (see Chapter 11), the intestine joins with the rectum. At the cau dal end of the recrum, a depression forms where the endo derm meets the overiymg ectoderm, and a thin cloacal membrane separates the two tissues. When the cloacal membrane eventually ruptures, the resultant opening becomes the anus. Specification of the gut tissue The digestive tube proceeds from the pharynx to the anus, differentiating along the way into the esophagus, stomach, duodenum, and intestines, and putting out branches that become (among other things) the thyroid, thymus, pan creas, and liver.

What tells the endodermal tube to become these tissues at particular places? Why do we never see a mouth opening directly into a stomach? There appear to be two major ways to specify the gut tube. First, there is a glob al specification by a retinoic acid gradient that leads to expression of regionally specific transcription factors in the endoderm. Second, signals from the lateral plate-derived mesenchymal cells help specify the endodermal regions. The gut appears to be regionally specified at a very early stage. Indeed, in the chick, the endoderm appears to be regionally specified even before it forms a tube. The gra dients of retinoic acid and FCF signals mentioned earlier in this chapter pattern the endoderm.

Studies on chick embryos using bea ds containing either retinok acid or inhibitors of its synthesis (Bayha e t al.

2009) show that the pharynx can develop only in areas containing little or no yngeal arches Thiyroid iu Pharyhnge al Phar Towanl soilfinpouch 2](ry '' 2 (jl aExteruhaditoneal.. : Etuaubsdetatchian Tympanic pTharyamtohuydsroid3 Autubdeitory 1 7 aa o Pbostbranchial body h h h h pouc I m pn r Um pr \Sl parathyrOid primordium \" r m e \ Postbranchial l m 4 n n l , (• P r thyr id III -'r\K y FIGURE 12.27 Formation of glandular primordia from the p a ryngeal pouches. The end of eac of the first p aryngeal pouches becomes the tympanic cavity of the middle ear and the eustach ian lube. The second pouc es receive aggregates of lymphoid tissue and become the tonsils. Tlle dorsal portion o(the third pharyngeal pouches forms part o(the parathyroid gland, while the ventral portion (orms the thymus. Both migrate cau da lly and meet with the (issue from the fourth pharyngeal pouches to form the rest of the parathyroid and the postbranchial body.

n,e thyroid, which had originated in the midline of the pharynx, also migrates caudally into the neck region. (After Carlson 1 981 .) 473 od io) ry 474 CHAPTER 1 2 E n d o d e r m (LfoacSatolirnzsecdinhterangdseochdroeipgrmtio)n i endod rm sPpyhloinricter Rsiegeneapltiobnyoenfdpoadraecrmine Spdoeifffeifirneedanotidoaentrimoand Paracrine factors (AJ MeReceptisoondeorfmSonic heidngmeeh sgodper mte Hoxingemeneseoxdperems ion oSfpmeecifsiocdateiromn memasode brym Difmeeresnotdiactrimon (B) ----- + - s t t - - + -- + i FIGURE 12.28 Regional spec f- cation of the gut endoderm and splanchnic mesoderm through reciprocal interactions. (A) Region al transcription facto rs of the (mature) chick gut endoderm.

Th ese factors are seen prior to interactions with the mesoderm, but they are not stabilized. (B) Pos sible course of interaCtions between the endoderm and the spl anchnic mesoderm. (C) Suggest ed mechanisms by which mes enchymal cells may i nduce gut endoderm to become either i ntes tine or stomach. (A a fter G rapi n Botton et al. t BMPs, FGFs 2001.) - .

of gene that is essential for the d uo den um, pancreas, and liver, as well as the cSox2 gene that is expressed in the pre and esophagus (Matsushita et al. 2002, 2008).

Still higher concentrations of RA d irec tl y induce the posterior gene CdxA in the intestinal e ndodenn .

This patterning of the axis by retinoic acid may represent cursors of the stomach D eSm Hox one of the oldest developmental patterning mechanisms edxC (see Figure 12.28A; SimOes-Costa et al. 2008). III edxA epInitetstlinem epStiohtmelaiuchm NoWnt ;l .I .I .I, .GI"' mhRlbltsors nm   Rarxl l Si ox9t. Nkx Sp inctermuscle r In addition to this direct mechanism of cell spec ifica tion, the endodermal epithelium is able to respond differ (C) th ;;; ""m e WI hy e e ently to signals from different regionally specific mes enchymes that are derived from the splanclmic mesoderm. i These responses enable the digestive tube and respiratory Wor -!>..... h EMP, i   : Wm (sF P) -2 h RA, while the RA gradient p at terns the pharyngeal arch a graded manner. This is probably accom plished by activating and repressing particular sets of tran scri p tion factor genes. Highe levels of RA specify the Pdxl endoderm in tube to develop their very different structmes. As the diges tive tube meets different mesenchymes, the mesenchyme cells instruct the endoderm to differentiate into esophagus, stomach, small intestine, and colon (Okada 1960; Gumpel Pinot et al. 1978; Fukumachi and Takayama 1980; Kedinger et a1. 1990). The endodenn and the splanchnic lateral plate mesoderm Wldergo a complicated set of interactions, and the signals for generating the different gut tissues appear to b e conserved throughout the vertebrate classes (see Wa l ace and Pack 2(03). The stabilization of the gut boundaries results from interaction with the mesoderm (Figure 12.28B) . As the gut tube begins to form at the anterior and p oste ior ends, it induces the splanchnic mesoderm to become regionally specific. Roberts and colleagues (1995, 1998) have impli cated Sonic hed gehog (Shh) in this specification. Shh is secreted in different concentrations at different sites, and its target appears to be the mesodenn surrounding the gut tube. The secretion of Shh by the hindgut endoderm induces a nested pattern of "posterior" Hox gene expres- r LATERAL PLATE MESODERM AND THE ENDODERM Da y er bu Stomach a (A) i Lv Day 30-35 (B) Bile duct ep d H (C) """G r\ Gal blad er Gal blad er e tr pbaundetrae tic pDobaundrcsraelatic Vpancreas rl n al in the mesoderm. As in the vertebrae (see Chapter 8), the anterior borders of Hox gene expression delineate the sian morphological boundaries of the regions tl,at will form the cloaca, large intestine, cecum, midcecum (at the midgut hindgut border), and posterior portion of the midgut (Roberts et at. 1995; (AcpDan)ceDasreoayrtyi4c2duct 40 tic • Ven Day Yokouchi et at. 1995). When Hox (B)yBy 475 duct Ventral pancreatic d t u c r c an i Ventra[ductanc e p a ti c reat c d uct p FIGURE 12.29 Pa n creat i c development in humans. (A) At 30 da s, the ventral pancreati c bud is close to the liver primordium. 35 days, it begi ns migrati ng posteriorly, and (C) comes i nto contact with the dorsal pa ncreati c bud during the sixth week of development. (D) In most individuals, the dorsal pancreatic bud loses its duct into the duodenum; however, in about 10% of the pop u lati on , the dual duct system p""ists.

expressing viruses cause the misexpression of these Hox genes in the mesodenn, the mesodermal celis alter the dif ferentiation of the adjacent endoderm (Rober ts et al. 1998). The Hox genes are thought to specify the mesoderm so that muscle that normally lines the stomach and intes tine it can interact with the endodermal tube and specify its (TIeodosiou and rabin 2005). Thus, a signal from the small are established, d ifferentiation can begin. The regional dif intestine instructs the mesoderm of the adjacent stomach ferentiation of the mesoderm (into smooth muscle types) Such communication allows the endoderm to form a and the regional differentiation of the endoderm (into dif with very exact regional compartments. regions. Once the botmdaries of the transcription factors tube to tell its mesoderm and endoderm how to differentiate. ferent functional units such as the stomach, duodenum, small intestine, etc.) are synchronized. The molecular signals by which the mesenchyme influ ences the gut tube are just beeoming known (Figure 12.28C). For instance, the mesenchyme lining the posterior (intes prote ins. 111ese Wnt proteins suppress genes such as Hhex tine-forming) region of tLte endodermal tube secretes Wnt Liver, pancreas, and gallbladder organs that develop immediately caudal to the The endoderm also forms the lining of three accessory stomach: divertic the liver, pancreas, and gall bladder. The hepa tic ulum is a bud of endoderm that extends out from the and prevent the stomach, liver, and pancreas from forming foregut into the surrounding mesenchyme. The endoderm (Bossard of this bud comes from two populations of ceUs-a later and Zaret 2000; Mclin et a1. 2007). [n the anterior regions of the gut tube (which form the thymus, pancreas, al group that exclusively forms liver cells, and ventml stom ach, and liver), Wnt Signaling must be blocked.

In the medial endoderm cells that form several midgut regions, stomach -forming regio, the mesen chy m e lining the gut including the liver (Tremblay and Zaret tube expresses the transcription factor Barxl, which act enchyme induces this endoderm to proliferate, branch, and vates production of Frzb-like Wnt-blocking proteins (sFRPI and sFRP2) . These Wnt antagonists block Wnt signaling in the vicinity of the stomach but not around the intestine.

Kim et aJ. 2005.) 2005). rhe mes form the glandular epithelium of the nver. A portion of the hepatic diverticulum (the region closest to the digesti ve branch from this duct produces the gallbladder (Fig 12.29). tube) continues to function as the drainage duct of the liver, (Indeed, BanI-deficient mice do not develop stomachs and and a express intestinal markers in that tissue; ure In addition to FGF and Wnt signaling, BMPs also act in TI,e pancreas develops from the fusion of distinct dorsal regional specification. The intestinal mesenchyme secretes and ventral diverticula. As they grow, they come closer BMP4, which instructs the mesoderm anterior to it to express the Sox9 and Nkx2-5 transcription factors (see Fig lire 12.28C). These factors tell the mesoderm to become the muscles of the pyloric sphincter rather than the smooth together and eventually fuse.

In humans, only the ventral to carry digestive enzymes into the intestine. In other species (such as the dog), both dorsal and ventral duct survives ducts empty int'O the intestine. 476 CHAPTER 1 2 SIDEL I G H TS SPECULATIONS Specification of Liver and Pancreas T here ;s an i ntimate relationship between the splanchnic lateral plate mesoderm and the foregut endoderm. Just as the foregut endo derm is critical in specifying the car diogenic mesoderm, the blood vessel endothelial cells induce the endoder mal tube to produce the liver primordi um and the pancreatiC rudiments.

liver formation The expression of liver-specific genes (such as the genes for a-fetoprotei n and albumin) can occur in any region of the gut tube that is exposed to car diogenic mesoderm.

However, this induction can occur only if the noto chord is removed.

If the notochord is placed by the portion of the endoderm normally i nduced by the cardiogenic mesoderm to become liver, the endo derm will not form liver (hepatic) tis sue. Therefore, the developi ng heart appears to induce the liver to form, while the presence of the notochord i n hi bits liver formation (Figure 12.30). This induction is probably due to FGFs secreted by the developing heart cells (Le Douarin 1 975; Gualdi et at. 1 996; Jung et a l . 1 999). However, Matsumoto and col leagues (200 1) found that the heart is not the only mesodermal derivative needed to form the l iver. Blood vessel endothel ial cells are also critical. If endothel ial cells are not present in the area around the hepatic region of the gut tube, the liver buds fail to form. This induction occurs even before the endothel ial cells have formed tubes, so it does not have anything to do with getting nutrients or oxygen into the region. Thus, the heart endothelial cells have a developmental function in addition to their circulatory roles: they induce the formation of the l iver bud by secreting FGFs. But in order to respond to the FGF signal, the endoderm has to become competent. This competence is given to the foregut endoderm by the fork head transcription factors. Forkhead Figure 12.30 Pos itive and negative signal i ng in the formation of the hepatic (liver) 26-Day embryo endoderm. The ectoderm and the notochord block the abil ity of the endoderm to express liver-specific genes. The cardiogenic meso- derm, probably through Fgf1 or Fgf2, pro motes liver-specific gene transcription by bl ocki ng the inhibitory fadors induced by the surrounding tissue. (After Gualdi et a l. 1 996.) 5 02 ": .r.Ew ICE!2B =:;;; Dorsal endode rm hepatogenesis blocked : ____ Hep'"i? region of ____ gut endoderm expressing a-fetal protein and albumin mesoderm transcription factors Foxa1 and Foxa2 are required to open the chromatin surrounding the liver-specific genes. These proteins d isplace nucleosomes from the regulatory regions surround ing these genes and are required before the FGF signal is given (Lee et a l . 2005). Mouse embryos lacking Foxa 1 and Foxa2 expression in their endoderm fai l to produce a liver bud or to express liver-specific enzymes. Once the Signal is given, other forkhead transcription factors, such as H N F4a, become critical. HNF4a is essential for the morphological and . biochemical differentiation of the hepatic bud i nto liver tissue (Parviz et al. 2003).

When conditional (floxed) mutants of HNF4o. were made such that this factor was absent only in the developing l i ver, neither the tissue arch itecture, cell u lar structu re, or Jiverwspecifi c enzymes formed i n the liver bud cells. Meanwhile, Odom and colleagues (2004) found that fork head transcription factors were also critical for the differentiation of the endocrine islands of the pancreas. H N F4a bound to the regulatory regions of almost half the actively transcribed pancreas-specific genes i n these

expression in the mesoderm surrounding the gut. The regional- 483 ized mesoderm then instructs the endodermal tube to become the different organs of the digestive tract. 17. The endoderm helps specify the splanchnic meso derm; the splanchnic mesoderm, especially the heart and the blood vessels, hel s specify the endoderm. p 18. The pancreas forms in a region of endoderm that lacks sOltic hedgehog expression. The Pdxl and PtEla transcription factors are expressed in this region. 19.
The endocrine and exocrine cells of the pancreas have a common origin. The Ngn3 transcription fac tor probably decides endocrine fate. outpocketing of the d igestive tube. The regional specificity of the mesenchyme it meets determines whether the tube 20. The respiratory tube is derived as an remains straight (as in the trachea) or branches (as in the bronchi and alveoli). 21. The chorion and amnion are made by the somato pleure. In birds and reptiles, the chorion abuts the shell and allows for gas exchange. The amnion in birds, reptiles, and mammals bathes the embryo in amnionic fluid. 22. The yolk sac and allantois are derived from the splanclmopleure. The yolk sac (in birds and reptiles) allows yolk nutrients to pass into the blood. The allantois collects nitrogenous wastes.
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j. Anderson. 1998. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. Cell 93: 741-753. 484 CHAPTER 1 2 Go Online WEBSITE 12.1 Coelom formation. Coelom formation is readily visualized by animations. The animation presented here shows the expansion of the mesoderm during chick devel opment. Vade Mecum Early heart development. The vertebrate heart begins to fwlction early in its development. You can see this in movies of the living chick embryo at early stages when the heart is little more than a looped tube. WEBSITE 12.2 Induction of the lung. The induction of the lung involves interplay between FGFs and Shh. How- ever, it appears to be different from the induction of either the pancreas or the liver. Development of the Tetrapod Li m b LIMB. Firs t, consider its polarity. It has fingers Or toes at one end, a humerus or femur at the other. You won't find anyone with fingers in the middle of their arm. Consider also the differences between your hands and your feet. The differences are subtle but obvious. If your fingers were replaced by toes, you would know it. But also consider how similar the bones of your feet are to the bones of your hand; it's easy to see that they share a common pattern. The bones of any tetrapod limb, be it arm or leg, wing or flipper, consist of a proximal stylopo d (humerus/femur) adjacent to the body wall; a zeugopod (radius-ulna/ tibia-fibula) in the middle region; and a distal aulopod (carpa ls- fin gers/tarsals-toes) (Figure 13.1). Last, consider the growth of your limbs.
Both hands are remarkably similar in si zei so are both your feet. So after about 20 years of growth, each of your feet turns out, independently, to be the same length. These commonplace phenomena present fascin a ting questions to the devel opmental biologist. How can growth be so precisely regulated? How is it that we have fouf linlbs and not six or eight? How is it that the fingers form at one end of the linlb and nowhere else' How is it that the little finger develops at one edge of the linlb and the thumb at the other?
How does the forelimb grow dif ferently than the hindlimb? All of these questions are really about pattern formation. Pattern formation is the set of processes by which embryome cells form ordered spatial arrange ments of differentiated tissues. Pa ttern formation is one of the most dramatic properties of a developing organism, and one that has provoked awe in scien tists and laypeople alike. It is one thing to differentiate the chondroblasts and osteoblasts that synthesize the cartilage and bone matrices, respectively, it is another thing to produce those cells-in a temporal-spatial orientation that gen erates a functional bone. It is still another thing to make that b one a hu merus and not a pelvis or a femur. The tissues of the finger bone, cartilage blood ves sels, nerves, dennis, and epidermis-arc the same in the toe and thigh; it is their arrangement that differs. The ability of limb cells to sense their relative positions and to differentiate with regard to those positions has been-and still is-the subject of intense study, ex pe rinlenta tion, and debate. The vertebrate limb is an extremely complex organ with an asymmetrical arrangement of parts. Tlle positional information needed to construct a limb has to function in a three-dimensional* coordinate system: CON S I DER YOUR - .. "Actually, it is a four-dimensional system, in which time is the fourth axis. Developmental biologists get used to seeing nature in four dimensions.
What can be more curious than tha t the hand of a man, form.ed for grasp ing, that of a mole for digging, the leg of a horse, the paddle of the porpoise, and the wing of the bat should all be constructed on the same pattern and should include similar bones, and in t.he same relative positions? CHARLES DARWTN (1859) DEBRA NTEHOFF (2005) If only you had realized how easy it would have been to create a limb when you were an inch-long embryo all you would have needed were the right words. Just in case you ever manage to turn back time, those right words are fibroblast growth faclOrs.' 486 CHAPTER 1 3 experimentally remove or lTansplant parts of the develop ing limb, or create limb-specific mutants, without interfer ing with the vital processes of the organism. Such experi ments have shown that the basic "morphogenetic rules" for forming a limb appear to be the same in all tetrapods. Grafted pieces of r p tile or mammalian limb buds can direct the formation of chick limbs, and regions of frog limb buds can direct the patterning of salamander limbs (Fal lon and Crosby 1977; Sessions et a1. 1989; see Hinchliffe 1991). Moreover, as will be detailed in Chapter 15, regener ating salamander limbs appear to follow the same rules as developing limbs (Mun oka and Bryant 1982). But what are these morphogenetic rules? e e FIGURE 13.1 Skeletal pattern of t e chick , the di t are u be e 2, 3, h wing. According to n on gi s n m r d and 4, The ca rti lage condensations forming the digits appear similar to those forming d igits 2, 3, and 4 of mice and humans; however, new evidence (discussed later in the chapter) suggests that the correct designa tion may be 1 , 2, and 3 . conve ti • The first dimension is the proximal-distal axis ("close-far"; shoulder finger or hip toe). The bones of the limb formed by endochondral ossifi a tion.
They are initially cartilaginous, but eventually most of the cartilage is replaced by bone. Somehow the limb cells develop dif ferently at early stages of development (when they make the stylopod) than at later stages (when they make the are c • The second autopod). dimension is the mlterior-posterior axis (thumb-pinkie). Our little fingers or toes mark the poste rior side, while our thumbs or big toes are at the a n teri or end. In humans, it is obvious that each hand develops as a mirror image of the other. One can im a gine other arrangements to exist-such as the thumb developing on the left side of both hands-but these patterns do not occur. Formation of the Limb Bud Specification of the limb fields Limbs do not form just anywhere along the body axis. Rather, there are discrete positions where limbs are gener ated. The mesodermal ce lls that give rise to a vertebrate limb can be identified by (1) removing certain groups of cells and observing that a limb does not d evelop in their absence (Detwiler 1918; Harrison 1918), (2) transplanting groups of cells to a new location and observing that they form a limb in this new place (Hertwig 1925), and (3) m rk ing groups of cells with dyes or radioactive precursors and observing that their descendants partake in limb develop ment (Rosenquist 1971). Figure 13.2 shows the prospective forelimb areas in the tailbud stage of salamander and the 10-somite stage of m use. The fate-mapping studies on salamanders, pio neered by Ross Granville Harrison's laboratory (see Har rison 1918, 1969), showed tha t the center of this of normally gives rise to the limb itself. Adjacent to it are the a a o a disc cells • Finally, limbs have a dorsal-ventral ' axis: our palms Somites Pronephric (ventral) are readily distinguishable fro ru ou knuckles (dorsal). During the past decade, proteins have been identified that play critical roles in the formation of each of the three limb axes. The proximal-distal axis (shoulder-finge r; hip toe) appears to be regulated by proteins of the fibroblast growth factor (FGF) fa mily. The a nte rior-pos t ri or axis regulated by Sonic lledgehog. The dorsal-ventral (knuckle-palm) is regulated, at least in part, by Wnt7a. The interactions of these proteins mutu ally support one another and determine the differentiation of cell types. The fundamental problem of morphogenesis-how spe structures arise in particular pla es-is exemplified in limb development. Since the limbs, unlike the heart or brain, are not essential for embryonic or feta l liie, one c n e (thumb-pinkie) seems to be axis cific e a flank tissue FIGURE 13.2 Prospective forelimb fields of the salamander and mouse. The forelimb field of the salamander Ambystoma macula tum. The central area contains cells destined to form the limb per se (the free limb). The celts surrounding the free limb give rise to the peribrachial flank tissue and the shoulder girdle. The ring of cells outside these regions usual ly is nol included in the limb, but can form a limb if the more central tissues are extirpated. (After Stocum and Fallon '1982.) DEVELOPMENT O F T H E TETRAPOD LIMB (A) Epaxial 487 (S) myotom e UU"�4f'� Spi al cn'-o ----, n dermatome \.,. Sclerotome myotome bud ,_Limb muscle ___ Notochord Pron "F'hrcm/ prec r ors us bud End der Limb skeletal o m precursors Lateral plate m soder e m FIGURE 13.3 Emergence of the limb bud.
IA) Proliferation of mesenchymal cells (arrows) from the somatic region of the lateral plate mesoderm causes the limb bud in the amphibian embryo to bulge outward. These cells generate the skeletal elements of the limb. Contributions of myoblasts from the lateral myotome pro vide the limb's musculature. (8) Entry of myoblasts (purple) into of myf5 mRNA found in developing muscle cells. If you can cross your eyes (or Iry to (B co rt J. cells that will form the peribrachial flank tissue and the arise, their position is constant with respect to the level of shoulder girdle. However, if all these cells are extirpated from the embryo, a limb will still form, albeit somewhat Hox gene expression along the ante ri or p osterior axis (see Chapter 8). For instance, in fish (in which the pectoral and l pelvic fins correspond to the anterior and posterior limbs, a ter, from an additional ring of ceUs that surrounds this a limb). If this the limb bud. This computer stereogram was created from sections an in situ hybridization to the fOCUSing "past" the page, looking through il your toes), the three-dimensionality of the stereogram will become apparent. u esy of Streicher and G. Muller.) - area (and which would not normally form respectively), amphibians, birds, and mammals, the fore last ring of cells is included in the extirpated tissue, no limb limb buds are found at the most anterior ex pression region will develop. This larger region, representing all the cells in the area capable of forming a limb, is called the limb field. of Hoxc6, the position of the first thoracic vertebra* (Oliv er et al. 1988; Molven et al. 1990; l3urke et a1. 1995). The lat eral plate mesoderm in the four limb fields is also special in Limb development begins when mesenchyme cells pro liferate from the somatic layer of the limb field lateral plate mesoderm (the li mb skeletal somites (the limb precursor cells) and from the muscle precursor cells) at the same level. These mesenchymal cells accumulate under the ectoder mal tissue to create a circular b ulge called a limb bud (Fig ure 13.3A). that it induces rnyoblasts to migra te out from the somites and enter the limb bud to become the limb mLlsculature (Figure 13.36). other regions of the lateral plate meso derm can do that (Hayashi and Ozawa 1995). The regulative ability of the limb bud has recently been highlighted by a rema rkable experiment of nature. In No numerous ponds in the United States, multilegged frogs When it first forms, the limb field has the ability to reg and salamanders have been found (F igure 13.4). The pres ulate for lost or added parts. In the tailbud stage of ence of these extra appendages has been linked to the infes new site (Harrison 1918). This The eggs of these worms apparently split the developing Ambystoma, any half of the limb disc is able to generate an entire limb when grafted to a tation of the larval abdomen by parasitic trematode worms.
potential can also be shown by splitting the limb disc ver tadpole limb buds in several places, and the ti a lly into ments develop as mLltiple limbs (Sessions and Ruth 1990; c two or more segments and placing thin barri ers between the segments to prevent their reunion. When Sessions et a l. limb bud frag 1999). this is done, each segment develops into a full limb. Thus, like an early sea urchin embryo, the limb field represents a "harmonious equipotential system" wherein a cell can be instructed to form any part of the limb. In land vertebrates, there are only four limb buds per embryo, and they are always opposite each other with respect to the midline. Although the limbs of different ver tebrates differ with respect to the somite level at which they Python) cre 488 CHAPTER 1 3 FIGURE 13.4 Multilimbed Pacific tree frog (Hy!a regilla), the result of infestation of the tadpole-stage developing limb buds by trematode cysts.
The pa rasi tic cysts apparent ly split the developing limb buds in several places result ing in exira limbs. In this adult frog's skeleton, the cartilage is stained bluei the bones are stained red. (Courtesy of S. Sessions.) \ \//-I I , a!. by the somites (Zhao et 2009). In this FCF-free zone, FgflO is produced throughout the lateral plate mesoderm. Immediately prior to limb formation, however, it becomes restricted to those regions of the lateral plate mesoderm where the limbs will form. This restriction in placement appears to result from the actions of Wnt proteins-Wnt2b in the chick fore limb region and Wnt8c in the chick hindlimb region-which stabilize FgflO expression at these sites (Figure 13.6; Ohuchi et a!. 1997; Kawakami et a!. 2001).
Induction of the early limb bud: Wnt proteins and fibroblast growth factors Molecular studies on the earliest stages of limb formation have shown that the signal for limb bud formation comes from the lateral plate mesoderm cells that will become the prospective skeleton. These cells secrete the paracrine lactor FgflO, which is capable of initiating the limb-form ing interactions between ectoderm and mesoclenn. If beads contairting FgflO are placed ectopically beneath the flank ectoderm, extra limbs emerge (Figure 1 3.5; Ohuchi et al. 1997; Sekine et al. 1999). After FgfB signaling has helped establish the anterior posterior axis, it is eliminated in the areas capable of form ing limbs. TIlis elimination is due to retinoic acid secreted limb FIGURE 13.5 n *1bx sta ds for T-box. The T (Brachyury) gene and its relatives have a sequence that encodes this specific DNA-binding domain. Humans heterozygous for the TBX5 gene have Holt-Oram syn drome, characterized by abnormalities of the heart and upper limbs (Basson et al. 1996; Li et al. 1996). (B) (A) FgflO expression and action in the developing chick limb. (A) FgflO becomes ex pressed in the lateral plate mesoderm in precisely those posi tions (arrows) where limbs normally form. (8) When transgenic cells that secrete Fgfl0 are placed in the flanks of a chick embryo, the FgflO can cause the formation of an ectopic limb (arrow). (From Ohuchi et al. 1 997, courtesy of S. N oj i.) Specification of forelimb or hindlimb The mechanisms by which instructions from the Hox code enable the production of FgflO is being investigated. Inter estingly, the question of how Hox gene expression causes FgflO expression may be intimately linked to the question of how the forelimbs and hindlimbs are distinguished.
One clue came from specific gene expression patterns. The gene encoding the Tbx5 transcription factor in mice is transcribed in the anterior lateral plate mesoderm and in the forelimbs, - DEVELOPMENT OF T H E TETRAPOD LIMB are expressed in Ule posterior lateral plate mesoderm 489 while the genes encoding the transcription factors Tbx4 and forelimb buds, the type of limb produced was determined Pitxl by the Tbx protein expressed. Limb buds induced by plac 1996; Gibson-Brown 1996; Takeuchi et al. 1999). Could these three transcrip and in the hindlimbs' (Chapman et a!. ing FGF beads close to the hindlimb (opposite somite 25) et al. expressed Tbx4 and became hindlimbs. Buds induced close 17) expressed Tbx5 and tion factors be causally involved in directing forelimb ver to the forelimb (opposite somite sus hindlimb specificity, and could they help bridge the gap developed as forelimbs (wings). Limb buds induced in the t between Hox and FgflO expression? In 1998 and Tbx5 in the anterior Tbx4 in the posterior portion of the center of the flank tissue expressed 1999, several laboratories (Logan et al. 1998; Ohuchi et al. 1998; Rodriguez-Esteban e 01. 1999; Takeuchi et aJ. 1999, among others) provided gain-of function evi dence that Tbx4 and TbxS help specify hindlimbs and fore limbs, respectively. First. if FGF-secreting beads were used embryo was made to express Tbx4 throughout the flank to induce an ectopic limb between the chick hindlimb and tissue (by infecting the tissue with a vuus that expressed portion of the limb and limb. These limbs developed as chimeric structures, with the anterior resembling a forelimb and the posterior resem bling a hindlimb (Figure 1 3.7). Moreover, when a chick FIGURE le lar model for initiation een 48 and rs gestation. (A) Fgfl expression is first ug u l t plate meso derm. It Wn 8c to the area where hindlimbs form.
(B) Fgf10 syn he- seen 13.6 Mo c u of the limb bud in the chick betw 54 hou of 0 th ro ho t the a era l becomes stabilized by tt i ti i t t i n i in 10 ' 15 Wnrt2b 1 5 Wn t .2b. . . (-ca-tenin) . . . . . R ::.
-ca-tenin) . . . . . R ::. FgfS . . .. F.. ·gtfJO 20 FgflO 20 .. .. I Smeomsoitdcerm meIntesormderdmiate FgflO Wn·ca te3nain) .FDFggfBflO (- Fgt10 ': FgflO Lmeatseordacl nplnate Secutrofdacerm I r' I si s stab i l i zed by the actions ofWntlb to the region where the forelimbs will form. (C) FgflO from these two regions of lateral plate mesoderm induces FgfS i n the ap cal ectoder mal ridge (AER). This nduc on is accom plished through a pathway involving Wn 3 a in the responding ectoderm. Fgf8 secreted from the AER nduces the continued mesodermal expression of Fgfl o. The roles of FgfB the in ermedi a e mesoderm in nd u c i g or maintaining Wnt expression are uncerta n . (After Kawakami et al. 2001 .) 48 h LJ ' . . . . \ WntBe · . . . · . . . · . . . .
. . .
- .
. . • '" AER ..
. Wnt3a . . . . . / \ I r , • (stabilized) ER IZl Wnt2b/8c .Wnt3a 490 CHAPTER 1 3 (early day Stage 14/15 3) (A) Normal ;;.:::... 8 0 0 0 0 0 (ll) g • llQJ 0 0 0 FGF • induced)FboUrdelimb)Hibl ndlimb) $UF G F ; 0 0 0 8 ..
W • o FIGURE 13.7 Specificalion of limb lype in the chick by Tbx4 and Tbx5.
(A) I n silu hybridizations show that during normal chick development, TbxS (blue) is found in the ante rior lateral plate mesoderm, while Tbx4 (red) is found in the posterior lateral plate mesoderm. TbxS-containing limb buds produce wings/ while Tbx4-containing limb buds generate legs. IS) If a new limb bud is induced with an FGF-secreting bead, the type of limb formed depends on which Tbx gene is expressed in the limb bud. If placed between the regions of Tbx4 and Tbx5 expression, the bead wi ll induce the expression of Tbx4 posteriorly and Tbx5 anteriorly. The resulting limb bud will also express Tbx5 anteriorly and Tbx4 pos teri orly and will generate a chimeric limb. (C) Expression of Tbx5 in the forelimb (w, wing) buds and in the anterior portion of a limb bud induced by an FGF-secreting bead.
(The somite level can be determined by staining for Mrf4 mRNA, which is localized to the myotomes.) (0) Expression of Tbx4 in the hindlimb (Ie, leg) buds and in the posterior portion of an FGF-incluced limb bud. IE,F) A chimeric limb induced by an FGF bead con tains anterior wing structures and posterior leg structures. (F) is at a later developmental stage, after feathers form. (A,B after Ohuchi et al. -1998, Ohuchi and Noji 1 999; C-F courtesy of s. Noji.) (C))) • • • • • • . • • (E) (F) Wing Leg Wing • Chimera • Leg DEVELOPMENT OF THE TETRAPOD LIMB Tbx4), limbs ind uced in the anterior region of the flank 491 bud and will become a major signaling center for the devel oping limb (Saunders limb specification. In mice, at least, 1948; Kieny 1960; Saunders and 1974; Fernandez-Teran and Ros 2008). Its roles include (1) maintaining the mesenchyme beneath it in a plastic, proliferating state that enables the linear (proxi mal-distal, shoulder-finger) growth of the limb; (2) main responsible for the outgrowth of taining the expression of those molecules that generate the often became legs instead of wings. Thus, Tbx4 and Tbx5 appear to be critical in instructing the limbs to become hindlimbs and forelimbs, respectively. However, the Tbx genes are not the complete story of Tbx4 and TbxS are the limbs but not their specification. When either Tbx5 or Tbx4 is knocked out, the lateral plate mesoderm fails to make FgflO n i the forelimb or hindlimb regions . Therefore, it seems likely that Hox proteins may activate Tbx genes and that Tbx proteins acti vate Fgf10. Moreover, Tbx4 can be replaced by Tbx5 with Reuss anterior-posterior (thumb-pinkie) axis; and (3) interacting with the proteins specifying the anterior-posterior and dor sal-ventral (knuckle-palin) axes so that each cell is given instructions on how to differentiateo The proximal-distal growth and differentiation of the out changing the limb from forelimb to hindlimb . Rather limb bud are made possible by a series of interactions in mice, it appears that Pitxl is critical in constructing the between the AER and the limb bud mesenchyme d i rectly hindlimbs. Indeed, misexpression of Pitxl in the mouse forelimb results in the transformation and translocation of acquires a hindlimb-like morphology (Minguillon et al. 2005; Delaurier et al. 2006). Tbx4 expressed in the fore limb will not do th is . Moreover, Pi tx1 protein activates hindlimb-specific genes in the forelimb, including Hoxc10 the muscles, bones, and tendons, such that the forelimb and Tbx4.
Ongoing research seeks t o discover the mecha nisms by which the forelimbs and hindlinlbs become dis tinct from one another. mesenchyme is called the zone (PZ) mesenchy me (and sometimes the undifferentiated zone), since its proliferative activity extends the limb bud (Harrison 1918; Saunders 1948; Tabin and Wolpert 2007). These interactions were demonstrated (200 m) by the results of several experiments on chick embryos (Figure The apical ectodermal ridge When mesenchyme cells enter the limb field, they secrete a struc ture called the apical ectodermal ridge, or AER (Figure Fgfl O that induces the overlying ectoderm to form 1 3 .8). The AER runs along the distal margin of the limb 13.9): 1 . I f the AER i s removed a t a n y time during limb devel opment, further development of distal limb skeletal ele ments ceases. 2. Generating the Proximal-Distal Axis of the Limb beneath it.
This distal p rogress II an extra AER is grafted onto an supernumerary structures are existing limb bud, formed, usually toward the distal end of the limb. 3. If leg mesenchyme is placed directly beneath the wing distal hindlimb structures (toes) develop at the end of the limb.
(However, if this mesenchyme i$ placed AER, farther from the AER, the hindlimb [leg] mesenchyme becomes integrated into wing structures.) 4. If limb mesenchyme is replaced by nonlimb mes enchyme beneath the AER, the AER regresses and limb development ceases. Thus, although the mesenchyme cells induce and sustain the AER and determine the type of limb to be formed, the for the sustained outgrowth and devel opment of the limb (Zwilling 1955; Saunders et aJ. 1957; Saunders 1972; Krabbenhoft and Fallon 1989). The AER AER is responSible keeps the mesenchyme cells directly beneath it in a state of mitotic proliferation and prevents them from forming cartilage. Hurle and co-workers (1989) found that if they cut away a small portion of the AER in a region that would normally fall between the digits of the chick leg, an extra digit emerged at that position ' See WEBSITE 1 3 .1 Induction of the AER to *When referring to the hand, one has an orderly set of names to and d. minim 115, respectively, from thumb little finger). No such nomenclature exists for the pedal digits, but the plan proposed by specify each digit (digitus poflicis, d. FIGURE 13.8 Scanning electron micrograph of an early chick forelimb bud, with the apical ectodermal ridge in the foreground. (Courtesy of K. W. Tosney.) il1dicis, d. medius, d. alH1ularis, much merit. The pedal digits, from hallux to named porcellus fori, p. domi, p. camivor!ls, p. lion vomtus, and p. plomns domi, respectively. small toe, would be Phillips (1991) has 492 CHAPTER 1 3 13.9 Summary of experiments demonstrating the effect c ectodermal ridge (AER) on the u nderlyi ng mes enchyme. A r FIGURE of the a p i a l (fte Wesselis 1 977.) See VADE MECUM Patterning the limb FGFs are critical for the induction, maintenance, and func tion of the apical ectodermal ridge. The principal protein inducing the AER is FgflO (Xu et al. 1998; Yonei-Tamura et aJ. 1999). FgflO is capable of inducing the AER in the com petent ectoderm between the dorsal and ventral sides of the embryo. The bOW1dary where dorsal and ventral ecto-derm meet is critical to the placement of the AER. In mutants in which the limb bud is dorsalized and there is no dorsal-ventral junction (as in the dlick mutant limbless), the AER fails to form and limb development ceases (C ar rington and Fallon 1988; Laufer et a!. 1997a; Rodriguez Esteban et aJ. 1997; Tanaka et al. 1997).
FgllO from the lat eral plate mesoderm acts by ind ucing a Wnt protei n (Wnt3a-in chicks; Wnt3 in humans and mice) i n the prosp ective limb bud ectoderm. The Wnt pro tein acts through the canonical -catenin pathway to induce Fgf8 expression and fonn the AER (Figure 1 3.1 0; see Figure 13.6; also see Fernande z-Teran and Ros 2008). Fgf8 stimulates mitosis in the mesenchyme cells beneath it auld causes these cells to keep expressing FgflO. Thus, a (B) J - Limb development ceases "" AER,'lb Wing FCFs in the induction of the AER (A) rem oved AER (C) regresses; development ases positive feedback loop is established: Fgfl0 in the mes enchyme induces Fgf8 in the AER, and F gf8 in the AER maintains FgflO expression in the mesenchym . Each FGF activates the synthesis of the other (see Figure 13.6; Mah mood et a!. 1995; Crossley et al. 1996; Vogel et al. 1996; Ohuchi et a!. 1997; Kawakami et a!. 2001). The continued expression of these FGFs mai ntains mitosis in the mes enchyme beneath the AER. e FIGURE 13.10 Fgf8 in the apical ecto dermal ridge. (A) In situ hybridization showing expression of fgfB message (stained dark purple) in the ectoderm as the limb bud begins to form. (B) Expres sion of fgf8 RNA in the AER, the source of mitotic signals to the underlying meso- derm . (e) In the normal 3-day chick embryo, fgf8 is expressed in theAER of both the forelimb and hindlimb buds. It is a lso expressed in several other places in the embryo, incl uding the pharyngeal arches. (A,S courtesy of J. C. tzpisua-Sel monte; C courtesy of A. Lopez-Martinez and J . F. Falion.) DEVELOPMENT OF T H E TETRAPOD LIMB (A) 493 FIGURE 13.1 1 The AER is necessory for wing development. (AI Skele ton of a normal chick wing (dorsal view), (6) Dorsal views of skeletal patterns after removal of the entire AER (rom the right wing bud of chick embryos at various stages. (From iten 1 982, courtesy of L. !ten.) (B) Specifying the limb mesoderm: Determining the proximal-distal polarity of the limb 1948, Jom) Saunders made a simple and profound obser vation: if the AER is removed sHght ly later, humerus, radius, and ulna form (Figure 1 3.1 1 ; Saunders 1948; Iten 1982; Rowe and Fallon 1982). Explain ing how tms happens has not been easy. First it had to be determined whether the positional information for proxi mal-distal polarity resided in U,e AER or in the progress zone mesenchyme. Through a series of reciprocal trans plantations, tltis specificity was found to reside in the mes enchyme. If the AER had provided the positional informa tion-somehow instntcting the undifferentiated mesoderm beneath it as to what structures to make-then older AERs combined with younger mesoderm should have produced limbs with deletions in the middle, wmle younger AERs combined with older mesenchyme should have produced duplications of shuctures. Tms was' not found to be the case; rather, normal limbs formed in both experiments (Rubin and Saunders 1972). But when the entire progress zone (including both the mesoderm and AER) from an early embryo was placed on the limb bud of a later-stage embryo, new proximal structures were produced beyond those already present.
Conversely, when old progress zones were added to young limb buds, distal structures devel oped immediately, so that digits were seen to emerge from the humerus without an intervening ulna and radius (Fig ure 1 3.1 2, Summerbell and Lewis 1975). But how does the mesenchyme specify the proximal distal axis? Three models have been proposed to account for tms phenomenon. One model emphasizes the dimenIn (A) (8) U)e FIGURE 13.12 Conlrol of proximal-dislal specification by Ihe progress wne mesenchyme. (A) An extra set of ulna and radius formed when an early wing bud progress zone was transplanted to a late wing bud that had already formed ulna and radius, (8) Lack of intermediate structures seen when a late wing-bud progress zone was transplanted to an early wing bud. (From Summerbell and Lewis 1 975, courlesy of D. 5ummerbel1.) 494 CHAPTER 1 3 . sian of time, one model emphasizes the dimension of space, and one model emphasizes generation of periodicity ..
The progress zone model postulates that each meso derm cell is specified by the amount of time it spends dividing in the progress zone. The longer a cell spends in the PZ, the more mitoses it achieves, and the more distal its specification becomes. Since the PZ remains the same size, cells must exit the PZ constantly as the limb grows; once they leave, they can form cartilage. Thus, the first cells leaving the PZ become the stylopod, and the last cells to leave it become the autopod (Figure 1 3.13A; Summerbell 1974; Wolpert et a!. 1979).
According to this model, remov ing the AER means that the progress zone cells can no longer divide and be further specified, so only the proxi mal structures form.
Although the progress zone model has been the prevail ing means of explaining limb polarity, it does not explain recent data from experiments in which the progress zone mesenchyme was prevented from dividing in mice lack ing both fgfB and fgf4 (both of which are expressed in the AER). In the limbs of these mice, the proximal elements were altogether missing, while the distal elements were present and often normaL This result could not be explained by a model wherein the specification of distal elements depended on the number of mitoses in the progress zone (Sun et a!. 2002). Moreover, when individ ual early PZ cells were marked with a dye, the progeny of each cell were limited to a particular segment of the limb. The tip of the early limb bud provided cells only to the autopod, for instance (Dudley et a1. 2002). This finding sug gested that specification was early and that the time spent in the progress zone did not specify cell position. In addi tion, cell division is seen throughout the limb bud, not sole ly in the progress zone. Gail Martin and Cliff Tabin have proposed an early allo cation and progenitor expansion model as an alternative (A) (B) Progres zone model Early al ocation and progenitor expansion model to the progress zone modeL Here, the cells of the entire early limb bud are already specified; subsequent cell divi sions simply expand these cell populations (Figure 13.13B). The effects of AER removal are explained by the observa tion of Rowe et a!.
(1982) that when the AER is taken off the limb bud, there is apoptosis for approximately 200 m. If one removes the AER of an early limb bud, before the mesenchymal cell populations have expanded, the cells specified to be zeugopod and autopod die, and only the cells already specified to be stylop ad remain. In later embryonic stages, the limb bud grows and the 200-llm region of apoptosis merely deletes the autopod. Research is presently underway to distinguish the contributions of these mechanisms to anterior-posterior axis specification in the limb. In recent years, the proponents of both models have got ten together and proclaimed that neither model fits the data generated over the past decade (Tabin and Wolpert 2007), and that new models must demonstrate how the progenitors of each region of the limb are generated and how they become specified to a particular segment. To this end, we turn to molecular and physical analyses of limb polarity. A reaction-diffusion model for limb specification In contrast to models wherein the proximal-distal axis is specified by paracrine-induced transcription factors, the activator-inhibitor model of Newman and Bhat (2007) por trays these factors as secondarily stabilizing patterns that have been initiated by a reaction-diffusion mechanism (Fig ure 1 3.1 4). According to this model, the limb bud becomes sequentially patterned into three areas. 1.n the first area, basically the progress zone, cells respond to FGFs from the AER that suppress the synthesis of fibronectin and there- 0 3 o zone o 3 0 4 FIGURE 13.13 mesodermal s ec f cat of Two models for p i i ion of the proximal-distal axis the limb. (A) Progress zone model, wherein the length of time a mesodermal cell remains in the progress zone speci fies its posi tion. (8) Early allocation and progenitor expansion model, wherein the territories of the limb bud are established very early and the cells grow in each of these areas. DEVELOPMENT O F T H E TETRAPOD LIMB "Froz.en A c _AER (Apical r=:"B." 0SS ercidtogd)erm to formcartilage CFibrGonFe-P1ctin Diinhffuibsitbolre t Active pi al I t L.
1 FIGURE 13.14 Reaclion-diffusion model for proximal-distal limb specification. The AER divides the limb into three domains. The apical domain (basically the progress zone) is a region where FGF signals from he AER prevent the expression of fibronedin, an extracellular matrix protein that assembles the mesenchymal cells into nodules. The second domain, outside the influence of the AER, is where the nodules actively form according to a reaction diffusion system (shown on the bottom of the figu re) Here each cell secretes and can respond to the paracrine factors of the TGF-p iamily. TGF stim u lates its own synthesis as well as that of fibronectin. TGF-p also stimulates the synthesis of a diffusible inhibitor of its own synthesis, thereby limiting the synthesis of ibronectin according to the geometry of the limb bud. (i.e., how many I'waves" of activator will be allowed.) The fibronectin -aggre gated nodules can now differentiate into cart age, freezing the configuration. This constitutes the third domain. 495 and laterally inhibit the formation of other foci of conden sahon. The number of foci, then, depends on the geome try of the tissue and the strength of the lateral inhibi tion. If the inhibition remains the same, tissue volume must increase in order to get two foci forming where one had been allowed before. This model predicts that stight size changes of the distal limb bud alter the number of digits.
This is indeed found to be the case, and it may be a very simple way of gaining or losing digits during evolution. According to this model, these standing waves of synthe sis and inhibition would constitute the original pattern of the limb. Such patterning of the limb into the regions of stylopod, zeugopod, and autopod by reaction-diffusion rnechanisms rather than by transcription factors has been demonstrated as being possible by computer modeling (see Forgacs and Newman 2005). Since no single model cur rently explains all the experimental phenomena, the gen eration of the proximodistal axis of the limb remains under intense investigation. . il " " by prevent condensation of the mesenchyme cells into car tilaginous nodules. In the area just beyond the reach of the AER Signals, each mesenChymal cell becomes responsive to TGF Signals produced by other mesenchymal cells and by themselves. This is the region of patterning activity that directs the formation of cartilaginous nodules. It is thought that the TGF acts (1) as a positive inducer of itself, (2) as a positive inducer of the extracellular matrix protein fibronectin (which induces cartilaginous nodule forma tion), and (3) as an inducer of its own soluble inhibitor (which has not yet been identified). This unidentified inhibitor diffuses more rapidly than TGF-J3 and prevents TGF-J3 synthesis as long as the in hibitor's concentration is above a certain level. When its concentration falls below that level TGF (and its inhibitor) are synthesized. This signaling creates a periodic pattern of regions alternately containing and lacking TGF-J3 and fibronectin. As the nodules form and coalesce, they become refractile to the Signals and are "hozen" in form. Also according to this model, the form of each bone depends on the physical shape of the bud. At different sizes of the limb, different numbers of cartilaginous units can form bones. First a single condensation can fit (huruerus), then two (ulna and radius), then several (wrist, digits). In this reaction-diffusion hypothesis, the aggregations of car hlage actively recruit more cells hom the surrounding area Specification of the Anterior-Posterior Axis The zone of polarizing activity The specification of the anterior-posterior axis of the limb is the earliest restriction in limb bud cell potency from the pluripotent condition. In the chick, this axis is specified shortly before a limb bud is recognizable. Hamburger (1938) showed that as early as the 16-sorrute stage, prospec tive wing mesoderm transplanted to the flank area devel ops into a limb with the anterior-posterior and dorsal ventral polarities of the donor graft, not those of the host tissue.
Several experiments (Saunders and Gasseling 1968; Tickle et a!. 1975) suggest that the anterior-posterior axis is specified by a small block of mesodermal tissue near the posterior junction of the young limb bud and the body wall. When tissue from this region is taken from a young limb bud and transplanted to a position on the anterior side of another limb bud, the number of digits of the result ing wing is doubled (Figure 1 3.1 5). Moreover the struc tures of the extra set of digits are mirror images of the normally produced structures. The polarity has been main tained, but the information is now coming from both an anterior and a posterior direction.
Thus this region of the mesoderm has been called the zone of polarizing activity (ZPA). , SONIC HEDGEHOG DEFINES THE ZPA 111e search for the mol ecule(s) conferring this polarizing activity on the ZPA became one of the most intensive quests in developmen tal biology. In 1993, Riddle and colleagues showed by in situ hybridization that sonic hedgehog (shh), a vertebrate homologue of the Drosophila hedgehog gene, was expressed specifically in that region of the limb bud known to be the ZP

was changed into a copy of digit 1. Similarly, when the webbing on the other side of digit 3 was removed, the third digit formed a copy of digit 2 (Figure B.22A-C). Moreover, the positional value of the webbing could be altered by changing the BMP level. When beads containing BMP antagonists such as Noggin a DEVELOPMENT OF THE TETRAPOD LIMB (A) (B) (C) • 2 • • • (D) 5 • 501 3 4 FIGURE 13.21 Shh secreting cells form digits 4 and 5 and contribute to the specification of digits 2 and 3 in the mouse limb. (A) In the early mouse hindlimb bud, the progenitors of digit 4 (green dot) and the progenitors of digit 5 (red dot) are both in the ZPA and express sonic hedgehog (light green). (B) At later stages of limb development, the cells (arming digit 5 are still expressing shh in the ZPA, but the cells forming digit 4 no longer do. (C) When the digits form, the cells in digit 5 will have high levels of Shh protein for a longer time than the cells in digit 4. (D) Schematic by which digits 4 and 5 are specified by the amount of time they were exposed to Shh in an autocrine fashion; digit 3 is specified by the amount of time the cells were exp sed to Shh both in an autocrine and paracrine fashion. Digit 2 is specified by the concentration of Shh its cells received by paracrine diffu sion, and digi 1 is specifoed independently Shh. (After Hane et al.

2004.) - DiDiggiitt 32:: andtcioconneceoneftnertxartpaitroiconsnion o f e x p r e s i o n dif usion descend.nts Digit J : Shh-independent ""...ll- Shh Shh m e seen ot Digits 4-5. Shh time o Shh (A) • Shh of Control (B) J 2-->1 3 '3 C,' 4 3 (1 C)/ .,2 r 2-->1 \ 3-->2 "C/. 3 of t i n I D 4) ? • I s 7 7 F Q-.• b-' 4 FIGURE 13.22 Regulation digit iden ti ty by BMP concentrations in the interdigital space anterior to the digit and by Gli3. (A) Scheme for removal of interdigital (10) regions. The results are shown in (B) and (C), respec ively. (B) Removal of ID region 2 between digit primordia 2 (p2) and 3 (p3) causes digit 2 to change to the structure of digit 1 . (C) Removing ID region 3 (between digit primordia 3 and causes digit 3 to form the struc tures digit 2. (D) Control digits and their ID spaces. (E,FIThe same transformations as in (B) and (C) can be obtained by adding of " 4 / I La • 32-->2 iii • t (E) 4 b (F) 1Q ..... •c 4 beads containing the BMP inhibitor Noggin to the ID regions. (E) When a Noggin-containing bead (green dot) is placed in I D region 2 , digit 2 i s transformed into a copy of digit 1. (F) When the Noggin bead is placed region .1, digit 3 is transformed into a copy of digit 2. (G)The forelimb of a mouse homozygous for deletions of both gli3 and shh is characterized by extra digits of no specific type. (After Dahn and Fallon 2000; litingtung et al. 2002; B-G, photographs courtesy of R. D. Dahn and J. F. Fallon.) 4 502 CHAPTER 1 3 were placed in the webb ing between digits 3 and 4, digit 3 was anteriorly transformed into digit 2 (F igure 1 3.22 D-F). digit has a characteristic array of nodules that form the digit skeleton, and S uz uki and olleagues (2008) have shown that the differen t levels of BMP signaling in the interdigital webbing regulate the recruitment of progress zone mesen hyma l cells into the nodules that make the Each (A) c e digits. (B) vp Generation of the Dorsal-Ventral Axis , The third axis of the limb distinguishes the dorsal half of (knuckles, nails) frolll the ventral half (pads, soles). In 1974 MacCabe and co-workers demonstrated that the dorsal-ventral polarity of the limb bud is determined by the ectoderm encasing it.

If the ectoderm is rotated 180 degrees with respect to the limb bud mesenchyme, the dor sal-ventral axis is partially reversed; the d i stal elements (digits) are "upside- c Dorsal-to-ventral tra nsformations of limb reglo " in mice deficient for both Wnl7a genes. (A) Histological sect ion (stained w i th hematoxyl in and eosi n) of w il d-type 1 5.5-day embryonic mouse forelimb paw. Tbe ventral tendons (vt) and tral footpads (vp) are readily seen. Numbers i ndicate digi t identi (8) Same section t h rou g h a mutant embryo deficient in Wnl7a.

Ventral tendons and footpads are d uplicated on what would nor mally be the dorsal surface of the paw-that the dorsal tendor: (dt) and dorsal footpads (dp). (From Parr and McMahon 1995; photographs courtesy of the auth ors.) FIGURE 13.23 ver is, Wnt7a is the first known dorsal-ventral axis gene expressed in limb development. lt induces activa tion of the Lim1 gene in the dorsal mesenchyme. Lind encodes a tran appears to be essential for spe ifying dorsal cell fates in the limb.

lf Liml protein is expressed in the ventral mesenchyme ceils, those cells develop a dorsal phenotype (Ridelle et a1. 1995; Vogel et al. 1995; Altabef and Tickle 2002).

Mutants of LimI in humans and mice also show this gene's importance for specifying dorsal limb fates. Knockouts n i mice produce a syndrome in which the dorsal limb phenotype is lacking, and loss-of function mutations in humans produce the nail patella syndrome, in which the dorsal sides of the limbs have been ventral ized (e.g ., no nails on the digits, no kneecap s) (Chen et a1.

1998; Dreyer et a1. 1998). Lim! protei n p robably specifies the cells to differentiate in a dorsal manner, and this is crit ical, as we have seen (Chapter 10), for the innervation of motor neurons, whose growth cones recognize inhibitory factors made differentially in the dorsal and ventral com partments of the limb bud . c scription factor that Coordinating the Three Axes The mechanisms specifyi ng U1e limb are interrela ted three axes of the tetrapod and coor dina ted . At first, when the limb bud is relatively small, an initial positive feedback loop between Fgfl 0 and Fgf8 is established (Figure 1 3.24A). As the limb bud grows, the ZPA is established and anoth er regul atory loop is created (Figure 13.24B). Sonic hedge hog in the ZPA activates Gremlin, which inhibits BMP, The BMPs (mostly BMP4) are able to inhibit the FGFs 0: the AER. Thus, Sonic hedgehog in the ZPA prevents down regulation of the FGF genes expressed in the AER: fgf4, fgF fgJ9, and fgf1 7 (N isw ander et a1. 1994; ZUiUga et a1. 1999Scherz et a1. 2004; Vokes et al.

2008). Alter this, the interac tivity gets complicated . Depending on the levels of c FGFs in the AER, the ZPA a n be either activated or sh ut down; two feedback loop> (Figure 13.25A; Benazet et al. 20()9; first, relatively low levels 0: AER FGFs activate Shh and keep the ZPA functioning (Fig ure 1 3.25B; also see Figure 13.24B). The FGF signals appear to repress the proteins Etv4 and Etv5, whid1 are represoo,," of Sonic hedgehog transcr iption (Mao et a1. 2009; Zhang et a1. 2009). Thus, the AER and ZPA mutually support each other through the positive loop of Sonic hedgehog and FGFs (Todt and Fallon 1987; Laufer et al. 1994; Niswander et a1. 1994). In this phase of limb development, levels of Gremlin (a powerful BMP antagonist) are high, and the positive FGF /Shh loop sustains the limb growth.

Howe,' er, as FGF levels rise (Figure 1 3.25C), they block G remli n expression in the d istal mesenchyme. This repression of have been demonstrated Verheyden and Sun 2008). At DEVELOPMENT OF T H E TETRAPOD L I M B (A) (B) • FgflO FIGURE 13.24 Early interactions between the AER and limb bud senchyme (A) In the limb bud, FgflO from esenchyme gener ated by the lateral plate mesoderm activates a Wnt (Wnt3a in chicks; Wnt3 in mice and humans) i n the ectoderm. Wnt activates the • catenin pathway, which induces synthesis of Fgf8 in the region near the AER. Fgf8 activates FgflO, causing a positive feed back loop. (B) As the limb bud grows, Sonic hedgehog in the pos terior mesenchyme creates a new signal ing center that induces posterior-anterior polarity, and it also activates Gremlin (Grem 1) to prevent 8MPs from blocking FGF synthesis in the AER. (After Fernandez-Teran and Ros 2008.) me Wnt3a(Wnt3) pgf8) . 503 Shh m lier lacked not only dorsal limb structures but also poste rior digits, suggesting that Wnt7a is also needed for the Ent anterior-posterior axis (Parr and McMahon 1995). Yang and Niswander (1995) made a similar set of observations in chick embryos. These investigators removed the dorsal ectoderm from developing limbs and found that tlus result ed in the loss of posterior skeletal elements D Ent D En t from the limbs. The reason these limbs lacked posterior digits was that shh DWnt3a(Wnt3) expression was greatly reduced.

Vlfal-induced expression Shh of Wnt7a was able to replace the dorsal ectoderm and restore shh expression and posterior phenotypes. These findings showed that the synthesis of Sonic hedgehog is stimulated by the combination of Fgf4 and Wnt7a proteins. Gremlin synthesis activates the inhibitory feedback loop Conversely, overactive Wnt signaling in the dorsal ecto derm causes an overgrowth of the AER and extra digits, and creates a zone of cells in the distal mesenchyme that do not secrete Gremlin. indicating tha t the proximal-distal patterning is not n i de pendent of dorsal-ventra l patterning either (Adamska et As long as the Gremlin signal can diffuse to the AER, FGFs will be made and the AER maintained. It is thought aL 2004). that as the limb bud expands, the Gremlin signal eventu Thus, at the end of limb patterning, BMPs are respon ally fails to reach the ectoderm. At that time, the BMPs sible for simultaneously shutting down the AER, indirect (with no FGFs to support it) is terminated.

The embryonic nal along the dorsal-ventral axis (Pizette et al. 2001). The abrogate FGF synthesis, the AER collapses, and the ZPA phase of limb development ends (F i gu re ly shutting down the ZPA, and inhibiting the Wnt7a sig 1 3.25D). The dorsal-ven tral axis is also coordinated with these other two. Indeed, the Wnl7n-deficient mice described ear- • • !Gremtl =+=! IBMPsl / • (A) FGF/Greml loop FGP/Shh loop Gr ml1--+ 1LOwFGF! (B) ! ; •J • Fgf4,9.17 FIGURE 13.25 _ .... .a e (D) I Later coordination of AER and ZPA signal ing. This is one of several models to explain the coordinated termination of limb outgrowth. (A) The two feedback loops linking the AER and ZPA. tn the positive feedback loop (bot tom), FGFs from the AER (specifically, Fgf4, 9, and 1 7) acti vate Shh transcription, stabilizing the ZPA. Reciprocally, in the inhibitory loop (top), Shh from the ZPA activates Grem lin (Grem1), which blocks the BMPs, thereby preventing the BMP-mediated inactivation of the FGFs in the AER. Thus, in stage (8), there is a mutual accelerated synthesis of both Shh (ZPA) and FGFs (AER).

However, as FGF concentration climbs (C), it eventually reaches a threshold wher it will inhibit Gremlin. This allows the BMP to begin repressing the AER FGFs. Eventually, as more cells multiply in the a ea not expressing Gremlin, the Gremlin signal near the AER is too weak to prevent the • BMPs from repressing the FGFs. At that po int (D), the AER disappears, removing the signal that stabilizes the ZPA. The : ZPA then disapp ars too. (Aher Verhey den and Sun 2008.) Gre.m11 - ILow'FGFI • •... .. , , r e 504 CHAPTER 1 3 BMF signal eliminates growth and patterning along all three axes. When exogenous BMP is applied to the AER, the elongated epithelium of the AER reverts to a cuboidal epithelium and ceases to produce FCFs, and when BMPs are inhibited by Noggin, the AER continues to persist days after it would normally have regressed (Ganan et al. 1998; Pizette and Niswander 1999). Cell Death and the Formation of Digits and Joints Sculpting the autopod Cell death plays a role in sculpting the tetrapod limb. Indeed, cell death is essential if our joints are to form and if our fingers are to become separate (Zaleske 1985; Zuzarte-Luis and Hurle 2005). The death (or lack of death) of specific cells in the vertebrate limb is genetically pro grammed and has been selected for over the course of evo lution. The difference between a chicken's foot and the webbed foot of a duck is the presence or absence of cell death between the digits (Figure 1 3.26).

Saunders and co workers have shown that after a certain stage, chick cells between the digit cartilage are destined to die, and will do so even if transplanted to another region of the embryo or placed in culture (Saunders et al. 1962; Saunders and Fal lon 1966). Before that time, however, transplantation to a duck limb will save them.

Between the time when the cell's death is determined and when death actually takes (A) MmlDUCKmalcel PdReaIMthORDIUM LEG Inetceriotrc•zone ECHIxtenCsKivLEGe cel PdReaIMthORDIUM Inetceredtigcizt•al e r• Annzoecteeortiocr nzoecnreotic D • If/l ! • JJ •I erioJj&J : nec tiezone r /) - /;J • (E) _/ Posterior : r . .1./ • .... n t \;) 0 0 } () r • place, levels of DNA, RNA, and protein synthesis in the cell decrease dramatically (Pollak and Fallon 1976). In addition to the interdigital necrotic zone, three other regions of the limb are "sculpted" by cell death.

The ulna and radius are separated from each other by an interior necrotic zone, and two other regions, the anterior and pos terior necrotic zones, further shape the end of the limb (see Figure 13.2613; Saunders and Fallon 1966). Although these zones are referred to as "necrotic," this term is a holdover from the days when no distinction was made between necrotic cell death and apoptotic cell death (see Chapter 3). These cells die by apoptosis, and the death of the inter digital tissue is associated with the fragmentation of their DNA (Mori et al. 1995). The signal for apoptosis in the autopod is probably pro vided by the BMP proteins.

BMP2, BMP4, and BMP7 are each expressed in the interdigital mesenchyme, and block ing BMP signaling (by infecting progress zone cells with retroviruses carrying dominant negative BMF receptors) prevents interdigital apoptosis (Zou and Niswander 1996; Yokouchi et al. 1996). Since these BMPs are expressed throughout the progress zone mesenchyme, it is thought that cell death would be the "default" state unless there were active suppression of the BMPs. This suppression may come from the Noggin protein, which is made in the developing cartilage of the digits and in the perichondri al cells surrounding it (Capdevila and Johnson 1998; Meri no et al. 1998). If Noggin is expressed throughout the limb bud, no apoptosis is seen. r; / FIGURE 13.26 Patterns of cell death in leg primordia of (A) duck and (B) chick embryos. Shading i ndicates areas of cell death. In the duck, the regions of cell death are very small, whereas there are extensive regions of cell death in the interdigital tiss e of he chick leg. (After Saunders and Fallon 1966.) u t DEVELOPMENT OF THE TETRAPOD LIMB 505 SIDEL IGHTS SPECULATIONS Limb Development and Evolution T he i mportance of development to the study of evolution was articu lated by C. H. Waddington. He wrote that when we say that a modern horse has one toe and that it came from an ancestor with five toes, what we are saying is that during the evolu tion of the horse lineage, developmen tal changes occurred in the cartilage deposition of the embryonic horses' feet. These changes were selected over time to the point of today's Eq(f(fs species. Wh i Ie we sti I I do not know much about the details of evolutionary change in horse l imbs, we have found several remarkable cases of limb evo lution caused by developmental changes. * *Earlier in this chapter, it was noted that developmental biologists get used to thinking in four dimensions. Evolution ary developmental biologists have to think in five d i mensio ns: the th ree stan dard dimensions of space, the dimension of developmental time (hours or days), and the dimension of evolutionary time (millions of years). Web-footed friends We can start with the sculpting of the -utopod. The regulation of BMPs is critical in creating the webbed feet of ducks (Laufer et al. 1 997b; Merino et al. 1 999). n,e interdi g ital regions of duck feet exhibit the same pattern of BMP expression as the webbing of chick feet . However, whereas the inter digital regions of the chick feet appear to undergo BMP-mediated apoptosis, developing duck feet synthesize the BMP inhibitor Gremlin and block this regional cell death (Figure 13.27). Moreover, the webbing of chick feet can be presĉlved if Gremlin-soaked beads are placed in the interdigital regions (Figure 13.28). Thus the evolu tion of web-footed birds probably involved the inhibition of BMP-mediat ed apoptosis in the interdigital regions. In Chapter 1 9, we will find that the bat embryo uses a similar mechanism to acquire its wings. Dinosaurs and chicken fingers Next, there is that postulated connec tion between birds and dinosaurs. Are birds really the descendants of dinosaurs? Although Thomas H u xley (1 868, '1 870) proposed in the late nineteenth century that the birds descended from dinosaurs, it was J.

H . Ostrom's 1 969 description o f the dinosaur Deinonychus antirrhopus and its similarities to the fossi l s of the first (Continued on next page) Fi gu re 13.27 Autopods of chicken feet (upper row) and duck feet (lower row) are shown al similar stages. Both show BMP4 expression (dark blue) in the interdigital webbing; BMP4 induces apoptosis. The duck foot (but not the chicken foot) expresses the BMP4-inhibitory protein Gremlin (dark brown; arrows) in th e i nterdigital webbing.

Thus the chicken foot undergoes interdig i ta ! apoptosis (as seen by neutral red dye accumulation in the dying cells), but the duck foot does not. (Courlesy of) . Hurle and E. Laufer.) Chick hindlimb Duck hindlimb BMP Gremlin Apoptosis Newborn 506 CHAPTER 1 3 SIDELIGHTS & SPECULATIONS (Continued) known bird, Archaeopteryx, that was critical in making the di nosaur-ta bird hypothesis acceptable. Ostrom l isted 22 simi larities between Oeinonychus and Archaeopteryx, simi larities found in no other groups and linking birds and dinosaurs. Without its feathers, Archaeopteryx looks exactly like a small coelurosaur (such as Jurassic Park's Ve/ociraptor). Indeed, one specimen of Archaeopteryx was m isidentified as a coelurosaur for over 1 00 years until its feathers were noticed by Peter Welln hofer (1 993). Gauth ier's cladistic work in the mid-1 980s (see Gauthier 1 986) provided systematic support for the theory that birds are the descen dants of coelurosaurian d i nosaurs. U n l i ke any other reptiles, both birds and theropod dinosaurs (of which the coelurosaurs are a group) have a 3-fin gered grasping hand and a 4-toed foot supported by three main toes. Thus Pad ian and Chiappe (1 998a) conclude that "in fact, living birds are nothing less than small, feathered, short-tailed theropod dinosaurs." However, whereas paleontologists were nearly unanimous in their appraisal that birds are the direct de scendants of dinosaurs, some develop mental biologists harbored serious doubts. Fossil evidence unambiguously identified the theropod-like birds as having wing digits 1 , 2 , and 3 (Pad ian and Chiappe 1 998b),' but embryologi cal evidence suggested that the wing digits of current birds are 2, 3, and 4. For instance, Bu rke and Feduccia (1 997) found that digit primordia in the fingers of early and present-day birds correspond to the index, middle, and ring fingers (2-3-4). Moreover, the arrangement of cartilaginous condensa tions is the one expected for the 2-3-4 pattern, not the 1 -2-3 pattern. This would mean that the simi larity of di nosaur and bird d igits is based on *For a detailed analysis of these transi tion forms, see httpjJv..rv.vw ucmp.berke ley.edu/diapsidslavians.html. Further speculations, based on the single fossil of a newly discovered Jurassic theropod, maintain that dinosaur digits may also have been 2-4 (see Xu et al. 2009). A least, the fossil's wrist appears 2-4, the digits appear -' 3. t (A) (B) Figure 13.28 Inhibition of cell death by in hi biti ng BMPs. (A) Control chick limb shows extensive apop tosis in the space between the digits, leading to the absence of webbing. (B) When beads soaked with Gremlin protein are placed in the interdigital meso derm, the webbing persists and generates a ducklike pattern. (From Merino et a1. 1 999, courtesy of E. Hurle.) independent selection for three digits (convergent evolution) and is not based on shared ancestry. This developmental critique of the bird-dinosaur l i n k has been made by other scientists studying chick limb development (see Galis 2005; Welten et al. 2005). They point out that bird feet have reversed toes used for perch ing on branches (some thing di nosaurs never developed), and that theropods had a characteristic joint in their lower jaw for grasping prey (someth ing never found i n birds). Alan Feduccia has called the notion that birds arose d i rectly from di nosaurs a "delusional fantasy by which one can vicariously study d inosaurs at the back yard bird feeder" (Feduccia 1 997). But a study by Vargas and Fallon (200Sa,b) suggests that embryologists have been wrong i n their assessment of bird digits. Although the condensa tions of the digits look like those expected for digits 2, 3, and 4, the Hox gene expression patterns suggest that the actual digits are indeed 1 , 2, and 3, just as in the theropod di nosaurs. Fa llon and Vargas claim that digit 1 (thumb!hallux) is uniquely c haracterized (at least i n the chicken hindlimb and the mouse forel imb and h i n d l i mb) by Hoxd13 expression i n the absence of Hoxd 12 expression (see Figure 1 3 . 1 9) .

A l l other digit pri mordia express both Hoxdl2 and Hoxd13. Thus, Vargas and Fallon pro pose that the wing digits of chickens are actually 1 -2-3, and that avian digit arrangement is further proof rather than a rebuttal of the idea that birds are the descendants of dinosaurs. Recent studies show some support for this "frameshift" model. The Hox expression of a l l igator digits also sug gests that i n the bird wing, digits 1 , 2 , and 3 develop from t h e embryological positions expected of digits 2, 3, and -l (Vargas et a l . 2008).

Furthermore, Var gas and Wagner (2009) showed that ii cyclopamine is used to i n h ibit Shh sig n a l i n g in chick l i mbs, the digit mor phologies that usually develop from positions 2 and 3 shift such that they develop from positions 3 and 4. Their findings l i n k the developmental analy sis of limb development to paleon tological studies by Wagner and Gauthier (1 999), who hypothesized that when digit 4 was lost in birds, a "homeotic frameshift" occurred, caus ing digits 2, 3, and 4 to develop from the embryological positions formerly giving rise to digits 2 , 3, and 4. If this developmental geneti C observation is confirmed, then the "greatest chal lenge to the theropod-bird link" (Zhou 2004) will have been eliminated. Developmental biologist Richard Hinchl iffe (1 994, 1 997) sees the argument in a larger context. While evolutionary biologists have reached consensus that birds and di nosaurs evolved from the same class of pre historic creatures, he says, "the only question we are arguing about is whether [birds] derived very late i n t i m e from a specific group o f theropod d i nosaurs, the so-called raptors, or are they derived from a common stem ancestor with d i nosaurs," 507 DEVELOPMENT OF THE TETRAPOD LIMB Forming the joints The function first ascribed to BMPs was the formation, not the destruction, of bone and cartilage tissue. In the devel oping limb, BMPs induce the mesenchymal cells either to tmdergo apoptosis or to become cartilage-producmg chon drocytes depending on the stage of development. The same BMPs can induce death or differentiation, depend ing on the age of the target cell. Trus "context dependen cy" of signal action is a critical concept m developmental biology.

It is also critical for the formation of joints. Macias and colleagues (1997) have shown that during early limb bud stages (before cartilage condensation), beads secret mg BMP2 or BMP7 cause apoptosis. Two days later, the same beads cause the limb bud cells to fonn cartilage. In the normally developing limb, BMPs use both of these properties to form joints. BMP7 is made m the peri chondrial cells surroundmg the condensing chondrocytes and promotes further cartilage formation (Figure 1 3.29A). Two other BMF proteins, BMP2 and GDF5, are expressed at the regions between the bones, where joints will fonn (Fig ure 1 3.298; Macias et al. 1997; Brunet et a1.

1998). Mouse mutations have suggested that the function of these pro teins in joint formation is critical. Mutations of Gdf5 pro duce brachypodia, a condition characterized by a lack of limb joints (Storm and Kingsley 1999). In mice homozy gous for loss-of function alleles of noggin, no joints form either. It appears that the BMP7-m these noggin-defective embry os is able to recruit nearly all the surrounding mes enchyme into the digits (Figure 1 3.29C). Wnt protems and blood vessels also appear to be critical in joint formation. The conversion of mesenchyme cells into nodules of cartilage-formmg tissue establishes where the bone boundaries are. The mesenchyme will not form such nodules In the presence of blood vessels, and one of the first indications of cartilage formation is the regression of blood vessels m the region wherem the nodule will form m and Pacifici 2(01). Wnt proteins are critical in sustain ing transcription of Gdf5, and p-catenin produced by the Wnts is able to suppress the Sox9 and collagen-2 genes that characterize pre-cartilage cells (Hartmann and Tabin 2001; Tufan and Tuan 2001). Joints are not merely just "absences" of bone. Rather, they are complex structures that incorporate a lubrication system, an immune system, and a ligament system, all joined to the proper articulation of the skeleton. One of the critical elements of joint formation that allows trus differen tiation is muscle contraction. In normal joint formation, the cells that will form the joint lose their chondrocyte charac teristics (such as the expression of collagen-2 and Sox9), and instead begin to express GDF5, Wnt4, and Wnt9a.

These cells will form the articula te cartilage and the syn ovium (wruch secretes the lubricating synovial fluid) (Paci fici et a1. 2005; Koyama et a1. 2008). Kahn and colleagues (2009) have shown that the movement of the bones is nec essary for maintaining this commitment to form joints. In mutant mice, where the muscles do not form or are para- (Al Wnt! p-catenin FGf •Dkk-l/ (from AER) Cartilage (BI (C) • FIGURE 13.29 Possible involvement of 8MPs in stabi l izi n car tilage a nd a p p t s i s . (A) Model for the dual role of 8MP si als in limb mesodermal cells. 8MP can be received in the p resen ce FGFs (to pr duce apoptosis) or Wnts induce bone). When FGFs from the AER a re present, Dickkopf (Okk) is activated. This protein mediates apoptosis and at the same time inhibits Wnt from aiding in s eleton iormation. The effects of No in. (6) 1 6 S day autopod from a wild-type mouse, showing GOFS expression (dark blue) at the joints. 1 6 . S day noggin-deficient mutant mouse autopod, showing GDF5 expres sion. Presumably, in the BMP7 was a le to convert nearly all the mesenchyme into cartilage. (A a fte Grote wold and Ruther 2002; 6,C from Brunet et al. 1 998, courtesy of A. P. McMa hon) g gn o o o .

- k (to (B,C) (C) neither joints nor absence of Noggin, of gg - b r . lyzed, the joint cells revert back to a cartilaginous pheno type. The discovery of the fossil Tiktaalik roseae, the "fish with fingers," highlights the importance of joint development m limb evolution. Fish fins, including those of some of the most primitive species, develop using the same three Hox gene expression phases as tetrapods use to form their limbs (Davis et a1. 2007; Ahn and Ho 2008). What may have allowed the independent modification of fin bones into limb bones are the joints. The joints of TiklaaUk's pectoral fins are very similar to those of amphibians and indicate that Tiktaalik had mobile wrists and a substrate-supported 508 CHAPTER 1 3 (A) stance in which the elbow and shoulder could flex (Figure 13.30; Shubin et al. 2006; Shubin 2008). In addition, the presence of wristlike structures and the loss of dermal scales in these regions suggest that this Devonian fish was able to propel itself on moist substrates. So Tiktaalik is though t to be a transition between fish and amphibians a "fishapod" (as one of its discoverers, Neil Shubin, called it), "capable of doing push-ups." Continued Limb Growth: Epi physeal Plates (B) (C) _ ---------. If all of our cartilage were turned into bone before birth, we could not grow any larger, and our bones would be only as large as the original cartilaginous model. However, as the ossification front nears the ends of the cartilage model the chondrocytes near the ossification front prolif erate prior to undergoing hypertrophy, pushing out the cartilaginOUS ends of the bone. In the long bones of many mammals (including humans), endochondral ossification spreads outward in both directions from the center of the bone. These cartilaginous areas at the ends of the long bones are called epiphyseal growth plates. As we saw in Chapter 11, these plates contain three regions: a region of chondrocyte proliferation, a region of mature chondrocytes, and a region of hypertrophic chondrocytes (see Figure 11.17; Chen et al. 1995). As the inner cartilage hypertro phies and the ossification front extends farther outvvard, the remaining cartilage in the epiphyseal growth plate pro liferates. As long as the epiphyseal growth plates are able to produce chondrocytes, the bone continues to grow. Fibroblast growth factor receptors: Dwarfism FIGURE 13.30 Tiktaa/ik, a fish with wrists and fingers, lived in shallow waters about 375 million years ago. (A) This reconstruc tion shows Tiktaalik's fishlike gills, fins, scales, and (lack of) neck. The external nostrils on its snout, however, indicate thaI it could breath air.

(8) Fossilized Tiktaalik bones reveal the beginnings of digits, wrists, elbows, and shoulders, and suggest that this amphibian-like fish could propel itself on stream bottoms and pel" haps live on land for small durations. The joints of the fin included a ball-and-socket joint in the shoulder and a planar joint that allowed the wrist to bend. Other joints allowed the animal to get purchase on its substrate. (C) Resistant contact with a substrate would allow flexion at the proxirnal joints (shoulder and elbow) and extension at the distal ones (wrist and digits). (A, rnodel and photograph © Tyler Keillor; B,C aher Shubin et al. 2006.) Recent discoveries of human and mouse mutations result ing in abnormal skeletal development have provided remarkable insights into how the differentiation, prolifer ation, and patterning of chondrocytes are regulated. The proliferation of the epiphyseal growth plate cells and facial cartilage can be halted by the presence of fibrob last growth factors, which appear to instruct the cartilage precursors to differentiate rather than divide (Deng et al. 1996; Webster and Donoghue 1996).

In humans, mutations of the receptors for PCFs can cause these receptors to become activated prematurely. Such mutations give rise to the major types of human dwarfism. Achondroplasia is a dominant condition caused by mutations in the transmem brane region of FGF receptor 3 (FgfR3). Roughly 95% of achondroplastic dwarfs have the same mutation of FgfR3, a base-pair substitution that converts glycine to arginine at position 380 in the transmembrane region of the protein. In addition, mutations in the extracellular portion of the FgfR3 protein or in the tyrosine kinase intracellular domain may result in thanatophoric dysplasia (see Figure 3.24), a lethal form of dwarfism that resembles homozygous achondroplasia (Bellus et aJ. 1995; Tavormina et aJ. 1995). DEVELOPMENT OF THE TETRAPOD LIMB As mentioned in Chapter 1, dachshlUlds have an achon droplastic mutation, but its cause is slightly different than that of the human form. Dachshunds have an extra copy of the fgf4 gene, which is also expressed in the developing limb (see Figure 1.21). Th i s causes Fgf4 to be made in excess, activating FgfR3 and accelerating the pathway that stops the growth of chondroblasts and hastens their differ entiation. The same extra fgf4 copy has been fOlUld in other short-limbed dogs such as corgis and basset hOlUlds (Park er et a1. 2009). Growth hormone and estrogen receptors The pubertal growth spurt and subsequent cessation of growth are induced by growth hormone; but growth hor mone can be induced by sex hormones (Kaplan and Grum bach 1990), and estrogen appears to be important for bone growth in both men and women. Estrogen receptors are found on the cells that regulate growth hormone produc tion, as well as n i all the cells of the human growth plate. Thus the effects of estrogen on growth may involve both the regulation of growth hormone and more local effects on the growth plate itself (Juul 2001). Relatively low levels of estrogen stimulate skeletal growth in men and women, while the higher levels that occur at the end of puberty induce apoptosis in the hyper trophic chondrocytes and simulate the invasion of bone forming osteoblasts into tl,e growth plate. Without any fur-ther cartilage formation, these bones stop growing, a process known as growth pl ate closure.

Different alleles of the estrogen receptors may play important roles in the genetic variations in growth rates and adult height (Lehrer et a1. 1994; Lorentzon et al. 1999). Estrogen is an important factor in regulating bone growth, even in men. There have been several document ed cases of men who do not produce estrogen (Smith 1994; Juul 2001). These men continue to grow even in adulthood and approach 7 feet in height. Their epiphyseal growth plates do not mature and thus remain full of chondroblasts.

ill most cases, this deficiency is d•e to a loss-af-function mutation in the gene encoding aromatase, the enzyme that 509 converts testosterone into estrogen; in such instances, ther apy with estrogens is able to stop the excessive growth. Parathyroid hormone-related peptide and Indian hedgehog ParathyrOid hormone-related peptide (PTHrP) maintains cell division of chondrocytes (by activating cyclin 01-syn thesis) and prevents their hypertrophy. In humans, loss of-function mutations in the protein encoding PUkP result i n severe growth defects due to the lack of limb growth (Provot and Schipiani 2005). l'THrP is stimulated in the cartilage by Indian hedgehog. Indian hedgehog also stimulates BMP production, coordinating the rates of cell divi sion and matrix deposition (Vortkamp et a1. 1996). Coda FGF proteins generate the proximal-distal axis of the verte brate limb; Shh and BMPs generate the anterior-posterior axiS; and Wnts appear to mediate formation of the dorsal ventral axis. Thus all the major paracrine factor families act in coordination to build the limb. While many of the "executives" of the limb bud formation have been identi fied, we still remain largely ignorant about how the orders of these paracrine factors and transcription factors are car ried out. Niswander (2002) writes: There is a very large gap in our understanding of how the activity of Shh, BMP, FGF, and Wnt genes influences, for example, where the cartilaginous condensation will form, how the elements are sculpted, how the number of phalangeal elements are specified, and where the tendon/muscle will insert.

Limb development is an exciting meeting place for developmental biology, evolutionary biology, and medi cine. Within the next decade, we can expect to know the bases for numerous congenital diseases of limb formation, and perhaps we will understand how limbs are modified into flippers, wings, hands, and legs. Snapshot Summary: The Tetrapod Limb 1. The positions where limbs emerge from the body axis depend upon Hox gene expression. 2. The proximal-distal axis of the developing limb is determined by the ind uction of the ectoderm a t the dorsal-ventral boundary by FgflO from the mes enchyme. This induction forms the apical ectoder mal ridge (AER). The AER secretes Fgf8, which keeps the underlying mesenchyme proliferative and undifferentiated. This area of mesenchyme is called the progress zone. 3. As the limb grows outward, the stylopod forms first, then the zeugopod, and the autopod is formed last. Each phase of limb development is characterized by a specific pattern of Hox gene expression. The evolu tion of the autoped involved a reversal of Hox gene expression that distinguishes fish fins from tetrapod limbs. 4. The anterior-posterior axis is defined by the expres sion of Sonic hedgehog in the posterior mesoderm of the limb bud. This region is called the zone of polar- CHAPTER 1 3 510 izing activity (ZPA). If ZPA or Sonic hedgehog secre ting cells Or beads are plac ed in the anterior margin of a limb bud, they establish a second, mir ror image pattern of Hox gene expression and a CO[respond ing mirror-image duplication of the digits. - 5. The ZPA is maintained by the interaction of FGFs from Ule AER with mesenchyme made competent to express Sonic hedgehog by its expression of Hand2 and particular Hox genes. Sonic hedgehog acts, probably in an indirec t manner, and probably through the Gli factors, to change the expression of ilie Hox genes in ilie limb bud .

6. The dorsal-ventral axis is formed in part by ilie expression of Wnt7a in ilie dorsal portion of ilie 11mb ectoderm. Wnt7a also maintains the expression level of Sonic hedgehog in ilie ZPA and of Fgf4 in ilie pos terior AER. Fgf4 and Sonic hedgehog reciprocally maintain each other's expression. 7. The levels of FGFs in the AER can eiilier support or inhibit the produc tion of Shh by ilie ZPA. As the limb bud grows and more FGFs are produced in the AER, inhibition of Shh occ urs. This ca uses the lower ing of FGFs, and eventually proximodistal out growth ceases. 8. Cell death in ilie limb is necessary for the formation of digits and joints. It is mediated by BMPs. Differ ences between ilie unwebbed chicken foot and the webbed duck foot can be explained by diiferences in the expression of Gremlin, a protein that anta gonizes BMPs. 9. The BMFs are involved boili in inducing apoptosis and in diiferentiating the mesenchymal cells into cartilage. The effects of BMPs can be regulated by the Noggin and Gremlin proteins. This is critical in forming the joints between the bones of the limb and regulating proximal-distal outgrowili. 10. The ends of the long bones of humans and other mammals contain cartilaginous regions called epi physeal growth plates. The cartilage in these regions proliferates SO that the bone grows la rger. Eventual ly, the cartilage is replaced by b one and growth stops .

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although it is not the only determining factor. (A) (B) R spondinl is also critical in ovary formation, since in human case studies several XX individuals with RSPOl gene mutations became males (Panna et aJ. 2006). Rspol acts in synergy with Wnt4 to produce J) catenin, and this p catenin appears to be critical both in activating further ovarian development and in blocking the synthesis of Sox9. Sox9 (as we will see below) is necessary in testis determina tion. In XY individuals with a duplication of the region on chromosome 1 that contains both the WNT4 and RSPOl genes, the pathways that make B catepin override the male pathway and caUSe a male-to female sex reversa1. Similar ly, if XY mice are induced to overexpress p catenin in their gonadal rudiments, they too will form ovaries rather than testes. Indeed, p catenin appears to be a key "pro ovarian" and "anti testis" Signaling molecule in all vertebrate groups, as it is seen in the female gonads (but not in the male gonads) of birds, mammals, and turtles. These groups have very different modes of sex detennination, yet Rspol and p catenin are made in the ovaries of each of them (Fig ure 14.5; Maatouk et al.
2008; Cool and Capel 2009; Smith et aJ. 2009). Certain transcription factors that appear to be activat ed by p catenin are found exclusively in the ovaries. One FIGURE 14.5 Localization of Rspol protein in embryoniC day , 4.5 mouse gonads.
Immunofluorescent probes were used to identify Rspol (green) and the meiotic germ cell marker, Scp3 (red). (A) Rspo'l was found on somati c cells (arrowhead) and at the germ cell surface (arrow) of the ovaries.
(B) These antibodies revealed neither Rspo 1 nor Scp) in the developing testes. (The germ cells in the male gonads have not entered meiosis a t this point in development, whereas the ovarian germ cells nave.) (From Smith et al. 2008; photograph courtesy of C Smith.) 518 CHAPTER 1 4 (1991) took the 14 kilobase region of DNA that includes The testis pathway SRY: THE Y CHROMOSOME SEX DETERMINANT In humans, the Sry gene (and presumably its regulatory elements) and microinjected this sequence into the pronuclei of newly fer the major gene for testis determination resides on the short tilized mouse zygotes. In several instances, arm of the injected with this sequence developed testes, male acces Y chromosome. Individuals born with the short but not the long arm of the Y chromosome are male, where as individuals born with the long arm of the Y chromo some but not the short arm are female. By analyzing the DNA of rare XX men and XY women, sory organs, and a perus' (Figure XX embryos 14.6). There are thus good Y reasons to think that Sry/SRY is the major gene on the chromosome for testis determination in mammals. the position of the 35,000- SOX9: AN AUTOSOMAL TESTIS-DETERMINING GENE The SRY base-pair region of the Y chromosome located near the tip gene is probably active for only a few hours. During this testis-determining gene was narrowed down to a of the short arm. In this region, Sinclair and colleagues time, it synthesizes the Sry transcription factor whose pri (1990) found a male-specific DNA sequence that encodes a peptide of 223 amino acids.
This gene is called SRY (sex mary (and perhaps only) role is to activate the (see Sekido and Lovell-Badge SOX9 gene 2008). SOX9 is an autosomal determining region of the Y chromosome), and there is gene involved in several developmental processes, most extensive evidence that it is indeed the gene that encodes notably bone formation. In the genital ridge, however, the human testis-determining factor. extra copy of SOX9 develop as males, even if they have no SOX9 induces testis formation. XX humans who have an SRY is found in normal XY males and also in the rare XX males; it is absent from normal XX females and from many XY females. Approximately 15% of XY females have the SRY gene, but their copies of the gene contain point or frameshift mutations that prevent the Sry protein from SRY gene, and XX mice transgenic for Sox9 develop testes (Figure 1 4.7; Huang et aJ. 1999; Qin and Bishop 2005).
Knocking out the Sox9 genes in the gonads of XY mice causes complete sex reversal (Barrionuevo et aJ. 2006). 1994; Werner et aJ. 1995).
Thus, even if Sry is present, mouse gonads cannot form If the SRY gene actually does encode the major testis-deter testes if Sox9 is absent so it appears that Sox9 can replace binding to DNA (Pontiggia et aJ. mining factor, one would expect that it would act in the Sry in testis formation. This is not altogether surprising: gemtal ridge immediately before or during testis differen although the tiation. This prediction has been found to be the case in studies of the homologous gene in mice. The mouse gene (Sry) also correlates with the presence of testes; it is pres ent in XX males and absent in XY females (Gubbay et aJ.
1990; Koopman et al.
1990). The Sry gene is expressed in the somatic cells of the bipotential mouse gonads of XY mice immediately before the differentiation of these cells into Sertoli cells; its expression then disappears (Hacker et al. 1995; Sekido et aJ. 2004).
The most impressive evidence for Sox9 appears to be the central male-determin in birds, frogs, and fish, it appears to b e activated by the dosage of Dmrtl (see Sidelights & Speculations, p. 521), and in those vertebrates with temperature-dependent sex determination, it is often activated (directly or indirectly) by the male-producing temperature. Sox9 may thus be the Sry being the gene for Sry induces testis formation, then inserting Sry DNA into the genome of a normal XX mouse zygote should cause that XX mouse to form testes. Koopman and colleagues WEBSITE 1 4.2 Finding the male-determining genes xx d' rest of the Y chromosome, which contains genes needed for sper formation in XXV mice and men, and the transgenic mice lacked the matogenesis. There is biodlemicai evidence (not yet tested in living catenin-mediated transcription (Bernard et al.
(Bl (A) The presence of two X chromosomes prevents sperm *These embryos did not form functional spenn but they were not expected to. mice) that Sry may have a second function, namely, to block . 2008; Lau et aL 2009). See xx 9 Indeed, ing gene in vertebrates. In mammals, it is activated by Sr)'; older and more central sex determination gene (Pask and testis-determining factor comes from transgenic mice. If Xy d' Sry gene is found specifically in mammals, Sox9 is fow1d throughout the vertebrate phyla. FIGURE 14.6 An XX mo use transgenic for Sry is male. (AI Polymerase chain reaction fol lowed by electrophoresis shows the presence of the Sry gene i n normal XY males a n d i n a transgenic XX/Sry mouse The gene is absent in a . Sry -+- 2 3 Control (autosomal) gene female XX littermale. (B) The exlernal genitalia of the transgenic mouse are male (right) and are essentially the same as Ihose in an XY male (left). (From Koopman el al. photographs courtesy of the authors.) 1991; SEX D ETERMINATION (A) (6) (C) (D) XY Wild-type XY Sox9 transgenic xx Wild-type xx Sox9 transgenic 519 Newborn gonadal morphology E I I.S Sox9 EI6.S AMF Adult gonad histology FIGURE 14.7 Abilily of 50x9 10 generate tesles. (A) A wild-type X V mouse embryo express in the genital ridge at 1 1 .5 days, anti-Mullerian factor (AMF) in the embryonic gonad Sertoli cells a(1 6.5 days, and eventually forms descended testes (T) with seminiferous lubules. (B) An XY embryo with the 50x9 I ransgene (a control for Ihe effecls of the transgene) also shows Sox9 expression, AMF expression, and descended testes with seminiferous lubules. (C) The wild-type xx embryo shows neilher 50x9 expression nor AMF. II conslructs ovaries with mature follicle cells. (0) An XX embryo with the Sox9 transgene expresses the Sox9 gene and has AMF in its 1 6.S day Sertol i cells. It has descended testes, but the seminif erous tubules lack sperm (because of the presence of two X chromosomes in the Sertoli cells). kidneys; A, adrenal glands; bladder; 1, testis; 0, ovary. (From Vidal et a l. 2001 ; photographs courtesy of A. Schedl.) es Sox9 K, B, Graves 1999). Expression of the Sox9 gene is specifically upregulated by the combined expression of Sry and Sfl proteins in Sertoli cell precursors (Pask and Graves 1999; Sekido et a1. 2004; Sekido and Lovell-Badge 2008). Thus, Sry may act merely as a "switch" operating during a very short time to activate Sox9, and the Sox9 protein may ini tiate the conserved evolutionary pathway to testis forma tion. So, borrOWing Eric Idle's phrase, Sekido and Lovell Badge (2009) propose that Sry initiates testis formation by "a wink and a nudge,1I Sox9 protein has several functions. First, it appears to be able to activate its own prounoter, thereby allowing it to tran scribe for long periods of time. Second, it blocks the ability of -catenin to induce ovary formation, possibly by block ing the FoxL2 gene (Wilhelm et aJ. 2009). Third, it binds to cis-regulatory regions of numerous genes necessary for testis production (Bradford et a1. 2009). Fourth, Sox9 binds to the promoter site on the gene for the anti-MUllerian factor, pro viding a critical link in the pathway toward a male pheno type (Arango et a1. 1999; de Santa Barbara et al. 2000). Fifth, Sox9 promotes the expression of the gene encoding Fgf9, a paracrine factor critical for establishing testis development. Fgf9 is essential for maintaining Sox9 transcription, thereby establishing a positive feed forward loop driving the male pathway (Kim et al' 2007).
fiBROBLAST GROWTH FACTOR 9 When the gene for fibrob last growth factor 9 (Fgf9) is knocked out in unice, the homozygous mutants are almost all female. Fgf9, whose expression is activated by Sox9 (Capel et al.
1999; Colvin et al. 2001), plays several roles. First, it causes proliferation of the Sertoli cell precursors and stimulates their differen tiation (Schmahl et a1. 2004; Willerton et al. 2004). Second, Fgf9 activates the migration of endothelial cells from the 520 CHAPTER 1 4 moe1m2-bDursayeyos o Gonad or Reurodmgimoenveiental gSmeoepnsaordnasetefprhormos XY (r XX + Sry) hcoulrtsure Recombine and 48 + [acZ C3 miSrgya: tAliolnowcfesl s cmeel sonephric (1) cel (2) - STY : No + (1) No mesonephric (C) FIGURE 14,8 M igration of mesoneph ric endothelial cells in10 STY+ gonadal rudiments. In the experiment diagrammed, urogenital ridges (containing both the mesonephric kidneys and bipotential gonadal rudiments) were collected from 1 2-day embryonic mice. Some of the mice were marked with a p galactosidase transgene (/acZ) that is active in every cell. Thus, every cell of these mice turned blue when stained for p galactosidase. The gonad and mesonephros were sepa rated and recombined, using gonadal tissue from unlabeled mice and mesonephros from labeled m ice.
(A) Migration of mesonephric cells into the gonad was seen (1) when the gonadal cells were XY or when they were XX wit h a Sry transgene. No m igrat on of mesonephric tissue into the gonad was seen (2) when the gonad contained either XX cells or XY cells in which the Y chromosome had a deletion in the STY gene. The sex chromosomes of the mesonephros did not affect the migration . (B) Gonadal rudiments for XX m ice could induce mesonephr c cell m igration if these rudiments had been incubat ed with Fgf9. (C) Intimate relation between the mesonephric duct and the developing gonad in a 1 6-day male mouse embryo. The duct tissue wi II form the efferent ducts of the testes and has been stained for cytokeratin 8. (A,B aher Capel et al. 1 999, photographs courtesy of B. Capel; C from Sariola and Saarma 1 999, courtesy of Sariala.) i XX XX or XY XX Xy + lacZ xx i H. '' e nep ric cels (2) Fgf9: Allows migration of m so h SEX DETERMI NATION adjacent mesonephros into the XY gonad.
While this is nor mally a male-specific process, incubating XX gonads in Fgf9 leads to the migration of endothelial cells into XX gonads (Figure 14.8). These endothelial cells form the major artery of the testis and play an instructive role in inducing the Sertoli cell precursors to form the testis cords; in their absence, these cords do not form (Brennan et aJ. 2002; Combes et al. 2009). sF1 : A CRITICAL LINK BETWEEN sRY AND THE MALE DEVElOP MENTAL PATHWAYS The transcription factor steroidogenic factor 1 (5f1) is necessary to make the bipotenlial gonad. Blit whereas Sfl levels decline in the genital ridge of XX mouse embryos, they remain high in the developing testis. It is thought that Sry (either directly or indirectly) maintains Sf} expression. Sf! protein appears to be active in masculin izing both the Leyclig and the Sertoli cells. In the Sertoli cells, Sf} works in collaboration with Sry to activate Sox9 (Seki do and Lovell-Badge 2008) and, working with 5ox9, elevates levels of anti-Milllerian factor transcription (Shen et al. 1994; Arango et aI. 1999).
In the Leyclig cells, Sf} aclivates genes encoding the enzymes that make testosterone 521 become an ovary (Bullejos and Koopman 2005; Wilhelm et al. 2009). This provides an impor tan t clue to how pr imary sex determination may take place.
Tlh11ing is critical.
There may be a brief window during which the testis-forming genes can flmction. If these genes are not turned on, the ovary-fonning pathway is activated.
Hermaphrodites are individuals in which both ovari an and testicular tissues exist; they have either ovotestes (gonads containing both ovarian and testicular tissue) or an ovary on one side and a testis on the other.'" In humans, hermaphroditism is a very rare condition that can result when a Y chromosome i s translocated to an X chromo some. In those tissues where the translocated Y is on the active X chromosome, the Y chromosome will be active and the SRY gene will be transcribed; in cells where the Y chro mosome is on the inactive X chromosome, the Y c1uomo some will also be inaclive (Berkovitz et al. 1992; Margarit et al. 2000). A gonadal mosaidor expression of SRY can develop into a testis, an ovary, or an ovotestis, depend ing on the percentage of cells expressing SRY in the Sertoli cell precursors (see Brennan and Capel 2004). . *Hermaphroditos was the yow,g man in Greek mythology whose wished to be united with him fo rever, and the gods, in their literal beauty inflamed the ardor of the water nymph Salmacis. She The right time and the right place Having the right genes doesn't necessarily mean you'll get the organ you expect. Studies of mice have shown that the Sry gene of some strains of mice failed to produce testes when bred onto a different genetic background (Eicher and Washburn 1983; Washburn and Eicher 1989; Eicher et aJ. 1996). This is attributed to a delay in Sry expression or to the failure of the protein to accumulate to the critical threshold level required to trigger Sox9 expression and launch the male pathway. By the time Sox9 gets turned on, it is too late the gonad is already following the path to fashion, granted her wish. The language used to group these condi tions is being debated.
Hermaphroditism has often been caUed an "intersex" phenotype; but some activists, physicians, and parents wish to eliminate the tenn "intersex" to avoid confusion of these They prefer to call these conditions "disorders of sex development." In contrast, other activists do not want to medicalize this condition anatomical conditions with identity s i sues such as homosexuality. not feel there is anything wrong with their health. For a more and find the "disorder" category offensive to individuals who do detailed Clnalysis of intersexuality, see Gilbert et a1. 2005 or the websites listed at the end of this chapter. SIDEL IGHTS SPECULATIONS Mysteries of Gonad Differentiation T he re are some genes that are defi nitely involved in primary sex determination, but we don't know how they act or what their nor mal functions are. DAXl: A potential testis suppressing gene on the X chromosome One of the most puzzling loci in the ca ta l og of sex determin i ng ge n es is - DAX 1 , locate d on the X ch romosome In XY humans having a duplica tio n of the WNT4 regi on, Daxl pro tei n is overproduced and the gonads develop into ovaries despite the presence of the Y ch rom osom e Uordan et a l . 2001).
Moreover, in XY individuals having a duplication of th e DAX7 gene on the i r X c h rom osom e the SRY signal is inhibited (Figure 1 4.9; Be rnstei n el al.
1 980; B ard on i et al. 1 994). tn XY embryos, DAX 7 normally would be . , suppressed, having two active copies of the gene, however, overrides this suppression. The mouse homologue, Oax 7, was cloned and shown to encode a mem ber of th e nuclear hormone receptor family (Muscatelli et al. 1 994; Zanaria 1 994). In mice, Dax 7 is initially exp ressed in the genital ridges of both male and female embryos prior to the (Continued on next page) S22 CHAPTER 1 4 Genotype DAXl DAXlInactive ofcDopAXlies SIDEL IGHTS & SPECULATIONS (Continued) X Y x X X Y 2 II Figure 14.9 Phenotyp i c sex reversal i n humans having two copies of the DAXl locus. DAXI (on the X chromosome) plus SRY (on the Y chromosome) produces testes. DAX I without SRY (since the other DAXI locus is on the inacti ve X chromosome) produces ovaries. Two active copies of DAX l (on the ac tive X chromosome) plus SRY (on the Y chromosome) lead to a poorly formed gonad.
Since the gonad makes neither anti-Mullerian factor nor testosterone, the phenotype is female. (From Genetics Review Group 1 995.) Sex-specific rnicroRNAs Gonads Tetst.is Ovtary Godysngtaednaesljs Phenotype Male Female Female time that sex determination occurs, but gradually its expression becomes female-specific. As predicted from the data in humans, overexpression of Dax 1 in male mice appears to antago n i ze the function of Sry and Sox9 and downregul ates Sf7 expression (Nachtigal et al. 1 998; Swain et al. 1 998; Iyer and McCabe 2004).
How ever, unexpectedly, the loss of Dax 7 does not disrupt ovary development, but instead impairs testis formation (Meeks et a l . 2003; Bauma et a l . 2005). The apparent contradictory roles of this protein are sti I I not understood. Dmrt genes: Highly conserved testis-deterrnining genes The Omrt (doubles ex and mab-3 relat ed transcription factor) genes are involved in male sex determination throughout the animal kingdom (Ray mond et al. 1 998). The male-specific Doublesex (DsxM) protein i n Drosophi la (which wi l l be discussed later i n the chapter) has a region of amino acids similar to the male-specific regulatory protein Mab-3 in Caenorhabditis e/e gans. Both proteins positively regulate male-specifiC development. Moreover, if the rna le-specific doublesex tran script from Drosophila is inserted into C. e/egans males lacking mab-3, the newly introduced Drosophila message will restore male-specific neurogenesis to the mutant C. elegans strain at levels the same as the introduction of mab-3. The female-specific doublesex tran script will not work. Omrt genes play a critical role i n t h e primary sex determination of Medaka fish, where sex is chromoso mally specified by the Y chromosome (Matsuda et al. 2002). Here a Omrt gene on the Y chromosomes encodes a protein that is made in the somatic cells of the developing testes (and not ovaries) and whose function is critical for testis formation. It probably binds to and activates the Sox9 gene (Bagheri-Fam et al. 2009). In the chicken (and other birds), the male is the homogametic sex (ZZ), and the female is the heterogametic sex (ZW). Smith and colleagues (2009) have shown that the Omrtl gene on the Z chromosome is the major male sex determining gene, and that a double dose of this gene is needed for the gonad to become a testis. Dmrl l, too, appears to activate (either directly or indirectly) the 50x9 gene. In mammals (including humans), Dmrt I , which i s autosomal, appears to function later in testis development. It is no longer involved in specifica tion of the testis, rather it is important in testis differentiation. Kim and col leagues (2007; Hong et al. 2007) showed that Dmrt l in mice is not crit ical for sex determination but is cru cial for Sertoli cel! differentiation and germ cell survival. There is evidence that sexual distinc tions exist even at the b lastocyst stage. Tavakoli and colleagues (2009) have found that XX embryonic stem cells appear to have greater differentiation potential (and slower growth rates) than XY stem cells. This difference may be the result of different populations of microRNAs found i n the XY and XX embryonic stem cells. Ciaudo and col leagues (2009) have found that xx and XY human embryonic stem cells tran scribe different populations of microR NAs, whose functions, though overlap ping, may differentially regulate genes involved in pluripotency and cell cycling. The apparent relationship betvveen cell division rates and sex determ ina tion is provocative. I ndeed, the rapid proliferation of Sertoli cell precursors has been hypothesized to be the uni fying feature in testis determination (Mittwoch 1 986; Schmahl and Capel 2003). In its most extreme form, this hypothesis predicts that anyth ing that increases the division rate of somatic cell precursors in the gonad will d irect gonadogenesis in the male direction.
While we do not know if this is true under a l l conditions, we do know that i n mice, one of the first distinguishing featu res of testis development is an increase in cell proliferation seen i mmediately after Sry expression. There appears to be a threshold n u m ber of Sertol i cells needed for mam malian testis development (Schmahl et 3 1 . 2000). In turtles and alligators, rapid division of Sertoli cell precursors is i n itiated during the critical stage for forming the males (Mittwoch 1 986; Schmahl et al. 2003).
From this per spective, anything that increases the division rate of Sertoli cell precursors might d i rect gonadogenesis in the male direction. SEX DETERMINATION Secondary Sex Determination in Mammals: Hormonal Regulation of the Sexual Phenotype Primary sex determination is the formation of either an ovary or a testis from the bipotential gonad. This process, however, does not give the complete sexual phen otype.
In mammals, secondary sex determination is the develop ment of the female and male ph en o types in response to h ormones secreted b y the ovaries and testes. Both female a nd male secondary sex determination have tw"o major temporal phases. The first phase occurs witrun the embryo during organogenesis; the second occurs at puberty. During embryonic development, hormones and paracrine coordinate the development of the gonads with the development of secondary sexual organs. In females, the Mullerian ducts persist and, through the actions of estrogen, will later differentiate to become the uterus, cervix, oviducts, and upper vagina (see Figure 14.3). The geni ta l lubercle becomes differentiated into the clitoris, and the labioscrotal folds become the labia.
The Wolfhan ducts atrophy in females, since they need testosterone to persist, and become the male accessory organs. As men tioned earlier, if the bipotential gonads are removed from an embryonic mammal, the female phenotype is realized: the Mullerian duct develops and the Wolffian ducts degen erate. This pattern is also seen in certain humans who are born without functional gonads. The coordination of the male phenotype involves the secretion of two testicular factors. The hrst of these is AMF, a BMP - like paracrine factor made b y the Sertoli cells that causes the degeneration of the Mullerian duct. The second \'\'0; Androgen (testosterone or Male: dihydrotestosterone) + androgen receptor Female: I] {Dickkopf. etc.) inhibiLOr Androgen (lC.'1to, . tllhydrolc wsterone) + • • • -t Wnt inhibitor (Dickkopf. etc.) The genetic analysis of secondary sex determination The existence of separate and independent AMF and testosterone pathways of masculinization is demonstrat ed by people with androgen insensitivity syndrome. ... acivity ' ' e-g' tEs r +llrO estrogen rect:pto r ;t Penis r / r Estrogen + ...t (''alenin estrogen receptor / / Urogenital swellings FIGURE 14.10 Maciel for th e iormation of external genitalia. In this schema, the mesenchyme in the urogenita l swellings secretes inhibitors of Wnt signal i ng. In the absence of Wnt signaling, estro- gen modifies the genital tubercle into the clitoris and the labio- scrotal folds into the labia majora surrounding the vagina. In males, however, androgens dihy. (such as testosterone and is the steroid hormone testosterone, which is secreted from the fetal Leydig cells. Testosterone callses the Wolffian ducts to differentiate into the epididymis, vas deferens, and seminal vesicles, and it causes the gerrita l tubercle to develop into the penis and the labioscrotal folds to devel op into the scrotum. The mechanism by which testosterone (and, as we shall see, its more powerful derivative, dihydrotestos terone) masculinizes the external genitalia is thought to involve its interaction with the Wnt pathway (Figu re 14.1 0). The Wnt pathway, which in the genital ridge acti vates the female trajectory, acts in the genital tubercle to activate male development. In one recent model, the mes enchyme in the XX urogenital swellings is seen to make inhibitors of the Wnt pathway (such as DickkopO. This prevents the activity of \Nn t in the mesenchy me and leads to the feminization of the geni ta l tube rc l e by estro gens (Holderegger and Keefer 1986; Miyagawa et aJ. 2009).
[n this case, the genital tubercle becomes the cli toris and the labioscrotal folds become the labia majora. In males, however, testosterone and dihydrotestosterone bind to the androgen (testosterone) receptor in the mes enchyme and prevent expression of the Wnt inhibitors. Thus, when Wnt expression is permitted in the mes enchyme, male urogenital swellings are converted into the penis and the scrotum. 'Vot 1f3-catenio . ... 523 scrot m Clitoris • labia majora drotestosterone) bind to the androgen receptor in the mesen c hy- mal cells and prevent the synthesis of the Wnt inhibitors. Wnt sig. naling is permitted, and it causes the genital tubercle to become the penis and the labioscrotal folds to beco me the scrotum. (After Miyagawa el aJ. 2009.) 524 These CHAPTER 1 4 XY individuals have the SRY gene and thus have testes that make testosterone and AMP. However, they have a mutation in the gene encoding the androgen recep tor protein that binds testosterone and brings it into the nucleus. Therefore these individuals cannot respond to the testosterone made by their testes (Meyer et al.
1975).
They can, however, respond to the estrogen made by their adre nal glands (which is normal for both XX and XY individ uals), so they develop female external sex characteristics (Figure 1 4.1 1) . Despite their distinctly female appearance, these individuals have testes, and even though they can not respond to testosterone, they produce and respond to AMF. Thus, their Milllerian ducts degenerate. Persons with androgen insenSitivity syndrome develop as normal appearing but sterile women, lacking a uterus and oviducts and having internal testes in the abdomen. Such phenotypes, in which male and female traits are seen in the same individual, are called intersex conditions.
Indeed, although most people have a reasonably good cor relation of their genetic and anatomical sexual phenotypes, about 0.4 1.7% of the population differs from the strictly 2000; Hull 2003). dimorphic condition (Blackless et al. Androgen insensitivity syndrome is one of several inter sex conditions that have traditionally been labeled pseudo-hermaphroditism. In pseudohermaphrodites, there is only one type of gonad, but the secondary sex characteristics differ from what would be expected from the gonadal sex. In humans, male pseudohermaphroditism (wherein the FIGURE 14.11 Androgen insensitivity syndrome. Despite havi ng karyotype, individuals with thi s syndrome appear female. They cannot respond to testosterone but can respond to estrogen. so they develop fema le secondary sex cha racteri stics (i.e., labia and a clitoris rather tha n a scrotum and a penis). Internal ly, they lack the MUllerian duct derivatives and have undescended testes. (Courtesy of C. B. Hammon d .) the XY gonadal sex is male and the secondary sex characteristics are female) can be caused by mutations in the androgen (testosterone) receptor or by mutations affecting testos 1994). Female pseudohermaphroditism (in which the gonadal terone synthesis (Geissler et al. The importance of DHT was demonstrated by Impera to-McGinley and her colleagues (1974). They found a small community in the Dominican Republic in which several sex is female but the person is outwardly male) can be inhabitants lacked a functional gene for the enzyme 5a in the ovary ketosteroid reductase 2-the enzyme that converts testos caused by the overproduction of androgens condition is congenital adren al h yp erpl asi a, or adrenal gland. The most common cause of this latter terone to DHT (Andersson et a1. 1991; Thigpen et al. 1992). in which Although XY children with this syndrome have function there is a genetic deficiency of an enzyme that metabolizes In the absence of this al testes, these remain inside the abdomen and do not cortisol steroids in the adrenal gland.
descend. Because they have a blind vaginal pouch and an enzyme, testosterone-like steroids accw:nulate and can bind to the androgen receptor, thus masculinizing the fetus (Migeon and Wisniewski 2001; Merke et a1. 2002). enlarged clitoris, these children appear to be girls, and are raised as such.
Their internal anatomy, however, is male: they have testes, Wolffian duct development, and Muller ian duct degeneration. Thus it appears that the formation TESTOSTERONE AND DIHYDROTESTOSTERONE Although of the external genitalia is under the control of dihy testosterone is one of the two primary masculinizing fac drotestosterone, while Wolffian duct differentiation is con tors, there is evidence that it might not be the active mas trolled by testosterone itself. culinizing hormone in certain tissues. Testosterone appears Interestingly, at puberty, when the testes of children to be responsible for promoting the formation of the male affected with this syndrome produce high levels of testos- reproductive structures (the epididymiS, seminal vesicles, terone, their external genitalia are able to respond to the an and vas deferens) that develop from the Wolffian duct pri hormone and differentiate. The penis enlarges, the scro mordium. However, it does not directly masculinize the Sa-dihydrotestosterone, or DHT 14.1 2). Siiteri and Wilson (1974) showed that testos male urethra, prostate, penis, or scrotum. These latter func tions are controlled by (Fi gu re terone is converted to DHT in the urogenital sinus and swellings, but not in the Wolffian duct. DHT appears to be a more potent hormone than testosterone. tum descends, and the person originally believed to be a girl is revealed to be a young man. See WEBSITE 1 4.3 Dihydrotestosterone in adult men See WEBSITE 1 4.4 Insulin-like hormone 3 SEX D ETERMINATION 525 Brain sex: Secondary sex determination through another pathway? vesicle Vas deferens Epididymis o Dihydrotestosterone-dependent • Testosterone-dependent FIGURE 14.12 Testosterone- and dihydrotestosterone-dependent regions of the human male genital system. (After Imperato McGinley et al . 1 974.) ANTI-MULLERIAN FACTOR Anti-Mullerian factor, or AMF, a member of the TGF family of growth and differenti factors. It is secreted from the fetal Sertoli cells and causes the degeneration of the MUllerian duct (Tran et al.
1977; Cate et al . 1986). AMF is thought to bind to the mes enchyme cells surrounding the Mullerian duct, causing these cells to secrete a paracrine factor that induces apop tosis in the duct's epithelium and breaks down the basal lamina surrounding the duct (Trelstad et al. 1982; Roberts et al.
1999). is ation ESTROGEN The steroid hormone estrogen is needed for complete postnatal development of both the MUllerian and the Wolffian ducts, and is necessary for fertility in both males and females. In females, estrogen induces the differ entiation of the Mullerian duct into the uterus, oviducts, cervix, and upper vagina. In female mice whose genes for estrogen receptors are knocked out, the germ cells die in the adult, and the granulosa cells that had enveloped them start developing into Sertoli-like cells (Couse et al. 1999). Male mice with knockouts of estrogen receptor genes pro duce few sperm. One of the functions of the male efferent duct (vas efferens) cells is to absorb most of the water from the lumen of the rete testis. This absorption of water, which is regulated by estrogen, concentrates the sperm, giving them a longer life span and providing more sperm per ejac ulate. [f estrogen or its receptor is absent in male mice, water is not absorbed and the mouse is sterile (Hess et al. 1997). Altho ugh, in general, blood concentrations of estrogen are higher in females than in males, the concentration of estro gen in the rete testis is even higher than in female blood. We have known for a long time that the brain, like other tissues, is responsive to the steroid hormones produced by the gonads. Now, recent evidence suggests that sex differ ences in the brain can b e observed even before the gonads mature, and that the brain may experience direct regula tion by the X and Y chromosomes (Arnold and Burgoyne 2004). The first indication that something besides the gonadal hormones testosterone and estTogen was imp orta nt in forming sexually different structures in the brain came from studies on Parkinson disease, during which embry onic rat brains were dissected before the gonads matured . These studies indicated that brains from XX embryos had more epinephrine-secreting neurons than XY embryonic brains (Beyer et al. 1991). Later studies, using microar rays and PCR, demonstrated that more than 50 genes in the mouse brain are expressed in sexually dimorphic patterns before gonad differentiation llLls occurreri (Dewing et al.
2003). Moreover, the mouse STY gene, in addition to being expressed in the embryOniC testes, is also expressed in the fetal and adult brain. The human SRY gene appears to be expressed n i the adult brain as well (Lahr et aI. 1995; Mayer et al. 1998, 2000). SRY is specifically active in th e substan tia nigra of the male hypothalamus, where it helps regu late the gene for tyrosine hydoxylase, an enzyme that is critical for the p rod uc tion of the neurotransmi tte r dopamine (Dewing et al. 2006). Stunning demonstrations that sexual d imorphism in the brain can be caused before gonadal hormone synthesis come from natural and experimental conditions in birds. One big difference between male and female finches is that large regions of the male brain are devoted to prodUCing songs. Male finches sing; the females do not. While testos terone is important in the formation of the song centers in finches (and, when added experimentally, can cause female birds to sing) , blocking those hormones in males does not prevent normal development of the song centers or singing. Genetically male birds form these brain regions even without male hormones (Mathews and Arnold 1990). A natural experiment presented itself in the form of a bird that was half male and half female, divided down the middle (Fi g ure 1 4.1 3).
Such anim als, where some body parts are male and others female, are called gynandro morphs (Greek gynos, "female "; andros, "male"; morplros, "form"). Agate and colleagues (2002) showed that the gynandromorph finch had ZZ (male) sex chromosomes on its right side and ZVV (female) sex chromosomes on its left. Its testes produced testosterone, and the bird sang like a male and copulated with females. However, although many brain structures were similar on both sides, some brain regions differed between the male and female halves. The song circuits on the right side had a more masculine phenotype than similar structures on the left, shOWing that both intrinsic and hormonal influences were important.
526 CHAPTER 1 4 . Gahr (2003) genera led his own avian sexual chimeras by surgically switching the forebrain regions (which con trol adult sexual beha viors) between ZZ and ZW quail embryos before their gonads had matured. If hormones were all th a t ma tt e red, the brains of the resu lting birds would be appropriate to the gonad that developed. For the females that received male forebrains, this was indeed the case : they looked and behaved like normal female quaiL However, male birds g iv en female forebrains did not act normally; they did not crow to attract mates, nor did they attempt copulation. Moreover, their testes failed to devel op nonmally, suggesting that (in quail, at least) a chromoso mally male brain is needed to complete development of the testes. Thus, although brain sex is usually correlated with gonadal sex, it seems probable that this harmony is creat ed both by intrinsic, cell-autonomous differences as wen as hormonal regLlation from outside the cell . *.*The sexual characteristics of the tammar wallaby (Macropll's eugenil) appear to be another case where secondary sex lTaits are controUed intrinsically by chromosomal genes rather than extrinsically by ciT cula ting hOimones. In this marsupial, the pouch and its mammary glands are found onJy in th e female, while the scrotum is made only in males. However, the ma rsupial pouch, mammary g].mds, and saohlm are eAch made before the gonads have matured and Right (cf) Left (9) Gynandromorph finch with U (male) cells on its r igh t side and ZW (female) cells on its left side, Since plumage is FIGURE 1 4.13 controlled by genes on the sex chromosom es, the adult fi nch has male plumage on its right and female plumage on its left.
Micro graphs show the difference in brain regions between the right and left sides, in d icated by stai n i ng of the neurons of the HVC nucleus (a neuron cluster involved in bird song production), (From Agate et al. 2002.) SIDEL IG H TS SPECULATIONS Brain Sex: Sex Determinants and Behaviors i n Mice and Rats D oeS p renata l (or neona ta l) expo sure to pa rt i cul ar steroid hor mones Impose permanent sex-specific cha nges on th e centra l nervous system ? Su c h sex-spec ifi c neural c hanges have been shown in regio n s of the brain thal regulate invol u ntary sexual p hys iology. The cyclic secretion of lu tei n izing hormone by the pituitary in adult female rats, for exa m p le, is dependent on the lack of testosterone d u ri ng the first week of the animal's l i fe. The l u te i ni zi ng hor mone secretion of female rats can be made noncyclical by giving them testosterone 4 days after birth. Con versely, the lutein i z i ng hormone secre tion of males can be made cyclical by remov ing their testes within a day of birth (B a rracl ough and Gorski 1 962). u Fetoprotein and the organi zation/activation hypothesis It is believed that sex hormones act during the fetal or neonatal stage of a mammal's l i fe to organize the nervous system in a sex-specifi c manner, and that c1uring adult life, the same hor mones may have transitory motivation al (or "ac tivational ") effects. Th is model of the hormonal basis of sex -sp ec i fic brai n deve lopment and behavior is called the organization/activation hypothesis. Ironical ly, the hormone chiefly responsible for d e term i n i ng the male neural pattern is estradiol, a type of estrogen. Testosterone from feta l or neonatal blood can be converted into estradiol by the enzyme aromatase (Figure 1 4.1 4A) ' This convers i on ""The terms estrogen and estradiol are often used interchangeably, However, estrogen refers to a c l ass of steroid hor mones responSible (among other func tions) for esta bl is h i ng and mai ntain i ng specific female characteristics. Estradiol is one of these hormones, and in most mammals (including humans) it is the most potent of the estrogens. The enzyme's name, aromatase, has n othi n g to do with aroma (although aromas are certainly crucial to rodenl sex), but refers to the destabil ization of hydrogen bonds in the steroid ring structure. SEX DETERMI NATION 527 SIDELIGHTS & SPECULA TIONS (Continued) (A) Aromatase conve rts androgens to estrogens OH Aromatase 0 • HO Estradiol Testosterone (B) OH 'i Ev 30 40 ." Figu re 14.14 Organization of brain devel opment by hormones. enzyme aromatase converts androgens (such as testos terone) into estrogens (such as estradiol).1he name of the enzyme comes from its ability to aromatize the six-carbon rin g by reduc ing Ihe ring-stabilizing keto group (=0) to a hydroxyl group (-OH).
This biochemical change allows the hormones to bind to different receptors (dormant transcription factors) and activate different genes. (B) Female lordosis behavior (in which the female mouse bends her spine so the male can readily mate with her) remains present in mice administered an inhibitor of aromatase; the behavior is abolished when the genes for a-fetoprotein are knocked out (AF-KO). However, when aromatase is blocked in female mice witho ut a-fetoprotei n (AFP-KO-ATD), lordosis behavior is restored . (After Bakker and Baum 2008.) (A) The 0 g. 20 . "2 10 to discriminate male pheromones) are also i mpa i red (Bakker and Baum 2008). 0 .., 0 Wild type Aromatase inhibited AFP- KO AFP-KO-ATD by ATD occurs i n the hypothalamus and limbic system two areas of the brain known to regulate hormone secretion and reprod uctive behavior (Reddy et al. 1 974; McEwen et al. 1 977). Thus, testosterone exerts its effects on the nervous system by being converted into estradiol i n the brain. But the fetal environment is rich in estrogens from the gonads and placen ta. What stops these estrogens from mase u l i nizing the nervous system of a female fetus? In both male and female rats, fetal estrogen is bound by a feto protein, which binds and inactivates estrogen, but not testosterone. Human fetuses, by contrast, do not make a strong estrogen-binding protein and have a much h i ghe r level of free estro gen than do rodent embryos (see Nagel and vom 5aal 2003).
50 although the organization/activation hypothesis explains many of the hor monal effects on rodent development, one of its fundamental assumptions that a-fetoprotei n strongly binds estro gens d u ri ng prenatal d eve lopme n t does not in fact hold true for human s . Relationsh i ps among estradiol, aro matase, and a-fetoprotei n have been analyzed by observing sexual behav iors in mice that have loss-of function mutations for aromatase and a-feto protein.
The brain and the behaviors of mice l acking a-fetoprotei n have been defeminized, showing that a-fetopro tein prevents the female brain from receiving circulating estrogens. I

playing a species-specific courtship song by vibrat ing his wings, licking the female, and finally, curling his abdomen so that he is in a position to mate. Each of these sex-specific courtship behaviors appear to be regulated by the products offruitless, a gene expressed in certain sets of "brain-sex" pathway in Drosophila that provides individ neurons involved with male sexual behaviors (Figure aggression behaviors. Among Drosophila, there are no par hearing, smell, and touel), and in total they represent about 2% of all the neurons in the adult male (Lee et aJ. 2000; Bil uals of each sex with the appropriate set of courtship and ents or other conspecifics to teach "proper" mating behav 1 4.21) . These include subsets of neurons involved in taste, ior, and mating takes place soon after the flies emerge from leter and Goodwin 2004; Stockinger et a\. 2005). Fruitless into the n i sect genome! rons in these circuits die during female development (and their pupal cases. So the behaviors must be "hard-wired" Among male Drosophila, there is one very simple rule of behavior: If the other fly is male, fight it; if the other fly is female, court it (Certel et aJ. 2007; Billeter et aJ. 2008; Wang and Anderson 2010). Although the outcomes of this algorithm are simple, the behaviors of Drosophila courtship and mating are quite complicated. A courting male must also retains certain male-specific neural circuits; the neu in frUitless mutants; see Kimura et aJ. 2005). As with doublesex pre-rnRNA, the Tra and Tra2 proteins splice fruitless pre-mRNA into a female-specific message; the default splicing pattern is male (see Figure 14.18). So the female makes Tra protein and processes thefmit/ess pre-mRNA in one way, whereas the male, lacking the Tra first confirm that the individual he is approaching is a protein, processes the fruitless message in another way. toward the female and follow a specific series of move- sequence in an early exon; therefore the female does not female. Once this is established, he must orient his body *This is not to say thflt flies don't leflrn; indeed, one thing they do learn is to avoid bad sexual encounters. A male who has been brushed off (quite literally) by a female because she has recently mated hesitates before starting to court another female (Siegel and Hall 1979; MacBride et al. l999). However, female fruitless mRNA includes a termination make functional Fruitless protein.

The male, however, makes an mRNA that does not contain the stop codon (Heinrichs et aJ. 1998), and the protein it transcribes is a zinc-finger transcription factor. Using homologous recom bination to force the transcription of particular splicing forms, Demir and Dickson (2005) showed that it is Fruit- 534 CHAPTER '1 4 less, and not the flies' anatomy, that controls their sexual behavior. When female flies were induced to make the male-specific Fruitless protein, they performed the entire male courtship ritual and tried to mate with other females. In normal females, the courtship ritual is not as involved as in males. However, females have the ability to be recep tive to a male's entreaties or to rebuff them. The product of the retained gene (rln) is critical in this female mating behavior. Both sexes express this gene, since it is also i.nvolved in axon pathfinding.

However, female flies with a loss-of-function allele of rln resist male courtship and are thus rend ered sterile by tlleir own behavior (Ditch et aJ. histone 4 (see Figure 2.4).

In this way, transcription factors gain access to the X chromosome at a much higher frequen cy in males than in females hence, "hyper transcription. " 2005). While the sex of most snakes and lizards is determined by sex chromosomes at the time of fertilization, the sex of most turtles anct all species of crocodilians is determined after fertilization, by the embrYOnic environment. In these rep tiles, the temperature of the eggs during a certain period of development is the deciding factor in determining sex, and small changes in temperature can cause dramatic The splicing of the/milless transcripts not only regulates sex-specific courtship patterns, it also regulates sex-specif ic aggression patterns as well. Female flies having a male Fruitless protein not only tend to court females, they also will fight males and try to establish themselves at the top of a dominance hierarchy. Male flies having a mutant/ruit less allele will show female-specific aggression against other females (Vronto u et aJ. 2006). Dosage Compensation In animals whose sex is determined by sex chromosomes, there has to be some mechanism by which the amount of X-chromosome gene expression is equalized for males and females.

This mechanism is known as dosage compensa tion. In Cha pter 2 we discu sse d mammalian X-chromo some inactivation, whereby one of the X chro mosomes is inactivated so that the transcrip tion product level is the same in both XX cells and XY cells. In the worm Cae/lorhab ditis elegans, dosage compensation occurs by lowering the transcription rates of both X chromosomes so that product levels are the same as those of XO individuals. In Drosophila, the female X chromosome s are not sup pressed; rather, the male's single X chromosome is hyper activated. This "hypertranscription" is accomplished at the level of translation, and it is mediated by the Sxl protein . Sxl protein (which you will recall is made by the female cells) binds to the 5' leader sequence arid the 3' untranslat ed regions (UTRs) of the mls2 message. The bound Sxl inhibits the attachment of msl2 mRNA to the ribosome and prevents the ribosome from getting to the mRNNs coding region (Beckman et aJ. 2005). The result is that female cells do not produce Ms12 protein (see Figure 14.17). However, Ms12 is made in male cells, in which Sxl is not present.

Mls2 is part of a protein-mRNA complex that targets the X chro mosome and loosens its chromatin structure by acetylating ENVIRONMENTAL SEX DETERMINATION Temperature-Dependent Sex Determination in Reptiles changes in the sex ratio (Bull 1980; Crews 2003). Often, eggs incubated at low temperatures produce one sex, whereas eggs incubated at higher temperatures produce the other.

There is only a small range of temperatures that p ermits both males and females to hatch from the same brood of eggs. ' Figure 14.22 sho ws the abrupt temp e rature-induced ch ange in sex ra tios for the red-eared slider turtle. If a brood of eggs is incubated at a temperature below 28°C, all the turtles hatching from the eggs will be male. Above 31°C, every egg gives rise to a female. At temperatures in between, the brood will give rise to individuals of both sexes. Variations on this theme also exist. The eggs of the *The evolutionary advantages and disadvantages of temperature dependent sex determination are discussed in Chapter 19. O? "iii • 100 0–0 Trachemys scripta 6 75 '*' " - .g ◆ Alligator mississippiensis 50 h X '" FIGURE 1 4. 22 Temperature-dependent sex determination in three species of reptiles: the American all igator (Alligator missis sippiensis), red-cared slider turtle (Trachemys scripta elegans), and alli gator snapping turtle (Macroclemys temminckl/). (After Crain and Guillette 1 998.) \ 0 u 25 0 0 22 24 26 28 30 Temperature (OC) 32 34 \ 36 SEX DETERMI NATION 535 snapping turtle Macroclemys, for instance, become female at either cool (22°C or lower) or hot (28°C or above) tem of Emys is very low at the male-promoting temperature of As we will see in Chapter 18, there can be multiple path period for sex determination (Desvages et al. 1993; Pieau normal temperature conditions, the sex of the lizard also seen in diamondback terrapins, and its inhibition mas peratures. Betvveen these extremes, males predominate. ways of sex determination in the same individuaL Under Bassiana duperreyi is determined by sex chromosomes (XY males; XX females). However, at low temperatures, the environmental component overrides the genetic sex-deter 25°C. At the female-promoting temperature of 30°C, aro matase activity increases dramatically during the critical et al. 1994). Temperature-dependent aromatase activity is culinizes their gonads (jeyasuria et al. 1994). Aromatase appears to be involved in the temperature-dependent dif ferentiation of lizards and salamanders as well (Sakata et mining mechanism, and all the offspring in cool nests are a1.

2005). It is possible that aromatase expression is activat 2008). matase protein itself may be temperature sensitive. In other male (even if their chromosomes are XX; Radder et al. One of the best-studied reptiles is the European pond turtle, Emys Emys obicularis. In laboratory studies, incubating eggs at temperatures above 30°C produces all females, while temperatures below 25°C produce all male broods. The threshold temperature (at which the sex ratio ed differently in different species. In some species, the aro species, the expression of the aromatase genc may be dif ferentially activated at high temperatures (see Murdock and Wibbels 2006; Shoemaker et aJ. 2007). When turtle gonads are taken out of the embryos and placed in culture, they become testes or ovaries depend is even) is 28.5°C (Pieau et al. 1994).

The developmental ing on the temperature at which they are incubated discovered by n i cubating eggs at the male-producing tem shows that sex determination is a local activity of the "window" during which sex determination occurs can be perature for a certain amount of time and then shifting them to an incubator at the female-prod ucing temperature (and vice versa). 1n Emys, the middle third of development appears to be the most critical for sex determination, and it is believed that the turtles cannot reverse their sex after this period. (Moreno-Mendoza et al. 2001; Porter et al. 2005). This gonadal primordia and not a global activity directed by the pituitary or the brain. By analyzing the timing and reg ulation of sex-determining genes in such cultured gonads, Shoemaker and colleagues (2005, 2007) proposed that female temperatures not only activate aromatase (which would cause ovary formation), but also activate the Wnt4 genc (which suppresses the formation of testes in mam The aromatase hypothesis for environmental sex determination The enzyme aromatase, which converts testosterone into estrogen (see Sidelights & Speculations, p. 528), appears to be a particularly important target for environmental trig gers.

Unlike the situation in mammals, whose primary sex mals). Conversely, male temperatures appear to activate Sox9 to a higher level in the male than in the female, pro moting testis development (Moreno-Mendoza et al. 2001). Estrogens, aromatase, sex reversal, and conservation biology primary sex determination in reptiles and birds is inilu Over the last two decades, data has emerged showing that several of the polychlorinated biphenyl compounds to develop. In reptiles, estrogen can override temperature, inducing ovarian differentiation even at masculinizing tem Bergeron et aJ. 1994, 1999). PCBs can reverse the sex of tur inhibitors of estrogen synthesis produces male offspring, important consequences in environmental conservation determination is a function of the X and Y chromosomes, enced by hormones, and esh"ogen is essential if ovaries are peratures. Similarly, experimentally e?Cposing eggs to even if the eggs are incubated at temperatures that usual (PCBs), a class of widespread pollutants introduced into the environment by humans, can act as estrogens (e.g., see tles raised at "male" temperatures.

This knowledge has efforts to protect endangered species (such as turtles, 1994). The sensitive time for the effects of .estrogens and amphibians, and crocodiles) in which hormones can effect changes in primary sex deternUnation. Indeed, some reptile nation usually occurs (Bull et al. 1988; Gutzke and Chymiy ments to elevate the percentage of females ly produce females (Dorizzi et al. 1 994; Rhen and Lang their inhibitors coincides with the time when sex determi conservation biologists advocate using hormonal treat n i endangered 1988). species (www.reptileconservation.org). ments mentioned above worked by blocking aromatase from herbicides that promote or destroy estrogens. One yield male offspring ' This correlation appears to hold demasculinized frogs after exposure to extremely low The estrogen-synthesis inhibitors used in the experi action, showing that experimentally low aromatase levels under natural conditions as well. The aromatase activity 1994). *One remarkable finding is that the injection of an aromatase inhibitor into the eggs of an all-female, parthenogenelic species of lizards causes the fonnation of males (Wibbels and Crews The survival of some amphibian species may be at risk such case involves the development of hermaphroditic and doses of the weed killer atrazine, the most widely used her bicide in the world (Figure 14.23). Hayes and colleagues (2002a) found that exposing tadpoles to atrazine concen trations as low as 0 . 1 part per billion (ppb) produced gonadal and other sexual anomalies in male frogs.

At 0.1 ppb and higher, many male tadpoles developed ovaries in 536 (A) CHAPTER 1 4 (B) FIGURE 14.23 Demasculinization of frogs by low amounts of atrazine, (A) Testis of a frog from a natural site having 0.5 parts per billion (ppb) atrazine. The testis con tains three lobules that are developing both sperm and an oocyte. (8) Two testes of a frog from a natural site contain ing 0.8 ppb atrazine. These organs show severe testicular dysgenesis, which characterized 28% of the frogs found at that site. (C) Effect of a 46-day exposure to 25 ppb atrazine on plasma testosterone levels in sexually mature male Xenopus. Levels in control males were some tenfold higher than i n control females; atrazine-treated males had plasma testosterone levels at or below those of control females. (A,B aher Hayes et al. 2003, photographs courtesy ofT. Hayes; aher Hayes et al. 2oo2a.) ---r-, .:. 5 (C) ◆ C E S 3 '" v ◆ 2 * ◆ 8 addition to testes.

At 1 ppb atrazine, the vocal sacs (which a male frog must have in order to signal and obtain a potential mate) failed to develop properly. Atrazme induces aromatase, and aromatase can con 2007). In laboratory experiments, the testosterone levels 90% (to levels of con trol females) by 46 days of exposure to 25 ppb atrazine U.s. drinking water is 3 ppb, and atrazine an ecologically relevant dose, since the allowable amount of atrazine in levels can reach 224 ppb in streams of the midwestern United States (Battaglin et al. 2000; Barbash et aJ. 2001). Given the amount of atrazine in the water supply and the sensitivity of frogs to this compound, the situation 0 Control Atrazinemale ces females males vert testosterone into estrogen (Crain et a1.1997; Fan et aJ. of adult male frogs were reduced by 2 tr at d Control location-Dependent Sex Determination Environmental factors other than temperature can also be sex determinants in some species. For example, it has been known since the nineteenth century that the sex of the echi uroid worm Bonellia viridis depends on where a larva set Bonellia larva lands on the ocean tles (Baltzer 1914). If a could be devastating to wild populations. In a field study, floor, it develops into a lO-cm-long female. If the larva is Hayes and his colleagues collected leop a rd frogs and attracted to a female's proboscis, it travels along the tube water at eight sites across the central United States until it enters the female's body. There it differentiates into a minute (1-3 rom long) male that is essentially a sperm (Hayes et al. 2002b, 2003). They sent the w a ter sa mples to two separate laboratories for the determination of atrazine, coding the frog specimens so that the techni cians dissecting the gonads did not know which site the animals came from. The results shoyved that all but one site contained atrazine-and this was the only site from which the frogs had no gonadal abnormalities. At con producing symbiont of the female (Figure 14.25). Another species in which sex determination is affected by the location of the organism is the slipper snail Crepidu la fornicata. In this species, individuals pile up on top of one another in a great mound. Young individuals are always male, but this phase is followed by the degenera centrations as low as 0 . 1 ppb, leopard frogs displayed tion of the male reproductive system and testicular dysgenesis (stunted growth of the testes) or ity. The next phase can be conversion to ovaries. found in the testes In many examples, oocytes were (see Figure 14.23A). a period of labil either male or female, depend ing on the animal's position in the mound. If the snail is attached to a female, it will become male. If such a snail is Concern over atrazine's apparent ability to disrupt sex removed from its attachment, it will become female. Sim- hormones in both wildlife and humans has resulted in bans ilarly, the presence of large numbers of males causes some way; Sweden, and Switzerland (Dalton 2002).

Many geo ual becomes female, i t will not revert to being male graphical and social concerns mediate the amount of 1936; Collin 1995; Warner et aJ. 1996). on the use of this herbicide by France, Germany, Italy; Nor of the males to become females. However, once an individ (Cae atrazine use (Figure 1 4.24), and the company making Many fish change sex based on social interactions; such atrazine has lobbied against the work of independent changes are mediated by the neuroendocrine system (God researchers whose research indicates that it might cause win et aJ. 2003, 2009). Interestingly, altllOugh ti,e trigger of reproductive malfunctions or cancers in wildlife and humans (see Blumenstyk 2003). Endocrine disruptors will be discussed in more detail in Chapter 17. the sex change may be stress hormones (e.g., cortisol) that induce sex-speCific neuropeptides, the effector of the change may be (once again) aromatase. There are two vari- SEX DETERMI NATION SociaJ and geological factors /r◆◆T◆ (soil qp◆'T:i, Hydrology Geology Curronl Past Zine use Meterology ◆ Atrazine exposure Molecular biology 537 FIGURE 14.24 Poss i ble chain of ca usa tion leading to the feminization of male frogs and the decline of frog populations in regions where atrazine has been used to control weed populations. Socia', geo logical, and bio log ica l agents are shown. CYP 1 9 is the gene encoding aromatase, and it has been shown that transcription of the human CYP T 9 gene is induced by atrazine (Sanderson et al. 2(00). (From Hayes 2005.1 CYP19 gene Biochemis[ry Biological factors Developmental endrocrinology ◆ / Feminization Demasculinization Physiological! behavioral organismal Evolution / ◆ Decreased reproductive success or in the in the gonads. Black and colleagues (2005) showed a str iking correlation between changes in brain aromatase Jevels and changes in sexual behavior during the sexual transitions of gobies, in which the school typi ants of aromatase in many animals, one expressed brain and one cally contains only one male and multiple females. The increase (>200%) in the aggressive behavior of the largest removal of the male from a stable group caused a rapid female, wruch then became a male in about a week's time. This transformation may have resulted from an increase in brain testosterone levels, since .within hours upon removal of the male, these dominant females developed a lower brain aromatase than the other females (Figure 14.26). The gonadal aromatase levels, however, stayed the same, and gonadal sex change came later. In porgy fish, I '" . ◆ ◆ 10 cm I ◆+ ' 1 Proboscis (may- extend meter) Male Bonel/in (lives symbiotically inside the reproductlve organs of the female) aromatase inhibitors can block the natural sex change and induce male development (Lee et al. 2002). Thus, changes in the social group, perceived by the nervous system, became expressed by the hormonal system within hours, thereby changing the behavioral phenotype of the female fish. Interestingly, when it comes to behaviors in fish, sex is in the brain before it is in the gonads.

See WEBSITE 14.5 Forms of hermaphrodilism FIGURE 14.25 Sex determ ination in Bonellia viridis. Larvae that settle on the ocean floor become female. The mature female's body is about 1-0 cm long, with a l-meter-long proboscis that emits chemicals which attract other B. viridis l a rvae. Larvae that land on the p(Qboscis are taken in to the female'S body, where they develop and live symbiotically as ti ny (1-3 mm longl males. (A) CHAPTER 1 4 538 • o • FIGURE 14.26 Aggressive behavior and aromatase activity (AA) in the brain and gonads of the goby Lylhrypnus dalli. (A) On day 4 (prior to male removal), there was no statistical difference in aver age daily displacements among the largest females.

On day 5, the male was removed and dominant females increased their aggres sive behavior. (Dominance¥phase fish have no day 5 data because they were sacrificed on day 5 just after.) (B,C) Brain (B) but not gonadal (C) AA was significantly lower in dominance-phase and sex changed individuals compared with control females. Estab lished males had lower brain AA than all other groups and lower gonadal AA than all groups except dominance-phase females. (After Black et al. 2005.) - (B) Brain 12 c e 5 20 r Coda Nature has many variations on her ma st rpiec In some species, including most mammals and insects, sex is deter mined by chromosomes, in other species, sex is a matter of environmental conditions. In yet other species, both environmental and genotypic sex determination function, often in different geographical areas. Different environ mental or genetic stLmuli may trigger sex determh1ation through a series of conserved pathways. As CTews and Bull (2009) have reflected, "it is possible that the develop mental decision of male versus female does not flow through a single gene but is instead determined by a 'par liamentary' system involving networks of genes that have sim ul taneous inpu ts to several components of the down stream cascade." We are finally beginning to understand the mechanisms by which this Umasterpiece of nature" is created. 4 Dominfemal ancee Sex-changed in'?di- vidualO phase Control female or Average for day (male present) Average for day Observation before sacrifice 8 . ◆ 4 - ◆◆◆_◆ [J_ Control DomlnanceSex-changed female phase female individual e O L◆ ◆ ◆ ◆_____(e) Gonads 180 Mal Control an ee ei d- hadn female phoaseinfemal D m c- d ge S x. c n ivi ual a M le Snapshot Summary; Sex Determination 1.

i In mammals, pr m ry sex determination (the deter mination of gonadal sex) is a function of the sex chromosomes. XX individuals are females, XY indi viduals are males. a 2. The Y chromosome plays a key role in male sex determination. XY and XX manunals both have a bipotential gonad. In XY animals, Sertoli cells differ entiate and enclose the germ cells within testis cords. The interstitial mesenchyme becomes the Leydig cells. 3. In XX individuals, the germ cells become surround ed by follicle cells in the cortex of the gonadal rudi ment. The epithelium of the folUdes becomes granulosa cells; the mesenchyme becomes the thecal cells. the 4. In humans, the SRY gene is the testis-determining factor on the Y chromosome. It synthesizes a nucleic acid-binding protein that may function as either a transcription factor or as an RNA splicing factor. It activates the evolutionarily conserved SOX9 gene. 5. The SQX9 gene product also initiate testes for mation. Functioning as a transcription factor, it binds to the gene encoding anti-Mullerian factor is responsible for activating FGF9. Fgf9 and Sox9 pro teins have a positive feedback loop that activates tes ticular development and suppresses ovarian devel opment. can 6. Wnt4 and Rspo-l are involved in ovary formation. These proteins upregulate production of ◆ catenin; the functions of p-ca tenin include promoting the ovarian p thw y of development while blocking testicular pathway of development a a the SEX DETERMINATION the factors produced by the developing gonads. In ent. The proteins translated from both dsx messages male mammals, the Miillerian duct is destroyed by of a set of genes involved in producing the sexually 7. Secondary sex determination in mammals involves are active, and they activate or inhibit transcription the AMF produced by the Sertoli cells, while testos terone produced by the Leydig cells enables the Wolffian duct to differentiate into the vas deferens and seminal vesicle. In female mammals, the Wolffi an duct degenerates with the lack of testosterone, whereas the MUllerian duct persists and is differenti ated by estrogen into the oviducts, uterus, cervix, and upper portion of the vagina . S. The conversion of testosterone to dihydrotestos terone in the genital rudiment and prostate gland precursor enables the differentiation of the penis, scrotum, and prostate gland. 9. 11. dimorphic traits of the fly. B. Sex determination of the brain may have different Drosophila Tra proteins also activate the fruitless gene downstream agents than in other regiOns of the body. in males (but not in females); in mammals, the Y chro mosome may activate brain sexual differentiation independently from the hormonal pathways. 14. Dosage compensation is critical for the regulation of gene expression in the embryo_ With the salne num ber of autosomes, the transcription from the X chro In mammals, one X chromosome of X X mosome must be equalized for XX females and X Y males. Individuals with mutations of these hormones or females is inactivated. I n Drosophiln, the single X thejr receptors may have a discordance between chromosome of XY males is hyperactivated. their primary and secondary sex characteristics. 10. 539 Drosophila, sex is determined by the number of X chromosomes in the cell; the Y chromosome does not In 15. In turtles and alligators, sex is often determined by the temperature experienced by the embryo during the time of gonad determination. Because estrogen is play a role in sex determination. There are no sex necessary for ovary development, it is possible that hormones, so each cell makes differing levels of aromatase (an enzyme that can a sex-determination "decision." However, paracrme factors play impor convert testosterone into estrogen) distinguish male tant roles in forming the genit'" struchlres. from female patterns of gonadal differentiation. The Drosophila Sex-lethal gene is activated in females (by the accumulation of proteins encoded on the X an RNA splicing factor to splice an inhibitory exon 16. Aromatase may be activated by enviroru nental com pounds, causing demasculinization of the male chromosomes) but repressed in males. SxJ protein acts gonads in those animals where primary sex determl- as na tion can be effected by hormones. transformer (tra) transcript. Therefore, female flies have an active Tra protein, while males d o not. from the 12. The Tra protein also acts as an RNA splicing factor to splice exons from the doublesex (dsx) transcript. The 17. In some species, such as Bonellia and Crepidu/a, sex is In determined by the position of the individual with regard to other individuals of the same species. fish, numerous environmental factors-especially dsx gene is transcribed in both XX and XY cells, but its pre-mRNA is processed to form different ent in the population- mRNAs, depending on whether Tra protein is pres- individua L temperatllre and the number of males already pres For Further Reading Complete bibliographical citations for all literature cited in this chapter can be found at the free-access website www.devbio.com . Arnold, A.

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25: 19-29. Stockinger, P., D. Kvitsiani, S. Rotkopf, L. Tirian and B. J. Dickson.

2005. Neural circuitry that governs Drosophila male courtship behavior. Cell 1 2 1 : 795-807. Go Online WEBSITE 14.1 Social critique of sex determination research. In numerous cultures, women are seen as the "default state" and men are seen as having /Isomething extra./I Historians and biologists show that, until recently, such biases characterized the scientific study of human sex determination. WEBSITE 14.2 Finding the male detennining genes. The mapping of the testis-determining factor to the SRY region took scientists mare than 50 years to accomplish.

More over, other testis-forming genes that act downstream of SRY have been found on autosomes. WEBSITE 14.5 Forms of hermaphroditism. In C. elegans and many other invertebrates, hermaphroditism is the gen eral rule. These animals may be born with both ovaries and testes, or they may develOp one set of gonads first and the other later (sequential hermaphroditism) . In some fish, sequential hermaphroditism is se◆n, with an individual fish being female in some seasons and male in others.

- WEBSITE 14.3 Dihydrotestosterone in adult men. The drug finasteride, which inhibits the conversion of testos terone to dihydrotestosterone, is being used to treat prostate growth and male pattern baldness. WEBSITE 14.4 Insulin-like hormone 3. [n addition to testosterone, the Leydig cens secrete insulin-like hormone 3 (INSL3). This hormone is required for the descent of the gonads into the scrotum.

Males lacking INSL3 are infertile because the testes do not descend. In females, lack of this hormone deregulates the menstrual cycle. Outside Sites For discussions of disorders of sexual development, indud ing intersex conditions, see the American Academy of Pedi atrics site at viewlPoint-of-Care-Quick-Reference/397188/allldisor- ders_oCsexual_development. The Intersex Society of North America website, , is an excellent resource for information on human intersex conditions. The Wikipedia entry on inter sexuality, has a good discussion about the language llsed to describe these conditions. Poste m b ryonic Development Metamorphosis, Regeneration, and Aging DEVELOPMENT NEVER CEASES. Throughout life, we continuously generate new blood celis, lymphocytes, keratinocytes, and digestive tract epithelium from stem cells. In addition to these continuous daily changes, there are instances in whidl postembryonic development is obviolls-sometimes even startling. One of these instances is metamorphosis, the transition from a larval stage to an adult stage.

In many species that undergo metamorphosis, a large proportion of the animal's structure changes, and the larva and the adult are unrecognizable as being the same individual (see Figure 1.4). Another startling type of postembryonic devel opment is regeneration, the creation of a new organ after the original one has been removed from an adult animaL Some adult salamanderS, for instance, can The earth-bound early stages built enormous digestive tracts and hauled them around on caterpillar treads. Later in the /.ife-history these assets could be liquidated and reinvested in the construction of an entirely new organis1Il-a flying-machine devoted to sex. CARROLL M. WILLIAMS (1958) regrow limbs and tails after these appendages have been amputated. The third category of postnatal developmental change encompasses those alterations of form and function associated with aging in adult organisms. This area is more controversial, with some scientists believing that the processes of tal biology. In this view, aging involves the random decay of normative proces> age-associated degeneration are not properly part of the study of developmen es. Others scientists claim that the genetically determined, species-specific pat terns of aging are an important part of the life cycle and believe that gerontology-the scientific study of aging is rightly part of developmental biology. As Peter Medawar (1957) noted, "That which we call 'development' when looked at from the birth and the life end becomes 'senescence' when looked at from its close." Whatever their relationship to embryonic development, meta morphosis, regeneration, and aging are critical topics for the biology of the twen ty-first century. METAMORPHOSIS: THE HORMONAL REACTIVATION OF DEVELOPMENT Animals (including humans) whose young are basically smaller versions of the adult are referred to as direct d evelope rs In most animal species, however, embryonic development includes a larval stage with characteristics very differ morphosis; these animals are indirect developers. ent from those of the adult organism, which emerges only after a period of meta Very often, larval forms are specialized for some function such as growth or dispersal, while the adult is specialized for reproduction. Cecropia moths, for example, hatch from eggs and develop as Wingless juveniles-caterpillars-for several months. After metamorphosis, the insects spend a day or so as fully f'd give my right arm to know the secret of regeneration. OSCAR E. SCHOITE (1 950) 542 CHAPTER 1 5 TABLE 1 5.1 Summary of some metamorphic changes in anurans System larva Adult Locomotory Aquatic; tail fins Terrestrial; tailless tetrapod Respiratory Gills, skin, lungs; larval hemoglobins Skin, lungs; adult hemoglobins Circulatory Aortic arches; aorta; anterior, posterior, and common jugular veins Carotid arch; systemic arch; cardinal veins Nutritional Herbivorous: long spiral gut; intestinal symbionts; small mouth, horny jaws, labial teeth Carnivorous: short gut; proteases; large moth with long tongue Nervous Lack of nictitating membrane; porphyropsin, lateral line system, Mauthner's neurons Development of ocular muscles, nictitating membrane, rhodopsin; loss of lateral line system, degeneration of Mauthner's neurons; tympanic membrane Excretory Largely amD1onia◆ some urea (ammonotelic) Largely urea; high activity of enzymes of ornithine-urea cycle (ureotelic) Integumental Thin, bilayered epidermis with thin dermis; no mucous glands or granular glands Stratified squamous epidermis with adult keratins; well-developed dermis contains mucous glands and granular glands secreting antimlcrobial pep tides Source: Data from Turner and Bagnara 1976 and Reilly et al. 1994. developed winged moths and must mate quickly before they die. The adults never eat, and in fact have no mouth parts during this brief reproductive phase of the life cycle. As might be expected, the juvenile and adult forms often live in different environments. During metamorphosis◆ developmental processes are reactivated by specific hor mones, and the entire organism changes morphologically, phYSiologically, and behaviorally to prepare itself for its new mode of existence.

Among indirect developers, there are two major types of larvae.* Secondary larvae are found among those ani mals whose larvae and adults possess the same basic body plan. Thus, despite the obvious differences between the caterpillar and the butterfly, these two life stages retain the same axes and develop by deleting and modifying old parts while adding new structures into a pre-existing framework. Similarly, the frog tadpole, although special ized for an aquatic environment, is a secondary larva, organized on the same pattern as the adult will be Uager sten 1972; Ralf and Ralf 2009). Larvae that represent dramatically different body plans than the adult form and that are morphologically distinct from the adult are called primary larvae. Sea urchin lar vae, for instance, are bilaterally symmetrical organisms that float among and collect food in the plankton of the *Although there is controversy on the subject , evolved after the adult form had larvae probably been established. In other words, animals evolved through direct development,, and larval forms came about as specializations for feeding or dispersal during the early part of the life cycle Genner 2000; Rouse 2000; Raff and Raff 2009). Even so, the biphasic life cycle may be a trait characteristic of metazoans (see Degnan and Degnan 2010). open ocean. The sea urchin adult is pentameral (organized on fivefold symmetry) and feeds by scraping algae from rocks on the seafloor. There is no trace of the adult form in the body plan of the juvenile (see Figure 5.22). Amphibian Metamorphosis Amphibians are named for their ability to undergo meta morphosis, their appellation coming from the Greek amph' ("double") and bios ("life"). Amphibian metamorphosis is associated with morpholOgical changes that prepare an aquatic organism for a primarily terrestrial existence. In urode.les (salamanders), these cllanges include the resorp tion of the tail fin, the destruction of the external gills, and a change in skin structure. In anUians (frogs and toads), the metamorphic changes are more dramatic, with almost even- organ subject to modification (Table 15.1 ; see also Figure 1.1). The changes in amphibian metamorphosis are initiat ed by thyrOid hormones such as thyroxine (T4) and trio-iodothyronine (T3) that travel through the blood to reach all the organs of the larva. When the larval organs encounter these thyroid hormones, they can respond in any of four ways: growth, death, remodeling, and respecification. Morphological changes associated with amphibian metamorphosis GROWTH OF NEW STRUCTURES The hormone tri-iodothy ranine induces certain adult-specific organs to form. The limbs of the adult frog emerge from specific sites on the metamorphosing tadpole, and in the eye, both nictitating membranes and eyelids emerge. Moreover, T3 induces the proliferation and differentiation of new neurons to serve POSTEMBRYONIC DEVELOPMENT 543 (B) these organs. As the limbs grow out from the body axis, new neurons proliferate and differentiate in the spinal cord. llese neurons send axons to the newly formed limb mus cu l ature (Marsh-Armstrong et al. 2004). Blocking T3 activ ity prevents these neurons from fOrming and causes paral ysis of the l.imbs. One readily observed consequence of anuran metamor from their originally lateral position (F igure 1 5.1).' The lat phosis is the movement of the eyes to the front of the head eral eyes of the tadpole are typical of preyed-upon herbi vores, whereas the frontally located eyes of the frog befit its more predatory lifestyle. To catch its prey, the frog needs to see in three dimensions, That is, it has to acquire a binoc (e) (D) ularfield ofVisiOI1, where inputs from both eyes converge in the brain (see Chapter 1 0). In the tadpole, the right eye innervates the left side of the brain, and vice versa; there are no ipSilateral (same-side) projections of the retinal neu rons. During metamorphosis, however, ipsilateral path ways emerge, enabling input from both eyes to reach the same area of the brain (Currie and Cowan 1974; Hoskins Xenopus, these new neuronal pathways result not from and Grobstein 1985a). In the remodelIng of existing neurons, but from the formation of new neurons tllat differentiate in response to thyroid hor mones (Hoskins and Grobstein 1985a,b).

The ability of tl,ese axons to project ipsilaterally results from the induction of ephrin B in the optic chiasm by the thyroid hormones (Nak agawa et al. 2000).

Ephrin B is also found in the optic chi asm of mammals (which have ipsilateral p rojections throughout life) but not in the chiasm of fish and birds (which have only contralateral projections). As shown in Chapter 10, ephrins can repel certain neurons, causing them to project in one direction rather than in another. CEll DEATH DURING METAMORPHOSIS The hormone T3 also induces certain larval-specific strue tures to die.

Thus, T3 FIGURE 15.1 Eye migration and associated neurona l changes during metamorphosis of the Xenopus faevis tad pol e. (A) The eyes of the tadpole a re laterall y placed, so there is relatively li«le binocular field of vision. (B) The eyes migrate oo rsa lly and rostral ly during metamorphosis, creating a l arge binocular field for the adu lt frog. (CD) Retinal projections of metamorphosing tadpole. The dye Dil was placed on a cut stump of the optic nerve to label the retinal projection . (C) In early and middle stages of metamor phosis, axons project across the midline (dashed line) from one side of the b rai n to the other. (0) In late metamorp hosis, ephri n B is produced in the o pti c chiasm as certain neurons (arrows) are formed that project i ps ilatera l ly. (A,S from Hoskins and Grobstein 1 984, cou rtesy of P. G ro ste i n ; C,D from Nakagawa et al. 2000, cou rtesy of C. E. Holt .) b causes the degeneration of the paddle-like tail and the oxy gen-procuring gills that were important for larval (but not adult) movement and respiration. While it is obvious that the tadpole'S tail muscles and skin die, is this death mur der or suicide? In other words, is T, telling the cells to kill themselves, or is T3 telling something else to kill the cells? jinla et al. 2005). This was confirmed by the demonstration that the apoptosis-inducing enzyme caspase-9 is lmpor◆ tant in causing cell death in the tadpole muscle cells (Rowe et al. 2005). However, later in metamorphosis, the tail mus cles are destroyed by phagocytOSiS, perhaps because the Recent evidence suggests that the first part of tail resorp- extracellular matrix that supported the muscle cells has tion is caused by suicide, but that the last remnants of the been digested by proteases. tadpole tail must be killed off by other means. When tad Death also comes to the tadpole'S red blood cells. Dur pole muscle cells were inject ed with a dominant negative ing metamorphosis, tadpole hemoglobin is changed into T3 re cep tor (and therefore could not respond to T,), the muscle cells survived, indicating that T3 told them to kill tllemselves by apoptosis (Nakajinla and Yaoita 2003; Naka- adult hemoglobin, which binds oxygen more slowly and releases it more rapidly (McCutcheon 1936; Riggs 1951). The red blood cells carrying the tadpole hemoglobin have a different shape than the adult red blood cells, and these 'One of the most spectacular movements of eyes during metamor phosis occurs in flatfish such as flounder. Originally, a flounder's eyes, like the lateral eyes of other fish species, are on opposite sides of its face. However, during metamorphosis, one of the eyes migrates larval red blood cells are specifically digested-"

takes place in a "larval" body. The Mexican axolotl, Ambystoma mex;canum, does not undergo meta morphosis i n nature because its pitu itary gland does not release the thy rotropin (thyroid-stim ulating ho rmone) (B) Figure 1 5.6 Metamo rphosis in Ambystoma. (Al Norma l adult Ambystoma, with prominent gills and broad tail. (B) Metamorphosed Ambystoma not seen in natu ral popul ation s. This individual was grown in water su ppl emented with thyroxi ne . Its gills have regressed, and its skin has changed significantly. (A © Mark BoultoniAlamy; B courtesy of K. Crawford.) that would activate T4 synthesis (Prahlad and Delanney 1 965; Norris et a l . 1 973; Taurog et al. 1 974). The axolotl does synthesize functional thy roid ho rmo ne receptors, however, and when investigators administered either thyrOid hormones or thyrotropin, they found that the salamander metamor phosed into an adult form not seen in nature (Figure 1 5.6; Huxley 1 920; Safi et al. 2004). Other species of Ambysloma, such as A. tigrinum, metamorphose only i n response to cues from the envi ron ment. In parts of its range, A tigrinum is neotenic: its gonads and germ cells mature and the salamander mates suc cessfully while the rest of the body retains its aquatic larval form. How ever, in other regions of its range, the l a rva l form is transitory, leading to the POSTEMBRYONIC DEVELOPM ENT 549 SIDEL IGHTS & SPECULATIONS (Continued) land-dwel ling adult tiger salamander. The abil ity to remain aquatic is highly adaptive i n locations where the terres trial environment is too dry to sustain the adult form of this salamander IDuellman and Trueb 1 986). Some salamanders are permanently neotenic, even in the labo ra tory Whereas T3 is able to produce the long l ost adult form of A. mexicanum, . t he neotenic species of Necturu5 and Siren remain u n responsive to thyroid hormones (Fri eden 1 98 1) .

Strangely, Necturus was recently found to have functional thyroid hormone receptors. It appears that these receptors do not bind to those genes that i nitiate and promote metamorphosis (Safl et a l . 2006; Vlaeminck Guil lem e t a l . 2006). De Beer (1 940) and Gould (1977) have spec u l ated that neoteny is a major factor in the evolution of more co mp lex taxa.

By retarding the devel opment of somatic tissues, neoteny may give natural selection a flexible substrate. Accord ing to Gould (1 9 77, p. 283), neoteny may Hprovide an escape from spec ialization. Animals can relinquish their highly spec ialized adult forms, return to the labil ity of youth, and prepare themselves for new evolutionary d i rections. - " Progenesis In progenesis, gonadal maturation is accelerated while the rest of the body develops norma l l y to a certain stage. Progenesis has enabled some salaman niches. BoNtag/ossa occidentalis is a tropical salamander that, u n l ike other der species to find new ecological members of its genus, l ives in trees. Th i s salamander's webbed feet and small body size suit it for arboreal exis tence, the webbed feet p rod u c i n g suc tion fo r c limbing and the small body making such traction efficient.

Alberch and Alberch (1 98 1) showed that B. occidentalis resembles juveniles of the related species B. subpalmata a nd B. rostrata (whose young are small, with digits that have not yet grown past their webbing). B. occidentalis reaches young at a much smaller size than its relatives, and this appears to have given it a phenotype that made tree-dwelling possible. D i rect development Wh i l e some animals have extended their larval life stage, others have " accelerated" their development by abandoning their larval form for d i rect development. Thus there are frog species that lack tadpoles and sea urchins that have no pluteus larvae. Elinson and his colleagues (del Pino an d Eli nson 1 983; Elinson 1 987) have studied Eleutherodactylus coqui, a small frog that is one of the most abundant vertebrates on the island of Puerto Rico. U n l ike the eggs of Rana and Xenopus, the eggs of E. coqui are ferti l ized while they are still in the female's body. Each egg is about 3 . 5 m m i n d i ameter (roughly 20 times the volume of a Xenopus egg). After the eggs are laid, the male gently sits on the developing embryos, protecting them from predators and desiccation (Taigen et al. 1 984).

Early E. coqui development is like that of most frogs.

Cleavage is holoblastic, gastrulation is i n i tiated at a subequatorial position, and the neu ral folds become elevated from the su rface. However, shortly after the neural tube closes, limb buds appear on the surface (F igure 1 5.7A,B). This early emergence of l i m b buds is the first indication that this animal w i l l not pass through the usual limbless tad pole stage. Moreover, the develop ment of E. caqui is modified such that the modeling of most of its featu res including its limbs does not depend on thyroid hormones. Its thyroid gland does develop, however, and thyroid hormones appear to be critical for the eventual resorption of the tail (which is used as a respiratory rather than a locomotor organ) and of the primitive kidney (Lynn and Peadon 1955). It appears that the thyroid-dependent phase has been pushed back into embryonic growth (Hanken et al. 1 992, Callery et a l . 200 1) . What emerges from the egg jelly 3 weeks after fert i l ization is not a tadpole but a tiny frog (Figure 15.7C) . (Continued on next page) Figu re 15.7 D i rect development of the frog Eleutherodactylus coqui.

IA) Limb buds a,e seen as the embryo develops on the yolk (B) As the yolk is used up, the limb buds are easily seen. (C) Three weeks after fertil ization, tiny froglets hatch . They are seen here in a petri dish and on a Canadian dime. (Courtesy of R. P. El inson .) . (A) (B) (C) 550 CHAPTER I S SIDELIGHTS & SPECULATIO NS (Co ntin ued) D irect developing frogs do not need ponds for their larval stages and can therefore colonize habitats that are inaccessible to other frogs. Direct development also occurs in other phyla, in which it is also correlated with a large egg.

It seemS that if nutri tion can be provided in the egg, the l ife cycle need not have a food gather ing larval stage. Tadpole-rearing behaviors Most temperate zone frogs do not invest time or energy in providing for their tadpoles. However, among tropi cal frogs, there are numerous species in which adult frogs take painstaking care of their tadpoles. An exampl e i s the poison arrow frog Dendrobates, found i n the rain forests of Central and South America. Most of the time, these highly toxic frogs live in the leaf l i tter of the forest floor. After the eggs (B) are laid in a damp leaf, a parent (sometimes the male, sometimes the female, according to the species) bers--or is informed about-where stands guard over the eggs. If the the tadpoles have been deposited. ground gets too dry, the frog w i l l uri Brooding frogs carry their develop ing eggs in depressions i n their skin. nate on the eggs to keep them moist. Some species brood their tadpoles i n When the eggs mature into tadpoles, their mouth and spit out their progeny the guarding parent a llows them to wriggle onto its back (Figu re 1 5.8A). when their tadpoles undergo meta The parent then c l i mbs into the morphosis. Even more impressive, the gastric-brooding frogs of Austral ia, canopy until it finds a bromeliad plant with a small pool of water in its leaf Rheobatrachus silus and R. vitellin us, eat their eggs. The eggs develop into base. Here it deposits one of its tad larvae, and the larvae undergo meta pole s, then goes back for a nother, and morphosis in the mother's stomach. so on until the entire brood has been placed into numerous small pools. The About 8 weeks after being swal lowed al ive, about two dozen small frogs female returns each day to these pools emerge from the female's mouth (Fig and deposits a sma l l number of Lmfer ure 1 5.8B; Corben et a l . 1 974; Tyler t i l i zed eggs into them, thus replenish ing the tadpoles' food supply until 1 983). What stops the Rheobatrachus they complete metamorphosis eggs from being digested or excreted?

It appears that the eggs secrete (Mitchell 1 988; van Wijngaarden and . prostaglandins that stop acid secretion Bolanos 1 992; Brust 1 993). It is not and prevent peristaltic contractions i n known how the female frog remem- Metamorphosis i n Insects Whereas amphibian metamorphosis is largely characterized by the remodeling of existing tissues, insect metamorpho sis primarily involves the destruction of larval tissues and their replacement by an ent:irely different population of cells. Insects grow by molting-shedding their cuticle-and form ing a new cuticle as their size increases. There are three Figure 15.8 Parental care of tadpoles. (A) Tadpoles of the poison arrow frog Den drobales are carried on their parent's back to small pools of water i n the Peruvian rain forest canopy. (6) This female Rheobatra- chus of Australia brooded more than a dozen tadpoles in her stomach . They emerged after com pl e tin g metamorphosis. Unfortunately, the last time anyone saw a Rheobarrachus frog alive was in 1 985. (A © Michael Dool ittl cJAl amy; B cou rtesy of M. Tyler.) the stomach (Tyler et a l . 1 983). During this time, the stomach is fundamental ly a uterus, and the frog does not eat. After the oral birth, the parent's stom ach morphology and function return to normal.

Unfortunately, both of these remarkable frog species are now feared extinct.

No member of either Rheobatrachus species has been seen since the mid-1 980s. Inajor patterns of insect development. A few insects, such as springtails, have no larval s tage and undergo direct devel opment. These are called the ametabolous i nsects (Figu re 1 5.9A) .

Immedia tely after they hatch, these insects have a pronymph stage bearing the structures that enabled it to get out of the egg. But after this h'ansitory stage, the insect looks like a small adult, it grows larger after each molt but is unchanged in form (Truman and Riddiford 1999). POSTEMBRYO N I C DEVELOPMENT (A) AMEDEVETWABPOMWUSENT (B)) DEIEMVEIMWEPTMABEONWUST DEHOLVEOWMEP'MIAEBNOTLWUSarva P r o n y m p h (i n s t r (,m"_ Ny(insmtaprh1) Larva ◆ m p h ◆Ny ◆ (i n s t a r) 2 ◆◆ (c) ◆ t ◆◆ t ◆ ◆ r ◆◆.◆ ◆ t I) (instar 2) t t p) molt J Metamorphic T (p u al 551 FIGURE 15.9 Modes of insect development. Molts are represented as arrows. (A) Ametabolous (direct) development in a silverfish.

After a brief pronymph stage, the insect looks like a small adult. (8) Hemimetabolous (gradual) metamor phosiS in a cockroach. After a very brief pronymph phase, the insect becomes a nymph. Aher each moll, the next nymphal instar looks more like an adult, gradually growing wings and genital organs. (C) Holometabolous (complete) meta, morphosis in a moth. After hatChing as a larva, the insect undergoes suc cessive larval molts until a metamor phic molt causes it to enter the pupal stage. Then an imaginal molt turns it into an adult.

j◆◆It Other insects, notably grasshoppers and bugs, undergo 15.9B). a gradual, hemimetabolous metamorphosis (Figure a pupa. The pupa does not feed, and its energy must come from those foods it ingested as a larva. During pupation, After spending a very brief period of time as a pronyrnph adult structures form and replace the larval structures. (whose cutide is often shed as the insect hatches), the insect Eventually, an imaginal molt enables the adult (imago) to looks like an immature adult and is called a nymph. The shed its pupal case and emerge. While the larva is said to rudiments of the wings, genital organs, and other adult hatch from an egg, the imago is said to ec/ose from the pupa. mature with each molt. At the final molt, the emerging insect Imaginal discs structures are present and become progressively more is a winged and sexually mature adult, or.imago. In the holometabolous insects such as flies, beetles, moths, and butterflies, there is no pronymph stage (Figure In holometabolous insects, the transformation from juve- nile into adult occurs within the pupal cuticle. Most of the 1 5.9C). The juvenile form that hatches from the egg is larval body is systematically destroyed by programmed cell a larva. The larva (a caterpillar, grub, or maggot) cell death, while new adult organs develop from relative undergoes a series of molts as it becomes larger. The stages ly undifferentiated nests of imaginal cells. Thus, within between these larval molts are called instars. The number any larva there are two distinct populations of cells: the of larval molts before becoming an adult is characteristic larval cells, which are used for the functions of the juve of a species, although environmental factors can increase nile insect, and thousands of imaginal cells, which lie with or decrease the number. The larval instars grow in a step wise fashion, each instar being larger than the previous one. Finally, there is a dramatic and sudden transforma tion between the larval and adult stages: after the final instar, the larva undergoes a metamorphic molt to become in the larva in dusters, awaiting the signal to differentiate. There are three main types of imaginal cells: 1. The cells of imaginal discs will form the cuticular struc tures of the adult, including the wings, legs, antelmae, eyes, head, thorax, and genitaUa (Figure 1 5.1 0), 552 CHAPTER 1 5 Discs for: Frontal plate and upper lip /-- Antenna _ _ _

undersides of pond p lant s The "head" con. ·The Hydra is another character from Greek mythology. Whenever one of this serpenl's many heads was chopped off, it regenerated two new ones. Hercules finally defeated the Hydra by cauterizing the stumps of its heads with fire. Hercules seems to have had a Sig nificant interest in regenera tion he also finally freed the bound Prometheus, thus stopping his daily hepatectomies (see p. 570). ·· (A) tion, l i miting Meis gene products to the proximal regio n of the limbs.·
Dur ing regeneration of the salamander limb, the Meis genes are activated by RA and also appear to be associated with the proximal identities of the limb bones. Overexpression of Meis genes in the d i stal blastema cells caus es these cells to relocalize in proximal locations, whereas antisense oligonu cleotides that b loc k Meis expression inhibit RA from proximalizing the regenerating limbs (Mercader et a l ·
2000, 2005).·
Hoxa a n d Meis genes appear to be the targets of RA's abili ty to spec ify proximal cell fate during regeneration. sists of a conical hypostome region (containing the mouth) and a ring of tentades (which catch food) beneath it. Hydra, like all cnidarians, has only ectoderm and endoderm; these animals lack a true mesoderm. Hydras can reproduce sex- ually, but they do so only under adverse conditions (such as crowding or cold weather).·
They usually multiply asex ually, by budding off a new individllal (Figure ls.25A) . The buds form about two-thirds of the way down the animal's body axis. A hydra's body is not particularly "stable." In humans and flies, for instance, a skin cell in the body's trwlk is not expected to migrate and eventually be sloughed off from the face or foot. But that is precisely what does happen in Hydra. The cells of the body column are constantly W\der- (B) Tentacle Budding in Hydra. (A) A new individual buds about two-thirds dow n the side of an adult hydra . (B) Cell movements in Hydra were traced by fol lowing the migration of labeled tissues. The arrows indicate the starting and leav ing positions of the labeled cells. The bracket indicates regio n s in which no net cell movement took place. Cell division takes place throughout the body co lu m n except at the tentacles and foot. (A CO Bio photo/Photo Researchers Inc., B after Steele 2002.) FIGURE 15.25 '"·,,"J.) ' ' Li ttl e or no cell movemellt 20daySi Foot (Little ------ or nO cell movement)
POSTEMBRYONIC DEVELOPMENT 567 If a hydra's body column is cut into several pieces, each piece will regenerate a head at its original apical end and a foot at its original basal end. No cell ctivision is required a sma ll hyd ra Since each cell retains its plasticity, each piece can re·form a smaller organism; this is the basis of morphallaxis. • for this to happen, and the result is . The head activation gradient Every portion of the hydra body column along the apical basal axis is potentially able to fonm both a head and a foot. (B) I n d u c e d b a s a l i s c / dBascal We a k a p i c a l i n d u c t i o n (C) Hyposlome / is I However, the polarity of the hydra is coordinated by a series of morphogenetic gradients that permit the head to form only at one place and the basal disc to form only at another. Evidence for such gradients was first obtained from grafting experiments begun by Ethel Browne in the early 1900s. When hypostome tissue from one hydra is transplanted into the middle of another hydra, the trans p l anted tissue forms a new apical-basal axis, with the hypostome extending outward (Figure 1 5.26A). When a basal disc is grafted to the middle of a host hydra, a new axis also forms, but with the opposite polarity, extending a basal disc (Figure 1 5.26B).·
When tissues from both ends are transplanted simultaneously into the middle of a host, no new axis is formed, or the new axis has Httle polarity (Figure 15.26C; Browne 1909; Newman 1974). These exper iments have been rnterpreted to indicate the existence of a head activation gradient (highest at the hypostome) and a foot activation gradient (highest at the basal disc). The head activation gradient can be measured b y djsc Basal implanting rings of tissue from various levels of a donor hydra into a particular region of the host trunk (Wilby and Webster 1970; Herlands and Bode 1974; MacWilliams 1983b). The higher the level of head activator in the donor tissue, the greater the percentage of implants that will induce the formation of new heads. The head activation elise.·
Three peptides have been associa ted factor is concentrated in the head and decreases linearly FIGURE 15.26 Di fferent morphogenetic capabilities in different regi ons of the Hydra Clpical basal axis. (A) ◆yposlome tissue graft ed onto a host trunk induces a secondary axis with an extended hypostom e. (B) Basal disc tissue grafted onto a host trunk induces a s econdary axis with an extended basal disc. (C) If hypostome and basal disc tissues are transplanted together, only weak (if any) inductions are seen. (After Newman 1 9 74.) toward the basal with this head activation gradient. Two of them, Heady and Head Activator, are critical for head fom1ation and the initiation of the bu d . The other, Hym301, regulates the number of tentacles formed (Takahashi et a!. 2005). The head inhibition gradient If the tissue of the Hydra body column is capable of lonm ing a head, why doesn't it do so? In 1926, Rand and col leagues showed that the nonmal regeneration 01 the hypos going mitosis and are eventually displaced to the extrem tome is inhibited when an intact hypostome is grafted ities of the column, from which they are shed (Figure adjacent to the amputation site. Moreover, if a graft of sub 1 5.25B; Campbell 1967a,b). Thus, each cell plays several hypostOlnal tissue (from the region just below the hypos roles, depending on how old it is; and the signals specify ing cell fate must be active all the time. In a sense, a hydra's tome, where there is a relatively high concentration of head body is always regenerating. second ary axis forms (Figure See VADE MECUM Planarian regeneration activator) is placed in the same region of a host hydra, no 1 5 .27A). The host head appears to make an inhibitor that prevents the grafted tis sue from forming a head and secondary axis. However, if one grafts subhypostomal tissue to a decapitated host 568 (A) CHAPTER 1 5 Intact host. No secondary axis induced ---- ◆ . ·,,. Host's head removed. e o da - G rafting experiments providing evidence for a head inhibition gradient. (A) Subhypostomal tissue does not gener- FIGURE 1 5.27 ate a new head when placed close to an existing host head. (B) Subhypostomal tissue generales a head if the existing host head is removed. A head also forms at the site where the host's head was ampulaled. (c) Subhypostomal lissue generates a new head when placed far away from an existing host head. (After Newman 1 974.) (B) hydra, a second axis does form (Figure 15.27B) . A gradient of this inhibitor appears to extend from the head down the body column, and can be measured by grafting subhypos tarnal tissue into various regions along the trunks of host - S c n ry axis induced ....·
◆ . · hydras. This tissue will not produce a head when implant a head if placed lower on the host (Figu re 1 5 .27C) . ed into the apical area of an intact host hydra, but it will form Thus, there is a gradient of head inhibitor as well as head activator (Wilby and Webster 1970; MacWilliams 1983a). (C) In tact host: Graft away from head The hypostome as an "organizer" Ethel Browne (1909; Lenhoff 1991) noted region induces secondary axis that the hypos tome acted as an Horganizer" of the hydra. This notion has been confirmed by Broun and Bode strated that (1) (2002), who demon • when transplanted, the hypostome can induce host tissue to form a second body axis, (2) the • hypostome produces both the head activation and head inhibition signals, (3) the hypostome is the only "self-dif ferentiating" region of the hydra, and (4) the head inhibi tion signal is actually a signal to inhibit the formation of new organizing centers.·
See WEBSITE 1 5 .7 Ethel Browne and the organizer ink (colloidal By inserting small pieces of hypostome tissue into a host hydra whose cells were labeled with India ing head tissue came from host tissue, not from the differ entiation of donor tissue (Figure 15.28A). In contrast, when tissues from other regions (such as the subhypostomal region) were grafted into a host trunk, the head and a pi carbon), Broun and Bode found that the hypostome cal trunk of the new hydra were made from the grafted induced a new body axis and that almost all of the result- dOlwr tissue (Figure 15.28B). In other words, only the hypos- .·
(A) FIGURE 15.28 formation of secondary axes following transplantation of head regions into the trunk of a hydra. The host endoderm was stained with India ink. (A) Hypostome tissue grafted onlo the trunk induces the host's own trunk tissue to become tentacles and head. (8) Subhy postomal donor tissue placed on the host trunk self-differentiates into a head and upper trunk. (From Broun and Bode 2002, courtesy of H. R. Bode.) (B) POSTEMBRYONIC DEVELOPMENT (A) (0) (C) (B) 569 , • FIGURE 15.29 Expression of a Wnr gene during Hydra budding. (A) Early bud. (B) Mid-stage bud. (() Bud with early tentacles. (0) Adult with late bud. (From Hobmayer et al. 2000, courtesy oiT. W. Holstein and B. Hobmeyer.) The basal disc activation and inhibition gradients Certain properties of the basal disc suggest that it is the source of both a foot inhibition and a foot activation gra tome region could alter the fates of the trunk cells and dient (MacWilliams et al. cause them to become head cells. Broun and Bode also Sclunidt and Schaller found that the signal did not have to emanate from a per 1999). The inhibition gradients for the head and the foot 1970; Hicklin and Wolpert 1973; 1976; Meinhardt 1993; Grens et al. manent graft. Even transient contact with the hypostome may be important in determining where and when a bud region was sufficient to induce a new axis from a host can form. hydra.·
In these cases, foot inhibitors appear to block bud fonmation. However, all the tissue of the new axis came In young adult hydras, the gradients of head and from the host. The head inhibitor appears to repress the as the hydra grows, the sources of these labile substances effect of the inducing signal from the donor hypostome, grow farther apart, creating a region of tissue about two and it normally functions to prevent other portions of the thirds down the trunk where levels of both inhibitors are hydra from having such organizing abilities. At least three genes are known to be active in the hypo minimal. This region is where the bud forms (Figure stome organizer area, and their expression in this cnidar 1 5.30A; Shostak 1974; Bode and Bode 1984; 1999). ian structure suggests an evolutionarily conserved set of signals that function as organizers throughout the animal kingdom . First, a set of Hydra Wnt proteins is seen in the Schiliro et aJ. Certain mutants of Hydra have defects in their ability to form buds, and these defects can be explained by alter apical end of the early bud, defining the hypostome region ations of the inhibition gradients. The L4 mutant of Hydra magnipapillata, for instance, forms buds very slowly, and as the bud elongates These proteins act to only after reaching a size about twice as long as wild-type form the head organizer: Signaling through the canonical individuals. The amow)t of head inhibitor in these mutants (Figure 1 5.29). Wnt pathway, they inhibit GSK3 to stabilize p-catenin in was found to be much greater than in wild-type the cell nucleus' (Hobmayer et at. (Takano and Sugiyama Lengfeld et at. 2000; Broun et at. 2005, 2009, also see Bode 2009).·
If GSK3 is inhib ited throughout the body axis, ectopic tentacles form at all Hydra 1983). Several small peptides have been found to activate foot formation, and researchers are just beginning to sort out levels, and each piece of the trunk has the ability to stim the mechanisms by which these proteins arise and fune ulate the outgrowth of new buds. The expression of anoth tion (see Harafuji et at. er vertebrate organ..izer molecule, Goosecoid, is restricted the specification of cells as they migrate from the basal to the Hydra hypostome region. Moreover, when the 2001; Siebert et aJ. 2005). However, region through the body column may be mediated by a gradient of tyrosine kinase. The product of the adult hydra, it induces expression of the gene Brachyury gene, sllinguard is a tyrosine kinase that extends in a gradient from hypostome is brought into contact with the trunk of an just as vertebrate organizers do- f!ven though hydras lack the ectoderm just above the basal disc through the lower mesoderm (Broun et al. region of the trunk. Buds appear to form where this gradi 1999; Broun and Bode 2002). ent fade, (Figure *Wnts and p-cateruns also appear to regulate anterior posterior polarity during planarian (flatvonn) regeneration (Gurley et al. 2008; tails), Petersen and Reddien 2008).·
Here, 1}-catenin is expressed (via Wnts) in the posterior-facing blastema (which generates and jj RNA interference against p-catenin eliminates this protein, the posterior facing blastema forms a head. A frizzled-like repressor of Wnt signal ing forms in the anterior-facing blastema (which forms heads). 15.30B). The s"inguard gene appears to be activated through the product of the manacle gene, a puta tive transcription factor that is expressed earlier in the basal disc ectoderm. The inhibition and activation gradients also inform the hydra "which end is up" and speCify positional values along the apical-basal axis. When the head is removed, the head inhibitor no longer is made, causing the head activa- 570 CHAPTER 1 5 FIGURE 15.30 Bud location as a function of head and foot inhi- bition gra dients. IA) Head inhibi tion (blue) and fool inhibition (red) gradients in newly dropped buds young adults, and budding adults. IB) Expression of the Shin guard protein in a gr ded fashion in a budding hydra. IA after Bode and Bode 1 984; B from Bridge et (B) (A) , a al. 2000.) \ NeWlY \(dropped bud / Young adult U Head inhibition Foot inhibition Head / l l Budding adult Foot tor to induce a new head. The region with the most head activator (i.e., those ceUs directly beneath the amputation The removal or injury of the liver is sensed through the bloodstream, as some liver-specific factors are lost while site) will form the head. Once the head is made, it gener others (such as bile acids and gut lipopolysaccharides) ates the head inhibitor, and thus equilibrium is restored. increase. These lipopolysaccharides activate two of the non-hepatocyte cells to secrete paracrine factors that allaH' the hepatocytes to re-enter the celi cycle. The Kupffer cell Compensatory Regeneration in the Mammalian liver secretes interleukin 6 (IL6) and tumor necrosis factor-a. (which are usually involved with activating the adult Compensatory regeneration wherein differentiated cells immune system), wlille the steUate ceUs secrete hepatocyte divide to recover the structure and function of an injured growth factor organ-has been demonstrated in the mammalian liver ever, hepatocytes that are still connected to one another in and in the zebrafish heart (poss et al. 2002). (HGF, or scatter factor) and TGF-p. How an epithelium cannot respond to HGF. The hepatocytes According to Greek mythology, Prometheus's punish activate cMet, the receptor for HGF, within an hour of par ment for bringing the gift of fire to humans was to be tial hepatectomy, and the blocking of cMet (by RNA inter chained to a rock and to have an eagle tear out and eat a ference or knockout) blocks liver regeneration (Borowiak portion of his liver each day. His liver then regenerated et al. 2004; Huh et 31. 2004; Paranjpe et al. 2007).·
each night. providing a continuous food supply for the The trauma of partial hepatectomy may activate metal eagle and eternal punishment for Prometheus. Today the loproteinases that digest the extracellular matrix and per standard assay for liver regeneration is a partial hepatec mit the hepatocytes to separate and proliferate. These tomy. In this procedure, specific lobes of the liver are enzymes also may cleave removed (after adnunistering anesthesia), leaving the other HGF to its active form (Mars e t al.·
1995). TogeU,er, the factors produced b y the Kupffer and hepa tic lobes intact. A1tllOugh the removed lobe does not stellate cells allow the hepatocytes to divide by prevent grow back, the remaining lobes enlarge to compensate for ing apoptosis, activating cyclins 0 and E, and repressing the loss of the missing tissue (Higgins and Anderson 1931). The amount of liver regenerated is equivalent to the amount of liver removed. cyclin inhibitors such as p27 (see Taub 2004). The liver stops growing at the appropriate size; the mechanism for how this is achieved is not yet known. One clue comes The human liver regenerates b y the proliferation of from parabiosis experiments, wherein the circulatory sys existing tissue.·
Surprisingly, the regenerating liver cells do tems of two rats are linked together. Here, partial hepa te not fuUy dedifferentiate when they re-enter the cell cycle. etomy in one rat will cause liver enlargement in the other No regeneration blastema is formed. Rather, the five types (Moolten and Bucher of liver ceUs-hepatocytes, duct ceils, fat-stOring (Ito) ceUs, is some factor or factors in the blood that are establishing 1967). Therefore, it seems like there endothelial cells, and Kupffer macrophages-all begin the size of the liver. Huang and coUeagues (2006) have pro dividing to produce more of themselves. Each cell type posed that these factors are bile acids produced by the liver retains its cellular identity, and the liver retains its ability and that stimulate hepatocyte growth. Partial hepatecto to synthesize the liver-specific enzymes necessary for glu my stimulates the release of bile acids into the blood, and cose regulation, toxin degradation, bile synthesis, albumin these bile production, and other hepatic functions (Michalopoulos vate the Fxr transcription factor. The Fxr protein promotes and DeFrances 1997). cell division, and mice without this protein cannot regen- acids are received by the hepatocytes and acti POSTEMBRYO N IC DEVELOPMENT erate their livers. Therefore, these bile acids (a relatively Enhances small percentage of secreted liver products) appear to reg • I[Somatic . of cells. ' Prevents patient's eliseased liver can be replaced by compatible liver tissue from a living donor (usually a relative). The donor's liver has always grown back. Human livers regenerate more slowly than those of mice, but function is restored quickly (Pascher et a!. 2002; Olthoff 2003). j" maUl 'Jance ulate the size of the liver and keep it at a particular volume Because human livers have the power to regenerate, a 571 I I Age-related cellular degeneration and disease in addition, mammalian livers possess a "second line" of regenerative ability.·
If the hepatocytes are lmable to regenerate O,e liver sufficiently within a certain amount of time, the oval cells divide to form new hepatocytes. Oval cells are a small pro genitor cell population that can produce hepatocytes and bile duct cells. They appear to be kept in reserve and to be used orily after the hepatocytes have attempted to heal the liver (Fausto and Campbell 2005; Knight et a!. FIGURE 15.31 Conceptual framework, first proposed by Kirk wood (1977), that organisms must effect a compromise between the energy allocated to reproduction and growth and the energy allocated to the maintenance and repair of bodily tissues. (After Vijg and Campisi 2008.) 2005). The molecular mechanisms by which these factors interact and by which the liver is first told to begin regenerating and then to stop regenerating after reaching the appropriate size remain to be discovered. both unknown but are estimated to be more than 150 years.·
The maximum We span of a domestic dog is about 20 years, and that of a laboratory mouse is 4.5 years. 11 a Drosophila fruit fly survives to eclose (in the wild, more than 90% die as larvae), i t has a maximum life span of 3 months.·
The species-specific life span appears to be determined AGING: THE BIOLOGY OF SENESCENCE 1 5.31). In other words, aging results from na tural selection operat Entropy always wins. A multicellular organism is able to develop and maintain its identity for orily so long before deterioration prevails over syntheSis, and the organism ages. Aging reproduction and somatic maintenance (F ig ure by genes tha t effect a trade-off between early growth and can be defined as the time-related deteriora ing more on early survival and reproduction than on hav ing a vigorous post-reproductive We. If longevity is a selec table trait, one should expect to find hereditable variation within populations. Recently, long-term studies of wild tion of the physiological functions necessary for survival populations of animals have proVided convincing data that and fertility. The characteristics of aging-as distinguished there is hereditable variation within a species for aging affect all the inelividuals 01·a species. The aging process has (Wilson et al. from diseases of aging, such as cancer and heart disease- two major facets.·
The first is simply how long an organism lives; the second concerns the physiological deterioration, or senescence, that characterizes old age These topics arc . often viewed as being interrelated. Aging and senescence have both genetic and envjron 2007). Molecular evidence (see Kenyon 2001; Vijg and Campisi 2008) indicates that certain genetic components of longeVi ty are conserved between species: flies, worms, mammals, and even yeast all appear to use the same set of genes to pro mote survival and longevity. There are MO sets of genes that arc well knO'vVTI to be involved in aging and its prevention, mental components. The interplay between mutatiuns, and both sets appear to be conserved between phyla and environmental factors, and random epigenetic change even kingdoms of organisms. These are the genes encoding makes these phenomena both fascinating and frustrating DNA repair enzymes and the genes encoding proteins to study. Moreover, in recent years, new molecular involved in the insulin signaling paO,way. approaches and stem cell technologies have allowed new approaches to these age-old questions (see Stocum 2006; Carlson 2007). Genes encoding DNA repair proteins DNA repair and synthesis may be important in prevent ing senescence. Individuals of species whose cells have Genes and Aging more efficient DNA repair enzymes live longer (Figure Genetic factors play roles both between species and with in species. The maximum lifespan, which is the maxlinum 1 5.32A; Hart and SeHow 1974). Certain premature aging syndromes (pro gerias) in humans appear to be caused by 1998; 2(01).·
[n humans, Hutchinson-Gilford prog number of years any member of a given species has been mutations in such DNA repair enzymes (Sun et a!. is characteristic of a species. The maxi mum human life span is estimated to be 121 years (Arking 1998). The life spans of some tortoises and lake trout are Shen and Loeb known to survive, eria is a rapid-aging syndrome; children born with this can- dition age rapidly, dying (usually of heart failure) as early CHAPTER 1 5 572 (A) (6) 100 Human . Elephant. 60 ! ◆ 30 • Cow 10 - Hamster Rat . 3 Mouse . ·Shrew I L- __ ◆ ◆ ◆ __ L__ L__ L- __ o 10 20 30 40 50 DNA repair (grains/nucleus) FIGURE 15.32 l i fe span and the aging phenotype. IA) Correia- tion between life span and the ability of fi brob l asts to repai r DNA in various mammalian species. Repai r capacity is rep resented in autoradiography by the number of grains from radioactive thym i dine per cell nucleus. Note that the y ax is (life span) is a logarith mic scale. (8) Hutchinson-Gilford progeria. A lthough they are not yet 8 years old, these children have a phenotype similar to that of an aged person. The hair loss, fat distribution, and skin transparen cy are characteristic of the normal aging pa ttern as seen in elderly adults, and may result from mutations in DNA repair enzymes (A aher Hart and Setlow 1 974; B © Associated P ress .) as 12 years of age (Figure 15.32B).·
Symptoms include thin skin with age spots◆ resorbed bone mass◆ hair loss, and arteriosclerosis-aU characteristics of the human senescent phenotype. Hutchinson-Gilford progeda is the result of a dominant mutation in the gene that encodes lamin A, a nuclear membrane protein, and these same mutations can be seen in age-related Se nesc ence (Scaffidi and Misteli 2006).·
Another intriguing progeria has been documented in mice and is caused by loss-of-function mutations of the Klollto' gene (Kuro-o et aJ. 1997). Conversely, the same gene's gain-of function phenotype (causing its overexpres sion) has been known to prolong a mouse's life by 30% (Kurosu et al. 2005). Klolho appears to encode a hormone that downregulates insulin signaling.·
As we shall soon see, the suppression of signaling by insulin and insulin-like '"The gene is named after one of the Fates of Greek mythology Klotho, who spun the thread of life. The other two Fates, Lachesis and Atropos, measured out and cut life's thread, respectively. growth factor 1 (IGF-1) is one of the ways life span can be many species. The pro tein p53 also appears to be important in aging. This transcription factor, one of the most important regula tors of cell division, has been called the "guardian of the genome " because of its ability to block cancer in several ways. It can stop the cell cycle, cause cellular senescence in rapidly dividing cells, instruct the Bax genes to initiate cellular apo ptosis, and activate DNA repair enzymes. In most cells, p53 is bound to another protein that keeps p53 inactive.·
However, ultraviolet radiation, oxidative stress, and other factors that cause DNA dama ge will also sepa rate and activate p53. The induction of apoptosis by p53 can be beneficial (when destroying cancer cells) or delete rious (when destroying, say, neurons). It is possib le that animals with high levels of p53 have increa sed pro tec tion a gainst cancer, but they may also age more rapidly (Tyner et aI. 2002). Indeed, p53 can be activated b y the a bsence of lamin A (Varela et al. 2005), thereby suggesting a mecha nism for Hutchinson-Gifford progeria.·
Another set of genes important in aging are the sirtuin genes, which encode histone deacetylation (chromatin silencing) enzymes.·
The sirtuin proteins guard the genome, preventing genes from being expressed at the wrong times and places, and blocking chromosomal rearrangements. They are usual ly found in regions of chromatin (especially repetitive DNA sequences) where such mistaken chromo somal rearrangements can occur. However, when DNA strands break (as inevitably happens as the body ages), sir tuin proteins are called on to fix them and earUlot attend to their usual functions. Thus genes that are usually silenced become active as the cells age. Sirtuin proteins have been fowld to prevent aging throughout the eukary otic kingdoms, including in yeasts and mammals (Howitz et al. 2003; Oberdoerffer et al. 2008). extended in POSTEMBRYO N I C DEVELOPMENT 573 Aging and the insulin signaling cascade increased resistance to oxygen radicals that can crosslink One of the criticisms of the idea of genetic "programs" for proteins and destroy DNA. The pathway that regulates aging asks how evolution could have selected for them. both dauer larva formation and longevity has been iden Once the organism has passed maturity and raised its off tified as the insulin signaling paulway (Kimura et al. 1997; spring, it is " an excrescence" on the tree of life (Rostand Guarente and Kenyon 2000; Gerisch et al.·
2001; Pierce et 1962); natural selection presumably cannot act on traits that al. 2001). Favorable environments signal the activation of affect an organism only after it has reproduced. But "How the insuli.n receptor homologue DAF-2, and this receptor can evolution select for a way to degenerate?" may be the stimulates the onset of adulthood (Figure 1 5 .33A) . Poor wrong question. Evolution probably can't select for such environments fail to activate the DAF-2 receptor, and dauer traits. The right question may be "How can evolution select in any formation ensues. While severe loss-of-function alleles in for phenotypes that can postpone reproduction or sexual this pathway cause the formation of dauer larvae maturity?" There is often a trade-off between reproduction envirorunent, weak mutations in the insulin signaling path and maintenance, and in many species reproduction and way enable the animals to reach adulthood and live four senescence are closely linked. times longer than wild-type animals. Recent studies of mice, Caenorhabditis elegans, a n d Drosophila suggest that there is a conserved genetic path has several other functions. First, it appears to influence Downregulation of the insulin signaling pathway also way that regulates aging, and that it can be selected for metabolism, decreasing mitochondria) electron transport. during evolution. This pathway involves the response to When the DAF-2 receptor is not active, organisms have insulin or insulin-like growth factors. In C. elegans, a larva decreased sensitivity to reactive oxygen species (ROS), meta proceeds through four larval stages, after which it becomes bolic by-products that can damage cell membranes and an adult. If the nematodes are overcrowded or if there is proteins and even destroy DNA (Feng et al. 2001; Scott et insufficient food, however, the larva can enter a metabol al. 2002; we discuss these in more detail on p. 577). Second, ically dormant dauer larva stage, a nonlee downregulating the insulin pathway increases the produc pause during which development and aging are suspend tion of enzymes that prevent oxidative damage, as well as i ed. The nematode can remain in the dauer larva stage for DNA repair enzymes (Honda and Honda 1999; Tran et al. up to 6 months, rather than becoming an adult that lives 2002). Third, this lack of insulin signaling decreases fertil only a few weeks. In this diapausal state, the nematode has ity (Gems et al. 1998). This increase n DNA synthetic (B) (A) c.·
elegaas Mouse (C) (insulin-like growth factor receptor) Drosophila IGF-IR I I PKB AGE- l (Forkheafdactor)transcription (o eadfactorYranscr on PAcrottivecattiioonn oagaifDNAnst repair enzymes DAF- 1 6 '" F rkh • • Foxo t ◆ ipti ROS t Increase life dspancellular t Increased the longevity of organism c io I Ch (Forkheadfactor transcripti) on dFoxo FIGURE 15.33 A passible pathway for regulating longevity. In the insulin signaling pathway inhibits the synthesiS of proteins that would otherwise protecl cells against oxidative dam age caused by reactive oxyge species (ROS) that crosslink pro- leins and can damage DNA.·
These protecti ve protein s may be particularly i mporta t in mitochondria, When insulin signaling is downregulated, forkhead transcription factors may activate DNA repair enzymes that may protect against mutations caused randomly by ROS or other agents. Such prolection against ROS and mutation may increase the functional life span of the cells and the longevity of the organism. each case, n n 574 CHAPTER 1 5 enzymes and in enzymes that protect against ROS is due to the DAF-16 transcription lactor. This lorkhead-type tran scription lac tor is inhibited by the insulin receptor (DAF2) signal. When that signal is absent, DAF-16 can function, and this factor appears to activate the genes encoding sev eral enzymes (such as catalase and superoxide dismutase) that are involved in reducing ROS, several enzymes that increase protein and lipid ttunover, and several stress pro teins (Lee et al. 2003; Libina et aJ.·
2003; Murphy et aJ.·
2003). It is possible that this system also operates in mammals, but the mammalian insulin and insulin-like growth factor pathways are so integrated with embryonic development and adult metabolism that mutations often have numer ous and deleterious effects (such as diabetes or Donahue syndrome). However, there is some evidence that the insulin signaling pathway does affect life span in mam mals (Figure 15.33B). Dog breeds with low levels of insulin like growth factor 1 (IGF-1) live longer than breeds with higher levels of this factor. Mice with loss-of function muta tions of the insulin signaling pathway live longer than their wild-type litterrnates (see Partridge and Gems 2002; Bliiher et al. 2004). Holzenberger and colleagues (2003) lound that mice heterozygous lor the insulin-like growth factor 1 receptor (IGF-1R) not only lived about 30% longer than their wild-type littermates, they also had greater resistance to oxidative stress. In addition, mice lacking one copy of their IGF-1R gene lived about 25% longer than wild-type Growths factors, + IIGF-IRI + DNA repair IGF in nlin r------◆ e Mitochondria and mitochondrial r spiratory chain Genotoxi stress c Cell cycle cellnlarrest, ar j I PTEN I ◆'---- + / ◆ ' ◆J ◆◆Translatit on I 1 i◆ APOPtOSiSI◆ FOXO ==FOXO ◆ Anlioxidant defense Gluconeogenesis + mice (and had higher ROS resistance, but otherwise nor mal physiology and lertility). The insulin signaling pathway also appears to regulate liie span in Drosophila (Figure 15.33C). Flies with weak loss ai-function mutations 01 the insulin receptor gene or genes in the insulin signaling pathway (such as chico) live near ly 85% longer than wild-type flies (Clancy et al. 2001; Tatar et a1. 2001).·
These long-lived mutants are sterile, and their metabolism resembles that of flies that are in diapause (Kenyon 2001). The insulin receptor in Drosophila is thought to regulate a Forkhead transcription factor (dFoxo) simi lar to the DAF-16 protein of C. elegans. When the Drosophi la dFoxo gene is activated in the fat body, it can lengthen the fly's life span (Giarrnakou et a1. 2004; Hwangbo et al. 2004). Although it has not been demonstrated that dFoxo regulates the enzymes that protect against ROS, iJldepend ent studies have shown that when these enzymes (such as superoxide dismutase) are downregulated by mutation or by RNA interference, the resulting flies die early, have increased oxidative stress, and display higher levels of DNA damage (Kirby et a1. 2002, Woodruff et aJ. 2004). Con versely, overexpression of superoxide disrnutase genes can lengthen the Drosophila life span (Parkes et a1. 1998). While some evidence points to a correlation between longer life span, lower insulin Signaling, and elevated ROS protection in Drosophila (Broughton et a1. 2005), other studies suggest that some flies and other insects can obtain longer life spans without increasing the enzymes kno\",,rn to protect ◆ senescence P53 ◆ l ◆ ◆.1J t BCL2 Apoptosis Cell survival ◆◆◆ s r- Fat mobilization Gluconeogenesis + pr I n Cell fate. stress resistance, metabolic functions FIGURE 15.34 Possible evol u ti o narily conserved aging pathways and their i nter- actions. The insulin signaling pathway and mitochondrial pathway for apoptosis are indicated. The pro aging activities of these pathways are conserved across phy a, and energy sensors, such as AMPK, are poten tia ly important integrators of these path ways. Many longevity signals converge on of and i rtu i n protein families, which can Note that sir tuin protein s act ivate and rep res depending on the context.·
TOR is a pos t ive regu la or of general translation al activity. Here, insulin-like factors and DNA-disrupting (llgenot x i c") chemicals i nitiate these pathways. (After Vijg and Campisi 2008.) l s members the FOXO interact. can both FOXO, i t o l s POSTEMBRYONIC DEVELOPMENT against oxidative stress (Le Bourg and Fournier 2004; Park er et a1. 2004). From an evolutionary point of view, the insulin path way may mediate a trade-off between reproduction and sw-vival/ maintenance. Many (although not aU) of the long lived mutants have reduced fertinty. Thus, it is interesting that another longevity signal originates in the gonad. When the germline cells are removed from C. elegans, the animals live longer. It is thought that the germline stem cells pro duce a substance that blocks the effects of a longevity inducing steroid hormone (Hsin and Kenyon 1999; Gerisch et a1. 2001; Arantes-Oliviera 2002). Conversely, ROS appears to promote germline development at the expense of somatic development in C. elegans. The oxidation of cer- tain lipids accelerates germ cell development, while those same lipids, in their unoxidized form, prevent germ cell proliferation (Shibata et al. 2003). Integrating the conserved aging pathways The proteins involved in the insulin signaling pathway and the DNA repair pathway interact with one another (Figure 1 5.34) . The p53 factor that induces cell cycle arrest also blocks the activity of the receptor for insulin-like growth factor 1. And sirtuin proteins, in adctition to activating Foxo proteins, can also block p53. In some cases, the same pro tein is involved in both the DNA repair and insulin signal ing pathways (Niederrihofer et a1. 2006). This is the case in the protein encoded by the XPF-ERCCl gene in humans. Most people with mutations of this gene have xeroderma on " . s: E ◆ = 0 ." ·.3 " "0 "◆ 0 = v ◆ v '" 10 /19 9 20 1 80 60 40 20 00 10 20 30 40 50 60 70 80 90 10 Age FIGURE 15.35 Survival curves for the United States population for the periocs 1 900-1 902, 1 949-1 951, and 1 999-2001 . The circles represent Msol the age at which 50% of individuals of that age survived (From COCINCHS, National Vital Statistics Reports 815108.) 575 pigmentosum, a defect in DNA repair that makes them susceptible to cancers, especially melanomas.·
However, if the mutation occurs in a different part of the same gene, the affected individuals have a premature aging syndrome in which the genes involved in the insulin Signaling path way are downregulated. It is possible that the enzyme encoded by this gene has two functions. Initially it may be used for DNA repair, but later it might act to prolong life by downregulating the insulin pathway. Environmental and Epigenetic Causes of Aging Most people cannot expect to live 121 years, and most mice in the wild do not live to celebrate even their first birthday. Life expectancy-the length of time an average individ ual of a given species can expect to live-is not character istic of species, but of populations. It is usually defined as the age at which half the population still survives. A baby born in England during the 1780s could expect to live to be 35 years old. In Massachusetts during that same time, life expectancy was 28 years. These ages represent the nor mal range of hlunan life expectancy for most of the hwnan race throughout recorded history (Arking 1998). Even today, in some areas of the world (Cambodia, Togo, Afghanistan, and several other countries) life expectancy is less than 40 years. In the United States, a male born in 1986 can expect to live 74 years, while females have a life expectancy of around 80 years. * Given that in most times and places people did not live much past the age of 40, OUI awareness of human aging is relatively new. In 1900, 50% of Americans were dead before the age of 60, in 1950, the comparable age was 72; by 2000, this "median survival" age had climbed to 80 years (Figure 15.35). A 70-year-old person was exceptional in 1900 but is commonplace today People n i 1900 did not have the "luxu ry" of dying from heart attacks or cancers, because these conditions are most likely to affect people over 50. Rather, people died (as they are still dying in many parts of the world) from microbial and viral infections (Arking 1998). Thus, the phenomena of senescence and the diseases of aging are much more common today than they were a cen tury ago. Until recently, relatively few people exhibited the general hwnan senescent phenotype: gray hair, sagging and wrinkling skID, stiff joints, osteoporosis (loss of bone calci um), loss of muscle fibers and muscular strength, memory loss, eyesight deterioration, and slowed sexual responsive ness. As the melancholy Jacques notes in Shakespeare's As 1935, *You can see why the funding of Social Security is problematic in the United States. When it was created in the average working citizen died before age 65.·
Thus, he (and it usually was a he) was not expected to get back as much as he had paid into the system. Similarly, marriage "until death do us part" was easier to achieve when death occurred in the third or fourth decade of life.·
Before antibiotics, the death rate of young women due to infections associ ated with childbirth was high throughout the world. CHAPTER 1 5 576 You Like It, those who did survive to senescence left the world "sa11s teeth, sans eyes, sans taste, sans everything ." The general senescent phenotype is characteristic of each species. But what causes it? This question can be asked at many levels. Here we wiJJ look primarily at the ceJJular level of organization. While there is not yet a con sensus on what causes aging (even at U,e oeJJular level) , a *There is a popular proposal that the shortening of telomeres repeated ONAsequences a t the ends of du-omosomes-is responsi ble for senescence. Telomere shortening has been connected to a decrease in the ability of cells to divide. However, no correlation betvvcen tc.lomere length and the life span of an animal (humans have ruuch shorter telomeres than mice) has been found, nor is there a correlation belveen human telomere length and a person's age (Cristofalo et al. 1998; Rudolph et a1. 1999; Karlseder et al. 2002).·
Nematodes can have mutations that extend or shorten longevity, and the length of the telomere does not correlate with the age in these rOLU1dworms (Raices et al. 2005). Telomeres appear to be criti cal in stem cell main tenance, and the telomere-dependent inhibition of cell division might serve primarily as a defense against cancer (see Blasco 2005; Flores et al. 2005). (A) theory is e me rging that includes oxidative stress, hor mones, and DNA da mage. ' WEAR-ANO-TEAR ANO GENETIC INSTABIliTY "Wear-and-tear" theories of aging are among the oldest hypotheses pro posed to account for the human senescent phenotype (Weismann 1891; Szilard 1959). As one gets older, small traumas to the b ody and its genome build up. At the molecular level, the number of point mutations increases, and the efficiency of the enzymes encoded by our genes decreases.·
If mutations occur in a part of the protein syn thetic apparatus, the cell produces a large percentage of faulty proteins (Orgel 1963). If mutations were to arise in the DNA-synthesizing enzymes, fue overall rate of muta tion in the organism would be expected to increase markedly; Murray and HeJJiday (1981) have documented such faulty DNA polymerases in senescent cells. A new variant of thls idea is the hypothesis of random epigenetic drift Given that appropriate methylation is essen tial for normal dev elopment, one can immediately see that diseases would result as a consequence of inappropriate epi- 50-year-old-nvins (B) 3-year-old twins 90 • , 80 • 70 .Q 60 -g<]= . . .◆ SO :i! '6 ◆ 40 0 ◆ u .Q E .t ,; ◆ Z 20 JO 0 t! 'P' 30 Chromosome 1 .Chromosome 1 3-year-old 50-year-old twins twins • .. • Chromosome 1 2 FIGURE 15.36 Differenlial DNA melhyla li on patterns i n aging twins. (A) In bisulfite sequence mapping, regions of DNA that are unmethylated w i l l be cut by restriction enzymes (because bisu lf

Fraga , M. F. and 20 others 2005. Epige netic differences arise during the life time of monozygotic twins. Proc. Nat/. Acad. Sci. USA 102: 10604-10609. Jiang, c., A. F. Lamblin, H. SteUer and C. S. Thumme!. 2000. A steroid-trig gered transcriptional hierarchy controls salivary gland ceU death during Drosophila metamorphosis. Mol. Cell S: 445-455. ! Kumar, A., j. W. Godwin, P. B. Gates, A. A. Garza-Garcia and J. P. Brockes.

2007. Molecular basis for the nerve depend ence of lirn regeneration in an adult vertebrate. Seier/ce 318: 772-777. Schubiger, M. and J. w. Truman. 2000. The RXR ortholog US!' suppresses early metamorph..ic processes in Drosopilila in the absence of ecdysteroids. Develop ment 127: 1151-]]59. Stocum, D. L.

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Ca mpisi . 2008. Puzzles, promises and a cure for ageing. Nature Vijg, J. and 454: 1065-1 071. Go Online WEBSITE 15.] The molecular biology of wing fonna tion. Formation of lhe Drosophila wing involves the interac tion of more than 200 genes . This site discusses some of these gene interactions. WEBSITE 15.2 Homologous specification. If a group of cells in one ima gina l disc are mutated such that they give rise to a structure characteristic of another imaginal disc (for instance, cells from a leg disc givi n g rise to antennal structures), the reg ional specification of those structures will be in accordance with their position in the original disc.

WEBSITE 15.3 Insect metamorphosis. The four links on this website discuss (I) the experiments of Wigglesworth and others who identified the hormones of metamorphosis and the gla nd s producing them; (2) the variations that Drosophila and other insects play on the general theme of metamorphosis; (3) the re mode l ing of the insect nervous system during me tamorphosis; and (4) a microarray analy sis of Drosophila metamorphosis wherein several thousand genes are Simultaneously screened. WEBSITE 15.4 Precocene s and syn theti c JH.

G iven the v oracity of insect larvae, it is amazirl.g that any plants sur vive. However, many plants get revenge on their preda tors by ma king compounds that alter insect metamorpho sis, thus p re ven ting the animals from developing or reproducing .

WEBSITE 15.5 Regeneration in annelid worms. An easy laboratory exercise can discover the rules by which worms regenerate their segments. This website details some of those experiments. 582 CHAPTER 1 5 WEBSITE 15.6 Polar coo rdi n ate and boundary mod els. The phenomena of epimorphic regeneration can be seen formally as events that reestablish continuity among tissues that the amputation has severed. The polar coordi nate and boundary models attempt to explain the numer ous phenomena of limb regeneration. WEBSITE 15.7 Ethel Browne and the organizer. As detailed in Chapter 7, Speroano and Mangold's work with amphibians brought the concept of the Organizer into Vade Mecum Amphibian metamorphosis and frog calls. For photo- graphs of amphibian metamorphosis (and for the sounds of the adult frogs), check out the metamorphosis and frog call sections of the Amphibian segment. Chronlosome squash. The Fmit Fly segments contains a sequence showing how to do a chromosome squash using th.e Drosophila larval salivary gland, embryology, and Spemann's laboratory helped make the Planari an regeneratio n idea a unHying principle of embryology. However, it has duce by splitting their body in half, The front can regener . Freshwater planarians can repro been argued that the concept actually had its origins in ate a back, and the back can regenerate a front.

This pro Ethel Browne's experiments on Hydra. vides a laboratory exercise in stem cells and potency that is easy to perform, The Saga of the Germ Line WE ARE ABOUTTO COME FULL CIRCLE. We began our analysis of animal devel opment by discussing fertilization, and we will finish Our studies of individual development by investigating gametogenesis, the processes by which the sperm and the egg are formed. In addition to forming its own body, an individual ani And the end of all our exploring Will be to a1Tive where we started And know the place for the first time. T.S. ELLIOT (1 9 42) mal must set aside cells that will provide the material and instructions for initi ating bodies in the next generation. Germ cells provide the continuity of life between generations, and the mitotic ancestors of our own germ cells once resided in the gonads of reptiles, amphibians.fish, and invertebrates. In many animals, including insects, roundworms, and vertebrates, there is a clear and early separation of germ cells from somatic cell types. In several other animal phyla (and throughout the entire plant kingdom), U,is division is not as well established. [n these animal species (which include enidarians, flatworms, and I1.micates), somatic cells readily form new organisms.

The zooids, buds, and polyps of many invertebrate phyla testify to the ability of somatic cells to give rise to new individuals (Liu and Berrill l948; Buss 1987). 1n those organisms in which there is an established germ line that separates from the somatic cells early in development, the germ cells often do not arise within the gonad itseU. Rather, the gamete progenitor cells-the primordial germ cells (PGCs)-arise elsewhere and migrate into the developing gonads. The first step in gametogenesis, then, involves forming the PGCs and getting them into the genital ridge as the gonad is forming. Therefore, our discussion of gametoge nesis will include: • Formation of the germ plasm and the determination of the primordial germ cells • Migration of the PGCs into the developing gonads • The process of meiosis and the modifications of meiosis for forming sperm and eggs • Differentiation of the sperm and egg cells (gametogenesis) • Hormonal control of gamete ma turation and ovulation Germ Plasm and the Determination of the Primordial Germ Cells All sexua lly reproducing animals arise from the fusion of gametes-sperm and eggs. All gametes arise from primordial germ cells. In most laboratory model organisms (incl uding frogs, nematodes, and flies), the primordial germ cells are specified autonomously by cytoplasmic determinants in the egg that are parceled When the spermatozoon enters the egg, it enters a cell system which has a/read)' achieved a certain degree of organ ization. ERNSf HADORN (1 9 5 5) 584 CHAPTER 1 6 (A) Animal Chromosome Germ plasm No chromosome diminution (B) diminutio n FIGURE 16.1 out to specific cells during cleavage. However, evidence suggests that in the majority of species (including salaman inter ders and mammals), the germ cells are specified by actions among neighboring cells (ExITavour and Akam 2003). In those species in which detemlina tion of the pri mordial germ cells is brought about by the autonomous localization of specific proteins and mRNAs, these cyto plasmic components are collectively referred to as the geffi1 Distribution of germ plasm during cleavage of nor mal and centrifuged zygotes of Parascaris.

(A) In normal cleavage, the germ plasm is l ocal ized in the vegetalmost blastomere, as shown by the lack of chromosomal diminution in that particular cell. Thus, at the 4-cell stage, the embryo has a single stem cell for its gametes. (B) When cen tri fugati on is used to di splace the first cleavage by 90 degrees, both of the resulting cells have vegetal germ plasm, and neither cell undergoes chromosome diminution. After the second cleavage, both of these two cells give rise to ger minal stem cel ls . (After Waddington 1 966.) plasm. Meanwhile, in the vegetal blastomere, the chromosomes Germ cell determination in nematodes ' BOVERI S EXPERIMENTS ON PA RASCARIS Theodor Boveri (1862-1915) was the first person to observe an organism's chromosomes throughout its developm nt. In so doing, he discovered a fascinating feature in the development of the remain normal. During second cleavage, the animal cell splits meridionally while the vegetal cell again divides equatorially. Both vegetally derived ceUs have normal duo mosomes. However, the chromosomes of the more anirnal ly located of these two vegetal blastomeres fragment before roundworm the third cleavage . Thus, at the 4 ceU stage, only one cell duomosomes per haploid cell, thereby allpwing detailed sive cleavages, nuclei with diminished chromosomes are Parascaris aequorum (formerly known as Ascaris megalocephala). This nematode worm has only two observations of its individual chromosomes. The cleavage plane of the first embryonic division is unusual in that it is equatorial, separating the animal half from the vegetal half of the zygote (Figure the most vegetal contains a full set of genes. At succes given off from this vegeta lm ost line until the 16-cell stage, when there are only two cells with undiminished chromo somes. One of these WO blastomeres gives rise to the germ 1 6.1 A) . More bizarre, however, is cells; the other eventually undergoes chromosome diminu sion of these first two blastomeres. The chromosomes in kept intact only in those cells destined to form the germ the behavior of the chromosomes in the subsequent divi the animal blastomere fragment into dozens of pieces just before this cell divides. This phenomenon is called chro mosome dimination, because only a portion of the original chromosome survives. Numerous genes are lost when the chromosomes fragment, and these genes are not included tion and forms more somatic cells. The chromosomes are line. If this were not the case, the genetic information would degenerate from one generation to the next. The cells that have undergone chromosome diminution gener ate the somatic cells. Boveri has been called the last of the great observers of in the newly fOffi1ed nuclei (Tobler et al. 1972; MUller et al. embryology and the first of the great experimenters. Not 1996). content with observing the retention of the full chromo- T H E SAGA O F T H E GERM L I N E 585 ferentiating into somatic cells, and germ cell differentia tion cannot commence until the d isappearance of PIE-1 in later embryonic stages.

Until that time, the germline nuclei are silenced (Figure 16 2). It is possible that these germ plasm proteins can aggregate into P granules only in the P1-P4 cells_ The MEX-5 and PAR-1 proteins inhibit P-gran ul e stability in the rem ining somatic cells (Brangwynne et al 2009). . a See WEBSITE 1 6.1 Germline sex determination in C. elegans See WEBSITE 1 6 2 Mechanisms of chromosome diminution i o of transcription in germ cell precursors of Caenorhabdilis e/egans. The photograph shows in situ hybridizaton to p-galactosidase mRNA expressed under control of t he pes- IO promoter. The pes- I O gene is one of the earliest genes expressed in C elegans. The P-blastomere that gives rise to the germ cells (far right) does not transcribe the gene. (From 5ey doux and Fire 1 994, courtesy of G. 5eydoux.1 FIGURE 16.2 I n h ibit i n some complement by the germ cell precursors, he set out to test whether a specific region of cytoplasm protects the nuclei within it from diminution. If so, any nucleus hap pening to reside in this region should remain undimin ished. In 1910, Boveri tested this hypothesis by centrifuging Parascaris eggs shortly before their first cleavage. This treat ment shifted the orientation of the mitotic spindle. When the spindle forms perpendicular to its normal orientation, both resulting blastomeres contain some of the vegetal cytoplasm (Figure 16_1 B). Baveri found that after the first division, neither nucleus unden-vent chromosomal diminu tion. However, the next division was equatorial along the animal-vegetal axis. Here the resulting animal blastomeres both underwent dlmination, whereas the two vegetal cells did not Baveri concluded that the vegetal cytoplasm con tains a factor (or factors) that protects nuclei from chromo somal diInination and determines germ cells. _ In the nematode CaenoJ'habditis elegans, the germline precursor cell is the P4 blastomere. The P gran ules that enter this cell are critical for instructing it to become the germ line precursor (see Figure 5.45). The P granule protein repertoire includes several transcription al inhibitors and RNA-binding proteins, including homo logues of Drosophila Vasa, Piwi, and Nanos, whose functions we will discuss below (Kawasaki et al. 1998; Sey doux and Strome 1999; Subramanian and Seydoux 1999). In addition, as discussed in Chapter 5, the C. elegans germ plasm contains the PIE-1 protein, which prevents the phos phorylation of RNA po lymerase II, thereby preventing transcription in the germ cell lineage (Ghosh and Seydoux 2008). This is critical for preventing the germ line from di!C. HEGANS Germ cell determination in insects In Drosophila, PGCs form as a group of p o l e cells at the posterior pole of the cellularizirlg blastoderm. These nuclei migrate into the posterior region at the ninth nuclear divi sion and become surrounded by the p o l e p l asm, a com plex collection of mitochondria, fibrils, and polar granul es (Figure 16.3; Mahowald 1971a,b; Schubiger and Wood 1977). If the pole cell nuclei are prevented from reaching the pole plasm, no germ cells will be made (Mahowald et al. 1979). The germ cells are responsible for formi ng the germline stem cells, each of which divides asymmetri cal ly to produce another stem cell and a differentiated da ugh ter cell called a cystobl as t Cystoblasts undergo four mitot ic divisions with incomp le te cytokineSiS to form a cluster of 16 cell s interconnected by cytoplasmic bridges called . (A) Ot .. .,. 1Jff , r "q i ' 1t) , , o t". (B) . • .7 · FIGURE 1 6.3 Pole p la Drosophila_ (A) Electr n of polar ranu les from particulate (raction of Drosophila pole cells (B) Scanning electron micrograph of a Drosophila just prior to completion The pole cells e an be seen at the right cou rtesy of Maho l) g . sm of of cleavage. of the photograph. (Photographs wa d o micrograph embryo A. P. 586 CHAPTER 1 6 ring canals.

Only those two cells having four interconnec tions are capable of developing into oocytes, and of those two, only one becomes the egg (the other begins meiosis but does not complete it). Thus, only one of the 16 cysto cytes beco mes an ovum; the remairring 15 cells becume nurse cells (Figure 16.4). As it turns out, the cell destined to become the oocyte is that cell residing at the most posterior tip of the egg chamber, or ovariole, enclOSing the 16-cell clone. Howev er, since the nurse cells are connected to the oocyte by the ring canals, the entire complex can be seen as one egg-pro ducing unit. The nurse cells produce numerous RNAs and pro teins that ultima tely are t ransported into the oocyte through the ring canals. (A) Di vid ng cystoblast Fusome t t i 2-cell cyst The component s of the germ plasm are responsible for specifying these cells to be germ cells and for inhibiting somatic gene expression in these cells. These two functions might be interrelated, since the inhibition 01 gene transcr.iP lion appears to be essential lor germ cell determination (see Santos and Lehmann 2004). One of the components of the pole plasm is the mRNA of the germ cell-less (gcl) gene. This gene was discovered by J ongens and his colleagues (1992) when they mutated Drosophila and screened lor lemales that did not have "grandoflspring." They assumed that if a female did not place functional pole plasm in her eggs, she could still have o llspring-b ut those ollspring would lack germ cells and would be sterile. The Wild-type gel gene is transcribed in the nurse cells of the fly's ovary, and its mRNA is transported into the egg.

Once inside the egg, it is transported to the posteriormost portion and nn n FIGURE 16.4 Formation of 16 interconnected cystocytes i n Drosophila. (A) Dia gram of an adult ovariole, sh owi g the sequence of oogenesis as younger germ nal cysts (germaria) mature.

(8) D ivisio of the cystocyte- formi g stem cells (cy5tO blasts), The cells are represe ted schematically as dividing i n a single plane. The cys tobl ast div des to produ ce another stem cell plus an oogoni u m that is to formi ng the cystocytes. When the oogonium divides, the ce ntriol e of dau ghter cystocyte 1 retains the fU50me (red), wh ich grows through the ring canal toward its mi totic sister. The arrow shows the po larity, pointing to the cell from which the fusome grew. After three more m itotic divisions, the 1 6-cell clone is formed. If i ntra cellular transport is coordinated by the fusome, the transport of mRNAs and proteins would be toward cystocyte 1 , which would thus become the oocyte. Ic) Section through germinal cysts showing ring canals (stained yel low). Here the actin is stai ned red. th rou gh center ofthree germinal cysts, showing the matura ti on of the oocyte. Actin is stained green, and th e nuclear DNA is stained red. (A i n (D) Section (B) committed after Ruohola et a l . 1 99 1 , B after Lin and p radl i ng 1 995; C courtesy of S. E. Rou l ier and S. Beckendorf, 0 co u rtesy of B.

M. Mech l er.) S Ring canals tl i (e) Two more divisions (D) T H E SAGA O F T H E GERM L I N E Mutant Wild type (A) gel mRNA Gel protei n resides in what will become the pole plasm. This message is translated into protein during the early stages of cleavage (Figure 1 6.5A).

The gel-encoded protein appears to enter the nucleus, and it is essential for pole celi production. Flies with mutations of this gene lack germ ceUs (Figure 1 6.5B). The gel gene encodes a nuclear envelope protein that pre vents gene transcription and is crihcaJ for specifying the pole cells (Leatherman et al. 2002). Homologues of the gel gene have been found in the germ cells of mice and humans, and the mouse Gel gene also represses transcrip tion. Human males with mutant GCL genes have defective spermatozoa and are often sterile (Nili et ail. 2001; Kleiman et a 2003). A second protein found in Drosophila pole plasm (and one that becomes localized in the polar granules) is the polar granule component (Pgc) .

Pgc mRNA is part of the original pole plasm and becomes translated there.

The Pgc protein inhibits transcription, and it does so (like PIE-l in C. elegans) by preventing the phosphorylation of RNA poly merase II (Martinho et al. 2004; Hanyu-Nakamura et 2008; see Figure 2.7). Without this phosphorylation, the RNA polymerase cannot transcribe any genes. If the mater nal Pgc gene is mutated, the germ cells begin expressing the genes characteristic of their neighboring somatic cells. A third set of pole plasm components are the posterior group determinants. The Oskar protein (see Chapter 6) appears to be the critical member this group, since expression of oskar mRNA in ectopic sites will cause the nuclei in those areas to form germ cells. The genes that restrict Oskar to the posterior pole are also necessary for germ cell formation (Ephrussi and Lehmann 1992; New mark et 1997; Riechmann et ail. 2002). Moreover, Oskar appears to be the limiting step of germ cell formation, since adding more oskar message to the oocyte causes more germ cells to form. Oskar functions by localizing the proteins and RNAs necessary for germ cell formation (such as germ cell-less) to the posterior pole (Ephrussi and Lehmann 1992; Snee and Macdonald 2004). 1. a1. a1. Loca li ati on of germ gene products in the posterior of t egg and embryo. The gel mRNA can be seen in he pos terior pole of early-cleavage embryos produced by wi ld-type females (A), bot not in embryos produced by gel deficient mutant females (B). The pro tein encoded by the gel gene can be detected in the germ cells at the cellu lar blastoderm stage of embryos pro duced by wild-type females, but not in em bryos from mutant females (arrows). (From Jongens et al. 1992, courtesy ofT. A. Jongens.) FIGURE 16 .5 z cell-less he Drosophila (B) of 587 One of the mRNAs localized by Oskar is the Nanos mes sage, whose product is essential for posterior segment for mation and germ cell specification. Pole cells lacking Nanos do not migrate into the gonads and fail to become gametes. While Gel and Pgc appear to be critical in regu lating transcription, Nanos appears to be essential for inhibiting the translation of certain messages. In embryos lacking Nanos, the germline cells usually die; but if inhib ited from dying, these germline cells can become somatic cells (see Chapter 6). Nanos thus prevents the pole cells from activating the pathway tilat would lead to the forma tion of somatic cells (Hayashi et al. Another of the posterior mRNAs encudes Vasa, an RNA binding protein. The mRNAs for this protein are seen in the germ plasm of many species, and Vasa is critical for initiat ing germ ceU differentiation and meiosis (Ghabriail and Schiip bach 1999). Two other nucleic acid-binding proteins, Piwi* and its relative Aubergine, are also found in the pole plasm. They, too, have the ability to repress transcription. Piwi will later become critical tn establishing the germ cell as stem cell in the gonad (Cox et a1. 1998; Megosh et ail. 2006). There are numerous components of the pole plasm that we know little about (see Santos and Lehmann 2004). For instance, mitochondrial ribosomes are seen transiently in 2004). a eration throughoul lhe e u karyo tic kingdoms. In addition to being *Piwi appears to be required for stem cell maintenance and prolif expressed in the totipo ten t stem cells of plana ria and regenerating presnt in germ stem cells, Piwi genes have also been found annelids. Inhibiting Piwi gene expression in the adult flatvworm exp ressed in the somatic stem cells of jellyfish and is upregulated blocks the worm's regeneration (Reddien 2004). Piwi is also expression of I'iwi i.n d iffe rentiated ceUs of jellyfish may underlie inunediately before transdifferentiation.

The continuous low their ability to remodel their bodies so profOlUldly (Seipel et aL across kingdoms: 1,\"0 Piwi genes in Arabidopsis are crucial for main 2004). Piwi may even be responsible for stem ceU maintenance taining meristem proliferation at the root and shoot of the plant (Bohmert et al. 1998; Moussian et al. 1998). 588 CHAPTER 1 6 the Dmsophila pole plasm. Kobayashi and Okada (1989) demonstrated ribosomal component's importance by showing that injecting mitochondrial RNA into embryos formed from ultraviolet-irradiated eggs restored their abil ity to form pole cells. It is possible that some of the pole plasm mRNAs are being translated by these mitochondri al ribosomes. Inhibiting protein synthesis by these ribo somes impairs production of the Gel protein (Amikura et a1. 2005). VADE MECUM Germ cells in the Drosophila embryo See WEBSITE 1 6.3 The insect germ plasm Germ cell determination in frogs and fish Cytoplasmic localization of germ cell determinants has also been observed in vertebrate embryos. Bounoure (1934) showed that the vegetal region of fertilized frog eggs contains material with staining properties similar to those of Drosophila pole plasm. He was able to trace this cortical cytoplasm into the few cells in the presumptive endoderm that would normally migrate into the genital ridge. By transplanting genetically marked cells from one embryo into another of a differently marked strain, B1ackler (1962) showed that these cells are the primordial germ cell pre cursors. The germ plasm of amphibians consists of germinal granules and a matrix around them. It contains many of tl,e same RNAs and proteins (including the large and small mitochondrial ribosomal RNAs) as the pole plasm of Drosophila, and they appear to repress transcription and translation (Kloc et a l . 2002). The early movements of amphibian germ plasm have been analyzed in detail by Savage and Danilchik (1993), who labeled the germ plasm with a fluorescent dye. They found that the germ plasm of unfertilized eggs consists of tiny "islands" that appear to be tethered to the yolk mass near the vegetal cortex. TI,ese islands move with the vegetal yolk mass during the corti cal rotation just after fertilization. After this rotation, the islands are released from the yolk mass and begin fusing together and migrating to the vegetal pole. Their aggrega tion depends on micro tubules, and their movement to the vegetal pole depends on a kinesin-like protein that may act as the motor for germ plasm movement (Robb et al. 1996; Quaas and Wylie 2002). Savage and Danilchik (1993) found that UV light prevents vegetal surface contractions and inhibits the migration of germ plasm to the v egetal pole. Furthermore, the Xenopus homologues of Nanos and Vasa messages are specifically localized to the vegetal region (Figure 1 6.6; Forristall et al. 1995; lkenishi et al. 1996; ZhOli and King 1996). FROGS In zebrafish, the germ plasm forms a dense structure characterized by polar granules, mitochondria, ZEBRAFISH Germ plasm at the vegetal pole of frog embryos situ hybridization to the mRNA for Xcat2 (the Xenopus homo l ogue of Nanos) l oca l izes the message in the vegetal cortex of first-cleavage (upper) and fourth-cleavage (lower) e bryos .

(After Kloc et a!. 1 998, courtesy of L. Etkin.) FIGURE 16.6 In . m and concentrated mRNAs. Two of these mRNAs are Vasa and Nanos. These messages are maternally supplied, and they appear to be associated w ith the cleavage furrows of the early dividing egg (Yoon et al. 1997).

Vasa mRNA and other components of the germ plasm form a compact struc ture that is inherited by only one of the two daughter cells at each division. Thus, at late cleavage (a round 1000 cells), only four cells have the germ plasm. However, after this stage, the germ plasm is distributed evenly at cell division, creating four clusters of primordial germ cells (see Figure 16.12). Germ cell determination in mammals In insects, frogs, nenlatodes, and flies, the geml cells are in mammals, there is no obvious germ plasm. Rather, germ cells are induced in the embryo (Wakahara 1996; Hayashi et al. 2007). In mice, the germ cells form at the posterior region of the epiblast, at the junction of the extraembryonic ecto derm, epiblast, primitive streak, and allantois (Fi g u re 1 6.7A, B). This is called the posterio r p roxi ma l epiblast because it is close (proxinlal) to the extraembryonic ecto derm, and it will be at the posterior of the embryo. Thus, the cells that become the PGCs in mice are not intrinsical ly different from the otl,er cells of the epiblast and contain no specific germ plasm. Rather, the posterior epiblast cells are induced by the extraembryonic tissue. Wnts from the visceral endoderm are p roba bly responsible for giving the posterior p roxi.mal epiblast cells the competence to determined by material i.n the egg cytoplasm. However, THE SAGA OF TH E GERM LINE (A) Anterior (S) _ ""i111 All _ 'c.c- T P e ndoder m FIGURE 16.7 Specification and migration of mammalian pri mordial germ cells. (A) I n the mouse embryo, BMP signals (blue) from the extraembryonic ectoderm induce neighboring epiblast cells (purple circles) to become precursors ot pees and extraem bryonic mesoderm. Duri g gastrulation (arrow), these cells come to reside in the posterior epi bl st (S) On embryonic day 7, these cells emerge from the posterior primitive streak. The PGc precur sors also express Prdm 14 and blimp 1 (red). (C) Late st eak Slage (approximately embryonic day 7.0), Nuclei are stained whit.e (with DAPI), and the expression of the Prdm 14 gene (havi ng been fused to the mRNA of a green fluorescent protein) is seen by the green fluorescence. Arrowhead shows expression in the pee in the extraembryon ic mesoderm. A, anterior; P, posterior.

(A,B after Hogan 2002; C from Yamaji et al.

2006, courtesy of M. Saitou.) n . - (C) s toi PGCs rim t e streak bud ,""-'L -W---' a an 589 i v r signals and BMP4 gave rise to PCCs. When such pecs from cultured male pib ast cells were transferred into tes e l ticular tubules, they produced viable sperm that could fer tilize mouse eggs (Oh nata et a1.

2009). respond to BMP signals provided by the extraembryonic ectoderm.' This happens during gastrulation (at about day i The inert genome hypothesis 6.5 in mice), before the g rm lay rs are established (Fig ure 16.7C; Pfist r et a1. 2007; Yamaji et a1. 2008; Ohinata et As indicated above, one of the critical events in spe ifying a1. 2009). germ cells because they are forbidden to become any other c e c expr ssi n Accordlng to this hypothesis, the cells become e The BMPs induce the expression of blimpl and Prdml4 in a small cluster of cells (about six cells in the 6 s-day . represses somatic-type gene expression, while activating mouse embryo). Blimp1 is a transcriptional regulator that those genes (such as Sox2 and Nanog) associated with pluripotency. BLimpl also activates the germline determi nant Nanos3, which protects the germ cells against apop c l ob al repression of gene germ cells appears to be the g o type of cell (Nieuwkoop and Sutasuxya 1981; Wylie 1999; Cinaill et al.

2(08). This suppression of transcription is seen in the germ cells of several species, including mammals, flies, frogs, and nematodes (see Figure 16.2). In mice, the germ cells undergo extensive chromatin modification (Seki et al 2(07), causing them to become transcriptionally inert . tosis during their migration (Tsuda et a1. 2003).

Prdml4 at embryoni day 8.5 (as they begin migrating). Many of it is critical for the chromatin modifications th t will later the components in the germ plasm (such as Gel, Pgc, Piwi, helps establish pluripotency by also activating 50x2, and a c and Nanos in Drosophila and PIE-1 in C. elegans) act by silence the genome of the germ cells (Yamaji et al. 2008). inhibiting either transcription or translation (Leatherman et germ cell fate (Saitou et al. 2002; Ohinata et a1.

2005). The requirement for germ cell induction was shown by aJ. 2002; de l s Heras et aJ. 2009). Many such proteins are fOlmd throughout th animal kingdom. It is interesting that Cells that express blimpl and PI'dm1 4 are restricted to the transplanting clumps of tissue from rhe distal portions of the epiblast to the proxi mal posterior portion of the epi blast. These cells then gave rise to PCCs (Tam and Zhou 1996).

Moreover, cultured epiblast cells exposed to Wnt occur only and antelior. e when animal germ cells are separated from the somatic cells-whether in chicks, mice, or flies-the germ cells are often specified olltside the developing body proper.

Per haps this exile into an extraembryonic "enclave" insulates the primordial germ cells from para rine Signaling taking c son 1994). Once the repression of somatic gene e pression place within the somatic cells of the growing embryo (Dick c rr epiblast, BrvrP antagonists prevent it from oc ""TIls induction can a u ing in the trunk in the posteriormost region of the x is accomplished, the germ cells can retUIn to the embryo and travel to the gonads. This germ cell migration will be our next topic. 590 CHAPTER 1 6 SIDEL I G H TS SPECULATIONS Pluripotency, Germ Cells, and Embryonic Stem Cells P rimordial germ cells and embry onic stem cells are both charac terized by their abil ity to gener-

ate any cell type in the embryo. Embryonic stem (ES) cells are derived from the inner cell masses of mam malian blastocysts and are bel ieved to be the functional equivalent of the inner cell mass (ICM) blastomeres (see Chapter 8). One of the best pieces of evidence for this equivalence is that when ES cells are i njected into the ICMs of mouse blastocysts, they behave like mouse blastocyst cells and contribute cells to the embryo. One of the interesting species-specific d iffer ences between human and mouse ES cells is that human ES cells appear to (A) contribute to the trophoblast, whereas mouse ES cells do not (Xu et al. 2002). Transcription factors associated with totipotency In mammals, the retention of totipoten cy or pluripotency has been correlated with the expression of three nuclear transcription factors: 0(t4, Stat3, and Nanog (see Chapter 8). Oct4 is a homeodomain transcription factor expressed in all early-cleavage blas tomere nuclei, but its expression becomes restricted to the {eM. During gastrulation, Oct4 becomes expressed solely in those posterior epiblast cells thought to give rise to the primordial germ cells. After that, Oct4 is seen (8) only in the primordial germ cells and, later, in oocytes (Figure 16.8; see also Figure 8:18). Oct4 is not seen in the developing sperm after the germ cells reach the testes and become commit ted to sperm production (Yeam et al. 1 996; Pesce et al. 1 998). Nanog is another homeodomain transcription factor found in the pluripotential cells of the mouse blastocyst, as well as in ES cells and germline tumors. Nanog expression is high in the PGCs of cer tain mouse embryos (Hatano et aL 2005; Yamaguchi et al. 2005). Knock out experiments indicate that Nanog is critical in maintaining the pluripotency of stem cells, and overexpression experiments demonstrate that elevated (C) ; . (D) - " Figure 1 6. 8 Expression of OC14 mRNA corre lates with totipotency and abi li ty to form germ cells in mammals. (A) Oct4 transcription (actor is stained green with a fluorescent antibody, while all cell nuclei are stained red with propidi um iodide. The overlap (yel low) shows that Oct4 is found on ly in the inner cell mass. (B,C) An Oe1411acZ transge ne driven by the Oct4 promot er region shows its expression (dark color) (6) in the posterior ep iblast of th e 8 . 5 -day mouse embryo and i n migrating pees in the 10.5day embryo. (O,E) Labeled antibody (brown) staining shows Oet4 protein in the nuclei of (0) spermatogonia in post nata l testes and (E) oogo nia in from Yeam et al. 1 996; O,E frolll Pesce et al . 1 998; cou rtesy of H. R. Sch6Ier.) (C) postnatal ovaries. (A C T H E SAGA OF T H E GERM L I N E 591 SIDELIGHTS & SPECULATIONS (Conti n u ed) Epithelium Keratinized cells Bone matrix Cartilage Figure 16.9 Photomicrograph of a secti on through a muse teratocarcinoma, showing numerous differe ntiated cell types. (From Gardner photograph by C. Graham, courtesy of R. L. Gardner.) 1 982; Nanog negates the need for Stat3 and by itself maintains Oct4 transcription in ES cells (Chambers et a l . 2003; Mit sui et al. 2003).

The same pluripotency and tran scription factor expression pattern is seen not only in the PGCs but in two derivatives of the PGCs: cultured embryonic germ cells, and t umorous germ cells called teratocarcinomas. Embryonic germ cells When PGCs are first placed into cul ture, they resemble ES cells. Stem cell factor increases the proliferation of migrating mouse primordial germ cells i n culture, and this proliferation can be further increased by adding another growth factor, leukemia inhibition fac tor (LIF). However, the life span of these PGCs is short, and the cells soon die. But if an additional mitotic regulator -':' basic fibroblast growth factor, Fgf2 is added, a remarkable change takes place. The cells continue to proliferate, producing pluripotent embryonic stem cells with characteristics resembling those of the inner cell mass (Matsui et al.

1 992; Resnick et a l .

1 992; Rohwedel et a l . 1 996). These PGC derived cells are called embryonic germ (EG) cel ls, and they have the potential to differentiate into a l l the cell types of the body. Connective tissue Keratinizing epithelium I n 1 998, researchers i n John Ger hart's laboratory cultured human EG cells (Shamblott et a l . 1 998). These cells were able to generate differenti ated cells from a l l three primary germ layers, so they are presumably pluripotent. Such cells could be used medically to create neural or hematopoietic stem cells, which might be used to regenerate damaged neural or blood tissues. EG cells are often considered ES cells, and the distinc tion of their origin is ignored. Embryonal carcinoma cells What happens if a PGC became malig nant? In one type of tumor, the germ cells become embryonic stem cells, much l i ke the Fgf2-treated PGCs in the experiment above.

This type of tumor is cal led a teratocarcinoma. Whether spontaneous or experimenta l l y pro duced, teratocarcinomas contain an undifferentiated stem cell population that has biochemical and developmen tal properties remarkably similar to those of the inner cell mass (Graham '1 977; see Parson 2(04). Moreover, these stem cells not only divide but can also differentiate into a wide vari ety of tissues, including gut and respi ratory epithelia, muscle, nerve, carti lage, and bone (Figure 1 6.9). These undifferentiated pluripotential stem cells are cal led embryonal carcinoma (EC) cells. Once differentiated, these cells no longer divide, and are there fore no longer mal ignant. Such tumors can give rise to most of the tissue types in the body (Stevens and Little 1 954; Kleinsmith and Pierce 1 964; Kahan and Ephrussi 1 970). Thus, the terato carcinoma stem cells mimic early mammalian development, but the tumor they form is characterized by random, haphazard development. In 1 98 1 , Stewart and Mintz formed a mouse from cells derived i n part from a teratocarcinoma stem cel l . Stem cells that had arisen i n a teratocarcino rna of an agouti (yel low-tipped) strain of mice were cultured for several cell generations and were seen to maintain the characteristic chromosome com plement of the parental mouse. Indi vidual stem cells descended from the tumor were injected into the blasto cysts of black-furred mice. The blasto cysts were then transferred to the uterus of a foster mother, and l ive mice were born. Some of these mice had coats of two colors, indicating that the tumor cell had integrated itself into the embryo. This, in itself, is a remarkable demonstration that the tissue context is critical for the phenotype of a cel l a malignant cell was made nonmalig nant. B u t the story does not end here. When these chimeric mice were mated to mice carrying alleles reces sive to those of the original tumor cell, the alleles of the tumor cell were expressed in many of the offspring.

This means that the originally malig nant tumor cell had produced many, if not a l l, types of normal somatic cells, and had even produced normal, func tional germ cells! When such mice (being heterozygous for tumor cell genes) were mated with each other, the resulting litter contained mice that were homozygous for a large number of genes from the tumor cell (Figure 16.10). Thus, germ cell tumors can retain their pluripotency. Germ cells and stem cells: Possible interactions One idea emerging from this study is that some descendants of the pluripo tent cells (such as the teratocarci noma or ES cells) form PGCs that can under go meiosis to form sperm or eggs. I ndeed, there is evidence that ES cells (Continued on next page) CHAPTER 1 6 592 Insert celb asls oIntocystemt SIDELIGHTS & SPECULATIONS (Conti n u ed) can develop into oogonia that enter meiosis and recruit adjacent cells into fol licle-like structures (Hubner al. 2003). There is also parallel evidence that mouse ES cells can be made to d i fferentiate into sperm atocytes that can become functional sperm when an planted into testes (Toyooka et a l . 2003). These studies need to b e exten sively confirmed and extended to humans if they are to provide a new way of curing inferti lity. et It is even possible that ES, EG, and presumptive PGe cells. Zwaka and Thomson (2005) hypothesize that the cells are actually the equivalent of PGes and not of the inner cell mass. Not every inner cell mass blastomere ES can become an ES cell, and Zwaka and Thomson suggest that perhaps the successful stem cells are those that have been positioned next to tro at the future posterior proxi mal region of the embryo. In other words, the blastomeres that become the ES cells might actua l l y be the pre sumptive PGes.

While this idea remains hypothetical, it would relate these four pluripotent cell t pe . phoblast y s s ' - l t breeding mice n y e 1 n , 1 98 1 .) - mto Figure 1 6.10 Protocol for whose genes are derived largely from tumor cells. Stem cells are isolated from a mouse teratocarcinoma and i nserted i to blastoc sts from a different strain of mouse. The chimeric blastocysts are implanted in a foster mother. If the tumor cells are integrated into a blastocyst, the mouse that dev lops will have many of its cells derived from the tumor. If the tumor has given rise to germ cells, the chimeric mouse can be mated to normal mice to p roduce an F I generation. Because F1 mice should be heterozygous for all the chro mosomes of the tumor cell, mati gs between F I m ice should produce F m ice that are homozygous for some genes derived from the tumor. Thus F2 mice should express many tumor cell genes. (After Stewart and Mi tz \" - r " Mosal C -q N t Wild type ' ,, .

" 0 - ' tt @ FI wheren tumor-deri the germ celvedls genes contai t 0' -q @ n "Newtwo strain" mi(Fz)ceformed when are mated FI repulsion. The products o f the Germ Cell Migration wunen genes appear to be responsible for directing the migration of the primordial Germ cell migration in Droso p hila During r 0'1 r)] t /4r (e'.-i ./, r\111 lli)8:.,"': (-)../ R) 10 IrlfM IncorpOlahon \ II Materatocarcmoma Ignan Isolahnete stem cell mass mner SUlglCal transfer cell mto foster mothel 0';;:. , ' tr s EC cells have a common origin i n the l germ cells from the endoderm into the mesoderm and their division into two streams (Figure 1 6. 1 1 C E). This protein Drosophila embryogenesis, the primordial germ is expressed in the endoderm immediately before PGC cells move from the posterior pole to the gonads. The first migration and in many other tissues that the germ cells step in this migration is a passive one, wherein the avoid, and it appears to be repelling the germ cells.

In loss 3O pole cells are displaced into the posterior midgut by the movements of gastrulation (Figu re 1 6.1 1A,B). The germ cells are actively prevented from migrating during this stage (jaglarz and Howard-of-function mutants of this gene, the PCCs wander ran domly (Zhang et a1. Sano et al. 1997; Hanyu-Nakamura et aJ.

2004, 2005). 1994; Li et aJ. 2003). In the sec HMC-CoA reductase, the product of the columbus gene, ond step, the gut endoderm

and the chromosomes are thus joined togeth er (Figure 16.1 7A,B).

Examinations of meiotic cell nuclei with the electron microscope (Moses 1968; Moens 1969) suggest that paired chromosomes are bound to the nuclear membrane, and Comings (1968) has suggested that the nuclear envelope helps bring together the homologous chromosomes.

The configuration formed by the four chromatids and the synap tonemal complex is referred to as a tetrad or a bivalent. During the next stage of meiotic prophase, pachytene (Greek, "thick thread"), the chromatids thicken and short en. Individual chromatids can now be distinguished under the light microscope, and crossing over may occur. Cross ing over represents an exchange of genetic material where by genes from one chromatid are exchanged with homol ogous genes from another. CrOSSing over may continue into the next stage, diplotene (Greek, "donble threads").

Here, the synaptonemal complex breaks down, and the two homologous chromosomes start to separate. Usually, however, they remain attached at various points called chi asmata, which are thought to represent regions where crossing over is occurring (Figure 1 6.1 7C). The diplotene stage is characterized by a high level of gene transcription. In some species, the chromosomes of both male and female germ cells take on the "lampbrush" appearance character istic of chromosomes tha ve been actively making RNA (see below). During the next stage, diakinesis (Greek, "moving apart"), the kinetochores move away from each other, and the chromosomes remain joined only at the tips of the chro matids. This last stage of meiotic prophase ends with the breakdown of the nuclear membrane and the migration of the chromosomes to the metaphase plate. Anaphase of meiosis I does not commence until the chromosomes are properly aligned on the mitotic spindle fibers. This align ment is accomplished by proteins that prevent cyclin B THE SAGA OF THE GERM LINE (8) (A) 599 (C) elements Transverse filament elements elements FIGURE 16.17 The synaptonemal complex and recombination. (Al I nterpretive diagram of the ladder l i ke sy naptonemal complex s tru c tu re (B) Homologous chromosomes held together in a synapto nemal com pl ex during the zygotene phase oi the first mei otie prophase in a Neortielfa (mushroom) oocyte. (C) Chiasmata in di p l o tene bivalent chromosomes of salam ander oocytes. Kineto . from being degraded until after all the chromosomes are securely fastened to micro tubules. If these proteins are defi cient, aneuploidies such as Down syndrome can occur (Homer et aJ. 2005; Steuerwald et al 2(05). During anaphase I, the homologous chromosomes are separated from each other in an independent fashion. This stage leads to telophase I, during which two daughter ceUs are formed, each cell containing one partner of each homol ogous chromosome pair. After a brief interkinesis, the sec ond division of mejosis takes place. During this division, the kinetochore of each chromosome divides during anaphase 50 that each of the new cells gets one of the two chromatids, the final result being the creation of four hap loid cells. Note that meiosis has also reassorted the chro mosomes into new groupings. First, each of the four hap loid cells has a different assortment of chromosomes. Humans have 23 different chromosome pairs; thus 223 (nearly 10 million) different haploid cells can be formed from the genome of a single person. In addition, the cross ing-over that occurs during the pachytene and diplotene stages of prophase I further increases genetic diversity and makes the number of potential different gametes incalcula bly large. See WEBSITE 1 6.4 Human meiosis Meiosis is the core of sexual reproduction, and it seems to have arisen in the ancestor of fungi, plants, and animals. Indeed, nearly all the genes and proteins used in yeast (fungus) meiosis also function in mammalian meiosis. This observation has allowed the identification of a "core mei otic recombination complex" used by plants, fungi, and animals. This meiotic recombination complex is built on rings of the cohesin proteins, which encircle the sister chromatids. The rings of cohesin resist the p ulling forces of the spindle micro tubules and thereby keep the sister chromatids attached together (Haering et aJ. 2008; Brar et chores are visible as darkly stained circles; the arrows point to the two ch iasmata . (A aher Schmekel and Dancholt 1 995; B from von Wettstein 1 9 7 1 , courtesy of von Wettstei n; C courtesy of J. Kezer) . D. aJ. 2009). This complex recruits another set of proteins that help promote pairing between the homologous chromo somes and allow recombination to occur (Pelttari et aJ. 2001; Villeneuve and Hillers 2(01). These recombination indUCing proteins are involved in making and repairing double-stranded DNA breaks. The cohesin proteins will be degraded in the second meiotic division. Although the relationship between the synaptonemal complex and the cohesin-recruited recombination complex is not clear, it appears in mammals that the synaptonemal complex stabilizes the associations initiated. by the recom bination complex, giving a morphological scaffolcling to the tenuous protein connections (Pelttari et aJ. 2(01). If the synaptonemal complex fails to form, the germ cells arrest a t the pachytene stage and their chromosomes fragment (Figure 1 6. 1 8). If the murine synaptonemal complex forms but lacks certain proteins, chiasmata formation fails, and the germ cells are often aneuploid (having multiple copies of one or more chromosomes) (Tay and Richter 2001; Yuan et a1. 2002). The events of meiosis appear to be coordinated through cytoplasmic connections between the dividing cells. Whereas the daughter cells formed by mitosis rou tinely separate from each other, the products of the meiot ic cell divisions remain coupled to each other by cytoplas mic bridges. Animals can modify meiosis in different ways. Some of the most interesting modifications have occurred in those animal species tha t have no males. In these species, meio sis is modified such that the resulting gamete is diploid 600 CHAPTER 1 6 (A) (ll) FIGURE 16.18 Importance of the synaptone ma l complex. The CPEB p rotei n is a c ons tituent of the synaptonemal complex, and when this gene is knocked out in mice, the synapto nema l complex fails to form. (A,B) Staining for synap tonemal complex proteins in wild-type (A) and CPEB-deficient (B) mice. The syna ptonema l complex is stained green, and the DNA is stained blu e. The synaptonema l complex is absent in the mutant nuclei, and their DNA is not organized into discrete chromosomes. ((,D) I n preparations stained with hem$\hat{}$toxylin-eosint the DNA can be seen to be in the pachytene stage in the wild-type cells IC) and fragmented in the mutant cells (0). (From Tay and Richter 2001 , cou rtesy of ich t r) (D)n.,,-.....,....,"... .:_ .. -. (C) J. D. R e .

and need not be fertilized to develop Such ani ma ls are said to be parthenogenetic . (Greek, virgin birth"). In the fly Drosophi la l/umgabeirai, one of the pol a r bodies (a " meiotic cell ha ving very little cy topl asm) acts as a spenn and fertilizes the oocyte after the second meiotic division. In some other insects and in the lizard Cnemidophorus uniparens, the Dag ama further double their chromosome number before meiosis, so that the halv ing of the chromosomes restores the diploid number. The germ cells of the grasshoppe r Pycnoscelus surinamensis dis pense with meiosis al together, forming diploid ova by two mi totic divisions (Swanson et aL 1981). All of these species consis t en ti rely of females. In other spec ies, hapl oi d parthenogenesis is widely used not onl y as a means of " " reproduction but also as a medlanism of sex determina tion. In the Hy m enoptera (bees, wasps, and ants), unfer tilized haploid eggs develop into males, whereas fertilized eggs are diploid and develop into femal es . The haploid males are ab le to produce sperm by abandoning the first meiotic division, thereby forming two sperm cells through second meiosis. SIDEL IG H TS SPECULATIONS Big Decisions: Mitosis or Meiosis? S perm o r Egg?

I n many spec ies, the germ cells m igrating into the gonad are bi po tential and can differentiate into either sperm or eggs, depe n d i ng on their gonadal environment. When the ovaries of salamanders are ex peri m en tally transformed into testes, the resi dent germ cells cease their ooge ni c differentiation and begin devel opi ng as sperm IBurns 1 930; H umph rey 1 93 1). Si m i l arly, i n the ho u sefl y and mouse, the gonad is able to direct the differen tiation of the germ cells (McLaren Th us, in most orga nisms the sex of the gonad and that of its germ cells is the 1 983; Inoue and Hiroyosh i 1 986). , sa m e But what about hermaphroditic animals, wh ere th e cha nge from sperm production to egg production is a naturally occ u rri ng p hys i o l ogi ca l event? How is the same animal capa ble of producing sperm during one part of its l ife and oocytes during another part?

U s i n g Caenorhabditis elega ns, Kimble and her co l l eagues . identified two "decisions" that pre sumpt ive germ cells have to ma k e The first is whet he r to enter meiosis or to remain a m i toti ca l l y dividing stem cell. The second is w h et her to become an egg or a sperm. There is evidence that these deci sions are intimately l i n ked. The mitosis/meiosis decision in C.

e/egans is co n tro lled by a single nondividing cell-the distal tip cel l-l oca ted at the end of eac h go n ad The germ cell-pre cursors near this cell divide mitotical.

. T H E SAGA O F TH E GERM L I N E Pachyt (A) Mitotic region Transition ene g on r zon e $\hat{}$ 601 SIDELIGHTS & SPECULATIONS (Continued) $\hat{}\hat{}$ e _ _ _ A ' _ _ _ " $\hat{}$; -..g4.::" .. $\hat{}$. i . m igure 16.19 Regulation of the mitosis/meiosis decision in the adult ger l ine of C. eJegans. (A) The distal tip cell and its long projections co prise the stem m cell niche. The distal lip of this adult worm is stained green (by attach i ng the GFP gene to the promoter of the lag2 gene). Germline cells are stained red, and the mitotic region, transition zone, and pachytene region are shown. (B) The balance of m itotic and mei otic regulator pr teins determines wh ther a cel l remains in mitosi s or enters meiosis. Vertical ycl low bars represent the relative number of mitotic nuclei at each position in the mitotic region. The transition from m ito sis o meiosis begins where 60% of nuclei have the crescent-shaped morphology typi cal of early meiotic p rophase (red crescents in A). Green bars represent the percentage of nuclei in meiotic prophase at a given position a l ng the d istal-proxi mal axis. Lev els of G LP-l and FBF mitotic regu lators are high t hrough u t the mitotic region a d dec rease ra ra ti l y as the germ cells enter meiosis {horizontal yell w bands}. Conversely, levels of most meiotic regu lat rs (horizontal green bands) grad ally increase in the proximal part of the m itotic region, reaching high levels as germ cells enter meiosis. (One ex eption is NOS-3, which is distributed un i formly throughout the germ line.) (C) Simplified summary of a network co n ro ll ng the mitosis/meiosis decision. Notch are very similar proteins whose nega tive feedback loop may explain the size of the mitotic region, as it negatively regu lates levels of GLD-l and GLD-3, i nhi bi ti ng meiosis in the distal region of the germ li n e. (After Kimble and Crit ten den 2005; A cour tesy of S. Cri enden and I. Ki mble.) o t o d mo l ca Mitotic nuclei $\hat{}\hat{}\hat{}

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A and B. Mintz. 1981. Suc cessful generations of mice produced from an established culture line of euploid teratocarcinoma cells. Proc. Nat!. Acad. Sci. USA 78: 6314-6318. Weidinger, G., U. Wolke, M. Koprunner, M. Klinger and E. Raz. 1999. Identifica tion of tissues and patterning events required for distinct steps in early migration of zebrafish primordial germ cells. Development 126: 5295-5307. Go Online WEBSITE 16.1 Germline sex determination in C. ele gans. The establishment of whether a germ cell is to become a sperm or an egg involves multiple levels of inhi bition. Translational regulation is seen in several of these steps. WEBSITE 16.2 Mechanisms of chromosome diminu tion. The somatic cells do not lose DNA randomly.
Rather, somes as somatic cells do. Gene repetition and gene ampli fication are both used to transcribe these enormous amounts of rRNA WEBSITE 16.7 Honnones and mammalian egg matura tion. To survive, the follicle and its oocyte have to "catch the wave" 01 gonadotropic hormone release. The hormones of the menstrual cycle synchronize egg maturation with specific regions of DNA are lost during chromosome dirninu tion. the anatomical changes of the uterus and cervix. WEBSITE 16.3 The insect germ plasm. The insect ger sis. The hormone-mediated disruption of communication minal cytoplasm was discovered as early as 1911, when between the oocyte and its surrounding follicle cells may Hegner found that removing the posterior pole cytoplasm be critical of beetle eggs caused sterility in the resulting adults. WEBSITE 16.4 Human meiosis. Nondisjunction-the fai1ure of chromosomes to sort properly during meiosis is not uncommon in humans. Its frequency increases with maternal age. WEBSITE 16.5 The Nebenkern. Sperm mltochondria are often highly modified to fit the streamlined cell. The mlto chondria of flies fuse together to form a structure called the Nebenkern; this fusion is controlled by the fuzzy onions gene. WEBSITE 16.6 Synthesizing oocyte ribosomes. Ribo somes are almost a "differentiated product" of the oocyte, and the Xenopus oocyte contains 20,000 times as many ribo- WEBSITE 16-8 The reinitiation of mammalian meio in the resumption of meiosis in female mammals. Vade Mecum Genn cells in the Drosophila embryo. In the Fruit Fly seg ment, a view of gametogenesis follows the primordial germ cells of the living Drosophila embryo from their for mation as pole cells through gastrulation as they move from the posterior end 01 the embryo into the region of the developing gonad. Gametogenesis in mammals. Stained sections of testis and ovary illustrate the process of gametogenesis, the stream lining of developing sperm, and the remarkable growth of the egg as it stores nutrients for its long journey. You can see this in movies and labeled photographs that take you at each step deeper into the mammalian gonad. PART SYSTEMS B I O LOGY Expanding Developmental Biology to Medicine, EcologYt and Evolution pter 17 Medical aspects of developmental biology pter 1 8 Developmental plasticity and symbiosis pter 19 Developmental mechanisms of evolutionary change Systems biology is an attempt to redefine the priorities of biology as a discipl ine, focusing on its complexity and integration. Throughout this book, we have focused on the physical, looking at development in terms of DNA sequences, cells, and organs.
Systems theory provides a complementary, if more abstract, way of look ing at development: it views development as the flow of information. This i nfor mation is embodied i n the genes, cells, organs, and external environment. Indeed, if i nformation flow i s the process unifying these disparate entities, one might be able to establish equations and models for developmental phenomena.
Such mod eling i s one of the goals of many systems developmental biologists (see Van Spey broeck et a l. 2005; Madar et al. 2009; Edelman 2 0 1 0). Different streams of systems biology emphasize different principles (see De Backer et al. 201 0). Some are extensions of molecular biology and aim to model the molec ular networks underlying phenotypic diversity within and between species (see the discussion of micromere specification in Chapter 5 or the discussion of Drosophi la segmentation genes i n Chapter 6). In such approaches, models are proposed based on bioinformatics (genome sequences, protein composition, etc.), and exper iments are set up confirm or deny the model and, as i n all science, to predict fur ther properties that can be verified (or not) by further experimentation. Thus, there is a continuing cycle of laboratory experimentation and computer modeling.
Another approach to systems biology is embodied in the "systems theory approach," which attempts to understand the principles behind biological organ ization and to relate the different levels of organization (gene, cell, tissue, organ, organ ism, ecosystem, etc.) to one another. Modern systems theory approach can be 618 PART IV said to have had its sta rt i n developmental biology, which was one of the first sciences to utilize these pr i nc i ples of causation, i ntegration, and context dependency. The demonstration of embryonic regulation, where one blastomere of a 4-cell sea urchin embryo could become an entire larva and where the fate of a newt blastomere depended on its neighbors, caused a crisis in the field of experimental embryology. I n response to the vitalism of Driesch (who held that there must be some non-physi cal wisdom directing development) and the reductionism of Raux (who held that the embryo was basically a machine), Oskar Hertwig (1 894) proposed a type of materi alist philosophy called wholist orga nici sm. This philosophy embraced the views that, first, the properties of the whole cannot be predicted solely from the properties of its component parts; and second, that the properties of the parts are informed by their relationship to the whole. Subsequently, several developmental biologists notably Paul Weiss (1 926) and Ludwig von Bertalanffy (1 928, 1 932) formalized these notions into a systems approach to development (Brauckmann 2000; Drack et al. 2007). Both the reductionist and organicist approaches are materialist i n that they do not invoke any extramaterial agent (entelechy, the soul; Bildungslrieb) as directing devel opment. However, while reductionism claims that a l l complex. entities (including pro teins, cells, organisms, and ecosystems) can be completely explained by the proper ties of the i r component parts, organicism claims that complex wholes are inherently greater than the sum of their parts in the sense that the properties of each part are dependent on the context of that part within the whole i n which it operates. Thus, when we try to explain how the whole system behaves, we ca nn o t get away with speaking only about the parts.
These explanations are no less materialistic than reduc tionism. As Denis Noble (2006) has stated, Systems biology . . . is about putting together rather than taking apart, integration rather than reduction. It requires that we develop ways of thinking about i n tegra tion that are as rigorous as our reductionist programmes, but different. ... It means changing our philosophy, i n the full sense of the term. Although the emphaSiS differs widely among systems biologists, the theoretical systems approach can be characterized as having fi ve principles that apply directly to the study of development. o o o Context-dependent properties. The meaning or role of an individual com ponent of a system is dependent on its context on what is before and after, above and below it. What BMP4 does, for instance, depends on the history and context of the cell that receives it. At one time and place, BMP4 may signal bone formation; at another time and place, it may signal apoptosis; at yet another, it may specify epidermis. Level-specific properties and emergence. The properties of a system at any given level of organization cannot be totally explained by those of levels "below" it. Thus, temperature is not a property of an atom, but a property that "emerges" from an aggregate of atoms. Similarly, voltage potential is a property of a biological membrane but not of any of its components. High er-level properties result from lower-level activities, but they must be under stood in the context of the whole. Heterogeneous causation and integration. I n biology, causation is seen as being both "upward," from the genes to the environment, and "downward," from the environment to the genes. What a cell is depends both on its genes and on the other cells surrounding it (i.e., on the input it receives from both internal and external sources). Systems biology demands the integration of different types of explanations. It also calls for the integration of analysis (taking things apart) and synthesis (putting things together). SYSTEMS BIOLOGY • Modularity and robustness. The orga nism develops as a system of modules. Raff (1 996) characterized developmental modules as having discrete genetic specification, hierarchical organization, interactions with other modules, a particular physical location within a developing organism, and the ability to undergo transformations on both developmental and evolutionary time scales.
Robustness (sometimes called canalization) refers to the abil ity of an organism to develop the same phenotype despite perturbations from the environment or from mutations. This is a function of interactions within and between modules. We w i l l look briefly at each of these in light of animal development. Context-Dependent Properties Language provides an analogy by which organicism can be understood (Co l l ier 1 985). Certai n combinations of letters form words, and certain organizations of words form sentences. The meaning of a sentence obviously depends on the meaning of each component wo rd (the parts define the whole). But most words have multiple and distinct meani ngs, and it is the context of the sentence that determines which specific meaning of a word is most appropriate (the whole defines the parts). Con sider the following three sentences: 1 . The party leaders were split on the platform. 2. The disc jockey was a black rock star. 3. The pitcher was driven home on a sacrifice fly. I n these sentences, each word's meaning is suspended until the sentence is com plete; the words and sentence mutually define each other. I n other words , when put into relation with each other, specific meanings of words are singled out, resulting i n a series of words with a particular ("emergent") meaning (i.e., a story is formed). Parts determine the whole, and the whole determines each of its parts. In embryology, we are constantly aware of the parts being determined by their con text within the whole (Needham 1 943; Haraway 1 976; Hamburger 1 988; Gilbert and Sarkar 2000). I ndeed, in vertebrate embryos, the fate of a cell is spec ified by its posi tion.
Simi larly, as said earlier, the function of a protein is determined by its context BMP can induce apoptosis or bone formation; 8-crysta l l i n can be a transparent lens protein or a l iver enzyme; -catenin can be a cofactor of transcription factors or adhe sion molecules. . Emergence In our l i nguistic metaphor, emergent properties can be seen in the relationship of letters to words. Standing alone, most letters usually have no intrinsic meaning. But when letters are grouped together in certain arrangements and according to certain rules, meaningful words emerge. In developmental biology, one often encounters these emergent properties. For example, in renal development, the nephron is formed by i nteractions between the ureteric bud and the metanephrogenic mesenchyme. If one cultures these two tis sues separately, neither develops any portion of the kidney However, if you place these tissues together, the mesenchyme cells form the 1 0 cell types cha rac teristic of the renal fi ltration apparatus, and the ureteric bud tissue branches j ust as it would in the intact organism. Thus, 1 0 new cell types "emerge" from the interactions between 2 cell types, neither of which had any of the specific properties of the proximal con voluted tubule cells, juxtaglomerular cells, or Bowman's capsule cells. Moreover, the . 619 620 PART IV shape of the filtration apparatus, with the tubules descending i nto Henle's loop before ascending into the glomerulus; the connection between the nephron and the collect ing duct; and the h istotypic arrays of the nephrons and their relationship to the col lecting ducts are all higher-order properties that are reproducibly seen from kidne) to kid ney. Yet none is a property predicted from the properties of the isolated metanephrogenic mesenchyme cells or the ureteric bud. Level-Specific Rules Parts are organized into wholes, and these wholes are often components of larger wholes. Moreover, at each biological level there are appropriate rules, and one can not necessarily "reduce" all the properties of body tissues to atomic phenomena. As mentioned above, temperature is not an appropriate concept for a n atom, nor is semipermeabi l ity a proper concept for a protein. When you have an entity as com plex as the cell, the fact that quarks have certain spins is i rrelevant. This is not to say, however, that each level is independent of those "below" it. To the contrary, laws at one level may be almost deterministically dependent on those at lower levels; but they may also be dependent on levels "above." This notion of level-specific interac tive modules forms the basis of many new computer programs. Indeed, the impor tance of level-specific rules to our conception of reality in general has been empha sized by philosophers and has been summarized by Dyke (1 988) and Wimsatt (1 995). The noted embryologist R. C. Harrison was firm on this point: that there were "integrative levels" of organization i n the embryo and that one could not homoge nize these levels. As Joseph Needham (1 943) wrote, The deadlock [between mechanism and vitalism) is overcome when it is realized that every level of organization has its own regularities and princi ples, not reducible to those appropriate to lower levels of organization, nor applicable to higher levels, but at the same time in no way inscrutable or immune from scien tific analysis and comprehension. Heterogeneous Causation What is the "cause" of a cell's fate or behavior? Often we depict this as a one-way arrow, from the nucleus to the cytoplasm, or from the "bottom up." But as we have seen, paracrine factors and extracellular matrix (i.e., "higher levels") also play roles i n determ i n i ng which genes are expressed. In the next three chapters, we will see that "top-down" causation-that is, from the environment to the genome-is criti cal i n medicine, ecology, and evol ution. We will see that a variety of substances present in the environment, both anthropogenic and natural, can adversely affect animal development. We will see that symbionts (organisms that live i n and on a second, host, organism) are often crucial to the host's development. And we will see how environmental forces i nteracting with developing organisms can drive the processes of biological evolution. To say that there is a "gene for" something is often poor shorthand for a large vari ety of factors (see Tauber and Sarkar 1 992; Moss 2003). As we have seen, gene prod ucts work together, and the genetic approach usually is able to determine only that a particular gene is necessary to make a particular cell type or tissue. A gene's loss of function means that an organ doesn't form or function. In terms of the whole organ ism, this is analogous to saying that a "gas pedal gene" is responsible for automo b i les, since cars do not function without one. Even when one has gain-of-function studies (such as those showing that three transcription fadors will convert an exocrine pancreatic cell into an insulin-secreting beta cell), the i n serted genes are working SYSTEMS BIOLOGY with a host of transcription factors, methylating enzymes, and other components that are the cell's heritage.
That causation occurs from below and from a bove is becom ing an important con cept in medicine and conservation biology. The specification of sex in turtles and many other reptiles is dependent not only on the genome (which provides the infor mation that sets the threshold temperature separating males and females) but also on the immediate temperature of the organism's environment. This knowledge is impor tant for biologists attempting to protect endangered turtle species. The link between an organism's development and the symbionts that naturally infect that organism is often so tight that development cannot take place without the symbionts being present. Such l i n kage is critical in development and evol ution, and even in medicine. One new way of treating i nfections of parasitic worms, for example, is to give patients antibiotics. These drugs do not destroy the worms directly; rather, they wipe out the prokaryote symbionts that provide factors essential for the worm's development (Hoer auf et al. 2003; Coulibaly et al. 2009).
Thinking of the host and symbiont as a devel oping system allows us to see development in a new way and al lows new questions to be asked.
Integration One of the chief components of systems biology is its insistence on synthesis as well as analysis. This is also one of the biggest sources of heterogeneity among those who consider themselves systems biologists. True to the notion of level-specific rules, there can be different systems biologies at different levels, but each of them will be synthetic. For i n stance, on a single level-that of gene transcription-Davidson and col leagues (see Chapter 5) have attempted to map a l l the gene inputs i nvolved i n the specification of the early sea urchin embryo. This systems approach creates a "wiring diagram" of a l l the inputs and outputs of each gene involved in cell specification, and finds the rules (such as the "double-negative gate"; see Figure 5 . 1 2 B) that are the "logic" of development. But this approach can be expanded by defining the "sys tem" not as the cell or the organism itself, but as the cell within an organism within a n ecosystem.
This is done routinely i n the subspecialty of obstetrics and gynecol ogy ca l led maternal-fetal medicine. Here, neither the mother nor the fetus is seen as an entity separate from the other. As we w i l l see, the field of teratology (birth defects) concerns the modulation of cell behavior during development (differential gene transcription, cell specification and adhesion, etc.) by environmental agents mediated through the mother. Similarly, cancer research now sees many epithelial tumors a s arising from defects in the mesenchymal cel ls surrounding the epitheli um, and these defects can be caused by environmental agents (Soto et al. 2008, 2009). In these cases, the explanation of cell behavior is as much a function of "top down causation" from the environment as it is "bottom-up causation" from the genome. In eu karyotic embryos, there are so many levels of complexity that it w i l l be dif ficult to get an i nventory of every component involved (as wel l as an i nventory of potential i nteractions between the components). The compilation of such an i nven tory is being undertaken by the "-omics ": genomics (the inventory of genes), pro teomics (the i n ventory of proteins), transcriptomics (the inventory of RNAs), and metabalomics (the inventory of the sma l l molecule end-products of gene expres sion). Comparisons between the genomes of different species, and between the tran sCriptomes and proteomes of the same cell type in different species, have become a necessary precondition for seeing how organisms can change as they develop and evolve (Ge et al. 2003; Bonneau et al. 2007; Vidal 2009). Indeed, we will see numer ous examples (such as the effects of symbionts or different environmental conditions 621 622 PART IV on cell transcription) where comparing i nventories of gene products reveals that the outside agent is actually changing gene expression patterns. However, the lack of technology to measure the exact (yet often fluctuating) quantity of these compo- nents, and to map their context sensitivity and their often short and rapid reactions in real time, remains a hurdle to this endeavor. Modules and Robustness Systems biology views the embryo as a complex adaptive system (Cell-Mann 1 964; Edelman et al. 2 0 1 0). I t is "complex" because of the diversity of parts and the m u l tiple interacting modules. I t is "adaptive" because the component modules can mod ify their behaviors in response to environmental or genetic perturbations. Modularity is one of the most important concepts in all biology, and it is funda mental to any discussion of development and evolution. According to Bolker (2000), discussions of modularity i n development have emphasized (1) that a module is a biological entity (e.g., a structure, a cis-regulatory region, a cell l ineage, a morpho- genetic field) characterized by more internal than external int gration (Wagner 1 996; Wagner and Altenberg 1 996; von Dassow and Munro 1 999), and (2) that modules are biological entities that can be delineated from their surroundings or context, and whose behavior or function reflects the integration of their parts, not simply the arith metical sum. living beings are organized according to what philosopher Chuck Dyke (1 988) calls "level-interactive modular arrays."This concept implies a nest of modular struc- tures. Each entity is an organized array of constituent modular parts, and is at the same time the constituent of a larger module. The components of each module (which are themselves modules) interact to form the coherent (larger) module. Moreover, the modules i n teract with the levels above and below them.
Just as each organelle must function to make a coherent cell, so each cell and cell type must function to make a coherent tissue.
Thus, the tissue architecture of the liver can regulate elements in the modules below it (the liver extrace l l u la r matrix and interce l l u lar i n teractions within the tissue regu lating gene expression within the cell); and the lower- level modules such as those of the cell obviously determine the function of the higher-level mod u les such as the tissue.
Development depends on modularity. Suppose (as Dyke does) that you bought a bicycle online, and at the bottom of the product description a note said "Some assem bly reqUired." Since you're good with your hands and the price is reasonable, you order it.
A week later a truck del ivers to your door a basket of iron ore, a large sack of l imestone, a box of bauxite, a bag of charcoal, and a container fu l l of sap from a . rubber tree. The packing slip says "One bicycle." The synthesis of a bicycle (and cer tainly the development of an animal) is a path-dependent process comprising a large number of steps, and the path involves the synthesis of modular intermediates (Dyke 1 988). Without the production of these i n termediates, there is no way that an organ ism can come into being. Tissues must be made from pre-assembled cells, and cells are made from pre-assembled components such as proteins. Proteins must be made from pre-assembled amino acids, and so forth. Simon (1 962) argues for the importance of modularity, which he offers both as a prescription for human designers and as a description of the complex systems we see in nature. To make the latter point, he offers the parable of the two watchmakers.
Tem pus and Hora are both expert watchmakers who make complicated watches from numerous parts. However, they are both interrupted frequently in their work. Tempus does not design his watches as modular systems. He puts every piece in one at a time, so every time he is interrupted and forced to set aside his work, the unfinished assem bly fal l s apart. By contrast, Hora first builds stable subassemblies that he can then put together in h ierarchic fashion into larger, stable subassemblies. Thus, when Hora is SYSTEMS BIOLOGY interrupted, only the last unfinished subassembly falls apart.
In a selective environ ment, such developmental stability would be rewarded with evo lutionary survival (Simon 1 962). What makes Tempus's unfinished watches so unstable is not the number of parts involved. Rather, it is the parts' interdependency. In a non-modular system, the success ful operation of any given part is l i kely to depend on the characteristics of many other components throughout the system. So when such a system has missing Or defective parts, the whole ceases to function. In a modular system, by contrast, the proper work ing of a given part depends primarily on the characteristics of other parts within its subassembly, although it depends to some degree on the characteristics of parts out side that subassembly. As a result, a modular system is usually able to function to some degree even if some subsystems are damaged or incomplete. If one adds an editing function such as redundancy or the abil ity to replace a defective module- then one has assurance that the entire system w i l l function. As we will see, modularity al lows anatomical change to occur in one module with out necessarily affecting another module. This change during development is predicat ed on the modularity of enhancer sequences, mentioned i n Chapter 2 . Modularity also al l ows for robustness: the abil ity to retain the phenotype despite environmental or genetic changes. Robustness is a characteristic of living systems (Kitano 2004) and is responsible for the normal phenotype developing so typically, despite the m i l lions of interactions that have to occur sequentially and at the right place during embryo genesis.
This robustness is due to redundancy in control and to feedback loops between modules (von Dassow et al. 2000).
Thus, Spemann (1 927) spoke about "double assurance" (which he likened to wear ing both a belt and suspenders) on the anatomical level. The lens could form in more than one way, and if one tissue didn't induce the lens, the other tissue would. If the operculum did not open i n its normal manner during frog metamorphosis, another system kicked in and opened that flap of skin. More recently, redundancy has been found at the level of genes. Muscles w i l l form when the muscle-forming MyoD gene is knocked out of a mouse embryo, even if the details of the muscle development are not perfect (Wang et a l . 1 996). This is because MyoD usually downregulates the syn thesis of Myf5, a MyoD-like gene that can also d i rect muscle development. In the absence of MyoD, Myf5 is syntheSized and can perform most of the functions of the absent molecule. The segment polarity genes of Drosophila are i nterconnected by a series of regulatory loops such that changing any one of them sti l l gave proper seg mentation 90% of the time. As C. H. Waddi ngton (1 957) painted out, however, studying systems biology i n development i s going t o b e more difficult than studying systems biology i n physiol ogy. Physiologists attempt to understand the interactions among pa rts that exist throughout the life of the organism.
But Waddington pointed out that the task of the developmental biologist is not to understand homeostasis-how the organism main tains itself. Rather, the task of the developmental biologist is to understand home orhesis-how the organism stabi l izes its different cell l i neages while i t is sti l l con structing itself. Donna Haraway points out that modularity depends on i n tegrated processes, not factors. "Relationships," she writes (2008, p. 228) "are the smallest possible pattern for analysis." This way of thinking may make systems biology different from what has come before, and new inSights are begi n n ing to emerge. Waddington, who made substantial contributions to both developmental biology and systems biology, viewed development as a mountainous landscape and the cel l types as basins.
Like a ball rol l i ng down a hill, a cel l eventua l l y finds itself i n one or another of these basins. Basins are constructed through the interactions of cells during development. Kauff man (1 969, 1 987, 1 993) and others (Huang 2009a,b) see these basins as "attractor states," where a stable and robust configuration of gene expression becomes possi- 623 624 PART I V FIGURE 1'1.1 An "epigenetic land scape" ror a complex network wherein N genes can interaCl with one another. The overal l slope «rom back to front) represents the progression of develop ment over lime.
Normal developmental trajectories (blue l ines) lead to attractors representing distinct cell types and are prevented from entering "abnormal anractors" (red dashed circl e) by ep ige- netic barriers (lavender hill), Mutations or methylat'ion differences can lower this barrier, opening access to attractors that encode an abnormal/ imma tu re ph en o- type, including cancerous cell types (red arrow). (After H uang et al. 2009b.) Each network of N genes is a point in N-dimensional state space State space (projected t o n 2D plane) with epigenetic l a dscape n Epigenetic barrier (can be lowered by mutation) "Abnormal" attractor ... ..i (preexisting or generated -by mutation; normally unused) " .... - Elevation reflects the stabi ty of a state Each poin t represents a network state Unstable = transient state ur Stable mat e cell cype Stable mature cell type ble (Figure IV.1). The ability of a set of transcription factors to rearrange transcrip tional networks to convert exocrine pancreas or liver cells i nto endocrine pancreas cells can be seen as an example of such an attractor state (Horb et a l . 2003; Zhou et al. 2008). Most combinations of transcription factors and enhancers are expected to be unstable; those combinations that are stable, however, can become cell types. Huang and COlleagues (2005, 2009a) have provided evidence based on systems analy sis that the neutroph i l (a white blood cell) is such a stable cell type. Their computer analysis connecting over 2750 genes into networks found that the neutrophil expres sion pattern is the most stable pattern, and that the only "rival" to it is that of neu trophilic leukemia. The formations of new cell types (such as the neural crest cells of vertebrates) can be looked at as constructions of new stable attraction states over the c.D urse of evolution. The stem cell state of cell division so important for cancer may be another stable attractor state (Huang et a l . 2 009b). Denis Noble (2006) stated that the "ultimate goal of systems biology" is "to recon nect physiology and developmental models to theories of evolution." He also noted that we have just begun this enterprise. I n the closing chapters of Ihis book, we wi ll examine the roles of development i n disease, ecosystem dynamics, and evolution, keeping in mind the properties of systems. Medical Aspects of Developmental Biology OUR UNDERSTANDING OF MAMMALIAN DEVELOPMENT promises to expand our medical capacities in this next century as much as microbiology allowed us to regulate common infectious diseases in the past century. Organ regeneration, cancer therapies, and even the prolongation of healthy lives may be possible through stem cell technologies and our new knowledge of paracrine factors and transcription factors.
However, our technolOgical society is constantly produc ing new chemical compounds that can produce developmental anomalies in humans and wildlife. In both cases, developmental biology is now critical to issues of medicine and public health.
Developmental biology has been the basis of numerous breakthroughs in medicine, including in vitro fertilization to cir cumvent infertility; bone marrow transplantation, which uses hematopoietic stem ceBs to treat anemia; and the administration of erythropoietin (a paracrine factor that stimulates red blood cell production) to patients whose cancer chemotherapy has led to anemia. This chapter will first focus on four major developmental aspects of disease: • Genetic syndromes, wherein mutations alter development in deleterious ways • Teratogenesis, • • wherein environmental substances cause birth defects Endocrine disruption, wherein environmental substances that alter the devel opment of the endocrine system result in pathologies later in life Cancer, wherein cells escape the normal regulation of their neighbors and regain embryonic properties such as cell division, migration, and invasiveness The chapter will conclude b y looking at how insights from developmental biol ogy are being used to cure and repair injured tissues.' These developmental ther apies include: • Anti-angiogenesis treatment for cancer, wherein tumor cells are prevented from • receiving necessary blood vessels Induced pluripotent stem cells, wherein normal adult body celis can be convert ed into pluripotent cells resembling embryonic stem cells that can generate any cell type in the body • RegelIernlive biology, wherein adult stem cells and paracrine factors can restore damaged or diseased organs "When one discusses the medical aspects of any science, one must take social issues as well as purely scientific concerns into account. Discussions concerning how genet ic diseases, birth defects, cancers, or infertility might be controlled also raise issues concerning social, political. and economic motives and opportunities (see Gilbert et al. 2005). While this chapter seeks to provide general information about medical topics, it is no!" intended to provide medical advice for specific persons or disorders. The amazing thing abollt mammalian development is not that it sometimes goes wrong, bill that it ever slIcceeds. VERONICA VAN HEYNINGEN (2000) The ft/wre is already here.
It's just not evenly distributed yet. WILLIAM GIBSON (1999) 626 CHAPTER 1 7 r tam genes on chromosome 21 are thought to encode tran DISEASES Of DEVELOPMENT Genetic Errors of Human Development probably causes an overproduction of these regulato y scription factors, and the extra copy of chromosome 21 proteins. Such overproduction would cause the misreg If you think it is amazing that any one of us survives to be ulation of genes necessary for heart, muscle, and nerve born, you are correct.
It is estimated that one-half to two thirds of all human conceptions do not develop Success Down syndrome is one of the few trisomies mild enough fully to term (Figure 1 7. 1) . Many of these embryos express to allow the fetus to be born and to survive beyond the their abnormality so early that they fail to implant in the first weeks of infancy.' uterus. Others implant but fail to establish a successful focused almost excl USively on inborn errors in metabolic pregnancy.
Thus, most embryos are spontaneously abort formation (Chang and Min 2009; Korbel et al. 2009).
Until recently, the molecular study of human genetics ed, often before a woman even knows she is pregnant and structural proteins. Thus, diseases of enzymes, colla (BoUl' et al. 1985). Using a sensitive immunological test that gens, and globins predominated. But these proteins are the detects the presence of human chorionic gonadotropin final products of differentiated cells. Errors in the proteins (hCG) as early as 8 or 9 days after fertilization, Edmonds involved and co-workers (1982) monitored ll2 pregnancies in nor paracrine factors, and elements of signal transduction path with development-transcription factors, mal women.
Of these hCG-determined pregnancies, 67 ways-were little understood. In the past decade, howev (about 59%) were not maintained. er, we have made great gains in our. knowledge of human Most early embryonic and fetal demise is probably due opmental processes. There are also defects that are not dele to chromosomal abnormalities that interfere with devel terious to the fetus (which does not depend on organs such development, and we now know that many of these mal formations are caused by mutations of genes encoding transcription factors and signal transduction proteins (Table 1 7.1) . Many of these mutations have been used to as the brain, kidneys, and lungs while inside the mother) discover the protein responsible for the deficient reaction, but that can threaten life once the baby is born. Winter and these have been mentioned throughout this book. (1996; Epstein 2008) has estimated that approximately 2.5% Indeed, Charles Epstein (2008) wrote that "Science has ("present at birth") abnormalities and losses of the fetus finally caught up with human malformations," and his ce edited 1600-page book documents that claim. of newborns have a recognizable birth defect. Congenital prior to birth have both intrinsic and extrinsic causes. Those abnormalities caused by genetic events may result from mutations, aneuploidies (improper chromosome See WEBSITE 1 7. 1 Human embryology and genetics number), and translocations (Opitz 1987).
Even an extra copy of the tiny chromosome 21 disrupts numerous developmental functions. This trisomy 21 caus es a set of anomalies among them facial muscle changes, heart and gut abnormalities, and cognitive problems collectively known as Down syn d rome (Figure 1 7.2). Cer- in contact with Successful fertilization Successful implantation Successful fourth weekdevelopment, Successfulweekdev l ent eighth Fetuses t Eggs *With proper medical care, some infants born with trisomies 13 and 18 (Patau syndrome and Edward syndrome, respectively) can live for years, although they usually suffer from lung and intestinal defects and heart malformations. sperm e opm coming to crm , 20 40 60 Percent 80 100 FIGURE 17. 1 Fate of hypothetical human eggs in United States and western Europe. U nder normal conditions, only 6.2 (or fewer) of the original 20 eggs would be expected to devel p s u ccessfu ll y to term. the (AherVolpe 1 987.) 20 o M E D I CAL ASPECTS OF DEVELOPME NTAL BIOLOGY 627 (B) TAB L E 1 7. 1 Some genes encoding human transcription factors and phenotypes resulting from their mutation Gene Mutation phenotype Androgen Androgen insensitivity syndrome AZFJ Azoospermia CBFAJ Cleidocranial dysplasia CSX Heart defects EMX2 Schizencephaly Estrogen receptor Grow th regulation problems, sterility receptor FIGURE 17.2 Down syndrome. (A) Down syndrome, caused by a third copy of chromosome 2 1 , is characterized by a particular facial pattern and by cognitive deficiencies, the absence of a nasal bone, and often heart and gastrointestinal defects. (6) The proce- dure shown here tests for chromosome number using fluorescent ly labeled probes that bind to DNA on chromosomes 21 (pink) and 13 (bl u e) . This person has Down syndrome (trisomy 2 1) but has the normal two copies of chromosome 1 3 . (A 0 Mood- Board/Alamy; 6 courtesy o/V}"is, Inc.) The Nature of Human Syndromes Forkhead-like 15 Thyroid agenesis, cleft palate GLi3 Crieg syndrome HOXA13 Hand-foot-genital syndrome HOXD13 Polysyndactyly LMXIB Nail-pateUa syndrome MITF Waardenburg syndrome word syndrome comes from the Greek sy"dromos, "running PAX2 Renal-coloboma syndrome together." Genetically based syndromes are caused either PAX3 by (1) a chromosomal event (such .as aneuploidies) where several genes are deleted or added, or (2) by one gene hav- li lg many effects. The production of several effects by one Waardenburg syndrome type l PAX6 Aniridia Human birth defects, which range from Ufe-threatening to relatively berngn, are often linked into syndromes, where there a re several abnormalities occ urring together. The type 2 PTX2 Reiger syndrome PLTX3 Congenital cataracts pleiotro py when the effects are produced independently POU3F4 Deafness and dystonia as a result of the gene being critical in different parts of the SOX9 body (Figure 17.3A). For instance, the KIT gene is expressed in blood stem cells, pigmen t stem cells, and germ stem Campomelic dysplasia , male sex reversal SRY Male sex reversal TBX3 Schinzel syndrome (ulna-mammary syndrome) TBX5 Holt-Oram syndrome TCOF Treacher-Collins syndrome TWIST Seathre-Chotzen syndrome WTl Urogenital anomalies gene or pair of genes is called pleiotropy (see Cruneberg 1938; Hadom 1955). Plei otropic effects a re ca lled mo saic When this gene is defective, the resulting syndrome of ane- mia (lack of red blood cells), sterility (lack of germ cells), and albinism (lack of pigment cells) is evidence of mosaic pleio tropy.
Syndromes are said to have rel ational pleiotropy when a defective gene in one part of the embryo causes a defect in another pa rt, even though the gene is not expressed in cells, where it is needed for their proliferation (se e p. 27).
628 CHAPTER 1 7 (A) Mosaic pleiotropy 8 lineAl -8 (B) Relational pleiotropy 18. Phenotypic heterogeneity comes about because genes are not autonomous agents. Rather, genes interact with other genes and gene products, becoming integrated into complex pathways and networks. BeJJus and colleagues the same mutation in the FGFR3 gene in 10 different, unre (1996), for instance, analyzed the phenotypes derived from lated human families. These phenotypes ranged from rel atively mild anomalies to potentiaJJy lethal malformations. Similarly, Freire-Maia (1975) reported that withln one fam FIGURE 17.3 Mosaic and relational pleiotropy.
(A) In mosaic pleiotropy, a epe de tly expressed i n several tissues. Each product and develops abnormally its absence. (8) I n relational pleiotropy, a gene product is needed by on ly particular tissue. However, a second tissue needs a from in order to develop properly. If the first tis e develops abnormal ly, the signal is not iven , so the tissue also deve l ops abnormally, gene is ind n n tissue needs the gene one the first tissue signal su g in second ily, the homozygous state of a mutant gene affecting hmb development caused phenotypes ra nging from severe phocomelia (Jack of limb development) to a mild abnor mality of the thumb.
The severity of a mu tant gene's effect often depends on the olher genes in the pathway as well as on environmental factors . Phenotypic heterogeneity in women can also be caused by statistical variation in X-chromosome inactivation (see Sidelights & Sp ec ulations, p. 501). Ore such case involved identical twins, each of whom carried one normal and one mutant allele for an X-linked blood clotting factor. One twin's random inactivation pattern resulted in the the second tissue (Figure 1 7.38). The failure of MITF expres sion in the pigmented retina prevents this structure from full y differentiating. This failure of pigmented retina growth, in tum, causes a malformation of the choroid fis sure of the eye, resulting in the drainage of vitreous humor. Without this fluid, the eye fails to enlarge (hence microph thalmia, or "small eye").
The lenses and corneas are small er, even though they themselves do not express MITF. Genetic Heterogeneity and Phenotypic Heterogeneity In pleiotropy, the same gene can produce different effects in different tissues. However, the op posite phenomenon is an equally important feature of gene tic syndrOll1€s: mutations in different genes can produce the same pheno type. If several genes are part of the same s ignal transduc tion pathway, a mutation in any of them often produces a similar phenotypic result. This production of similar phe notypes by muta tions in different genes is called genetic he te rog ene ity. The syndrome of sterility, anemia, and albinism caused by the absence of Kit protein (discussed above) can also be caused by the absence. of its paracrine ia, a phenotype that can be produced by mutations in the sonic hedgehog gene (see Chapter 3) or by mutations in the ligand, stem cell factor (SCF). Another example is cyclop genes activated by hedgehog or in the genes controlling cholesterol synthesis (since cholesterol is essential for loss of a large percentage of the X chromosomes that car ried the normal allele, and she had severe hemophilia (ina bility of the blood to clot after injury). The other twin, with a lower percentage of her normal X chromosomes inactivated, did not have the disease (Tiberio 1994; Valleix et aJ. 2002). Teratogenesis: Environmental Assaults on H uman Development In addition to gene tic mutations, we now know th a t numerous env ironmental factors can disrupt develop ment. The summer of 1962 brought two portentious dis coveries. The first was the disclosure by Rachel Carson (1962) that the pesticide DDT was destroying bird eggs and preventing reproduction in several species (see Chap ter 18). The second (Lenz 1962) was the discovery that thalidomide, a sedative used to help manage pregnan cies, could cause limb and ear abnormalities in the fetus (see Chapter 1). These two discoveries showed that the embryo was vulnerable to environmental agents.* This was underscored in 1964, when an epidemic of rubella (German measles) spread across the United States. Adults showed relatively mild symptoms when infected by this virus, but over 20,000 fetuses infected by rubella were born blind, deaf, or both. Many of these infants were also born wjth heart defects and/or mental retardation (CDC 2002). hedgehog signaling). Not only can different mutations produce the same phenotype, but the same mutation can produce a differ ent phenotype in different individuals (Wolf 1995, 1997; Nij hout and Paulsen 1997). This phenomenon, called phe notypic heterogeneity, is discussed at length in Chapter "'Indeed, Rachel Carson realized the connection, commenting that "It is all of a piece, thalidomide and pesticides. They represent our \villingness to rush ahead and use something without knowing what the results will be" (Carson 1962). MEDICA L ASPECTS OF DEVELOPMENTAL BIOLOGY 629 SIDEL IGHTS SPECULATIONS Prenatal Diagnosis and Preimplantation Genetics O One of the consequences of in vitro fertil ization (IVF) and the ability to detect ge neti C muta tions early i n deve l op men t is a new area of medicine called preimplanta tion genetic diagnosis (PGD). Preim plantation ge net ics see

acid (RA) is vitamin A derivative that is importan t positions along the axis. RA is secreted from particular is then degraded by other cells. Howev if RA is present in large amount s, the g radient is per turbed, and cells that norm a l ly would not receive hi gh concentrations of this mo lecu le are exposed to it and will respond to it.
In its pharmaceutical fo rm, 13 cis-retinoic acid (also called isotretinoin and sold under the trademark Accutane) has been useful in trea ting severe cystic acne and has been available for this purpose since 1982. The deleterious effects of administering large amounts of vitamin A or its anacells, diffuses, and er/ CHAPTER 1 7 634 logues to pregnant animals have been known since the 1950s (Cohlan 1953; Giroud and Martinet 1959; Kochhar et Endocrine disruptors 1984). The drug now carries a strong warning against its use by pregnant women. However, about 160,000 women of childbearing age (15-45 years) have taken the A specialized area of tera togenesis involves the misregu drug since it was introduced, and some have used it dur ally active compounds n i the environment endocrine dis a1. ing pregnancy. Lammer and co-workers (1985) latian of the endocrine system during development. According to the endocrine disruptor hypothesis, hormon studied a ruptors have a significant impact on the health of hnman group of women who inadvertently exposed themselves and wildlife populations. The vast amounts of these sub to retinoic acid and who elected to remain pregnant. Of stances being released into the environment by human their 59 fetuses, anomalies, 12 26 were born without any noticeable activities have resulted in increased incidences of disease aborted spontaneously, and 21 were born and physiological dysfunction.
The phenotypic changes with obvious anomalies. The affected infants had a char produced by endocrine disruptors are not the obvious acteristic pattern of anomalies, including absent or defec anatomical changes produced by classic teratogens. Rather, tive ears, absent or small jaws, cleft palate, aortic arch the anatomical alterations induced by endocrine disrup abnormalities, thymic deficiencies, and abnormalities of tors are often seen only microscopically, and the major the central nervous system. changes are physiological. These functional changes are This pattern of multiple congenital anomalies is similar more snbtle than those produced by the teratogens, but to that seen in rat and mouse embryos whose pregnant they can be extremely important phenotypic alterations. mothers were given these drugs. Goulding and Pratt (1986) Moreover, they may persist in generations after the expo placed 8-day mouse embryos in a solution containing 13- sure to the disruptor. cis-retinoic acid at a very low concentration (2 x Even at this concentration, approximately one-third of the born, Frederic vom Saal, and Ana Soto in embryos developed a specific pattern of anomalies, includ compounds are also known by many other names: hor 1 0 6 M). ing dramatic reduction in the size of the first and second pharyngeal arches (see Figure 8.33). The term endocrine disruptor was coined by Thea Col 1993, but these mone mimics, environmental signal modulators, environ In normal mice, the mental estrogens, or hormonally active agents. Endocrine first arch eventually forms the maxilla and mandible of the disruptors are exogenous (coming from outside the body) jaw and two ossicles of the middle ear, while the second chemicals that interfere with the normal functions of hor arch forms the third ossicle of the middle ear as well as mones, and consequently disrupt development (Colborn other facial bones. et aI. The basis for this developmental insult appears to reside in the ability of RA to alter Hox gene expression and thereby respecify portions of the anterior-posterior 1. et al. 1994). (DES), which binds to the estrogen receptor and mim ics the sex hormone estradiol, a common form of estro neural crest cells and arrests both their proliferation and 1986). 1985; Goulding and Pratt The teratogenic period during which cranial neu ral crest cells are affected occurS on days 20-35 in humans (days 8-10 in mice). They can be agonists, mimicking the effect of a natural paradigmatic endocrine disruptor diethylstilbestrol 1994; Studer Radioactively labeled RA binds to the cranial their migration Oohnston et al. These chemicals can interfere with hor hormone and bindi.ng to its receptors. An example is ti1e axis and inhibit neural crest cell migration from the cra nial region of the neural tube (Moroni et al. 1993, 1997). monal functions in many ways.
gen. 2. They can act as antagonists and inhibit the binding of a hormone to its receptor or block the syntheSiS of a hor mone. DOE, a metabolic product of the insecticide DDT, can act as an anti-testosterone, binding to the androgen receptor and preventing normal testosterone from func tioni.ng properly. *Retinoic acid is a critical public health concern precisely because there is significant overlap between the population using acne med icine and the population of women of childbearing age, and because it is estimated that half of the pregnancies in the U.S. are unplalU1ed (N'ulman et al. 1997). Vitamin A is itself teratogenic in megadose amounts. Rothman and colleagues (1995) found that pregnantwomen who took more than 10,000 international units of vitamin A per day (in the form of vitamin supplements) had a 2% chance of having a baby born with disimptions similar to those pro duced by RA. According to the new rules ot the U.s. Food and Drug Administration, each patient using isotretinoin, each physi- cian prescribing it, and each pharmacy selling it must sign a reg ishy Moreover, each woman using this drug is expected to take a pregnancy test within 7 days betore filling her prescription, agree to use two methods of birth control, and adhere to pregnancy testing on a monthly basis. 3. They can affect the syntheSiS, elimination, or transporta tion of a hormone in the body. One of the ways that polychlorinated biphenyls (PBCs) disrupt the endocrine system is by interfering with the elimination and degra dation of thyroid hormones. The herbicide atrazine ele vates the synthesis of estrogen.
4. Some endocrine disruptors can " prime" the organism to be more sensitive to hormones later in life.
For instance, bisphenol A experienced in utero causes the embryonic mammary gland to make more estrogen receptors. These extra receptors cause the mammary gland to have altered growth responses to natural estro gen later in life, predisposing it to form cancers (see Wadia el al. 2007). MED ICAL ASPECTS OF DEVELOPMENTAL B I OLOGY Given the paradigm of teratogens, it had been thought that there were only a few "bad" agents, and that the only people who received these were pregnant women who 635 infertility or sub fertility (Robboy et aJ. 1982; Newbold et 1983). Moreover, the distal Mullerian ducts often fail to come together to form a single cervical canal (Figure 1 7.9). aJ. inadvertently exposed their embryos to high doses of these Symptoms similar to the human DES syndrome occur chemicals. However, we now recognize that hormone dis in mice exposed to DES in utero, allowing the mechanisms ruptors are everywhere in our technological society (and of this endocrine disruptor to be uncovered. Normally, the even in rural areas where pesticides and herbicides are regions of the female reproductive tract are specified by abundant), and that low-dose exposure can be enough to the HoxA genes, which are expressed in a nested fashion (Figure 1 7.1 0). Ma and col (1998) showed that the effects of DES on the female produce significant disabilities later in life. Endocrine dis throughout the Mullerian duct ruptors include chemicals that line baby bottles and the leagues brightly colored plastic containers from which we drink mouse reproductive tract could be explained as the result our water; chemicals used in cosmetics, sunblocks, and hair of altered dyes; and chemicals used to give cars their "new car smell" was injected under the skin of pregnant mice, and the and to prevent clothing from being highly flammable . fetuses were allowed to develop almost to birth. When the DIETHYlSTIBESTROl One of the most potent environmental fetuses from mothers that had not received DES, it was estrogens is diethylstilbestrol, or scen that DES almost completely repressed the expression Hoxal O expression in the Mullerian duct. DES fetuses from the DES-injected mothers were compared with DES. This drug was thought to ease pregnancy and prevent miscarriages, and it is estimated that in the United States over 1 million pregnant women and fetus 1947 and 1971. es were exposed'lO DES between Oviduct (Fallopian tube) This is probably a small fraction of exposures worldwide ' R&search from the 1950s showed that DES actually had no beneficial effects on pregnancy, but it was still prescribed until the FDA banned it in 1971. The ban was imposed when a specific type of tumor (clear-cell adeno carcinoma) was discovered in the reproductive tracts of women whose mothers took DES dur ing pregnancy. DES interferes with sexual and gonadal development by causing cell type changes i n the female reproductive tract (the derivatives of the Mullerian duct, which forms the upper portion DES-exposed of the vagina, cervix, uterus, and oviducts; see Chapter 14). In many cases, DES causes the boundary betvveen the oviduct and the uterus (the uterotubal junction) to be lost, resulting i n *In addition to the DES that was administered to preg nant women individually, biologically relevant levels of DES were found in meat; it had been fed to cattle to accelerate their growth (see Knight 1980). FIGURE 17.9 Gen ita l anomalies can occur in women exposed to DES in utero . ! n these "DES daughters," the cervical tissue (red) is often displaced into the vagina. Such individuals may have a uterus that is T-shaped and constricted, as well as adenosis of the cervix and vagina (where the lining differentiates into mucosa! cells), precancerous cells, ectopic preg nancies, adenocarcinomas, and other effects. Adenosis of cervix and vagina (abnormal tissue) Cervical dysplasia (preca n cerous growth) Tissue "hood" of cervix 636 (A) CHAPTER 1 7 HO (8) C,Hs HsC, OH \, Hoxa9 expression border '\. throughout most "' of oviduct Hoxal l high expression horder at uterus/ OViductbO expression border at uterus/oviduct boundary f ---i Hoxal3 I HoxaJ 1 HoxaIO However, DES, acting through the estrogen receptor, represses the WI1t7a gene. This repression prevents the maintenance of the Hox gene expression pattern, and also prevents the activation of another Wnt gene, Wnt5a, which encodes a protein necessary for cell proliferation (Miller et al. 1998; Carta and Sassoon 2004). The DES tragedy is a complex story of public polic)" medicine, and developmental biology (Bell 1986; Palmlund 1996). However, according to biologists Frederick vom Saal, Ana Soto, and others, the public and the pharmaceutical industry have learned little from the DES tragedy, and we may be experiencing the same endocrine disruption today, only on a much larger scale. These researchers claim that some of the major constituents of plastics aTe estrogenic compounds, and that they are present in doses large enough to have profound effects on sexual development and behavior. Two of these products are bisphenal A and nanylphenol. (A) (8) cvx OV Hoxa9 FIGURE 17.10 Effects of DES exposu re on the femal e reproduc tive system. (A) The chemical structure of DES. (B) S_ HoxA gene express i on in the rep rodu ctive system of a normal 1 6.S-day embryon ic female mouse. A whole mount in situ hybridization of the Hoxa 7] probe is shown (red) alon with a p robe for Haxa 10 (purpl e) Hoxa9 expression exten ds th rou hout the uterus and about through much of the presumptive oviduct Hoxa 10 expres sion has a sharp anterior border at the transition betvveen the pre sumptive uterus and the oviduct. Hoxa 1 1 as the same anterior border as Hoxa 10, but its expression diminishes closer to the cervix. Hoxa 13 expression is found on ly in the cervix and upper vagina. (After Ma et aJ. 7 998.) md Control - g , g QES (D) (C) . h of HoxaIO in the Miillerian duct (Figure 1 7.1 1). This repres sion was most pronounced in the stroma (mesenchyme) of the duct, where experimental embryologists had localized the effect of DES (Boutin and Cunha 1997). The case for DES acting through repression of HoxaIO is strengthened by the phenotype of the HoxaIO knockout mouse (Benson et 81. 1996; Ma et a1. 1998), in which there is a transforma tion of the proximal quarter of the uterus into oviduct tis sue, as well as abnormalities of the uterotubal junction. The link between Hox gene expression and uterine mor phology is the Wnt proteins which are associated with cell proliferation and protection against apoptosis. The repro ductive tracts 01 DES-exposed female mice resemble those of Wnt7a knockout mice. The Hox genes and the Wnt genes conunnkate to keep each other activated.
Moreover, the Hox and Wnt proteins are involved in the specification and morphogenesis of the reproductive tissues (Figure 1 7.1 2) . , control FIGURE 17.11 In situ hyb ridization of a Haxa70 probe shows that DES expos re represses Haxa 70. (A) Normal 1 6.5-day embry onic femal e mice show Hoxa 10 expression from the boundary of u the cervix through the uterus p ri mordi u m and most of the oviduct (cvx, cervix; md, Mullerian d uct; ov, ovary). (8) In mice exposed prenatally to DES, this expression is severely repressed.
(C) In con trol female mice at 5 d ays after birth (when reproductive tissues are still form i ng), a section through the uterus shows abundant expression of Hoxa 1 O in the uterine mesenchy me . (0) In fem al e mice that are given high doses oi DES 5 days after birth, Haxa 10 expression i n the mesenc hy me is almost completely suppressed (ie, luminal epitheliu m; s, stroma). (After Ma et aI.
1 998.) MEDICAL ASPECTS OF DEVELO PMENTAL BIOLOGY (B) "DES daughter" (A) Normal Wnt7a Epithel ium Mesenchyme 637 FIGURE 17.12 Misreguiation of Mullerian duct morphogen esis by DES.
(A) During n ormal morphogenesis, the Hoxa 1 0 and Hoxa 1 1 genes in the mesenchy me are acti vated an d maintained by Wnt7a from the epithelium .
Wnt7a also induces WntSa in t he mesenchyme, and WntSa protein both maintains Wnt7a express ion and causes mesenchymal cell prol i ferati on Together, these fac tors specify and order th e morp ho genesis of the uterus. (8) DES, acti ng through the estrogen receptor, blocks Wnt7a exp ressio n . P roper activation of the Hox genes and Wnt5a in the mesenchyme does not occur, leading to a radically altered morphol ogy of the fema le gen i tal i a. (After Kitajewsky and 5a5500n 2000.) . Improper cell specification; disorganized smooth muscle tissue PLASTICS AND PLASTICITY: NONYIPHENOL Estrogenic com of hormone research, the steroid hormones were very dif pounds are ficult to isolate, so chemists manufactured synthetic ana in the food we eat and in the plastic wrapping that surrounds them. The discovery of the estrogenic effect logues that would accomplish the same tasks. BPA was of plastic stabilizers was made in a particularly alarming synthesized by Dodds, who demonstrated it to be estro way. Investigators at Tufts University Medical School had been studying estrogen-responsive tumor cells, which geme in 1936. (Two years la ter, Dodds synthesized DES.) Later, polymer chemists realized that BPA could be used require estrogen in order to proliferate. Their studies were in plastic production, and today it is one of the top 50 going well until 1987, when the experiments suddenly chemicals went awry. Their control ceUs began to show high growth States make almost 2 billion pounds of it each year for use rates, suggesting stimulation comparable to that of the in the resin lining in most cans, the polycarbonate plastic in production. Four corporations in the United estrogen-treated cells. It was as if someone had contami in baby bottles and children's toys, and dental sealant. BPA nated the control culture medium by adding estrogen to is also used in making the brightly colored polycarbonate it. What was the source of contamination? After spending water bottles.
Its modified form, tetrabromo bisphenol A, 4 months testing aU the components of their experimental system, the researchers discovered that the source of estro gen was the plastic containers that held their water and is the major flame retardant on the world's fabrics. However, as severa] laboratories have shown (Krishnan et a l . 1993; vom Saa1 2000; Howdeshell et al. 2003), BPA is serum. The company that made the containers refused to not fixed in plastic forever. If you let water sit in an old describe its new process for stabilizing the polystyrene polycarbonate rat cage at room temperature for a week, plastic, so the scientists had to discover it themselves. yOll can measure arollnd 300 µg per liter of BPA The culprit turned out to be p-nonylphenoL a com in the water. That is a biulogkally active amount-a concentra pound that is also used to harden the plastiC of the pipes tion that will reverse the sex of a frog and cause weight that bring us water and to stabilize the polystyrene plas tics that hold water, milk, orange juice, and other common changes in the uterus of a young mouse. It also can cause chromosome anomalies. When a laboratory technician mis. liquid food products (Soto et aJ. 1991; Colborn et takenly rinsed some polycarbonate cages aJ. 1996). This compound is also the degradation product of deter in an alkaline detergent, the female mice housed in the cages had meiot gents, household cleaners, and contraceptive creams. ic abnormalities in 40% of their oocytes (the normal Nonyphenol has been shown to alter reproductive physi amount is about 1.5%). When BPA was adm inistered to ology in female mice and to disrupt sperm function. It is pregnant mice under controlled circumstances, Hunt and also correlated with developmental anomalies in wildlife her colleagues (2003) showed that a short, low-dose expo (Fairchild et aJ. 1999; Hill et al. 2002; Kim et al. 2002; sure to BPA was sufficient to cause meiotic defects in Adeoya Osiguwa et al. 2003; Kurihara et al.
2007). maturing mouse oocytes (Figure 1 7.1 3). BISPHENOL A Bisphenol A (BPA) was actuaUy synthesized calise abnonnalities in fetal gonads, low spenn counts, and as an estrogenic compound in the 1930s. In the early years behavioral changes when these fetuses become adults BFA at environment" lly relevant concentrations can 638 CHAPTER 1 7 (A) (B) do et al . 2007; Vandenberg et a1. 2007). It is thought that exposure to BPA induces conditions that can lead to tumors w hen a second exposure of estrogenic hor mones or mutation-producing agents is experienced later in life. The lowest dose of BPA to alter mammary develop ment was 25 par ts per trillion-2000 times lower than the U.s. Environmental Protection Agency reference dose.
But is there any evidence that bisphenol A reaches the human fetus in concentrations that matter? Unfortunate ly, BPA in the human placenta is neither eliminated nor metabolized into an inactive compoW1d, rather, it acctunu lates to concentrations that can alter development in lab oratory animals (Ikezuki et al.
2002; Schonfelder et-al. 2002). Moreover, recent survey studies have found that 95% of urine samples of people in the U.s. and Japan have meas urable BPA levels (Calafat et al.
2005). BPA ingested by pregnant rats appears to pass readily into the fetus and is not hindered by steroid-binding hormones. Within an hour of ingestion, BPA is found in the fetus at the same doses it had been in the mother (Miyakoda et al. 1990). Females are not the only ones affected by BPA. As men tioned above, BPA has been inlplicated (along with other estrogenic compow1ds) in the decline in sperm quality and number and in the increase in prostate enlargement seen in men. When vom Saal and colleagues (1997) gave preg nant mice 2 p arts per billion BPA-that is, 2 nanograms per gram of body weight-for the 7 days at the end of pregnancy (equivalent to the pe riod when human repro- ductive organs a re developing), treated males showed an increase in p rostate size of about 30% (Wetherill 2002; TImms et al. 2005).
The plastics industry has cOW1tered these claims, say ing the evidence is meager and that the experiments can not be repeated (Cagen et a1. 1999; see also Lamb 2002). However, a review of the in dustry S own studies (claim ing that mice exposed in utero to BPA do not have enlarged prostates or low sperm COW1tS) points out that the positive control of the industry-sponsored research did not produce the expected effects. Indeed, when one study (Lerna th et al.
2008) showed that BPA disrupted monkey brain devel opment (at concentrations lower than what the U.s. EPA considers safe), the American Chemical COW1eil rep lied that "there is no direct evidence that exposure to bisphe nol-A a dversel y affects human reproduction or develop ment" (see Layton and Lee 2008; Gilbert and Epel 2009).* Reviewin g the literature, vom Saat and Hughes (2005; Chapel Hill Consensus 2007) conclude that BPA is one of the most dangerous chemicals known and that govern ments should consider banning its use in products contain ing liquids tha t humans and animals might drink. gestational FIGURE 17.13 Bisphenol A causes meiotic defects in maturing mouse oocytes (A) Ch romosom es (red) normally l i ne up at the center of the spindle during first meiotic metaphase. (B) Short expos u res to BPA cause chromosomes to align randomly on the spindle. Different numbers of chromosomes then enter the egg and polar body, resulting i n aneu ploi dy and infertility. (From Hunt et al. 2003, courtesy of P. Hunt.) . (vom Saal et al. 1998; Palanza et a1. 2002; Kubo et a1. 2003). Indeed, recent research has implicated BPA (and PCBs) in a suite of trends, including the lowering of human sperm counts, an increase in prostate enlargement, and a lower ing of the age of female sexual maturation (vom Saal 2000; vom Saal and Hall 2005). Research from several laboratories showed that female mice exposed in utero to low doses of BPA wlderwent sex ual maturity faster than w1exposed mice. They also showed altered mammary development at puberty, a l terations in the organization of their breast tissue and ovaries, and altered estrous cyclicity as adults (Howdeshell et a1. 1999, 2000; Markey et a1.
2003). Each mammary gland produced more terminal buds and was more sensitive to estrogen. This may predispose these mice toward breast cancer as adults (Munoz-de-Toro et a1. 2(05). What is remarkable in these studies is that the dosage of BPA was 2000 times lower than the dosage set as "safe" by the U.S. government. See WEBSITE 1 7.3 Our stolen future BPA also appears to make breast tissue more sensitive to estrogens later in life, predisposing women exposed to BPA in utero to breast cancer.
Petal exposure to BPA caused the development of ea rly-stage cancer in the mammary glands of one-third of the mice exposed to environmental ly relevant doses of BPA (F igure 1 7. 14; Munoz-de-Toro et a1. 2005; Murray et a1. 2007).
None of the control mice developed such tumors.
Fetal exposure to BPA also mereased the number of "preneoplastic lesions" (areas of rapid cell growth in the ducts) three- to fourfold. Further more, daily gestational exposure to as little as 25 ng/kg body weight BPA followed at puberty by a "subcarcino genic dose" of a chemical mutagen resulted in the forma tion of tumors only in the animals exposed to BPA (Duran- ' *The catch is that "direct evidence" would mean testing the drugs human fetuses. Interestingly, in the absence of government regulation, Nalgene and Wal-Mart volwltar ily stopped making and selling BPA-containing bottles. in known concentrations on MEDICAL ASPECTS O F DEVELOPMENTAL BIOLOGY (A) Abnormal terminal bud Ducts Co nt rol 25 nglkg BPA  tJ u .Sl  t . 250 nglkg BPA (C) (B)  639 30 20 10 o o 2.5 25 250 1000 BPA dose (glkglday) / Control 1 BPA-exposed fiGURE 17.14 Sisphenol A induces mammary lesions in mice. (A) Photomicrographs of mammary gland tissue show profound differences between a control animal and animals exposed perinatally to nanogram levels of BPA. The increase in terminal end buds and branching in the BPA-exposed mammary glands predi sposes the animals to cancer later in life. (S) The percentage of mammary gla nds showing intraductal hyperplas ia (indicating a cancer-prone state) is significantly increased at postnatal day 50 in BPA-treated animals. (C) later in life, BPA-exposed animals display significant cancer in situ. (From Murray et a l . 2007 and Soto et al. 2008, courtesy of Ana Soto.) Other teratogenic agents In addition to natural chemicals, hundreds of new artifi cial compounds come into general use each year in our industrial society. Pesticides and organic mercury com pounds have caused neurological and behavioral abnor malities in infants whose mothers have ingested them dur ing pregnancy. Moreover, drugs that are used to control diseases in adults may have deleterious effects on fetuses. Such drugs include cortisone, warfarin, tetracydine, and valproic acid (see Table 17.2). Over 87,000 artificial chemicals are currently licensed for use in the United States, and around 500 new com pounds are being made each year. The Toxic Substances Control Act of the United States assumes d1emicals are safe unless demonstrated to be otherwise, and only around 8,000 chemicals have been tested for their potential health effects (Johnson 1980; EPA 2008).
Although teratogenic compowlds have always been with us, the risks increase as more and more untested compounds enter our environ- ment. Most industrial chemicals have not been screened for their teratogenic effects. Standard screening protocols are expensive, long, and subject to interspecies differences in metabolism. There is still no consensus on how to test a substance's teratogenicity for human embryos. HEAVY METALS Heavy metals such as zinc, lead, and mer cury are powerful teratogens. Industrial polIUtion has resulted in high concentratiOll5 of heavy metals in the envi ronment in many places (see Gilbert and Epel 2009). In the fonner Soviet Union, the unregulated "industrial produc tion at all costs" approach left behind a legacy of soaring birth defect rates. In some regions of Kazakhstan, heavy metals are found in high concentrations in drinking water, vegetables, and the air. In such locations, nearly half the people tested have extensive chromosome breakage, and in some areas the incidence of birth defects has doubled since 1980 (Edwards 1994). In the United States, lax enforcement of antipollution laws has led to the contami nation of most lakes in the country by heavy metals. This 640 CHAPTER 1 7 SIDEL IGH TS SPECULATIONS Testicular Dysgenesis Syndrome and the Transgenerational Effects of Endocrine Disruptors A s noted ea rl i er, o u r tech no logi cal environment has increasing ly become an estrogenic envi ronment. As such, one would expect not only females but also males to have reproductive problems, and they do. Laboratories from a l l over the world have reported cases of a "testicular dysgenesis* syndrome" i n men. This syndrome encompasses low sperm count, poorly formed testes a n d penises, and tes tic ul ar tumors (Figure 1 7.1 5). *Dysgenesis is from the Greek for "bad begi n ni ng" and denotes d efec ts in devel opment. (A)  Testicular cancer c 5 -g -g -3 .S go In the p ast 3 decades, there has been an increase in testicular cancers and a decrease in sperm concentra tion throughou t the industrial izcd world (Carlsen et a l . , 992; Ai tken et al. 2004). The sperm count (number of sperm per ml) has dropped precipi tously throughout much of E u rope and the Americas. Skakkebaek and his team at Copenhagen University reviewed 61 international st udies done between ' 938 and ' 992, i nvolv ing 1 4,94 7 men. They found the aver age sperm count had fallen from ' 1 3 m i l l ion per m l in 1 940 to 66 m i l l ion in 1 990 (Carlsen et a l . 1 992). In addi tion, the number of "normal" sperm fel l from 60 m i l l ion per ml to 20 mil lion in the same period. Subsequent studies have confirmed and extended Ska.kkebaek's findings (Merzenich et a l . 201 0). A survey of i Figure 17.15 Developmental estrogen syndrome is man ifest in c l imb ng rates of breast cancer and testicular dysgenes is. The rise of testicular cancer (A) paral lels the rise of breast cancer (BI and anomalies of penis development (C) such as hypospadias (failure to complcle [y close the penis) . Sperm counts (D) among North American males have declined nearly 50% with in the past century, and the decline has even been steeper among European men. (After Sharpe and Irvine 2004.) (B) Breast 6.0 150 5.5 140 5.0 130 'U OB 0 4.5 cancer 120  O 00 t  4.0 -< 0.. 3.5 1973 110 100 1977 1981 1985 1989 1993 1997 1973 1977 1981 Year CD) (C) Hypospadias 40  c5 1989 1993 200 35 -.§ .E 0 0 0 Sperm counts Year 1985  0 - North 1997 America - Europe 150 " 100 0 c 30  25 0 u §  0. 20 1970 1973 1976 1979 t982 1985 1988 1991 Year SO 1920 1940 1960 Year 1980 2000 MEDICAL ASPECTS OF DEVELOPMENTAL BIOLOGY 641 SIDEL IGHTS & SPECULATIONS (Continued) (A) (ll) Figure 17.16 Cross-section of seminifer ous tubu les from the testes of (AI a control rat and (B) a rat whose grandfather was born from a mother that had been i nj ected with vinclozolin. This rat was infertile. The arrow in (A) shows the tails of the sperm. The arrow in (B) shows the lack of germ cells i n the much smaller tubule. (From Anway et al.
2005, courtesy of M.
K. Skinner.) 1 ,350 sperm donors i n Pa r i s found that sperm counts decl i ned by around 2% each year from the 1 970s through the mid-1 990s,with younger men hav ing the poorest quality semen (Auger et al. 1 995). (Average sperm count was 90 m i l lion sperm per ml in 1 973 and only 60 m i l lion per ml in 1 992.) A study from Finland showed a similar decl ine, and also showed that the average weight of the testes had decreased, while the proportion of fibrous testicu lar tissue had increased at the expense of the sperm-producing seminiferous tubules (Pajarinen et a l .
1 997). A Scottish study showed that men born i n the ' 9705 were produc ing some 24% fewer motile sperm i n their ejaculate than men born i n the 1 950s (Irvine et al. 1 996). In addition to the drop in sperm count document ed in these sludies, there has also been an increase in testicular cancers over recent decades (see Figure 1 7.1 SA,C). Sharpe (1 994) has suggested that testicular dysgenesis syndrome may be due in large part to endocrine dis ruptors.
While a chain of causation has not been completely establ ished (see Sharpe and Irvine 2004), there is evidence that the pathologies of this syndrome can be caused by environ mentally relevant concenlrations of endocrine disru ptors.
I ndeed, a l l the developmental anomalies (but not the testicular tumors) can be i nduced by administering phthalate derivatives to pregnant rats (Fisher et a l . 2003). Among male mice exposed in utero to dibutyl phthalate, more than 60% exhibited cryptorchidism (undescend ed testicles), hypospadias (misplaced urinary aperture), low sperm count, and testis abnormalities very similar to conditions found i n human testicu lar dysgenesis syndrome. Phthalates are ubiquitous in i ndus trialized society and are widely used i n p l astics and in cosmetics (that great "new car smell" consists largely of volati lizing phthalates). Evidence obtained from newborns showed that male babies exposed in utero to rela tively high phthalate levels had some morphological changes in their testes (Duty et a l . 2003). Other endocrine disruptors that adversely affect sperm are dioxins, nonylphenol, bisphenol A, acry [amide, and certai n pesticides and herbicides' (see Aitken et al. 2004; Newbold et a l . 2006). The Sunscreen 4-MBC, a camphor derivative, has been found to decrease the size of the testes and prostate glands, and it can delay male puberty in rats (Schlumpf et a l . 2004).
Pesticides may be critical ly important in impairing male fertility. The link betvveen pesticides and infer til ity has been known for a long time (Carson 1 962; Colborn et a l . 1 996). "'Genistein, the estrogenic compound found in soy (and soy products such as tofu) is also being scrutinized (Newbold et al. 200 1 ; Cederroth et al. 2 0 1 0). For people eating an omnivorous diet with soy supplementation, genistein should not be a concern (indeed, this comJX>und may protect against certain cancers). However, researchers at the National Institute of Environmental Health Science are worried that infants born to vegan or vegetarian mothers and who are fed sole ly soy-based formula may develop abnor malities of the reproductive system and thyroid glands. One of the most important endocrine disrupting pesticides is DDT. I n humans, DDT 'has been linked t o pre term births and immature babies, and it is banned in the United States (Longnecker et a l . 2 00 1). DDT is probably not the active compound, though. It breaks down into DDE (dichlorodiphenyldichloroethylene), and DDE binds to the a ndrogen receptor, preventing testosterone bind ing (Xu et al. 2006). The fungicide vinclozolin (used extensively in grape farming) also works as an anti-androgen, inserting itself into the androgen receptor and preventing testosterone from binding there (Grey et al. 1 999; Monosson et al. 1 999). Vinclozolin administered to rats around the time of birth causes penis malformations, absent sex accessory glands, and very low (around 20% of normal) sperm pro duction (Figure 1 7. 1 6). Male rats born to mothers injected with vinclozolin late in pregnancy are sterile. This impaired fertility affects not only the generation exposed in utero but has been observed in males for at least four generations afterward. The sons of rats injected with vinclozolin dur ing m id pregnancy were able to reproduce, but their testis cells under went apoptosis more than usual, their sperm count dropped 20%, and the sperm that remained had significantly lowered motility. When affected males were mated with normal females, the male offspring also had this testicular dysgenesis syndrome. Some of the offspring were sterile, and some had reduced fert i l i ty. The (Continued all next page) 642 CHAPTER 1 7 SIDELIGHTS & study (Anway et a l . 2005) ended afte r th e fo u rt h ge n e rati o n of males continued to show low sperm count, low sperm moti l ity, prostate disease, and high te st i cu lar cell a po ptosis . This transgener ational effect is thought to be the re su l t of methylation of genes involved in sperm a toge n e sis (C h a ng et al. 2006). Some scientists argue that these claims a re exagge rat ed and that their tests on mice i ndicate that lit ter size, sperm concentration, and development are not affected by e nv i ro nm e n t ally relevant concen trations of environmental estro gens.
H owe ve r, investiga t i ons by Spea row and colleagues (1 999) have shown a rem arkab l e gen et i c d i ffere n ce i n se ns itivi ty to estrogen among d i fferent mouse strains. The strai n that was used for testing environmental estrogens, the CO-1 stra i n of l abo ratory m i ce, is at least 1 6 times more resistant to endo crine dis r upti o n than the most sen sitive strains, such as 86. When estroge n -co nta i n i n g pellets were i mpl a nted beneath the skin of young male CO-1 m i ce, very l i ttle ha ppe ned . However, when the same pellets were pl a ced beneath the skin of B6 m i ce, their testes shrank and the number of sperm seen in the seminiferous tubules d rop ped dra mati ca l l y (Figure 1 7.1 7).
This widespread range of sensitivities has impo rta nt conse quences for determ i n i n g safety l i m its for humans.
PECU LATIONS (Conti n u ed) - 100 S CD-I  -" .E .3 on .3 80 60 stram  40 0 u 0- Sh  "en 20 B6 strain 0 40 20 Estradiol dose (.g) 2.5 gSensitive strain 40 g (B6) Figure 1 7.17 Effects of estrogen imp l ants on different strains of mice. The graph shows the percentage of sem i n iferous tub ules conta i ning elongated spermatozoa. (The mean +/- stan dard error is for an average of six individuals.) The micrographs show cross sections of the testicles and are all at the same magnification. Forty g of estradiol did not affect spermato genesis in the CO-1 strain, but as l ittle as 2.5 g almost comp l etely abolished spermatogene sis in the 86 strain . (After Spearow et al . 1 999, photographs cou rtesy of J .
L Spearow.) contamination is especially prevalent where mining inter ests have been allowed to disgorge metal-containing slag into streams feeding the lakes (USGS 2009). An Interna tional Joint Commission of the u.s. and Canada had warned that pregnant women should not eat fish caught in the Great Lakes. Lead and mercury can damage the developing nervous system (Bellinger 2005). The polluting of Minamata Bay, Japan, with mercury in 1956 produced brain and eye defi ciencies both by transmission of the mercury across the placenta and by its transmission through mother's milk. Mercury is selectively absorbed by regions of the develop ing cerebral cortex (Eta 2000; Kondo 2000; Eto et a1. 2001). When pregnant mice are given mercury on day 9 of gesta tion, nearly half the pups are born with small brains or small eyes' (O'Hara et a1. 2002). *At the same time in America, the lead industry lobbied to assure consumers that lead was safe to use in paints. The U.s. banned paint in 1977-52 years after it was banned in Europe (see Steingraber 2003). leaded MED ICAL ASPECTS O F DEVELOPMENTAL BIOLOGY PATHOGENS Another class of teratogens includes viruses and other pathogens. Gregg (1941) first documented the fact that women who contracted rubella (German measles) during the first h'imester of their pregnancy had a 1 in 6 chance of giving birth to an infant WW, eye cataracls, heart malformations, or deafness. This study provided the first evidence tha t the mother could not fully protect the fetus hom the outside environment. The rubella virus is able to enter many cell types, where it produces a protei.n that pre vents mitosis by blocking kinases that allow the cell cycle to progress (Atreya et a!. 20W). Thus, numerous organs are affected, and the earlier in pregnancy the rubella infection occurs, the greater the risk that the embryo will be mal formed. The first 6 weeks of development appear to be the most critical, because that is when the heart, eyes, and ears are formed (see Figure 17.5). The rubella epidemic of 1963-1965 probably resulted in over 10,000 fetal deaths and 20,000 infants with birth defects in the United States (CDC 2002b). Two other viruses, cytomegalovirus and the her pes simplex virus, are also teratogenic. Cytomegalovirus infection 01 early embryos is nearly always fatal; infection 01 later embryos can lead to blindness, deafness, cerebral palsy, and mental retardation. Bacteria and protists are rarely teratogenic, but some of them are known to damage human embryos. Toxoplasma gondii, a protist carried by rabbits and cats (and their leces), can cross the placenta and cause brain and eye defects in the fetus. Treponema pallidllm, the bacterium that causes syphilis, can kill early letuses and produce congenital deaf- ness and facial damage in older letuses. Cancer as a D isease of Development Many endocrine disruptors are known to cause tumors as well as developmental abnormalities.. and cancer is increas ingly being studied as a disease of development. Although the genetics-centered approach to malignancy, which sees cancers as resulting from an accumulation of mutations, explains the formation of numerous tumor types, it is not the whole story. As Folkman and colleagues (2000) noted, the genetic approach to cancer therapy must be comple mented by a developmental perspective seeing that "epige netic, cell-cell, and extracellular interactions are also piv otal in tumor progression." Indeed, both carcinogenesis and teratogenesis can be seen as diseases of tissue organization and intercellular communication. Some defects in signaling pathways can cause cancers, while other defects in the same pathways (even the same protein, but at different sites) can cause malfonnations (see Cohen 2(03). There are many reasons to view malignancy and metastasis in terms of development. Four of them wil be discussed here: • Context-dependent tumor formation • Deficient stem cell regulation in tumor formation • Reactivation of embryonic migration pathways • Epigenetic reprogramming of cancer ceUs 643 Context-dependent tumors Many tumor cells have normal genomes, and whether or not tl'lese tumors are malignant depends On their environ ment. The most remarkable of these cases is the teratocar cinoma, which is a tumor of germ cells or stem cells (see Chapter 16; !llmensee and Mintz 1976; Stewart and Mintz 1981). Teratocarcinomas are malignant growths of cells that resemble the inner ceB mass of the mammalian blastocyst, and they can kill the organism. However, if a teratocarci noma cell is placed on the inner cell mass of a mouse blas tocyst, it will integrate into the blastocyst, lose its malig nancy, and divide normally.
Its cellular progeny can become part of numerous embryonic organs. Should its progeny form part of the germ line, sperm or egg cells formed from the tumor ceB will transmit the tumor genome to the next generation (see Figure 16.10). Thus, whether the cell becomes a tumor or part of the embryo can depend on its surrounding cells. DEFECTS IN CELL-CELL COMMUNICATION This brings uS to the idea that cancer can be caused by miscommunication between cells. In many cases, tissue interactions are required to prevent cells from dividing. Thus, tumors can arise through defects in tissue architecture, and the sur roundings of a cell are critical in determining malignancy (Sonnenschein and Soto 1999, 2000; Bissell et a!. 2(02).
Stud ies have shown that tumors can be caused by altering the structure of the tissue, and that these tumors can be sup pressed by restoring an appropriate tissue environment (Coleman et a!. 1997; Weaver et a!. 1997; Sternlicht et a!. 1999). In particular, whereas 80% 01 hwnan tumors are from epithelial cells,

bu rsti n g idly div i ding tissue, needs oxygen and nutrients to sur vive. Withou t a b lood supply, potential tumors eW,er die or remain as dormant "microtumors," stable cell popula tions wherein dying cells are replaced by new cells. Thus one important area in which knowledge of developlnent (Figure 1 7.22; Mu thu kkaruppan and A uerba ch the eye. Other adult solid tissues do not induce blood vessels to form. It might therefore be pOSSible to block tumor devel opmen t by blocking a ngiogenesis . Numerous chem icals are bei ng tested as natura l and artificial angiogenesis inhibitors. These compounds act by preventing endothe- MEDICAL ASPECTS OF DEVELOPMENTAL BIOLOGY (A) 649 (B) Vein Growing tumor Artery / Tumor 2 Days —.. 6 Days o .. . . I•ii>r 8 Days —.. 12 Days FIGURE 17.22 New blood vessel growth to the site of a mam nlary tUnlor transplanted into the cornea of an albino mouse. (A) Sequence of events leading to vascularization of the tumor on days 2, 6, 8, and 1 2 . Both the veins and the arteries in the limbus surrounding the cornea supply blood vessels to the tumor. (B) Photograph of living cornea of an albino mouse, with new blood vessels from the limbus approaching the tumor graft. (From Muthukkaruppan and Auerbach 1 979, B courtesy of R. Auerbach.) lial cells from responding to the angiogenetic signal of the tumor.' One of the advantages of these compounds is that the tumor cells are unlikely to evolve resistance to them, since the tumor cell itself is not the target of these agents (Boehm et al. 1997, Kerbel and Polkman 2002). In one set of clinical trials, an antibody against VEGF A was fOWld to be successful against colon cancer but not against breast cancer. This is probably because colon cancer is more dependent on VEGP-induced angiogenesis than are mammary tumors (Whisenant and Bergsland 2005). Antibodies against a placental form of VEGP were able to inhibit the growth of tumors without affecting healthy blood vessels (Fischer et al. 2007). Blocking the VEGP receptor VEGFR3 prevents the angiogenic sprouting needed for new blood vessels (Tammela et al. 2008) and stops tumors from getting blood-borne nutrients and oxygen. Interestingly, thalidomide, the tenitogen responsible for birth defects in the 1960s, is now being used to block l1.unor-induced blood vessels. Thalidomide has been found to be a potent anti-angiogenesis factor tha t can reduce the growth of cancers in rats and mice (0' Amato et aJ. 1994; Dredge et a l.

2002). Cancer and congenital maJiormations are opposite sides of the san1e coin. Both involve disruptions of normal devel opment. Thus, as we have seen, agents that have been known to cause congenital malformations-thalidomide, retinoic acid, and cyclopamine-can be used as drugs to *This is the flip side of differentiation therapy discussed above. In differentiation therapy, paracrine factors or hormones are added to promote differentiation. Here, the substances being administered block paracrine signals in order to prevent tissue (Le., blood vessel) formation. prevent cancers. Just as they disrupt normal development, these substances can disrupt the caricature of development that is caused by tumor cells. When angiogenesis is blocked, the tumor cells can be starved. Stem Cells and Tissue Regeneration Embryonic stem cells As we discussed in Chapter 8, the inner cell mass of the mammalian blastocyst generates the entire embryo. 1den tical twins and chimeric individuals show that each of the cells of the inner cell mass are pluripotent. When cultured, the cells of the inner cell mass blastomeres can become embryonic stem (ES) cells, which remain pluripotent.

Cur rently, pluripotent stem cells are obtained by two major techniques (Figure 17.23A). They can be derived from the inner cell mass of blastocysts, such as those left over from in vitro fertilization (Thomson et al. 1998), and they can also be generated from germ cells derived from sponta neously aborted fetuses. The latter are generally referred to as embryonic germ (EG) cells (Gearhart 1998). Some experimental evidence (Stre1chenko et aJ. 2004) suggests that it may also be possible to derive embryonic stem cells from late morulae, befoTe they form blastocysts. While adult stem cells are rare and do not usually remain undif ferentiated in culture, embryonic stem cells can be readily harvested and retain their undifferentiated state for years of culturing. The IDlportance of pluripotent stem cells in medicine is potentially enormous. The hope is that human ES cells can be used to produce new neurons for people with degener ative brain disorders (such as Alzheimer and Parkinson dis ease) or spinal cord injmies, and new pancreatic cells for people with diabetes.

People with deteriorating hearts might be able to have damaged tissu e repla ced with new heart cells, and those suffering from immune deficiencies might be able to replenish their failing immune systems. Such ther apies have already worked in mice. Murine ES cells have been cultured under conditions causing them to form insulin-secreting cells, muscle stern cells, glial stem cells, and CHAPTER 1 7 650 . (A) Q@ @ -:. . • . • .

G.::I . . . . . B lastocyst-stage em b ryo Cells from inner cell mass Fetus Embryonic stem cells; I Primordial germ mass cultured pluripotent stem cells Lineage-specific stem ceUs FIGURE 17.23 E mb ryo n i c stem ce l l ther a pe u ti cs IA) ES cel l s can be obtained from the inner cell mass of the blastocyst or from primordial germ cells and cul tured in different ways to produce lineage- spec ifi c stem cells. These cells (or the pre cursor cells derived from them) can then be tran sp lan ted into a host. (B) Differentia- tion of mo u se ES cells into l ineage-restrict ed (neuronal and glial) stem cells can be accomplished by al tering the media in which the ES cells grow. (A after Gearhart 1 998; B, photographs from Brustle et al. 1 999 and Wi cke l gren 1 999, courtesy of O. Brustle and J . W. McDonald.) neural stem cells (Figure McDonald et al. 1999). 1 7.236; Brustle et aJ. 1999; Dopaminergic neurons derived from ES cells have been shown to signifi- cantly reduce the symptoms of Parkinson disease Heart cell precursor • (B) • ES cell . . tI • ..... Neural precursor I in rodents (Bjorklund et al. 2002; Kim et al.

2002). Wheu neural stem cells derived from germ cell- derived ES cells were transplanted into the injured \ " ; / ' , , Transplantation therapy brains of mice, the neural stem cells replaced neu rol15 and gUal ceUs in the forebrains of the newborn 2005). However, in most cases mice (Mueller et al. when human ES cells have been transplanted into the brains of immunosuppressed animals, the results have been less encouraging. Typically, trans plantation or thousands of ES cell-generated neu Medium .

. contammg basic fibroblast growth factor and platelet-derived growth factor Glial stem cells I ansp!antation t mto mICe rons into animals results in very few surviving Medium containing retinoic acid • t_ dopaminergic neurons and a high frequency of tumors (Li et aJ. 2006). For such transp!antatiol15 to work, one must be able to find the neural stem cells made by the ES cells and to prevent undifferentiat ed ES cells from being transplanted. Human embryonic stem cells differ from their murine counterparts in their growth requirements. In most ways, however, they are very similar, and have a similar, if not identical, pluripotency. Like mouse ES cells, human ES cells can be directed Neural stem cells Kaufman and his colleagues down specific developmental paths.

For example, (2001) directed human ES cells to become blood-forming stem ceUs by plac ing them on mouse bone marrow or endothelial cells.

These ES-derived hematopoietic stem cells could further differentiate into numerous types of blood cells (Figure 1 7.24) . Human embryonic germ in cells were able to cure virus-induced paraplegia rats. These stem cells appear to do thls both by dif ferentiating into new neurons and by producing paracrine factors (BDNF and TGF-a) that prevent the death of the existing neurOI15 (Kerr et aJ. Functional glial cells 2003). Similarly, ES cells from monkey blastocysts have Functional neurons been able to CUIe a Parkinson-like condition in adult MEDICA L ASPECTS OF DEVELOPMENTAL BIOLOGY FIGURE 17.24 Differentiated blood cells developing from human ES cel l s cultured on mouse bone marrow. (Courtesy of The University of Wisconsin.) monkeys whose dopaminergic neurons had been destroyed (Tagaki et al. 2005). Research is now being done to find ways of directing the differen tia tion of ES cells by using small molecules (rather than paracrine factors). Chen and colleagues (2009), for instance, have found tha t indolactam V molecules can induce Prix1 expression in ES cells, di.recting them into the pancreatic lineage. Thus, ES cells may be able to provide a reusable and readily available source of cells to heal dam aged tissue in adult men and worn,en. See WEBSITE 1 7.4 Therapeutic cloning 651 The newfound knowledge of the transcription factors needed to maintain pluripotency has illuminated a star tlingly easy way to generate embryonic stem cells that have the exact genotype of the patient.' In 2006, Kazutoshi Taka hashi and Shinya Yamanaka of Kyoto University demon strated that by inserting activated copies of four genes that encoded some of these critical transcription factors, nearly any cell in the adult mouse body could be made into a cell with the pluripotency of embryonic stem cells.

Such cells are called induced pluripotent stem (iPS) cells. Using a strategy very similar to the one used to identi fy the active components of Spemann's organizer (see Chapter 7), Takahashi and Yamanaka obtained mRNA from mouse ES cells and made these into cDNAs, which they placed onto active viral promoters. They transfected sets of 24 of these recombinant viruses into cultured fibrob lasts that had a neomycin-resistance gene placed onto an Fbx15 regulatory region. The Fbx15 gene is usually turned on in ES cells, so if the transfected genes activated the Fbx15 gene, then the neomycin-resistance gene would be activat ed and the cells would survive in neomycin-mntaining cul ture medium (Figure 1 7.25A). U a cohort of 24 active genes was found to activate the Fbx15 promoter (as shown by the cells' growing in neomycin-containing medium), then the group of cloned genes was further split. Eventually, Taka hashi and Yamanaka discovered that only four active genes were needed to tum on the Fbx15 gene. These genes encoded the transcription factors Oct4, Sox2, .Klf4, and c-Myc (Figure 1 7.256). When the researchers cultured those cells whose Fbx15 promoter was activated, they found that in many cases the cells had become pluripotent, as demon strated in a series of tests: • When the cells were aggregated together, they formed a teratoma-a tumorlike amalgam containing cell types of all three germ layers.

Induced pluripotent stem cells The mammalian inner cell mass is known to be character ized by certain transcription fadors, including Nanog, Oct4, and Sox2. Knocking out these. genes in mice abolish es the pluripotency and self-renewal of the inner mass blas tomeres, eventually leading to the demise of the embryo (see Boyer et aJ. 2006; Niwa 2007).

Sox2 and Oct4 can dimerize to form a transcription factor complex tha t acti vates thei.r own genes (Oe14 and Sox2) as well as the Nnnog gene. It appears that Oct4 not only promotes the synthesis of Sox2 and Klf4, but it also blocks the production of the miR145 microRNA that would otherwise prevent the trans lation of Oct4, Sox2, and Klf4 messages (Chivukula and Mendell 2009; Xu et al. 2009). These transcription factors (and microRNAs) initiate a transcription network in the inner cell mass and ES cells that is essential for pluripoten cy (see Welstead et al. 2008). This fi.rst set of transcription factors probably acts by activating other transcription fac tors, such as Nanog and FbxlS, which are critical in main taining the pluripotent and dividing state. • • When injected onto the blastocyst of a normal mouse, the induced cells showed that they could contribute to the production of cells in each of the three germ layers (Figure 1 7.25C; Maherali et a1. 2007; Okita et a1. 2007; Wernig et a!.

2007). 'When the inner cell masses of mouse embryos were C0111pletely compo;;ed of induced pluripotential cells, normal mice were generated (Boland et al. 2009; Zhao et al. 2009). • The transcription and DNA methylation pattern of the induced pluripotential cells was found to be almost iden tical to that of actual mouse embryonic stem cells. The genes for Nanos, Sox2, and other ES-cell transcription factors were hypomethylated, as they are in ES cells.

Interestingly, the methylation problems that plague -tIn order to be a successful therapeutic agent embryonic stem cells have to be the same genotype as the patient, SO that their differenti ated products will not be rejected. In fact, however they are not quite exact, because the viruses used to induce pluripotency are integrated into the chromosomes, as we'll see next. , CHAPTER 1 7 652 (A) Pbx 15 Ii enhan er X F I enhancer " ' &) " N _ Fibroblast iPS cell _ Pbx 15 II (B) C=I , • " N, 1Retroviral infection and neomycin selection 5' LTR II Viral II I 3'LTR promoter N Control (C) comyc -resistant in cDNA FIGURE 1 7.25 Produ ction of i nduced pluripotent stem cells by expression of four transc ri pti on factors in adult mouse tail fibro blasts.

(A) A mouse was produ ced wherein the 5' regul a to ry region of the Fbx7 5 gene was attached to a neomycin-resistance (N eo RI gene. When Fbx 15 gene is activated (as it is in ES cellsl, th e n eomycin-res stan ce gene is a ctivated and the cells are able to grow in medium containing neomycin. Various combinations of genes encoding mRNAs found in ES cells were added to the fibroblasts, and the fibroblasts grown in neomycin to see if a ny combination of genes activated the Fbx 15 promo ter. (8) Some neomyc in-resistant colonies survived . These resistant colonies were found to have genes encoding Sox2, Oct4, Klf4, and c-Myc. (C) The pluripotency of these cells was shown by their i nsertion into the inner cell mass of a mouse blastocyst. GFP-labeled iPS cells were subsequ e tly found in all organs of the embryo. (After Takahashi and Yamanaka 2006.1 _ i n normal cells. However, Nakagawa and colleagues (2007) showed that by altering the culturing techniques, one could circumvent the need for c-Myc.

In other words, one should be able to obtain induced pluripotent stem cells by adding merely three active genes to a patient's cell. Another way of "getting rid of c-Myc" was to place the genes for the key ES transcription factors onto episomal viral vectors (Kaji et a1. 2009; Yu et a1. 2009). Episomal vectors are derived from viruses (such as the Epstein-Barr virus that causes mononucleosis) that do not insert themselves into host DNA. rn this manner, the genes from the vector generated the transcription factors that converted human fibroblasts into iPS cells. Once the iPS cells were produced, the cul ture media could be changed so that the vector would be eliminated.

This technique generated human iPS cells that did not have a v.irus inserted in it. Thus, not only was the cloned animals do not appear to be a problem for embry on.ic stem cells derived by somatic cell nuclear transfer. It appears that the nuclei in the small population of ES cells that survive in culture have had their methylation pat terns erased, thus enabling them to redifferentiate (Jaenisch 2004; Rugg-Gwm et a1. 2005). problem of c-Myc circumvented, but so was the problem of the viral insertion capable of causing a gene mutation. Recently, adult stem cells have been found that are already "part of the way" to becoming pluripotent and do not need as many factors to make them so. Giorgetti and colleagues (2009) showed that blood stem cells from the human umbilical cord can be converted into pluripoten tial stem cells merely by adding activated OCT4 and SOX2 This technique does nothing less than transform any cell genes, and Kim and colleagues (2009) found that they in the body into an embryonic stem cell. By 2007, the could transform human neural stem cells into iPS cells by Yamanaka laboratory (Takahashi et a1. 2007) had used the adding only a single activated gene, OCT4. same set of four transcription factors to induce pluripoten The therapeutic potential of iPS cells was demonstrat tiality in adult human fibroblast cells.

These induced cells ed by the ability of iPS-derived hematopoietic stem cells formed teratomas containing cells of all three germ layers, to correct a sickle-cell anemia phenotype in mice and they had the same transcription profile as the human (Figure 17.26; Hanna et a1. 2007). Here, tail-tip fibroblasts from a ES cells. mouse with sickle-cell hemoglobin were made into iPS cells Yamanaka (2007) proposed that c-Myc helped "immor b y being infected in culture by viruses containing Oct4, talize" the cell, keeping it in an undifferentiated state of Sox2, Klf4, and c-Myc. The iPS cells were selected, then proliferation, and that Klf4 prevented apoptosis and senes electroporated with DNA containing wild-type globin cence. But this situation would have created a tumor, were genes. These genetically corrected iPS cells were cultured in it not for Oct4 and Sox2, which redirected growth and gave media that promoted the production of hematopoietic stem the cell properties similar to those of germ cells. The need cells, and the hematopoietic stem cells were injected back for c-Myc was disconcer ting, however, since this is a well into the mice with sickle-cell anemia. By known oncogene, capable of initiating tumor formation in mia had been cured. 2 months, the ane MEDICAL ASPECTS OF DEVELOPMENTAL BIOLOGY The combination of iPS cells and genetic engineering may be able to cure certain genetic diseases. Raya and colleagues (2009) have cultured dermal fibroblast cells from patients 653 paracrine factors that appear to activate the heart's own stem cells to repair the damage (Cho et al. 2007; Mirotsou 2007). et at. with the genetic disease Fanconi illemia and have added to them a good copy of the defective gene. These cells were BONE REGENERATION Several stem cell therapies can be es bearing the activated forms of OCT4, SOX2, KLF4, and c seen then converted into iPS cells by the addition of retrovirus MYC. Moreover, these iPS cells could be directed to become can become normal parts of in an incredibly important area of regenerative med icine: forming new adult bone. VVhile fractured bones can heal, bone cells in adults usually do not regrow to bridge the blood stem cells needed by the patients. However, we wide gaps. The finding that the same paracrine and still do not know if these cells endocrine factors involved in endochondral ossification the patients and not produce tumors themselves. are also involved in fracture repair (Vortkamp et al. 1998) raises the pOSSibility that new bone could grow if the prop er paracrine factors and extracelJular environment were Adult stem cells and regeneration therapy provided. Several methods are now being tried to devel In the introduction to Part 1II, we mentioned the potential op new functional bone in patients with severely fractured these new therapies.

Bonadio and his colleagues medical uses of adult stem cells, especially the mesenchy mal stem cells. We will now provide details about some of or broken bones. One solution to the problem of delivery was devised by (1999), who developed a col lagen gel containing plasmids carrying the human parathy CARDIAC REGENERATION When mesenchymal stem cells roid hormone gene. The plasmid-impregnated gel was from the bone marrow of heart attack patients are injected placed in the gap be tween the ends of a broken dog tibia into their own hearts, these cells can differentiate into heart or femur.

As cells migrated into the collagen matrix, they and vessel cells and can significantly m i prove the patients' incorporated the plaSmid and made parathyroid hormone. outcomes (Yousef et al.

2009). Interestingly, in many A dose-dependent increase in new bone formation was 1 7.27). This type of treat instances the stem ceUs do not create new structures them seen in about a month (Figure selves to circumvent the blockage. Rather, they secrete ment has the potential to help people with large bone frac tures as well as those with osteoporosis. Humanized sickle ceU (HbSIHbS) anemia mouse model Uo , o Transplant corrected hematopoetic progenitors back into irradiated mice t " I \ c Differentiate into embryoid bodjes t, y HbAlHbS iPS clones , in IPS cells by speCific gene targeting E,y\ FIGURE 1 7.26 Protocol for curing a "human" disease in a mouse, using iPS cells fibroblas t plus recombinant genetiCS. (1) Tail-tip fibroblasts are taken from a mouse whose genome contains the human alleles for f) Infect with Oct4, sickle cell anemia (HbS) and no mouse Sox2, Klf4 and genes for this protein. (2) The cells are cultured and infected with viruses con taining the four transcription factors known to induce pluripotentiality. (3) The iPS cells are identified by their distinctive shapes and are given DNA containing the wild-type al lel e of human g l ob in (HbA). (4) The embryos are allowed to differenti ate in culture . They form Ilernbryoid bod ies" that contain blood-forming stem ce lls. (5) Hematopoietic progenitor and stem cells from these embryoid bodies are injected into ./ mouse-derived the original mouse and cure its sickl e-ce ll aneiPS dones mia. (After Hanna et aJ. 2007.) o .rrect sick1e-ceU utation · Harvest tail tIp fibroblasts I @ 654 CHAPTER 1 7 b (e) Whole on e, 53 wk (A) Treated fracture FIGURE 17.27 Bone formalion from collagen matrix containing plasm ids bearing the human parathyroid hormone. (A) A 1 .6 cm gap was made in a dog femur and stabilized with screws. Plasmid-containing gel was placed on the edges of the break. Radiographs of Ihe area at 2, 8, 1 2, 1 6, and 1 8 weeks afler the sur gery show the formalion of bone bridging the gap at 1 8 weeks. (B) Control fracture (no plasmid in Ihe gel) al 24 weeks (C) Whole bone a year after surgery, showing repaired region. (From Bonadio el aJ. 1 999, courtesy of J. Bonadio.) . and mechanical engi neering, is called tissue engineering. Li and collea gue s (2005) made scaffolds of ma terial that resembles normal extracellular matrix and which can be molded to form the shape of bone needed. The bone (B) Untreat.ed fracture, 24 wk Another approach is to find the right mixture of paracrine factors to recruit stem cells and produce normal bone. Peng and colleagues (2002) genetically modified muscle stem cells to secrete BMP4 or VEGF-A. These cells were placed in gel matrix discs, which were implanted in wowlds made in mouse skulls.

The researchers found that certain ratios of BMP4 and VEGFs were able to heal the wounds by making new bone. Similarly, BMP2 has been used to heal large fractures of primate mandibles and rab bit femurs (Li et a1. 2002; Marukawa et a1. 2002). A third approach is to make sca ffolds that resemble those of the bone and seed them with bone nlarrow stem cells. This approach, combining developmental biology marrow stem cells can be placed in these scaf- folds and plac ed into the existing bone. These stem cells are told how to differentiate by the local conditions, and histological and gene expression studies have shown that these cells form bone with the appropriate amounts of osteocytes and chondrocytes. Similarly, when cells are removed from the tracheae of recent cadavers, they can be reseeded with bone marrow stem cells of patients with damaged tracheae. The stem cells dif ferentiate into chondrocytes, and the newly reseeded tra cheae can be substituted for ille damaged tracheae and pro vide a normal airway (M acchi arini et a1. 2008). A fourth ap proach has been to let the body do the work. While bones can't regenerate if wide gaps appear, many bones can undergo normal healing of small wounds. Here, cells in the periosteum (the sheath of cells that surrounds the bone) will differentiate into new cartilage, bone, and ligaments to fill the crack. Stevens and colleagues (2005) have used this normal healing process to make new bones. They injected saline solutions between the rabbit tibial bone and its periosteum, mimicking a fracture, and kept this space open by adding a gel containing calcium (to push the per iosteal cells to differentiate into bone rather than cartilage). Within a few w eeks, these cav ities filled with new bone, which could be tra nspl a nted into sites where b one had been damaged.

This technique might pro vide a r ela tively painless way to produce new bone for fus ing vertebrae and other surgical techniques. NEURONAL REGENERATION While the central nervous sys tem is characterized by its ability to change and make new connections, it has very little regenerative capacity. The motor neurons of the peripheral nervous system, however, have Significant regenerative powers, even in adult mam- mals. The regeneration of motor neurons involves the MEDICAL ASPECTS OF D EVELOPMENTAL B I OLOGY J£ the cell body of a motor neu 655 regrowth of a severed axon, not the replacement of a miss myelination, and these transcription factors appear to be ing or diseased cell body. repressed by high levels of Wnt signaling (Arnett et a1. ron is destroyed, it cannot be replaced. 2004; Fancy et al. 2009). Therefore, the use ofWnt inhibitors The myelin sheath that covers the axon of a motor neu (such as Frzb and Dickkopf) may be a mechanism for treat ron is necessary for its regeneration. This sheath is made ing this disease. Research into CNS axon regeneration may by the Schwann cells, a type of glial cell in the peripheral become one of the most important contributions of devel nervous system (see Chapter opmental biology to medicine. 9). When an axon is severed, the Schwann cells divide to form a pathway along which the axon can regrow from the proximal stump . This pro liferation of the Schwann cells is critical for directing the Direct transdifferentiation regenerating axon to the original Schwann cell basal lam One of the newest developmental therapies involves using ina. If the regrowing axon can find that basal lamina, it can transcription factors to convert one cell type into another be guided to its target and restore the original connection. without the intermediary of stem cells, a procedure known In turn, the regenerating neuron secretes mitogens that as transdifferentiation. In Chapter allow the Schwann cells to divide. Some of these utitogens transdifferentiation of exocrine pancreatic cells into insulin 2, we discussed the are specific to the developing or regenerating nervous sys secreting pancreatic p cells by three transcription factors tem (Livesey et a1. (Ngn3, Mafal, and Pdxl).

More recently, Kajiyama and col 1997). The neurons of the central nervous system cannot regenerate their axons under normal conditions.

Thus, leagues (2010) have transfected .one of these factors, Pdxl, into mouse adipose-derived stem cells and converted the spinal cord injuries can cause permanent paralysis. One stem cells into insulin-producing pancreatic cells. These strategy to get around this block is to find ways of enlarg induced pancreatic cells were able to reduce the hyper- ing the population of adult neural stem cells and to direct glycemia in diabetic mice. their development in ways that circumvent the lesions Vierbuchen and colleagues (2010) found that ti,e viral caused by disease or trauma. The neural stem cells found insertion of three active transcription factor genes (Ascll, .in adult mammals may be very similar to embryonic neu Bm2, ral stem cells and may respond to the same growth factors dermal fibroblasts into fmctional neurons in vitro. These Oohe et al. 1996; Johansson et a1. 1999; Kerr et a1. 2003). Another strategy for CNS neural regeneration is to cre and My tll) sufficed to effiCiently convert mouse induced neuronal cells expressed several neuron-specif ic proteins, were able to generate action potentials, and ate environments that encourage axonal growth. Unlike formed functional synapses (resembling excitatory neu the Schwann cells of the peripheral nervous system, the rons of the forebrain). It remains to be seen if this trans myelinating glial cells of the central nervous system, the differentiation is stable and whether it will ameliorate oligodendrocytes, produce substances that inhibit axon disease in vivo. However, the ability to generate pancre regeneration (Schwab and Caroni atic cells and neural cells that are genetically identical 1988).

Schwann cells transplanted from the peripheral nervous system into a to a patient holds the promise of significant therapies for CNS lesion are able to encourage the growth of CNS axons patients with diabetes and degenerative neural diseases. to their targets (Keirstead et a1. 1999; Weidner et a1. 1999). Three substances that inhibit axonal outgrowth have been isolated from oligodendrocyte myelin: myelin-associated glycoprotein, Nogo-l, and oligodendrocyte-myelin glyco 1994; (hen et a1. 2000; Grand 2002). Each of these substances protein (Mukhopadyay et a1. Pre et al. 2000; Wang et al. Coda Developmental biology is gaining increasing importance in modern medicine. First, preventive medicine, pubHc health, and conservation biology demand that we learn binds to the Nogo receptor (NgR). Thus, NgR may be the more about the mechanisms by which industrial chemi critical target for therapies allowing regeneration. Chen cals and drugs can damage embryos. The ability to effec and colleagues (2009) IISed RNA interference to block tIle tively and inexpensively assay compounds for potential synthesis of NgR and found that this helped rat optic neu harm is critical. Second, developmental biology is pro rons to regenerate. The axons regenerated even better viding us new ways of preventing and curing cancers. when this therapy was combined with nutrients and pos Third, developmental biology is providing the explana itive growth factors.

tions for how mutated genes and aneuploidies cause their As mentioned in the case with bone, there is a dose rela aberrant phenotypes. Fourth, the field of regenerative tionship between wound healing and regeneration.

medicine is using developmental biology to provide Patients with multiple sclerosis suffer from the demyeli induced pluripotent stem cells as well as the knowledge nation of axons of paracrine factors needed to form new cells, tissues, and in the brain and spinal cord. Several tran scription factors are needed to reinitiate the pathway to organs in adults. 656 CHAPTER 1 7 Snapshot Summary: Medical Aspects of Developmental Biology 1. Pleiotropy occurs when several different effects are produced by a single gene, In mosaic pleiotropy, each effect is caused independently by the expres sion of the same gene in different tissues . In relation al pleiotropy, abnormal gene expression in one tissue influences other tissues, even though those oilier tis sues do not express that gene, 2. type, in different individuals, can produce different defects (or differing severities 4, Preimplantation genetics involves testing for genetic abnormalities in early embryos in vitro, and implanting only those embryos that may develop normally. Selection for sex is also possible using preimplantation genetics, 5. Teratogenic agents include certain chemicals such as alcohol and retinoic acid, as well as heavy metals, certain pathogens, and ionizing radiation. These agents adversely affect normal development, yield ing malformations and functional deficits. 6. Fetal alcohol syndrome is completely preventable. There may be multiple effects of alcohol on cells and tissues that result in this syndrome of cognitive and physical abnormalities. 7, 10, Cancer can be seen as a disease of altered develop ment. Some tumors revert to non-malignancy when placed in environments that fail to support rap id cell 11.

Cancers can arise from errors in cell-cell communica tion. These errors include alterations of paracrine factor syn thesis. Phenotypic heterogeneity arises when the same gene of the same defect) by turning genes all, methylation can alter metabolism and development clivision. Genetic heterogeneity occurs when mutations in more than one gene can produce the same pheno 3, inherited from one generation to the next. Such Endocrine disruptors can bind to or block hormone receptors or block the synthesis, transport, or excre tion of hormones. DES is a powerful endocrine dis ruptor, Presently, bisphenol A and other compounds are being considered as possible agents of low sperm counts in men and a predisposition to breast cancer in women. 8. Environmental estrogens can caus reproductive system anomalies by suppressing Hox gene expres sion and Wnt pathways, 9. In some instances, endocrine disruptors methylate DNA, and these patterns 01 methylation can be 12.

Cancers metastasize in manners similar to embryon ic cell movement. 13. In many instances, tumors have a rapidly dividing as well as more quiescent and differentiated cells. stem cell population which produces more stem cells 14, The methylation pattern 01 cancer cells is often aber rant and these methylation differences can cause cancer by inappropriately inactivating tumor sup pressor genes or activating oncogenes. 15, Disrup ting tumor-induced angiogenesis may become an important means of stopping tumor progression. 16, Skin fibroblasts, and perhaps any normal adult cell, can be induced to form pluripotent stem cells by the incorporation of activated genes encoding certain transcription factors. These pluripotent stem cells would not be rejected by the patient from whom they were formed, 17, By altering conditions to resemble those in the embryo and by providing surfaces on which adult stem cells might grow, some adult stem cells might be directed to form numerous cell types. 19. Neurons in the central nervous system can be aided in regenerating by blocking the glial-derived paracrine factors that stabilize neurons and prevent their growth. 20. Transdifferentiation is the viral insertion of tran scription factors (or transcription factor activation by small molecules), which can convert one stable cell type into another, For Further Reading Complete bib liographical citations for all literature cited in this chapter can be found at the free-access website www.devbio.com Anway, M, D" A. S. Cupp, M. Uzumcu and M, K. Skipper. 2005, Epigene tic transgeneration effects of endocrine dis ruptors and male fertility, Science 308: 146&-1469. Baksh, D" L Song and R. S, Tuan. 2004, Adult mesenchymal stem cells: Charac terization differentiation, and applica tion in cell and gene therapy, j, Cell Mol, Med. 8: 301-316, Bissell, M, j" D.

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Outside Sites Go Online WEBSITE 17.1 Human embryology and genetics. This links to other websites that will connect you to tutorials in human development, as well as to the Online Mendelian Inheritance in Man (OMIM), which details all human genetic conditions. WEBSITE 17.2 Association of Reproductive Health Professionals: malfonned arms and legs, and it provided the first major resources for health care providers and their Endocrine disruptor exchange: Reproductive Toxicology website: www.reprotox.org has summaries of over 5000 agents, exposure levels, and their effects on development and evidence that drugs could induce congenital anomalies. The mechanism of i ts action is still hotly debated. reproduction. WEBSITE 17.3 The NIH website on stem cell education. Our stolen future. nus website monitors the environmental effects of endocrine disruptors. It is a political and consumer action site as well as a scientific clearinghouse for endocrine disruption. Run by the authors of the book Our Stolen Future, it also provides links to the websites of people who disagree with them. t echniqu e, thera peu tic the immune rejection Therapeutic cloning. Prior to induced pluripotent stem ceils, another cloning, was seen as a way around of stem cell-derived differentiated cells. Vade Mecum Somites and thalidomide. These movies are from the lab oratory of Jay Lash, whose insightful work on cartilage for mation resulted in some of our first insights into the mechanisms b y which the drug thalidomide halts limb growth. clients. www.arhp,orgltopics/enviro-repro health contains www.endocrinedisruption.com Thalidomide as a teratogen.

The drug thalidomide caused thousands of babies to be born with WEBSITE 17.4 657 Stern cell basics: "All things stem cell." An informative stem cell blog: . allthingsstemcell.com For information on fetal alcohol syndrome: ,gov/ncbddd/fasdidata.htmi . NIH, FASD: AboutFAS.htm Substance Abuse and M ental Health Services Administration (SAMHSA): Developm ental Plasticity and Sym biosis iT WAS LONG THOUGHT THAT THE ENVIRONMENT played only a minor role in development. Nearly all developmental phenomena were believed to be a "read out" of nuclear genes, and those organisms whose development was significant ly controlled by the environment were considered interesting odd ities. When environmental agents played roles in development, they appeared to be destruc tive, such as the roles played by teratogens and endocrine disruptors (see Chap ter 17). However, recent s tudies have shown that the environmental context plays significant roles in the narmaJ d evelopment of almost aU species, and that animal genomes have evolved to respond to environmental conditions. Moreover, symbiotic associations, wherein the genes of one organism are regulated by the products 01 another organism, appear to be the rule rather than the exception. One reason developmental biologists have largely ignored the environment's effects is that a criterion for selecting wh.ich animals to study has been their abil C. elegans, Drosapl.i/fl, zebra fish, Xenopus, ity to develop in the laboratory (Bolker 1995). Given adequate nutrition and tem perature, all "model organisms " - ch.icks, and laboratory mice develop independently 01 their particular environ ment, leaving us with tl,e erroneous impression that everythlng needed to form the embryo is wW1in the fertilized egg. Today, with new concerns about the loss of organismal diversity and the effects of environmenta l pollutants, there is renewed interest in the regulation of development by the environment (see van der Weele 1999; Gilbert and EpeI 2009). The Environment as a Normal Agent in Producing Phenotypes Although the nucleus and cytoplasm of the zygote contribute a majority of phe notypic instructions, everything needed for producing a pa rticula r phenotype is not pre-packaged in the fertilized egg. Rather, crucial parts of phenotypiC deter mination are regulated by environmental facton, outside the organism. Phenotyp ic plasticity is the ability 01 an organism to react to an env ironmental input with seen in embryonic or larval stages of animals or p la nts , this ability to change a change n i form, state, movement, or rate of activity (West-Eberhard 2003). When phenotype is often called developmental plasticity. We have already enco untered several exampl es of developmental plasticity in this book. When we discussed environmental sex determination in turtles, fish, and ech.iuroid worms (see Chapter 14), we were aware that the sexual phe notype was being instructed not by the genome but by the environment. When we discussed in Chapter 12 the ability of shear stress to activate gene expression in capillary, heart, and bone tissue, we Similarly were studying the effect of an We may now tum to consider adapw dons towards the extenlOl environment, and fimly the direct adaptations .

.. in which an animal, during irs deuelop mem, becomes mod ified by exremal factors in such a way as to increase its effiCiency in dea li ng Wilh them. c. H.

WADDINGTON (1 957))IAN xu AND)EFFREY I. GORDON (2003) HOllor thy sY11bionts. 660 CHA PTER 1 8 (A) (6) IC) (D) Spring morph among catkins Summer morph on twig environmental agent on phenotype . While studies of phe notypic plasticity play a central role in plant developmen (E) lal biology, the mechanisms of plasticity have only recent ly been studied in animals.

These studies are showing that the integra tion of animals into ecological communities is accomplished largely through developmental interactions. autonomous enti ties but rather that we

in preda tor-free environments. In some species, phenotypic plas ticity is reversible, and removing the predators can restore the non-induced phenotype (Relyea 2003a). The metabolism of predator-induced morphs may differ significantly from that of the uninduced morphs, and this has important consequences. Relyea (2003b, 2004) has found tha t in the presence of the chemical cues emitted by predators, the toxicity of pes ticides such as carbaryl (Sevin®j can become up to 46 times more lethal than it is without the predator cues. Bullfrog and green frog tadpoles were espeCially sensitive to carbaryl when exposed to pred ator chemicals. Relyea has related these findings to the global decline of amphibian populations, saying that gov ernments should test the toxicity of the chemicals under more natural conditions, including that of predator stress. He concludes (Relyea 2003b) that "ignoring the relevant ecology can cause incorrect estimates of a pesticide's lethal ity in nature, yet it is the lethality of pesticides under nat ural condi tions that is of utmost interest. The accumulat ed evidence strongly suggests that pesticides in na ture could be playing a role in the decline of amphibians ." VIBRATIONAL CUES ALTER DEVELOPMENTAL TIMING The phe notypiC changes induced by environmental cues are not confined to structure. They can also include the timing of developmental processes. The embryos of the Costa Rican red-eyed treefrog (Agnlychnis callidryas) use vibrations trans mitted through their egg masses to escape egg-eating snakes. These egg masses (shown on this book's cover) are laid on leaves that overhang ponds. Usually, the embryos develop into tadpoles within 7 days, and these tadpoles wiggle out of the egg mass and fall into the pond wa ter. However, when snakes feed on the eggs, the vibrations they produce cue the remaining embryos inside the egg mass to begin the twitcl' ing movements that initiate their hatching (within seconds!) and dropping into the pond. The embryos are competent to begin these hatching movements a t day 5 (Figu re 1 8.6).

Interes tingly, the embryos have evolved to respond this way only to vibrations given at a certain fre quency and interval (Warkentin et al. 2005, 2006). Up to 80% of the remaining embryos can escape snake predation in this way, and research has shown that these vibrations alone (and not smell or sight) cue these hatching movements in the embryos. There is a trade-off here, too. Although these embryos have escaped their snake predators, they are now a t greater risk from waterborne predators than are fully of the early developed embryos, because the musculature hatchers has not developed fully. Temperature as an environmental agent TEMPERATURE AND SEX There are many species in which temperature does control whether testes or ovaries devel op. Indeed, among the cold-blooded vertebrates such as fish, tur tles, and all igators there are many species in which DEVELOPME NTAL PLASTICITY A N D SYMBIOSIS (A) 665 (B) (e) FIGURE 18.6 Preda tor- i nd uced polyphenism in the red-eyed tree frog Agalychnis callidryas. (A) When a snake eats a clutch of Aga/ychnis eggs, most of the remaining embryos inside the egg mass respond to the vibrations by hatching prematurely (arrow) and falling into the water. (8) I mmature tadpole, induced to hatch at day S. (C) Normal tadpol es hatch at day 7 and have better-developed musculature. (Cou rtesy of K. Warkentin.) the environment determines whether an individual is male or female (Crews and Bull 2009). This type of environmen tal sex determination has advantages and disadvantages. One advantage is that it probably gives the species the ben efits of sexual reproduction without tying the species to a 1 : 1 sex ratio. In crocociles, in which extreme temperatures produce females while moderate temperatures produce males, the sex ratio may be as great as 10 females to each male (Woodward and Murray" 1993). 1n such instances, where the number of females limits the population size; this ratio is better for survival than the 1:1 ratio demand ed by genotypic sex determination. The major disadvantage of temperature-dependent sex determination may be its narrowing of the temperature lim its within which a species can persist. Thus thermal poUu tion (either locally or due to global warming) could con ceivably eliminate a species in a given area Uanzen and Paukstis 1991). Researchers (Ferguson and Joanen 1982; Miller et al. 2004) have speculated that dinosaurs may have had temperature-dependent sex determination and that their sudden demise may have been caused by a slight dlange in temperature creating conditions wherein only males or only females hatched. (Unlike many turtle species, whose members have long reproductive lives, can hiber nate for years, and whose females can store sperm, dinosaurs may have had a relatively narrow time to repro duce and no ability to hibernate through prolonged bad times.) See WEBSITE 1 8.2 Volvox: When heat brings out sex Charnov and Bull (1977) argued that environmental sex determination would be adaptive in those habitats ellar acterized b y patchiness-that is, a habitat having some regions where it is more advantageous to be male and other regions where it is more advantageous to be female. Conover and Heins (1987) provided evidence for this hypothesis. In certain fish species, females benefit from being larger, since larger size translates into higher fecun dity. If you are a female Atlantic silverside fish (Menidia menidia), it is advantageous to be born early in the breed ing season, because you have a longer feeding season and thus can grow larger. (The size of males in this species doesn't influence mating success or outcomes.) In the southern range of Menidia, females are .indeed born early in the breeding season, and temperature appears to play a major role in this pattern. However, .in the northern reach es of its range, the species shows no environmental sex determination. Rather, a 1:1 sex ratio is generated at all tempera tures.

Conover and Heins speculated tha t the more north.em populations have a very short feeding season, so there is no advantage for females in being born earlier. Thus, this fish has environmental sex determination in those regions where it is adaptive and genotypic sex deter mination in those regions where it is not. BUTTERFLY WINGS 1n tropical parts of the world, there is often a hot wet season and a cooler dry season. In Africa, a polyphenism of the dimorphic Malawian butterfly (Bielj ellis anljnafla) is adaptive to seasonal changes. The dry (cool) season morph is a mottled brown butterfly that sur vives by hiding in dead leaves on the forest floor. In con trast, the wet (hot) season morph, which routinely flies, has prominent ventral eyespots that deflect attacks from predatory birds and lizards (Figure 1 8.7). 666 CHAPTER 1 8 Dry-seas on form Decreased amount of20-hydroxyecdysone OH HO ' 24-C Larva OH o Increased amount of 20-hydroxyecdysone OH HO ' o Distal-less expression in imaginal disc OH OH HO ' Wet-season form OH _ o FIGURE 18.7 Phenotypi c plasticity in Bicyclus anynana is regu lated by temperature during pupation. High temperature (either i n the wild o r in c ontroll ed laboratory conditions) allows t h e accu mulation of 20 hydrox yecdysone (20E), a hormone that is able to sustain Distal-less expression i n the pupal imaginal disc. The region of Distal-less expression becomes the focus of each eye spot. In cooler weather, 20E is not formed, Distal-Jess expression in the imaginal disc begins but is n ot sustained, and eyespots fa il to form.

(Courtesy of S. Ca rrol l and P. Brakefield.) The importance of hormones such as 20E for mediating env ironmental signals controlling wing phenotypes has been docmnented in the Araschnin butterfly (Figure 1 8.8). Araschnia develops aIternative phenoty pes depending on whether the fourth and fifth instars experience a photope riod (hours of daylight) that is longer or shorter than a par ticular critical day length. Below tnis critical day length, ecdysone levels are low and the butterfly has the orange wings characteristic of spring butterflies. Above the criti The factor determining the seasonal pigmentation of B. nnynal1a is not diet, but the temperature d uring pupation. Low temperatures produce the dry-season morph; higher temperatures produce the wet-season morph (Brakefield and Reitsma 1991). The mechanism by which temperature regulates the Bicyclus phenotype is becoming known. In the late larval stages, transcription of the distal-less gene in the wing imagin al discs is restri cted to a set of cells that will become the signaling center of each eyespot. In the early pupa, higher temperatures elevate the formation of 20-hydroxyecdysone (20E; see Chap ter)5). This hormone sustains and expands the expression of distal-less in those regions of the wing imaginal disc, result.ing in prominent eyespots. In dry season, the cooler temperatures prevent the accumula tion of 20E in the pupa, and the foci of Dis tal-less signaling are not sus tained . In the absence of the Distal-less signal, th e eyespots do not form (Brakefield et at. 1996; Koch et at.

1996). Distal-less protein is believed to be the activating signal that determines the size of the eye spot (see Figure 18.7). FIGURE 18.8 Environmentally induced morphs ofthe European map butterfly (Araschnia levana). Th e orange morph (bottom) forms i n the spri ng when levels of ecdysone in the la rva are low. The dark morph with a white stripe (top) forms i n summer, when h igher temperatures and longer photoperiods induce greater ecdysone p roduction in the larva. Linnaeus classified the two morph s as different species. (Courtesy of H. F. Nijhout.) , cal point, ecdysone is made and the sum mer pigmenta tion forms. The summer form can be ind uced in spring pupae by injecting 20E into the pupae. Moreover, by altering tne timing of 20E injections, one can generate a series of inter mediate forms not seen in the wild (Kodl and BUckmann 1987; Nijhout 2003).

DEVELOPMENTAL PLASTICITY A N D SYMBIOSIS Environmental I nduction of Behavioral Phenotypes 667 • Low maternal care • Ell Plentiful maternal care In many instances, the morphological phenotype is accom panied by a behavioral phenotype. This is obvious in the l environunental determination of sex, where an individual's sexual behavior generally matches the gonads and geni 100 90 " 80 70 .g 60 > talia. This is also seen in the cases of butterfly wings (fliers vs. crawlers) and dung beetle horns (fighters vs. "sneak-5 50 E 40 .S 30 8-20 10 o ' u, .t ---.t . ' '' ers").

Sometimes, however, the behavior is the major devel opmental phenotype induced by the environment. Adult anxiety and environmen tally regulated DNA methylation G EnVironmentally derived methylation differences near birth may lead to important behavioral differences in 5'- GTG adults. In rats, behavioral differences in the response to . . 3' stressful situations have been correlated with the number Binding site for Egr I transcription factor of glucorticoid receptors in the brain's hippocampus. The more g lucocorticoid receptors, the better the adult rat is able to downregulate these adrenal hormones and deal with stress. The number of glucocortiCOid receptors appears to depend on the quality of grooming and licking the rat pup experiences duri.ng the first week after birth. How is the adult phenotype regulated by these perina tal (near the time of birth) experiences? Weaver and col leagues (2004) have shown that the behavioral difference involves the methylation of a particular site in the enhancer region on the glucocorticoid receptor gene. Before birth, there is no methylation at this site; 1 day after birth, this site is methylated in all rat pups. However, in those pups tl,at experience intensive grooming and licking during tile first week after birth, this site loses its methylation; but methylation is retained in those rats that do not have such FIGURE 18.9 Differential DNA methylation due to beh av ioral differences in parental care. A portion of an enhancer sequence of the rat glucocorticoid receptor (GR) gene is shown, indicating the binding s i te for the Egr1 transcription factor. Two cytosine residues within this bindi ng site have the potential to be methylated. The cytosine at the 5' end is completely methylated in the brains of pups that did not receive extensive licking and grooming from their mothers (red bar). The Egr1 Iranscription faclor did not bind these methylated sites, and thus the CR gen e remained inactive. If the pups received proper maternal care, this same site was largely unmethylated (ora nge bar), and the gene was transcribed in the brain. The cytosine at the 3' end of the enhancer (blue bars) was always methylaled and had no effect on Egr1 binding. (After Weaver et al. 2004.) extensive care.

Moreover, this methylation difference is not seen at other sites in or near the gene (Figure 1 8.9). demonstrated that tl1is metllylation difference was dependent B y switching pups and parents, Weaver and colleagues ent on the mother 's care, and was not the result of differences in tile pups themselves. When unmetllylated, this enhancer learning: The Developmentally Plastic Nervous System site binds tile Egrl transcription factor and is associated witll Learning provides remarkable examples of phenotypiC "active" acetylated nucleosomes. The transcrip tion factor plasticity. Since neurons, once formed, do not divide, the does not bind to tile metllylated site, and tile chromatin in "birthday" of a neuron can be identified by treating the such cases is not activated. These chromatin differences, organism with radioactive thymidine. Normally, very lit established during the first week after birth, are retained tle radioactive thymidine is taken up into the DNA of a throughout the life of tile rat. Thus, adult rats that received neuron that has already been formed. However, if a neu extensive perinatal grooming have more glucocortiCOid ral precursor ceU divides during the treatment, it will incor receptors and are able to deal witll stress better tl1an rats tllat porate radioactive thymidine into its DNA. received less care.' Just how grooming can alter DNA metlly Such new neurons are seen to be generated when male lation patterns, however, remains to be discovered.

songbirds first learn their songs. Juvenile zebra finches -Does this relate to humans? Using an "extreme" set of cases, memorize a model song and tllen learn tile pattern of mus of the hippocampus-specific glucocorticoid receptor is more highly erated in the hyperstriatum of the finch's brain. Many of McGowan and coUeagues (2009) showed that the Cis-regulatory region methylated in the brains of suicide victims with a history of dUldhood abuse than in suicide victims with no childhood abuse, or in controls. cle contractions necessary to sing a particular phrase. In this learn.llig and repetition process, new neurons are gen these new neurons send axons to the archistriatum, which is responSible for controlling the vocal musculature 668 CHAPTER 1 8 Right eye Left eye Opti nervec Lateral geni nucleculusate Optic radiatio",;'\ I from rivental ght eyepathways (vi e w i s surface offrombrain) Visual cortex Visual the (Nordeen and Nordeen 1988). These changes ale not seen males that are too old to learn the song, nor are they seen in j uvenile females (which do not sing these phrases). In white-crowned sparrows (Zana!richia /eucaphrys), whose song is regulated by photoperiod and hormones, exposing adult males to long hours of light and to testosterone induces over 50,000 new neurons in their vocal centers (Tramontin et a1. 2000). The neural cl.rcuitry of these birds' brains shows seasonal plasticity. Testosterone is believed to increase the level of brain-derived neurotro pic factor (BDNF) in the song-producing vocal centers. If female birds are given BDNF, they also produce more neurons there (Rasika et a1. 1999). The cerebral cortices of young ra"ts reared in stimulat ing environments are packed with more neurons, synapses, and dendrites than are found in rats reared in isolation (Turner and Greenough 1983). Even the adult brain con tinues to develop in response to new experiences.

When adult canaries learn new songs, they generate new neu rons whose axons project from one vocal region of the brain to another (Alvarez-Buylla et al. 1990). Studies on adult rats and mice indicate that environmental stimula tion can increase the number of new neurons in the den tate gyrus of the hippocampus (Kern perma nn et a1. 1997a,b; Gould et a1. 1999; van Praag et al. 1999). Similar ly, when adult rats learn to keep their balance on dowels, their cerebellar Purkinje neurons develop new synapses (Black et a1. 1990). In addition to indUCing the formation of new neurons, learning and new ex periences can a lso remodel old neu rons into new patterns of connections. When students were taught the classic three-ball cascade juggling routine (which takes months to get right), the neurons in a specific area of the temporal lobe of the brain took on a new pattern-a pattern not seen in students who were not taught this skill in Vifromsuallepathways ft eye ned left and right viCornbi sual pathways Major pathways h a ni nnuclul etusnucleus.

Onuthe visual ms FIGURE 1 8.1 0 of the mammalian visual system. In mammals, the optic nerve from each eye branc es, sending nerve fibers to lateral geniculate nucleus on each side of the brain. On the ipsilateral side, a particular part of the retina proj ects to a particular part of the lateral ge ic a e contralateral side, the lateral ge culate receives input from all parts of the retina. Neurons from each lateral gen ic l a te i n nervate the cortex on the sa e ide. nucleus (Oraganski et a1. 2004).

Similarly, mice reared in cages expe rienced cha nges in their neural circuitry when they were placed into more natural environments (Polley et a1 . 2004). Thus, the pattern of nemonal connections is a product of inherited patterning and patterning produced by experi ences. This interplay between innate and experiential development has been detailed most dramatically in stud ies on mammalian vision.

Experiential changes in mammalian visual pathways Some of the most interesting research on manunalian neu ronal patterning concerns the effects of sensory depriva tion on the developing visual system in kittens and mon keys. The pa ths by which electric impulses pass from the retina to the brain in manunals are shown in Figure 1 8.1 0. Axons from the retinal ga nglion cells form the two optic nerves, which meet at the optic chiasm. As in Xenapus tad poles, some axons go to the opposite (contralateral) side of the brain, but unlike in most other vertebrates, mammalian retinal ganglion cells also send inputs into the same (ipsi lateral) side of the brain (see Chapter 10). These axons end at the two lateral geniculate nuclei. Here the input from each eye is kept separate, with the uppermost and anteri or layers recei ving the axons from the contralateral eye, and the middle of the layers receiving input from the ipsi- DEVELOPME NTAL PLASTICITY AND SYMBIOSIS lateral eye. The situation becomes even more complex as neLlrons from the lateral geniculate nuclei connect with the neurons of the visual cortex. Over 80% of the neural cells in the visual cortex receive input from both eyes. The result is binocular vision and depth perception. A remarkable finding is that the retinocortical projec tion pattern is the same for both eyes. If a certain cortical neuron is stimulated by light flashing across a region of the left eye 5° above and 1° to the left of the fovea,' it will also be stimulated by a light flashing across a region of the right eye 5° above and 1° to the left of the fovea. Moreover, . the response evoked in the cortical neuron when both ey are stimulated is greater than the response when either reti na is stimulated alone. Hubel, Wiesel, and their co-workers demonstrated that the development of the nervous system depends to some degree on the experience of the individual during a criti cal period of development (see Hubel 1967). In other words, not all neuronal development is encoded in the genome; some is the result of learning. Experience appears to strengthen or stabilize some neuronal connections that are already present at birth and to weaken or eliminate oth ers . These conclusions Con€ from studies of partial senso ry deprivation.

Hubel and Wiesel (1962, 1963) sewed shut the right eyelids of newborn kittens and left them closed for 3 months. After this time, they unsewed the right eye lids. The cortical neurons of these kittens could not be stim ula ted by shining light into the right eye.

Almost all the inputs into the visual cortex came from the left eye only. The behavior of the kittens revealed the inadequacy of their right eyes; when the left eyes of these kittens were covered, they became functionally blind. Because the lateral genic ulate neuronS appeared to be stimulated by input from both right and left eyes, the physiological defect appeared to be in the connections between the lateral geniculate nuclei and the visual cortex. Similar phenomena have been observed in rhesus monkeys, where the defect has been correlated with a lack of protein syntheSiS i n the lateral geniculate neurons innervated by the covered eye (Kennedy et a1. 1981). Although it would be tempting to conclude that the blindness resulting from these experiments was the result of failure to form the proper visual connections.. this is not the case. Rather, when a kitten or monkey is born, axons from lateral geniculate neLlrons receiving input from each eye overlap extensively in the visual cortex (Hubel and Wiesel 1963; Crair et aJ. 1998). However, when one eye is covered early in the animal's life, its connections in the visual cortex are taken over by those of the other eye (Fig ure 1 8.1 1). The axons compete for connections, and experi ence plays a role in strengthening and stabilizing the con nections that are made. Thus, when both eyes of a kitten are sewn shut for 3 months, most cortical neurons can still be stimulated by appropriate illumination of one eye or the other. The critical time in kitten development for this *Thejovea is a depression in the center of the retina where only cones are present (rods and blood vessels are absent). In this instance, it serves CIS-CI convenient landmark. (A) 669 FIGURE 18.11 Experience al-ters neural connections. (A,BI Dark-field autora diographs of monkey striate (visual) cortex 2 weeks after one eye was i n jected with 13HJproline in the vitreous humor. Each retinal neuron lakes up the radioactive label and transfers it to th e cells with which it forms synapses. (A) Normal l abeli ng pattern. The white stripes indicate that roughly half the columns took up the label, while the other half did not-a pattern refl ect i ng that half the cells were innervated by the labeled eye and half by the unla beled eye (8) Labeling pattern when the unlabeled eye was sutured shut for 1 8 months. Axonal projections from the normal (labeled) eye have ta ken over the regions that would normally have been innervated by the sutured eye. (e,D) Drawings of axons from the lateral genic ul ate nuclei of kittens in which one eye was occluded for 33 days. The terminal branching ofaxons receiving input from the occ lu ded eye (cl was far less extensive than that ofaxons receiving input from the nonoccluded eye (0). (A,S from Wiesel 1 982, cour tesy ofT. Wiesel; e,D aher Anlonini and Stryker 1 993.) . (e) (D) Cortical layer 3 Cortical layer 4 670 CHAPTER 1 B validation of neuronal connections begins between 4 and 6 weeks aiter birth. Monocular deprivation up to the fourth week produces little or no physiological deficit, but through the sixth week it produces all the characteristic neuronal changes. If a kitten has had normal visual experi ence for the first 3 months, any subsequent monocular dep rivation (even for a year or more) has no effect. At that point, the synapses have been stabilized. Two principles, then, can be seen in the patterning of the mammalian visual system.

First, the neuronal connec tions involved in vision are present even before the animal sees. Second, experience plays an important role in deter mining whether or not certain connections persist. ... Just as experience refines the original neuromuscular cOIUlections, experience plays a role in refining and improving the visu al connections . It is possible, then, that adult functions such as learning and memory arise from the establishment and/ or strengthening of different synapses by experience. As Purves and Lichtman (1985) remark: The interaction of individual animals and their world continues to shape the nervous system throughout life in ways that could never have been programmed. Modifi cation of the nervous system by experience is thus the last and most subtle developmental strategy.

embryo, entering diapause just before segmentation. The gypsy moth Lymantria dispal' initiates diapause as a larva, and needs an extended period of cold weather to end dia pause, which is why this pest is not found in the southern regions of Europe or the United States. Over 100 mammalian species undergo diapause. The two most common mammalian strategies are delayed fer tilization (the sperm are stored for later use) and delayed implantation (the blastocyst remains unimplanted in the uterus, and the rate of cell divisions diminishes or vanish es). Some species have seasonal diapause, so embryos con ceived in auhtmn will be born in spring rather than win ter; in other species, diapause is induced by the presence of a newborn that is still getting mille In the tammar kan garoo (Macropus euge/1ii), diapause can be a response to suckling-induced prolactin release, but it can also be induced by prolactin synthesized in response to changes in day length. In both cases, progesterone seems to be the signal that restores implantation and embryonic growth. Different groups of mammals use different hormones to induce or break diapause, but the result is the same: dia pause lengthens the gestation period, allowing mating to OCClli and young to be born at times and seasons appro priate to the habitat of that species (Renfree and Shaw 2000). See WEBSITE 1 8.3 Mechanisms of diapause Life Cycles and Polyphenisms Diapause Larval settlement Throughout nature, organisms are able to respond to the environment by changing their developmental strategy. One of these strategies is to delay development to a better time, and many species of insects and mammals have evolved a developmental strategy called diapause to sur vive periOdically harsh conditions, Diapause is a suspen sion of development that can occur at the embryOniC, lar val, pupal, or adult stage, depending on the species (see Chapter 15). Diapause is not a phYSiological response brought about by harsh conditions. Rather, it is induced by stimuli (such as changes in the duration of daylight) that presage a change in the environment---cues beginning before the severe conditions actually arise, Diapause is especially iruportant for temperate-zone insects, enabling them to survive the winter. The overwintering eggs of the hickory aphid provide an example of this strategy. The development in the egg is suspended over the winter, so the larvae do not hatch when food is unavailable. In this case, diapause occurs during early development. The silk worm moth Bornbyx moti Similarly overwinters as an The ability of marine larvae to suspend development lmtil they experience a particular environmental cue is called larval settlement. A free-swimming marine larva often needs to settle near a source of food or on a firm substrate on which it can metamorphose. Among the molluscs, there are often very specific cues for settlement (Hadfield 1977; Hadfield and Paul 2001; Zardus et al. 2008). In some cases, the prey supply the cues, while in other cases the substrate itself gives off molecules used by the larvae to initiate set-tlement.

These cues may not be constant, but they need to be part of the environment if further development is to occur' (Pechenik et al. 1998). ·Studies have shown that differences in neurotransmitter release result in changes i n synaptic adhesivity ilnd cause the withdrawal of the axon providing the weaker stimulation (Colman et al. 1997). Studies in mice suggest that BDNF is cnlcial during the critical period (Huang et al. 1999; Katz 1999, Waterhouse and Xl1 2009; Cowansage et a/. 2010). tThe importance of substrates for larv al settlement and metamor phosis was first demonstrated in 1880, when William Keith Brooks, an embryologist at Johns Hopkins UniverSity, was asked to help the ailing oyster industry of Chesapeake Bay. For decades, oysters had been dredged from the bay, and there had always been a new crop to take their place. But by J880, each year brought fewer oysters. What was responsible for the decline? Experimenting with larval oysters, Brooks discovered that the American oyster (unlike its bet ter-studied European relative) needs a hard substrate on which to metamorphose. For years, oystermen had thrown the shells back into the sea, but with the advent of suburban sidewalks, they start ed selling the shells to cement factories. Brooks's solution: throw the shells back into the bay. The oyster population responded, and the Baltimore wharves s ill t seJl their descendants. DEVELOPMENTAL PLASTICITY A N D SYMBIOSIS Jaw-hyoidean muscle group (eats other CARNIVORE tadpoles) Ventral surface ry 671 . terhYOi mnscle dean coiIntesti ls nal !, Ventral OMNIVORE (eats brine shrimp, algae) surface FIGURE 18.12 Polyphenism in tadpoles of the spad efoot toad (Scaphiopus CQuchil). The typical morph (right) is an omnivore, feeding on arthropods and algae. When ponds are d ing out q uickly, however, a carnivorous (cannibalistic) morph forms (left). It develops a wider mouth, l arger jaw muscles, and an i ntesti ne modified for a carnivorous diet. The center photograph shows a cann ibalist ic tadpole eating a smaller pondmate. (Photograph © Thomas Wi ewandt; drawings courtesy of R. Ruibel.) In many marine llwertebrate species, larval settlement is regulated by mats of bacteria called biofilms that help determine the clistribution of the invertebrate populations. Hwnans are changing this distribution, however, with OUI desire to place large objects in the oceans. Such objects readily acquire biofilms and the resultant marine fauna that attach to them. As early as 1854, Charles Darwin spec ulated tl,at barnacles were transported to new locales when their larvae settled on the hulls of ships. Indeed, the abili ty of biofilms to aid invertebrate larval settlement and colony formation explains the ability of barnacles and tube worms ("biofouling invertebrales") to accumulate on ship keels, clog sewer pipes, and deteriorate underwater struc tures (Zardus et al. 2008). The spadefoot toad: A hard life The spadefoot toad (Scaphiopus couchii) has a remarkable strategy for coping with a harsh environment. These toads are called out from hibernation by the tllunder that accom panies the fil'st spring stonm in the Sonoran desert. (Unfor tunately, motorcycles produce the same sounds, causing the toads to come out of hibernation only to die in the scorching Arizona sun.) The toads breed in temporary ponds formed by the rain, and the embryos develop quick ly into larvae. After the larvae metamorphose, the young toads return to the desert, burrowing into the sand until the next year's stonns bring them out. Desert ponds are ephemeral pools that can either dry up quickly or persist, depending on their irUtial depth and the frequency of rainfall. One might envision two alterna tive scenarios confronting a tadpole in such a pond: either (1) the pond persists until you have time to fully metamor- phose, and you live; or (2) the pond dries up before your metamorphosis is complete, and you die.

S. couchii (and several other amphibians), however, have evolved a third alternative. The timing of their metamorphosis is controlled by the pond. If the pond persists at a viable level, devel opment continues at its normal rate, and the algae-eating tadpoles develop into juvenile toads. However, if the pond is drying out and getting smaller, some of the tadpoles embark on an alternative developmental pathway. They develop a wider mouth and powerful jaw muscles, which enables them to eat (among other things) other Scaphiopus tadpoles (F igu re 1 8.1 2) . These carnivorous tadpoles meta morphose quickly, albeit into a smaller version of tl,e juve nile spadefoot toad. But they survive while other Scaphio pus tadpoles perish from desiccation (Newman 1989, 1992). The signal for accelerated metamorphosis appears to be tile change in water volume. In the laboratory, Scaphiopus tadpoles are able to sense the removal of water from aquar ia, and tlleir acceleration of metamorphosis depends on the rate at which the water is removed. The stress-induced cor ticotropin-releasing hormone signaling system appears to modulate this effect (Denver et al. 1998, 2(09).

This increase in brain corticotropin-releasing hOrmone is thought to be responSible for the subsequent elevation of the thyroid hor mones that initiate metamorphosis (Boorse and Denver 2003). As in many other cases of polyphenism, the devel opmental cl)anges are mecliated through the endocrine sys tem. Sensory organs send a neural signal to regulate hor mone release. The hormones then can alter gene expression in a coordinated and relatively rapid fashion. See WEBSITE 1 8.4 Pressure as a developmental agent 672 CHAPTER 1 8 SIDEL IG H TS SPECULATIONS Life Cycle Choices: Dictyostelium O f the many deve lopm ental cha nges triggered by the envi ronment, few are as profound as the effect of nutrient starvation on Dic ryostelium discoideum. * Oictyostelium is a multicellular o rganism only when environmental cond i ti ons ciemand it. The life cycle of this fasci nati ng organ- ism is i l lustrated in Figure 18.13. In its asexual cycle, solitary haploid amoe- 'Although colloquially called a "cellular slime mold," Dictyostelium is not a mold, nor is it co ns i stent l y slimy. It is perhaps best to think of Oictyostelium as a social amoeba. bac (called myxamoebae or lisocial amoebae" to d i st i nguish them from amoeba species that always remain solitary) live on decaying logs, eati ng bacteria and reproducing by binary fis sion. When they have exhausted their food supply, their gene expression changes, and they begin expressing cell adhesion molecules.

Moreover, they start migrating to a common p lace. Tens of thousands of these myxamoe bae join together to form moving streams of cells that converge at a cen tral point. Here they synthesize new adhesion molecules and pile atop one another to produce a conical mound called a tight aggregate. Subsequently, a tip arises at the top of this mound, and the tight aggregate bends over to produce the migrati ng s l ug (with the tip at the front). The slug (often given the more dignified title of pseudoplasrnodi urn-or grex) is usually 2-4 mm long and is encased in a slimy sheath. The grex begins to migrate (if the environ ment is dark and moist) with its anterior tip slightl y raised. When it reaches an illuminateci area, migration ceases, and the culmination stages of the life cycle take place as the grex differentiates into cud . s hOllrs01 16 hour A '' '1IiiIi ...I!!I!Ii /15 U 17hours / 14hOurs 20 hours MIGRATION .Y U . s 12 hours AGGREGATrON b": ght •aTiggregatC:;, 9 hours .,,#. "'t.tJt$&,>' \ CULMINATION v 23 hours sporesooo", o hours (Mature '", 6 hours / ru ng Loose ") body aggregate \ \ • s _ ;Myxamoebae /7]' 't:. = ... = . (1) a fruiting body composed of spore cells and (2) a stalk that supports the fruit i ng body (Raper 1 940; Bonner 1 957). The entire cellular popula tion, form the tubed stalk. This process begins as some of the central anterior cells, the prestalk cells, begin secreting an extracellular cellUlose coat and extending a tube through the grex.

As the prestalk cells differentiate, they form vacuoles and enlarge, l ifti ng up the mass of prespore cells that made lip the posterior four-fifths of the grex Oermyn and Williams 1 99 1) . The stalk cells die, but the prespore cells, elevat ed above the stalk, become spore cells. TI)ese spore cells disperse, each one becoming a new myxamoeba.

Aggregation of Dictyostelium cells What causes the myxamoebae to aggregate? 'TIme-lapse videomi croscopy has shown that no directed movement occurs during the first 4-5 hours following nutrient starvation. During the next 5 hours, however, the cells can be seen moving at about 2-0 mm/min (or 1-00 seconds. This move ment ceases for about 4 minutes, then resumes. Although the movement is directed toward a central point, it is not a simple radial movement. Rather, cells join with one another to form streams; the streams converge into larger streams, and eventually all streams merge a t the center. Bonner (1 947) and Shaffer (1 953) showed that this movement is a res ult of chemo tax is : the cells are guided to aggrega tion centers by a soluble substance. This substance was later identified as cydic adenosine 3',5'-monophos phate (cAMP; Konijn et a l. 1 967; Bon ner et al. 1 969), t h e chemical structure of which is shown in Figure 1 8.14A. See WEBSITE 1 8.5 The Dictyostelium life cycle: Variations within variations See VADE MECUM Slime mold life cyde (Continued on next page) CHAPTER 1 8 674 SIDELIGHTS & SPECULATIONS (Continued) (A) BIAS g Myxamoebae Grex ' v c " cells j (I Cr0 (B) LABILE COMMITMENT Prestalk (C) STABLE COMMITMENT AND DIFFERENTIATION p Aggregation is initiated as each of the myxamoebae begins to synthesize cAMP. There are no dominant cells that begin the secretion or control the others. Rather, the sites of aggregation are determined by the distribution of the myxamoebae (Keller and Segal 1 970; Tyson and M urray 1 989). Neigh bo ri ng cells respond to cAMP in two INays: they initiate a movement toward the cAMP pulse for about a m inute, and they release cAMP of their own (Robertson et al. 1 972; Shaffer 1 975). The movement of each myxamoeba is caused by the change in cytoskeletal polarity brought about by the cAMp · (Parent et al. 1 998; lijima et al. 2002). After this happens, the cell is unre sponsive to further cAMP pulses for several m i nutes. During this time, an extracellular membrane-associated phosphodiesterase then cleaves the remaining cAMP from the environ ment, allowing the receptors to get ready to receive another pulse. The result is a rotating sp i ral wave of cAMP that is propagated throughout the pop u lation of cells (Figure 1 B.14B-D). A s each wave arrives, the cel ls take anoth er step toward the center. The differentiation of individua l myxamoebae into either stalk (somat ic) or spore (reproductive) cells is a complex matter. In Dictyostelium, d i f ferentiation involves a d ichotomous decision. There appears to be a pro-) upper cup (E) (F) (G) Prestalk and stalk cell strain Spore sac (prespore ceUs) Lower cup --- Pres ore cells r (D) -Stalk Prespore and spore cell strain BaSal diSC '' FIGURE 18.15 Alternative cell fates in Dictyostelium discoideum . (A-C) Progressive com mitment of cells to become either spore or stalk cells. (A) Myxamoebae may have biases toward stalk or spore formation due to the stage of the cell cycle they were in when starved. (B) As the grex migrates, most presta l k cells are in the anterior third of the grex, while most of the posterior consists of prespore cells. Some presta l k cells are also seen in the posterior, and these cells will contribute to the cups of the spore sac and to the basal disc at the bot tom of the stalk. The cell fates are not yet fixed, however, and if the stalk-forming anterior is cut off, the anteriormost cells remaining will convert from stem to stalk. (e) AI cu l m i nation, the spore-forming cells are massed together in the spore sac. The stalk cells form the cups of the spore sac, as well as the stalk and basal disc.

(D,E) Grex and culminant stained with dye that recognizes the extracellular matrix of the preslalk and stalk cells. (F,G) Grex and culmi nant stained with dye that recognizes the extracellular matrix of prespore and spore cells. (After Esca la nte and Vicente 2000; photographs courtesy of R. Esca l ante .) gressive commitment to one of the two alternative pathways (Figure 1B.1 5). At first there is a bias toward one path or another. For i nstance, cells starved in the 5 and early G2 phases of the cell cycle have rela ti vely high levels of calcium and display a tendency to become stalk cells, while those starved i n mid or late G2 have lower calcium levels and tend to become spore cells (Nanjundiah 1 997; Azhar et a l . 2001).

Then there is a labile specification, a time when the cell wi l l normal ly become either a spore cell or a sta l k cell, but when i t can still change its fate i f placed i n a d ifferent position i n the organism. Due to the biases in these cells, cAMP i s used i n d ifferent ways by the prespore and prestal k cells (see Kimmel and Fir tel 2004).

In the prespore cells of the grex, extracellular cAMP i nitiates the expression of spore-specific mRNAs. It does this by inducing f3-catenin, which enters the nucleus to activate certai n spore-specific genes (Ginsburg and Kimmel 1 997; Plyte et a l . 1 999; Kim et a l . 2002).

In the prestal k cells that are in the anterior tip of the grex, cAMP suppresses this pathway and causes these cells to become prestalk cells. Another group of prestalk cells are formed by a secreted chlorinated l i p i d, DIF-1 , which is made by the prespore cells (Fukuzawa et al. 2003; Thompson et a l . 2004). The third and fourth stages are a determination to a specific fate, followed by the cell's dif ferentiation i nto a particular cell type, either a sta l k cell or a spore cell. Several proteins, including Tris hanku and spore differentiation fac tors SDF1 and SDF2, appear to be important in the final differentiation DEVELOPMENTAL PLASTICITY AND SYMBIOSIS 675 SIDELIGHTS & SPECULATI ONS (Continued) of t he prespore cells into encapsulat ed spores (Anjard et a l . 1 998a,b; Mujumdar et a l . 2009). SDF1 and Trishanku are important in initiating culmination, while SDF2 seems to cause the prespore cells (but not pre stalk cells) to become spores. The pre spore cells appear to have a receptor that enables them to respond to SDF2, while the prestalk cells lack this receptor (Wang et al. 1 999). Cul m ination is also brought about by declining ammonia concentrations (Follstaedt et a l . 2003). Ammonia is released preferentially i n the anterior portion of the grex, and it appears to he l p regu late chemotaxis of the pre- stalk cells as well as aid in the pro duction of spore cells (Oyama and Blumberg 1 986; Feit et al. 200 -1). The formation of stalk cells from prestalk cells is s i m i larly complicated and may involve several factors working synergistically (E a rl y 1 999). Indeed, prestalk cells from different parts of the grex pass through different inter mediary cell types before reaching the final stage of stalk cell. Thus, the stalk cells that cover the spores have a slightly different history than those stalk cells that hold the ball of spores above the ground. The differentiation of stalk cells appears to need a signal from the intracellular enzym e PKA, and at least one type of stalk cell is induced by the DIF-1 lipid (Thomp son and Kay 2000; Fukuzawa et aJ. 200 1). $0 there are many environmental factors triggering a lternative develop mental pathways in Diclyoslelium. First, nutrient depletion causes a wholesale change in gene expression and cell adhesivity; second, cAMP ori ents the migration of individual cells toward a common center; third, the neighboring cells determine whether any particular cell is to be stalk or spore; and fourth, molecules such as ammonia can help complete the processes of differentiation. Developmental Symbioses are discovering that few symbiotic relationships are truly Contrary to the popular use of the term to mean a mutu neutral with respect to either party.

ally beneficial relationship, the word symbiosis (Greek, In addition, the term endosymbiosis ("living insid e") in whi dl one cell sym, "together"; bios, "life") can refer to any close associa is widely used to describe the situation tion between organisms of different species (see Sapp 1994). In lives inside another cell, a circumstance thought to aa:ount than the

ed for that particular environment. Enviroru nent is there fore considered to play a role in the generation of phenotypes in addition to its well-established role in the selection of phenotypes. The fact that we co-develop with other organisms is an important concept for developmen tal biology. It also calls into question the notions of auton omy and independent development. Moreover, if we are not truly "individuals," but have a phenotype based on conununity interactions, what exactly is natural selection selecting? Can natural selection select teams or relation ships? (See Gilbert and Epel 2009; Gilbert et al. 2010.) The ramifications of developmental plasticity and develop mental symbiosis on the rest of biology are just beginning to become appreciated.

DEVELOPMENTAL PLASTICITY AN D SYMBIOSIS 681 Snapshot Summary: Developmental Plasticity and Symbiosis 1. The environnlent plays critical roles during normal 7. There are several routes through which gene expres development. These agents include temperature, sion can be influenced by the environment. Environ diet, crowding, and the presence of predators. mental factors can methylate genes differentially; 2. Developmental plasticity makes it possible for envi ronmental circumstances to elicit different pheno types from the same genotype.

The genome encodes a repertoire of possible phenotypes. The environ ment often selects which of those phenotypes will they can ind uce gene expression in surrounding cells; and they can be monitored by the nervous sys tem, which then produces hormones that affect gene expression, 8. Behavioral phenotypes can also be induced by the environment. Learning results from changes in the become expressed. 3. Reaction norms are phenotypes that quantitatively respond to enviroru nental conditions, such that the phenotype reflects small differences in the enviro ment al conditions . nervous system brought about by experiences with the environment. 9. Organisms usually develop with symbiotic organ isms, and signals from the symbionts can be critical 4. Polyphenisms represent "eitherlor" phenotypes; that is, one set of conditions cheits one phenotype while another set of conditions elicits another. 5 . Seasonal cues such as photoperiod, temperature, or for normal development. 10.

Symbionts can be acquired horizontally (through infection) or vertically (through the oocyte). 11. In an obligate mutalism, both partners are needed type of food can alter development in ways that for the survival of the other, in an obligate develop make the organism more fit under the conditions it mental mutualism, at least one partner is needed for encounters. Changes in temperature also are respon the proper development of another. sible for determining sex in several organisms, including many reptiles and fish . 12. The mammalian gut contains symbionts that active ly regulate intestinal gene expression to generate 6. Predator-induced polyphemsms have evolved such proteins that are normal physiological components that prey species can respond morphologically to the of intestinal development and function. Without presence of a specific predator. these symbionts, the intestinal blood vessels and In some instances, this induced adaptation can be transmitted to the gut-associated lymphoid tissue of some mammalian progeny of the prey, species fail to form properly.

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CHAPTER 1 8 682 Go Online WEBSITE 18.1 Inducible caste determination in ant colonies. In some species of ants, the loss of soldier ants cre ates conditions that induce more workers to become soldiers . WEBSITE 18.2 Volvox: When heat brings out sex. Dur ing most of its life cycle, Volvox (a "green algae") repro duces asexually. However, when the ponds Volvox inhabits become hot a signal that they may soon dry up the next generation becomes sexual and produces sperm and eggs that unite in zygotes that can survive desiccation. WEBSITE 18.3 Mechanisms of event. WEBSITE 18.4 Pressure as a developmental agent. Mechanica l stress is critica l for gene expression in numer ous tissues, including bone, heart, and muscle. Without physical force, we would not develop our kneecaps. WEBSITE 18.5 The Dictyostelilltn life cycle: Variations within va_riations Dictyostelillm has some fascinating varia tions on its Ufe cycle, including the ability to become sexual. WEBSITE 18.6 Developmental symbioses and para sitism. Some embryos acquire protection and nutrients b)' forming symbiotic associations with other organisms. The mechanisms by which these associations form are now being elucidated. In other situations, one species uses mate rial from another to support its development Blood-suck . ing mosquitoes are examples of such parasites. Vade Mecum Slime mold life cycle. The life cycle of this fascinating organism is show n in movies. There is also original footage Dr. Bonner, whose pioneering work demon of some of John Tyler Bonner's experiments, along with an interview with strated the major principles of Dictyoslelilll1l development. Outside Sites Karen Warkentin's Boston University website includes an amazing movie of the snake-induced rapid development of red-eyed tree frogs. (KWvideoSMALLER.html) Developmental Mechan isms of Evolutionary Change WHEN WILHELM RaUX ANNOUNCED the creation of experimental embryology in 1894, he broke many of the ties that linked embryology to evolutionary biol ogy. However, he promised that embryology would someday return to evolu tionary biology, bringing with it new knowledge of how animals are genera ted and how evolutionary changes might occur. He predicted that once we knew how bodies are formed, we would be able to analyze alterations in development that lead to new body plans. A little mOre than a century later, we are at the point of fulfilling Roux's prophecy. Developmental biology is returning to evolutionary biology, forging a new discipline, evolutionary dev el opmental biology ("evo-devo"), and producing How does newness come into the world? How is it born' Of what fusion.s, translations, conjoinings is it made? How does it survive, extreme and dangerous as it is? What compm mises, what deals, what betrayals of its secret nature must it make to stalle off the wrecking crew, the exumninat ing angel, the guillotine? SALMAN RUSHDIE a new model of evolution that integrates developmental genetics and popula explain and define the diversity of hfe on Earth (Raff 1996; Hall 1999; Arthur 2004; Carroll et al. 2005; Kirschner and Gerhart 2005; Gilbert and Epel 2009). Contemporary evolu tionary developmental biology is analyzing how changes in develop ment can create the diverse variation that nahlfal selection can act on. Rather than concentrating on the "survival of the fittest," evolution tion genetics to ary developmental biology gives us new insights into the "arrival of the fittest" (Gilbert and EpeI2009). "Unity of Type" and "Conditions of Existence": Charles Darwin's Synthesis In the nineteenth century, debates over the origin of species pitted two ways of viewing nature against each other. One view, championed by Georges Cuvier and Charles Bell, focused on the differences among species that allowed each species to adapt to its environment. Thus, the hand of the human, the flipper of the seal, and the wings of birds and bats were seen as marvelous contrivances, each fash ioned by the Creator, to allow these animals to adapt to their "conditions of exis tence." The other view, championed by Etienne Geoffroy Saint-Hilaire and Richard "unity of type" (the similarities among organisms, which Owen and the wings of bats and birds were all modifications of the same basic plan (see Figure 1.19). In discovering that plan, one could find the form upon which the Creator designed these animals. The adaptations were secondary. Darwin acknowledged his debt to these earlier debates when he wrote in 1859, "It is generally acknowledged that all organic beings have been formed on two Owen, was that called "homologies") was critical. The human hand, the seal's flipper, great laws-Unity of Type, and Conditions of Existence." Darwi.n went on to explain that his theory would explain wuty of type by descent from a common ancestor. The changes creating the marvelous adaptations to the conditions of (1 988) study of the effects of genes during development is as essential for an understanding of evolution as are the study of mutation and that of selection. A JULIAN HUXLEY (1942) 684 CHAPTER 1 9 existence would be explained by natural selection. Danvin called this concept descent with modification. Darwin noted that the homologies betvveen the embryonic and lar val structures of different phyla provided excellent evi dence for descent with modification. He also argued that adaptations that depart from the "type" and allow an organism to survive in the "conditions" of its particular environment develop late in the embryo. Thus, Darwin recognized two ways of looking at descent with modifica tion. One could emphasize common descent in the embry onic homologies betvveen two or more groups of animals, or one could emphasize the modifications by shoWUJg how development was altered to produce diverse adaptive structures (Gilbert 2003). Or, as Darwin's friend Thomas Huxley aptly remarked, "Evolution is not a speculation but a fact, and it takes place by epigenesis" (Huxley 1893, p. 202). See WEBSITE 1 9.1 Relating evolution to development in the nineteenth century Preconditions for Evolution through Developmental Change Darwin was greatly perplexed about variation. After all, if natural selection could only operate on existing variants, where was all that variation corning from? He concluded (in his next books) that it would have to come from changes in development, but how could the development of an embryo change when development is so finely tuned and complex?

How could such change occur without destroying the entire organism? Darwin's German col league, Ernst Haeckel, proposed that the major way to evolve was to add a step to the end of embryonic develop ment. But there turned out to be so many exceptions to that rule that it fell into disrepute.

When the molecular biology of protein synthesis became understood, the problem did not go away. If a pro tein-encoding gene were mutated, the .abnormal protein would be made in all the places where the protein was nor mally expressed. There was no way the mutation could cause the protein to be made in one place and not anoth er. The matter lay unsolved until evolutionary develop mental biologists demonstrated that large morphological changes could be made during development because of two conditions in the ways that organisms develop: mod ularity and molecular parsimony. Modularity: Divergence through dissociation We now know that even early stages of development can be altered to produce evolutionary novelties. Such changes can occur because development occurs through a series of discrete and interacting modules (Riedl 1978; Bonner 1988; Kuratani 2009). Examples of developmental modules include morphogenetic fields (for example, those described for the limb or eye), signal transduction pathways (described throughout this book), imaginal discs, cell lin eages (such as the iuner cell mass or trophoblast), insect para segments, and vertebrate organ rudiments (Gilbert et al. 1996; Raff 1996; Wagner 1996; Schlosser and Wagner 2004).

The ability of one module to develop differenth from other modules (a phenomenon sometimes called dis sociation) was well known to early experimental embry ologists. For instance, when Victor Twitty (Twitty and Schwind 1931; Twitty and Elliott 1934) grafted the limb bud from the early larva of a large salamander onto the embry onic trunk of a small salamander larva, the limb grew to its normal large dimensions within the small larva, indi cating that the limb field module was independent from the global growth patterning of the embryo.

The same independence was seen for the eye field.

Modular units allow certain parts of the body to change without interfer ing with the functions of other parts. One of the most important discoveries of evolutionary developmental biology is that not only are the anaiomical units modular (such that one part of the body can devel op differently than the others), but that the DNA regions that form the enhancers of genes are modular. This was shown in Figure 2.10. The modularity of enhancer elements allows particular sets of genes to be activated together and permits a particular gene to become expressed in several discrete places. TIUIS, if a particular gene loses or gains a modular enhancer element, the organism containing that particular allele will express that gene in different places or at different times than those organisms retaining the original allele. This mutability can result in the develop ment of different anatomical and physiological morpholo gies (Sucena and Stem 2000; Shapiro et al. 2004), and major morphological changes can proceed through a mutation in a DNA regulatory region. Thus, the modularity of enhancers can be critical in providing selectable variation.

DUFFY BLOOD GROUP SU BSTANCE Enhancer modularity has been known from studies of selectable traits in human populations. Plasmodium vivax is a protozoan parasite that causes about 75 million cases of malaria each year. This form of malaria is not as lethal as jalciparum malaria, but it can be incapacitatiIlg, causing severe pain, diarrhea, and fever. Some African populations are immune to P. vivax because their red blood cells lack a protein, the Duffy gly coprotein, that P. vivax needs to attach itself to the host's red blood cells. The Duffy glycoprotein is probably one of several receptors for interleukin 8 (IL8, a paracrine factor involved in the circulatory system and in the cerebellum), and it is found on Purkinje neurons, veins, and erythro cytes (red blood cells). People who lack Duffy glycopro tein on their fed blood cells still have it on their veins and Purkinje neurons.

So if one asks, "Why don't these peo ple have Duffy glycoprotein on their red blood cells?" the ultimate answer is probably that the lack of the Duffy gly coprotein was selected in these populations because it gives these people resistance to vivax malaria, given that DEVELOPMENTAL MECHANISMS OF EVO LUTIONARY CHANGE Plasmodillm uses the protein to infect the red blood cells. 111e proximate answer is that the lack of Duffy glycopro tein is caused by a mutation in the erythrocyte enhancer, a C-to-G substitution at position -36, which prevents the binding of the GATA 1 transcription factor present in red blood cell precursors (Tournamille et a1. 1995). Thus, the mutation blocks one 01 the enhancers (the one for expres sion in red blood cells) from func tioni ng, but allows the enhancers that permit the gene's expression in veins and Purkinje neurons to function. PITX1 AND STICKLEBACK EVOLUTION The importance of enhancer modularity has been dramatically demonstrated by the analysis 01 evolution in the threespine stickleback fish (Gas/erostells aCII/eatlls).

Freshwater sticklebacks evolved from marine sticklebacks about 12,000 years ago, when marine popula tions colonized the newly formed Ireshwater lakes at the end of the last ice age. Marine stick lebacks (Figure 19.1 A) have pelvic spines that serve as pro tection against predation, l acera ting the mouths of pred a tory fish that try to eat the stickleback. (Indeed, the scientific name of the fish transla tes as "bony stomach with spines.") Freshwater sticklebacks, however, do not have pelvic spines (Figure 1 9.1 B). This may be because the fresh water fish lack the piscine p red a tors that the marine fish face, but instead must d eal with invertebrate pred a tors that can easily capture them by grasping ont o such s pines. Thus, a pelvis without spines was selected in freshwater populations of this species To determine which genes might be involved in this pelvic difference, researchers mated individuals from marine (with spines) and freshwater (no spines) po pu l a tions. The resulting offspring were bred to each other and produced numerous progeny, some of which had pelvic spines and some of which didn't. Using molecular mark. FIGURE 19.1 Modularity of development: enhancers. Loss of Pit.< l gene expressio n in the pel vic region of freshwater popu lations o f the threespine stickleback (Gasterosteils aculealus). Bony plates and pelvic spines characteri ze marine populations of this spec ies (A). I n freshwater populations (B), Ihe pelvic spines are absent, as is much of the bony armor. In magnified ventral views of em bryos (i nset photos), in situ hybridization reveals Pitx r expression (purp le) in the pelvic area (as well as 685 ers to identify specific regions of the parenta l chromo somes, Shapiro and co-workers (2004) found that the major gene lor pelvic spine development mapped to the distal end of chromosome 7. That is to say, nearly all the fish with pelvic spines inherited this "hindfin-encoding" chromosomal region from the marine parent, while fish lack i ng pel vic spines obtained this region from the fresh water parent. The researchers then tested numerous can didate genes (genes known to be present in the hindlimb structures of mice, for instance) and found tha t the gene encoding transcription factor Pitx1 was located on this region of chromosome 7. When they compared the amino acid sequences of the Pitxl protein between marine and freshwater sticklebacks, there were no differences. However, there was a critically important difference when they compared the exp ression patterns 01 Pitxl between these populations. In both pop ulations, Pi/xl was expressed ◆1 the precursors of the thy mus, nose, and sensory ne ur ons In the marine popula tions, Pitxl was also expressed in the pelvic region But in the Ireshwater populations, the pe l vic expression of Pitxl was a bsent or severely reduced (Figure 1 9.1 C). Sinc e the coding region of Pitxl was not mutated (and since the gene involved in the pelvic spine differences maps to the site of the Pi/x1 gene, and the di fference between the freshwater and marine populations involves the expression of this gene at a particular site), it was reasonable to conclude that the enhancer region allowing expression of Pi/xl in the pelvic area (i.e., the pelvic spine enhancer) no l onger func tions in the lreshwater populations.

. . (A) Marine Pitxl expression (ventral view) / Pelvic spine (B) Freshwater in sensory neurons, thymic cells, and nasal regions) of the marine population.

The staining in the pelvi c region is absent in freshwater populafions, although it is still seen in the other - . , areas (the arrow points to one of several expression points). (C) Model for Ihe evolulion of pel vic spine los . ' - ' ' - ' ITr◆nscription Sensory neuron enhancer P;txl Nasal pil enhancer 686 CHAPTER 1 9 This conclusion was confirmed when high-resolution genetic mapping showed that the DNA of the "hindlimb" enhancer of Pitxl differed between sticklebacks with pelvic spines and those without pelvic spines' (Chan et a1. 2010). When this 2.5-kb DNA fragment from marine (spined) fish was fused to a gene for green fluorescent protein (GFP) and inserted into fertilized freshwater stickleback eggs, it caused the GFP protein to be expressed in the pelvis. More over, when this region from the marine sticklebacks was placed next to the Pitxl coding sequence from the fresh water (spine-deficient) fish and then injected into fertilized eggs of the spine-deficient fish, pelvic spines were proper ly formed in the freshwater fish. See WEBSITE 1 9.2 Correlated progression Molecular parsimony: Gene duplication and divergence The second precondition for macroevolution through developmental change is molecular parsimony, sometimes called the "small toolkit." In other words, although devel opment differs enormously from lineage to lineage, devel opment within all lineages uses the same types of mole cules. The transcription factors, paracrine factors, adhesion molecules, and Signal transduction cascades are remark ably simllar from one phylum to another. Certain transcrip tion factors such as Pax and Hox genes are found in all aru mal phyla, including cnidarians, insects, and primates. In fact, some "toolkit genes" appear to play the same roles in all animal lineages. Thus, Pax6 appears to be involved in specifying light-sensing organs, irrespective of whether the eye is that of a mollusc, an insect, or a primate (Figure 1 9.2; Gehring 2005). Hox genes appear to specify the anterior-posterior body axis, even though the way these genes are activated differs enormously among nematodes, flies, and birds. Similarly, homologues of Otx specify head formation in both verte brates and invertebrates; and, though insect and vertebrate hearts are very different, both are specified by tinman/Nkx2-5 (see Erwin 1999). The Wnt and TGF-◆ paracrine factors and their signaling pathways can be found in the primitive cnidarians as well as in primitive bilaterians such as flat worms (Finnerty et a1. 2004; Carroll et a1. 2005). There is descent with modification for the signal transduction path ways and transcription factors, just as there is descent with modification of whole organisms. *Interestingly, the Joss of the pelvic spines in several stickleback populations appears to have been the result of independent losses of this Pitxl expression domain.

This finding suggests that if the loss of Pitxl expression in the pelvis occurs, this trait can be readily Here we see that by combining pop selected (Colosimo et a1. ulation genetics approaches and developmental genetics approach es, one can determine the mechanisms by which evolution can ocelll. 2004). FIGURE 19.2 Th e Pax6 gene for eye development is an example of a gene ancestral to both protostomes and deuterostomes. The micrograp h shows ommatidia emerging in the leg of a fruit fly (a protostome) in which mouse (deuterostome) Pax6 eDNA was expressed in the leg disc. (From Halder et al. 1 995, courtesy of W). Geh ri ng and G. Halder.) One theme that echoes through this book is that Hox gene expression provides the basis for anterior-posterior axis specification throughout the animals. This means that the enormous variation of morphological form among ani mals is underlain by a common set of instructions. Indeed, Hox genes provide one of the most remarkable pieces of evidence for deep evolutionary homologies among all the animals of the world.

The similarity of all the Hox genes is best explained by descent from a common ancestor. First, the different Hox genes in the horneotic gene cluster each originated from duplications of an ancestral gene. This would mean that in Drosophila, the Deformed, Ultrabithorax, and Antennapedia genes all emerged as duplications of an original gene. The sequence patterns of these three genes (especially in the homeodomain region) are extremely well conserved. Such tandem gene duplications are thought to be the result of errors in DNA replication, and they are very common. Many genomes have at least two genes (usually located close to each other) that resulted from a replication error. Once replicated, the gene copies can diverge by ran dom mutations in their coding sequences and enhancers, developing different expression patterns and new functions (Lynch and Conery 2000; Damen 2002; Locascio et a1. 2002). This scenario of duplication and divergence is seen in the Hox genes, the globin genes, the collagen genes, the Distal-less genes, and in many paracrine factor families (e.g., the Wnt genes). Each member of such a gene family is homologous to the others (that is, their sequence similari ties are due to descent from a common ancestor and are not the result of convergence for a particular function), and they are called paralogues. Thus, the Antennnpedia gene of Drosophila is a paralogue of Drosophila Ultrabithorax. Susumu 0000 (1970), one of the founders of the gene fam- DEVELOPMENTAL MECHANISMS OF EVOLUTIONARY CHANGE ily concept, likened gene duplication to a method used by a sneaky criminal to circumvent surveillance.

(A) 687 (8) While the "po l ice force" of natural selection makes certain that there is a "good" gene properly perform ing its function, that gene's duplicate, unencumbered by the constraints of selection, can mutate and under take new functions. One of the most Significant fates for duplicated genes is the subclivision of the ancestral expression and function (Force et al. 2005).

Thus, the original Distal less gene was probably expressed in many places (as it still is in insects, which have only one Distal-less gene), but in vertebrates, which have numerous Distal-less genes, the original domains have become subdivided so that the different Distal-less paralogues regulate dif ferent functions. Distlll-Iess-3, for instance, is necessary for epidermal differentiation, while Distal-less-5 helps specify neural crest cells (Panganiban and Rubenstein 2002). Such subfunctionalization has since been shown to be the case for many genes, including Hox genes. Each Hox gene in Drosophila has a homologue in FIGURE 19.3 Evidence of the evol ion ary of regulatory ge es (A) Th e enidarian homologue of the vertebrate Bmp4 Drosophila Decapemap/egic ge es is ex pre se at the edge of th e b a opore (marked with an asterisk) in the embryo of the ge e represents an ancestral form of the protostome of the gene. (6) The Hox gene Anthox6, a cnidarian member of the paral.ogue 1 group of Hox genes, expressed at the blastopore of the anemone. (From 01. 2004, of n ut . conservation and s d asymmetrically n l st anemone Nematoste/la. This n and deuteroslome forms is side larval sea Finnerty et courtesy M. Martindale.) sea vertebrates. In some cases, the homologies go very deep and can also be seen in the gene's functions. Not only is the vertebrate Hoxb4 gene similar in sequence to i ts tral Hox genes emerged as a duplication from one of the earlier genes (de Rosa et al.

1999). Drosophila homologue, Deformed (Ofd), b u t the human In the deuterostome chordate lineage, two large-scale duced into Dfd-deficient Drosophila embryos (Malicki et a1. 1992). As mentioned in Chapter 8, the Hox genes in insects duplications of the entire Hox cluster took place, such that HOXB4 gene can perform the functions of Dfd when intro their respective chromosomes. Their expres and humans are not just homologous-they occur in the same order on sion patterns are also remarkably similar: the more 3' Hox genes have more anterior expression boundaries* (see Fig vertebrates have four Hox clusters per haploid genome instead of just one. Thus, instead of having a single Hox4 have Hoxa4, Hoxb4, Hoxc4, and Hoxd4. This constitutes the gene (orthologous to Deformed in Drosophila), vertebrates Hox4 paralogue group in vertebrates. Such large-scale duplications have had several consequences. First, as seen ure 8.30). Thus they are homologous genes between species in Chapters 11 and 13, these duplications create much (as opposed to members of a gene family being homolo redundancy. It is difficult to obtain a loss-of function between species are called o rth ol ogues . mutant phenotype, since to do so fungi the genes have become specialized. Hoxdll, for instance, gous within a species). Genes that are homologous All multicellular organisms-animals, plants, and have Hox-like genes, so it is most likely that there - all copies of these par alogue group genes must be deleted or made nonfunction al (WeUik and Capecchi 2003). However, in some instances, was an ancestral Hox gene that encoded a basic helix-loop plays an important role in the mammalian limb bud but helix transcription factor in protoz.oans. In the earliest ani mal groups, this gene became replicated. One of the two Hox genes present in some extant en idarians (e.g., jelly not in the reproductive system. Mammalian Hoxall, how ever, plays roles in both the limb (where it is critical in spec genes (and is expressed in the anterior portion of the Wong et al. 2004). cnidarian larva), while sequences in the other cnidarian the vertebrates took place during fish evolution. In the ancestral group that gave rise to the teleost fishes (such as fish) corresponds to the anterior set of vertebrate Hox gene are a posterior class Hox gene (Figure 19.3i Yanze et al. 2001; Hill et al. 2003; Finnerty et al. 2004). Perhaps even the ancient midarians used Hox genes to distinguish their anterior and posterior tissues.

In bilateral phyla, the cen -*The conservation of Hox genes and their colinearity demands an explanation. One recent proposal (Kmita et al. 2000, 2(02) contends that the Hox genes "compete" for fl remote enhancer that recog nizes the Hox genes in a polar fashion.

This enhancer most efficient ly activates Hox genes at the 5' end. If the pOSitions of the genes are d1anged by recombinfltion or deletion, then different genes are acti vated in different regions of the body, and morphology manges. ifying the zeugopod) and in the female reproductive tract (where it helps construct the uterus; Lynch et aJ. 2004; The second duplication event of the Hox genes within zebrafish), the entire genome was duplicated. After this duplication, some clusters were lost. But most teleosts still have six or seven Hox clusters, whereas the other verte brates have four. Again, there is much redundancy, caus ing a stabilization of phenotype.

But the fish have also used some of these genes for different functions. For instance, ti.e expression pattern of one zebrafish HoxcI3 paralogue, HoxcI 30, is different from the second paralogue, HoxcI3b, (Thumme l et al. 2004). indicating their roles cliverged after the second duplication 688 CHAPTER 19 In summary, then, gene duplication and divergence are extremely important mechanisms for evolution. Duplica tion allows the fannation of redundant structures, and divergence allows these structures to assume new roles. While one gene copy maintains its original role, the other copies are free to mutate and diverge functionally. NUIner ous transcription factors and paracrine factors are mem bers of such paralogue families. Hox genes are used to pat tern the body and limb axes, Distal-less genes are used to extend appendages and to pattern the vertebrate skull, and members of the MyoD family specify different stages of muscle development. These genes, each family derived from a single ancestral gene, are active in different tissues and provide instructions for the formation of different cell types.'" Thus one of the most important differences between the genome of a fruit fly and that of a human "is not that the human has new genes but that where the fly only has one gene, our species has mnltigene families" (Morange 2001, p. 33). Deep Homology One of the most exciting findings of evolutionary devel opmental biology has been the discovery not only of homologous regulatory genes, but also of homologous sig nal transduction pathways, many of which have been men tioned earlier in this book. In different organisms, these pathways are composed of homologous proteins arranged in a homologous maImer (Zuckerkandl 1994; Gilbert 1996; Gilbert et a!. 1996). This shows a level of parsimony even deeper than that of the individual genes. In some instances, homologous pathways made of homologous parts are used for the same function in both protostomes and deuterostomes. This has been called deep homology (Shubin et a!. 1997, 2009). Conserved similari ties in both the pathway and its function over millions of years of phylogenetic divergence are considered to be evi dence of deep homology between these structures (Shubin et a1. 1997). One example is the chardin/BMP4 pathway discussed in Chapter 7. In both verteb.rates and inverte brates, chardin/Shart-gastrulation (Sag) inhibits the later alizing effects of Bmp4/Decapentaplegic (Dpp), thereby allOWing the ectoderm protected by chordin/Sog to become the neurogenic ectoderm. These reactions are so similar that Drosophila Dpp protein can induce ventral fates in Xenopus and can substitute for Sog' (Figure 19.4; Holley et a!. 1995). According to this scheme, the central nervous system (eNS) only originated once, and i ts ventral and dorsal posi tion was a later occurrence. This idea has been supported by evidence that annelid worms and cephalochordates also *The duplication and divergence scheme for generating paralogous and orthologolls homologies was first described in the 1840s by Sir Richard Owen, a friend (and later rival) of Charles Darwin (see Gilbert 1980). 'The relationship between the number of genes or gene families and complexity is not Simple. Horseshoe crabs and teleost fishes have more Hox clusters than humans.

Drosophila Sag Chrd \ Mouse FIGURE 19.4 The same set of instructions forms the nervous sys tems of both protostomes and deuterostomes . In the fruit fly (pro tostome), the TGF-◆ lamily member Dpp (Decapentaplegic) is expressed dorsally and is opposed by Sag ventrally. In the mouse (deuterostome), the TGF-◆ family member Bmp4 is expressed ven trally and is countered dorsally by chore/in (Chrdj . The h i ghest concentration of chordi n/Sog becomes the midline (M). The mid line is dorsal in vertebrates and ventral in insects, and the concen tration gradient of the TGF-◆ family protein (Bmp4 or Dpp) acti vates the genes specifying the regions of the nervous system in the same order in both groups: vndlNkx2, followed by indlesx, and finally MshlMsx. These genes have also been scen to be expressed in a similar fashion in cnidarians. (After B use the inhibition of the BMP pathway to make their CNS (Denes et a!. 2007; Yu et a1. 2007). Thus the protostOlne and deuterostome nervous systems, despite their obvious differences, seem to be formed by the same set of instructions. The plan for specifying the animal nervous system may have been laid down only once. See WEBSITE 1 9.3 The search for the Urbilaterian ancestor "In addition to this central inhibitory reaction, there are other reac tions that add to the deep homology of the instructions for fomling the protostome and deuterostome neural tube. For instance, the spread of Dpp in Drosophila is aided by Tolloid, a metalloprotease that degrades Sog. TIle gradien t of Dpp concentration from dorsal to ventral is created by the opposing actions of Tolloid (increasing Dpp) and Sog (decreasing it) (Marques et aL 1997). In Xenoplls and zebrafish, the homologues of Tolloid (Xolloid and BMPl, respec tively) have the same function: they degrade chordin. The gradient of BMP4 from venb'al to dorsal is established by the antagonistic interactions of Xolloid or 13MPI (increasing BMP4) and chordin (decreasing BMP4) (Blader et a1. 1997; Piccolo et a1. 1997). Even the regulators of BMP stability are conserved between these two groups of animals and function in the same way (Larrain et a1. 2001). DEVELOPMENTAL MECHANISMS OF EVOLUTIONARY CHANGE 689 Mechanisms of Evolutionary Change Heterotopy In 1940, Richard Goldschmidt wrote that the accumulation One important way of creating new structures is to alter of small genetic changes was not sufficient to generate evo the lutionarily novel structures such as the neural crest, teeth, tor is expressed. This spatial alteration of gene expression turtle shells, feathers, or cnidocysts.

He claimed that such is caUed heterotopy (Greek, "clifferent place"). Heterotopy evolution could occur only through inheritable changes in location where a transcription factor or paracrine fac allows different cells to take on a new identity (as sea the genes that regulated development. This idea was urchin micromeres did when they recruited the genes for brought up again in 1975, when Mary-Claire King and the skeleton formation; see Chapter 5) or to activate or Alan Wilson published a paper titled "Evolution at Two inhibit a paracrine factor-mediated process in a new area Levels in Humans and Chimpanzees." This study showed of the body (as when Gremlin inhibits BMP-mediated that despite the large anatomical differences between chim apoptosis in the webbing between digits; see Figure 13.27). panzees and humans, their DNA was almost identical. The describe next. differences would be found in the regulatory genes that acted during development: The organismal differences between chimpanzees and humans would . . . result chiefly from genetic changes in a few regulatory systems, while amino acid substitutions in general would rarely be a key factor in major adaptive shifts. In other words, the allelic substitutions of the genes that encode protein sequences which seem to be pretty much There are many other examples, some of which we will HOW THE BAT GOT ITS WINGS AND THE TURTLE GOT ITS SHELL that the bat evolved its wing In Chapter 1, we mentioned by changing the development pf the forelimb such that the cells in the interdigital webbing did not die. It turns out that the bat retains its forelimb webbing in a manner very similar to how the duck embryo retains its hindlimb web bing by blocking the BMPs that would otherwise cause the interdigital ceUs to undergo apoptosis (see Figures 1.20 the same for chimpanzees and humans were not seen and 13.28).

Both Gremlin and FGF signaling appears to as being important. The important differences are where, when, and how much the genes are activated.

In 1977, the block BMP functions in the bat wing. Unlike other mam lution was extended by Franc;ois Jacob, the Nobel laure this protein is critical for maintaining the ceUs there. Signaling is inhibited (by drugs such as SU5402), BMPs can idea that change within regulatory genes is critical to evo If FGF mals, bats express Fgf8 in their interdigital webbing, and ate who helped establish the operon model of gene reg ind LIce apoptosis of the forelimb webbing, just as in other ulation. First, Jacob said, evolution works with what i t mammals (Laufer et al. 1997; Weatherbee et al. 2006). The has: it combines existing parts i n new ways rather than Fgf8 in the webbing is probably also responSible for pro kering" would be most likely to occur in those genes that viding the mitotic signal that extends the digits of the bat, creating new parts. Second, he predicted that such "tin construct the embryo, not in the genes that function in adults (Jacob 1977). Wallace Arthur (2004) has catalogued four ways in which Jacob's "tinkering" can take place at the level of gene thereby expanding its wing (Hockman et al. 2(08). FGFs, but in different ways.

What distinguishes turtles The formation of the turtle shell also L1ses BMPs and from other vertebrates are their ribs-they migra te later ally into the dermis instead of forming a rib cage (Figure expression to generate phenotypic variation available for 1 9.5) . Certain regions of the turtle dermis attract rib pre natural selection: cursor cells, and these dermal regions differ from those of other vertebrates because they synthesize FgflO. FgflO • Heterotopy (change in location) • Heterochrony (change in time) • Heterometry (change in amount) Thomas et aJ. 2005). The lateral growth of the ribs causes • Heterotypy (change in kind) es the scapula (shoulder blades) to reside inside the ribs. These changes can only be accomplished if the gene seems to attract the ribs, since tile ribs do not enter the der mis if the FgflO signal is blocked (Burke 1989; Cebra some muscles to establish new attachment sites and caus This phenomenon is seen only in the turtles (Nagashima expression patterns are modular, that is, if they are con et al. 2009). Once inside the dermis, the rib cells do what trolled by different enhancer elements.

The modularity of rib ceUs are expected to do they undergo endochondral this, BMPs are made. But the rib is embedded in der development allows one part of the organism to change ossification wherein the cartilage ceUs are replaced by bone. without necessarily affecting the other parts.' To do "'This chapter concentrates on transcriptional-level changes that can generate new morphological forms, but morphological changes can be instigated at these leve1s as well. Abzhanov and Kaufman (1999), for instance have shown that translational differences in the Sex combs reduced gene are critical in converting legs into maxillipeds in the crustacean pill bug Porcellio scnber. each of the newly positioned ribs instructs the dermis , mis, and the dermal ceUs can also respond to the BMPs by becoming bone (Cebra-Thomas et al. 2005). In this way, around it to become bone, and the turtle gets its shell. HOW BIRDS GOT THEIR FEATHERS Although it had long been accepted that feathers emerged as modified reptilian scales 690 CHAPTER 1 9 (C) (A I (BI FIGURE 19.5 H e tero topy on severa l levels in turtle devel- opment. The carapace (dorsal shejl) of the turtle is formed through seq u e n tial layers of h eterotopi es . Fgll0 expresso ion in certai n regions of th e dermis impels rib precursor cells to migrate l atera l ly into the dermis instead of forming a rib cage .

(A,B) Cross section of early turtle em bryo as the rib enters the dermis (A, brightfield; B, autoradiograph stai n in g for Fgll0). (C) Half cross section of a sl i gh tl y later tu rt l e em bryo, showing a rib ex tend ing from the vertebra into th e (see Maderson 1972; Prum et al. 1999; Maderson and Alibardi 2000), the mechanism that produces feathers has remained e l usive . Harris and his colleagues (2002) have a develop mental mechanism for feather evolu provided region of the dermis that wiJl expand to form the shell. (D) Hatchling turtle sta i ned with alizarin to show bones. Bones can be seen in the dermis around the ribs that entered into it. Heterotopies i nc lude Fgf70 exp ressi on, rib placement, and bone location. (After Loredo et al. 2001.) tion, showing that the feather most likely evolved from the archosa urian (dinosaur/bird ancestor) scale through an alteration of the expression pattern of the Sonic hedgehog (Shh) and BMP2 proteins. serially around the proximal-distal axis. The interaction ScaJes and feathers start off the same way, with Bmp2 and Shh expressed in separate domains. However, in the feather, both expression domains shift to the distal region Scale bud Feather €bud ?:7◆__◆ of the appendage. This feather-specific pattern is repeated between BMP2 and Shh proteins then causes each of these regions to form its own axis-the barbs of the feather ure 1 9.6). Moreover, when this serially ◆ ◆ Tubular feather, from scale FIGURE 19.6 Mod el for the evolution of the feather by changes in the patlern of Bmp2 and Shh expressio n . Stage 0 shows the Shh and Bmp2 exp ression in ◆- Stage: 0 (F ig repeated pa ttern was 2-3a the scale (above) and feather bud (below). Stage ' represen ts a tubular feather as evolved from an archosaurian scale.

The Shh and Bmp2 expression patterns are post u lated to be at the tip . Stage 2 represents the emergence of a branched feather evo lved by fu rth er changing the expression patterns of Bmp2 and Shh to iorm rows a l ong th e proxima l -d islal axis. In stage 3a, changes in feather morphol ogy evolved by altering the pattern to produce a cen- tral rachis. (Aher Harris et al. 2002.) DEVELOPM ENTAL MECHANISMS OF EVOLUTIONARY CHANGE experimentally modified, the feather pattern was modified in a predictable manner (Harris et al. 2002; Yu et al. 2(02). python development, Hoxc6 is not expres""d in the absence of Hoxc8, so the forelinlbs do not form. Rather, the combi nation of HOW S

eventually becomes a change in qual ity .

It has been postulated that this type of mechanism produced the unique cheek pouches of pocket gophers and kangaroo rats that live in deserts. These external pouches differ from internal ones in that they are fur-lined and have no inter nal connection to the mouth. They allow these animals to ""The lack of such transitional forms is often cited by creationists as evidence against evolution, For instance, in the tra nsition from rep tiles to mammals, three of the bones of the reptilian jaw became the incus jaw (see Chapter 1) Gish (1973), a creationist, says th a t this is an impossible situation, since no fossil has been discovered showing two or three jaw bones and tvvo or three ear ossic1es. Such an aru mal, he claims, would have dragged its jaw on the ground. Howev er, such a specific transitional form (and there are over a dozen doc wnented h'ansitionaJ forms between reptilian and mammalian skulls) need never have existed. Hopson (1966) has shown on embryological grounds how the bones of the jaw could have divid ed and been Llsed for differen t functions, and Romer (1970) has found reptilian fossils whereiJ1 the new jaw articulation was already functional while the older bones were becoming useless. Several spec e therapsid reptiles had two jaw articulations. with the stapes brought into close proximity with the upper portion of the quadrate bone (which would become the incus). i s of 696 CHAPTER 1 9 Heterotypy In heterochrony, heterotopy, and heterometrYI mutations affect the regulatory regions of the gene. The gene's prod uct the protein remains the same, although it may be synthesized in a new place, at a different time, or in differ ent amounts. The changes of heterotypy affect the actual coding region of the gene, and thus can change the ftmc tional properties of the protein being synthesized. WHY INSECTS HAVE ONLY SIX LEGS Insects have six legs, but most other arthropod groups (think of spiders, millipedes, centipedes, and shrimp) have many more. How is it that the insects came to form legs only in their three thoracic segments?

The answer seems to reside in the relationship, mentioned earlier, betvveen Ultrabithorax protein and the Distal-less gene. In most arthropod groups, Ubx protein does not inhib it the Distal-less gene. However, in the insect lineage, a mutation occurred in the Ubx gene wherein the origina1 3' end of the protein-coding region was replaced by a group of nuc1eotides encoding a stretch of about 10 alanine residues (Figure 19.13; Galant and Carro11 2002; Ronshau gen et a!. 2002). This polyalanine region represses Dista/ less transcription. When a shrimp Ubx gene is experimen tally modified to encode this polyalanine region, it too represses the Distal-less gene. The ability of insect Ubx to inhibit Distal-less thus appears to be the result of a gain-of ftmction mutation that characterizes the insect lineage. HOW PREGNANCY MAY HAVE EVOLVED IN MAMMALS One of the most amazing features of marrunals is the female uterus, a structure that can hold, nourish, and protect a develop ing fetus within its mother's body. One of the key proteins in FIGURE 19.13 Changes Ubx prote in associated with the insect clade in the evolution of arth ropods Of all arthropods only the insects have Ubx p rotei n that is a b l e to repress Distal less gene expression and thereby inhibit abdominal legs. This ability to repress Distal-less is due to a mutation that is seen only in the insect Ubx gene. (Ailer Galanl and Carroll 2002; Ronshaugen et al. 2002.) . , enabling this internal gestation is prolactin. Prolactin pro motes differentiation of the uterine epithelial cells, regu lates trophoblast growth, allows blood vessels to spread toward the embryo, and helps downregulate the immune and inflammatory responses (so the mother's body does not perceive the embryo as a "foreign body" and reject it). At about the same time the mammalian uterus and preg nancy evolved" one of the mammalian Hox genes Hoxall appears to have undergone intensive mutation and selection in the lineage that gave rise to placental mam mals. Analysis shows that the sequence of the Hoxall pro tein changed in mammals such that it associates and inter acts with another transcription factor, FoxolA (Figure 1 9.1 4, Lynch et a!. 2004, 2008). This association with FoxolA enables Hoxall to upregulate prolactin expression from the enhancer used in uterine epithelial cells. Hoxall from non eutherian mammals (opossum and platypus) and from chickens does not upregulate prolactin.

If morpholinos knock out the Hoxall mRNA in mouse uterine celis, no pro lactin is expressed. Therefore, one of the most important evolutionary changes in the lineage leading to mammals involved the heterotypic alteration of the Hoxall sequence.

HETEROTOPY AND THE EMERGENCE OF CORN Heterotypy is also responsible for one of the most important regulatory mutations in agriculture the exposure of the kernels on the ears of com (Zea mays). The gene Tgal (teosinte glume architec ture-l) encodes a transcription factor that reduces the area of the glume (the sheath of the ear of corn), enabling the ker nels to be exposed and harvested (Wang et a1.

2005). As in the other cases, the new phenotype cannot be pro duced by a single genetic change. One model that has been proposed numerous times (but which is now receiving experimental support from examples such as those above) is that one particular mutation (i.e., Tgal or the hindlimb enhancer allele of Pitxl) makes a big change and then other mutations fine-tune the change. This type of evolution has been seen in bacteria (see Chouard 2010). By looking at changes in gene expression, evolutionary developmental biology is now able to explore the origin of numerous peptide Insects ����' Fly Mosquito Po ya a e Butterfly Moth Beetle Springtail p Spi d er Common pede ancestor L.,--- Centi Onycophoran l l ni n \ 1- L,. L,. Homeodomain UbdA Polyalanine AQAQK • VQGGHICDQ _ _ _ _ FQNRRMKLKKE IQAIKELNEQEKQAQAAKAGLFINLGGLIANSF ... _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Shrim WFQNRRMKLKKE LQAI KELNEQIIKQAQNAKQANATAVTPGATTDSTPTP. FQNRRMKLKKE IQAIKELNEQE QAQAAKTA---STSTVS SNSNSNN. WFQNRRMKLKKE I QAIKELNEQ KRITPSKLHSNC-SSPTGILVTMKKM WFQNRRMKLKKE ' Q IK LNEQEm DEVELOPMENTAL MECHAN ISMS OF EVOLUTIONARY CHANGE FIGURE 19.14 Ability of the mam malian Hoxal 1 prote in, in combination with FoxolA, to promote expression of the uterine prolactin enhancer. The acti vated luciferinase reporter gene (d3321luc3), activated human Hoxa l I gene (HsaHoxA 1 1 1, and activated human Foxo lA (HsaFoxo1 Al each failed to activate the prolactin gene from the enhancer transcription. Mammalian (but not opossum, platypus, or chicken) Hoxa l 1 increased transcription from this enhancer, but only in the presence of Foxo l A. IIEutherian A1 1 " indicates generalized HoxA 1 1 from placental mammals. Therian A1 1 " indicates the consensus HoxA 7 1 sequence from all mammals. (After lynch et al. 2008.1 697 4.5 v • 1.5 • _" u "" -0 u.. 0.5 " adaptive One of Darw in 's greatest puzzles or "the arrival of the fittest"-is now capabl e of being so l ved . variations. the origins of selectable variation, Developmental Constraints on Evolution There are only about three dozen major arumal l ineages, different bo dy plans seen in the a nimal kingdom. One can easily envision other body plans by imagining animals that do not exis t (science fic tion writers do it all the time). So why don't we see more body plans among the living animals? To answer this, we have to consider the constraints that development impos es on evoilition. The number and forms of pOSSible phenotypes that can be created are limited by the interactions that are possible among molecules and between modules. ' These interactions a lso allow change to occur in certain directions more easily than n i others. Collectively, the restraints on phenotype produc tion are called deve lopmental con• straints. Constraints on evolution fall into three major categories: physical, morpho genetic, and phyletic (see Richardson and Chipman 2(03). and they encompass all the Sev WEBSITE 1 9.4 How the chordates got a head Physical constraints of diffusion, hydraulics, and phYSical support are immutable and will permit only cerlain physical pheno- The laws *G. W. Leibniz, who roay have been the philosopher who most jn{)uenced Darwin, noted that existence must be limited nol only to types to arise. For e xa mple, blood cannot circulate to a rotating organ; thus a vertebrate on wheeled appendages (of the sort that Doro t hy saw in Oz) cannot exist, and this entire evolu tionary avenue is closed off. Similarly, struc tural parameters and fluid dynamics would prohi bit the existence of 6-foot-tall mosquitoes or 25-foot-long leeches. The elasticity and tensile strength of tissues are also physical constraints. In Drosophila. sperm, for example, the type of tubulin that can be use d in the ax oneme is con strained by the need for certa in physica l properties in the exceptionally long flagellu m (Nielsen and Raa 2002). The six cell behaviors used n i morphogenesis (division, growth, shape change, migra tion, death, and matrix secretion) are each limited by physical parameters, and thereby provide limits on what structures animals can form. Interactions between different se ts of tissues involve coordinating the behaviors of sheets, rods, and tubes of cells in a limited number of ways (Larsen 1992). Morphogenetic constrain ts: The reaction-diffusion model (1894) and Alberch (1989) noted that when organ isms depart from their normal development, they do so in only a limited number of ways. Although there have been many modifications of the vertebrate limb over 300 mil lion years, some modifications (such as a middle digit shorter than its su rround ing digits) are never seen (HoldBateson into the rest of the organism (or that can cause a compensatory change in the rest of the organism) will be seen. Developmental the possible but to the compossible that is, whereas numerous biologists see constraints as limiHng the appearance of certain phe th.ij1gs con come into existence, onJy those that are mutually com notypes, whereas population gcneticists see constraints as limiting patible will ac tual ly exist (see Lovejoy 1964). So, even though many developmental changes are posslbJc, only those that can integrate "ideal" adaptation (such as constraints on optimaJ foraging) (Amundson 1994, 20051. 698 CHAPTER 1 9 er 1983; Wake and Larson 1987). These observations sug from fOnning. But which of the many P peaks will survive? gest a limb construction scheme that follows certain rules That depends on the size and shape of the tissues in which (see Figure 13.14; Oster et a1. 1988; Newman and Miiller the oscillating reaction is occurring.

This pattern is analo 2005).

One set of rules constraining limb development may gous to the harmonics of vibrating strings, as in a guitar: be the mathematics of the reaction-diffusion mechanism, only certain resonance vibrations are permitted, based on a model that can be extended throughout development. the boundaries of the string. The mathematics describing The reaction-diffusion model for developmental pat terning was formulated by Alan Turing (1952), one of the which particular wavelengths are selected consist of com plex polynomial equations. founders of computer science (and the mathematician who Turing's mathematics have been used to model the spi cracked the German "Enigma" code during World War ll). ral patterning of slime molds, the polar organization of the He proposed a model wherein two homogeneously dis limb, and the pigment patterns of mammals, fish, and tributed substances ("substance P" and "substance S") snails (Kondo and Asai 1995, Meinhardt interact to produce stable patterns during morphogenesis. most successful of these attempts applied the model to the These patterns would represent regional differences in the evolution of mammalian teeth. 2004). One of the concentrations of the two substances. Their interactions would produce an ordered structure out of random chaos. In Turing's model, substance P promotes the produc tion of more substance P as well as substance S. Substance P, S, however, inhibits the production of substance P. Turing's mathematics show that if S diffuses more readily than sharp waves of concentration differences will be generated THE EVOlUTION OF MAMMALIAN TEETH Stephen J. Gould (1989) once joked that paleontologists believe mammalian evolution occurs when two teeth mate to produce slightly altered descendant teeth. Since tooth enamel is far more durable than ordinary bone, teeth often remain after all the other bones have decayed, and the study of tooth morphol P (Figure 1 9.1 5). The reaction-diffusion model ogy has been critical to mammalian systematics and ecol predicts alternating areas of high and low concentrations ogy. Changes in the cusp pattern of molars are regarded as for substance of some substance. When the concentration of the sub especially impo rtant in allowing the radiation of mammals stance is above a certain threshold level, a cell (or group of cells) can be instructed to differentiate in a certain way. An .into new ecological niches. What mechanism allows molars will be suppressed. As local concentrations of P increase, 2004) pioneered important feature of Turing's model is that particular chemical wavelengths will be amplified while all others the values of S form a peak centering on the P peak, but to change their form so rapidly? 2000, 2008; Salazar-Ciudad and Jernvall 2002, Jukka Jernvall and colleagues (Jernvall et a1. Salazar-Ciudad a compu ter-based approach to pheno type production using Geographic Information Systems becoming broader and shallower because substance S dif (GIS) to map gene expression patterns in incipient tooth P peaks buds. Their studies showed that gene expression patterns fuses more rapidly. These S peaks inhibit other (A) (B) Time I Activator (P) stimulates production of inhibitor (5) 5 diffuses quickly and inhibits autocatalysis of P Slowly diffusing activator (P) Time 2 P FIGURE 19.15 Reaction-diffusion (Turing) model of pattern generation . (A) Genera tion of periodic s patial heterogeneity can come about spontaneously when wo reac tants, 5 and P. are mixed together under the conditions that 5 inhibits P; P catalyzes production of both 5 and P; and 5 diffu ses faster than P. These conditions yield a peak of P and a lower peak of 5 at the same p lace (6) The distribution of the reactants is initially random, and their concentrations fluctuate over a given average. As P increases l ocal ly it produces more 5, which diffuses to inhibit more peaks of P from forming in the vicinity of its production. The resul t is a series of P peaks ("standing wavesH) at regular intervals.

• F=< Time I In hibitor (5) C\.AJ V cz: c• • '" Activator (P) T i me 2 .8 ;;: b 8 8 j _ Time 3 . ' , a Position DEVELOPMENTAL MECHANISMS OF EVOLUTIONARY CHANGE Buccal (toward cheek) 699 t FIGURE 19.1 6 GIS analysis of gene activity in h e formation of the first set of cusps i n mouse and vole molars. (The first two cusps are the proconid, labeled Prd, and t e metaconid, labeled Med).

For both species, GIS map ping of molar shape is shown on the leh, and the expression of fgf4 and Shh (two genes expressed from the enamel knots) is shown at the right. The gene expression patlern on embl)'onic day 1 5 predicts the formation of the new cusps seen on day 1 6; the gene expression pattern on day 1 6 predicts the formation of cusps i n those areas on day 1 7. Similarly, in the vole molar, whose cusps are diagona l to one a nother, gene expression predicts cusp formation. (After Jernvall et al. 2000, cou rtesy of J. Jernvall.) h Anteriodr (towa r lips) forecast the exact location of the tooth cusps in mice and voles based on differences in gene exp ression pa tterns (Figure 1 9.1 6). Salazar-Ciudad and Jernva ll (2004, 2010) also showed how reaction-diffusion models can exp ain the differences ing the "constants" of diffusion as development progress center of the tooth, the enamel knot, secretes BMPs, FGFs, (see Chapter 10). Shh and FGFs inhibit BMP pro duction, while BMPs stimulate both the production of more BMPs and the synthe si s of their own inhibitors. BMPs also induce epithelial differentiation, while FGFs paracrine factors.) l (This is because the •• versa BMP4 1 p21 • DAN _____ L...,_ ' (BMP inhibitor) Mesenchyme proliferation The result is a pattern of gene ac tiv ity (Figure 1 9 . 1 7). Under this model, the l arge differ by small changes in the binding constants and diffusion rates of the BMP and Shh proteins.

A small increase in the diffusion rate of BMP4 and a stronger binding constant of involved (1) chang- ---------. the ences between mouse and vole molars can be generated induce epithelial growth. For this study, two additions to MSX2(?) ex tracellular matrix cha nge s as that change s as the shape of the tooth changes, and vice and Shh (A) associated cells differentiate, altering tissue shape and the diffusion of in gene expression between mice and voles. The Signaling the classic reaction-diffusion equation e lastici ty with the extracellular matrix as development progresses. es, and (2) changing the amount of (B) Predicted Observed Mouse • • .. po .ef" • (. FIGURE 19.17 Basic model for cusp development i n mice and voles. (A) Experimentally derived gene network wherein BMPs activate their own production as well as the production of their inhibitors, Shh and FGFs. The FGFs and Shh stimulate cell proliferation; the BMPs inhibit it. (B) Predicted and observed results from this m odel. The model can generate the final and intermediate forms o(molar development in mice and voles, and the difference between mouse and vol e molars can be reproduced by slight alterations in the rate of BMP diffusion and bi nding to inhibitors. (After Salazar-Ciudad and Jernvall 2002; photographs courtesy of J . Jernvall.) Vole Predicted Observed ..

4I!P C1 1#* { _ • • cr.r 700 CHAPTER 1 9 FIGURE 1 9.18 Mammalian tooth cusp patlern modeled by reaction-diffusion equations.

(A) The reaction-diffusion mechanism serves as a motor regu l ati ng the genes respo ns ibl e for slowing the growth of the enamel-forming cells and promoting the proliferation of the dentin pulp-forming cells. (B) Poss i bl e forma tion of the four-cusped tooth of Hyracotherium (a fossil horse from 55 million years ago) from the three-cusped tooth of lnxO/OphU5, a mammal that may be an earlier member of th e horse lineage. This transition in tooth sha pe can be achieved by modifying a si ngle parameter of the reaction-diffusion equation.

(Courtesy of J.

Jernvall.) (A) heliaoln prolEpiifterati (enamel; cusp) ! Mesenchymal prol(denti iferatin;on pulp) Phyletic constraints historical restrictions based on the genetics of an organism's Ph yle tic constraints on the evolution of new structures are Fossils .. Loxoloph us development (Could and Lewontin 1979). In other words, once a structure comes to be established by inlnctive inter actions, it is difficult to start over again. The notochord, for exa mple, is functional in where it specifies the neural tube and sclerotome. Similar ly, Wadding ton (1938) noted that, although the pronephric .. growth into that of the mouse. The work on mouse and vole molars showed how large morphological changes can result from very small changes in initial conclitions The set of equations that emerged also modeled the observed tooth shape variation seen in natu ral p opu ations of seals, thereby relating small changes in devel opment with microevo lutionary variation within a species. Not only clid the "virtual" teeth resemble real seals' tee th; the progression of cusps in the - i start off with the same basic set of instructions, and spec f ic instructions will emerge as the cells interac t. The Turing model also predicts that some types of teeth are much more likely to evolve in certain ways than i.n oth ers (Figure 19.18). Moreover, the ecological context of tooth use (herbivory versus carnivory, for instance) would select certain variants and not others, and this mexiel can predict i both the nwnber and size of molars kidney of the chick embryo is considered ves tigia l (since it has no ability to concentrate urine), i t i s the source of the ureteric bud that induces the formation of a functional kid ney during chick deve opment (see Chapter 11).

One fascina ting example of a phyle tic constraint is the lack of variation among marsupial limbs. Although euther ian lin-tbs show a dramatic range of diversity (claws, wings, paddles, flippers, hands), limbs among marsupial species are all pretty much the same. Sea rs (2004) has docwnent ed that the necessity for the marsup al fetus to crawl into its mother 's pouch has constrained limb development such that the limb musculature and cart la ge must develop very ear ly into a str uct re that can grasp and crawl. Any vari ation in this trait has effectively been eliminated. l its inNbitor is sufficient to change the vole pattern of tooth i under different ecolog (Kavanagh et al.

2007; Polly 2007).

The pre dic t ons conform to what p aleontol og sts have observed ical conditions such as brates.

Yet it is transiently necessary in vertebrate embryos• Hyracotheriuln Reaction-diffusion model l adult pr,otochordates Amphioxus (Berrill 1987) but degenerates in adul t verte about manunalian evow tion. These stuclies show the power of mathematical modeling to integrate deve lopment, cell biology, and genetics into a preclictive model for evo lution. i u l i i As genes acquire new functions during the course of evo ution, they may become active in more than one mod l i ule, mak ng change difficult. Calis and colleagues provide evidence that the reason the segment p olarity gene net work is conserved in al types of nsects is that these genes play roles in several different pathways (Calis et aJ. 2002). Such pleiotropy constrains the possibilities for alternative i the ability of a gene to play different roles in different ceils, mechanisms, since i t makes change difficult. Pleiotropy, of modularity• involving the cormections between parts rather than their independence.

Pleiotropies may underlie the constraints seen in mam malian de velopment Calis speculates that mamma s have only seven cervical vertebrae (while birds may have dozens) because the Hox genes that spec ify these vertebrae is the " oppos te " . l have become linked to stem cell proliferation in mammals i (Calis 1999; Calis and Metz 2001; Abramovich et aJ.

2002; Schiedlrneier et aJ. 2007). Thus, changes in Hox gene expres sion that might fac litate evol lrtionary changes in the skele ton might also misregulate cell proliferation and lead to can cers. Calis sLIpports this speculation with epidemiological DEVELOPMENTA L MECHANISMS OF EVOLUTIONARY CHAN G E 701 (B) (A) 35 30 - FIGURE 19.19 Extra cervical ribs are associaled with childhood cancers. (A) Radiogram showing an extra cervical rib. (8) N earl y 80% of fetuses with extra cervical ribs clie before birth. Those sur viving often develop cancers very early in life. This indicates strong selection against changes in the number of mammalian cervical ribs, (After Garis et al.

2006, c urtesy of F. Galis.) o evidence showing that changes in skeletal morphology cor relate with childhood cancer. The intraembryonic selection against having more or fewer than seven cervical ribs appears to be remarkably strong. At least 78% of human Figure embryos with an extra anterior rjb (i.e., six cenrical verte tebrates arrive at the pharyngula stage, they do so by very brae) die before birth, and first different means. Birds, reptiles, and fish arrive there after year. These deaths appear to be caused by multiple congen meroblastic deavages of different sorts; amphibians get ital anomalies or cancers 83% die by the end of the (Figure 19.1 9; Calis et aJ. 2006). Until recently, it was thought that the earliest stages of development would be the most resistant to change, because 1.10; Slack etal.

1993). And in fact, while all the ver there by way of radial holoblastic cleavage, and mammals reach the same stage after constructing a blastocyst, chori on, and amnion (see Chapters 7 and 8).

altering them would either destroy the embryo or generate Before the vertebrate pharyngula stage, there are few a radically new phenolype. But recent work (and the reap inductive events, and most of them are on global scales praisal of older work; see Raff et aJ. 1991) has shown that is a great deal of regulation, so small changes (involving axis specification). in these early stages of devel certain alterations can be made to •arly cleavage without opment, there upsetting the organism's final form. Evolutionary modifi in morphogen distributions or the position of cleavage cations of cytoplasntic determinants in molluse embryos can planes can be accommodated (Heruy et al.

give rise to new lypes of larvae that still metamorphose into pharyngula stage, there are a great many inductive events, molluscs, and changes in sea urchin cytoplasntic determi but almost all of them occur within discrete modules (Fig 1989). After the nants can generate sea urchins that develop directly (with ure 1 9.20). The lens induces the cornea, but if it fails to do no larval stage) but still become sea urchins. so, only the eye is affected. But du ring the phylotypic The ear liest stages of development, then, appear to be pharyngula stage, the modules interact. Failure to have the extremely plastic. The later stages are very different from heart in a certain place can affect the induction of eyes. Fail species to species, as the different phenotypes of adult ver ure to induce the mesoderm in a certain region leads to tebrates amply demonstrate.

There is something in the malformations of the kidneys, limbs, and tail. By search middle of development, however, ti1at appears to be invari ing the literature on congenital anomalies, Calis and Me12 ant. Sander (2001) have documented that the pharyngula is much more mation of new body plans (1983) and Raff (1994) have argued that the for-(Ba"pline) is inhibited by the vulnerable than any other stage. Moreover, based on pat global consequences of induction during the phylotypic terns of multiple organ anomalies in the same person, they stage the stage that typifies a phylum. For instance, the concluded that the multiple malformations were due to late neurula, also known as the pharyngula, is the phylo the interactivity of the modules at this stage. Thus, this lypic stage that appears to be critical for all vertebrates (see phylotypic stage that typifies the vertebrate phylum 702 CHAPTER 1 9 FIGURE 19.20 Mechanism for the bottleneck at the pharyngula stage of vertebrate devel opment. (A) In the c leaving embryo, global interactions exist, but there are very rew of them (mai nly to specify the axes of the organism). (6) At the neurula to pharyngula stages, there are many global i nductive interactions. (C) After the pharyngula stage, there are even more inductive interactions, but they are primarily local in effect con fined to their own modules. (After Raff 1 994.) (A) (C) (B) /E• D F B•C G A appears to constrain its evolntion. Once an organism becomes a vertebrate, it is probably impossible for it to evolve into anything else. inheritance systems function in parallel with the genetic S e lectable Epigenetic Variation inheritance systems of the nucleus and mitochondria. Changes in development provide the raw ma terial of vari Daphnia and locusts. Here, environmental conditions expe rienced by the juveniles (predators III Daphnia; population Early in Chapter ation. But we have seen earlier n i the book (especially in Chapter 18) that developmental signals can come from the varia ti on be inherit environment as weJ] as from the nuclei and cytoplasm. Might this environmentally induced in environmentally induced traits could be inherited 18, we discussed the polyphenisms of denSity in lcensts) alter the adult phenotype.

But in both cases, the altered phenotype is transmitted to the offspring, even in the absence of the environmental cues that initiat ed and selectable? This idea smacks of Lamarckism, where ed it. The mechanism by which predator-induced poly wrong in thinking that phenotypes acquired by use or rus not known (Agrawal et a!. through the germ line. We now know that Lamarck was use could be transmitted. Chlldren of weight lifters don't will be inherit their parents' physiques, and acddent victims who have lost limbs can rest assured that their children born with normal arms and legs. phenisms in Daphn.ia are transmitted to their progeny is 1999); the case of the locust, is better understood. The gregarious brown, long-Winged migratory morph of the locust Schistocerca gregoria is retained for several genera tions after the crowding stimulus initiates the transforma tion from the solitary morpho This transgenera tional effect is however, But what if an environmental agent were to cause now known to be mediated during oviposition (egg laying) changes not only in the somatic DNA but also in the by a chemical agent introduced into the foarn surrounding DNA? Then the effect might be able to be trans the eggs. Part of the gregarious female's phenotype involves germline mitted from one generation to the next. There are dozens the production of a particular chemical that she introduces of such examples where the environmentally induced phe nto the foam surrounding her eggs. This chemical agent the absence of an environmental inducer Oablonka and Raz ter L dopa) is thought to be syntheSized notype can be inherited from one generation to the next in (which appears to be a modified fonn of the neurotransmit in the accessory 2(09). So this is another example of developmental changes generating variation. The question becomes: "How impor tant is phenotypic plasticity for evolu.tion?" Some scien glands of the female reproductive tract and to act at the time tists say it is of minor concern, valid for a few special cases. females to egg masses produced by solitary females, the soli of many, if not most, species on the planet. Evolutionary off, the gregarious state reverts to the solitary phenotype after Other scientists say this is a critical agent n i the evolution developmental biology is still a very young science. It will take time to find out. 2000; Miller et al. 2008). tary eggs turn into gregarious locusts. If the foam is washed Simpson and Miller 2(07). a few mol t s (Figure 19.21 ; McCaffery and Simpson 1998; These examples show that environmentally induced phenotypes can be inherited. There are two major epige Transgenerational inheritance of environmental/y induced traits A third process, genetic assimilation, shows that some em• netic inheritance systems known: epialleles and symbionts. So far we have discussed the roles of genetic variation in producing variations that can be tested through natural selection. But developmental plasticity also plays a role If foam is transferred froru egg masses laid by gregarious of egg laying (Hagele and Simpson in producing variation. in some instances, this epigenetic vari ation is stable between generations, and the processes cre called epi ge netic inheritance systems. These epigenetic ating such transmissible epigenetic inheritance have been ronmentally induced traits, when continually selected, are stabilized genetically so that the trait is inherited without having to be induced in each genera tion. ic inheritance system are variants of the DNA sequence, the EPIAllELES While the alleles that are the basis of the genet epialleles of epigenetic inheritance systems are variants of DEVE LOPMENTAL MECHANISMS OF EVOLUTIONARY CHANG E Hatchlings' Mother's phase l ita FIGURE 19.21 S u m ma ry of experiments demonstrating that the source of the trans ge nerat i o na l gregarizing agent in Schisto cerca is a substance found in the egg froth produced by gregarious females. (Aner Simpson and Miller 2007; Simpson and So ry Sword 200B.) _ _ _ Gregarious chromatin structure that can be inher ited between generations. In most knowvn casesf epiaJJeles are differences ---1L _I• I mother _ _ _ _ gregarious are able to affect the germ line and Froth washed off The asymmetrical peloria variant of the L Ext reapplractled (Linaria vllgaris; Figure 1 9.22) was first described by Linnaeus 1742 as a stably inherited form. Coen's laboratory showed that this variant was due not to a distinctive allele, but rather to a stable epiallele (Cubas et al. 1999).

Instead of this gene was hypermethylated. It does not matter to the of carrying a mutation in the cycloidea gene, U,e peloria form developing system whether a gene has been inactivated by effect is the same. There are dozens of examples of epiaUelic inheritance 2009; Jablonka and Raz • ferences affect coat color and obesity. When a pregnant (-100%) Gregarious (-100%) I Solitary (-72%) I I Gregarious (--88%) 1'-. . , Enzymatic and metabolic phenotypes are established in utero by protein-restricted diets in rats when protein restriction during a grandmother rat's pregnancy leads discussed some epialleles in their developmental context. female Gregarious and Skinner 2(07). 2009). We have • In the viable Agouti phenotype in mice, methylation dil Solitary (-95%) the progeny of those mice and to their progeny Oirtle a mutation or by an altered chromatin configuration. The (Gilbert and Epel :I L _ _ _ _ ---, _ • thereby be transmitted to offspring. in phase Froth added from in DNA methylation patterning that toad flax plant - 703 to a specilic methylation pattern pups (Burdge et al. • in her pups and grand 2007) . The endocrine disruptors vinclozolin, methoxychlor, and is fed a diet high in methyl donors, the specific bisphenol A have the ability to alter DNA methylation methylation pattern at the Agouti locus is transmitted patterns in the germ line, thereby causing developmen not only to the progeny developing in utero, but also to tal anomalies and predispositions to diseases in the (A) (B) f fiGURE 19.22 Epigenetic forms o toadflax. (A) Typica l Linaria, with a re l ative l y unmethylated cycloidea gene. (6) The cycloidea gene of the pe oria variant is rel atively heavi ly methy l ated . The ep i a l l e les that c rea te the different ph enotypes of / this species are stably inherited. (Courtesy of R. Grant-Downton.) 704 CHAPTER 1 9 grandpups of mice exposed to these chemicals in utero • Stress-res istant behavior of rats was shown to be due to (Anway et al. 2005, 2006a,b; Chang et al. 2006; Newbold et al. 2006; Crews et aJ. 2007). methylation patterns, induced by maternal care, in the glucocorticoid receptor genes. Meaney (2001) found that rats that received extensive maternal care had less stress induced anxiety and, if female, developed into mothers that gave their offspring similar levels of maternal care. Because of this interdependence, aphids are highly con strained to the ecological tolerances of Buchnem. A recent study (Dunbar et aJ. 2007) showed that heat tolerance of pea aphids and Buchnera could be destroyed with a single nucleotide deletion in a heat shock gene promoter. They discovered that a Single-base deletion in the promoter of the Buchneria ibpA gene can lower the thenmotolerance of the aphid/ symbiont organism. This microbial gene encodes a small heat shock protein, and the deletion eliminates the transcriptional response of SYMBIONT VARIATION As we explored in Chapter 18, one important aspect of phenotypic plasticity involves inter ibpA to heat. Buchnera with the short Although pea aphids harboring ibpA promoter allele suffer from decreased therrnotoler actions with an expected population of symbionts. When ance, they experience increased reproductive rates under symbionts are transmitted through the germ line (as Wof cooler temperatures (1.;"'20'C). Aphid lines containing the bachia bacteria are in many insects), the symbionts provide during the first 6 days of reproduction compared with that have fast growth rates and can thus change more rap between thenmotolerance and fecundity allows the pea idly under el'wirorunental stresses than invertebrates or ver aphids and tebrates. Rosenberg et aJ. (2007) describe four mechanisms have pointed out, advantageous mutations will spread by which microorganisms may confer greater adaptive more qUickly potential to the whole organism than can the host genome because of the rapid reproductive rates of bacteria. This alone.

First, the relative abundance of microorganisms asso may be especialJy important in species such as aphids that a second system of inheritance (Gilbert and EpeI 2009). Most symbiotic relationships involve microorganisms short-promoter Buchnera produce more nymphs per day aphid lines containing long-alJele Buchnera. This trade-off Buchnera to diversify. As Rosenberg et al. (2007) in bacterial genomes than in host genomes ciated with the host can be changed due to environmental are produced parthenogenetically (without males) and pressure. Secondr adaptive variation can result from the therefore are essentialJY a clonal population. The symbionts introduction of a new symbiont to the community.

Third, can be responsible for the variation needed to survive in changes to the microbial genome can occur tluough recom different environments. Such partnerships, where thermo bination or random mutation, and these changes can occur in a microbial symbiont more rapidly than in the host. And fourth, there is the possibility of horizontal gene transfer between members of the symbiotic community.

tolerance is provided by the symbiont, are also seen in coral and Christmas cactuses (see Gilbert et aJ. 2010). In addition to providing selectable epigenetic variation for homeostatic functions (i.e., thermotolerance), experi One example of symbionts conferring variation to the ments on mice indicate there may be instances where dif entire organism involves pea aphids and their symbiotic ferent symbiotic bacterial populations alter the develop bacteria. The pea aphid Acrylhosiphon pisllm and its bacte ment of an organism. When mice with mutations in their rial symbiont Bllchnera aphidicofa have a mutualJy obligate symbiosis. That is, neither the aphids nor the bacteria will leptin genes become obese, their guts contain a 50% higher proportion of Firmicutes bacteria and a 50% reduction in flourish without their partner. Pea aphids rely on Buchnera Bacteroidetes bacteria than wild-type mouse guts. More to provide essential amino acids that are absent from the over, when the gut symbionts from the obese mice were pea aphids' phloem sap diet (Baumann 2005). In exchange, transplanted into genetically wild-type germ-free mice, the pea aphids supply nutrients and l)tracellular niches these mice gained 20% more weight than those germ-free that permit the mice receiving gut microbes from wild-type mice (Figure Buchnera to reproduce (Sabeter et aJ. 2(01). 1 9.23). 11,US, there may be an interaction between the genotype of the host and the types of micro bial symbionts that are selected by that host envi ronment (Ley et aJ. 2005; Turnbaugh et aJ. 2006). Together, they generate a particular phenotype in this case, obesity. FIGURE 19.23 M ice with mutations in the feptin genes are gen eti cal ly obese.

Their gut m i crobes are also different from those of wild-type mice, having 50% more Firmicutes and 50% less Bacteroidetes. The mix of microbes in the obese mouse's intestine is more effective at releasing calories from food. When the bacteria from obese mice are transferred into wild type mice, the wild-type mice also become obese. (Photograph © Science VU Visuals Unlimited.) 705 DEVELOPMENTAL MECHANISMS OF EVOLUTIONARY (HANGE with broken crossveins induced by heat shock at the pupal Genetic assimilation In the early 1900s, some evolutionary biologists speculated stage rose to above 90%. Moreover (and Significantly), by tha t the environment could select one of a variety of envi viduals were crossveinless even among flies of this line that generation 14 of such inbreeding, a small proportion of indi In had not been exposed to temperature shock. When other words, the environment could both induce and select the adults that had developed the abnormality without heat ronmentally induced phenotypes, and that this phenotype would then become the dominant one for the species. Waddington extended this artificial selection by breeding a phenotype. But these scientists had no theory of develop shock, the frequency of crossveinless individuals among ment or genetics to provide mechanisms for their hypothe untreated flies became very high, reaching 100% in some ses. This idea was revisited in the middle of the twentieth lines. The phenotypically induced trait had become "genet "fix" a particular envi century, and at that time several models were proposed to explain how constant selection could ically assimilated" into the population.' In addition to finding the crossveil1.1ess phenotype on ronmentally induced phenotype in a population. exposure to heat shock, Waddington showed that his lab One of the most important hypotheses of such plastic oratory strains of Drosophi/a had a particular reaction norm ity-driven adap tation schemes is the concept of genetic in their response to ether. Embryos exposed to ether at a assimilation, defined as the process by which a phenotyp particular stage developed a phenotype similar to the bitho ic character initially produced only in response to some rax mutation and had four wings instead of two. The flies' environmental influence becomes, through a process of haiteres-balancing structures. on the third thoracic seg selection, taken over by the genotype so that it is formed ment-were transformed into wings . Generation after gen eration was exposed to ether, and individuals shOWing the even in the absence of the environmental influence that had first been necessary (King and Stanfield 1985). The idea four-winged state were selectively bred each time. After of genetic assimilation was introduced independently by 20 generations, the mated Drosophila produced the mutant Waddington (1942, 1953, 1961) and Schmalhausen (1949) phenotype even when no ether was applied (Figure 19.24; to explain the remarkable outcomes of artificial selection Waddington 1953, 1956). experiments in which an environunentally induced pheno-Subsequen t experiments have borne out Waddington's type became expressed even in the absence of the external findings (see Bateman 1959a,b; Matsuda 1982; Ho et al. stimulus that was initially necessary to induce it. 1983). In 1996, Gibson and Hogness repeated Wadding GENETIC ASSIMILATION IN THE LABORATORY Genetic assim 1996). Moreover, they found that four alleles of the Ultra ilation is readily demonstrated in the laboratory, as bit/lOrax (Ubx) gene had existed in the population and were ton's bithorax experiments and got similar results (Gibson Waddington's many experiments proved.

For example, Drosophila melanogaster were exposed to a heat shock of 4QOC, when pupae from a laboratory population of wild-type FIGURE 19.24 Phenocopy of the bithor.x mutation. (A) A bitho rax (four-winged) phenotype produced aher treatment of Ihe embryo with ether. The forew i ngs have been removed to show the aberrant metathorax. This particular individual is actua l ly from th e "assimilated" stock that produced this phenotype without being exposed to ether. (B) Sel ection experiments for or against the bilhorax-like response to ether treatment. Two experiments are shown (red and blue l i nes). In both cases, one group was selected for the trait and the other group was selected again st the trait. (After Waddington 1 956.) some of the emerging adults exhibited in their wings a gap in the posterior crossveins. This gap is not normally present in untreated flies (Waddington 1952, 1953). Two selection regimens were followed, one in which only the aberrant flies (termed crossvein/ess) were bred to one another, and another in which only non-aberrant flies were mated. After some generations of selection, when only' the individuals show ing this gap were allowed to breed, the proportion of adults *Note that in these artificial selection experiments, the orig inal phenotype induced by the environe.nt was not intrinsi cally adaptive. Only the hand of the experimenter choosing which flies mated made it so. (A) (B) 70 ::R " - 60 .; b on c l 50 = • 30 • = 20 -• 10

correlate with major transitions 14. Co-option (recruitment) of existing genes and path sion can explain the origms of novel structures such the limbless phenotypes of snakes. 11. in the evo only six legs is one example; the evolution of the ing genes. Changes 8. in properties that may have significant developmental 6. Evolution can occur through the "tinkering" of exist 7. in the timing of gene development at the level of gene expression are: 22. Genetic assimilation, wherein a phenotypiC charac ter initially induced by the environment becomes, though a process of selection, produced by the geno type in all permissive environments, has been well documented in the laboratory. 23. Evolutionary developmental biology is able to show ate large phenotypiC changes and enable the produc how small genetic or epigenetic changes can gener tion of new anatomical struChlres. DEVELOPM ENTAL MECHANISMS OF EVOLUTIONARY CHANGE For Further Reading Complete bibliograph.ical citations found at the free access website 711 for aU literature cited in thi> chapter can be www.devbio.com Abzhanov, A., W. P. Kuo, C. Hartmann, P. R Grant, R Grant and C. Tabin. 2006. The calmodulin pathway and evolution of beak morphology in Darwin's finch es. Nature 442: 563-567. Epigenetics, Medicine alld Evolutian. Sin auer Associates, Sunderland, tvtA. Amundson, R 2005. The Changing Role of the Embryo ill Evolutionary Thought: Roots of Evo-Devo. Cambridge Universi Lynch, V. j., A. Tanzer, Y. Wang, F. C. Leung, B. Gelelrsen, D. Emera and G. P. Wagner. 2008. Adaptive changes in the transcription f,letar HoxA-l1 are essen tial for the evol ution of pregnancy in mammals. Pmc. Natl. Acad. Sci. USA. 105: 14928-14933. ty Press, New York. Carroll, S. B ., 2006. Endless Forms Most Beautiful: The New Science of Eva-Dew. Norton, New York. Cohn, M. j. and C. Tickle. 1999. Devel opmental basis of limblessness and axial patterning in snakes. Nature 399: 474-479. Gilbert, S. F. and D. Epel. 2009. Ecologi cal Developmental Biology: lntegrating , Kuratani, S. 2009. Modularity, compara live embryology and eva-cleva: Devel opmental dissection of body plans. Dev. BioI. 332: 61-69. Merino, R,j. Rodriguez-Leon, D. MaCias, Y. Ganan, A. N. &onomides and j. M. Hurle. 1999. The BMP antago nist Gremlin regulates outgrowth, chon drogenesis and programmed cell death in the developing limb. Development 126: 5515-5522. Rockman, M. v., M. W. Hahn, N. Soran zo, D. B. Goldstein and G. A. Wray. 2003. Positive selection on a lurnan specific transcription factor binding site regulating IL4 expression. Curro BioI. 13: 2118-2123. Shapiro, M. D. and 7 others. 2004. Genetic and developmental basis of evolutionary pelvic reduction in truce spine sticklebacks. Natllre 428: 717-723. Shubin, N., C. Tabin and S. B. Carroll. 2009. Deep homology and the origins of evolutionary"novelty. Nature 457: 818-823. Smith, K. 2003. Time's arrow: Hetero chrony and the evolution of develop ment. Int. J. Dev. BioI. 47: 613-621. Go Online Relating evolution to development in have been the precursor of both protostomes and deuteros the nineteenth century. Immediately after Darwin's Ori tomes. This organism may have resembled the planula lar gin of Species biologists attempted to relate evolution to vae of contemporary cnidarians. WEBSITE 19.1 changes in development.

Here the attempts of three such scientists-Frank Lillie, Edmund B. Wilson, and Ernst Haeckel-are highlighted. WEBSITE 19.2 Correlated progression. In many cases, modules must co-evolve. The upper and lower jaws, for WEBSITE 19.4 How the chordates got a head. The neu ral crest is responsible for forming the heads of chordates. But how did the neural crest come into existence? It is probable that ancestral deuterostomes had all the requisite genes, but only in the chordates did these genes become instance, have to fit together properly. If one changes, so linked together into the network that became the neural must the other. If the sperm-binding proteins on the egg crest cell. change, then so must the egg-binding proteins on the sperm. This site looks at correlated changes during evolu tion. WEBSITE 19.5 "Intelligent design" and evolutionary developmental biology.

Evolutionary developmental biol ogy explains many of the "problems" (such as the evolu The seach for the Urbilaterian ancestor. tion of the vertebrate eye and the evolution of turtle shells) Homologous genes specifying the formation of the eye, that proponents of "intelligent design" and other creation heart, body axis, and nervous system enable biologists to ists claimed were impossible to explain by evolution. WEBSITE 19.3 intuit an "Urbilaterian ancestor"-an organism that may G lossary Allantois A Achondroplasic d wa rfism Condition wherein chondrocytes stop proliferat ing earlier than usual, resulting in short limbs. Often caused by muta tions that activate FgJR3 prematurely. Drosophila head; includes the brain. Acrosome (acrosomal vesicle) Cap Aeron The terminal portion of the like organeUe containing proteolytic enzymes that, together with the sperm nude us, (orms the sperm head. The Ca2+ -depend ent fusion of the acrOSOlfle with the sperm cell membrane, resulting i n exo cytosis and release of proteolytic enzymes that allow the sperm to pene tTate the egg extraceUular matrix and Acrosome reaction fertilize the egg. Ade pith cl ial cells Cells that migrate into the insect imaginal disc early in development. During the pupal stage, these cells give rise to the muscles and nerves that serve the leg.

Attachment between cells or between a cell and its extracellular substrate. The latter provides a surface Adhesion for migrating cells to travel along. Adult stem cells Stem cells found in the tissues of organs after the organ has matured. Adult stem celJs are usually involved in replacing and repairing tis sues of that particular organ. and can form only a subset of ceU types. Afferent ne u ron s Neurons that carry inIonnation from sensory receptor cells (e.g .• sound waves from the ear, light signals from the retina. touch or pain sensations from the skin) to the central nervous system (spinal cord and brain). Agi n g The time-related deterioration of the physiological functions necessary for survival and fertility. In aITUl.iote species, extraem bryonic membrane that stores urinary wastes and helps mediate gas exchange. It is derived from splanchnopleure at the caudal end of the primitive streak. ln mammaLs, the size of the allantois depends on how well nitrogenous wastes can be removed by the chorionic placenta. In l'ep l'i les and birds, the allantois becomes a large sac, as there is no other way to keep the toxic by-prod ucts of metabolism away from the developing embryo. Allometry Developmental changes that occur when diHerent parts of an organ ism grow at different rates. Alternative splicing A means of pro ducing multiple different proteins encoded by a single gene by splicing together different sets of exons to gen erate different types of mRNAs. AmetaboloU5 A pattern of insect devel opment in which there is no larval stage and the insect undergoes direct development to a small adult form fol10wh'g a transitory pronymph stage. A rn n i ocent.esis Medical proced ure that removes a sample of amnionic fluid around the fourth or fifth month of pregnancy.

Fetal ceUs from the fluid are cultured and analyzed for the pres ence or absence of certain chrol1lo somes, genes, or enzymes.

" Wa ter sac." A membrane enclosing and protecting the embryo Amnion and its surrounding amnionic fluid.

Defining the "aITUl.iote vertebrates," this epithelium is derived from somatopleure. Etodermcil tissue sup plies epithelial cells, and the l1lesoclenn generates the essential blood supply. fluid A secretion that serves as a shock absorber for the developing embryo while preventing it from dry ing out. Amnionic Vertebrates whose embryos form an am.n.ion: the reptiles, birds, Amniotes and mammals. Latin, "flask." The segment of the mammalian oviduct, distal to the uterus and near the ovary, where fertil ization takes place. Ampulla A n al ogo us SLTuctures and/or their respective components whose similari ty arises from their performing a simi lar function rather than their ariSing from a common ancestor (e.g., the wing of a butterfly vs. the wing of a bird). Compare with homologous. The cell connecting the overlying gonad to the vulval precur sor celJs in C. degans. If the anchor cell is destroyed.. the VPCs will not form a Anchor cell vulva. but instead become part of the hypodermis (skin). Androgen insensitivity syndrome lntersex condition in which an XY individual has a mutation i n the gene encoding the androgen receptor pro tein that binds testosterone. This results in a female external phenotype, lack of a uterus and oviducts and pres ence of abdominal testes. Anencephaly A lethal congenital defect resulting from failure to dose the anterior neuropore. The forebrain remains in contact with the am.n.ionic fluid and subsequently degenerates, so the vault of the skull fails to form. Angioblasts From allgio. blood vessel, and blnst, a rapidly dividing cell (usu ally a stem cell). The progenitor ceUs of blood vessels.

Angiogenesis Process by which the primary network of blood vessels cre ated by vasculogenesis is remodeled and pruned into a distinct capillary bed, arteries, and veins. G-2 GLOSSARY Angiopoietins P�racrine factors that mediate the interaction between endothelial cells and pcricy les.

Animal cap In a mph i bia ns, the roof of the blastocoel (in the animal hemi sphere). Animal hemisphere The non-yolk con taining (upper) haJJ of the amphibian egg. During embryogenesis, cells in the animal hemisphere divide rapidly and become actively mobile ("nimated"). Anoikis Rap id apoplosis that occurs when ep ithelia l ceUs lose their attach ment to the extracellular matrix. Antennapedia complex A region of Drosophila chromosome 3 containing the homeotic genes labial (lab), Anlell /fopet/in (Antp), sex combs reduced (sa), defonned (dfd), and probescipedia (Pb), specify head and thorade seg ment identities.

Anterior heart lield Cells 01 the heart field forming the outflow tract (conus and ITuncus arteriosus, right ventricle). Anterior intestinal portal (AlP) The posterior opening of the developing foregut region of the primitive gut tube; it opens into the future midgut region which is contiguous with the yolk sac at this stage. Anterior visceral endoderm (AVE) Mammalian equivalent to the chick hypoblast and similar to the head POI Han of the amphibian organizer, i t cre ates an anterior region by secreting antagonists of Nodal. Anterior-posterior (anteroposterior) axis The body axis extending from head to tail (or mouth 1"0 anus in those organisms that lack a head and tail). When referring to the limb, this refers . to the thumb (anterior)-pinkie (posteri or) axis. Anti-Mullerian factor (AMF) TGF-� family paracrine faclor secreted by the embryoniC testes thai induces apopto sis of the epithelium c'd destruction of the basal lamina of the Mullerian duct, preventing formation of the uterus and oviducts. Fonnerly known as anti-MUllerian hormone, or AMH. Anurans Frogs dJld toads. Aorta-gonad-mesonephro5 region (AGM) A mesnchymal area in the lateral plate splanchnopleure near the aorta that produces the controversial putative definitive hematopoietic stem cells of the fetus and adult. Aortic arches These begin as symmet ri ca lly arranged, pelired vessels that develop within the paired pharyngeal arches and link the ascending and descending/d orsal paired aortae.

Some arches degenerate. Left aortic arch IV becomes the Arch of the Aorta which and aortic arch VI is the ductus arterio sus on the left.

Apical ectodermal ridge (AER) A ridge along the distal margin of the limb bud that will become a major sig naling center for the developing limb. Its roles include (1) maintaining the mesenchyme beneath it in a plastic, proliferating state that enables the lin ear (proximal-distal) growth 01 the limb; (2) main taining the expression of those molecules that generate the ante rior-posterior axis; factors regulate gene expression to direct the cell n i to a particular path of development. Autopod The distal limb bones of any vertebrate limb, consisting of carpals and metacarpals (forelimb), tarsals and metatar""ls (hindlimb) and the pha langes (fingers and toes). Axon Continuous extension of the nerve cell body. Transmits signals (action potentials) to targets in the cen LTal and peripheral nervouS systems. Axonal migration is crucial to develop ment of the vertebrate nervouS system. B Basal disc The "foot" of a hydra, which enables the animal to stick to rocks or t.he undersides of pond plants. Basal lamina Specialized, closely knit sheets of extracellular matrix that underlie epithelia, composed of largely of laminin and type IV collagen. Epithelial cells adhere to the basal lam ina in part via binding between inte grins and laminin. Sometimes called the basement membrane. Basal layer (stratum germinativum) The inner layer of both the embryOniC and adult epidermis, it contains epider mal stem cens attached to a basement membrane that they help to make. p-Catenin A protein that can act as an anchor for cadherins or as a transcrip tion factor (induced by the Wnt path way). It is important in the specifica tion of germ layers throughout the animal phyla. bHLH proteins The basic helix-loop helix family of tran�ription factors. It includes such proteins as scleraxis, the MRFs (MyoO, My15, and myogenin), and c-Myc. Bicoid Anterior morphogen critical for establishing anterior-posterior polarity in the Drosophila embryo. Functions as a transcription factor to GLOSSARY Bipotential gonad (indifferent go nad) A common precursor, derived from the genital ridge, from which both the male and female gonads diverge. Bisphenol A (BPA) Synthetic estrogenic chemical compound used in plastics ing contact with the outside surface by way of a slender neck. Brain-derived neurotrophic factor (BDNF) A paracrine factor that regu l(ltes neural activity e1.nd ap pears to be critical for synapse formation by inducing local translation of neur al and flame retardants. BPA has been assodated with meiotic defects, repro ductive abnormalities, and precancer ous conditions in rodents. messages in the dendrites. BONF is required for the survival of a particular subset of neurons in the slT ia rum (a region of the brain involved in movement). Bithorax complex The second region of Drosophila chromosome 3 containin g the homeolic genes Ultrabithorax (Ubx), which is requi red for the identity of the third thoracic segment, and the nbdominal A (a/JdA) and Abdominal B (AbdB) genes, which are respunsible for the segmental identities of the abdominal segments. Blastocoel A fluid-filled cavity that forms n i the animal hemisphere of early amphibian and echinoderm embryos, or between the epiblas t and hypoblast of avian, rep til i an and mam malian blas toderm stage embryos. Blastocyst A mammalian blastula. The blastocoel is expanded and the inner cell mass is positioned on one slde of the ring of trophOblast cells. Blastodisc Small region at the arumal pole of the telolecithal eggs of fish and chicks, conta ining the yolk- free cy to plasm where cleavage can occur and that gives rise to the embryo. Follow ing clea vage, the blastodisc becomes the blastoderm.

Blastomere A cleavage-stage cell resulting fmm mitosis. Blastopore The invagination point whe re gas tru la tion begins.

irl deute rostomes, this marks the site Of the anus. In protostomes, this marks the site of the mouth. Blastula Ear ly-stage embryo consisting of a sphere of ceUs surrounding an inner Ouid-filled cavity, the blastocoel. Blood islands Aggregations of heman: giobl asts in the s planchni c mesoderm. It is gene ral ly thought that the inner cells of these blood islands become blood progenitor celis, while the outer cells become angioblasts. Bone marrow-derived stem cells (BMDCs) See Mesend1yma l stem cells. Bone morphogenetic proteins (BMPs) Members of the TGF-p superfaenily. OdginaUy identified by their ability to ind uce bone formation, they are extremely mul ti fwlctiona t having been found to regulate cell djvision, a poptosis, cell migration, and differen tiation. Bottle cells ln vaginating cells during amphibian gastrulation, the main body of each cell is displ aced toward the inside of the embryo while maintain- Bulge A regi on of the hair follicle that selV'es as a niche for adLlit stem cells. C Cadherins Calcium-dependent adhe sion molecules. Transmembrane pro- teins thai interact with other cadherins on adjacen t cells and are critical for establishing and maintaining intercel lular connections, spa tial segregation of cell types, and the organ iza tion of animal form. Calorie restriction Dietary restriction as a means of ex tend i ng mammalian longevity (at the expense of fertility). Cancer stem cell hypothesis The hypothesis that the malignan t part of a tumor is eHher an adult stem cell that has escaped the control of its ruche or a more differentiated cell that has regained stem cell properties. Cap sequence A 5' cap is added to nuclear RNA before it leaves the nucle us and is necessary for the binding of mRNA to the ribosome and for subse quent translation. Capacitation The set of phYSiological changes by which spe rm become capa Cardia bifida A condi ti on in which two sepa rate hea rts form, resulting from ma nip ulation of the embryo or ble of fertilizing an egg. genetic defects that prevent fusion of the two endoca rdial tubes. Cardiac neural crest S ubregion of the cranial neural crest that extends from the otic (ea r) placodes to the third somites. Cardiac neural crest cells d evelop into mclanocytes, neurons, cartilage, and connective tissue. Car diac neural crest also ciontribu tes to the muscular-contlective tissue wa l l of the large arteries (the "outflow tracts") of the heart, as well as contributing to the septum that separates pulmonary circulation iTom the aorta. Cardiogenic mesoderm (heart field) Two groups of cardiac cells in the later al plate mesoderm, at the level of the node. The cardia.c ce1Js of the heart field migrate through the primitive streak during gastrulation such that - the medial-lateral a rrangement of these early cells will become the ante- G-3 rior-posteriur (rostra l-cauda l) axis uf the developing heart tu be . Cardiomyocytes Cardiac cells derived from cardiogeruc mesoderm that form the muscular layers of the heart and i ts inflow and outflow tracts.

Catenins A compl ex of pruteins that anchor cadherins inside the cell The i cadher n-catenin complex forms the classic adherens j unc tions that help hold epi thel i al cells together and, by binding to the actin (mi crofilament) cy toskeleton of the cell, integrate the epithelial cells into a mechanical unit. One of them, p-catenjn, can also be a tran Caudal intestinal portal (ClP) The anterior opening of the developing hi.ndgut region of the prim.itive gut tube; it opens into the future midgut region which is contiguous with the yolk sac at this s tage. Caudal Referring to the tail. Cavitation A process whereby the tro phob last cells secrete fluid into the mOrulel to create a blastocoel. The membranes of trophoblast cells pump Na ions into the c entral cavi ty, which draw in water osmotical ly, thus creat ing and en l arging the blastocooL Cell lin eage The series of cell types starting from an undifferentiated, p luripoten t stem cell through stages of increasing differentiation, to the te nni nally d ifferentiated cell type. Cellular blastoderm Stage of Drosophi- ia deve lopment .in which all the ceJIs are arrange d in a single-layered jacket around the yol ky core of the egg Centrolecithal Type of egg. such as those of insects, which have yolk in the center an d un dergo superficial cleavage. Centromere A region of DNA where chromatids are at tached to each other by the kine tochore. Centrosome- attracting body (CAB) Cellular structure thaI', in some inver tebrate blastomeres, positions the cen b·osomes asymmetrically and recruits par ticular mRNAs so tha t the resu l ting daughter cells have different proper ties. A transverse furrow formed during gastrulation in Drosophila that separates the future head region (procephalon) from the germ band, which will form the thorax and abdome n . Cephalic furrow Cephalic Referring to the head. Chemoatlractant A biochemical tha t causes cells to move toward it.

Chemotaxis Movement of a cell down a chemical gradient, such as sperm fol lowing a chemical (chemoattractant) secreted by the egg. G-4 G LOSSARY Chiasmata Points of attachment between homologous chromosomes during meiosis which are thought to represent regions where crossing�over is occurring. Chimera an organism consisting of a mixture of cells from two individuals. Chimeric e mbryo Embryo made from tissues of more than one genetic source. Chondrocyte-like osteoblasts Cranial neural crest cells llldergoing early stages of intramembranous ossifica tion. These cells downregulate RlUlX2 and begin expressing the osteopontin gene, giving them a phenotype similar to a developing chondrocyte. Chondrocytes Cartilage cells. Chordamesoderm Axial mesoderm that produces the notochord and head process. Chordate An animal that has, at some stage of its life cycle, a notochord and a dorsal nerve cord or neural tube. Chardin A paracrine factor with organ izer activity. Chordin binds directly to BMP4 and BMP2 and prevents their compl exing with their receptors, thus inducing dorsal ectoderm to form neu ral tissue. Chorioallantoic membrane Fo rms in SOme amniote species, such as chick ens, by fusion of the mesodermal layer of the allantoic membrane with the mesodennal layer of the chorion. This extremely vascular envelope is crucial for chick development and is responsi ble for transporting calcium from the eggshell into the embryo for bone pro duction. Chorion An extraembryonic mem brane essential for gas exchange in amniote embryos. It is generated from trophoblast and extraembryonic meso derm (somatopleure). The chorion adheres to the shell in birds and rep tiles, allowing the exchange of gases between the egg and the environment.

It forms the embryonic/fetal portion of the placenta in mammals. Chorionic villus sampling Taking a sample from the placenta at 8-10 weeks of gestation to grow fetal cells to be analyzed for the presence or absence of certain chromosomes, genes, or enzymes. Chromatid Half of a mitotic prophase chromosome, which consists of dupli cate "sister" chromatids that are attached to each other by the kineto chore. Chromatin The complex of DNA and protein in which eukaryotic genes are contained. Chromosome diminution The frag mentation of chromosomes just prior to cell division, resulting in cells in which only a portion of the original chromosome survives. Chromosome dinllmution occurs during cleavage in Parascaris aequorum in the cells that will generate the somatic cells while the future germ cells are protected from this phenomenon and maintain an intact genome. Ciliary body A vascular structure at the junction between the neural retina and the iris that secretes the aqueous humor. cis-regulatory elements Regula tory elements (promoters and enhancers) that reside on the same strand of DNA. Cleavage furrow A groove formed in the cell membrane in a dividing cell due to tightening of the microfilamen taus ring. Cleavage A series of rapid mitotic cell divisions following fertilization in many early embryos; cleavage divides the embryo without increasing its mass. Cloaca Latin, "sewer." An endoder mally lined chamber at the caudal end of the embryo that will become the waste receptacle for both the intestine and the kidney. Amphibians, reptiles, and birds retain this organ and use it to void both liquid and solid wastes. In mammals, the cloaca becomes divided by a septum into the urogenital sinus and the rectum. Cloacal membrane Caudal end of the primitive streak formed by closely apposed endoderm and ectoderm, future site of the anus. Cloning transfer. See Somatic cell nuclear Coelom Space between the somatic mesoderm and splanchnic mesoderm that becomes the body cavity. In mam- mals, the coelom becomes subdivided into the pleural, pericardial, and peri toneal cavities, enveloping the thorax, heart, and abdomen, respectively. Coherence Scientific evidence that fits into a system of other findings and is therefore more readily accepted. Cohesin proteins Protein rings that encircle the sister chromatids during meiosis, provide a scaffold for the assembly of the meiotic recombination complex, resist the pulling forces of the spindle micro tubules, and thereby keep the sister chromatids attached together and promote recombina tion.

Combinatorial association In devel opmental genetics, the principle that enhancers contain regions of DNA that bind transcription factors, and it is this combination of transcription factors that activates the gene. Commensalism A symbiotic relation ship that is beneficial to one partner and neither beneficial nor harmful to the other partner. Commitment Describes a state in which a cell's developmental fate has become restricted even though it is not yet displaying overt changes in cellu lar biochemistry and function.

Committed stem cells Describes the multipotent and WLipotent stem cells, which have the potential to become relatively few cell types. Compaction A wtique feature of mam malian cleavage, mediated by the cell adhesion molecule E-cadherin. The cells in the early (around eight-cell) embryo change their adhesive proper ties and become tightly opposed to each other.

Comparative embryology Study of how anatomy changes during the development of different organisms. Compensatory regeneration Form of regeneration in which the differentiat ed cells divide but maintain their dif ferentiated functions (e.g., mammalian liver).

Competence The ability to respond to a specific inductive signal. Conditional specifi cation The ability of cells to achieve their respective fates by interactions with other cells. What a cell becomes is in large measure speci fied by paracrine factors secreted by its neighbors. Congenital adrenal hyperplasia A condition causing female pseudoher- maphroditism due to the presence of excess testosterone. Congenital defect Any defect that an animal or person is born with. Con genital defects can be hereditary or they may have an environmental cause (e.g., exposure to teratogenic plants, drugs, chemicals, radiation, etc.). They may also be "idiopathic" (we don't know the cause). Consensus sequence Located at the 5' and 3' ends of the introns that signal the "splice sites" of the intron. Contact inhibition The mechanism for directional cell movement wherein cells are prohibited from moving "backwards" due to interactions with th e cell membranes of other migrating cells. Context-dependent properties A principle of the theoretical systems app roach: The meaning or role of an individual component of a system js dependent on its context. Conus arteriosus Cardiac outflow tract, precursor of both ventricles. Convergent extension A phenomenon wherein cells intercalate to narrow the GLOSSARY tissue and at the same time move it forward. Mechanism used for elonga tion of the archenteron in the sea urchin embryo, notochord of the tuni cate embryo, and involuting meso derm of the amphibian. This move ment is reminiscent of traffic on a highway when severa l lanes must merge to form a single lane . Cornified layer (stratum corneum) The outer layer of the epidermis, con sisting of kerafulocytes that are now dead, flattened sacs of keratin protein with their nuclei pushed to one edge of the cell. These cells are continually shed throughout IHe and are replaced by new ceUs. Corona radiata The innermost l ayer of cumulus ceils, immediately adjacent to the zona pellucida. Corpora alia ta Insect glands that secrete juvenile hormone during larval molts. Correlative evidence Evidence based on the association of events. An outer structure (in con trast with. medulla, an inner structure). Cortex Cortical cytoplasm A thin layer of ge1like cytoplasm ly ing i mmed ia tely beneath the cell membrane of most eggs. The cortex contains high concen trations of globular actin molecules that will polymerize to form microfila ments and microvilli during fer tiliza tion . Cortical gra n u le reaction The basis of the slow block to polyspermy in many animal species, incl uding sea urchins and most mammaJs. A mechamcal b lock to polyspermy that becomes active about a minute after successful- sperm-egg fusion, in which enzymes from the egg's corticaJ granules con tribu te to the forma tion of a fertiliza tion envelope that blocks further spenn entry. Cortical gran ules Membrane-bound, Golgi-derived structures located in the egg's cortex that c on tain proteolytic enzymes and are thus homologous to the acrosomal vesicle of the sperm. CpG islands Regions of DNA rich in cytosine and guanosine that flank many promoter TATA box regions. Cranial (cephalic) neural crest cells Neural crest cells in the future head region that migrate to produce the craniofacial mesenchyme, which dif ferentiates into the cartilage, bone, cra nial neurons, glia, and connective tis sues of the face. These cells also enter the pharyngeal arches and pouches to give rise to thymic cells, the odonto blasts of the tooth primordia, and the bones of the middle ear and jaw. Cra ni a l ecto de r mal pi a codes Epider mal thkkenings that form neurons and sensory epitheli a. Cranium The vertebrate skull, com posed of the neurocranium (skull vault and base) and the viscerocranium (jaws and other pharyngeal arch deriv a tives) . Crossing over The exchange of genetic material d uring meiosis, whereby genes from one chromatid are excha.nged with hn mnlogous genes from another. Crystallins Transparent, lens-specific proteins. Cumulus oophorus A l ayer of cens surrounding the mammalian egg.

which is made up of the innermost layer of ovarian follicular (granulosa) cells that were nurturing the egg a t the time of its release hom the ovary. Cutaneous appendages Species-spe cific epidermal modifications that include hairs, scales, feathers, hooves, claws, and homs. Cyclic adenosine 3',S'-monophosphate (cNvtP) An important component of severa l intraceUular signaling cascades and the soluble chem otac tic substance tha t directs the aggregati on of the myxa moebae of Dictyosteliunl to fonn a grex. The larger subunit of mitosis promoting factor, shows the cyclical behavior that is key to mitotic regula tion, accumulating during S and being degraded after the ceUs have reached M.

Cyc lin B regulates the small sub unit of MPF, the cyclin- Cyclin B Cyclin-dependent kinase Small sub wtit or MPF, activates mitosis by phos phorylating several target proteins, includhlg rustones, the nuclear enve lope Jamin proteins. and the regulatory subwut or cytoplasmic myosin result ing in chroma it n condensation, nuclear envelope depolymerization, and the organiza �ion or the mitotic spindle. Requires cyclin D to function. Cyclooxygenase-2 (COX2) An enzyme that generates prostaglandins from the faH.y acid, arachidonic acid. Cyclopia Congenital defect character ized by a single eye. caused by muta genes that encode either Sonic i tions n hedgehog or the enzymes that synthe size cholesterol and can be induced by certain chemicals that interfere \with the cholesterol biosynthetic enzymes Cytoblasts/cystocytes Derived from the asymmetric division of the germJine stem cells or Drosophila, a cys toblast undergoes four mitotic divi sions wi th incomp lete cytokinesis to form a cluster of 16 cys tocytes (one G-S ovum and 15 nurse cells) interconnect ed by ring canals. Cytokines Paracrine factors that are collected and concentrated by the extracellular matrix of the stromal (mesenchymal) ceUs at the sites of hematopoiesis and are involved in blood ceU and lymphocyte formation. The division of the cell cytopllsm into two daughter cells. The mechaluca l agenl of cytokinesis is a Cytokinesis contractile ring of microfilaments made of actin. Each da ugh ter cell receives one of the nuclei produced nuclear division (ka ryokhlesis). by Cytoplasmic bridges Continuity between adjacent cells that results from incomplete cytokinesis, e .g ., during gametogenesis. Cytotrophoblast Mammalian extra embryonic epithelium composed of the original trophoblast ceUs, it adheres to the endometrium through adhesion molecules and, in species with invasive placentation such as the mouse and hu man, secretes pro teolytic enzymes that enables the cytotro phoblast to enter the uterine wall and remodel the uterine blood vessels so that the maternal blood ba thes fetal blood vessels. D A metabolically dormant larval stage in C. elegans. See also diapause. Dauer larva The maternal portion of the placenta, made from the endometrium of the u terus. Decidua Deep cells A population of coUs in the zebrafish blastula between the EVL and the YSL tha t give rise to the embryo proper. Deep homology Signal transduction pathways composed of homologous proteins arranged in a homologous manner tha t are used for the same function in both protostomes and deuteroslomt:.s. Delamination The splitting of one cel lular sheet into two more or l ess paral lel sheets. CeU-surface ligand for Notch, participates in juxtacrine inter action and activation of the Notch pathway. Della protein The fine, branching exten sions or the neuron that are used to pick up electric impulses from other cells. Dendrites Dermal bone Bone that forms in the dermis of the skin, such as most of the bones of the �kull and face. They can be derived from head mesoderm or cranial neural cres t-der ived m esenchy mal cells. G-6 G LOSSARY Dermal papilla A component of mes enchymal-epithelial induction during hair formation; A small node formed by dermal fibroblasts beneath the epi dermal hair germ that stimulates pro Hferation of the overlying epidermal basal stem cells. Oermamyotome Dorsolateral portion of the somite that contains skeletal muscle progenitor cells, (including those that migrate into the limbs) and the cells that generate the dermis of the back. Dermatome The central portion of the dermamyotome that produces the pre cursors of the dennis of the back and a third population of muscle ceUs. Descent with modification Darwin's theory to explain unity of type by descent from a common ancestor with changes creating the adaptations to the conditions of particular environments. Determination The second, and irre versible, stage of cell or tissue commit ment in which the cell or tissue is capable of differentiating autonomous ly even when placed into a non-neutral environment. Deuterostomes In the deuterostome animal phyla (echinoderms, hmicates, cephalochordates, and vertebrates), the first opening (i.e., the blastopore) becomes the anus while the second opening becomes the mouth (hence, deuteto stoma, "mouth second"). Com pare with protostomes. Development The process of progres sive and continuous change that gen erates a complex muJticellular organ ism from a si.ngle cell. Development occws throughout embryogenesis, maturation to the adult form, and con ' tinues into senescence. Developmental constraints on evolu tion The number and forms of possi ble phenotypes that can be created are li.mited by the interactions that are possible among molecules and between modules. Deve lopm en ta l plasticity The ability of an embryo or larva to react to an environmental input with a change in form, state, movement or rate of activ ity (i.e., phenotypic change).

Diacylglycero l (DAG) Second messen ger generated in the lPJ pathway from membrane phospholipid phos phatidylinositoI 4,5-bisphosphate (PlP2), along with]P3. DAG activates protein kinase C, which in tum acti vates a protein that exchanges sodium ions for hydrogen ions, raising the pH. Diapa use A metabolically dormant, nonfeeding stage, during which devel opment and aging are suspended, that can occur at the embryonic, larval, pupal, or Dickkopf German, "thick head," "stubborn." A protein that interacts directly with the Wnt receptors, pre venting Wnt signaling.

Di en cephalo n The caudal subdivision of the prosencephalon, will form the optic vesides, retinas,and the thalamic and hypothalamic brain regions, which receive neural input from the retina. Diethylst ilbestrol (DES) A potent enviroruental estrogen. DES adminis tration to pregnant women interferes with sexual and gonadal development in their female offspring resulting in infertility, sub fertility, ectopic pregnan cies, adenocarcinomas, and other effects.

Differential adhesion hypothesis A model explaining patterns of ceU sort ing based on thermodynamic princi ples. CeUs interact so as to form an aggregate with the smallest interfacial free energy and therefore, the most thermodynamically stable pattern. Differential expressio n A basic princi ple of developmental genetics; In spite of the fact that all the cells of an indi vidual body contain the same genome, the specific proteins expressed by the different cell types are widely diverse. Differential gene expression, differen tial nRNA processing, differential mRNA translation, and differential protein modification all work to allow the extensive differentiation of cell types.

Differentiation The process by which an unspecialized ceU becomes special ized into one of the many cell types that make up the body.

Digestive tube The primitive gut of the embryo, which extends the length of the body from the pharynx to the cloaca. Buds from the djgestive tube form the liver, gallbladder, and pan creas.

Sa-Dihydrotestosterone (DHT) A steroid hormone derived from testos terone by the action of the enzyme 50, ketosteroid reductase 2. DHT is required for masculinization of the male urethra, prostate, penis, or scro tum.

Diplobla st ic Refers to "two-layer" ani mals of certain phyla, such as the poriferans (sponges) and ctenophores (comb jelUes), that lack a true meso derm, in contrast to triploblastic ani mals. Direct development Embryogenesis characterized by the lack of a larval stage, where the embryo proceeds to construct a small adult. Discoidal Meroblastic cleavage pattern for telolecithal eggs, in which the cell divisions occur only in the small blas todisc, as in birds, reptiles, and fish. Disru ption Abnonnality or congenital defect caused by exogenous agents (teratogens) such as plants, chemicals,. viruses, radiation, or hyperthermia. Dissociation The ability of one module to develop differently from other mod ules, Distal tip cell A single nondividing cell located at the end of each gonad in C. elegans which maintains the nearest germ cells in mitosis by i.nhibiting their meiotic differentiation, D izygotic "Two eggs." Describes twins that result from two separate fertiliza tion events (fraternal twins). Compare with monozygotic. DNA-binding domain Transcription factor domain tha t recognizes a partic ular DNA sequence. DNA me thyla tio n A method of con trolling the level of gene transcription in vertebrates by the enzymatic methy lation of the promoters of inactive genes. Certain cytosine residues that are followed by guanosine residues are methylated and the resulting methyl cytOSine stabilizes nucleosomes and prevents transcription factors from binding.

Important in X chromosome inactivation and DNA imprinting. Dorsal blastopore li p Location of the involuting marginal zone cells of amphibian gash-ulation. Migrating marginal ceDs sequentially become the dorsal lip of the blastopore, tum inward and travel along the inner sur face of the outer animal hemisphere cells (Le., the blastocoel roof). Dorsal closure A process that brings together the two sides of the epidermis of the Drosophila embryo together at the dorsal surface. Dorsal root ganglia (DRG) Sensory spinal ganglia derived from the neural crest lying laterally paired and dorsal ly to the spinal cord. Sensory neurons of the ORG connect centrally with neu rons i.n the dorsal horn of tile spinal cord. Dorsal-ventral (dorsoventral) axis The line extending from back (dorsL1m) to belly (ventrum).

When referring to the limb, this axis refers to the knuckles (dorsal) and palms (ventral). Dorsolateral hinge points (DlHPs) Two hinge regions, besides the MHP, that fonn furrows near the connection of the avian and mammalian neural plate with the remainder of the ecto derm. Dosage co m p ensatio n Equalization of expression of X chromosome-encoded gene products in male and female cells. Achieved by either (1) doubling GLOSSARY the transcription rate of the male X chromosomes (Drosophila), (2) partially repressing both X chromosomes (C ele gans), or (3) inactivating one X chromo some in each female cell (mammals). Double-negative gate A mechanism whereby a repressor locks the genes of specification, and these genes can be wuocked by the repressor of that repressor.

(In other words, activation by the repression of a repressor.) Ductus arteriosus A vessel that forms from left aortic arch VI, serves as a shtUlt between the embryonic/fetal pulmonary artery and the descending aorta. It normally closes at birth (if not, a pathological condition results called patent ductus arteriosus). Duplication and divergence Tandem gene duplications resulting from repli cation errors. Once replicated, the gene copies can diverge by random mula tions developing different expression patterns and new functions. Dynein The protein attached to the axoneme microtubules that provides the force for sperm propulsion. Dynein is an ATPase, an enzyme that hydrolyzes ATP, converting the released chemical energy into mechan ical energy to allow the active sliding of the outer doublet microtubuies, causing the flagellum to bend.

Dysgenesis Greek, "bad begiIU1ing." Defective development. E Early allocation and progenitor expan sion model An alternative to the progress zone model of proximal-dis tal specification of the limb, wherein the cells of the entire early limb bud are already specified; subsequent cell divisions simply expand these cell populations.

Ecdysone 1J1Sect steroid hormone, secreted by the prothoracic glands,. that is modified in peripheral tissues to become the active molting hormone 20-hydroxyecdysone.

Crucial to insect metamorphosis. Ectoderm Greek ektos, "outside." The cells that remain on either the outside (amphibian) or dorsal (avian, mam malian) surface of the embryo follow- ing gas trulation. Of the 3 germ layers, the ectoderm is the one that forms the nervous system from the neural tube and neural crest and also generates the epidermis covering the embryo. Ectodysplasin (EDA) cascade A gene cascade specific for cutaneous appendage formation. Vertebrates with dysfunctional EDA proteins exhibit a syndrome called anhidrotic ectodermal dysplasia characterized by absent or malformed cutaneous appendages (hair, teeth, and sweat glands). Efferent ducts Ducts that link the rete testis to the Wolffian duct, formed from remodeled tubules of the mesonephric kidney. Efferent neurons Neurons (most often motor neurons) that carry information away from the central nervous system (brain and spinal cord) to be acted on by the peripheral nervous system (muscles). Compare with afferent neurons. Egg chamber The ovary in which the Drosophila oocyte will develop, con taining 15 intercormected nurse cells and a single oocyte. Egg jelly A glycoprotein meshwork outside the vitelline envelope in many species, most commonly it is used to attract and/or to activate sperm. Embryo A developing organism prior to birth or hatching. 111 mammals, the term embryo generally refers to the early stages of development, starting with the fertilized egg until the end of

determinants. Glia Supportive cells of the central nervous system, derived from neu roepithelial cells. Glial-derived neurotrophic factor (GDNF) A paracrine lactor that binds to the Ret receptor tyrosine kinase.

It is produced by the gut mes enchyme that attracts vagal and sacral neural crest cells, and it is produced by the metanephrogenic mesenchyme to inducelhe formation and branching of the ureteric buds. Glial guidance A mechanism impor tant for positioning young neurons in the developing mammalian brain (e.g., the granule neuron precursors travel on the long processes of the Bergmann glia in the cerebellum). Glycogen synthase kinase 3 (GSK3) Targets ẞ-catenin for destruction. Glycosaminoglycans (GAGs) Complex acidic polysaccharides consisting of unbranched chains assembled from many repeats of a two-sugar unit. The carbohydrate component of proteogly cans. Gonadotropin-releasing hormone (GRH; GnRH) Peptide hormone released from the hypothalamus that stimulates the pituitary to release the gonadotropins follicle-stimulating hor mone and luteiniZing hormone, which are reqUired for mammalian gameto genesis and steroidogenesis. Gonocytes Mammalian PGCs that have arrived at the genital ridge of a male embryo and have become incor porated into the sex cords. G protein A protein that binds GTP and is activated or inactivated by GTP modifying enzymes (such as GTPases.) They play important roles in the RTK pathway and in cytoskeletal mainte nance. Granule neurons Derived from neuroblasts of the external granule layer of the developing cerebellum. Granule neurons migrate back toward the ven tricular (ependymal) zone, where they produce a region called the internal granule layer. Granulosa cells Cortical epithelial cells of the fetal ovary, granulosa cells sur row1d individual germ cells that will become the ova and will form, with thecal cells, the follicles that envelop the germ cells and secrete steroid hor mones. The number of granulosa cells increase and form concentric layers aroW1d the oocyte as the oocyte matures prior to ovulation. A band of inner gray cytoplasm that appears following a rotation of the cortical cytoplasm with respect to the internal cytoplasm in the marginal region of the I-cell amphib ian embryo. Gastrulation starts h1 this location. Gray crescent Green fluorescent protein (GFP) A protein that occurs naturally in certain jellyfish. It emits bright green fluores cence when exposed to ultraviolet light. The GFP gene is widely used as a transgenic label for cells in develop mental and other research, since cells that express GFP are easily identified by a bright green glow.

Growth and differentiation factors (GDFs) See Paracrine factors. Growth cone The motile tip of a neu ronal axon, leads nerve outgrowth. Growth factor A secreted protein that binds to a receptor and initiates signals to promote or retard cell division and growth. Growth plate closure Causes the ces sation of bone growth at the end of puberty. High levels of estrogen induce apoptosis in the hypertrophic chondrocytes and stimulate the inva sion of bone-forming osteoblasts into the growth plate. Gynandromorph Greek gynos, "female"; ndros, "male." An animal in which some body parts are male and others are female. Compare with hermaphrodite. H A pair of balancers on the third thoracic segment in Drosoph.iln.

Halteres Haptotaxis Migration on preferred substra,tes. Hatched blastula Free-swimming sea urchin embryo, after the cells of the animaJ hemisphere synthesize and G-1 1 GLOSSARY secrete a hatching enzyme that digests the fertilization envelope. Head activation gradient A morpho genetic gradient in Hydra that is high est at the hypostome and permits the head only to form in one place. Head mesoderm Mesoderm located anterior to the trunk mesoderm con sisting of the unsegmented par xial mesodenn and prechordal mesoderm. This region provides the head mes enchyme that forms much of the con nective tissues and musculature of the face and eyes. ẞ Head process See Chordamesoderm. Heart field See Cardiogenic mesoderm. Hedgehog A family of paracrine fac- tors used by the embryo to induce par ticular cell types and to create bOlUld aries ?etween tissues. Hedgehog proteins must become complexed with a molecule of cholesterol in order to function. Vertebrates have at least three homologues of the Drosophila hedgehog gene: sonic hedgehog (shh), desert hedgehog (dhl]), and indian hedge hog (illh). Hedgehog pathway Proteins activated by the binding of a Hedgehog protein to the Patched receptor. When Hedge hog binds to Patched, the Patched pro tein's shape is altered such that it no longer inhibits Smoothened. Smoothened acts to release the Ci pro tein f.r0m ẞe microtubules and to pre vent Its bemg cleaved. The intact Ci protein can now enter the nucleus where it acts as a transcriptional a liva tor of the same genes it used to repress.

ẞ Hemangioblasts Greek " blood vessel" plus "blast," a rapidly dividing cell, usually a stem cell. These are the stem cells that form blood vessels and blood cells.

Hematopoesis The generation of Hematopoietic inductive microenvi ronments (HIMs) Cell regions that mduce different sets of transcription factors in mul tipipotent hematopoietic stem cells, and these transcription fac tors specify the developmental path taken by the descendents of those cells. A multipotent stem cell type that gener ates a series of intermediate progenitor cells whose potency is restricted to cer tain blood cell lineages . These lineages are then capable of producing all the blood cells and lymphocytes of the body. Hemimetabolous A form of insect metamorphosis that includes pronymph, nymph, and imago stages. Hemogenic endothelial cell hematopoietic stem cells. Hensen's node (primitive k not) A r@gional thickening of cells at the ante nor end of the primitive streak. The center of Hensen's node contains a funnel-shaped depression (sometimes called the primitive pit) through which cells can enter the embryo to fonn the notochord and prechordal plate.

Hensen's node is the functional equiv alent of the dorsal lip of the amphibian blastopore (i.e., Spema.rtn's organizer) and the fish embryonic shield. Hepatic diverticulum The liver pre cursor, a bud of endoderm that extends out hom the foregut into the surrounding mesenchyme. Hepatocyte growth factor (HGF) A paracrine factor secreted by the stellate cells of the liver that allows the hepa tocytes to re-enter the cell cycle during compensatory regeneration. Also called scatter factor. Hermaphrodite An individual in which both ovarian and testicular tis sues exists, having either ovotestes (gonads containing both ovarian and testicular tissue) or an ovary on one side and a testis on the other. Compare with gynandromorph. Heterochromatin Chromatin that remains condensed throughout most of the cell cycle and replicates later than most of the other chromatin. Usu ally transcriptionally inactive. Heterochrony The phenomenon wherein animals change the relative time of appearance and rate of devel opment of characters present in their ancestors. Heterogeneous causation blood cells. Hematopoietic stem cell (HSC) that are derived from angioblasts that @igra.ted from the sclerotome. They g1Ve nse to the hematopoietic stem cells that migrate to the liver and bone marrow and become the adult Primary endothelial cells of the dorsal aorta especially those in the ventral area : A principle of the @eo@etical systems approach: Causahon IS seen as being both "upward," from the genes to the envi rorunent, and "downward," from the envirorunent to the genes. Heterochrony Greek, "different time." A shift in the relative timing of tw'o developmental processes as a mecha nis@ to generate phenotypic variation available for natural sel@tion. One module changes its time of expression or growth rate relative to the other modules of the embryo. paracrine factors) as a mechanism to generate phenotypic val·iation avail able for natural selection.

Heterotypy Creek, "cliJfferent kind." Th@ alteration of the acrna! coding regIOn of the gene, changing the func tional p.operties of the protein being synthesized, as a mechanism to gener ate phenotypic variation available for natural selection. Histoblast nests CLusters of imaginal cells that will form the adult abdomen in holometabolous insects.

Histone Positively charged proteins that are the major protein component of chromatin .

See also nucleosome. Histone acetylation The addition of n@gatively c@arged acetyl groups to histones wh lch neutralizes the basic charge of lysine and Loosens the his tones, and thus activates transcription. Histone acetyltransferases Enzymes that place acetyl groups on histones (especially on lysines in histones H3 and H4), destabilizing the nucleo somes s? tha t they come apart easily, . thus faCJhtal1ng transcrip tion. Histone deace tylases Enzymes that remove acetyl groups, stabilize the @ucleosomes, and prevent transcrip tion. Histone methylation The addition of methyl groups to histones. Can either activate or further repress transcrip tion, depending on the amino acid that is methylated and the presence of o@her methyl or acetyl groups in the . . VICUUty. Histone methyltransferases Enzymes tha t add methyl groups to histones and either activate or repress thanscription. Holoblastic Greek halos, "complete." Refe@ s to a ceU cUvision (cleavage) pat tern lCl the embryo in which the entire egg is divided into smaller cells' as i t is in frogs and mammals. Holometabolous The type of insect metamorphOSis found in flies, beetles, moths, and butterflies. There is no pronymph stage. The insect hatches as a lalva (a caterpiliar, grub, or maggot) and progresses through instar stages as it gets bigger between larval moits a ll@eta @orphic molt to become a pu a, an Imagmal molt and finally the emer gence (eclose) of the adult (imago). p Ho me @ rhe@ is How the organism stabj .

@LZe@ Its different cell lineages while it Heterometry Greek, "different meas ure." A change in the amount of a gene product as a mechanism to generate Homeostasis Heterotopy Homeotic complex (Hom-C) The region of Drosophila chromosome 3 phenotypic variation avaiJable for nat ural selection. Greek, "'different place." @e spahai a l teration of gene expres Sion (e.g., transcription factors or IS stiU constructing itseU. Maintenance of a stable physiological state by means of feedH back responses. C-12 GLOSSARY containing both the Antennapedia complex and the bi thorax complex. Homeotic selector genes A class of Drosophila genes regula ted by the pro tein products of the gap, pair-rule, and segment pola ri ty genes whose tran scription determines the developmen tal fate of each segment. Homeotic m uta nts Result from muta tions of homeotic selector genes, n i which one structure is replaced by another (as where an antenna is replaced by a leg). Two identica l protein molecules bound together. Homodimer Homologous Structures and/or their respective components whose similari ty arises from their being derived from a common ancestral structure. For example, the wing of a bird and the forelimb of a human. Compare with analogous. Horizontal transmission When a host that is born free of symbionts but sub sequently becomes infected, either by its environment or by other members of the species. Can also refer to the transfer of genes from one bacterium to another. Compa re with vertical transmission. The larger organism in a symbio tic rela tionsltip in which one of the organisms involved is much larger than the other, and the sm aller organ ism may live on the surface or inside the body of the larger. Host genes Abbreviation of "homeo box" genes . Large family of rela ted Hox genes that dictate (at least in part) regi onal identity i.n the embryo, pa r tic ula rly along the anterior-posterior axis. Hox genes encode transcripHon factors that regu la te the expression of other genes. All known mammalian genomes contain four copies of the Hox compl ex per haploid set, located on four different chromosomes (Hoxa through Hoxd in the mouse, HOXA tllfough HOXD in humans). The mam malian Hox/HOX genes are numbered from 1 to 13, sta rting from the end of each complex that is expressed most anteriorly. A regulatory microenvironment in Drosophila testes where the stem cells Hub for sperm reside. A coa ting around the sea urchin egg formed by the cortical gran Hyaline layer ule protem hyalin. The hyaline layer provides support for the blastomeres during cleavage. Hydatidiform mole A human tumor whidl resembles placental tissue, arise when a haploid sperm fertilizes an egg in which the female pronucleus is absent and the entire genome is derived from the sperm which pre- c1udes normal development and is cited as evidence for genomic imprint ing. An insect hormone that initiates and coordinates each molt, regulates the changes in 20-hydroxyecdysone (20E) gene expression that occur during metamorphosis, and signals imaginal disc differen tiation. Hyperactivation Describes the type of motility displayed by capacitated sperm of some mammalian species. HyperacHvation has been proposed to help detach capacitated sperm from the oviductal epithelium, allow sperm to travel more effectively through vis cous oviductal fluids, and facilitate penetration of the extracellular matrix of the cumulus cells.

Hypertrophic chondrocytes Formed during the fourth phase of endochon dral ossification, when the chondro cytes, under the inn Llence of the tran scription factor Runx2, s top dividing and n i crease their volume dramatical ly The inner layer of the thick end margin of the epiboHzing blasto derm in the gastrulating zebrafish embryo or the lower layer of the bil aminar embryonic blastoderm in birds and mammals. The hypoblast in fish Hypoblast (but not in birds and mammals) con tains the precursors of the endodenn and mesoderm. Hypoblast islands (p r ima ry hypoblast) Derived from area pellucida ceUs of the avian blastoderm that migrate individua lly into the subgerminal cavi ty to form individual disconnected clusters conta ining 5-20 cells each.

Does not contribute to the embryo proper. A conical region of the "head" of a hydra that contains the mouth . Hypostome Induced pluripotent stem (iPS) cells Adult mouse or human cells that have been converled 10 cells with the plu ripotency of embryonic stem cells. Usually this is accomplished by the activation of certain transcription fac tors. Inducer Tissue that produces a Signal (or signals) that induces a cellular behavior in some other tissue. The process by wltich one cell population influences the develop ment of neigh boring cells via interac tions at close range. Induction Migra tion of indi vidual cells from the surface layer into the interior of the embryo.

The cells become mesenchymal (Le., they sepa Ingression rate from one another) and migrate independently. Inner cell mass (IeM) A small group of in ternal cells within a mammalian morula or blastocyst that wi ll eventu ally develop into the embryo proper and its associated yolk sac, allantois, and alIUli on. Inosilol l,4,5-trisphosphate (IP,) A second messenger generated by the phospholipase C enzyme that releases intracellular Ca21 stores. Important in the initiation of both cortical granule release and sea urchin development. Instructive interaction A mode of inductive interaction in which a Signal from the inducing cell is necessary for initi a ting new gene expression in the responding cell .

Insulin-like growth factors (IGFs) Growth factors that initiate an FGF-like signal transduction cascade that interferes with the Signal transduction pa thways of both BMPs and Wnts. IGFs are required for the formation of the anterior neural tube, including the brain and sensory placodes of amphib ians. A principle of the theoreti cal systems app roach: How the parts Integration I maginal discs CJ usters of relatively undifferentiated cells set asi de to pro duce adult structures. Imaginal discs will form the culicular structures of the adult, induding the wings, legs, anten nae, eyes, head, thorax, and genitalia in hol ometabolous insects. Develops from the left coelomic sac of the pluteus lalVa and will fonn many of the struchrres of the adult sea urchin. Imaginal rudiment Imago A winged and sexuaUy mature a.d ult insect. An.imals for which embryonic development incl udes a larval stage with character istics very different from those of the adult organism, which emerges only after a period of metamorphosis. Indirect developers are put together and how they interact to form the whole. A family of receptor p roteins so named because they integrate the Integrins extracellular and intracellular scaf folds, allowing them to work together. On the extracell ular side, integrins bind to the sequence arginine-glycine aspartate (RGD), found in several adhesive protei ns in extracellular matrices, including fibronectin, vit ronectin (found in the baJ;al la m ina of the eye), and laminin. On the cytoplas mic side, in tegrins bind to talin and a ccnin, two proteins that connect to actin microfi laments. This dual bind ing enables the cell to move by con tracting the actin ruicrofilaments against the fixed extracellular matrix.

GLOSSARY Intermediate mesoderm Mesodenn immediately lateral to the paraxial mesoderm. It forms the outer (cortical) portion of the (ldrenal gland and the urogenjtal system, consisting of the kidneys, gonads, and their associated Inte rm ed iate ducts. p roge n itor cells (lpes) Neuroblast precursor cells of the sub- ventricular zone derived from radial glial cells.

l11e fust committed stem ce ll type of the mam- malian testis, they are committed to Intermediate spermatogonia I ntersex A condition in which male becoming spermatozoa. and female trclitS are observed in the same individual. Intracytoplasmic sperm injection (lCSI) A method of assisted reprod uction for the treatment of infertility. A sperm is J J agge d p rote in Intramembranous ossification Bone fonnation directly from mesenchyme. There are three main types of int'ramembranous bone: sesamoid bone and periostal bone, which come from mesoderm, and dermal bone which originate from cranial neural cres l'-deriv ed mesenchymal cells. Introns Non-protein-coding regions of DNA within a gene. I nvagi n ati on The infolding of a region of cells, much like the indenting of a soft rubber bali when it is poked. Involuting margin al zone (IMZ) Cells that involute during Xenopus gastru la Involution Inturning or inward move- ment of an expanding outer layer so that it spreads over the internal surface . o(the remaining external cells. A compoWld tha t allows the diffusion of ions such as Cah acIOSS lipid membranes, permi tting lonophore them to traverse otherwise imperme able barriers. A pigmented ring of muscular tis sue in the eye that controls the size of the pupil and determines eye color. Iris Greek, "equal yolk." Describes eggs with sparse, equaUy distributed yolk particles, as in sea Isolecithal urchins, marrun The narrow segment of the mammaJj(ul ov iduct adjacen t to the Isthmus uterus. Janus kinase prote ns. Linked to JAK-STAT cas ca d e A pathway activat ed by paracri ne factors binding to receptors that are linked to JAK (Janus kinase) proteins. The binding of ligand to the receptor phosphorylates the STAT (s gnal transducers and activa tors of transcription) family of tran scr ption factors.

i i J uven ile hormone OH) A lipid hor mone in in sects that prevents the ecdysone-i.nduced changes in gene expression that are necessary for meta morphosis.

TIlUS, its presence during a molt ensures that the result of that molt is another larval instar, not a pupa or an adult.

Ju xtacrine interactions VVhen ceU membrane proteins on one cell surf K j Kairomones Chemicals that are released by a predator and can induce defenses in its prey. Karyo ki nesis The mitotic div ision of the cell's nucleus. The mechanical i Differentiated epider mal cells that are bound t ghtly togeth er and produce a water-impermeable Keratinocytes tion, includes precursors of the pha ryngeal endoderm, head mesoderm, notochord, somites, heart, kidney, and ventra l mesoderms. i FGF receptors in the JAK-STAT cas cade. JAK directly injected into the oocyte cyto plasm, bypassing any interaction with the egg plasma membrane. j Ligand for Notch, par ticipates in uxtacrine inter seal of lipid and protein. Kit RTK Cell-sudace receptor tyl'osine kinase for stem ligand. Dind ing of stem cell factor to kit libits . apoptosis and promotes prohfer cell fllclor @ See Primitive streak. Koller's sickle Kupffer's vesicle Transient fluid-fil1ed organ housing the cilia thai control left-righl asymmetry in zebrafish.

L Labioscrotal folds i Folds surroWlding the cloacal membr.me in the indiffer ent stage of d fferen tiation of mam malian external geni talia. They will form the lab ia in the female and the scrotwn in the male. Also called ure thral lolds or genital sweUings. The E.

coli gene for @ galac tosidase; commonly used as a reporter facl gene Laminin in A large glycopro te and major component of the basal lc1mina, plays a role in assembling the extr@cel lular matrix, promoting cell-adheSIOn and growth, changing cell shape, and penniting cell migration. gene. G-13 lampbrush ch ro m osom es Chromo somes dUJ'ing the diplotene st.lge of amphibian oocyte meiosis th.lt stretch out large loops of Ot :A that represent sites of upregulated R.'\!A synthesis. Large micromeres A tier of cells pro duced by the fifth cleavage in the sea urchin embryo when the micromeres divi de. Larva The sexually immature stage of an organism, often of significantly dif ferent appearance than the adult and frequently the stage that liv@ the . longest and is used for feedmg or dIS persal.

Laryngotracheal groove An oul pouching of endodermal epithelium in the center of the pharyngeal floor, between the fourth pair of pharyngeal pouches, that extends ventrally. The laryngotracheal groove then bifurcates in to the branches that form the paired bronchi and lungs. Late ra l p late mesoderm Mesodermal sheet lateral to the inter media te meso derm. Gives rise to appendicular bones, cOlmective tissues of the limb buds, circulatory system (heart, blood vessels, and blood cells), muscles and cOMective tissues of the digestive and respiratory tracts, and lining of coelom and its derivatives. It also helps form a series of extraembryonic membranes thai are importan t for transporting nutrients to the embryo.

Lateral somitic fro n ti er The boundary between the primaxial and abaxial muscles and between the somile de- rived and lateral plate-derived dermis. Lens placode Paired epidermal th.ick erungs induced by the underlying optic cups that invaginates to form the transparent lens that allows light to impinge on the retina. leve l-specific p roperti es and emer gence A pr ciple of the theoretical in systems approach : The properties of a system at any given level of orgaruza tion calu10t be totally explained by those of levels "below" it.

Leyd ig cells Testis cells derived from the interstitial mesenchyme cells sur roWl.ding the testis cords that make the testosterone required for secondary sex de termination and, in the adult, required to support spermatogenesis. ẞ TIle length of time an average individual o a @iven specie@ life expectancy can expect to live which IS charactens tic populations, not of species. It is usually defined as the age at which limb bud A dreuJar b u lge that will form the future limb. The limb bud is formed by the proliferation of mes- enchyme cel ls from the somatic layer of the limb field lateral plate mesohalf the population still survives. G-1 4 G LOSSARY deem (the limb skeletal preewsor cells) and from the somites (the Limb ml/sele precursor cells) . limb field An area of the embryo con taining al\ of the cells capable of form ing a limb. lineage- restricted stem ce lls Stem cens derived from multi pote nt stem cells, and which can now generate only a p articular cell type or set of ceU types. loss-of-function evidence (negative inference ev id e nce) The absence of the postulated cause is associated with the absence of the postulated effect.

While stronger than correlative evi dence, loss-of-function evidence still does not exclude other explana tions. Lu teinizing hormone (LH) A peptide hormone secreted by the mammalian pituitary that stimulates the produc tion of steroid hormones, such as estrogen from the ovarian follicle ceUs and testosterone from the testicuJar leydig cells. A surge in LH levels caus es the primary oocyte to complete meiosis I and prepa res the follicle for ovulation. M Macromeres Larger cells generated by asymmetrica l cleavage, e.g., the four large cells generated by the fourth cleavage when the vegetal tier of the sea urchin embryo lmdergoes an unequal equatorial cleavage.

Male pronucleus of the sperm. The haploid nucleus Malformation Abnormalities caused by genetic events such flS gene muta tions, chromosomal aneuploidies, and translocations. Mantle (inlermediate) zone Second layer of the developing spinal cord and medulla that forms around the original neural tube. Because it con tains neuronal cell bodjes and has a grayish appearance grossly, it will form the gray ma tter. M a rgina l zone (1) The third and outer zone of the developing spinal cord and medulla composed of a cell-poor region composed of a xons ex tending from neurons residing in the mantle zone. Will form the white matter as glial ceUs cover the axons with myelin sheaths, which have a whitish appeal ance. (2) In amphibians: Where gastru lation begins, the region surrounding the €G uator of the blastula, where the animal and vegetal hemispheres meet. (3) ln birds and reptiles (= marginal belt), a t!tin layer of cells between the area pe.llucida and the area opaca is a thin layer of cells, important in deter mining cell fate during early e!tick development. Malernal effect genes Encode mes senger RNAs that are placed in differ ent regions of the Drosophila egg.

Medial hi nge point (MHP) Derived from the portion of the avian and mammaUan neural plate just anterior to Hensen's node and from the anteri or midline of Hensen's node. MHP ceUs become anchored to the noto chord beneath them and fonn a hinge, which forms a furrow a t the dorsal midline. Medullary cord Forms by condensa tion of mesenchYIlle cells and then mese.nchymal-to-epithelial transition in the caudal region of the embryo during the process of secondary neu rulation.

It will then cavitate to form the caudal section of the neuraj tube. Meiosis A unique division process occurring only in germ ceUs, to reduce the number of chromosomes to a hap loid complement. All other cells divide by m.itosis. Meiosis differs from mito sis in that (1) meio tic cells undergo t"\vo cell divisions without an intervening period of DNA replication, and (2) homologous chromosomes (each con sisting of two sister chroma tids j Oi ned at a kinetochore) pair toge ther and recombine genetic mCl terial. Mela nocytes Cells cont(l ining the pig ment melanin. Derived from ne ural crest cells and undergo extensive migration to all regions of the epider mis, Melanocyte stem cell Adult stem cell derived from melanocyte tnmk neural crest cells that resides in the bulge niche of the hair or feather follicle and which gives rise to the pigment of the skin, hair, and feathers. Meroblastic Greek meres, " part." Refers to the cell division (cleavage) pCl1tern in zygotes containing large ammmts of yolk, wherein only a por- tion of the cytoplasm is cleaved. The cleavage furrow does not penetrate the yolky portion of the cytoplasm because the yolk platelets impede membrane formation there. Only part of the egg is destined to become the embryo, while the other portion the yolk-serves as nutrition for the elllbryo, as in insects, fishl reptiles, and birds. Meroistic oogeneSiS Type of oogeneSis found in certain insects (including Drosophila and moths), in which cyto plasmic connections remain between the cells produced by the oogonium. Mesencephalon The midbrain, the middle vesicle of the dev eloping verte- brate brain; major derivatives incl ude optic tectum and tegmen hun. Its lumen becomes the cerebra1 aqueduct. Mesenchymal stem cells (MSCs) Also caUed bone marrow.-derived stem cells, or BMOCs. Multipotent stem cells that originate in the bone marrow, MSCs are able to give rise to numerous bone, cartilage, muscle, and fat lineages. Mesenchyme Loosely organized embryonic connective tissue consisting of scattered fibroblast-like and some times migratory mesenchymal cells separated by large amounts of extra ceUular ma trix.

Mesentoblasts [n snail embryos, the cells derived from the 4d blastomere that give rise to both the mesodermal (heart) and endodermal (intestine) organs. Mesoderm Greek mesos, "between." The middle of the three embrYOnic germ layers, lying between the ecto derm and the endoderm. nle meso derm gives rise 'to muscles and skeleton; connective tissue; the repro ductive organs; and to kidneys, blood, and most of the cardiovascular tissue. Mesodermal mantle TIle cells that involute through the ventral and later al blastopore lips during am phibian gastrulation and will form t he heart, kidneys, bones, and parts of several other organs. Mesomeres The eight cells generated in the sea urchin embryo by the fourth cleavage when the four cells of the ani mal tier divide meridionally into eight blastomeres, each with the same vol ume. Mesonephros The second kidney of the allniote embryo, induced in the adjacen t mesenchyme by the middle portion of the nephric duct. It func tions briefly in urine filtration in some mammalian species and mesonephri c tubules form the tubes that transport the sperm from the testes to the urethra (the epididymis and vas deferens). Mesonephric duct See Wolffian duct. Meta ll o pro tein ases Matrix metallo proteinases (MMP) Enzymes that digest extracellular matrices and are important in many types of tissue remodeling in disease and develop ment, induding metastasis, branching morphogenesis of epithelial organs, placental detachment at birth, and arthritis.

Metamorphosis Changing from one form to another, such as the transfor mation of an insect larva to a sexually mature adult or a tadpole toa frog. Metaneph roge nic mesenchyme An area of mesenchyme, derived from posterior regions of the intermediate mesoderm, involved in mesenchymal- epithelial interactions that generate the GLOSSARY metanephric kidney and will form the secretory nephrons. Metanephros/metanephric kidney The thjrd kidney of the embryo and the permanent kidney of amniotes. Metaphase plate A structure present during mitosis or meiosis in which the chromosomes are attached via their kinetochores to the microtubule spin dle and are lined up midway between the two pales of the cell.

Metastasis The invasion of a malignant cell into other tissues. Metencephalon The anterior subdivi sion of the rhombencephalon; gives rise to the cerebellum, whjch coordi nates movements, posture, and balance. Microfilaments Long cables of poLy merized actin necessary for cytokinesis and also formed during fertilization in the egg's cortex where they are used to form microvilli. Micromeres Small cells created by asymmetrical cleavage, e.g., four small cells generated by the fourth cleavage at the vegetal pole when the vegetal tier of the sea urchin embryo under goes an unequal equatorial cleavage. Micropyle The only place where Drosophila sperm can enter the egg, at the future dorsal anterior region of the embryo, a tunnel in the chorion (eggshell) that allows sperm to pass through i t one at a time. MicroRNA (miRNA) A small (about 22 nucleotide) RNA complementary to a portion of a pcll-ticLlJar mRNA that reg ulates translation of a specific message. MicroRNAs often bind to the 3'lffR of mRNAs. Microspikes Essential for neuronal pathfinding, mkrofilament-containing pointed fjlopodia of the growth cone that eJongate and contract to allow axonal migration. Microspikes also . sample the microenvironment and sends signals back to the soma. Microvilli Small projections that extend from the egg surface during fertiliza tion that may aid sperm entry into the cell. Mid-blastula transition The transition from the early rapid biphasic (only M and S phases) mitoses of the embryo to a stage characterized by (1) mitoses tha t include the "gap" stages (Gl and G2) of the cell cycl e, (2) loss of syncronicity of cell division, and (3) transcription of new (zygotic) mRNAs needed for gas trulation and cell specification. Mid-piece Section of sperm flageUum near the head that contains rings of mitochondria that provide the ATP needed to fuel the dynein ATPases and support sperm motility. Mitosis-promoting factor (MPF) Con sists of cyclin B and p34/Cdkl cyclin, required to initiate entry into the mitotic (M) phase of the cell cycle in both meiosis and mitosis. mRNA cyto p lasmic localization The spatial regulation of mRNA transJa tion, mediated by (1) diffusion and local anchoring, (2) localized protec hon, and (3) active transport along the cytoskeleton. Model systems Species that are easily studied in the laboratory and have special properties that allow their mechanisms of development to be readily observed {e.g'l sea urchins, snails, ascidians, and C. elegans}. Modularity A principle of the theoreti cal systems approach. The organism develops as a system of discrete and interacting modules.

Module A biological entity character ized by more n i ternal than external n i tegration. Molecular parsimony (the "small toolkit") Development within all line ages uses the same types of molecules: transcription factors, paracrine factorS, adhesion molecules, and signal trans duction cascades are remarkably simi lar from one phylum to another. Monospermy Only one sperm enters the egg, and a haploid sperm nucleus and a haplOid egg nucleus combine to form the diplOid nucleus of the fertil ized egg (zygote), thus restoring the chromosome number appropriate for the species. Monozygotic Greek, one egg." Describes twi.ns that form from a sin gle embryo whose cells become disso ciated from one another, either by the separation of early blastomeres, or by the separation of the inner cell mass into hvO regions within the same blas tocyst. "Identical" hvins. Compare with dizygotic. Morph One of several different poten tial phenotypes produced by environ mental conditions.

Morphallax is Type of regeneration that occurs through the repatterning of existing tissues with little new growth (e.g., Hydra). Morphogens Greek, " form-givers." Substances that, by their differing con centrations, differentially specify cell fates. Morphogens are made in specific sites in the embryo, diffuse over long distances, and form concentration SJa dients where the highest concentration s i a t the point of syn thesis, becoming lower as the morphogen diffuses away from its source and degrades over time. Morphogenesis The organization of the cells of the body into hmctional " G-1 5 structures via coordinated cell growth, cell migration, and cell dea tho Mo rphogenetic determinants Tran- scription factors or their mRNAs that will uUuence the cell's development. Morpholino An antisense oligonu cleotide against an mRNA used to inhibit protein expression. Morula Latin, "muJberry." Vertebrate embryo of 16--64 cells;, precedes the blastula or blastocyst stage. Mam malian morula occurs a t the l@celJ slelge, consists of a small group of internal cells (I:he inner cell mass) sur rounded by a larger group of external (trophoblast) cells. Mosaic embryos Embryos in which most of the cells are determined by autonomous specification, with each cell receiving its instructions n i de pendently and without cell-cell inter action. Mosaic pleiotropy A syndrom.e char acterized by the expression of multi ple, independently produced effects resulting from a gene being critical in different parts of the body. Mullerian duct (paramesonephric duct) Duct running lateral to the mesonephric duct in both male and female mammalian embryos. These ducts regress in the mate fetus, but form the oviducts, uterus, cervix, and upper part of the vagina in the female fetus. See also Wolffian duct. Multipotent cardiac progenitor cells Progenitor cells of the heart field thai form cardiomyocytes, endocardiwnr epicardium, and the Purkinje neural fibers of the heart. Multipotent stem cells Adult stem cells whose commitment is limited to a relatively small subset of all the possi ble cells of tile body. Mutualism A form of symbiosis in which the relationship benefits both partners. Myelencephalon The posterior sub division of the rhombencephalon; becomes the meduUa oblongata. Myelin sheath Modllied oligodendro cyte or schwarm cell pll1sma mem brane that surrounds nerve axons, pro viding insulation that confines electrical impulses transmitted along axons, and is necessary for normal neural transmission. Myoblast Muscle precursor cell. Myocardium Heart muscles. Myogenic regulatory factors (MRFs) bHLH transcription factors (such as MyoD and Myf5, that are critical regu lators of m.uscle development. Myostatin (Greek, "muscle stopper") A member of the TGF-p family of pl11'acril1c factors that negatively regu- G-16 GLOSSARY lates muscle developmen t. Genetic defects in the gene or its negative regu latory miRNA cause h uge muscles in some mammals, including humans. Myotome Portion of the somite that gives rise to skeletal muscles.

The myotome has two components: the primaxial component, closes t to the neural tube, which (orms the muscula ture of the back and rib cage, and the abaxial component, away from the neural tube, which forms the muscles of the ventral body waJI. N NAD+ kinase Neural groove U-shaped groove that forms in the center of the neural plate during primary neurulation. Neural plate The region of the dorsal ectoderm that is specified to become neural ectoderm. The cells of this region have a columnar appearance. Neural restrictive silencer element (NRSE) A regulatory DNA sequence found in several mouse genes which prevents their expression in adult neu rons. Neural restrictive silencer factor (NRSF) A zinc finger transcription factor that binds the NRSE and is expressed in every cell that is a mature neuron. Activated during the early response of the sea urchin egg to the sperm, converts NAD'" to NADP+ which can be used as a coenzyme for lipid biosynthesis and may be impor Neural retina Derived from the n i ner layer of the optic cup, composed of a tant in the construction of the many new cell membranes required during cleavage. NADp- is also used to make NAADP. ies of the ganglion cells; bipolar interneurons that transmit electric Nanos Protein critical for the establish ment of anterior-posterior polarity of the Drosophila embryo. Nanos mRNA is localized to the posterior pole. Negative inference evidence Loss-of-function evidence. Neoteny See Retention of the juvenile fonn as a result of retarded body develop ment relative to the development of the germ cells and gonads (which achieve maturity at the normal time). See also progenesis. ephric duct See Wolffian duct. Nephron Functional unit of the kidney. Nerve growth factor (NGF) Neu rotrophin released from potential tar get tissues that works at short ranges as either a chemotactic factor or chemorepulsive factor for axonal guid ance. Also important in the selective survival of different subsets of neu rons. Netrin-l, Netrin-2 Paracrine factors found in a gradient that guide axonal growth cones. They a re important in commissural axon migration and reti nal axon migration. Neural crest A transient band of cells, ariSing from the lateral edges of the neural plate, that joins the neural tube to the epidermis. It gives rise to a cell population the neural crest cells that detach during formation of the neural tube and migrate to form a vari ety of cell types and structures, includ ing sensory neurons, enteric neurons, glia, pigment ceils, and (in the head) bone and car tilage. Neural folds Thickened edges of the neural plate that move upward during neurulation and migrate toward the midline and eventually fuse to form the neural rube. not layered array of cells that include the light- and color-sensitive photorecep tor cells (rods and cones); the cell bod stimuli from the rods and cones to the ganglion cells, Miiller glial cells that maintain its integrity, amacrine neu rons (which lack large axons), and hor izontal neurons that transmit electric impulses in the plane of the retina. Neural tube The embryonic precursor to the central nervous system (brain and spinal cord). Neuroblast A neural precursor cell.

Neuron Nerve cell; neurons are derived from neuroepithelial cells. Neuropore The two open ends (anteri or neuropore and posterior neuropore) of the neural rube that later dose. Neurotransmitters Chemical messen gers molecules secreted at the ends of axons that cross the synaptic deft and are received by the adjacent neuron, thus relaying the neural signal. Neurotrophin Greek trophikos, "nour ish." Neurotropins supply factors (usuaUy growth factors) that keep the neuron alive. Neurotropin Greek tropikos, "turn." Chemoattractant of neurons.

Neurula Refers to an embryo during neurulation (i.e., while the neural tube s i forming). Neurulation Process of folding of the neural plate and dosing of the cranial and caudal neuropores to form the neural tube. Neural crest effectors TranSCription factors activated by neural crest speci fiers that give the neural crest cells their migratory properties and some of thei r differentiated p roperties. A set of tran scription factors (e.g., FoxD3, $ox9, Id, Twist, and Snail) induced by the bor der-specifying transcription factors, Neural crest specifiers that specify the cells that are to become the neural crest. A set of transcription factors induced by the neural plate inductive signals. These Neural plate border specifiers factors, including DistaUess-5, Pax3, and Pax7, collectively prevent the bor der region from becoming either neu ral plate or epidermis. Neural plate inductive sign a ls BMPs and Wnts secreted from the ventraJ ectoderm and paraxial mesoderm interact to specify the boundaries between neural and non-neural ecto derm during chick gastru lation . Rela tive concentrations of the same signals also specify neural crest and cranial placode cells.

Nieuwkoop center The dorsalmos t vegetal blastomeres of the amphibian blastula, formec;i as a consequence of the cortical rotation initiated by the spenn enlry; an impor tan t signaling center on the dorsal side of the embryo.

One of its main functions is to induce Spemann's Organizer. Nodal A paracrine factor and member of the transfonning growth factor @ (TGF-@) family involved in establish ing left-right asymmetry in vertebrates and n i vertebrates.

Nodal vesicular parcels (NVPs) Small, membrane-bound particles in the mammalian embryo that contain Sonic hedgehog protein and retinoic acid. Secreted from the node cells under the influence or FGF signals, GUary flow carries the NVPs to the left side of the body, delivering paracrine factors for the establishment of left-right asym metry. Node The mammalian homologue of Hensen's node. Noggin A soluble BMP antagonist that blocks BMP signaltng. Noninvoluting marginal zone (NIMZ) Region of cells on the exterior of the gas trulating amphibian embryo that expan d by epiboly to cover the entire embryo, e ventually forming the sur face ectoderm. Non@skeletogenic mesenchyme Formed from the veg2 layer of the 60cell sea urchin embryo, it generates pigment cells, immunocytes, and mus cle cells. Also called seconda ry mes enchyme. Notch protein Receptor for Delta, Jagged, or Serrate, participan ts in jux tacrine interactions. Ligand-binding causes Notch to undergo a conforma tional change that enables a part of its cytoplasmic domain to be cut off by the Preseni1in-l protease. The cleaved portion enters the nucleus and binds to a dormant transcription factor of the CSL family. When bound to the Notch G LOSSARY protein, the CSL transcription factors activate their target Notochord A transien t mesodermal rod in the most dorsal portion of the embryo that plays an important role in inducing and pat1:erning the nervous system.

compac ted through rusulfide bonds, that package the DNA of the sperm nucleus. Protamines Basic proteins, tightly Prote o glyca n s Large extracellular matrix molecules con sisting of core proteins (such as syndecan) with cova lent ly attached glycosaminoglycan G-20 G LOSSARY polysaccharide side chains. Two of the most widespread proteoglycans are heparan sulfate and chondroiton sul fate. The number and type of proteins encoded by the genome.

Proteome Prothoracicotropic hormone (PTTH) A type of phenotypic plasticity in which the genome encodes the potential for a continuous rfHIge of potential phenotypes, and the environment the individual encoun ters determines the phenotype. Com pare with polyp henism. A peptide hormone that initiates the molting process in insects when it is released by neurosecretory ceUs Tn the brain in response to neural, hormonal, or environmental signals. PTTH stimu lates the production of ecdysone by the pro thoracic gland. Reaction norm A class of cadherins that lack the attachment to the actin skeleton through catenins. They are an important means of keeping migrating epithelia together, and they are impor tant in separating the notochord from surrounding tissues during its forma tion. Reactive oxygen species (ROS) Greek, "mouth first." Animals that fann their mouth regions from the blastopore, SUdl as molluscs.

Compare with deuterostomes. Receptor tyrosine kinase Protocadherins Protostomes The close-far axis of the limb, e.g., shoulder-finger or hip-toe. Proximal-distal axis Intersex conditions in which the secondary sex characteristics differ from what would be expected from the gonadal sex. Male pseudohermaphroditism (e.g., androgen insensi tivity syndrome) describes conditions wherein the gonadal sex is male and the secondary sex characteristics are female while Female pseudohermaphroditism describes the reverse situation (e.g., congenital adrenal hyperplasia). Pseudohermaphroditism R stances (an activator that activates itself as well as forming its own, faster diffuSing inhibitor) interact to produce stable patterns during morphogenesis. These patterns would represent regional differences in the concentra tions of the two substances. Meta bolic by-products that can damage cell membranes and proteins and destroy DNA. ROS are generated by mitochon dria due to insufficient reduction of oxygen atoms and include superoxide ions, hydroxyl ("free") radicals, and hydrogen peroxide. A receptor that spans the cell membrane and has an extracellular region, a transmem brane region, and a cytoplasmic region. Ligand (paracrine factor) bind ing to the extracellular domain causes a conformational change in the recep tor's cytoplasmic domains, activating kinase activity that uses ATP to phos phorylate specific tyrosine residues of particular proteins. Reciprocal inductions A common sequential feature of induction: An induced tissue in tum induces other tissues, including the original inducer. Regeneration The ability to reform an adult structure or organ that has been damaged or destroyed by trauma or disease. An aggrega tion of relatively dedifferentiated cells derived from the originally differenti ated tissue, which then proliferates and redifferentiates into the new limb parts during epimorphic regeneration. Regeneration blastema Neural pro genitor cells found in the ventricular zone (VZ) of the developing brain. At each division, they generate another VZ cell and a more comntitted cell type that leaves the VZ to differentiate. Radial glial cells (RGCs) Cleavage pattern in echinoderms. The cleavage planes are parallel and perpendicular to the animal-vegetal axis of the egg. Radial holoblastic deavage The hypoth esis that the chance accumulation of inappropriate epigenetic methylation due to errors made by the DNA methylating and demethylating enzymes could be the critical factor in aging and cancers. Random epigenetic drift The ability to respecify cells so that the removal of cells des tined to become a particular structure can be compensated for by other ceUs producing that structure. This is seen when an entire embryo is produced by cells thatwould have contributed only certain parts to the original embryo. It Regulation is also seen in the ability of two or more early embryos to form one chime ric individual rather than twins, tri ple ts, or a multiheaded individual. Describes syn dromes in which a defective gene in one part of the embryo causes a defect in another part, eveu though the gene is not expressed in the second tissue. A G-protein in the RTK pathway.

Mutations in the RAS gene account for Relational pleiotropy Model for developmental patterning wherein two homogeneously distributed sub- Reporter gene Ras a large proportion of cancerouS human tumors. Reaction-diffusion model A gene with a product that is readily identifiable and not usu ally made in the cells of interest. Can be fused to regulatory elements from a gene of interest, inserted into embryos, and then monitored for reporter gene expression If the sequence contains an enhancer, the reporter gene should become active at particular times and places. The genes for green fluorescent protein (GFP) and p-galactosidase (lacZ) a re common examples. A 1 4-amino-acid peptide that has been isolated from the egg jelly of Resact the sea urchin Arbacia pllllctulata Ulat acts as a chemotactic factor and sperm activating peptide for Arbacin sperm. Occurs during forma tion of the vertebrae from sclerotomes; the rostral segment of each sclerotome recombines with the caudal segment of the next anterior sclerotome to form the vertebral rudiment and this enables the muscles to coordinate the movement of the skeleton, permitting the body to move laterally. Resegmentation The future respirato ry tract, which forms as an epithelial outpocketing of the pharynx, and eventually bifurcates into the two lungs. Respiratory tube Responder During induction, the tis sue being ind uced. Cells of the responding tissue must have a recep tor protein for the paracrine factor and have the competence to respond to it . A network of thin canals located near, and eventually connect Rete testis ing with, the mesonephric duct that connects with the testis cords of the developing testis and conveys sperma tozoa from the seminiferous tubules to the mesonephric duct derivatives, the epididymi s and ductus deferens (vas deferens) in the adult. Cell that derived from the mammalian erythroblast that has expelled its nucleus. Although reticu locytes, lacking a nucleus, can no longer synthesize globin mRNA, they can translate existing messages into globins. Reticulocyte Retina See Neural retina. Retinal hom eo box (Rx) Gene expressed in the central b ulge region of the diencepha lon ; required for optic Retinoic acid (RA) A derivative of vita min A and morphogen involved i n cup and retina formation. anterior–posterior axis formation. Retinoic acid 4-hydroxylase An enzyme that degrades retinoic acid.

The map of retinal connections to the optic tectum. Point–for–point correspondence between the cells of the retina and the cells of the tectum that enables the ani mal to see an unbroken image. Retinotectal projection G LOSSARY the hydrolysis of this phosphate bond, return.ing Ras to its inac tive state. The active Ras activates the Raf protein kinase C (PKC), which in turn p hos phoryla tes a series of kinases. Eventu ally, an activ Rhombencep ha lon The hindbrain, the most caudal vesicle of the developing vertebrate brain; wiU form two second ary brain vesicles, the metencephalon and myelencephalon. Rhombomeres Periodic swellings that divide the rhombencephalon into smaller compartments, each with a differen t fate and different associated cranial nerve ganglia.

Right-left axis A line between the two lateral sides of the body. transl Ring canals The cytoplasmic intercon between the cystocytes that nections become the ovum and nurse cells in Drosophil. RNA-induced silencing complex (RISC) A complex containing several proteins and a microRNA which can then bind to the 3' UTR of messages and inhibit their translation. Robustness Also caUed canalization; a principle of the theoretical systems approach: Refers to the ability of an organism to develop the $-"l.me pheno type despite perturbations from the environment or from mutations. Roof plate Dorsal region of the neural tube import ant in the eSfablishment of dorsal-ventral polarity. The adjacent epidermis induces a secondary Signal- ing center and BMP4 expression in the roof pla te cells of the neural tube that in turn induces a cascade of TGF- proteins m adjacent cens. Rostral-caudal Latin, "beak-tail." An anterior-posterior posi tional axis, espe cia lly when referrmg to chick embryos or the head and brain of mammals. Rotational cleavage The cleavage pat tern for mammalian and nematode embryos. In mamm als, the first cleav age is a normal meridional division while in the second cleavage, one of the two blastomeres divides meridion aUy and the other divides equatorially. l.n C. elegltrls, each asyrrunet.rical divi sion produces one founder cell that produces differentiated descendants, one stem cell. The stem celi lille age always undergoes meridional divi sion to produce (1) an anterior founder cell and (2) a posterior cell that will continue the stem cell lineage. and R-spondinl (Rspol) Small, soluble protein that upregulates the Wnt pa th way and is critical for ovary formation. The receptor tyrosine kinase (RTK) is dimerized by Ugand, which causes au toph osphory lation of RTK pa thway the receptor. An adaptor protein recog nizes the phosphorylated tyrosines on tJle RTK and ac tivCl tes an intermediate protein, GNRP, which activCltes the Ras G protein by allowing the phosphory lation of lhe G OP-bound Ras.

At the same time, the GAP protein stimulates cases, this pathway is reinforced by the release of Ca2+ .

s Sacral neural Cfost Neural crest cells that lie posterior to chick somite 28 tha t generate the parClsympathetic (enteric) ganglia of the gut that are required for peristaltic movement in the bowels. Satellite cell Putative muscle stem cell found within the basal lamina of mature myofibers that can respond to injury or exercise b y proliferating into myogenic cells that fuse and form new muscle fibers. Sclerotomes Blocks of mesodermal cel ls in the ventromedial half of each somite thClt will differentiate into the vertebral bodies and intervertebral discs of the spine and ribs. They are also critical in patterning the neural crest and motor neurons. Secondary hypoblast UnderUes the epiblast in the bilaminar amniotic blas toderm. A sheet of cel ls derived. from deep)'olky cells at the posterior mar gin of the blastoderm that migrates the hypoblast islands (primary hypoblast). Hypoblast cells do not contribute to the primary germ layers of the embryo proper, but instead form portions of a nteriorly, d isplacing G-21 side the gonads. This inclu des the ma le or female duct systems and exter nal genita l ia, and, in many species, sex-specific body size, vocal carhJage, and musculature.

A pair of cells derived from the first meiotic division of a primary sperma tocyte, Secondary spermatocytes which then complete the second divi sion of meiosis lo generate the four haploid spermatids.

Seg m ental plate The ba nds of paraxial mesoderm in chkk embryos. Segment polarity genes Drosophila zygotic genes activated by the proteins encoded by the pair-rule genes whose mRNA and protein products divide the emb ryo into 14 segment-wide wli's, establishing the periodicity of the embryo. Segment polarity mutants showed defects (deletions, duplica tions, polarity reversals) in every seg ment. Sel ective affinity Principle that explains why disaggregated cells reag gregate to rence! their embryonic posi tions. Specifically, the inner surface of the ectoderm has a positive affinity for mesodermal cells and a nega tive affini ty fol' the endoderm, while the meso derm has positive affinities for both ectodermal and endodermal cells. Extracellular matrix pro teins thai repel migrating neural crest cells and axonal growth cones . Semaphorins Seminiferous tubules Form at puberty from the testis cords, containing the sertoll cells and t.he gonocytes/sper ma togonial stem cells, by the initiation of s perm atogenesi s, prOli fera tion and of the spermatogenic epithelium, and formation of a f1 ui d filled lumen. differentiation Senescence The phySiological deteri oration that characterizes old age. the external membranes and provide chemica! signals that specify the migration of epiblast cells. Also called endob last. Septum the same basic body plan as the adult, caterpillars and tadpoles are examples. Compare wi th pri mary laYVac. the seminiferous tubules of the testes involved in spermatogeneSiS in the adult through their role in nourish ing and maintaining the developing sperm cells. They secrete AMF in the fetus and prov ide a niche for the incoming germ cells. They are derived. from somatic cells, which are in tum derived from the genita l tidge epitheli um. Sesamoid bone Small bones at joints that form as a result of mechanical stress (such as the patel la). They are derived from mesoderm via intramem mones or factors produced by the Shield Secondary larvae Larvae that possess Secondary neu rula tion The process that forms the posterior portion of the neural ttlbe by the coalescence of mes enchyme cells n i to a soLid cord tha t subsequent1y forms cavities that coa lesce to create a hoUow tube. Secondary oocyte The etiploid oocyte following the first meiotic division, which also generates the first polar body. Secondary sex determination Devel opmental events, directed by hoc- gonads, that affect the phenotype out. A partition that divides a chamber, such as the ClfTial septa that split the devel oping atrium into left and righ t a tria. Sertoli cells Large secretory support cells in brall0us ossification. See Embryonic shield. G-22 G LOSSARY Short neural precursors (SNPs) Neu roblast precursor cells of the ventricu lar zone derived from radial glial cells. Signal transduction casca des Path ways of response whereby paracrine factors bind to a receptor that initiates a series of enzymatic reactions within the cell which in turn have as their end point either the regulation of transcrip tion factors (such that different genes are expressed in the cells reacting to these paracrine factors) or the regula tion of the cytoskeleton (such that the cells responding to the paracrine fac tors alter their shape or are permitted to migrate). Silencer A DNA regula tor y element that binds transcription factors that actively repress the transcription of a particular gene. Sinus venosus The posterior region of the developing heart, where the two major veins fuse. Inflow tract to the a trial area of the heart. Sirtuin genes Encode histone deacety lahon (chromatin-silencing) enzymes that guard the genome, preventing genes from being expressed at the wrong times and places, and blocking chromosomal rearrangements. They may be important defenses against premature aging. Skeletogenic mesenchyme Also called primary mesenchyme, formed from the first tier of micromeres (the large micromeres) of the 6Q-cell sea urchin embryo, forms the larval skele ton. Slow block to polyspermy See Corti cal granule reaction. SMAD pathway The pathway activat . ed by members of the TGF- super family. The TGF- ligand binds to a type II TGF- receptor, which allows that receptor to bind to a type I TGF- receptor. Once the two receptors are in close contact, the type II receptor phos phorylates a serine or threonine on the type I receptor, thereby activating it. The activa ted type I receptor can now phosphorylate the Smad proteins. Smads 1 and 5 are activated by the BMP family of TGF- factors, while the receptors binding activin, Nodal and the TGF-p family phosphorylate Smads 2 and 3. These phosphorylated Smads bind to Smad4 and fonn the transcription factor complex that will enter the nucleus. Small-interfering RNA (siRNA) Simi lar to miRNA, is the basis of RNA interference technique. siRNA is made from double-stranded RNA and is also packaged into RISCs. Small micromeres A cluster of cells produced by the fifth cleavage at the vegetal pole in the sea urchin embryo when the micromeres divide. Somatic cells Greek soma, body. CeUs that form the body; aU cells in the organism that are not germ cel ls.

Somatic cell nuclear transfer (SCNn Better but less accurately known as "cloning," this is the procedure by which a cell nucle us is transferred into an activated enucleated egg and directs the development of a complete organism with the same genome as the donor cell. Somatic mesoderm (parietal meso derm) Derived from la teral meso derm closest to the ectoderm (dorsal) and separated from other component of lateral mesoderm (splanchnic, near endoderm, ventral) by the intraembry ONC coelom. It forms the somatopleure with the overlying ectoderm, and it will form the body wall, and the lining of the coelom. Somite Segmental block or ball of mesoderm formed from paraxial mesoderm adjacent to notochord (the axial mesoderm). Differentiates to form, initially, sclerotome and der mamyotome; the latter goes on to form dermotome and muscle. Somites will form the axial skeleton (vertebrae, ribs), all skeletal muscle, and the dor sal dermis, tendons, joints, and dorsal aortic cells. Somitic mesodenn See Paraxial meso deml.

Somitogenesis the process of segmen tation of the paraxial mesoderm to form somites, beginning cranially and extending caudall y. Its components are (1) period icity, (2) fissure formation (to separate the somites), (3) epithelializa tion, (4) specification, and (5) differen tiation. Somitomeres Early pre-somites, con sisting of paraxial mesoderm cells organized into whorls of cells. Sonic hedgehog (SHH) The major hedgehog family paracrine factor. SHH has distinct functions in different tissues of the embryo. For example, it is secreted by the notochord and ven tralizes the neural tube. It is also involved in the establishment of left right asymmetry, primitive gut tube differentiation, proper placement of feather formation in chicks, and in the differentiation of the sclerotome and the epaxial myotome.

Specification The first stage of com mitment of ceU or tissue fate during which the cell or tissue is capable of differentiating autonomously (Le., by itself) when placed in an environment that is ne utral with respect to the developmen tal pathway. At the stage of specification, ceU cornm.itment is still capable of being reversed. Spemann's Organizer More correctly, the Spemann-Mangold Organizer. In amphibians, the dorsal lip cells of the blastopore and their derivatives (noto chord and head endomesoderrn).

Function ally equivalent to Hensen's node in chick, the node in mammals, and the shield in fish.

Organizer action establishes the basic body plan of the early embryo.

Spermatids HaplOid sperm ceUs gen erated by meiosis that are still connect ed to one another through their cyto plasmic bridges, and are therefore functionally diploid due to the diffu sion of gene products across the cyto plaSmiC bridges. Spermatogenesis The production of sperm.

Spermatozoa The male gamete or mature sperlll cell. Sperm head Consists of the nucleus, acrosome, and minimal cytoplasm Spermiogenesis The differentiation of the mature spermatozoa from the hap loid round spermatid. Sperm-surface galactosyltransferase A mammalian sperm surface protein that recognizes carbollydrate residues on ZP3 and may contribute to sperm zona binding. Spina bifida A congenital defect result ing from failure to close the posterior neuropore, the severity of which depends on how much of the spinal cord remains exposed.

Spiral holoblastic cleavage Character istic of several animal groups, includ ing arulelid worms, some flatworms, and most molluscs. Cleavage is at obliqlle angles, forming a "spiral" arrangement of daughter blastomeres, the cells touch one another at more places than do those of rad ia lly cleaving embryos, assuming the most thermodynamically stable packing orientation. Splanchnic mesoderm Also called the visceral mesoderm; derived from later al mesoderm closest to the endoderm . (ventral) and separated from other component of lateral mesoderm (somatic, near ectoderm, dorsal) by the intraembryonic coelom. It forms the splanchnopleure together with the underlying endoderm. It will form the heart, capillaries, gonads, the visceral mesothelial and serous membranes that cover the organs, the mesenteries, and blood cells. Spliceosome A comp–ex, made up of small nuclear RNAs (snRNAs) and splicing factors, that bind to splice sites and mediates the splicing of nRNA. G LOSSARY Splicing enhancer A cis-acting sequence on nRNA that promotes the assembly of spl iceosomes at RNA cleavage sites. Proteins that bind to splice sites or to the a re(1S adj acen t to S plici ng factors them. Splicing isoforms Different proteins encoded by the same gene and gener ated by alternative splicing. Splicing silencer A cis-acting sequence on nRNA that acts to exclude exons from an mRNA sequence. SRY Sex-determining region of the Y chromosome. Gene encoding the mammalian testis-determining factor. The SRY gene is probably (lctive for only a few hours n i the genital ridge, during which time, it synthesizes the Sry transcription factor. whose pri mary role is to activate the SOX9 gene required for testis formation. STAT Signal transducers and activators of transcription. A family of transcrip tion factors. part of U1e JAK-STAT pathway. Important in the regulation of human fetal bone growth. cel l A rela tively undifferentiated cell from the embryo, fetus, or adult that.

when it divides, produces (l) one cell that retains its undifferentiated character and remains in the stem cell niche, and (2) a second cell that leaves the niche and can u n dergo one or more paths of differentiation. Stem Stem cell mediated regene ration Stem cells allow an organism to regrow certain organs or tissues (e.g., hair, blood cens) that h ave been lost. Stem cel l niche All environment (regulatory microenvirorunent) that provides a milieu of extracellular matrices and paracrine factors that allows cells reSiding within it to remain relatively lUdifferentiated. Regulates stem cell proliferation and differentiation. Stereoblastulae Blastulae produced by spiral cleavage; have no blastocoeL Stomodeum See Oral plate. Stratum corneum See Cornified layer. Stratum germ inati vu m See Basal layer. Stylopod The proximal limb bones of any vert'ebrate limb. adjacen t to the body wall, consisting of eilher humerus (forelimb) or femur (hindlim b). Subendothelial cells A mino r cell pop ula tion in the bone marrow that may be critical for forming the hematopoi etic inductive microenvironment.

Subgermi na l cavity A space bet\veen the blastodenn and the yolk of avian eggs which is created when the blasto derm cells absorb water from the albu men ("egg white") and secrete the fluid between themselves and the yolk. A longitudinal groove that divides the developing spinal cord Sulcus l i m itans and medulla into dorsal (receives sen sory input) and ventral (initiates motor functions) halves.

Superficial cleavage The divisions of the cytoplasm of centrolecithal zygotes occur only in U1e rim of cytoplasm around the periphery of the cell due to the presence of a large (lmowtt of cen trally located yolk.

as in insects. Surfactant A secretion of specific pro teins and phospholipids SUdl as sphin gomyelin and lecithin produced by the type II alveolar cells of the IWlgS very late in gestation. The surfactant enables the alveolar cells to touch one another without sticking together.

Symbiont The smaller organism in a sytnbiotic relations hip in which one of U,e organisms i.nvolved is much larger than the other, and the smalJer organ ism may live on the surface Or inside the body of the larger. Symbiosis Greek, "Uving together." Refers to any close aSSOCiation between organisms of different species. Syncyti al blastoderm Describes the Drosophila embry o Since no cell mem branes exist other than that of the egg itself. Syncytial spe cifi cation The interac tions of nuclei and lTanscription fac tors, which eventua l ly result in cell specification, lake place in a conunon cytoplasm. Syncytiotrophoblast A population of cells from the m urine and primate tro phoblast thal undergoes mitosis with o ut cytokinesis resul ting in multinu cleate cells. The syncytiotrophoblast tissue is thought to further the pro gression of the emb ryo into the uterine wall b y digesting ute rine tissue. Syncytium Many nudei reside tn a conunon cytuplasm , results either from karyokinesis without cytokinesis or from cell fUl;ion. Greek SY", "connected." Delived from the most darsal sclero tome cells, which express the scleraxis gene and generate the tendons . Syndelome Syndrome Greek. " happen ing togeth er." Several malformations or patholo gies that occur concurrently. Genetical ly based syndromes are C(used either by (l) a chromosomal event (such as trisomy 21) where several genes are deleted or added. or (2) by one gene having many effects. G-23 T T-box (Tbx) A s pecific DNA-binding domain found in certain transcription factors, incl uding the T (8rachyllry) gene. lbx4 and lbxS. Tbx4 and lbxS help specify hindlimbs and forelimbs. respectively. The second step in the specification of axonal connection, wherein the axons, once they reach the correct area, recognize and bind to a set of cells with which they may form stable connections. Ta rget selection The sequence TATA within the promoter, to which RNA pOly merase will be bound. Usually occurs about 30 base pairs upstream from the TATA box first transcribed base Te lenceph a lon The iterior subdivi Sion 'Of the prosencephalon. wil l even tually form the cerebral hemispheres. Telolecithal Describes the eggs of bij ds and fish which have only one small area of the egg that is free of yolk. Repeated DNA sequences at Ule ends of chromosomes. Telomeres Telson Tail of Drosophila and other dipteran insects. Teratocarcinoma A tumor derived from malignant primordial germ cells and containing an undifferentiated stem cell pop'ulation (embryonal carci noma. or EC cells) that has biochemi cal and develop mental properties simi lar to those of the inner cell mass. EC cells can differentiate into a wide vari ety of tissues. incl uding gut and respi ratory epithelia. muscJe, nerve.

carti lage, and bone. Teratogen s Greek, "monster-formers." Exogenous agents lhat cause disrup tions in development res uJ ling in ter atogenesi s. the formation of congenital defects.

Tera tology is the study of birth defects and of how envirunmental agents djsrupt normal developmen t. Loops in the medullary (central) region of the developing testis formed by the developing Sertoli celis Testis cords and the incomi ng germ cells. Wtl l become the seminiferous tubules and site of spermatogenesis. Testis-determining factor A protein encoded by the SRY (sex determining region on Y) gene on the mammalian Y i chromosome that organ.zes the gonad into a testis rather than an ovary. Testosterone A steroid hormone secreted by the fetal testes which mas culinizes the fetus, stimulating the for mation of the penis, male duct system, scrotum, and other portions of the male anatomy, as weB as inhibiting development of the breast primordia. G-24 G LOSSARY TGF- su pe r fa m il y Transforming growth factor, More than 30 structural ly related members of a group of paracrine factors. The proteins encod ed by TGF-p superfamily genes are processed such that the carboxy-termi nal region contains tlle mature pep tide. These peptides are dimerized into homodlrners (with themselves) or het erodimers (with other TGF-p peptides) and are secreted from the ceiL The TGF-p superfam il y includes the TGF-p family, the activin family, the bone morphogenetic proteins (BMPs), the Vgl family, and other proterns, includ ing gtiaJ-derived neurotrophic factor (GDNF; necessary for kidney and enteric neuron differentiation) and Mullerian inhibitory factor (which is involved in mammalian sex determi nation).

Thecal cells Steroid hormone-secreting ovarian cells surrounding the follicle that differentiate from mesenchyme cells of the ovary. Thermotaxis Migration towards an area of higher temperature. Capacitat ed sperm of some mammalian spedes may sense U,e 2°C temperature differ ence between the isthmus of the oviduct and the warmer ampullary region and preferentially swim from cooler to warmer sites. Threshold model A model of develop ment wherein the different events of are triggered by different concentra tions of morphogens or hormones. Tip ce l ls Certain endothelial cells that can respond to the VEGF signal and begin "sprouting" to form a new ves sel during angiogenesis. Tissue engineering A regenerative medicine approach whereby a scaffold is generated from material that resem bles extracellular matrix or decellular ized extracellular matrix from a donor, is seeded with stem cells, and is used to replace an organ or part of an organ.

Totipotent Latin, "capable of aiL" Describes the earliest manunaJian blas tomeres (such as each blastomere of an S-cell embryo), which can form both trophoblasl cel ls and the embryo pre cursor cells.

Compare with pluripo t'ent. trans-activating domain The tran scription factor domain that activates or suppresses the transcription of the gene whose promoter or enhancer it has bound, usually by enabling the transcription factor to interact with the proteins involved in binding RNA polymerase or with enzymes that modify histones. lrans-regulatory elements Soluble molecules whose genes are located elsewhere in the genome and which g a dorsolat bind to the cis-regulatory dements. They are usually transcription factors. Transcription-associated factors (TAFs) Proteins required to facilitate efficient binding of eukaryotic RNA polymer.s bel ly if they migrate alon eral pathway. Tubulin A dimeric protein that poly merizes to form the microtubules that are the basis of the sperm flagellar axoneme and U,e mitotic spindle. es to the TATA sequence within the promoter. Transcription factor A protein that binds to enhancer DNA to alter gene expression. Tumor angiogenesis factors Factors secected by microtumors, incl uding VEGFs, Fgf2, placenta-like growth fac tor, and others, which stimulate mito sis in endothelial cells and direct the cell differentia tion into blood vessels Transcription faclor domains The three major domains are a DNA-bind ing doma i n, a trans-activating domain and a protein-protein interaction domain. Transcription initiation site Nucleotide sequence of at the 5' end signaling the start of gene transcrip tion. Also called the cap sequence because it encodes the 5' end of the RNA, which will receive a "cap" of modified nucleotides soon after it is transcribed. Tra nscription termination sequence Transcription continues beyond the AATAAA site for about 1000 nucleotides before being terminated.

Transdifferentiation The use of tran scription factors to directly transform one differentiated cell type into anoth er. Transforming growth factor See TGF-p. Transgene Exogenous DNA or gene introduced through experimental manipulation into a cell's genome. Trefoil stage A lhree-lobed stage of certain spirally cleaving embryos, resulting from the extrusion of a par ticularly large pola r lobe at first cleav age.

Triploblastic "three-layer" animals containing tissues derived from the three germ layers, ectodeml, meso- derm, and endoderm, as opposed to diploblastic animals that lack meso derm tissue. Trophoblast The external cells of the early mammalian embryo (i.e., the morula and the blastocyst) that bind to the uterus. Trophoblast cells form the dl0rion (the embryonic portion of the placenta). Also caJJed trophectoderm. Truncus arteriosus Cardiac outflow tract precursor that will (orm the roots and proximal portionof the aorta and pulmonary artery. Trunk neural crest Neural crest ceUs migrating from this region become the dorsal root ganglia containing the sen sory neurons, the adrenal medulla, the nerve dusters surrounding the aorta, and SchwaM cells if they migrate along a ventral pathway, and they gen erate melanocytes of the dorsum and in the direction of the tumor. Tumor suppressor genes Regulatory genes that inhibit cell division and increase the adhesion between cells; they can also induce apoptosis of rap idly dividing cells.

Cancer can result from either mutations or inappropriate methylations that inactivate tumor suppressor genes. Tunica albuginea A thick, whitish cap sule of extracellular matrix that encas es the testis. Type B sper m atogon ia Precursors of the spermatocytes and the last cens to undergo mitosis, they are derived from the mitotic division of intermediate spermatogonia. U Umbilical cord Connecting cord derived from the allantois that brings the embryoniC blood circulation to the uterine vessels of the mother. Unipotent stem cells Stem cells from particular tissues that are involved in regenerating a particular type of cell. Unsegmented mesoderm The bands of paraxial mesoderm in non-avian vertebrat'e embryos. 3' Untranslated region (3' UTR) A region of a eukaryotic gene and RNA following the translation termination codon that, although transcribed, is not translated into protein. It often contains regulatory regions that con trol the rate of translation. 5' Un translated region (5' UTR) A region of a eukaryotic gene or RNA: the sequence of base pairs i.ntervening between the initiation points of tran scription and translation. These are not translated into protein, but may con tain import..'lnt regulatory sequences. Upstream promoter elements DNA sequences near the TATA box and usu aUy upstream from it thai bind 10 the transcription-associated factors (TAFs). Ureteric bu ds Paired epithelial branches induced by the metanephro genic mesenchyme to branch from each of the paired nephric ducts. Ureteric buds will form the colJecting ducts, renal pelvis, and ureters that take the u rine to the bladder.

GLOSSARY Urodeles Salamanders. Uterine cycle A componen t of the menstrual cyde, the function of the uterine cycle is to provide the appro priate environment for the developing blastocyst. V Vagal neural crest N eura l crest cells from the neck region, which overlaps the o'a nial/ trunk o'est boundary. Gen erates th e parasympathetic (enteric) ganglia of the gut, which are req uired for peristaltic movement of the bowels. Vascular endothelial growth factors (VEGFs) A lamily proteins involved in vasculogenesis which 01 includes several VEGFs, as well as pla cental growth factor.

Each VEGF appears to enable the differentiation of the angioblasts and their multiplica- tion to form endothelial tubes.

Vasculogenesis The de novo creation of a nehvork of blood vessels from the lateral pla te mesoderm. Vas (ductus) deferens Derived from the WoLffian duct, the tube through which sperm pass from U,e epididymis to the urethra. VegT pathway lnvolved in dorsal-ven tral-pola rity and specification of the emb ryo. Tlle VegT pathway activates organizer cells in tbe amphibian the expression of Vgl and other Nodal-related paracrine factors, which in tum activate the Smad2/4 transcrip tion factor in the mesod ermal cells above them, activating genes that give these cells their "organizer" properties. Vegetal hemisphere The bottom por tion of the amphi bian egg, containing yolk, which serves as food for the developing embryo. The yolk-filled cells constituting the ventral midline of the embryo, at the onset of gastrula tion in Drosophila. Ventricular zone (VZ) Inner layer of the developing spinal cord and brain tion of the egg or embryo. Vegetal rotation During frog gastrula tion, internaJ cel l rea rrangements p lace the prospective pharyngeal endoderm cells adjacen t to the blastocoel and immediately above the involuting mesoderm. Vellus Short and silky hair of the fetus and neonate that remains on many pa rts of the hwnan body tha t are usu al ly considered hairless, such as the forehead and eyelids. In o ther areas of the body, vellus hair gives way to l onger and thicker " termin al" hair. Ventral furrow Invagmation of the prospective mesoderm, about lOOO gets p-catenin for degradation. How ever, when the Wnt signal is present and GSK3 is inh.ibited, – catenin can dissociate from the APC protein and enter the nucleus. Once inside Ule nucleus, it can (orm a heterodimer with another DNA-bin ding protem, becoming a transcription factor.

This complex binds to and ac tivates the Vital dyes Stains used to tabel living cells w i thout kil l ing them. When applied to embryos, vital dyes have been used to foUow cell migration dur ing development and generate fate maps of specific regions of the embryo. Vitelline envelope In inverteb rates, the extracellular matrix that forms a fibrous mat around the egg outside the cell membrane and is often involved in sperm-egg reCOgnition and is essential for the species-specific binding of sperm. The vitelline envelope contains several different glycoproteins. It is supplemen ted by extensions of mem brane glycoproteins from the cell Wnt-responsive genes. Wnt pathway (non-canonical) Disheveled protein can interact with a Rho GTPase. This GTPase can activate the kinases tha t phosphoryla te cytoskeletal proteins and thereby alter cell shape, cell polarity (where the upper and lower portions of the cell differ), an d motil i ty. A third Wnt pa th way diverges earlier than Disheveled. Here, the Frizzled receptor pro tein activates a phospho lipase (PLC) that synthesiZes a compound that rel ea ses calcium ions from the endoplas mic Vitelline veins The veins, continuous with the endocardiwn, that carry nutrients nom the yolk sac into the sinus venosus of the developing heart. These veins fonn from yolk sac blood islands, and bring nu trients to the avian embryo and transport gases to and from the sites of respiratory exchange.

Vitellogenesis Tlle accumulation of yolk proteins in tel oleci thal and mesolecithal eggs. Six cells in the larval stage of C. elegans th at will form the vulva via inductive signals. reticulum. ll1e releClsed calcium can activate enzymes, transcription factors, and translati on factors. Wolffian duct (nephric duct) Derived from the caudal portions of the pronephric d uct . In mammals, it is the central component of the excretory system throughout devel opmen t. Degenerates in fema les; in males, becomes Ule epid idymis and vas deferens. x Wnts A gene family of cysteine-rich glycoprotein paracrine factors. Their name is a fusion of the name of the Drosophila segruent polari ty gene W;Ilg less with the name of one of its verte- bral'e homologues, integrated. Wnt pro teins are critical in esta blishing the polarity of insect and vertebrate limbs; promoting the proliferation of steru Mecha nism of dosage compensa tion in mam X ch rom oso m e in activation mals; the irreversible conversion of the chroma tin of one X chromosome in each female (XX) cell Lnto highly con Wholist organicism Philosophical notion stating that the properties of the whole cannot be predicted solely from the properties of its component parts, and tha t the properties of the parts are informed by their relationship to the whole. It was very influential in the construction of developmental biology. When Wnt activates Disheveled, the membrane and by proteinaceous " posts" that adhere the vitelline enve- lope to the membrane. W The yolk con taining por GSK-1, if it were active, would prevent the dissociation of the – catenin pro tein from the APC protein, which tar- one generation to lhe next through the germ cells.

Can refer to the transfer of symbionts via the germ cells, usually the eggs. tula. Vegetal pole zled to activate the Disheveled protein. Once Disheveled is activated, it inhibits the activity of the glycogen synthase kinase 3 (GSK3) enzyme. Transfer from Vulval precursor cells (VPCs) Area of thickened cells at the vegetal pole of the sea urchin blas Wnt pathway (canonical) Binding of Wn t pro teins to Frizzled causes Friz tube and will eventually form the ependyma. cells are divide more slowly and undergo less movement during embryogenesis (and hence are like p lants, or "vegetal"). Vegetal plate reUs; and in several steps of urogen ital system devel opme nt. medulla.

Forms from the germinal neuroepitheJiwn of the original neural Vertical transmission G-2S y densed heterochromatin (Barr bodies), thus preventing excessive transcrip tion of X-chromosome genes. See also dosage compensation. Yellow crescent Region of the tunicate zygote cy toplasm containing yellow l ipid inclusions that wj]) become meso derm. Yolk cell The uncleaved, yolk-contain ing portion of the telolecithal egg that underlies the blastoderm. Yol k plug The large endodermal cells that remain exposed on the vegetal G-26 GLOSSARY surface surrounded by the blastopore of the amphibian gastrulating embryo. Yolk sac The hrst extrClembryon.ic membrane to form, derived from splanchnopleure that grows over the yolk to enclose it. The yolk sac medi ates nutrition in developing birds and reptiles. It is connected to the midgut by the yolk duct (vitelline duct), so that the walls of the yolk sac and the Yolk syncytial layer (YSl) A cell popu lation in the zebrafish cleav(lge stage walls of the gut are continuous. embryo formed at the ninth or tenth cell cycle, when the cells at the vegetal edge of the blastoderm fuse with the underlying yolk cell producing a ring Z of nuc1ei in the part of the yolk cell cytoplasm that sits just beneath the blastoderm . Lnportant for directing some of the ceU movements of gastru la tion.

Zeugopod The middle limb bones 01 any vertebrate limb, consisting of either radius and ulna (forelimb) or tibia and fibula (hindlimb). Zona peHucida Glycoprotein coat (extracellular matrix) aroW1d the mam malian egg, synthesized and secreted by the growing oocyte. Zo na protein s (ZP1, ZP2, ZP3) The three major glycoproteins of the mouse zona pellucida . ZP3 binds the sperm and, once the sperm is bOLUld, initiates the acrosome reaction. ZP2 binds to acrosome-reacted sperm. ZPl holds the zona pelJucida together. Zone of polarizing activity (ZPA) A small b l ock of mesodermal tissue near the posterior j unction of the yOW1g limb bud and the body wall that speci fies the anterior-posterior ax.is of the developing limb tJuough the action of the paracrine factor, sOllie hedgehog. A fertilized egg with a diploid chromosomal complement in its zygote nucleus generated by fusion of the haploid male and female pronuclei. Zygote