19th International *Xenopus* Conference

XIX IXC

Cambridge, MD USA - August 20-24, 2023

Hyatt Regency, Chesapeake Bay
Cambridge, Maryland USA
August 20-24, 2023

Conference Organizers:

Matt Good	University of Pennsylvania
Ann Miller	University of Michigan
Amy Sater	University of Houston
Aaron Zorn	Cincinnati Children's Hospital

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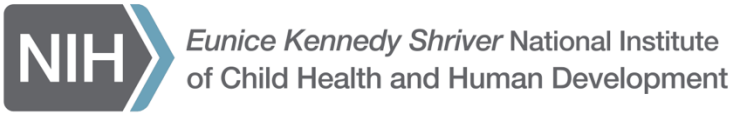

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19th International *Xenopus* Conference

Cambridge, MD USA - August 20-24, 2023

Invited speaker talks: 15 minutes followed by 5 minute question and answer time

Selected abstract talks: 10 minutes followed by 5 minute question and answer time (indicated in blue)

*** Trainee Presentation**

Each session will include a mix of topics across developmental biology, cell biology, genomics, biophysics, etc.

Online program link: https://www.19thxenopusconference.org/program

Sunday August 20, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
12:00 - 16:00	**Check-in / Registration**
16:00	**Welcome**
16:15 - 17:00	**Keynote 1 - Roberto Mayor**, Univ. College London, UK *Xenopus embryos: ideal for developmental mechanics* Chair: **Elena Silva**, Georgetown Univ., USA **Sponsored by the International Xenopus Board**
17:00 - 17:30	**Break**
17:30 - 19:25	**Session 1** Chair: **Sally Moody**, George Washington Univ., USA
17:30 - 17:50	**Laurent Kodjabachian**, IBDM Marseilles, France *Ciliated epithelium patterning and the emergence of fluid flows*
17:50 - 18:10	**Jenny Gallop**, Gurdon Inst., UK *Understanding the molecular origins of filopdial dynamics*
*18:10 - 18:30**	**Soichiro Kato**, Kumamoto Univ., Japan *Blastopore gating mechanism for temporal control of archenteron fluid excretion*
*18:30 - 18:50**	**Sourabh Sengupta (Levy Lab)**, Univ. of Wyoming, USA *Regulation of nuclear morphology under stress conditions in Xenopus laevis egg extracts*

Sunday August 20, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
18:50 - 19:05	**Nydia Tejeda-Munoz**, UCLA, USA *Lysosomes are Required for Early Dorsal Signaling in the Xenopus Embryo*
19:05-19:25	**Todd Stukenberg**, Univ. of Virginia, USA *Combining systems cancer approaches and frogs to understand how breast tumors generate Chromosomal Instability*
19:30	**Mixer / Dinner**

Monday August 21, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
08:00 - 08:30	**Breakfast**
08:30 - 10:45	**Session 2** Chair: **Mustafa Khokha**, Yale Univ., USA
08:30 - 08:50	**Soeren Lienkamp**, Univ. of Zurich, Switzerland *Genetic models for kidney disease*
*08:50 - 09:10**	**Venecia Valdez (Petry Lab)**, Princeton Univ., USA *Molecular Insights into how the protein HURP facilitates spindle microtubule formation*
09:10 - 09:30	**Chenbei Chang**, Univ. Alabama at Birmingham, USA *Functional and biochemical characterization of disease-associated YWHAZ variants in Xenopus*
09:30 - 09:45	**Betsy Pownall**, Univ. of York, UK *FGF regulates the switch from pluripotency to commitment to the myogenic lineage*
*09:45 - 10:00**	**Natalie Mosqueda, (Brownlee Lab),** Stonybrook Univ., USA *The Role of Palmitoylated Importin a in Ciliogenesis*
*10:10 - 10:25**	**Yuuri Yasuoka**, RIKEN, Japan *Genomic, transcriptomic, and proteomic approaches toward understanding mitochondrial genome evolution in frogs*

Monday August 21, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
10:25 - 10:45	**Leon Peshkin**, Harvard Univ., USA *Tabula Rana: Cell Atlas of larval and adult Xenopus tissues*
10:45 - 11:00	**Break**
11:00 - 12:55	**Session 3** Chair: **Sergei Sokol**, Icahn School of Medicine at Mt. Sinai, USA
11:00 - 11:20	**Ken Cho**, Univ. of California Irvine, USA *Interplay of maternal transcription factors and epigenetic modifiers in gene expression stability*
11:20 - 11:40	**Sarah Woolner**, Univ. of Manchester, UK *Mechanical regulation of cell division in health and disease*
11:40 - 12:00	**Stefan Hoppler**, Univ. of Aberdeen, UK *Gene regulatory networks downstream of Wnt signaling in early embryogenesis and heart muscle development*
12:00 - 12:15	**Adam Session**, Binghamton Univ., USA *Long-read Xenopus genome assemblies enable identification of subtelomeres and comparative analysis of tetrapod chromosome evolution*
*12:15 - 12:35**	**Florina-Alexandra Toma (Sweeney Lab)**, Inst. of Science and Technology, Austria *Spinal interneuron function in swim and limb behaviour over frog metamorphosis*
12:35 - 12:55	**Amy Sater**, Univ. of Houston, USA *Modeling traumatic brain injury in Xenopus tadpoles*
13:00 - 14:30	**Lunch**
13:30 - 14:30	*Panel on Emerging Practices in Publishing*
14:30 - 16:30	**Poster Session 1 and Sponsor Exhibit - odd numbered abstracts - Sponsored by Xenopus1**

Monday August 21, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
16:30 - 17:15	**Keynote 2 - Helen Willsey**, Univ. of California San Francisco, USA *Using frogs to dissect psychiatric disorders* Chair: **Amy Sater**, Univ. of Houston, USA **Sponsored by the International Xenopus Board**
17:15 - 19:20	**Session 4 - Sponsored by Xenopus1** Chair: **Susannah Rankin,** Oklahoma Medical Research Foundation, USA
17:15 - 17:35	**James Ferrell**, Stanford Univ., USA *Protein homeostasis from diffusion-dependent control of protein synthesis and degradation*
17:35 - 17:55	**Hui Chen**, Univ. South Carolina, USA *Spatiotemporal Regulation of Genome Activation In Early Embryogenesis*
17:55 - 18:15	**Karel Dorey**, Univ. of Manchester, UK *Reconstructing the spinal cord after injury*
*18:15 - 18:30**	**Janet Tait (Rupp Lab)**, Ludwig Maximilians Univ., Munich, Germany *The histone H4K20 methyltransferase SUV4-20H1/KMT5B is required for multiciliated cell differentiation in Xenopus*
18:30 - 18:45	**Hui Zhao**, Chinese Univ. of Hong Kong, China *ZSWIM4 regulates early embryonic patterning and BMP signaling pathway by promoting nuclear Smad1 degradation*
*18:45 - 19:00**	**Thomas Naert (Lienkamp Lab)**, Univ. of Zurich, Switzerland *XenoVision, steps towards fully automated 3D phenotyping of whole-embryo development and disease using advanced microscopy and artificial intelligence*
19:00 - 19:20	**Darcy Kelley**, Columbia Univ., USA *Identifying genomic loci responsible for innate, species-specific acoustic communication in Xenopus; CNS vocal pattern generation (rhythm) and laryngeal sound production (timbre)*
19:30	**Dinner**
21:00 - 23:00	**Mixer / Poster Session 1 and Sponsor Exhibit - Sponsored by Xenopus1**

Tuesday August 22, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
08:00 - 08:30	**Breakfast**
08:30 - 10:15	**Session 5 - Sponsored by Xenopus1** Chair: **Dan Weeks**, Univ. of Iowa, USA
08:30 - 08:50	**Peter Walentek**, Univ. of Freiberg, Germany *Self-organization in mucociliary epithelia - signaling control of stem cells and cell identities in development and disease*
*08:50 - 09:10**	**Coral Zhou (Heald Lab)**, Univ. of California Berkeley, USA *Mechanisms of genome scaling in embryogenesis and evolution*
09:10 - 09:30	**Kerstin Feistel**, Univ. of Hohenheim, Germany *Receptor-mediated endocytosis orchestrates anterior neural tube closure*
*09:30 - 09:45**	**Katharina Till (Borchers Lab)**, Philipps Univ. Marburg, Germany *The RhoGEF Trio functions downstream of Ptk7 in NC cell contact inhibition of locomotion*
09:45 - 10:00	**Abraham Fainsod**, Hebrew Univ. of Jerusalem, Israel *How folic acid prevents the formation of neural tube defects*
*10:00 - 10:20**	**Nicole Edwards (Zorn Lab)**, Cincinnati Children's Hospital, USA *Disrupted endosomal trafficking of polarity proteins causes congenital trachea-esophageal separation defects*
10:25 - 10:45	**Break**
10:45 - 12:45	**Session 6 - Sponsored by Molecular Instruments** Chair: **Jeremy Green**, King's College London, UK
10:45 - 11:05	**Anna Philpott**, Univ. of Cambridge, UK *To be or not to be (a neuron)-that is the question*
11:05 - 11:25	**Mark Corkins**, Icahn School of Medicine at Mt. Sinai, USA *Comparative analysis of Xenopus mesonephric transcriptomics: Conservation of the developmental lineage of nephron stages*
11:25 - 11:45	**Eva Hoermanseder**, Helmholtz Univ., Germany *Remember or Forget: Reprogramming Cellular Memories*
11:45 - 12:05	**Mike Sheets**, Univ. of Wisconsin Madison, USA

Tuesday August 22, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
	Post-transcriptional mechanisms and the control of embryonic cell fates
12:05 - 12:20	**Emmanuel Tadjuidje**, Alabama State Univ., USA *Embryotoxicity of Statins and other Prescribed Drugs that Inhibit Cholesterol Biosynthesis*
*12:20 - 12:35**	**Valentina Kostiuk (Khokha Lab)**, Yale Univ. Sch. of Medicine, USA *Nup107 contributes to maternal to zygotic transition by regulating the nuclear export of pri-miR427*
12:35 - 12:45	**Aneesh Acharya** - Molecular Instruments *HCR™ RNA-FISH: The benchmark in multiplexed RNA imaging*
13:00 - 14:30	**Lunch**
14:30 - 16:30	**Poster Session 2 and Sponsor Exhibit - even numbered abstracts - Sponsored by Iwaki Aquatic**
16:30 - 17:15	**Keynote 3 - Bill Bement**, Univ. of Wisconsin Madison, USA *Self-organizing waves of Rho GTPase activity as a conserved mechanism for cell shape control* Chair: **Ann Miller**, Univ. of Michigan, USA **Sponsored by the International Xenopus Board**
17:15 - 19:20	**Session 7** Chair: **Aaron Zorn**, Cincinnati Children's Hospital Medical Center, USA
*17:15 - 17:35**	**Edward Cruz (Wuhr Lab)**, Princeton Univ., USA *Turnover Proteomics in Xenopus Embryogenesis*
*17:35 - 17:55**	**Hyeyoon Lee (Niehrs Lab)**, DKFZ Heidelberg, Germany *R-Spondin 2 governs Xenopus left-right body axis formation*
*17:55 - 18:10**	**Tara Loughery (Gomez lab)**, Univ. Wisconsin-Madison, USA *Role of EGF signaling in peripheral axon exiting the spinal cord*
18:10 - 18:30	**Kelly Tseng**, Univ. of Nevada Las Vegas, USA *Understanding mechanisms of eye regrowth using the frog embryo*

Tuesday August 22, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
*18:30 - 18:45**	**Gembu Maryu (Yang lab)**, Univ. Michigan Ann Arbor, USA *Nuclear-cytoplasmic compartmentalization of cyclin B1-Cdk1 promotes robust timing of mitotic events*
18:45 - 19:00	**John Young**, Simmons Univ., Boston, USA *Insights into the mechanism of hind limb initiation in Xenopus laevis*
19:00 - 19:20	**Andrea Wills**, Univ. of Washington, USA *Defining the metabolic requirements for appendage regeneration*
19:30	**Dinner**
21:00 - 23:00	**Mixer / Poster Session 2 and Sponsor Exhibit - Sponsored by Iwaki Aquatic**

Wednesday August 23, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
07:30 - 08:30	**Breakfast**
08:30 - 10:30	**Session 8** Chair: **Jean Pierre Saint-Jeannet**, New York Univ., USA
08:30 - 08:50	**Taejoon Kwon**, UNIST, S. Korea *Evidence-based integration and improvement of Xenopus genome annotation*
08:50 - 09:10	**Romain Gibeaux**, Univ. Rennes, CNRS, France *Changes in seam number and location induce holes within microtubules assembled from porcin brain tubulin and in Xenopus egg cytoplasmic extracts*
09:10 - 09:30	**Muriel Perron**, CNRS-Paris, France *A roadmap for retinal regeneration*
09:30 - 09:45	**Kara Pratt**, Univ. of Wyoming, USA *microCT scans of the Xenopus laevis tadpole*

Wednesday August 23, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
09:45 - 10:00	**John Measey**, Yunnan Univ, China, and Stellenbosch Univ, South Africa *Home and away: the core gut microbiome of Xenopus laevis is modified by its environment*
10:00 - 10:15	**Takuya Nakayama**, Univ. of Virginia, USA *Novel insights regarding the role of transcription factor Six3 in retina development are revealed by a single-nucleus RNA-sequencing method for comparing expression in wild-type and mutant embryos of Xenopus tropicalis*
10:15 - 10:30	**Yuki Shibata**, Nippon Medical School, Japan *NEXTrans: Simple transgenesis at a novel safe harbor site by using CRIPSR-Cas9 in Xenopus laevis*
10:30 - 11:00	**Break**
11:00 - 12:55	**Session 9** Chair: **Rachel Miller**, Univ. of Texas Houston, USA
11:00 - 11:15	**Haruki Ochi**, Yamagata University, Japan *Unraveling the Mechanisms of Kidney Regeneration through Damage-Response/Regeneration Enhancers*
11:15 - 11:35	**Miler Lee**, Univ. of Pittsburgh, USA *Differential regulation of gene copies in the allotetraploid Xenopus*
11:35 - 11:55	**Qiong Yang**, Univ. of Michigan, USA *Single-cell analysis of mitotic cycles and energy flow: insights from cell-free Xenopus extracts*
11:55 - 12:15	**Paula Slater-Guzman**, Univ. San Sebastian, Chile *Mitochondrial function during Xenopus laevis spinal cord regeneration*
*12:15 - 12:35**	**Jaeho Yoon (Daar Lab)**, National Cancer Institute, USA *Wnt4 and ephrinB2 instruct apical constriction via Dishevelled and non-canonical signaling*
12:35 - 12:55	**Carole LaBonne**, Northwestern Univ., USA *The Evolution of Gene Regulatory Networks: A View From the Crest*
13:00 - 14:30	**Lunch / Afternoon Break**
13:30 - 14:30	*Roundtable on use of Xenopus in teaching*

Wednesday August 23, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
15:00 - 16:55	**Session 10** Chair: **Ira Daar**, National Cancer Institute, USA
15:00 - 15:20	**Richard Harland**, Univ. of California Berkeley, USA *The Rho GEF Plekhg3 is required specifically for involution movements in Xenopus gastrulation*
15:20 - 15:40	**Shuo Wei**, Univ. of Delaware, USA *Diphthamide deficiency promotes association of eukaryotic translation elongation factor 2 with p53 to induce p21 expression and neural crest defects*
15:40 - 16:00	**Dan Weeks**, Univ. of Iowa, USA *Nucleolar Domain Decisions: properties that contribute to dense fibrillar component localization*
*16:00 - 16:20**	**Shinuo Weng (Wallingford Lab)**, Univ of Texas Austin, USA *Planar polarized force propagation links cell intercalation to tissue-scale convergent extension*
16:20 - 16:35	**Jeremy Green**, King's College, London, UK *Assessment of a double ovulation protocol for Xenopus laevis: moderately transiently elevated corticosterone levels without loss of egg quality are associated with doubled fertilisation yield*
16:35 - 16:55	**Kris Vleminckx**, Ghent Univ., Belgium *Identification of cooperating cancer driver genes using CRISPR multiplexing in Xenopus tropicalis*
17:00 - 17:20	**Break**
17:20 - 18:55	**Session 11** Chair: **Matt Good**, Univ. of Pennsylvania, USA
17:20 - 17:40	**Brian Mitchell**, Northwestern Univ. USA *The role of macropinocytosis in tissue homeostasis*
17:40 - 18:00	**Shuyi Nie**, Georgia Tech, USA *Cdc42ep1 coordinates neural crest cell migration by interacting with Cdc42 and septic filaments*

Wednesday August 23, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
18:00 - 18:20	**Xianrui Cheng**, Univ. of Southern California, USA *De novo generation of spatially organized cytoplasm*
*18:20 - 18:35**	**Takayoshi Yamamoto**, Univ. of Tokyo, Japan *Robust and quick shaping of morphogen gradient through receptor feedback and heparan sulfate interactions*
18:35 - 18:55	**Jerome Jullien**, ISTERM, France *Sperm derived H2AK119ub1 is required for embryonic gene expression regulation in Xenopus laevis*
19:00 - 19:30	**Awards**
19:30	**Reception / Banquet**
21:00 - ??	**Dance**

Thursday August 24, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
07:30- 08:30	**Breakfast**
08:30 - 10:20	**Session 12** Chair: **Kim Mowry**, Brown Univ., USA
08:30 - 08:50	**Asako Shindo**, Kumamoto Univ. IMEG, Japan *Nutrients control thyroid morphogenesis*
08:50 - 09:10	**Peter Nemes**, Univ. of Maryland, USA *Metabolic Patterning of the X. laevis Embryo*

Thursday August 24, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
09:10- 09:25	**Beatrice Durand**, CNRS Sorbonne, France *Cerebellar granular neuron progenitors exit their germinative niche by way of Barhl1 mediated silencing of T-Cell Factor transcriptional activity*
09:25 - 09:40	**Christopher Thompson**, Virginia Tech Univ., USA *Overexpression of transthyretin and β-trace in the Xenopus laevis tadpole choroid plexus ameliorates the effects of lead poisoning on thyroid hormone mediated changes in brain development*
09:40 - 10:00	**Saurabh Kulkarni**, Univ. of Virginia, USA *Control of centriole number in multiciliated cells*
10:00 - 10:20	**Jakub Sedzinski**, Novo Nordisk Foundation Center of Stem Cell Medicine, reNEW, Univ. Copenhagen, Denmark *Dissecting the mechanism of basal bodies ascent, distribution, and patterning*
10:30 - 10:50	**Break**
10:50 - 12:10	**Session 13** Chair: **Andrea Wills**, Univ. of Washington, USA
10:50 - 11:10	**Yonglong Chen**, SUSTech, China *Activation of P53 pathway contributes to Xenopus hybrid inviability*
11:10 - 11:30	**Jen Landino**, Geisel School of Medicine at Dartmouth, USA *Self-organized Rho and F-actin patterning in an artificial cortex*
11:30 - 11:50	**Rachel Miller**, Univ. of Texas Houston, USA *Insights into Kidney Development and Disease: Perspectives from the Xenopus Model*
11:50 - 12:10	**Gerhardt Schlosser**, Univ. of Galway, Ireland *MARCKS and MARCKSL1 promote proliferation and neurite outgrowth during Xenopus spinal cord development and regeneration*
12:30 - 13:30	**Lunch**

Thursday August 24, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
13:30 - 16:00	**<u>Session 14</u> - Community Resources - Sponsored by Xenopus 1 and DSHB** Chairs: Mustafa Khokha, Yale Univ., USA and Aaron Zorn, Cincinnati Children's Hospital, USA
13:30 - 13:35	***Reagents*:** **Doug Houston**, University of Iowa, USA *Developmental Studies Hybridoma Bank (DSHB) Update*
13:35 - 13:40	**Todd Stukenberg**, University of Virginia, USA *ORFeome Update*
13:40 - 13:55	***Reagents Panel Q&A***
13:55 - 14:25	**James Coulombe,** Chief, Developmental Biology and Congenital Anomalies Branch, NIH, USA *Eunice Kennedy Shriver* National Institute of Child Health and Human Development *NICHD Opportunities*
14:25 - 14:30	***Stock Centers*:** **Marko Horb**, MBL, USA *National Xenopus Resource (NXR) Update*
14:30 - 14:35	**Matt Guille**, University of Portsmouth, UK *European Xenopus Resource Centre (EXRC) Update*
14:35 - 14:40	**Hajime Ogino**, Amphibian Research Center, Hiroshima Univ., Japan *National BioResource Project (NBRP) Update*
14:40 - 14:45	**Jaques Robert**, Xenopus Immunology Center, Univ. of Rochester, USA *Xenopus laevis Research Resource for Immunobiology (XLRRI) Update*
14:45 - 14:50	**Robert Weymouth**, Xenopus 1 *A collaboration-driven initiative for growth and sustainability of the Xenopus model organism*
14:50 - 15:25	***Stock Centers Panel Q&A***
15:25 - 15:30	***Genomes and Genomics*:** **Peter Vize**, University of Calgary, Canada *Xenbase Update*

Thursday August 24, 2023	Talks: Chesapeake Ballroom A-E Posters: Chesapeake Ballroom E-G Meals: Choptank
15:30 - 15:35	**Taejoon Kwon**, UNIST, S. Korea *The community platform for Xenopus genomic resource*
15:35 - 15:40	**Aaron Zorn**, Cincinnati Children's Hospital Medical Center, USA *Xenbase and the Alliance of Genomic Resources*
15:40 - 16:00	***Genomes and Genomics Panel Q&A***
16:00 - 16:15	**Closing**
	Conference Organizers: **Matt Good**, Univ. of Pennsylvania, USA **Ann Miller**, Univ. of Michigan, USA **Amy Sater**, Univ. of Houston, USA **Aaron Zorn**, Cincinnati Children's Hospital Medical Center, USA

Abstracts for Platform Presentations

Keynote 1:
Chair: **Elena Silva**, Georgetown Univ., USA
Sponsored by the International Xenopus Board

K1
Roberto Mayor, Univ. College London, UK
Xenopus embryos: ideal for developmental mechanics

Session 1:
Chair: **Sally Moody**, George Washington Univ., USA

S1.T1
Laurent Kodjabachian, IBDM Marseilles, France
Ciliated epithelium patterning and the emergence of fluid flows

Authors: Athullya Baby 1,2,3, Alexandre Chuyen 1, Aude Nommick 1, Etienne Loiseau 2, Camille Boutin 1, Virginie Thomé 1, Isabelle Cheylan 3, Julien Favier 3, Annie Viallat 2, Laurent Kodjabachian 1.

1. Marseille Developmental Biology Institute (IBDM), Turing Center for Life Sciences (Centuri), CNRS, Aix-Marseille University, France.
2. Marseille Interdisciplinary Nanoscience Center (CINaM), Turing Center for Life Sciences (Centuri), CNRS, Aix-Marseille University, France.
3. Laboratory of Mechanics, Modelling and Processes (M2P2), Turing Center for Life Sciences (Centuri), CNRS, Aix-Marseille University, France.

Ciliated epithelia are present throughout metazoans and serve functions ranging from locomotion of aquatic organisms, to mucociliary clearance of pathogens from airways in mammals. These functions are supported by the coordinated beating of myriads of motile cilia harbored by multiciliated cells (MCCs), which generates robust polarized hydrodynamic forces.

The Xenopus embryonic epidermis offers a tractable system that has provided key discoveries in the field of ciliated epithelium biology. It consists of thousands of regularly spaced MCCs that generate water currents that help clearing pathogens from the surface of the young tadpole. The production of efficient directional fluid flows depends on several parameters integrated across nanometric to millimetric scales during embryonic development, including cilia and MCC density, cilia and MCC distribution, cilia and MCC polarity.

In this presentation, I will review our recent works aimed at understanding the individual contribution of such parameters on cilia-powered fluid flows.
First, I will address how self-organized movement generates a regular pattern of MCC distribution.

Second, I will present our efforts to use computational modelling to evaluate the contribution of individual parameters to the production of cilia-powered flows, which can be complicated to achieve through experiments in the embryo.

S1.T2
Jenny Gallop
Understanding the molecular origins of filopodial dynamics

Authors: Jonathan R Gadsby 1, Pantelis Savvas Ioannou 1, Alison Smith 2, Claire Dobson 2, Jennifer L Gallop 1.

1 Gurdon Institute and Department of Biochemistry, University of Cambridge, Cambridge, UK.
2 Discovery Sciences, AstraZeneca, Granta Park, Cambridge, UK.

Filopodia are long, thin actin-rich projections from cells that are involved in sensing the environment and cell motility. They can reach across long distances to sense and influence embryonic movements. The actin in filopodia is dynamically separable from actin at the cell cortex or at the leading edge of migrating cells, with actin monomer incorporation occurring at the distal tips of filopodia. We have found that filopodia lengths are exponentially distributed and they display intriguing dynamic properties. To better understand the dominant contributors and molecular basis of filopodial length regulation we performed a phage display phenotypic screen isolating antibodies that perturb the lengths of actin bundles in a cell-free system of filopodia-like structures. By identifying and mapping the antigen of the antibodies we have found that the antibodies recognise a specific conformation of the actin filament and act to prevent actin disassembly. We propose that this conformational switch in the actin filament plays a key role in determining the length of filopodia, which may have implications for our broader understanding of cell motility.

S1.T3
Soichiro Kato
Blastopore gating mechanism for temporal control of archenteron fluid excretion

Authors: Soichiro Kato 1, Hidehiko Inomata 2

1. Institute of Molecular Embryology and Genetics, Kumamoto University
2. Center for Biosystems Dynamics Research, RIKEN

Abstract
Fluid uptake and efflux play roles not only in adult homeostasis but also in the patterning and morphogenesis of early embryos. Pathways for extracellular fluid movement in multicellular organisms are broadly classified into two types: cellular-level pathways, such as the paracellular and intercellular pathways, and tissue-level pathways, such as the multicellular pore or tube in which fluid movement is controlled by muscle contraction. Previous studies have reported that in early embryos with immature functional muscles, fluid movement is primarily regulated through cellular-level pathways. Interestingly, however, early Xenopus embryos rapidly excrete the archenteron fluid by opening of the tissue-level blastopore, the mechanism of which remains elusive. We revealed that the blastopore temporally regulates fluid excretion through actomyosin-mediated apical constriction (Kato and Inomata, 2023). Fluid pressure measurements using microelectrodes in vivo showed that the pressure in the archenteron is constant, whereas the upper limit of pressure resistance of the blastopore decreases with development. By combining physical perturbations and imaging analysis, we found that the pushing force exerted by the circumblastoporal collars (CBCs) at the slit periphery regulates pressure resistance. The pushing force is regulated by actomyosin-mediated apical constriction at both dorsoventral ends of the blastopore, and we found that release of the ventral constriction decreases the upper limit of pressure resistance, leading to the archenteron fluid excretion. These results demonstrate that early embryos, lacking muscle, can regulate blastopore gating via apical constriction to control the temporal fluid excretion, similar to adult urethral gating via the sphincter.

S1.T4
Sourabh Sengupta, PD (Levy Lab)
Regulation of nuclear morphology under stress conditions in Xenopus laevis egg extracts

Dr. Sourabh Sengupta, Prof. Daniel L. Levy

Department of Molecular Biology, University of Wyoming, Laramie, WY

Normal cells have a nuclear size within a defined range, while aberrations in nuclear morphology are often associated with disease, for instance cancers and laminopathies. The mechanisms by which a cell maintains nuclear size, shape, and structure are not well understood. We are investigating the impact of osmotic (mannitol), oxidative (hydrogen peroxide), and proteotoxic (proteasome inhibitor MG132) stress on

nuclear morphology, using de novo nuclear assembly in Xenopus egg extract. This system allows us to investigate transcription-independent stress responses, and the extract's biochemical composition can be easily manipulated. We found that MG132 treatment reduced the stability of nuclei during assembly, as observed by frequent ruptures, while osmotic and oxidative stress had little effect on the assembly process. All stress conditions induced changes in pre-assembled nuclei, typically a reduction in nuclear size and wrinkling of the nuclear envelope, accompanied by delocalization of Lamin B3 and the appearance of ring-like structures within the nucleus that stained for Aquaporin 2. Mass spectrometry based proteomic studies using stressed and unstressed egg extracts identified differentially abundant proteins, implicating microtubules in stress-induced nuclear morphology changes. We also observe altered nuclear morphology in response to stress conditions in Xenopus A6 and HeLa cells, as well as the presence of nuclear aquaporins. Our future work will elucidate the mechanisms for such stress-induced changes in nuclear morphology.

S1.T5
Nydia Tejeda-Munoz
Lysosomes are Required for Early Dorsal Signaling in the Xenopus Embryo

Nydia Tejeda Munoz 1,2, Edward M. De Robertis 1

1 UNIVERSITY OF CALIFORNIA, LOS ANGELES
2 UNIVERSITY of OKLAHOMA

Lysosomes are the digestive center of the cell and play important roles in human diseases, including cancer. Previous work has suggested that late endosomes, also known as multivesicular bodies (MVBs), and lysosomes are essential for canonical Wnt pathway signaling. Sequestration of Glycogen Synthase 3 (GSK3) and of β-catenin destruction complex components in MVBs is required for sustained canonical Wnt signaling. Little is known about the role of lysosomes during early development. In the Xenopus egg, a Wnt-like cytoplasmic determinant signal initiates the formation of the body axis following a cortical rotation triggered by sperm entry. Here we report that cathepsin D was activated in lysosomes specifically on the dorsal marginal zone of the embryo at the 64-cell stage, long before zygotic transcription starts. Expansion of the multivesicular body (MVB) compartment with low-dose Hydroxychloroquine (HCQ) greatly potentiated the dorsalizing effects of the Wnt agonist Lithium chloride (LiCl) in embryos, and this effect required macropinocytosis. Formation of the dorsal axis required lysosomes, as indicated by brief treatments with the vacuolar ATPase (V-ATPase) inhibitors Bafilomycin A1 or Concanamycin A at the 32-cell stage. Inhibiting the MVB-forming machinery with a dominant-negative point mutation in Vacuolar Protein Sorting 4 (Vps4-EQ) interfered with the endogenous dorsal axis. The Wnt-like activity of the dorsal cytoplasmic determinant Huluwa (Hwa), and that of microinjected xWnt8 mRNA, also required lysosome acidification and the MVB-forming machinery. We conclude that lysosome function is required for early dorsal axis development in Xenopus. The results highlight the intertwining between membrane trafficking, lysosomes, and vertebrate axis formation.

S1.T6
Todd Stukenberg
Combining systems cancer approaches and frogs to understand how breast tumors generate Chromosomal Instability

Catalina Alvarez Yela 2, Katherine Pfister 1, Jasraj Raghuwanshi 2, Justyna Pipka 1, Ira Hall 1, Kevin Janes 2, P. Todd Stukenberg 1

1 University of Virginia, School of Medicine, 2 University of Virginia, Department of Biomedical Engineering.

Approximately 40% of all breast cancer cells lower the fidelity of mitosis to become highly aneuploid. To understand how breast tumors lower the fidelity of mitosis to become aneuploid we developed a simple computational method that measures the degree of aneuploidy or structural rearrangements of large chromosome regions of 522 human breast tumors from The Cancer Genome Atlas (TCGA). Highly aneuploid tumors overexpress activators of mitotic transcription and the genes encoding proteins that

segregate chromosomes. TP53 mutations co-associate with the overexpression of mitotic transcriptional activators, suggesting that these events work together to provide fitness to breast tumors. Overexpression of three mitotic transcriptional regulators, E2F1, MYBL2, or FOXM1, in Xenopus animal caps is sufficient to lower the fidelity of mitosis at rates seen in tumors. To understand how the overexpression of transcription factors lowers the fidelity of mitosis we have built a reaction-diffusion model of a set of MybL2, FoxM1 targets that are central mitotic regulators and are overexpressed in aneuploid tumors. Inputting the RNA levels from breast cancer tumors into the computation model enables accurate predictions of the aneuploidy levels of these tumors. We are using this model to develop a personalized medicine tool to decipher how each patient's tumor dysregulates mitotic regulation to lower the fidelity of mitosis and drive breast tumor progression.

Session 2:
Chair: **Mustafa Khokha**, Yale Univ., USA

S2.T1
Soeren Lienkamp
Genetic Models for Kidney Disease

Paulina Ogar, Taiyo Yamamoto, Thomas Naert, Julia Traversari, Maike Getwan, Melanie Horn, Daniel Invernot-Perez, Soeren Lienkamp

Institute of Anatomy and Zurich Kidney Center (ZKC), University of Zurich, Switzerland

Genetic renal diseases are a major cause for end-stage kidney disease and the need for renal replacement therapy in both the pediatric and adult population. The underlying mechanisms of inherited conditions that result in cystic kidney disease are poorly understood, but often manifest by disrupting normal kidney development.

In the last few years, we have established various models for conditions, such as autosomal polycystic kidney disease (ADPKD), congenital malformations of the kidney and urinary tract (CAKUT) and ciliopathies with a renal manifestation using gene editing in Xenopus tropicalis. To phenotypically characterize the resulting phenotypes and underlying molecular mechanisms, we employ in toto light sheet imaging (mesoSPIM) and live imaging. Automation has accelerated the generation of knock out and transgenic lines and is heavily employed in genotyping. I will highlight some of our recent results. In summary, we aim to achieve a comprehensive comparison on disease mechanisms of various forms of cystic kidney disease.

S2.T2
Venecia Valdez, GS (Sabine Petry Lab)
Molecular Insights into how the protein HURP facilitates spindle microtubule formation

Venecia A. Valdez 1, Collin McManus 1, and Sabine Petry 1

1 Molecular Biology Department, Princeton University, Princeton, NJ, USA.

To divide, cells assemble a spindle made of microtubules. In this work, we investigate how the hepatoma upregulated protein, HURP, helps generate spindle microtubules. In Xenopus laevis egg extract, HURP has previously been found to be necessary for microtubule nucleation from chromatin. Moreover, it was identified in a large complex that contained TPX2, the nucleation factor XMAP215, and other microtubule associated proteins. However, how HURP aids in generating spindle microtubules remained unknown.

Our study reveals that that HURP is required for branching microtubule nucleation. In this pathway, new microtubules nucleate from the side of pre-existing microtubules while preserving direction of growth, allowing for amplification of microtubule structures. In X.laevis egg extract, this process is RanGTP dependent and requires the microtubule-associated protein TPX2 and the augmin complex. In addition, we

investigate the relationship between HURP and other proteins involved in branching microtubule nucleation, such as TPX2, augmin and the nucleation factor γ-TuRC.

In sum, this work sheds light on the role of HURP in directly promoting microtubule nucleation and provides a better understanding of the microtubule nucleation mechanisms at play during spindle assembly.

S2.T3
Chenbei Chang
Functional and biochemical characterization of disease-associated YWHAZ variants in Xenopus

Popov, I.K., Tao, J., Hiatt, S.M., Whalen, S., Keren, B., Ruivenkamp, C., van Haeringen, A., Chen, M.J., Cooper, G.M., Korf, B.R. and Chang, C

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Recent advances in genomic medicine technology greatly accelerate identification of human gene variants associated with congenital disorders. However, functional confirmation of pathogenicity of variants and mechanistic understanding of pathogenic mutations have been lagging behind. Rapid and scalable assays are needed to investigate disease-associated variants. In this study, we use the Xenopus model to analyze variants in YWHAZ that have been identified from individuals with congenital birth defects. We show that the variants have the distinct ability from the wild type gene to affect early Xenopus development. Biochemical analysis reveals that several variants with mutations in residues located within or near the phosphopeptide binding pocket of YWHAZ display reduced binding to key client proteins Craf/Raf1 and Braf, whereas other variants demonstrate enhanced binding to the Raf proteins. Survey of association of the wild type and variant YWHAZ with other client proteins demonstrates that mutations can alter substrate binding preference of YWHAZ. Our data implicate YWHAZ variants in pathogenesis of human diseases and uncover potential mechanisms for distinct phenotypes linked with the variants.

S2.T4
Betsy Pownall
FGF regulates the switch from pluripotency to commitment to the myogenic lineage

Laura Cowell 1, Lawrence Bates 2, Jenny Nichols 2, Harry V Isaacs 1, and Mary E (Betsy) Pownall 1

1 Biology Department, University of York, UK

2 MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, UK

The transcription factor MyoD has been referred to as the ‘master regulator’ of skeletal muscle development due to its ability to convert some non-muscle cell lines to muscle. However, overexpression of MyoD in Xenopus animal caps or mouse embryonic stem cells is not sufficient for muscle differentiation. This indicates additional factors are needed for pluripotent cells to become competent to form muscle. Our hypothesis is that FGF signalling promotes the activation of genes required to allow cells to transition from pluripotency to muscle cell lineage commitment. A skeletal muscle-inducing protocol was developed in animal cap organoids using low-level FGF4 treatment and MyoD expression and was analysed across a developmental time series using RNA-seq. This unbiased assay allowed the identification of some potential genetic players in this process, including tcf12, smyd1 and rbfox2. We will present evidence of a role for Tcf12 in a feedforward transcriptional pathway with MyoD, downstream of FGF, to activate the myogenic gene regulatory network. Preliminary studies suggest this mechanism is conserved during myogenesis in human embryonic stem cells, confirming the relevance and power of using efficient explant studies in frogs to dissect fundamental mechanisms of development.

S2.T5
Natalie Mosqueda, (Brownlee Lab)
The Role of Palmitoylated Importin a in Ciliogenesis

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Cilia are microtubule-based plasma membrane protrusions that sense the extracellular environment for signals such as growth factors, morphogens, hormones, and other biological signaling molecules. Ciliogenesis is known as the formation of cilia and dysfunction during ciliogenesis can lead human diseases known as ciliopathies. Proper formation and maintenance of cilia is dependent on protein transport to its base, yet this process remains poorly understood.

Nuclear import protein importin a has recently been identified to be palmitoylated to drive its localization to the cell cortex where it can act as a sensor of cell size to regulate nuclear and spindle scaling. In the present study, we have conducted a bioinformatics screen for plasma membrane proteins exhibiting NLS sequences and identified a group of ciliogenesis proteins as potential binding partners of palmitoylated importin a. The potential role for importin a in either a palmitoylated or unpalmitoylated state in ciliogenesis has yet to be explored therefore, to address this we have utilized in-vitro and in-vivo systems, hTERT immortalized cells and the Xenopus laevis epidermal multiciliated cells, respectively. Immunofluorescence staining in cell culture has shown importin a's localization near the cilium base, and upon pharmacological inhibition of palmitoylation of importin a, there is a decreased amount of epidermal multiciliated cells. These preliminary findings suggest a novel pathway for ciliogenesis as well as furthering our understanding of the etiology of ciliopathies and providing new potential targets for ciliopathy interventions.

S2.T6
Yuuri Yasuoka
Genomic, transcriptomic, and proteomic approaches toward understanding mitochondrial genome evolution in frogs

Authors: Yuuri Yasuoka 1 and Yasushi Okazaki 1

Affiliations: 1 RIKEN Center for Integrative Medical Sciences, Japan

Abstract: The mitochondrial genome (Mito-genome) of almost all bilaterian animals encodes 37 genes (13 proteins, 2 rRNAs, and 22 tRNAs). However, why this gene repertoire has been ultra-conserved for over 500 million years remains unsolved. To obtain clues to solving the mystery of Mito-genome evolution, here we focus on the common tree frog, Polypedates leucomystax, of which Mito-genome have lost the sequence encoding a N-terminal MPQL motif of Atp8 conserved from yeast to human. This frog is the only vertebrate reported to have lost the atp8 gene and is designated as an invasive alien species spreading in the Ryukyu islands. We obtained larval samples from exterminators and performed genomic, transcriptomic, and proteomic analyses. The RNA-seq data revealed that the Polypedates atp8 gene was indeed transcribed. Homology searches to genome and transcriptome assemblies suggested that the atp8 gene had not migrated to the nuclear genome. Presence of Polypedates Atp8 protein in the functional respiratory chain complex V (ATP synthase) was experimentally validated using blue-native PAGE and label-free quantitative mass spectrometry. These results indicate that the apparently abnormal Atp8 protein functions normally in mitochondria of Polypedates cells. Assuming the possible benefit of the mutated ATP8 to render the frog resistant to environmental stresses, now we are comparing the heat tolerance of the complex V between human, Xenopus, and Polypedates. To further infer the functional background of the Polypedates atp8 gene evolution, we are also conducting Mito-genome editing with a modified CRISPR-Cas9 system using Xenopus embryos and rescue experiments using human mitochondrial disease cells.

S2.T7
Leon Peshkin, Harvard Univ., USA
Tabula Rana: Cell Atlas of larval and adult Xenopus tissues

Session 3:
Chair: **Sergei Sokol**, Icahn School of Medicine at Mt. Sinai, USA

S3.T1
Ken Cho, Univ. of California Irvine, USA
Interplay of maternal transcription factors and epigenetic modifiers in gene expression stability

S3.T2
Sarah Woolner
Mechanical regulation of cell division in health and disease

Sarah Woolner, University of Manchester, UK

Living tissues are constantly being pushed and pulled and must sense and respond to these mechanical forces appropriately, for example by modifying patterns of cell division. Whilst we are beginning to understand the cellular mechanisms that link cell behaviour and force in single cells, a major gap in our knowledge is understanding how mechanical force is transmitted and sensed across complex tissues. Bridging this gap is important considering that many common diseases, such as cancer, alter the mechanical properties of our tissues. We are using Xenopus laevis and a combination of biological and mathematical approaches to investigate how cell division is regulated by mechanical force in complex tissue environments. We have developed methods to stretch and image animal caps and combined these with new mathematical models for inferring mechanical stress across a tissue. In my talk, I will describe how we have used these approaches to show that tissue stretch increases cell division rate and reorients divisions along the stretch axis. We find that whilst division orientation is predominantly sensitive to changes in cell shape, division rate is sensitive to the speed of stretch via modulation of nuclei shape and mitotic entry. A great strength of Xenopus is the ability to take what we learn ex vivo back in vivo. I will describe how findings in stretched animal caps relate to a model for early-stage cancer in Xenopus embryos, where we find that oncogenic cells pull on their wild-type neighbours and coerce them to divide aberrantly, contributing to tumour growth.

S3.T3
Stefan Hoppler
Gene Regulatory Networks downstream of Wnt signalling in early embryogenesis and heart muscle development

Stefan Hoppler 1, Ewa Kopec 1, Takayoshi Yamamoto 2, and Claudiu Giuraniuc 1

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2 Graduate School of Arts and Sciences, The University of Tokyo (Komaba), Japan

The Xenopus model system has been instrumental for unravelling molecular mechanisms of the Wnt pathway and for studying Wnt signalling function in vertebrate embryonic development; our lab has previously contributed to studying Wnt signalling function in early axis establishment, germ layer patterning and heart organ development.

Recognising that Wnt signalling mechanisms function in the embryo in a context with other biochemical mechanisms and while dependent on autoregulatory loops (and with access to further laboratory experiments somewhat restricted during the COVID pandemic), we have explored computational modelling to test our logic of an integrated Gene Regulatory Network response to maternal and zygotic Wnt signalling in the early embryo, and of autoregulatory feed-back loops in heart development. These now provide new

hypotheses to investigate relevant mechanisms for regulation of stage- and tissue-specific direct Wnt target genes, and for patterning of embryonic tissues.

S3.T4
Adam Session
Long-read Xenopus genome assemblies enable identification of subtelomeres and comparative analysis of tetrapod chromosome evolution

Authors: Adam Session, Jessen Bredeson, Sofia Medina-Ruiz, Austin B. Mudd, Richard Harland, Daniel Rokhsar

Long-read sequencing has enabled high-quality genome assemblies for a fraction of the cost. Recently, analysis of the Xenopus tropicalis v10 assembly showed that these high-quality genomes enable analysis of complicated pericentromeric and subtelomeric repeats not present in earlier builds of the X. tropicalis assembly. Subtelomeres are dynamic, repeat-rich regions adjacent to the telomere that are understudied outside of humans and yeast. Due to human chromosomes sharing identical segmental duplications in these regions, it has been hypothesized that non-homologous end joining between chromosomes is the mechanism by which the chromosomes share the same sequence.

We present the improved assembly of allotetraploid Xenopus laevis. The X. laevis v10 assembly has more protein-coding genes, more sequence in chromosomes, closing gaps often associated with repetitive regions, as well as improved contiguity. We develop a novel k-mer method to identify the subtelomeres of X. laevis and show that the two subgenomes of the allotetraploid have distinct subtelomeric repeats, suggesting that non-homologous end joining is not happening between the subgenomes of the allotetraploid. We also present on how the comparative analysis of these repeats can improve our understanding of vertebrate genome evolution.

S3.T5
Florina-Alexandra Toma (Sweeney Lab)
Spinal interneuron function in swim and limb behaviour over frog metamorphosis

Florina A. Toma 1, Zoe P. M. Harrington 1, Mara J. Julseth 1, David Vijatovic 1, Christoph Sommer 1, Robert Hauschild1, Lora B. Sweeney 1

1 Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria.

The Xenopus laevis frog shows a wide range of motor behaviors during metamorphosis, transitioning from tail-based escape swimming, to free swimming, and finally, limb-based movement. This swim-to-limb transition allows us to study in a single organism how motor and interneurons in the spinal cord generate these behaviors. Here, utilizing advances in genetic manipulation and behavioral tracking, we dissect the molecular features and contribution of each spinal cord cardinal class to swim versus limb behavior over frog metamorphosis.

We devise a high-throughput CRISPR screen, in which we knock out primary markers for twenty-five cardinal classes or subclasses of ventral and dorsal spinal neurons. We utilize the lineage map of frog embryos to generate whole and half mutants, in which only one side of the animal is mutant.

First, we visualize the molecular and cellular changes in the spinal cord of each CRISPR mutant, using immunohistochemistry and multiplexed in situ hybridization to pinpoint the role of each gene in determining cell-type identity and the resulting changes in spinal cord architecture.

We then apply the machine-learning-based SLEAP software as a high-throughput behavioral analysis tool [1] to track tail and limbs and quantify movement of Xenopus and its body parts across metamorphosis. Wildtype behavioral profiles are then compared to whole/half mutant animals to evaluate the contribution of each neuron subtype to the range of motor repertoires.

Our study uses frog metamorphosis to build a comprehensive understanding of the contribution of each spinal neuron cell-type to swim-to-limb behavior.

[1] T. D. Pereira, N. Tabris, A. Matsliah, D. M. Turner, J. Li, S. Ravindranath,E. S. Papadoyannis, E. Normand, D. S. Deutsch, Z. Y. Wang, G. C. McKenzie-Smith, C. C. Mitelut, M. D. Castro, J. D'Uva, M. Kislin, D. H. Sanes, S. D. Kocher, S. S.H. Wang, A. L. Falkner, J. W. Shaevitz, and M. Murthy, "SLEAP: A deep learning system for mulW-animal pose tracking," Nature Methods, vol. 19, no. 4, pp. 486–495, 2022.

S3.T6
Amy Sater
Modeling Traumatic Brain Injury in Xenopus Tadpoles

Sydnee Spruiell Eldridge, Jonathan F. K. Teetsel, Vrutant V. Shah, Alex Ferrer, and Amy K. Sater

Department of Biology and Biochemistry, University of Houston, Houston TX

Traumatic brain Injury (TBI), a major cause of death and disability across the globe, establishes a persistent disease state, which is maintained via interactions between neuronal, glial, and immune cell types. We seek to develop the Xenopus tadpole as a scalable model for TBI, using a focal impact (FI) injury to the dorsolateral midbrain, site of the optic tectum. This FI injury leads to axonal damage, cell death, edema, microglial activation, and reactive astrogliosis; it also disrupts visually directed behaviors, which resolve within 7 days. These responses to injury extend well beyond the site of initial impact: axonal damage is visible in the ventral midbrain, reactive astrogliosis extends to the contralateral side, and microglial accumulation is detected in the forebrain. Intraventricular administration of the Selective Estrogen Receptor Modulator tamoxifen (TMX) immediately after FI injury reduces edema and reactive astrogliosis; it also minimizes impairment of visually directed behaviors. Moreover, TMX treatment after injury produces elevated expression of neuroprotective genes, which persists for over 4 weeks. Bulk transcriptional profiling of the response to injury in the presence or absence of TMX suggests several pathways as candidate mediators of neuroprotection. Our findings illustrate the potential of Xenopus as a model for therapeutic discovery in TBI and suggest mechanisms of neuroprotection by TMX.

Keynote 2:
Chair: **Amy Sater**, Univ. of Houston, USA
Sponsored by the International Xenopus Board

K2
Helen Willsey
Using frogs to dissect psychiatric disorders

Helen Willsey 1,2

1 Department of Psychiatry and Behavioral Sciences, Weill Institute for Neurosciences,
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One of the most promising breakthroughs in psychiatric disorders is the recent discovery of high-confidence risk genes from patient DNA sequencing studies, as these genes offer a potential avenue to understanding and treating these conditions. However, it is also clear that hundreds of genes are involved for each disorder, raising the possibility of an impenetrably complex biology in which treatments will need to be found for each patient, each risk gene, or small groups of genes or patients, rather than for each disorder. However, multiple independent analyses suggest that these genes converge on a smaller number of biological pathways, developmental stages, and brain regions, specific to each disorder. This work has often relied on datasets from post-mortem human brain, providing important insights into human biology, but also suffering from inherent experimental limitations associated with ex vivo computational approaches. This work is similarly challenging in mammalian models where it is often not feasible to study dozens of

genes in parallel, where pleiotropy can be misleading, and where the scope of potential phenotypes is vast, so analyses are often underpowered. To address this critical need, I use Xenopus tropicalis (diploid frogs) as a high-throughput vertebrate model organism to perturb many psychiatric disorder risk genes in parallel and, in a hypothesis-free manner, identify phenotypes or functions in common as an entré to the core biology underlying
these conditions. In this talk I will summarize our work in Autism Spectrum Disorder, which has identified convergent biology during forebrain neurogenesis, and more specifically in tubulin-related biology. I will also present the vision for my nascent laboratory, using frogs to meet the call of functional genomics in psychiatric disorders.

Session 4:
Chair: **Susannah Rankin**, Oklahoma Medical Research Foundation, USA

S4.T1
James Ferrell, Stanford Univ., USA
Protein homeostasis from diffusion-dependent control of protein synthesis and degradation

S4.T2
Hui Chen (Good Lab), Univ. of Pennsylvania, USA
Spatiotemporal Regulation of Genome Activation In Early Embryogenesis

S4.T3
Karel Dorey
Reconstructing the spinal cord after injury

Lauren Phipps 1,2 Diane Pelzer 1, Shinhyeok Chae 1,3, Carlos Gallardo Dodd 1,2, Taejoon Kwon 3 and Karel Dorey 1

1 University of Manchester, School of Medicine, Division of Developmental Biology and Medicine
2 Present address: Karolinska Institute, Sweden
3 Ulsan National Institute of Science & Technology, Korea
Understanding how some organisms successfully achieve spinal cord regeneration is a longstanding question with profound implications for human health. This is especially true for the spinal cord given the poor ability of mammals to regenerate their central nervous system. About 27 million people worldwide suffer long-term disability following SC injury (SCI). In contrast, many vertebrates such as axolotl, reptiles and amphibians such as Xenopus tadpoles can regenerate a fully functional tail, including their spinal cord, following amputation. Interestingly, different species will deploy distinct strategies to achieve this objective. For example, Axolotl regenerates a perfect replica of their original tail and spinal cord, whilst lizards regenerate morphologically distinct structures that only recapitulates some of the function of the intact tail.

How is the spinal cord rebuilt after injury in Xenopus is still poorly understood. We have identified a regeneration-specific population of Neural Progenitor Cells (NPCs) that is required for efficient spinal cord outgrowth and de novo neurogenesis. In particular, we have shown that the transcription factor Foxm1 is essential for cell fate decision of NPCs during regeneration. Recent work as also revealed new and unexpected role for Foxm1 as regenerates from foxm1-/- animals exhibit global impairment in tissue oxidation and defects in mesenchymal proliferation. Furthermore, scRNAseq and labelling experiments indicate that the regenerating spinal cord does not recover its original patterning whilst regaining its function. Taken together, these data indicate that Xenopus is using a unique strategy to recover a functional tail after injury.

S4.T4
Janet Tait (Rupp Lab)
The histone H4K20 methyltransferase SUV4-20H1/KMT5B is required for multiciliated cell differentiation in Xenopus

Authors: Janet Tait, Alessandro Angerilli, Tamas Schauer, Ralph Rupp

Affiliations: Department of Molecular Biology, Biomedical Center, Ludwig-Maximilians- Universität München

Histone post-translatonal modificatons (PTM) greatly influence gene expression and are widely considered to regulate progression through development. However, the function of some PTMs remain elusive. H4K20 is sequentially methylated in concert with the cell cycle. In proliferating cells, SET8/KTM5A writes the monomethyl mark in G2/M phase, which then is converted to the di- and trimethylated states by SUV4-20H1/H2 (KMT5B/KMT5C) methyltransferases in the next G1 and S phase. In quiescent, differentiated cells, H4K20me2 represents the most abundant histone modification present in vertebrate chromatin. To address the function of H4K20 methyl states in development, we blocked the deposition of H4K20me2 and H4K20me3 by depleting the SUV4-20H1/2 enzymes in Xenopus embryos. This results in a severe ciliogenic defect in multiciliated cells (MCCs), as well as the repression of hundreds of cytoskeleton and cilium related genes. Further, we demonstrate that this defect can be rescued by wildtype, but not catalytically inactive Suv-4-20h1, as well as by overexpressing PHF8, an H4K20me1-specific histone demethylase. Taken together, this indicates that the conversion of H4K20me1 to H4K20me2 by SUV4-20H1 is critical to synchronize expression of multiciliogenic genes.

S4.T5
Hui Zhao (SA)
ZSWIM4 regulates early embryonic patterning and BMP signaling pathway by promoting nuclear Smad1 degradation

The dorsoventral gradient of BMP signaling plays an essential role in embryonic patterning. We found that Zinc Finger SWIM-Type Containing 4 (zswim4) is expressed in the Spemann-Mangold organizer at the onset of Xenopus gastrulation and then enriched in the developing neuroectoderm at the mid-gastrula stages. Knockdown or knockout of zswim4 caused ventralization. Overexpression of zswim4 decreased, whereas knockdown of zswim4 increased, and the expression levels of ventrolateral mesoderm marker genes. Mechanistically Zswim4 attenuates the BMP signal by reducing the protein stability of Smad1 in the nucleus. Stable isotope labeling by amino acids in cell culture (SILAC) identifies Elongin B (ELOB) and Elongin C (ELOC) as the interaction partners of Zswim4. Indeed, Zswim4 forms a complex with the Cul2-RING ubiquitin ligase and ELOB and ELOC, promoting the ubiquitination and degradation of Smad1 in the nucleus. Our study identified a novel mechanism that restricts BMP signaling in the nucleus.

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S4.T6
Thomas Naert (Lienkamp Lab)
XenoVision, steps towards fully automated 3D phenotyping of whole-embryo development and disease using advanced microscopy and artificial intelligence

Thomas Naert 1, Fabian Voigt 2,3, Nikita Vladimirov 4,5,6, Patricia Schmid 1, Daniel Walther 1, Fritjof Helmche n2,4,5, Soeren Lienkamp1

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Manual phenotyping of embryonic development and disease can rapidly become labor- intensive and usually exhibits a bias towards specific tissues, potentially driven by hypothesis pre-selection. Concurrently, artificial intelligence has witnessed an extraordinary growth and development, prompting us to envision automated phenotyping platforms as feasible (1).
In our pursuit to materialize this vision, we have pioneered several technological advancements. We developed a uniquely modular "SPIM-tower" holder that can accommodate four samples per level on a new generation of light-sheet microscope, the Benchtop mesoSPIM (2). Levels can be conveniently stacked using magnets, allowing us to simultaneously image 16 tadpoles (across four stacked levels), each embedded in its individual agarose block and cleared with BABB (imaging time 20 minutes). Standardized automated whole-embryo imaging after experimental intervention, such as chemical treatment or genetic manipulation (e.g., CRISPR/Cas9), has the potential to facilitate screening efforts by distinguishing between intrinsic variability and genuine phenotypes. The robustness of these uniformly large datasets makes them a prime candidate for future initiatives revolving around deep learning-based automated phenotyping.
Further, we introduce a ground-breaking type of microscopy using Schmidt mirror technology (3). This novel objective allows high-resolution three-dimensional imaging of Xenopus tadpoles. The depth and precision of this imaging allow for thorough examination of any morphological defects.

(1) Deep learning is widely applicable to phenotyping embryonic development and disease. Naert et al, Development 2021
(2). The Benchtop mesoSPIM: a next-generation open-source light-sheet microscope for large cleared samples. Vladimirov, Voigt, Naert et al, BioRxiv 2023
(3) Reflective multi-immersion microscope objectives inspired by the Schmidt telescope.
Voigt, Reuss, Naert et al; Nature Biotechnology 2023

S4.T7
Darcy Kelley
Identifying genomic loci responsible for innate, species-specific acoustic communication in Xenopus; CNS vocal pattern generation (rhythm) and laryngeal sound production (timbre)

Identifying genomic loci that support species-specific male advertisement calls in L clade Xenopus species: laevis, petersii, powerii, victorianus.

Darcy B. Kelley, Elizabeth Bagnato-Cohen, Eythan Jiang, Young Mi Kwon, Andres Bendesky*, Clementine Vignal and Avelyne Villain

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In each Xenopus species, adult males produce an advertisement call that attracts gravid females. The combination of temporal (sound pulse rate and pattern) and spectral (sound frequencies or pitches) in each pulse provides a unique species ID (Tobias et al., 2011). These acoustic features are heritable and thus genetically determined. The CNS circuit that produces vocal patterns has been mapped (Kelley et al., 2020). A specific neuronal cell type -the FTN or past trill neuron- in the hindbrain Parabrachial Nucleus controls sound rhythms (Barkan et al., 2018; Rhodes et al, 2007). Sound pulse spectral features are produced entirely by the larynx (Kwong-Brown et al., 2019). To explore genetic architectures that support innate neural architectures as well as laryngeal sound pulse features, during the recent pandemic we generated several thousand F2 (laevis/petersii) male and female hybrids; now adult. To perform QTL analyses of genomic loci that support the production and perception of innate vocal communication we recorded male advertisement calls (with replication) at sexual maturity and are quantifying female acoustic preferences.

Session 5:
Chair: **Dan Weeks**, Univ. of Iowa, USA

S5.T1
Peter Walentek, Univ. of Freiberg, Germany
Self-organization in mucociliary epithelia - signaling control of stem cells and cell identities in development and disease

S5.T2
Coral Zhou (Heald Lab)
Mitotic chromosomes scale to nuclear-cytoplasmic ratio and cell size in Xenopus

Coral Y. Zhou 1, Bastiaan Dekker 2, Ziyuan Liu 1, Hilda Cabrera 1, Joel Ryan 3, Job Dekker 2,4, Rebecca Heald 1

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During the rapid and reductive cleavage divisions of early embryogenesis, subcellular structures such as the nucleus and mitotic spindle scale to decreasing cell size. Mitotic chromosomes also decrease in size during development, presumably to scale coordinately with mitotic spindles, but underlying mechanisms are unclear. Here we combine in vivo and in vitro approaches using eggs and embryos from the frog Xenopus laevis to show that mitotic chromosome scaling is mechanistically distinct from other forms of subcellular scaling. We found that mitotic chromosomes scale continuously with cell, spindle and nuclear size in vivo. However, unlike for spindles and nuclei, mitotic chromosome size cannot be re-set by cytoplasmic factors from earlier developmental stages. In vitro, increasing nuclear-cytoplasmic (N/C) ratio is sufficient to recapitulate mitotic chromosome scaling, but not nuclear or spindle scaling, through differential loading of maternal factors during interphase. An additional pathway involving importin alpha scales mitotic chromosomes to cell surface area/volume ratio (SA/V) during metaphase. Finally, single-chromosome immunofluorescence and Hi-C data suggest that mitotic chromosomes shrink during embryogenesis through decreased recruitment of condensin I, resulting in major rearrangements of DNA loop architecture to accommodate the same amount of DNA on a shorter axis. Together, our findings demonstrate how mitotic chromosome size is set by spatially and temporally distinct developmental cues in the early embryo.

S5.T3
Kerstin Feistel
Receptor-mediated endocytosis orchestrates anterior neural tube closure

The endocytic receptor Lrp2 orchestrates apical constriction and cell polarity to drive cranial neural tube closure

Josefine Hoeren 1, Annette Hammes 2 and Kerstin Feistel 1

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Neural tube closure defects (NTDs) are developmental malformations occurring upon impairment of morphogenetic movements during neurulation. While caudal neurulation, forming the spinal cord, is dominated by planar cell polarity (PCP)-mediated convergent extension (CE), apical constriction (AC) is essential for neural tube closure in the cranial region, which gives rise to the brain.

Pathogenic variants of LRP2 have been identified in humans with NTDs. Patients carrying autosomal-recessive mutations in LRP2 develop Donnai-Barrow-Syndrome which includes severe forebrain defects. Modelling of lrp2-related NTDs in Xenopus and mouse showed that Lrp2 was essential for cranial neural tube closure. While loss of Lrp2 delayed or entirely inhibited neurulation, editing of Lrp2's C-terminal PDZ-binding domain (PBD) strikingly accelerated neural tube closure. We found that Lrp2 controlled AC - and that velocity and spatial distribution of AC determined the correct timing of cranial neural tube closure. In addition, Lrp2 connected AC to cell polarity. Despite the absence of PCP-driven CE in the cranial region, cells expressed the core PCP protein Vangl2 and acquired planar polarity during cranial neurulation. Lrp2 regulated the dynamic subcellular localization of Vangl2. Our data suggest that interaction of the Lrp2- / Vangl2-PBDs via PDZ-containing proteins is key to orchestrate the occurrence and mode of AC. Thus, Lrp2-mediated endocytosis emerges as a major regulator of morphogenetic movements essential for cranial neural tube closure, shedding new light on NTD etiology.

S5.T4
Katharina Till (Borchers Lab)
The RhoGEF Trio functions downstream of Ptk7 in NC cell contact inhibition of locomotion

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Neural crest (NC) cells are highly migratory cells contributing to a broad range of vertebrate tissues and their migration behavior resembles cancer cell invasion. The directional migration of NC cells is controlled by various mechanisms including information exchange via dynamic NC cell-cell contacts. A transmembrane protein that is likely involved in this process is PTK7 (protein tyrosine kinase 7), an evolutionary conserved Wnt/PCP co-receptor, which is required for Xenopus NC migration. Our data demonstrate that Ptk7 is dynamically localized at NC cell- cell contacts and plays a role in contact inhibition of locomotion (CIL), a phenomenon whereby NC cells change their polarity and directionality upon cell-cell contact. Recently, we identified the Rho guanine exchange factor (GEF) Trio as an interaction partner and downstream effector of Ptk7 during NC cell migration. Trio is especially well suited to relay signals, as it features two GEF domains, the GEF1 domain which activates the small GTPase Rac1 and the GEF2 domain which specifically acts on RhoA. Interestingly, our data suggest that the interaction of Ptk7 and Trio occurs via the GEF1 domain, while the GEF2 domain, but not the GEF1 domain, is sufficient to rescue the Ptk7 morphant phenotype. Like Ptk7, Trio is also required for NC migration and CIL. Thus, our data suggest a model in which Ptk7 interacts with and inhibits the Trio GEF1 domain at cell-cell contact sites, thereby limiting Trio activity to the activation of RhoA and mediating CIL.

S5.T5
Abraham Fainsod
How folic acid prevents the formation of neural tube defects

Tamir Edri, Dor Cohen, Yehuda Shabtai and Abraham Fainsod

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Neural tube defects (NTDs) are among the most debilitating and common developmental defects in humans. Abnormal folic acid (vitamin B9, FA) metabolism and excess RA signaling have been linked to NTD formation. We show that reducing RA signaling levels using RALDH inhibitors (ethanol, DEAB, and citral) or CYP26A1 overexpression efficiently induced NTDs. The NTDs are rescued by providing exogenous RA sources supporting the involvement of reduced RA signaling in their formation. We mapped the requirement for RA signaling to early gastrula stages and showed abnormal regulation of the neural plate stem cell maintenance genes, geminin, and foxd4l1.1, increased proliferation and expansion of the neural plate. NTDs induced by reduced RA are also rescued by FA compensating for the loss of RA. FA up-regulates the expression of known RA-responsive genes. We studied the 10- formyltetrahydrofolate dehydrogenase encoded by the aldh1l1 gene as an FA up- regulated activity capable of producing RA to

rescue NTDs. We generated aldh1l1 CRISPant embryos to study its contribution to the FA-promoted NTD rescue. FA efficiently rescues NTDs induced by reduced RA (ethanol, DEAB), but this rescue requires a functional aldh1l1 gene. We established an additional FA rescuable NTD model based on pax3 CRISPants. RA precursors rescue the NTDs in pax3 CRISPants. Also, FA is unable to rescue the NTDs in double pax3+aldh1l1 CRISPants. We show the importance of RA signaling in the formation of the neural plate and the formation of NTDs and provide evidence that FA promotes the production of RA to prevent NTD formation.

S5.S6
Nicole Edwards, PD (Zorn Lab)
Disrupted endosomal trafficking of polarity proteins causes congenital trachea-esophageal separation defects

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CLEARconsortium.org

Trachea-esophageal birth defects are life-threatening congenital anomalies that arise when the trachea and esophagus do not correctly separate from the common anterior foregut tube. The genetic causes of trachea-esophageal defects are not well known, and how the trachea and esophagus normally separate during embryonic development is not well understood. In a collaborative network called the CLEAR Consortium, we are using animal models and patient genome sequencing to discover new trachea-esophageal defect-related genetic mutations and determine their disease mechanisms. Using the complementary advantages of mouse and Xenopus embryos, we recently defined a novel endosome-mediated epithelial remodeling process that is critical for separation of the foregut into tracheal and esophageal tubes. We aimed to determine the cellular mechanisms regulated by endosomal trafficking by mutating the key endosomal pathway proteins Dynamin, Rab5a, and Rab11a in Xenopus. We found that disrupting endosome trafficking leads to trachea-esophageal fistulas, occluded trachea-esophageal lumens, and epithelial cell disorganization in the transient septum connecting the trachea and esophagus. We also find significantly altered localization of the polarity proteins laminin and Vangl2 in endosomal mutant embryos, suggesting that these proteins may be trafficked by recycling endosomes to maintain epithelial apical-basal cell polarity during trachea-esophageal separation. We also observed disrupted polarity in Xenopus mutants of novel patient variants in genes predicted to function in endocytosis, suggesting that disrupted endosomal-mediated epithelial remodeling may be a common disease mechanism underlying human trachea-esophageal anomalies. This work is funded by NICHD P01 HD093363.

Session 6:
Chair: **Jeremy Green**, King's College London, UK
Sponsored by Molecular Instruments

S6.T1
Anna Philpott
To be or not to be (a neuron)-that is the question

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** Nantes Universite

Mechanisms that lead to the establishment and maintenance of cell identity are paramount for organismal health. They also underpin successful cellular reprogramming for disease modelling and cell replacement therapies. We have been using Xenopus embryos to investigate the roles played by the epigenome in regulating lineage transcription factor-mediated establishment and stabilisation of cell fate in vivo.

We have used inducible versions of the master regulator transcription factors Ascl1 and MyoD to challenge cell identity in the developing frog embryo at different embryonic stages. To do this, we have been using genome-wide approaches to compare transcriptional profiles and probing the chromatin landscape in "permissive" tissues that respond to transcription factor over-expression by undergoing full lineage reprogramming and "non-permissive" tissues that resist full reprogramming. There are clearly considerable differences in the ability of tissues to respond even to potent transcription factors that have been reported to have strong chromatin opening "pioneering" activity. We have also been developing a new single cell multiplexing platform to explore heterogeneity of cell response to reprogramming factors in more detail. Furthermore, to understand the molecular mechanism of "permissiveness" to reprogramming, we are manipulating histone demethylase levels in the developing embryo to investigate the role of individual histone marks in determining the transcription factor responsiveness of different tissues. These studies, together with insights we are obtaining from mammalian embryonic stem cell systems, are beginning to shed light on the "rules" that govern how cells maintain lineage fidelity and plasticity in the face of fate challenge.

S6.T2

Mark Corkins

Comparative analysis of Xenopus mesonephric transcriptomics: Conservation of the developmental lineage of nephron stages

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The mammalian kidney develops in three sequential, stages referred to as the pronephros, mesonephros and metanephros, each developing from the preceding form. All three phases of kidney development utilize epithelized tubules called nephrons, which function to take in filtrate from the blood or coelom and selectively reabsorb solutes the organism needs, leaving waste products to be excreted as urine. The pronephros is heavily studied in aquatic organisms such as zebrafish and Xenopus, as it develops quickly and is functional. The metanephros is a preferred mammalian kidney model, as it best recapitulates human disease. However, very little is known about the mesonephric stage of kidney development in any organism. The pronephros extends to form the mesonephric duct, which ultimately develops into the Wolffian duct in male amniotes. Whereas, in organisms that lay their eggs in aquatic environments, the mesonephric kidney is the final form that is generated. Therefore, further understanding of the development and physiology of these kidneys will provide insight into the urogenital system as well as its evolutionary conservation. To gain a better understanding of its structure and cell types, we analyzed the developing mesonephros by in situ and single-cell mRNA sequencing of cells that make up the developing mesonephros. By comparing these data to those published for the Xenopus pronephros and mammalian metanephros, we were able to evaluate nephron conservation between the three kidney stages.

S6.T3
Eva Hoermanseder, Helmholtz Univ., Germany
Remember or Forget: Reprogramming Cellular Memories

S6.T4
Mike Sheets
Post-transcriptional mechanisms and the control of embryonic cell fates

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and Michael D. Sheets

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Post-transcriptional control of mRNAs is vital for embryonic development. Prior to zygotic genome activation, embryos rely entirely on maternally deposited mRNAs to express genes in the correct location and at the correct time. Much of this control is due to translational repression by RNA binding proteins, which select and repress specific target mRNAs. We have focused on Bicaudal-C (Bicc1), a conserved RNA binding protein that drives translational regulation essential for vertebrate development. To address how Bicc1 selects target mRNAs and controls their expression to guide specific cell-fate decisions, we examined the conservation of Bicc1 RNA binding functions, by comparing N-terminal sequences of 44 different Bicc1 proteins. This revealed that the KH2 domain and its GXXG motif (GKGG) were the most highly conserved features of the Bicc1 protein family. To examine functional conservation, recombinant Bicc1 proteins from multiple species were analyzed in RNA binding assays. We observed that all proteins bound efficiently to the cripto1 RNA, a well characterized Bicc1 target from Xenopus demonstrating a conserved mechanism of RNA recognition. The nucleotide sequences important for forming the Bicc1 RNA-protein interface were also analyzed by comparing Bicc1 binding to target RNAs Mm dand5 and Xl cripto1. We observed that Bicc1 RNA substrates share a common architecture: a single GAC motif upstream of a stem-loop structure. Together, our results support a model where the Bicc1-RNA interface forms between the conserved KH2 domain and key features of target RNA substrates. Research supported by NICHD (R01HD091921) and the Biotechnology Training Program NIGMS (T32GM135066).

S6.T5
Emmanuel Tadjuidje
Embryotoxicity of Statins and other Prescribed Drugs that Inhibit Cholesterol Biosynthesis

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Cholesterol plays pivotal cellular functions ranging from maintaining membrane fluidity to regulating cell-cell signaling. High cholesterol causes cardiovascular diseases, low cholesterol is linked to neuropsychiatric disorders, and inborn errors of cholesterol synthesis cause multisystem malformation syndromes. Statins lower cholesterol levels by inhibiting the first, rate-limiting reaction of the cholesterol biosynthesis pathway catalyzed by hydroxymethyl-glutaryl-Coenzyme A reductase (HMGCR). However, they have also been shown to interfere with cellular pathways that are unrelated to cholesterol synthesis. One of the last enzymes of cholesterol biosynthesis, 7-dehydrocholesterol reductase (DHCR7), is often mutated in the Smith-Lemli-Opitz syndrome (SLOS), a multisystem malformation syndrome. Strikingly, recent studies have shown that some prescribed psychotropic pharmaceuticals inhibit its activity. In this study, we used Xenopus laevis as a model organism to test the effects of 8 FDA-approved statins and selected psychotropic drugs on the developing vertebrate embryo. Drugs were tested at concentrations ranging from 0.1μM to 50μM. Embryos were exposed to the drugs from the blastula stage (stage 9) through the swimming tadpole stage (stage 45-46) will daily medium change. Our data show that statins are heterogenous with

respect their ability to cause embryonic lethality, with simvastatin and lovastatin being the most toxic ones. Observed phenotypes included delayed development, shortened body axis and pericardiac edema. On the other hand, psychotropic drugs were less embryonic lethal than statins, and mainly caused delayed development, and pericardiac edema. Our finding suggest that the proximal and distal inhibition of cholesterol biosynthesis have different but overlapping effects on embryonic development.

S6.T6
Valentina Kostiuk (Khokha Lab)
Nup107 contributes to maternal to zygotic transition by regulating the nuclear export of pri-miR427

Valentyna Kostiuk 1, Rakib Kabir 1, Anthony Isenhour 1, Nick D. L. Owens 2, C. Patrick Lusk 1, Mustafa Khokha 1

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Congenital heart disease (CHD) is the most common birth defect and one of the leading infant mortality causes in the US. A candidate gene, nucleoporin 107 (NUP107), was identified in CHD patients. NUP107 is part of the nuclear pore complex, which mediates nucleocytoplasmic transport, but its role in embryo development remains unknown. NUP107 mutations were described in patients with nephrotic syndrome, microcephaly, and gonadal dysgenesis. Due to this broad birth defects spectrum, NUP107 could affect early embryonic patterning leading to downstream organogenesis defects. NUP107 depletion in X. tropicalis caused abnormal germ layer specification and subsequent defects in gastrulation, left-right patterning, and cardiac looping. Interestingly, NUP107 mRNA and protein expression is robust at early stages and becomes almost undetectable after gastrulation. Such biased pattern further supports the crucial role of NUP107 in early embryonic patterning. To identify the mechanism of NUP107 action, I used an RNA-Sequencing time-course to detect mRNA transcripts regulated by NUP107 starting with early cleavage stages (32-cell) and through gastrulation (stage 12). Using RNASeq, I identified a candidate gene REST that affects ectodermal patterning in a manner consistent with my results in NUP107 depletion studies. NUP107 reduction increased REST mRNA levels, which could be explained by the delayed maternal transcript clearance. Since microRNA427 (miR427) is crucial for the maternal mRNA clearance, I tested if NUP107 depletion resulted in miR427 reduction and found that NUP107 depletion reduces miR427 levels through the abnormal nuclear export of miR427 primary transcript. This subsequently delays REST maternal mRNA clearance and disrupts ectodermal patterning.

S6.T7
Aneesh Acharya - Molecular Instruments
HCR(TM) RNA-FISH: The benchmark in multiplexed RNA imaging

Keynote 3:
Chair: **Ann Miller**, Univ. of Michigan, USA
Sponsored by the International Xenopus Board

K3
Bill Bement
Self-organizing waves of Rho GTPase ac7vity as a conserved mechanism for cell shape control

Bill Bement, Univ. Wisconsin - Madison

The Rho GTPases—Rho, Rac, and Cdc42—control a broad variety of events involving the cytoskeleton. Recently we discovered that these GTPases are deployed as self-organizing waves based on an activation-inhibition mechanism. This mechanism requires autoaactivation (ie positive feedback) from the active GTPase to its upstream guanine nucleo7de exchange factor (GEF) and delated autoinhibition (ie negative feedback) from the active GTPase through actin filaments and its downstream GTPase activating protein

(GAP). The molecular and cellular mechanisms that generate these waves, and their cellular roles will be discussed with (of course) a near-absolute emphasis on Xenopus oocytes, eggs, and embryos.

Session 7:
Chair: **Aaron Zorn**, Cincinnati Children's Hospital Medical Center, USA

S7.T1
Edward Cruz (Wuhr Lab)
Breaking Up is Hard to Do: The Role of Protein Turnover in Xenopus Embryogenesis

Edward Cruz, Argit Marishta, Alex Johnson, Aleigha Reynolds, Eric Wieschaus, Martin Wühr

Embryogenesis starts with a single fertilized egg which gives rise to a multitude of cell types, each bearing unique proteomes essential for lineage specification and organogenesis. Recent technological advances allow us to analyze the dynamic protein and mRNA levels underlying these processes; however, these levels often correlate poorly, suggesting an important role for post-transcriptional regulation via translational efficiency and protein turnover. While recent advances in ribosomal profiling have highlighted the significance of translational efficiency, the role of protein turnover in setting proteomic balance remains disputed and unexplored by direct systems-level developmental studies.

Here, we have developed a novel technique to measure protein turnover in embryogenesis by merging 18O-water labeling with multiplexed proteomics. When applying these approaches to Xenopus embryos, we find an enrichment of protein turnover in E3 ligases along with microtubule- and cell-cycle-associated proteins. Interestingly, when we compare protein half lives in Xenopus and Drosophila embryos, we find an even more pronounced role for turnover in Drosophila compared to Xenopus. These results support a drastic and global species-specific adaptation of protein abundance control.

Thus, we present a widely applicable technique for the quantification of protein half-lives in embryogenesis and a valuable resource of protein half-lives in frog and fly embryos that enriches our understanding of how protein turnover influences protein abundances to execute their biological functions in development.

S7.T2
Hyeyoon Lee (Niehrs Lab), DKFZ Heidelberg, Germany
R-Spondin 2 governs Xenopus left-right body axis formation

S7.T3
Tara Loughery (Gomez lab)
Role of EGF signaling in peripheral axon exiting the spinal cord

Authors: Tara Loughery, Caitlin Warlick-Short, Rohit Nagarimadugu, Elisa Keefner, Trenton Davig Huesmann, Timothy Gomez

Peripheral axons of motoneurons (MNs) and Rohon Beard sensory neurons must exit the Xenopus spinal cord to reach their targets. Our lab was the first to show that spinal neuron growth cones form invadosome-like protrusions, which are necessary for MN axon exiting. Invadosomes are f-actin-rich protrusions, first characterized in metastatic cancer cells, which release matrix metalloproteases (MMPs) to degrade and remodel the extracellular matrix, allowing penetration of surrounding tissue. While the factors that promote invadosome formation by growth cones are unknown, epidermal growth factor (EGF) was shown to induce invadosome formation by several cell types. Surprisingly, we found that EGF receptor ligands also induce invadosome formation in Xenopus spinal neuron growth cones, and ongoing work aims to clarify the specific receptors and ligands, downstream signals, and functional effects of these ligands in vitro and in vivo. Using 2D and 3D in vitro live and fixed cell assays, we find that EGF receptor ligands activate downstream signals including tyrosine kinase signaling, to promote invadosome formation and MMP-dependent collagen degradation. Using HCR RNA-FISH paired with immunohistochemistry and organic clearing methods, we are characterizing the expression pattern of EGF receptors within the spinal cord during key stages of

development. We find that ErbB4 appears to be specifically expressed spinal MNs and are currently conducting gain and loss of function experiments in vivo. Future experiments will utilize CRISPR/Cas9 gene editing to create knockouts and GFP knock-ins to further understand how EGF signaling guides peripheral axon exiting.

S7.T5
Kelly Tseng
Understanding Eye Regrowth Mechanisms Using the Frog Embryo

Kelly Tseng

School of Life Sciences, University of Nevada, Las Vegas, USA

Mammals have a limited ability to regrow tissues whereas some animals including frogs can restore lost body parts. Xenopus laevis has high regenerative ability and can regenerate eye tissues including the retina and lens. We found that tailbud embryos readily regrew eyes after surgical removal of ~85% or more of the tissues and overall development was normal. The regrown eye has normal morphology and complement of cell types, connects to the brain, and is functional. During regrowth, proliferation of retinal progenitor cells was extended by one day with a concomitant delay in retinal layer formation, which was largely restored by three days after injury. An examination of retinogenesis during regrowth showed that the retinal birth order was consistent with that observed for eye development. Thus differentiation and patterning during regrowth largely recapitulated endogenous eye development. This model allowed us to assess the role of known mechanisms in development versus regrowth. Loss-of-function studies showed that Pax6 is required for both eye development and regrowth. In contrast, apoptosis and the H+ pump V-ATPase are required for regrowth but not development. Apoptosis and V-ATPase are also required for appendage regeneration, suggesting that there are conserved pathways for promoting regrowth. Together, our findings indicate that X. laevis embryos can re-initiate the eye development process after considerable tissue loss and that this process requires regenerative and developmental mechanisms. Our study highlights this eye regrowth model as a robust platform to systematically define the molecular mechanisms required for regeneration.

S7.T6
Gembu Maryu (Yang lab)
Nuclear-cytoplasmic compartmentalization of cyclin B1-Cdk1 promotes robust timing of mitotic events

Gembu Maryu 1 , Qiong Yang 1,2

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The cyclin-dependent kinase 1 (Cdk1) oscillator has been extensively studied in homogenized cytosolic extracts, but the influence of nucleocytoplasmic compartmentalization on this process is unclear. To address this, we developed a Förster (or fluorescence) resonance energy transfer (FRET) biosensor to investigate Cdk1 spatiotemporal dynamics in reconstituted cells with and without a nucleus. I will present our discoveries that indicate a significant impact of compartmentalization on the oscillator properties previously reported in bulk studies. While cells without a nucleus have oscillation frequencies highly tunable to cyclin levels, those with nuclei maintain a constant frequency. Interestingly, all cyclin proteins degrade within the same mitotic duration, ensuring robust timing despite high expression variability. Furthermore, Cdk1 and cyclin B1 oscillate rigorously out-of-phase, generating broad phase-plane orbits crucial for oscillation robustness. In contrast to the well-known delayed spiky activation of homogenized Cdk1, we observed a steady nuclear accumulation of activated cyclin B1-Cdk1 until nuclear envelope breakdown (NEB), followed by another abrupt activation to initiate anaphase. The biphasic activation and spatial compartmentalization of Cdk1 may coordinate the precise sequencing of downstream events. I will also discuss the potential molecular mechanism behind this period's robustness.

S7.T7
John Young
Insights into the mechanism of hind limb initiation in Xenopus laevis

Samantha Royle1 ,2, Olive Lucanish 1, Vibhuti Naik 1, Milena Chaufan 1, Michelle Balcarcel-Monzon 1, ChangHee Lee 2, Clifford J. Tabin 2, and John J. Young 1

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The vertebrate limb has provided a deep understanding of the cellular, genetic, and molecular mechanisms that generate an appendage. The majority of limb experimentation has been in animals where the limb forms early in embryogenesis. Yet, several tetrapods, most notably frogs, form their limbs well after embryonic patterning and differentiation have occurred. Surprisingly, we know very little about the processes that direct limb formation in amphibian tadpoles since most limb research in these animals has focused on regeneration. Here we use both molecular methods and classical transplant experiments to investigate the earliest steps in Xenopus hindlimb initiation. We found that posterior lateral plate mesoderm from the late neurula contributes to the hind limb and that this tissue expresses Pitx1 and Tbx4 in the early tadpole, both key genes involved in limb initiation. Surprisingly, Fgf10, a major contributor limb formation in amniotes, is not expressed until well after bud formation. These results suggest that, unlike amniotes, Fgf10 is dispensable for bud formation. Single-cell sequencing confirmed these observations and revealed several factors consistent with cell motility and migration in the limb. Histological analyses revealed the limb generating cells are mesenchymal at stage 40 and appear to condense into a bud by stage 46. Together, these data suggest a model that resembles zebrafish fin formation whereby the limb-forming mesenchyme is specified early. However, bud formation occurs several days later via cell migration and condensation. This work presents new insights into how limb development varies across tetrapods.

S7.T8
Andrea Wills
Defining the metabolic requirements for appendage regeneration

Andrea E. Wills, University of Washington Department of Biochemistry
Jeet H. Patel, University of Pennsylvania
Morgan McCartney, University of Washington
Audrey O'Neill, University of Washington

Regeneration of complex tissues is a property distributed diversely and rather unpredictably across the metazoan phylogenetic tree, as sometimes even closely related species have markedly different healing outcomes. This leads us to wonder what the fundamental distinctions are between animals that can regenerate and those that cannot. Anuran frogs like Xenopus tropicalis are highly regenerative as tadpoles, but transiently lose this capability during the transition to independent feeding, and permanently at metamorphosis. We have interrogated these context-specific changes to identify the contributions of factors like nutrient mobilization, glucose utilization, and progenitor cell fate to regeneration. A principal focus in our lab is metabolic reprogramming during appendage regeneration. Under regenerative conditions, we have seen that redirecting glucose to the pentose phosphate pathway, rather than oxidative phosphorylation, contributes to cell proliferation and tissue regrowth. We hypothesize that one function for this change in glucose flux is that it directs carbon from glucose to biosynthesis, rather than being lost as carbon dioxide. Specifically, the pentose phosphate pathway favors production of ribulose-5-phosphate, a limiting reagent and common precursor for nucleotide production. In keeping with this role, our recent work shows a requirement for IMPDH2 during regeneration. This enzyme catalyzes the rate limiting step of purine nucleotide biosynthesis and has a remarkable allosteric regulation in which it forms cell-scale superstructures upon stress.

Session 8:
Chair: **Jean Pierre Saint-Jeannet**, New York Univ., USA

S8.T1
Taejoon Kwon
Evidence-based integration and improvement of Xenopus genome annotation

Taejoon Kwon

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The genomes of both Xenopus laevis and Xenopus tropicalis (version 10) have been updated in 2019 and 2021, respectively, with the support of long-read sequencing, which helps to fill many gaps and corrects misassembled regions from the initial chromosome-scale genome assembly (version 9). However, the different annotation versions for these genomes, mainly from three different groups (NCBI, Ensembl, and JGI/UC Berkeley), were not fully integrated, hindering the use of these improved genomes. Furthermore, recent applications of the single-cell transcriptome analysis, which mainly captures the 3'-end of transcripts, revealed that the incorrect annotation of 3'-UTR could significantly affect the estimation of gene expression, which may miss important signals to analyze the dynamics of the cell atlas. Here, I present the integrated genome annotations of X. laevis and X. tropicalis, evaluating gene models from different annotation sources with multiple high-throughput sequencing data. After then, I revised the 3'-UTR regions of each gene model to maximize the coverage of published single-cell data. This integrated annotation results with experimental evidence will make Xenopus a more attractive model system in genomics and help to annotate other amphibian genomes.

S8.T2
Romain Gibeaux
Changes in seam number and location induce holes within microtubules assembled from porcine brain tubulin and in Xenopus egg cytoplasmic extracts

Charlotte Guyomar 1, Clément Bousquet 1, Siou Ku 1, John Heumann 2, Gabriel Guilloux 1, Natacha Gaillard 3, Claire Heichette 1, Laurence Duchesne 1, Michel O. Steinmetz 3,4, Romain Gibeaux 1, Denis Chrétien 1*

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Microtubules (MTs) are major components of the eukaryotic cytoskeleton. Our current knowledge about MT structure is essentially derived from studies that have been performed in vitro on MTs assembled from purified tubulin. However, little is known concerning the structure of MTs in cells, and more specifically on the organization of the tubulin heterodimers within their lattice. To tackle this issue, we take advantage of the versatility, the biochemical traceability and cell-free nature of Xenopus egg extracts. Here, we used the motor domain of kinesin that binds every tubulin dimer to decorate MTs in the egg cytoplasm. Dual-axis cryo-electron tomography data were taken so that all MTs could be analyzed independently of their orientation with respect to the tilt axes. We then developed a segmented sub-tomogram averaging approach to analyze the organization of the tubulin molecules within all MTs. We find that the vast majority of the MTs are made of 13-protofilament B-type lattices with 3-start lateral helices and a unique seam of the A-type. Yet, we find exceptions such as MTs segments built from different protofilaments number (12

& 14), as well as fully helical 13 protofilaments MT segments with 4-start lateral helices. We also find that the seam location can vary within individual MTs, leaving holes inside their wall. In parallel, we have analyzed the structure of MTs assembled in vitro from pure tubulin and discovered that these are much more structurally heterogeneous, in terms of protofilaments, seam numbers, and frequency of lattice type transitions. Altogether, our data indicate that MT structural instability is tightly regulated by cytoplasmic factors and we further propose that this regulation could be a mechanism modulating MT dynamic instability in cells.

S8.T3
Muriel Perron
A roadmap for retinal regeneration

Muriel Perron
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Our lab is interested in a complex and intriguing issue in neurobiology, namely the tremendous variability of neural tissue regeneration efficiency among vertebrates. Regarding the retina, mammalian Müller glial cells possess remnants of stemness but are unable to sustain retinal regeneration, contrasting with their teleost or amphibian counterparts. We are studying the molecular mechanisms that underlie such divergent regenerative properties, taking advantage of both Xenopus and mouse models. I will focus my talk on the coupling between inflammatory signaling and regeneration.

S8.T4
Kara Pratt
microCT scans of the Xenopus laevis tadpole

Title: A comparative study on the development of the Xenopus tadpole retinotectal and retinotegmental projections

Uwemedimo G. Udoh, Kaiyuan Zheng, Kara G. Pratt

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How neurons self-assemble into circuits that give rise to behaviors is a fundamental question in neuroscience. The Xenopus tadpole retinotectal projection – the synapse between the retinal ganglion cells (RGCs) in the eye and the midbrain optic tectum – has been used as a model to study this question. But this projection is only one component of the amphibian visual system. Recently, we identified a retinotegmental projection, a direct projection from the RGCs to the ventral midbrain tegmentum. To compare these visual projections, RGC axons were activated by placing a stimulation electrode on the optic chiasm of an isolated brain preparation. RGC-evoked synaptic currents were recorded from tectal and tegmental neurons in whole cell configuration. Recordings were carried out at three key stages of retinotectal development: stage 42 (5 days post-fertilization; dpf), 44-46 (7-9 dpf) and 48/49 (12-21 dpf). We found that the maximum strength of RGC input onto tectal neurons peaked during stage 44-46 then sharply declined by stage 48/49. The decline was observed to be NMDA receptor- dependent, suggesting activity-dependent plasticity. In contrast, RGC input onto tegmental neurons did not display a transient peak, remained constant across the three developmental stages, and was unaltered by NMDAR blockade. Paired pulse recordings indicate that RGC axons display a higher probability of transmitter release onto tegmental neurons compared to tectal neurons, suggesting that different types of RGCs innervate the different visual centers. These findings suggest that these two visual projections are built differently and likely carry out different roles in processing visual stimuli.

S8.T5
John Measey
Home and away: the core gut microbiome of Xenopus laevis is modified by its environment

Authors: Measey, J., Ersin, M., Guille, M., Almojil, D., Araspin, L., Wagener, C., Boissinot, S., Watts, J., Robson, S.

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Abstract: The vertebrate gut microbiome is a community largely composed of bacterial, fungal and viral components, whose molecular component equal that of the host. The influence of the microbiome is known to be significant both on an individual basis, and also on population scales in a wide range of host organisms. The gut microbiome is known to be involved with key attributes of animal health, including assimilation of nutrients, immuno-defensive functions and host behavior. In this study, we used bacterial 16S rRNA amplicon-based sequencing for metataxonomic classification of the gut microbiome of individuals from eight populations of Xenopus laevis. These populations were selected to represent an altitudinal gradient in of the host species (0 to 3000 m asl). From the 16S rRNA community profiles, we determine the components of the core microbiome of X. laevis, and ask whether deviations from the core are associated with the environmental context in which they live. In addition, we sampled four European invasive populations and a laboratory population from the European Xenopus Resource Centre (EXRC) in the UK, to determine what aspects of the core microbiome are retained by non-native populations. This represents the first time that the microbiome of X. laevis has been assessed across such diverse conditions, and provides data that will help understand the role played by the environment and inform monitoring of health within this model organism.

S8.T6

Takuya Nakayama

Novel insights regarding the role of transcription factor Six3 in retina development are revealed by a single-nucleus RNA-sequencing method for comparing expression in wild-type and mutant embryos of Xenopus tropicalis

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Single-cell RNA-sequencing should be a very productive approach for comparing mutant and wild-type embryos at pre-differentiation stages to determine effects of mutations on developmental trajectories. We need genotyping to distinguish mutant and wild-type embryos at these stages but using single “cells” is challenging: embryos would need to be frozen to capture a given developmental stage until genotyping is complete, but intact cells cannot be isolated from frozen samples. We instead developed a protocol for isolation of high-quality nuclei from frozen cell suspensions that does allow genotyping individual embryos from a small fraction of a single embryo suspension, and the remaining suspension is frozen to use for single-nucleus RNA-sequencing. We applied this technique to examine st.18 six3 mutants, since the transcription factor six3 is essential for brain and eye formation, creating a high-resolution whole-embryo 60-cluster UMAP of wild-type and mutant embryos. Focusing here on the eye, we identified two retina clusters, finding, strikingly, that one of these is largely missing in the six3 mutant. Among the changes in gene expression in the mutant is the premature expression of several “late” genes essential for photoreceptor formation leading to the recognition of a previously unappreciated role of six3 (known to have repressor activity), as a key regulator of the eye-forming program by preventing premature expression of the photoreceptor program during early eye development. This data reveals critical effects of the six3 mutation as a key regulator of retina formation and highlights the general utility of this approach for studying early developmental processes.

S8.T7

Yuki Shibata

NEXTrans: Simple transgenesis at a novel safe harbor site by using CRIPSR-Cas9 in Xenopus laevis

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Abstract
Recently, we have identified for the first time a novel safe harbor site in Xenopus laevis, transforming growth factor beta receptor 2-like (tgfbr2l) locus and established a new target transgenesis method based on CRISPR-Cas9 in X. laevis, New and Easy Xenopus Transgenesis (NEXTrans). This improved strategy allows us to simply generate transgenic animals to stably express the transgene by only co-injecting a single guide RNA (sgRNA) targeting tgfbr2l, a preset donor plasmid containing the tgfbr2l.L fragment and a tissue-specific promoter/enhancer driving a reporter gene, and recombinant Cas9 protein into fertilized eggs. To evaluate the efficiency of target integration into the Xenopus genome, we tested three NEXTrans plasmids carrying reporter gene driven by tissue-specific promoters, the fgk promoter (fin and gill), the cmv promoter (whole body), and the gamma-crystallin promoter (lens). Approximately 7-12 % of faithful reporter expression was observed in all F0 transgenic animals in a tissue-specific manner. Genotyping analysis confirmed that all NEXTrans plasmids were integrated into the tgfbr2l loci. Importantly, we confirmed the germline transmission of the transgenes in the F1 siblings of all transgenic animal cases within one year. Thus, the target transgenesis at the novel safe harbor site was achieved using NEXTrans, and we expect that NEXTrans become a powerful tool for X. laevis functional genomics research by controlling the copy number of the transgene.

Session 9:
Chair: **Rachel Miller**, Univ. of Texas Houston, USA

S9.T1
Haruki Ochi
Unraveling the Mechanisms of Kidney Regeneration through Damage-Response/Regeneration Enhancers

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The kidney is a crucial organ that filters metabolic waste products, and its malfunction can lead to debilitating diseases. One potential solution for kidney dysfunction is to regenerate functional tissue by activating intrinsic genetic programs responsible for kidney development. Our team previously discovered damage-response/regeneration enhancers for kidney regeneration and explained their activation mechanisms (Suzuki N., eLife, 2019). However, our earlier study only focused on non-coding DNA regions for Lhx1, leaving genetic programs connected by regeneration enhancers unresolved. To address this issue, we conducted a comprehensive analysis of enhancers and associated genes in regenerating nephric tubules of Xenopus laevis. Putative enhancers were identified using ATAC-seq and H3K27ac ChIP-seq analyses, while target genes were predicted based on the proximity of enhancers to genomic DNA and the consistency of their transcriptome profiles with ATAC-seq/ChIP-seq profiles of the enhancers. Motif enrichment analysis revealed the central role of Krüppel-like factors (Klf) in enhancers. Klf15, a member of the Klf family, directly binds enhancers and stimulates the expression of regenerative genes, including alpha-1A adrenergic receptor (adra1a). Inhibition of Klf15 activity results in the failure of nephric tubule regeneration. Moreover, pharmacological inhibition of Adra1a-signaling suppresses nephric tubule regeneration, while its activation promotes nephric tubule regeneration and restores organ size. Therefore,

we discovered that Klf15-dependent adrenergic receptor signaling through regeneration enhancers plays a central role in the genetic network for kidney regeneration (Suzuki N., PNAS, 2022).

S9.T2
Miler Lee
Differential regulation of gene copies in the allotetraploid Xenopus

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Xenopus laevis is an allotetraploid with two non-recombining versions of each chromosome, having arisen from hybridization of two ancestral Xenopus species ~18 million years ago. Today, the X. laevis genome encodes two copies (homeologs) of many genes, one each from the so-called “L” and “S” subgenomes. Most of these gene pairs are thought to be functionally redundant; however, their relative expression levels in a given tissue context often differ, implying regulatory divergence that likely evolved post hybridization to maintain appropriate gene dosage. We seek to characterize gene regulatory divergence between homeologs in the early embryo during the maternal-to-zygotic transition, when egg-inherited maternal mRNA are supplanted by mRNA transcribed from the newly activated embryonic genome, leading to the induction of pluripotent stem cells and gastrulation. We find substantial asymmetric homeolog activation, which appears to be driven by extensive loss/gain of enhancers bound by maternally provided transcription factors Pou5f3 and Sox3; as well as asymmetric post-transcriptional regulation of maternal mRNA stability. However, composite embryonic transcriptomes are largely concordant between laevis and other Xenopus species; thus, our findings demonstrate that divergent gene regulatory landscape in the early embryo can lead to convergent developmental outcomes.

S9.T3
Qiong Yang
Single-cell analysis of mitotic cycles and energy flow: insights from cell-free Xenopus extracts

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Early embryonic development relies on precise intracellular timing driven by complex biochemical and genetic networks known as biological clocks. These clocks coordinate through cell-cell communications to form collective patterns. Yet, how clocks and developmental patterns maintain precision despite the prevailing intrinsic and extrinsic stochasticity remains unclear. The accuracy of far-from-equilibrium oscillatory systems, like information processing and kinetic proofreading, comes at an energetic cost. While theoretical studies have proposed a quantitative relationship between oscillator accuracy and energy dissipation, experimental characterization of the role of energy in oscillation dynamics at the single-cell level presents challenges.

We formulated droplet microfluidics to create thousands of oscillators encompassing diverse energy landscapes and cell-cycle dynamics with orthogonal network perturbations, enabling high throughput, multi-dimensional continuous mapping at the single-cell level. These single-cell oscillators, composed of phosphorylation/de-phosphorylation (PdP) networks centered on the cyclin-dependent kinase (Cdk1) from Xenopus egg extracts, were quantified using a newly developed Cdk1 FRET sensor. Systematic manipulation of ATP levels and network topology in each droplet revealed a non-monotonic response of cell cycle behavior to ATP. Specifically, as ATP levels increase or decrease from an intermediate level, oscillation slows down until arresting at high and low ATP boundaries, accompanied by a faster de-phasing rate. These highlight the complex coupling between the free energy budget and mitotic oscillator performance, suggesting the necessity of an optimal energy budget for fast and precise mitotic cycles. Additionally, manipulating the mitotic circuit design by removing PdP regulations on Cdk1 affected energy

dissipation during mitotic entry and the clock performance. Our results underscore the crucial role of intracellular energy flow and dissipation in maintaining cell cycle speed and precision.

S9.T4
Paula Slater-Guzman
Mitochondrial function during Xenopus laevis spinal cord regeneration

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Spinal cord injury (SCI) is a permanent affliction affecting the central nervous system motor and sensory nerves, resulting in paralysis beneath the injury site. It is estimated that SCI affects between 250.000 – 500.000 new cases worldwide every year. There is an absence of therapies allowing functional recovery, and humans and mammals in general, present limited regenerative capacity. Nevertheless, some non-mammalian organisms can regenerate. Xenopus laevis can regenerate in larvae stages, a capacity that is lost after metamorphosis, making possible the comparison of regenerative versus no regenerative capacity, and allowing the study of the cellular and molecular mechanisms underlying the successful regenerative processes, and those that are responsible for the lack of regenerative capacities.

Interestingly, mitochondria have extensively appeared in seminal works playing a pivotal role during SCI events: i) mitochondrial dysfunction is the common event prior to neuronal and glial death; ii) mitochondrial metabolism regulates immune response; iii) mitochondrial number and localization correlates with axonal regenerative capacity and iv) mitochondrial abundance and metabolism regulate neural stem progenitor cells proliferation, and differentiation. This evidence suggests that a more in-depth study of mitochondrial function and regulation is needed to identify potential targets for SCI therapeutic intervention. We studied mitochondrial morphology, dynamics, and function in Xenopus regenerative stages by using electron microscopy, confocal microscopy, RT qPCR, western blot, and enzymatic function. We determined that SCI resulted in decreased number and increased mitochondrial area, accompanied by a change in mitochondrial morphology, prevailing a swollen phenotype, which correlates with an observed increase in glycolytic transcripts and enzymatic activity.

S9.T5
Jaeho Yoon, PD (Daar Lab)
Wnt4 and ephrinB2 instruct apical constriction via Dishevelled and non-canonical signaling

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* Presenter
Apical constriction is a cell shape change along the apical-basal axis that supports tissue morphogenesis, such as neural tube formation. Actin-myosin networks are key components in the generation of the contracting force required for tissue remodeling, but the signaling cue that instructs apical constriction remains unknown. Previously, we showed that ephrinB2 is necessary for neural tube closure, however, the precise mechanism remained elusive. Using a blend of biochemistry, live and fixed cell imaging, gain-of-function and loss-of-function along with rescue experiments using wild-type and mutant constructs in vivo, we provide mechanistic insight into how ephrinB2 plays an instructive role in neural tube closure. These experiments led us to the identification of a signaling complex consisting of Wnt4, EphrinB2, Ror2, Dsh2, and Shroom3 (termed WERDS) that is responsible for this ephrinB2-driven process. Moreover, as part of this mechanism, we made the exciting revelation that ephrinB2 antagonizes Wnt/β-catenin signaling through a conformational change in the main Wnt signaling scaffold, dishevelled. This interaction switches dishevelled from canonical to non-canonical Wnt signaling that is required for apical constriction in the neural tube. We believe that these findings provide the profound understanding of how cross-talk occurs

between two seemingly separate major signal transduction pathways, Eph/ephrin and Wnt, to coordinate a major morphogenetic event, neural tube formation.

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S9.T6
Carole LaBonne, Northwestern Univ., USA
The Evolution of Gene Regulatory Networks: A View From the Crest

Session 10:
Chair: **Ira Daar**, National Cancer Institute, USA

S10.T1
Richard Harland
The Rho GEF Plekhg3 is required specifically for involution movements in Xenopus Gastrulation

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The coordination of cytoskeletal activity with cell surface and adhesion behavior underlies the shape changes that occur in embryonic development. Here, we focus on regulators of Rho, the Rho GTP exchange factors (Rho GEFs) that regulate the actin cytoskeleton. Precedents for stage-specific regulation of the actin cytoskeleton in Xenopus morphogenesis include the Rho GEF, Plekhg5 and Shroom3, which mediate apical constriction during gastrulation and neurulation. Like plekhg5, the Rho GEF plekhg3 peaks in expression during gastrulation, is elevated in expression in the marginal zone, and plekhg3 crispants show defects in the strength and persistence of involution movements. Frequently, despite aberrant early gastrulation, the hoop stresses driven by normal convergence of the neural plate buckle the marginal zone, and snap the blastopore closed. Indeed, the loss of plekhg3 does not prevent apical constriction, or convergence-extension movements, demonstrating the independence of regulatory mechanisms. Interestingly, overexpression of Plekhg3, but not Plekhg5, led to failure of cytokinesis, producing multinucleated cells, where LifeAct, and mCherryPlekhg3 colocalize at abortive cleavage furrows. Plekhg3, actin and Anillin assemble both at cleavage furrows and the cell cortex. We propose that Plekhg3 forms a complex with Actin, Anillin, and Rho to mediate stiffness of the cellular cortex in interphase, with consequences to fibronectin assembly and involution.
We acknowledge the critical contributions of our colleagues in the Ray Keller, Doug DeSimone, David Shook, Aaron Straight, Ann Miller, Lance Davidson, Christine Field and Marc Kirschner groups and funding from NIH R35GM127069, 1R21HD107363 and the Choh Hao Li Distinguished Chair.

S10.T2
Shuo Wei
Diphthamide deficiency promotes association of eukaryotic translation elongation factor 2 with p53 to induce p21 expression and neural crest defects

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Diphthamide is a modified histidine residue unique for eukaryotic translation elongation factor 2 (eEF2), a key ribosomal protein. Loss of this evolutionarily conserved modification causes developmental defects

through unknown mechanisms. In a patient with compound heterozygous mutations in Diphthamide Biosynthesis 1 (DPH1) and impaired eEF2 diphthamide modification, we observed developmental defects in craniofacial structures and other neural crest (NC)-derived tissues. To understand the mechanisms through which diphthamide deficiency causes NC defects, we depleted Dph1 in Xenopus tropicalis embryos using both CRISPR/Cas9 genome editing and translation-blocking morpholinos. Dph1 depletion led to decreased cell proliferation in the neuroepithelium and reduction of NC markers, which were rescued by an eEF2 mutant mimicking diphthamide modification but not wild-type eEF2. Loss of DPH1 facilitated dissociation of eEF2 from ribosomes and association with p53 to promote transcription of the cell cycle inhibitor p21, resulting in inhibited proliferation. Knockin mice harboring the patient's mutations also displayed reduced cell proliferation and craniofacial defects that could be rescued by knockout of one allele of p21. These findings uncover an unexpected role for eEF2 as a transcriptional coactivator for p53 to induce p21 expression and NC defects, which is regulated by diphthamide modification.

S10.3
Dan Weeks
Nucleolar Domain Decisions: properties that contribute to dense fibrillar component localization

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Abstract

The nucleolus is a non-membrane bound organelle central to ribosome biogenesis. The nucleolus contains a mix of proteins and RNA and has three known nucleolar compartments: the fibrillar center, the dense fibrillar component, and the granular component. The spatial organization of the nucleolus is influenced by the phase separation properties of nucleolar proteins, the presence of RNA, protein modification, and cellular activity. Here we investigated the role of intrinsically disordered regions in nucleolar compartment localization and found that the disordered regions are not sufficient to direct specific domain localization. We also investigated the importance of complex binding for members of the box H/ACA pseudouridylation complex in dense fibrillar component localization. We found that the accumulation of Gar1 and Nhp2 in the dense fibrillar component depended on their ability to bind to their box H/ACA complex binding partners, as site-directed mutations that block this binding alter compartment localization. Using a nanobody to introduce novel binding to a different dense fibrillar component localized protein, we restored the localization of the mutated forms of Gar1 and Nhp2.

S10.T4
Shinuo Weng, PD (Wallingford Lab)
Planar polarized force propagation links cell intercalation to tissue-scale convergent extension

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Convergent extension is an evolutionarily conserved collective cell movement that elongates the body axis and several organ systems during development. It is a multiscale process that requires coordination across multiple scales, from the molecular, to the cellular and tissue-level behaviors to achieve robust tissue shape change. However, the mechanisms linking stereotypic behaviors across scales is not well understood. To shed light on this, we developed and applied new image-based non-invasive methods to assess mechanical forces at different scales during development. We found that local cell movements work in synergy to propagate the tissue-scale convergent extension, and this synergy depends on cellular forces to regulate the cell packing configuration, allowing planar polarized force propagation across multiple cells. We further

found that when a disease-related gene is disrupted, even subtle changes to cellular forces can cause defects to escalate over time and affect morphogenesis at larger scales. Our data suggest a multiscale mechanical system that supports tissue morphogenesis in an efficient manner. It provides new cell biological and biomechanical insights into this fundamental morphogenetic process and its implication in congenital anomalies.

S10.T5
Jeremy Green
Assessment of a double ovulation protocol for Xenopus laevis: moderately transiently elevated corticosterone levels without loss of egg quality are associated with doubled fertilisation yield

Authors
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Xenopus laevis, is a successful model for the biology of fertilisation and embryonic, larval and metamorphic development. It has a long history and continues to attract significant funding, publication and citation. For such research, X. laevis females are usually induced to ovulate by chorionic gonadotropin injection. After three months rest, animals are re-used. Re-use is repeated for up to several years. Adult females can lay much larger egg numbers in a short period: egg numbers following several weekly gonadotropin injections decline only gradually and field observations suggested multiple egg-layings in a short season. We therefore compared the standard protocol with "double ovulation" in which females were re-ovulated after just seven days before three month's rest and re-use. We quantified egg number, fertilisation and cleavage (egg quality), and corticosterone secretion rate as a measure of stress response. Over seven 3-month cycles, we found no differences in egg number per ovulation or egg quality between the groups, and no long-term changes in either group. Corticosterone was slightly higher for the second ovulation of the double ovulation protocol compared to the first or single-ovulation group. Both groups exhibited the same baseline secretion rates before the subsequent cycle. We suggest that the benefits of a doubling in egg yield per cycle per animal without loss of egg quality or signs of acute or long-term harm outweigh the relatively modest and transient corticosterone elevation we observed. Double-ovulation therefore represents a potential new standard practice for Xenopus research.

S10.T6
Kris Vleminckx, Ghent Univ., Belgium
Identification of cooperating cancer driver genes using CRISPR multiplexing in Xenopus tropicalis

Session 11:
Chair: **Matt Good**, Univ. of Pennsylvania, USA

S11.T1
Brian Mitchell
The role of macropinocytosis in tissue homeostasis

Epithelial remodeling is an important biological process that involves cell division, cell death, live cell extrusion, cell rearrangements and cell shape changes. How these processes are interwoven to maintain tissue homeostasis during morphogenesis has been the focus of a wide range of studies in many model systems. Here we investigate the role of macropinocytosis as a regulator of membrane remodeling.

Macropinocytosis is the process by which cells non discriminately engulf large amounts of extracellular material. It has been implicated in nutrient uptake and receptor recycling. However, our data indicates that it also has an important role in regulating force distribution across the epithelium. Macropinocytosis is induced by low levels of membrane tension and is inhibited by high membrane tension. Furthermore, micropinocytosis significantly decreases apical cell size leading to an increase in relative levels of junctional tension. Blocking micropinocytosis alters cells size and tissue organization and increases mechanosensory regulated cell extrusion events. We propose that micropinocytosis is an important regulator of epithelial homeostasis.

S11.T2
Shuyi Nie
Cdc42ep1 coordinates neural crest cell migration by interacting with Cdc42 and septin filaments

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We have previously identified a Cdc42 effector protein, Cdc42ep1, which is selectively expressed in neural crest cells and is required for neural crest cell migration. Interestingly, Cdc42ep1 knockdown affected both the extension of cell protrusions and the retraction of cell body, the two major events during cell migration. Consistently, we found that Cdc42ep1 localizes to two subcellular locations in neural crest cells, at the cell front and in the cell center. At the cell front, Cdc42ep1 colocalizes with Cdc42 and regulates the formation of cell protrusions by interacting with Cdc42. Recently, we found that Cdc42ep1 interacts with another cytoskeletal component, septin filament, at the cell center. The cell center organizations of Cdc42ep1 and septin filaments are interdependent. When Cdc42ep1 is lost, the formation of septin filaments is disrupted, and septin forms fragmented filaments or tiny ring structures. When septin filaments are inhibited by either the loss of a key subunit Septin7 or by chemical inhibitors, the formation of filamentous Cdc42ep1 structure is also lost. This coordination between Septin filaments and Cdc42ep1 is important for the organization of actin filaments. The loss of septin filaments leads to impaired stability and contractility of actin fibers, resulting in decreased migration. The septin filaments also support the persistent migration of neural crest cells, likely serve as scaffolds for actin fibers. In summary, our results demonstrate a critical role of Cdc42ep1 - septin complex in the regulation of actin stress fibers, which, together with the activity of Cdc42ep1 - Cdc42 complex at cell protrusions, promotes coordinated migration of neural crest cells.

S11.T3
Xianrui Cheng
De novo generation of spatially organized cytoplasm

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Functional cytoplasm is spatially organized. How such organization may be generated de novo remains an open question. Cell-free Xenopus laevis egg extracts, essentially a scrambled pool of cytoplasmic components, self-organize into cell-like units ~200 micrometers in size in a one-pot reaction. Thus, egg extracts carry out de novo generation spontaneously and serve as a powerful model for identifying the underlying mechanisms. We first investigate what breaks the spatial homogeneity in extracts at the beginning of self-organization. We found that symmetry-breaking occurs through the formation of an unconventional type of microtubule aster. The microtubule organizing center (MTOC) focuses the plus ends of microtubules, forming a ‘plus end inside, minus end outside’ radial array that has the opposite polarity of a conventional centrosome aster. The MTOC shows properties of biomolecular condensates. In extracts,

the MTOCs localize to the edge of a plane previously occupied by the metaphase plate, where they accumulate pre-formed F-actin, a phenomenon typically seen near the cytokinesis furrow in cells. We propose that these polarity-reversed asters promote spatial organization of the cytoplasm and may be involved in cytokinesis signaling.

S11.T4
Takayoshi Yamamoto
Robust and quick shaping of Wnt gradient by Wnt-receptor feedback revealed by complementary wet and dry experiments

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Morphogens govern tissue patterning in a concentration-dependent manner. However, it is still unclear how reproducible patterning can be achieved with diffusing molecules, especially when that patterning concerns differentiation of thin tissues. Wnt6 patterns cardiogenic mesoderm to induce differentiation of a thin tissue, the pericardium, in Xenopus. In this study, we revealed that a Wnt receptor, frizzled-7, is expressed in a Wnt- dependent manner. With a combination of experiments and mathematical modeling, this receptor-feedback appears essential to shape a steep gradient of Wnt signaling. In addition, computer simulation revealed that this feedback imparts robustness against variations of Wnt ligand production and allows the system to reach a steady state quickly. We also found that a Wnt antagonist sFRP1, which is expressed on the opposite side of the Wnt source, accumulates on N-acetyl-rich heparan sulfate (HS). N-acetyl-rich HS concentration is high between the sources of Wnt and sFRP1, achieving local inhibition of Wnt signaling via restriction of sFRP1 spreading. These integrated regulatory systems restrict the Wnt signaling range and ensure reproducible patterning of the thin pericardium (Yamamoto et al., eLife 2022).
Antagonists such as sFRP1 expand Wnt ligand distribution. However, they do not always broaden the signal activation range. We are extending the present simulation to analyze the mechanisms behind the differences in ligand distribution and signal activation ranges.

S11.T5
Jerome Jullien, ISTERM, France
Sperm derived H2AK119ub1 is required for embryonic gene expression regulation in Xenopus laevis

Session 12:
Chair: **Kim Mowry**, Brown Univ., USA

S12.T1
Asako Shindo
Nutrients control thyroid morphogenesis

Asako Shindo
Institute of Molecular Embryology and Genetics, Kumamoto University, Japan

The thyroid consists of numerous follicles, and the luminal and spherical architecture of the follicles is widely recognized as essential for hormonal functions of the thyroid. However, the cellular and molecular mechanisms governing the simultaneous formation of multiple follicles remain poorly understood. We investigat the process of follicle formation in the thyroid using Xenopus laevis larvae and uncover intriguing connections between nutrient intake and thyroid morphogenesis. We observe that follicle formation begins with feeding, suggesting that it is a process regulated by nutritional cues. When feeding is absent, follicle formation is arrested and resumes upon the initiation of feeding. Exploiting this phenomenon, we have

identified important molecules involved in follicle formation and found that feeding reduces cell adhesion molecules while increasing extracellular matrix (ECM) in the thyroid. Interestingly, inhibiting cell adhesion or augmenting ECM levels promotes thyroid follicle formation without feeding. Live imaging of the developing thyroid reveals active movements of cells and lumina following feeding. In this talk, I will introduce the behavior of cells and lumina during thyroid development and discuss the connection between nutrient intake and the molecular responses underlying thyroid morphogenesis.

S12.T2
Peter Nemes
Mitotic chromosomes scale to nuclear-cytoplasmic ratio and cell size in Xenopus

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During the rapid and reductive cleavage divisions of early embryogenesis, subcellular structures such as the nucleus and mitotic spindle scale to decreasing cell size. Mitotic chromosomes also decrease in size during development, presumably to scale coordinately with mitotic spindles, but underlying mechanisms are unclear. Here we combine in vivo and in vitro approaches using eggs and embryos from the frog Xenopus laevis to show that mitotic chromosome scaling is mechanistically distinct from other forms of subcellular scaling. We found that mitotic chromosomes scale continuously with cell, spindle and nuclear size in vivo. However, unlike for spindles and nuclei, mitotic chromosome size cannot be re-set by cytoplasmic factors from earlier developmental stages. In vitro, increasing nuclear-cytoplasmic (N/C) ratio is sufficient to recapitulate mitotic chromosome scaling, but not nuclear or spindle scaling, through differential loading of maternal factors during interphase. An additional pathway involving importin α scales mitotic chromosomes to cell surface area/volume ratio (SA/V) during metaphase. Finally, single chromosome immunofluorescence and Hi-C data suggest that mitotic chromosomes shrink during embryogenesis through decreased recruitment of condensin I, resulting in major rearrangements of DNA loop architecture to accommodate the same amount of DNA on a shorter axis. Together, our findings demonstrate how mitotic chromosome size is set by spatially and temporally distinct developmental cues in the early embryo.

S12.T3
Beatrice Durand
Cerebellar granular neuron progenitors exit their germinative niche by way of Barhl1 mediated silencing of T-Cell Factor transcriptional activity

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SUMMARY

T-Cell Factors (TCF) are the main transcriptional effectors of Wnt/β-catenin signaling. TCF responsiveness is a hallmark of self-renewal in mouse embryonic, and adult, neural stem cells (NSC). However, in vivo contribution(s) of TCF activities in long-lived NSC biology are poorly understood. Cerebellar granule neurons arise from Atoh1-expressing granule neuron progenitors (GNP) in the upper rhombic lip (URL). Using functional and transcriptomic approaches in amphibian, we demonstrate that TCF are active in the URL, and strictly necessary for the emergence and maintenance of the GNP germinative zone. We identify BarH-like 1 (Barhl1), a direct target of Atoh1, as a gate keeper for GNP exit from the URL, through silencing of TCF transcriptional activity. Our transcriptomic and in silico analysis identifies Barhl1/TCF URL target genes, and confirms our functional data. Our study provides in vivo evidence that inhibition of TCF repressive activity is necessary for maintenance of the URL, a long-lived neural germinative niche.

KEYWORDS
Granule Neuron Progenitors, Cerebellum, Upper Rhombic Lip, Neural Stem Cell, Germinative niche, Wnt signaling, TCF/Lef, BarH-like 1.

S12.T4
Christopher Thompson
Overexpression of transthyretin and β-trace in the Xenopus laevis tadpole choroid plexus ameliorates the effects of lead poisoning on thyroid hormone mediated changes in brain development

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Despite decades of regulation, lead (Pb) poisoning is a persistent health issue in the United States due to legacy environmental contamination. Children are most susceptible to Pb poisoning, which causes persistent cognitive deficits and low IQ. The specific mechanisms by which Pb poisoning impairs brain development are not fully understood, however. Previous research showed that Pb interferes with several thyroid hormone (TH)-related processes, including decreases in expression of TH distributor proteins (THDPs) in the choroid plexus. Given that TH is a critical regulator of brain development in all vertebrates, we conducted several experiments to test if Pb impairs TH-mediated changes in brain development in Xenopus laevis tadpoles. We exposed stage 47 tadpoles to a range of concentrations (10ppb – 10,000ppb) of Pb for up to seven days and found that Pb interferes with TH-mediated changes in brain morphology, neurogenesis, and cell death. We also found that treatment with Pb decreases expression of two THDPs, transthyretin and β-trace. To determine if overexpression of these THDPs can ameliorate the deleterious effects of Pb, we generated two custom plasmids to overexpress transthyretin and β-trace in the choroid plexus. We found that overexpression nearly completely blocked the neurotoxic effects of Pb on TH-mediated changes in neurogenesis. These results show that Pb-poisoning may dysregulate expression of TTR and β-trace in the choroid plexus, leading to insufficient TH-signaling in the developing brain.

S12.T5
Saurabh Kulkarni, Univ. of Virginia, USA
Control of centriole number in multiciliated cells

S12.T6
Jakub Sedzinski
Dissecting the mechanism of basal bodies ascent, distribution, and patterning

Raghavan Thiagarajan 1, Younes Farhangibarooji 2, Poul Martin Bendix 2, Mandar Inamdar 3 & Jakub Sedzinski 1

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Motile cilia play a crucial role in properly distributing mucus and other cell secretions in the respiratory tract. Nucleation and anchoring of these motile cilia are carried out by centriole-like structures called basal bodies at the cell cortex on the apical side. These basal bodies are brought to the apical surface, distributed, and patterned, while the apical domain of the cell is expanding. But how these complex processes are executed simultaneously is not understood. Here, using a combination of imaging techniques and Xenopus embryonic epithelia as a model, we find actin to be the major driver of these processes. We show that actin reorganizes into cables and meshwork to perform such diverse tasks. Cables transport basal bodies from the basal to the apical side, and meshwork distributes the basal bodies across the apical domain of the cell while simultaneously expanding the apical domain. Results from high-resolution and high-speed imaging show a correlation between basal body dynamics and actin meshwork reorganization. While the basal bodies undergo diffusive behavior locally, they exhibit directional motion at the scale of the apical domain. We hypothesize that this complicated behavior could result from actin polymerization and cross-linking that generate pushing force for the apical domain expansion. We are currently developing a theoretical model to test this hypothesis and to understand the physical aspects of simultaneous force generation and patterning.

Session 13:
Chair: **Andrea Wills**, Univ. of Washington, USA

S13.T1
Yonglong Chen
Activation of P53 pathway contributes to Xenopus hybrid inviability

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Hybrid incompatibility as a kind of reproductive isolation contributes to speciation. The nucleocytoplasmic incompatibility between Xenopus tropicalis eggs and Xenopus laevis sperm (te×ls) leads to specific loss of paternal chromosomes 3L and 4L. The hybrids die before gastrulation, of which the lethal causes remain largely unclear. Here we show that the activation of the tumor suppressor protein P53 at late blastula stage contributes to this early lethality. We find that in stage 9 embryos, P53 binding motif is the most enriched one in the up-regulated ATAC-seq peaks between te×ls and wild-type Xenopus tropicalis controls, which correlates with an abrupt stabilization of P53 protein in te×ls hybrids at stage 9. Inhibition of P53 activity via either tp53 knockout or overexpression of a dominant-negative P53 mutant or Mdm2, a negative regulator of P53, by mRNA injection can rescue the te×ls early lethality. Our results suggest a causal function of P53 on hybrid lethality prior to gastrulation.

S13.T2
Jen Landino
Self-organized Rho and F-actin patterning in an artificial cortex

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The cell cortex, comprised of the plasma membrane and an underlying meshwork of filamentous actin (F-actin), is remodeled during a variety of essential biological processes including cell division. Previous work has shown that the cell cortex is dynamically patterned during cell division with subcellular waves of F-actin assembly and disassembly, a phenomenon termed “cortical excitability”, generated through coupled positive and negative feedback regulation of the small GTPase Rho. Investigating the mechanisms that support and regulate cortical patterning is currently limited by a lack of technical approaches that can bridge our understanding of biochemical feedback signaling and cortical pattern formation, including the molecular

regulation of signaling molecules, membrane dynamics, and cytoskeletal remodeling. A breakthrough in this gap in knowledge has been the development of an “artificial cortex”, made from supported lipid bilayers (SLBs) and Xenopus egg extract, which successfully reconstitutes active Rho and F-actin dynamics in a cell-free system. This reconstituted system spontaneously develops two distinct types of self-organized cortical dynamics: singular excitable Rho and F-actin waves, and non-traveling oscillatory Rho and F-actin patches. Like in vivo cortical excitability, patterning in the artificial cortex depends on Rho activity and F-actin polymerization. We find that SLB fluidity directly influences the propensity for pattern formation in the artificial cortex, which suggests membrane dynamics regulate cortical patterning. Additionally, altering SLB composition impacts the organization and dynamics of excitable waves. These findings reveal that the cell cortex is a self-organizing structure and present a novel approach for investigating mechanisms of Rho-GTPase-mediated cortical dynamics.

S13.T3
Rachel Miller
Insights into Kidney Development and Disease: Perspectives from the Xenopus Model

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Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common cause of pediatric end-stage renal disease. Many CAKUT cases result from defects in the formation of nephrons, which are composed of epithelial tubules that are required for the proper function of the kidney. The fundamental mechanisms driving nephron development therefore underly the establishment of CAKUT. Through studies using the frog (Xenopus laevis) embryonic kidney, the pronephros, in vivo time-lapse imaging of the developing kidney has uncovered dynamic cell biological processes that govern junction formation in vivo. Furthermore, comparative single-cell mRNA analysis of functional embryonic nephrons in Xenopus demonstrates that the patterning of the nephron is highly conserved with that of mammalian metanephric nephrons. These studies evaluating the fundamental mechanisms driving pronephros development in conjunction with studies modeling the effects of CAKUT-associated human mutations on pronephric development support the utility of the frog embryonic kidney in understanding mechanisms underlying metanephric nephron development and CAKUT.

Studies of pronephros development have further implications in the formation of the genitourinary (GU) tract. The embryonic pronephros is the precursor to the mesonephric and metanephric kidney in mammals, and subsequent GU development is dependent upon this structure. Specifically, as the pronephros extends toward the cloaca, mesonephric nephrons form adjacent to the elongating nephric duct, also known as the Wolffian duct. The Wolffian duct is required for Müllerian duct elongation, and these ducts give rise to the male and female reproductive tract, respectively. The pleiotropic GU phenotypes are observed in CAKUT patients indicate that studies of the Xenopus pronephros have implications to both renal and GU development in mammals.

S13.T4
Gerhardt Schlosser
MARCKS and MARCKSL1 promote proliferation and neurite outgrowth during Xenopus spinal cord development and regeneration

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The myristoylated alanine-rich C-kinase substrate (MARCKS) and MARCKS-like 1 (MARCKSL1) were identified in a screen for proteins upregulated during Xenopus laevis spinal cord regeneration. We now show that marcks and marcksl1 are expressed in the spinal cord throughout embryonic development. Knockdown of MARCKS and MARCKSL1 using both CRISPR/Cas9 and morpholinos results in a significant reduction in neurite outgrowth, cell proliferation, and activity of Sox2+ neural progenitor cells, indicating that these proteins have essential and redundant functions during normal spinal cord development. Pharmacological activation and inhibition of various signaling pathways in MARCKS and MARCKSL1 CRISPR mutants suggests that the proteins may promote cell proliferation and neurite outgrowth through a PIP2-dependent mechanism that is inhibited by PKC and, thus, probably involves unphosphorylated forms of these proteins. However, targets of PKC-phosphorylation, which may include phosphorylated MARCKS/MARCKSL1, can also promote proliferation and neurite outgrowth through additional mechanisms.

Our study further indicates that MARCKS and MARCKSL1 are upregulated after spinal cord transection in tadpoles in parallel to a general increase in cell proliferation, Sox2+ neural progenitor cells and neurite regrowth. Following CRISPR-mediated knockdown of MARCKS and MARCKSL1, tadpoles show significant deficiencies in behavioural recovery, injury gap closure, proliferative response, and neural progenitor activation, indicating that MARCKS and MARCKSL1 are required for these processes during spinal cord regeneration.

Taken together, we provide evidence for essential roles of MARCKS and MARCKSL1 for neurite outgrowth and proliferation of neural progenitor cells during spinal cord development and regeneration, potentially opening new avenues for promoting spinal cord regeneration in humans.

Session 14:
Community Resources - Sponsored by Xenopus 1 and DSHB
Chairs: **Mustafa Khokha**, Yale Univ., USA and **Aaron Zorn**, Cincinnati Children's Hospital, USA

Reagents:

S14.T1
Doug Houston
Developmental Studies Hybridoma Bank (DSHB) Update
S14.T2
Todd Stukenberg
ORFeome Update

NICHD:

S14.T3
James Couloumbe
NICHD Opportunities

Stock Centers:

S14.T4
Marko Horb
National Xenopus Resource (NXR) Update
S14.T5
Matt Guille
European Xenopus Resource Centre (EXRC) Update
S14.T6
Hajime Ogino
Amphibian Research Center Update
S14.T7
Jaques Robert
Xenopus laevis Research Resource for Immunobiology (XLRRI) update

University of Rochester Medical Center, Department of Microbiology & Immunology, and Environmental Medicine.

With its fifth successful competing renewal, the XLRRI is entering in its 21 years of support by the NIH/NIAID funding for continuing to safeguard, promote and develope Xenopus laevis as a relevant experimental organism for fundamental and medical immunology. Over the last 4 years, we have distributed MHC-defined inbred or cloned animals and reagents (e.g., antibodies, cell lines, recombinant ranavirus) to many laboratories worldwide. We also provided technical assistance, training and hosting numerous investigators. We joined efforts with NXR to generate Xenopus transgenic lines deficient for important immune genes. We have also leveraged X. laevis as an sensitive and reliable comparative experimental systems for Immuntoxicology by investigating the overlooked risk for immune human health of exposure to water pollutants with endocrine disruption activity and microplastics; as well as for infectious diseases by studying chronic infection and pathogenesis of emerging nontuberculosis mycobacterium (NTM) pathogens (M. marinus and M. abscessus) infecting humans. Finally, XLRRI in collaboration with Xenbase and multiple investigators has led a major concerted effort to validate, define, improve the annotation and Xenbase pages of immune genes in the two Xenopus genomes.

S14.T8
Robert Weymouth
A collaboration-driven initiative for growth and sustainability of the Xenopus model organism

Genomes and Genomics:

S14.T9
Peter Vize
Xenbase Update
S14.T10
Taejoon Kwon
The community platform for Xenopus genomic resource

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Here I will present a recent update of the Xenbase GitHub repository, which organizes up-to-date gene annotation information like gene symbols, gene names, and protein IDs. Furthermore, it will provide the source codes and scripts used for genome analysis in the Xenbase team, such as the RNA-seq and ChIP-seq analysis pipeline and exercises of the Xenopus bioinformatics workshop. It will become a new platform for education on genomic resources and for collaboration who want to use Xenopus genomic resources in their research.

S14.T11
Aaron Zorn
Xenbase and the Alliance of Genomic Resources

Poster Presentation Abstracts

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#1
Abreu, Jose

Title: Anterior-posterior oriented cell division contributes to developing neural plate

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Affiliations:

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Abstract: Discoveries regarding Xenopus laevis early embryogenesis has contributed greatly to the understanding of neural induction in vertebrates. Our work aims to identify cellular behavior patterns that are specific to the forming neural plate in Xenopus laevis and therefore contribute to the ongoing dispute on how much notochord signal is needed for early neural induction. Using mutant embryos and light sheet microscope technologies we were able to follow the GFP nucleus at the non-involuting marginal zone (DNIMZ) during gastrulation and neurulation. Using the data generated by this analysis, we could draw a pattern of cell behavior regarding cellular division throughout the non-involuting marginal zone (NIMZ) of the embryo as it is transformed into neural ectoderm and epidermis. We found out that dorsal NIMZ (DNIMZ) holds up a high cellular division rate throughout a larger duration than the rest of NIMZ and that most of the cell divisions in DNIMZ are oriented along the animal/anterior-vegetal/posterior axis. This tendency is more intense at the DNIMZ half that is closer to the dorsal blastopore lip, indicating that the orientation signaling could come from the dorsal blastopore lip. We also showed that blocking cellular division affects the natural morphogenesis of the neural plate and the formation of the head. Finally, we propose a model in which A-P-oriented cell division within the epithelial posterior DNIMZ is responsible for translating the radial intercalation of deep DNIMZ cells into an A-P elongation.

Support: CNPq, CAPES and FAPERJ

#2
Adhikary, Babli

TRPV4-mediated mechanosensitive calcium signaling regulates repair of tight junction breaks in the Xenopus epithelium

Babli Adhikary, Sara Varadarajan, and Ann L. Miller

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Epithelial cells adhere to one another through cell-cell junctions and maintain a selectively permeable seal, despite significant cell shape changes during development and tissue homeostasis. Polarized epithelial cells possess tight junctions (TJs) that regulate paracellular permeability and adherens junctions (AJs) that promote cell-cell adhesion. As epithelial cells change shape, they exert forces on neighboring cells, often causing junction elongation and necessitating remodeling of these junction complexes to preserve barrier function. Previous studies from our lab have demonstrated that in response to local barrier leaks, localized and transient activation of the small GTPase RhoA, which we termed a "Rho flare," occurs via mechanosensitive calcium influx at the site of barrier disruption. This mechanism enables rapid repair and reinforcement of cell-cell junctions following damage. However, it remains unclear which mechanosensitive channel (MSC) responds to TJ breaks. In this study, we are investigating which candidate MSC is responsible for calcium influx necessary for sustained activation of Rho flares. Our initial focus was on Piezo1, but we found that knockdown of Piezo1 did not replicate the effects observed with the MSC blocker GsMTx4 (e.g., barrier function was affected with GsMTx4, but remained unaffected with Piezo1 knockdown). We have now shifted our focus to TRPV4, a MSC that interacts with cell-cell junctions and cytoskeletal proteins. TRPV4 is highly expressed in gastrula-stage Xenopus epithelial cells, and our findings demonstrate that TRPV4 is localized along the lateral cell-cell membrane. Preliminary data shows changes in TRPV4 localization upon laser-induced junction contraction, which induces TRPV4-decorated finger-like protrusions from the membrane. Furthermore, treatment with a TRPV4 antagonist results in a reduction of Rho flares induced by CN03 (a Rho activator), suggesting that TRPV4 may be needed for the Rho flare TJ repair mechanism. In work-in-progress, we are quantifying ZO-1 reinforcement at TJ break sites and performing a barrier function assay when TRPV4 is perturbed. Understanding the role of TRPV4 in TJ remodeling will contribute to the identification of signaling pathways regulating TJ repair and potential therapeutic targets for epithelial barrier disorders such as inflammatory bowel disease and polycystic kidney disorder.

#3
Afouda, Boni

Genome-wide transcriptomics analysis of genes regulated by GATA4, 5 and 6 during cardiomyogenesis

Boni A. Afouda, Adam T. Lynch, Eduardo de Paiva Alves, Stefan Hoppler

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Abstract: The transcription factors GATA4, GATA5 and GATA6 are key regulators of vertebrate heart muscle differentiation (cardiomyogenesis), but specific target genes regulated by these individual cardiogenic GATA factors remain unknown. We have identified genes that are specifically regulated by each of them, as well as those regulated by either of them using genome-wide transcriptomics analysis in Xenopus laevis. The genes regulated by gata4 are particularly interesting because GATA4 is able to induce differentiation of beating cardiomyocytes in Xenopus and in mammalian systems. Among the specifically gata4-regulated transcripts we identified two SoxF family members, sox7 and sox18. Experimental reinstatement of gata4 restores sox7 and sox18 expression, and loss of cardiomyocyte differentiation due to gata4 knockdown is partially restored by reinstating sox7 or sox18 expression, while (as previously reported) knockdown of sox7 or sox18 interferers with heart muscle formation. In order to test for conservation in mammalian cardiomyogenesis, we confirmed in mouse embryonic stem cells (ESCs) undergoing cardiomyogenesis that knockdown of Gata4 leads to reduced Sox7 (and Sox 18) expression and that Gata4 is also uniquely capable of promptly inducing Sox7 expression. Our genome-wide transcriptomics analysis therefore identifies an important and conserved gene regulatory axis from gata4 to the SoxF paralogs sox7 and sox18 and further to heart muscle cell differentiation. Our identification of genes that are differentially regulated by each of cardiogenic gata factors also provides a platform for future investigations on the gene regulatory network underpinning embryonic cardiomyogenesis.

#4
Alhomouz, Mahmoud

A role for retinoic acid in the initiation of gliogenesis in Xenopus laevis.

Mahmoud M. Alhomouz, Christina H. Ulrich, and Amy K. Sater

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Astrocytes play vital roles in the central nervous system, such as maintaining neuronal homeostasis, blood-brain-barrier modulation, and synapse regulation. During neural development, glial lineages typically arise later than neuronal lineages, and the capacity to initiate glial development is often referred to as the gliogenic switch. Explants of neural plate ectoderm isolated from midgastrula embryos (NP) will later express both neuronal and astroglial genes, while animal caps overexpressing noggin (NOG AC) will only initiate expression of neuronal genes. We have previously used these explant systems to show that overexpression of the transcription factors associated with the initiation of mammalian gliogenesis, Sox9 and Nfia, is insufficient to activate expression of the astroglial glutamate transporter glast (aka slc1a3) in animal ectoderm overexpressing noggin. Our finding that expression of glast is first detected in the anterior spinal cord suggested a relationship between anteroposterior regionalization and the initiation of gliogenesis. We tested the hypothesis that the anteroposterior patterning factor retinoic acid (RA) could regulate expression of glast. Treatment of late gastrula embryos with all-trans-RA led to upregulation of glast and sox9; in NOG AC, all-trans-RA elicited expression of glast, as well as a reduction in the expression of neurod1. Pharmacological inhibition of RA signaling using either DEAB or the more specific inhibitor AGN193109 in whole embryos led to increased NeuroD1 expression in whole embryos and decreased expression of both neuronal and astroglial-associated genes in NPs. Our findings suggest that RA signaling is a crucial regulator of astroglial development in X. laevis.

#5
Anderson, Carl

An Improved Methodology for Sperm Cryopreservation in Xenopus

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Cryopreservation of sperm from X. tropicalis and X. laevis improves the utility and availability of transgenic, mutant, and wild-type lines. Preserving distinct genetically modified lines through cryopreservation expands the ability of the National Xenopus Resource (NXR) to generate new lines by reducing demand for space. Using new methods developed in collaboration with the Aquatic Germplasm and Genetic Resources Center (AGGRC), we analyze Xenopus sperm to assess concentration, viability, and motility, along with testing fertilization rate. Prior to our shift in practices focusing on early process quality control and correction, we saw greater variation in sample quality, and a bottleneck resulting from testing fertilization rate. Changes to equipment, including the addition of a controlled-rate freezer, use of French straws, and a simplified cryoprotectant media, allow us to closely control crucial stages of the cryopreservation process. By adjusting samples prior to freezing based on these assessment tools we can ensure consistency and efficacy for users. Differences we have identified in sperm concentration between individuals further reinforces the importance of assessment metrics. Through use of these tools, we increase throughput by eliminating time spent producing and testing samples with subpar concentration, low viability, or poor motility. With the incorporation of these new practices, the Xenopus community will have increased access to cryopreserved lines, greater success using cryopreserved samples, and improved ability to generate new lines at the NXR. Steps we have taken to improve our cryopreservation program are a necessary part of addressing the ever-expanding demand of Xenopus as a research model.

#6
Aslam, Faiza

Abca2 depletion associated with gastrulation and neurological defects in Xenopus tropicalis

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ABCA2 is a member of membrane associated transport proteins known to transport molecules across extracellular and intracellular membranes. ABCA2 encoded protein, highly expressed in the brain tissue, maintains the homoeostasis of lipids, especially sphingolipids, and sterols. Abca2 knockout mice have neurological defects due to aberrant myelination. We identified recessively inherited frameshift variants of ABCA2 in individuals of consanguineous families with neurological disease. Patients presented with developmental delay, intellectual disabilities, ataxia and seizures. In order to understand the molecular mechanism of ABCA2, we depleted Abca2 in Xenopus tropicalis (X. tropicalis) by targeting the gene with CRISPR Cas9 using three nonoverlapping guide RNAs. Depletion of Abca2 caused severe gastrulation defects. Embryos that survived past gastrulation had motility problems in later embryonic stages. We assayed motility in X. tropicalis Abca2 depleted tadpoles by stimulating stage 45 tadpoles near the head and measuring the distance traveled. Spontaneous seizures were also observed in the form of a ‘C’ shaped curve in the tails of embryonic stage 45+ tadpoles. Human wild-type ABCA2 overexpression rescued the gastrulation, motility and seizure phenotypes in Abca2 depleted embryos but patient variants failed to do so, verifying the detrimental nature of the variants. Hence, X. tropicalis knockout of Abca2 recapitulated the human phenotypes and also demonstrated an important role of the gene in early development. This work was supported by NIH/NICHD (R01hd102186) and IRSIP funding to FA from HEC, Pakistan.

#7
Berger, Florian

A testis ex vivo model to study and interfere with Xenopus laevis sperm epigenetic programming

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Sperm deliver to the embryos epigenetic information contributing to embryonic development (Teperek et al. 2016; Siklenka et al. 2015). However, the sperm epigenetic cues involved in this process are poorly defined. To better understand how sperm is epigenetically programmed for development we are combining scRNA-seq and RNA FISH approach to: (i) accurately characterize spermatogenesis intermediates and accessory cell types in Xenopus laevis testis and (ii) Identify chromatin pathways associated with male germ cells transition towards mature sperm. We use this in vivo spermatogenesis cell atlas as a benchmark to evaluate spermatogenesis in ex-vivo cellular explant (Risley et al. 1987). We aim to evaluate how spermatogenesis progress in such explant by tracking appearance of spermatogenesis marker from labelled progenitors. Once validated we plan to use this ex-vivo assay to interfere with sperm epigenetic programming and evaluate consequences on embryos development.

#8
Bowden, Sarah

Epigenetic integration of signaling to determine transcriptional outcome

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During development, cell fate decisions are determined by a handful of signaling pathways. As these pathways are reiteratively employed during development in different tissues, there must be an additional measure to regulate the use of these same signaling pathways to induce alternative transcriptional outcomes.
Signaling pathways can be induced in alternative fashions at temporal, cell, and transcriptional levels. One means of regulating signaling pathways is the transcriptional co- repressor Groucho. Groucho binds to the signaling pathways such as Wnt and Notch, to maintain gene repression in the absence of expression. As a secondary means of repression, it also can recruit HDACs, which cause the condensation of chromatin at these loci and therefore long-term repression of target genes. This means that Groucho has the capacity to modify which genes in a cell can respond to signaling and thus modifies the decisions that can be made by a cell.
To assess the effect Groucho plays on developmental pathways, we must first discover what is accessible. Using mucociliary epithelium as a model tissue for this mechanism, we perform a temporal study of accessibility using ATAC-seq, to demonstrate that chromatin is dynamic over time. We then additionally employ in silico analyses to demonstrate regions of accessibility within the genome that contribute to specific developmental processes, and how the chromatin changes allow for developmental decisions during specification and development of this tissue.

#9
Chomchai, Dominic

Positive Feedback is Essential for Rho Amplification in Cortical Excitability

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Recent studies have shown that the cell cortex – the plasma membrane and the cytoskeleton-rich layer of cytoplasm just beneath it – acts as an excitable medium. During cytokinesis, waves of Rho GTPase activity and F-actin assembly and disassembly are concentrated in the equatorial cortex, directing the formation of the contractile ring. These dynamics are termed cortical excitability and are proposed to result from an excitable circuit of positive and negative feedback loops. While positive feedback is suggested to depend on the Rho GEF Ect2, negative feedback relies on the Rho GAP RGA-3/4.
Modeling of cortical excitability predicts that positive feedback is essential for Rho wave generation, but this has yet to be directly tested in vivo. To test the predictions of the model, we used immature Xenopus laevis oocytes as an in vivo model for cortical excitability. We found that two Rho GEFs capable of positive feedback, GEF-H1 and Net1, can support cortical excitability in place of Ect2, whereas two others that lack positive feedback capacity did not. We also found that Rho wave generation is all-or-none; cells with low expression of Ect2 generated waves with the same characteristics as those with high expression, and there is a threshold of ~50ng/uL Ect2 expression necessary to produce Rho waves. Additionally, we found that Ect2 modified to lack the capacity to participate in positive feedback did not support cortical excitability.
Together, these findings provide direct in vivo evidence that Rho amplification by positive feedback is a feature of cortical excitability.

#10
Coppenrath, Kelsey

Xenopus models to study genetic function in human pathology

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The Xenopus Mutant Resource (XMR) provides an extensive genetic library for the scientific community to conduct tractable experiments on gene function and the development of human diseases. A recent collaborative publication demonstrated that one such gene, wnt11b in X. laevis, is required maternally for proper axis formation and zygotically to maintain left-right asymmetry. In X. tropicalis, we have recently characterized a null mutant line six1, a gene of which has been associated with the human disease Branchio-oto-renal (BOR) syndrome. Mutations induced in the protein- protein interacting domain (SD) result in tadpoles with limited expression patterns of placode genes and craniofacial defects compared to six1 heterozygous tadpoles. Toxicology studies on X. tropicalis Aryl Hydrocarbon Receptor-null (ahr-/-) tadpoles were utilized in experiments assessing the exposure effects of dioxin-like compounds on thyroid hormone activity and metamorphosis. Additionally, we are currently developing knock-out mutant X. tropicalis lines such as pigp, ripply3, and cbs, which are located along chromosome 2 and syntenic to human chromosome 21. Studies conducted on these genes allow us to compare and relate potential phenotypes to human diseases, including Down Syndrome. Here we present our work in generating knockout mutant lines to study a disease phenotype in various tissues.

#11
Davilavaladez, Alejandra

Role of p53 in Kidney Development: Modeling Renal Anomalies of Li-Fraumeni Patients

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Li-Fraumeni syndrome arises from gene mutations in p53, a crucial tumor suppressor gene. These mutations increase patient susceptibility to multiple types of cancers. Prior studies in mice have indicated that p53 is involved in kidney development. However, there is no confirmed link between p53 mutations in Li-Fraumeni patients and kidney abnormalities. However, preliminary MRI data indicate that these patients have an increased prevalence of urogenital abnormalities as compared with the general public. My objective is to use Xenopus laevis embryos to investigate the role of p53 in kidney development. I will do this by first determining the spatiotemporal expression of p53 using hybridization chain reaction (HCR) to show p53's subcellular expression as well as whole mount in situ to evaluate the tissue level expression of p53 in developing kidneys. We analyzed the expression of p53 by whole-mount in situ and HCR and found that p53 is expressed in the pronephric kidney, brain, otic vesicle, neural tube, and epithelial cells. Furthermore, our preliminary examination indicates that p53 mutations in Li-Fraumeni patients cause renal anomalies. Ultimately, these experiments will enrich our understanding of p53's overall influence on nephron development and how p53 mutations in Li-Fraumeni patients influence this process.

#12
Diab, Nicholas

An ancient cation channel affects cardiac patterning in Xenopus tropicalis

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Congenital heart disease (CHD) is the most prevalent congenital defect and arises from genetic and environmental triggers that push cardiac development off its normal course. Genomic screens of patient cohorts have revealed many candidate CHD genes and follow up studies in model organisms like Xenopus tropicalis (X. Tropicalis) can validate the disease relevance of candidates and assess their developmental functions. X. tropicalis has been particularly useful in defining mechanisms for cardiac heterotaxy, a form of CHD characterized by abnormal asymmetry of the heart in relation to the Left-Right (LR) axis. Golgi anti-apoptotic protein (GAAP) has emerged as a candidate CHD/heterotaxy gene from human genetic screens and multiple patients to-date have been identified with predicted damaging mutations in GAAP. GAAP is a 6-7 pass transmembrane protein that can act as a cation channel and protect cells from apoptotic stimuli. GAAP orthologues occur across evolutionarily distant groups including viruses, vertebrates, plants, and bacteria. The conservation of GAAP at the amino acid level between viruses, humans, and amphibians is ~ 70% and is uncharacteristic compared to the typical conservation of proteins from these groups, positing GAAP as an exciting CHD candidate. We sought to identify the role of GAAP in cardiac left-right patterning and reconstitute the human phenotypes of GAAP depletion in X. Tropicalis. Moreover, we report defects at gastrulation and during stages of the left-right patterning pathway. Moreover, we demonstrate that exogenous human and viral GAAP rescue cardiac phenotypes of GAAP depletion in frogs, suggesting evolutionary conservation of GAAP functions.

#13
Diarra, Salimata

AP2A2 mutation in Malian family causes early-onset hereditary spastic paraplegia with epilepsy and defective clathrin-mediated endocytosis

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Abstract

Hereditary spastic paraplegia (HSP) comprises a large group of neurogenetic disorders characterized by lower extremity spasticity. We identified a variant in a novel gene, AP2A2, in a Malian family with early-onset HSP. Three children with unaffected consanguineous parents presented with symptoms consistent with complicated HSP. Neurological exam found lower limb weakness, equine feet, spastic gait, seizures, and cognitive decline. Brain MRI studies showed thin corpus callosum with cortical and spinal cord atrophy, and EEG detected slow background in proband. WES identified a homozygous missense variant in the adaptor protein (AP) complex 2 alpha-2 subunit (AP2A2). Variant segregated with the disease phenotype and wasn't present in SNP databases. Taken together, the variant in AP2A2 is classified as pathogenic according to ACMG recommendations. Mutation of AP2A2 has not previously been associated with disease, but the protein is a member of the AP complex family known to be implicated in other forms of HSP. The AP2A2 protein is highly expressed in brain and spinal cord and serves a pivotal role in clathrin-mediated endocytosis. Western blot analysis showed reduced levels of AP2A2 in patient-iNeuron cells. Similarly, endocytosis of transferrin receptor (TfR) was decreased in patient iNeurons. In addition, we observed increased axon initial segment length in patient-iNeurons. Xenopus tropicalis with AP2A2 knockout showed progressive seizures. Immunoprecipitation of the mutant AP-2-appendage alpha-C construct showed defective binding to accessory proteins including AP180, auxilin, amphiphysin and EPS15.We report AP2A2 as a novel genetic entity associated with HSP and provide functional data in patient-iNeurons and a frog model.

Key words: Hereditary spastic paraplegia, AP2A2, endocytosis, Xenopus tropicalis, Mali.

#14
El Mir, Joudi

Modeling Xenopus laevis for studying the pigmentary abnormalities behind xeroderma pigmentosum type C

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Xeroderma pigmentosum type C (XP-C) is a rare autosomal recessive disorder, characterized by an extreme sensitivity to ultraviolet B rays (UVB), leading to photoaging, macules, and skin cancer. UVB can affect genomic DNA by creating cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). These lesions are mainly repaired by the nucleotide excision repair (NER) system, however, photolyase enzymes activated by blue light are also known to perform this function in Xenopus. Our main goal was to validate the use of Xenopus laevis as an in vivo model for investigating the impact of UVB, the main cause of XP-C clinical features, on skin physiology. The mRNA expression levels of xpc and six other NER genes and CPD/6–4PP photolyases were found at all stages of embryonic development and in all adult tissues tested. When examining embryos at different time points after UVB irradiation, we observed a gradual decrease in CPD levels, increased number of apoptotic cells, epidermal thickening, and increased dendricity of melanocytes. Quick removal of CPDs when embryos are exposed to blue light versus in the dark has also been witnessed, confirming the efficient activation of photolyases. A decrease in the number of apoptotic cells and an accelerated return to normal proliferation rate was noted in blue light-exposed embryos compared with their control counterparts. This mimics the human skin responses to UVB and support Xenopus as an appropriate and alternative model for such studies. Additionally, XPC deficient Xenopus embryos showed interesting observations related to the clinical features of XP-C.

#15
Engelhardt, Magdalena

Temporal Notch signaling regulates mucociliary cell fates through Hes-mediated competitive de-repression

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Tissue functions are determined by the types and ratios of cells present, but little is known about self-organizing principles establishing correct cell type compositions. Mucociliary airway clearance relies on the correct balance between secretory and ciliated cells, which is regulated by Notch signaling across mucociliary systems. Using the Xenopus epidermis model, we investigate how cell fates depend on signaling, how signaling levels are controlled, and how Hes transcription factors regulate cell fates. We show that four mucociliary cell types each require different Notch levels and that they are specified sequentially by a temporal Notch gradient. We describe a novel role for Foxi1 in the generation of Delta-expressing multipotent progenitors through Hes7.1. Hes7.1 is a weak repressor of mucociliary genes and overcomes maternal repression by the strong repressor Hes2 to initiate mucociliary development. Increasing Notch signaling then inhibits Hes7.1 and activates first Hes4, then Hes5.10, which selectively repress cell fates. We have uncovered a self-organization mechanism of mucociliary cell type composition by competitive de-repression of cell fates by a set of differentially acting repressors. We also model this process mathematically to recapitulate cell ratio regulation in silico.

#16
Estiri, Bahareh

Context-dependent effects of Eya1 in sensory neurogenesis

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Eya1 plays important roles for the development of sensory neurons acting as a cofactor of transcription factor Six1. Previous studies have established a context-dependent role of the Eya1/Six1 complex in cranial sensory neurogenesis with high levels promoting proliferation and low levels promoting differentiation. The main goal of this study is to clarify how Eya1 exerts its context-specific functions in progenitor cells versus differentiated sensory neurons. In a first step, we are currently identifying the target genes of Eya1 specific for progenitors and differentiating sensory neurons using RNA-Seq. We use specific promoter-GFP constructs (e.g. with promoters for Neurog1 and NTubulin) to selectively drive GFP expression in either progenitors or differentiated sensory neurons of Xenopus laevis embryos. Promoter-GFP constructs are generated by pTransgenesis and are integrated into the genome using I-SceI meganuclease. Overexpression of Eya1 in embryos transgenic for the different promoter-GFP constructs followed by FACS-sorting of GFP-positive cells and RNA sequencing allows us to specifically identify Eya1 target genes in progenitor cells versus differentiating neurons. In a second step, we will further characterize these target genes in gain and loss of function studies. This approach will help us to elucidate the context-specific role of Eya1 in progenitors and differentiating neurons during the development of sensory organs. Our study also promises new insights into the etiology of sensorineural disorders in human patients after mutations in Eya1 (e.g. Branchiootorenal syndrome).

#17
Ferrer, Alexander

Title: The Transcriptional Analysis of Response to Focal Impact Injury in the Xenopus tadpole midbrain

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We seek to develop the Xenopus tadpole as a scalable model for investigation and therapeutic discovery for Traumatic Brain Injury (TBI). As a proof-of-concept, we have shown that tamoxifen (TMX), a Selective Estrogen Receptor Modulator that is neuroprotective in mammalian spinal cord injury, promotes recovery from focal impact injury in our TBI model, based on cellular and behavioral assays. We carried out transcriptome profiling to evaluate the time course of transcriptional responses to Focal Impact Injury and the effects of tamoxifen in the tadpole midbrain. Metamorphic tadpoles at st. 56 were subjected to either focal impact injury to the dorsolateral midbrain or sham treatment, and injured tadpoles were injected intraventricularly with either TMX or ethanol (vehicle). Tadpoles were allowed to recover over a 7-day period, and euthanized for retrieval of midbrains at 3 hours, 24 hours, 48 hours, and 7 days following injury. Midbrain RNA was isolated for library preparation and examined via bulk transcriptome sequencing, followed by analysis with a pipeline incorporating Kallisto and DESeq2. The most extensive differences were observed at 3 hours and at 7 days. Targets of injury-dependent transcriptional changes included genes involved in inflammation, phagocytosis, and DNA repair. Notably, TMX treatment led to rapid upregulation of c-fos and jun family members and selected cytokines, as well as reductions in the expression of sphingosine-1-phosphate receptor 1 (s1pr1) and key inflammation pathway components. Ongoing studies will investigate the mechanisms by which TMX mediates neural repair in the Xenopus midbrain.

#18
Hendrickson, Clark

Clark Hendrickson, Ken Cho Lab

Maternal Foxi2 and Sox3 control over early ectoderm gene programming and establishing epigenetic states

During embryogenesis, maternal transcription factors (TFs) occupy cis-regulatory modules (CRMs) which activate zygotic gene expression through interplay with histone modifications and the chromatin architecture. Although maternal TFs governing mesendoderm specification have been characterized, analogous TFs controlling early ectoderm specification aren't well known. Previous work demonstrates that Foxi2 and Sox3 are animally graded maternal TFs involved in ectoderm gene regulation, thus we hypothesized their cooperation in early epidermal and neuroectoderm specification. We show that Sox3 and Foxi2 complex dynamically at target sequences in both cis-regulatory modules (CRMs) and promoters to activate zygotic gene expression. We demonstrate their direct co-activation of animal cap gene targets influencing neural progenitor formation and epidermal specification. Each factor independently represses unique mesendodermal targets, highlighting their role as dual-function TFs in the ectoderm. In regions of open chromatin, we find that Ep300 colocalizes with Foxi2 and Sox3 in co-regulated target gene CRMs. These CRMs overlap with significant animal cap specific enrichment of H3K4me1 and H3K27ac, suggesting Fox/Sox interplay with the establishment of the epigenetic state. Finally, early gastrula single nuclei sequencing analysis demonstrates Foxi2 and Sox3 target gene expression localized to unique sub-clusters of epidermis and neural ectoderm, revealing maternal Fox/Sox control over ectoderm bifurcation at the highest resolution. Taken together, we propose a model where localized maternal TFs bind to ectodermal CRMs, establish the epigenetic state and influence early ectoderm specification.

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#19
Hilton, Moira

Identifying genomic loci that support species-specific male advertisement calls in L clade Xenopus species: laevis, petersii, powerii, victorianus.

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In each Xenopus species, adult males produce an advertisement call that attracts gravid females. The combination of temporal (sound pulse rate and pattern) and spectral (sound frequencies or pitches) in each pulse provides a unique species ID (Tobias et al., 2011). These acoustic features are heritable and thus genetically determined. The CNS circuit that produces vocal patterns has been mapped (Kelley et al., 2020). A specific neuronal cell type -the FTN or past trill neuron- in the hindbrain Parabrachial Nucleus controls sound rhythms (Barkan et al., 2018; Rhodes et al, 2007). Sound pulse spectral features are produced entirely by the larynx (Kwong-Brown et al., 2019). To explore genetic architectures that support innate neural architectures as well as laryngeal sound pulse features, during the recent pandemic we generated several thousand F2 (laevis/petersii) male and female hybrids; now adult. To perform QTL analyses of genomic loci that support the production and perception of innate vocal communication we recorded male advertisement calls (with replication) at sexual maturity and are quantifying female acoustic preferences.

#20
Hoachlander-Hobby, Lila

Cdc42 Signaling Confines Rho Activity in Single Cell Wound Repair

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Single cell wound repair is conserved among eukaryotes and crucial for survival, yet there is much we do not know about the molecular mechanisms involved. Repair occurs in two steps initiated by Ca2+ influx through the wound: 1) intracellular vesicles fuse with each other and the plasma membrane (PM) to patch the wound and 2) a Rho GTPase signaling cascade restores the cortical cytoskeleton and expels the patch. We investigate this Rho GTPase signaling by laser wounding Xenopus laevis oocytes and visualizing repair via confocal microscopy. At least two Rho GTPases, Cdc42 and Rho, are activated around wounds to direct formation of an actomyosin contractile array that closes the wound. Active Cdc42 and Rho form concentric activity zones, with Cdc42 circumscribing Rho, which is confined to the wound edge through an unknown mechanism. I hypothesize that Cdc42 signaling, via N-WASP, Arp2/3, F-actin, and cortactin, recruits P190 RhoGAP to inactivate Rho in the Cdc42 zone, thereby confining active Rho to the wound edge. To test this hypothesis, I use the C2 domain of PKCbeta, which binds to Ca2+ and PM phosphatidylserine to recruit signaling modulators to wounds in a spatiotemporally controlled manner. I found that cortactin and P190 are recruited to the Cdc42 zone in a Cdc42-dependent manner, and that P190 is necessary for confining active Rho to the wound edge. I will investigate the role of N-WASP, Arp2/3, and F-actin in this pathway to develop a full picture of how Cdc42 signaling confines Rho activity in single cell wound repair.

#21
Hoeren, Josefine

The endocytic receptor Lrp2 orchestrates apical constriction and cell polarity to drive cranial neural tube closure

Neural tube closure defects (NTDs) are common malformations affecting embryogenesis. Impaired morphogenetic movements such as cell intercalation or apical constriction can cause NTDs. Convergent extension (CE) is the dominant morphogenetic movement of caudal neurulation. It requires cell polarity mediated by the planar cell polarity (PCP) pathway and is brought about by mediolateral intercalation of neural plate cells. Apical constriction (AC), on the other hand, creates hinge points that facilitate neural fold elevation and is essential for neural tube closure in the cranial region.

Cranial NTDs occur in human patients with pathogenic variants of the endocytic receptor Lrp2. Using Xenopus laevis and mice, we have shown that Lrp2 is essential for neural fold elevation and neural tube closure in the forebrain area. Lrp2-mediated endocytosis drives AC by interacting with scaffold proteins and removing excess apical membrane in constricting cells. At the same time, Lrp2 is required for cell polarity. Despite the lack of PCP-mediated CE in the cranial region, cells exhibit planar polarity involving the core PCP protein Vangl2. During cranial neurulation, the subcellular localization of the Vangl2 changes dynamically. Lrp2 regulates Vangl2 localization in a temporospatial manner, suggesting a direct interaction between the two proteins. Lrp2 and Vangl2 both contain a C-terminal PDZ-binding domain (PBD). Since PBD-containing proteins often form multiprotein complexes coupled by PDZ domain- containing scaffold proteins, the functional interaction between Lrp2 and Vangl2 may be mediated by such a complex.
To test this hypothesis, CRISPR/Cas9 was used to either induce loss of the entire protein or to eliminate the C-terminal PBD of lrp2. Loss of the entire protein, either using a translation blocking morpholino oligomer or N-terminal CRISPR/Cas9, impaired AC and disturbed dynamic cell polarization. CRISPR/Cas9-mediated deletion of the C-terminal PBD of Lrp2, however, increased AC while cell polarization was unaffected. Taken together, these results suggest that protein interactions via the C-terminal PBD of Lrp2 drive scaffold accumulation to regulate AC. The C-terminal PBD is dispensable for cell polarization, suggesting that the influence of Lrp2 on PCP-dependent cell polarity must be mediated by additional motifs in the intracellular domain of Lrp2. Our preliminary functional approaches suggest that an additional PBD acts as a regulator of cell polarity.

#22
Horn, Melanie

Non-invasive, scalable and automatic genotyping of Xenopus embryos

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The generation of different knock-out lines is an important method to model inherited diseases or study the influence of proteins on development. For efficient colony management it is crucial to identify transgenic animals in early stages to be able to only raise the necessary animals with a desired genotype to adulthood. Since most established genotyping methods either use invasive methods to collect tissue, are only applicable on older animals or are very time and labor intensive, we here describe a protocol to extract DNA from living Xenopus tropicalis embryos from stage 20 on via limited digestion with proteinase K. Despite the sub-optimal reaction temperature for proteinase K, the obtained DNA concentrations are sufficient for following high resolution melting analysis (HRMA) or Sanger sequencing of PCR amplicons. The survival rate of the tadpoles is very high (98%) and no morphological changes of the epidermis or ciliogenesis by the low proteinase K concentration could be detected. To make the protocol more efficient and less error-prone we adapted it to be performed on a robotic pipetting platform. This method offers a genotyping protocol which is cost-effective, fast, scalable and can be performed at early embryonic stages and therefore provides an alternative to other DNA extraction methods for higher throughput genetic analysis.

#23
Huffstetler, Carley

In vivo and in silico left-right asymmetries in ECM shape stomach curvature.

Carley Huffstetler 1,2, Julio Belmonte 1,4, and Nanette Nascone-Yoder 1,2,3

Embryonic left-right (L-R) asymmetry sculpts the proper anatomy of many organs; thus, laterality-related birth defects are often catastrophic. Understanding the morphogenetic mechanisms that shape simple L-R asymmetries, such as the leftward curvature of the stomach, can provide etiological insights into such defects. We previously established that conserved LR patterning cues orchestrate cell rearrangements in the left stomach endoderm to promote proper curvature. However, the stomach is comprised of multiple tissue layers, including mesoderm and extracellular matrix (ECM), and the potential influence of these additional tissue layers on curvature morphogenesis is unknown.
To investigate the potential role of ECM in stomach curvature, we conducted immunohistochemical analyses on sections of the Xenopus stomach at stages before, during, and after stomach curvature. On the left (i.e., convex) side of the stomach, fibronectin (FN) fibrils become aligned and localized within a compact basement membrane between the endoderm and mesoderm layers. However, on the right side of the stomach, FN is broadly distributed and disorganized, and forms a concavity with an irregular endoderm-mesoderm border. Importantly, these FN asymmetries are reversed or bilateral in embryos with experimentally-induced stomach curvature defects, indicating that LR asymmetric ECM distribution patterns locally influence organ topology.
We hypothesized that L-R differences in FN generate distinct left vs right tissue topologies by modulating interfacial tension between endoderm and mesoderm on the contralateral sides of the stomach. To directly test this idea, we developed a unique, multi-layer, multi-scale computational model of stomach curvature. In silico simulations confirm that L-R asymmetries in ECM surface tension can mimic in vivo morphologies. Overall, our results indicate that the morphogenetic events that shape stomach curvature involve complex, interdependent L-R asymmetries in multiple tissue layers, and highlight the power of combining in vivo experimentation with in silico modeling.

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#24
Jevitt, Allison

Studying Chromosome Cohesion in Real-Time in the Early Xenopus Embryo

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In metazoans, the cohesin complex plays an essential role in maintaining proper genome stability, gene expression, and development. Although in-vitro and cell culture methods have contributed to our understanding of cohesin regulation, they have limitations in replicating relevant physiological conditions that involve tissue architecture, cellular forces, and differentiation. The Xenopus embryo serves as a uniquely tractable model system for studying chromosome dynamics and cohesion in real-time, allowing us to assess the impact of chromosome cohesion regulation on cell cycle progression and development. We used expression of fluorescently labeled histones to visualize chromosome dynamics using time-lapse confocal imaging of live embryos, which enabled us to observe chromatin condensation, sister chromatid cohesion, and chromosome movements during early development. To validate this approach, we are targeting known regulators of the cohesin complex which allows us to assess the impact of cohesion strength on cell cycle progression and developmental outcomes. Our goal is to exploit the power of live imaging techniques to elucidate the impacts of altered cohesion in the cellular context in the early Xenopus embryo. We are developing a valuable toolkit for future work to identify mechanisms regulating chromosome cohesion in a physiological context, offering insights into the fundamental processes that govern accurate chromosome segregation and genome stability during development.

#25
Jiang, Eythan

Identfiying genomic loci that support species-specific male advertisement calls in L clade Xenopus species: laevis, petersii, powerii, victorianus.

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In each Xenopus species, adult males produce an advertisement call that attracts gravid females. The combination of temporal (sound pulse rate and pattern) and spectral (sound frequencies or pitches) in each pulse provides a unique species ID (Tobias et al., 2011). These acoustic features are heritable and thus genetically determined. The CNS circuit that produces vocal patterns has been mapped (Kelley et al., 2020). A specific neuronal cell type -the FTN or past trill neuron- in the hindbrain Parabrachial Nucleus controls sound rhythms (Barkan et al., 2018; Rhodes et al, 2007). Sound pulse spectral features are produced entirely by the larynx (Kwong-Brown et al., 2019). To explore genetic architectures that support innate neural architectures as well as laryngeal sound pulse features, during the recent pandemic we generated several thousand F2 (laevis/petersii) male and female hybrids; now adult. To perform QTL analyses of genomic loci that support the production and perception of innate vocal communication we recorded male advertisement calls (with replication) at sexual maturity and are quantifying female acoustic preferences.

#26
Jin, Minjun

Mechanisms underlying the cell cycle termination in cycling Xenopus extracts

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Xenopus extracts have been extensively utilized as a powerful in vitro model system for studying cell cycles for several decades. In previous studies, we have successfully reconstituted mitotic oscillations in microfluidic droplet cells using cycling Xenopus extracts. However, it was observed that these self-sustaining oscillators do not persist forever. Moreover, variations in the duration of oscillations were observed among extracts prepared from different frogs on different days: some extracts maintained oscillations for over two days, while others ceased after only a few cycles, arresting at a low level of CDK1 steady state. These differences among extracts indicate the presence of inherent factors that may regulate the termination of oscillations.

I explored a few hypotheses to explain these observations. First of all, considering the importance of cyclin B mRNA in the cell cycles and its depletion after the mid-blastula transition (MBT), it is reasonable to assume that there exists a concentration threshold of cyclin B mRNA, below which the oscillation halts. Using standard PCR, I have observed a gradual decline in cyclin B mRNA levels over time and variations in initial mRNA levels among extracts from different frogs. To further substantiate this hypothesis, I will quantitatively validate it using RT-qPCR or RNA-sequencing. Another possible explanation for the differences in termination time is the activation of the apoptotic cascade. I plan to employ the Caspase-3/7 probes to examine the onset of apoptosis. To establish a causal relationship, I will also induce apoptosis in the extracts at various time points. Additionally, I have found that the introduction of ATP slows down the protein aggregation when extracts die. By tuning ATP, we also observed the increase of lifespan of oscillations. Further investigation is required to elucidate the specific mechanism involved. Addressing these hypotheses will help us understand the factors influencing the longevity of mitotic oscillations in extracts.

#27
Kaneshiama, Toki

Mechanical force is necessary for neural crest specification in Xenopus

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Abstract

In vertebrate development, fertilized eggs divide into smaller cells, and these cells are specified into ectoderm, mesoderm, and endoderm. Ectoderm is specified into neural plate (NP), neural plate border (NPB), and epidermis. Although such tissue patterning is thought to be achieved by concentration gradients of secreted molecules including morphogens, it has been revealed, mainly by in-vitro analysis, that mechanical force can regulate cell specification. During in-vivo patterning, cells deform and migrate, and this applies mechanical force to surrounding tissues, shaping the embryo. However, the role of minute in-vivo force for cell specification is largely unknown.

In this study, we demonstrated that there is a tension gradient in lateral direction of ectoderm during Xenopus early neurula stage, and NP and NPB are under greater tension than epidermis, with an aspiration assay and atomic force microscopy. Ectopically applied force by cytoskeletal contraction laterally expanded the neural crest (NC) region, a derivative of NPB, whereas force relaxation by myosin inhibition or phospholipid scrambling suppressed it. In addition, force application activated both FGF and Wnt pathways, which are required for NC specification. Taken together, mechanical force is necessary for NC specification to regulate signaling pathways.

Furthermore, molecule-dependent signals specify NP, and NP generates mechanical force on adjacent tissue, NPB, with its closure, and this force locally activates signals, possibly specifying NC only in a narrow zone. This mechanical regulation can be employed in other developmental processes that have region-specific force.

#28
Kho, Mary

Coordinated regulation of Cdc42ep1, actin, and septin filaments during neural crest cell migration.

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The septin cytoskeleton has been demonstrated to interact with other cytoskeletal components to regulate various cellular processes, including cell migration. However, the mechanisms of how septin regulates cell migration are not fully understood. In this study, we use the highly migratory neural crest cells of frog embryos to examine the role of septin filaments in cell migration. We found that septin filaments are required for the proper migration of neural crest cells by controlling both the speed and the direction of cell migration. We further determined that septin filaments regulate these features of cell migration by interacting with actin stress fibers. In neural crest cells, septin filaments coalign with actin stress fibers, and the loss of septin filaments leads to impaired stability and contractility of actin stress fibers. In addition, we showed that a partial loss of septin filaments leads to drastic changes in the orientations of newly formed actin stress fibers, suggesting that septin filaments help maintain the persistent orientation of actin stress fibers during directed cell migration. Lastly, our study revealed that these activities of septin filaments depend on Cdc42ep1, which colocalizes with septin filaments in the center of neural crest cells. Cdc42ep1 interacts with septin filaments in a reciprocal manner, with septin filaments recruiting Cdc42ep1 to the cell center and Cdc42ep1 supporting the formation of s septin filaments.

#29
Kim, Songeun

Structure and function analysis of RGA-3/4, a cytokine c Rho GAP

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Traditionally, cytokinesis has been viewed as essentially linear, with Rho being activated in the beginning of the process to drive contractile ring formation, and then inactivated once cytokinesis is complete. However, propagating waves of Rho activation and inactivation and complementary waves of F-actin assembly and disassembly - referred to as cortical excitability - are focused at the equatorial cortex during cytokinesis in amphibian and echinoderm embryos, indicating that Rho is undergoing continual flux through the GTPase cycle. Cortical excitability is proposed to result from Ect2-dependent Rho activation coupled with delayed F-actin-dependent Rho inactivation. RGA-3/4, a GTPase activating protein (GAP) for RhoA, is an excellent candidate as a link between F-actin and Rho inactivation during cortical excitability. RGA-3/4 is required for cytokinesis and gets recruited to the equatorial cortex prior to the onset of furrowing. It colocalizes with F- actin in the cytokinetic apparatus and depends on F-actin for its cortical localization. In spite of its importance in cytokinesis and implication in cortical excitability, the mechanism to how RGA-3/4 interacts with F-actin and how it is being regulated is completely unknown. To further our knowledge about RGA-3/4, we have performed structure and function analysis. RGA-3/4, when expressed alone in Xenopus oocytes, localizes to and reorganizes the cortical actin network into interlocking cables. When co-expressed with Ect2 in Xenopus oocytes, RGA-3/4 induces cortical excitability. Sites of RGA-3/4 important for F-actin reorganization and cortical excitability have been characterized. Further study about RGA-3/4 will ultimately help answer how Rho is being regulated during cytokinesis.

#30
Kinnear, Mila

Mila Kinnear, Stephen Viviano, Engin Deniz

Mapping the ciliary ependymal surfaces in the developing Xenopus tropicalis brain
Yale University, USA

Cerebrospinal fluid (CSF) is an ultrafiltrate of plasma filling the brain ventricles, subarachnoid space, and spine. CSF is essential to maintain brain homeostasis and is primarily secreted by the specialized ventricular ependymal cells. CSF continuously moves within the ventricles, yet the role of this circulation, notably during embryonic development, is not well understood. Current data suggest two discrete systems that drive CSF circulation; pulsatile cardiac forces and the motile ependymal cilia, an apical membrane-bound organelle that propels CSF in the brain. Interestingly, we recently showed that ependymal cilia are the sole driver of the CSF circulation in Xenopus tropicalis tadpoles during early brain development. The role of this cilia-driven CSF circulation is yet to be determined. For this purpose, we must first assemble a comprehensive map of the ependymal cilia in relation to the CSF circulation. We used scanning electron microscopy (SEM) and immunohistochemistry (IHC) to map the ciliary types and locations along the entire ventricular system. Then we coupled our data with optical coherence tomography (OCT) imaging enabling real-time visualization of the CSF circulation in Xenopus. By building this structural and functional atlas of the embryonic Xenopus ependymal surface, we aim to understand the role of cilia during brain patterning and disease states such as human hydrocephalus.

#31
Kitamura, Kazuki

Identification of tumor-related genes by gene expression analysis of spontaneous tumor tissue in Xenopus skin

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Abstract

It is known that tumorigenesis occurred by multiple mutations. Although genomic studies have identified many oncogenes and tumor suppressor genes, experimental analyses of crucial gene sets involved in tumorigenesis have not been sufficiently performed. Here, we found many tumor individuals in the Nigerian H strain of Xenopus tropicalis. Histological analysis revealed that the dermis clearly increased in the tumor tissues. In addition, the dermis was meandering layered structure, which is similar to dermatofibromas in humans. Next, we analyzed difference in gene expression between tumor tissues and normal tissues of three individuals via RNA-seq and identified 82 genes that showed different expression (DEGs). Gene ontology and pathway analysis showed that upregulated genes of 82 genes in the tumor tissues were enriched in terms related to the extracellular matrix and collagen formation whereas the downregulated genes were enriched in terms related to muscle tissues. Additionally, hierarchical clustering for Xenopus tumor tissues and human cancer patients showed that gene expression patterns of tumor tissues in X. tropicalis were comparable to those of human connective, soft, and subcutaneous tissue-derived cancers. We also found that the expression tendency of some DEGs that have not been well analyzed in the human cancer field clearly determines the prognosis of cancer patients. This study provides a remarkable reference for future experimental work to identify gene sets involved in human cancer using Xenopus.

#32
Koike, Ryota

Title: Functional analysis of a zinc finger protein that promotes the formation of neural tissue in Xenopus embryos

Authors: Ryota Koike, Regina P. Virgirinia, Makoto Nakamura, Kimiko Takebayashi-Suzuki, and Atsushi Suzuki

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In vertebrates, BMP and Wnt signaling pathways play important roles in the formation of neural tissue during embryonic development. The transcription factors FoxB1 and Zbtb14 have been shown to regulate neural development in Xenopus by modulating morphogen signaling pathways (Takebayashi-Suzuki et al., 2011, 2018, 2020). In addition to these regulators, we report here that the zinc finger protein Znf281 induces the formation of neural tissue. RT-PCR analysis indicated that znf281 was expressed maternally and throughout early development in X. tropicalis. Spatiotemporal expression analysis of znf281 by whole-mount in situ hybridization showed that znf281 mRNA was present in the animal hemisphere of the blastula and gastrula embryos but was restricted to the neural plate after gastrulation. At the tailbud stage, znf281 was strongly expressed in the brain, eyes, and tailbud region. In an animal cap assay, overexpression of Znf281 induced expression of the early neural markers sip1 and sox3, but did not induce expression of the differentiated neural markers ncam and n-tubulin or of mesodermal markers. To examine the role of endogenous Znf281 during early Xenopus development, we performed a knockdown (KD) experiment using an antisense morpholino oligonucleotide (MO). When analyzed by quantitative RT-PCR, Znf281 KD reduced expression of sip1, sox3, ncam, and n-tubulin. The reduction of neural markers was rescued by 11-mis znf281 mRNA, which has mutations in the target site of Znf281 MO. These results suggest that znf281 is essential for the formation of neural tissue during early Xenopus development.

#33
Kostyanovskaya, Elina

Pleiotropy of SYNGAP1 on cilia

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SYNGAP1 is a neurodevelopmental disorder risk gene with well-established functions at the synapse. However, we know that proteins play pleiotropic functions during development, with many synaptic proteins also functioning on cilia. Cilia are small membrane protrusions essential for many signaling pathways as well as movement of mucus and cerebrospinal fluid. Here we show that SYNGAP1 is expressed in highly ciliated tissues during development and localizes to cilia in a wide variety of Xenopus and human cell types. Using CRISPR/Cas9 genome editing in X. tropicalis, we show that loss of syngap1 leads to ciliogenesis defects in vivo. We also investigate the effect of SYNGAP1 haploinsufficiency on ciliogenesis in 3 patient-derived iPSC lines. Finally, we describe clinical work investigating ciliary function in patients with de novo variants in SYNGAP1. In this way, we are using basic experiments in Xenopus to uncover novel mechanisms of disease that are clinically relevant.

#34
Kult Perry, Shiri

Studying the regenerative capacity of the respiratory system in Xenopus.

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2 National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA

Abstract:

The respiratory system takes a central role in animal biology by allowing vertebrates to breathe air. Our understanding of the embryonic development and regeneration of the respiratory system has increased substantially. However, these studies have been limited, focusing on a single species, mainly mammalian, such as humans or mice. Here, we hypothesized that some vertebrates, such as amphibians, can regenerate their respiratory system and asked what are the underlying molecular mechanisms that regulate it. To this end, we have established a protocol for lung injury in Xenopus tropicalis (X. tropicalis) and followed the healing process. Using chemically induced injury of the lungs with bleomycin, known to damage the lung through oxidative stress, we aimed to identify the cell populations that contribute to organ recovery post-injury. Combining various imaging techniques, such as histology, in situ hybridization, and immunostaining, we compared the healing process of lungs post-injury in X. tropicalis. Preliminary results show an elevation in collagen deposition and alveolar enlargement in X. tropicalis lungs post-injury, indicating bleomycin's effect, to cause lung injury and fibrosis in Xenopus lungs. The decrease of Sox9 expressing cells post-injury suggests the onset of differentiation in the distal part of the epithelium. These results show for the first time the physiological similarities between the amphibian and the mammalian lung following chemically induced organ injury, suggesting X. tropicalis as a potential model to study respiratory pathologies and possibly regeneration processes, and therefore may have significant implications for both the biomedicine and evolutionary biology fields.

#35
Lawrence, Merin

Title: Analysing the role of Sox9 and Sox10 in Xenopus neural crest and cranial placode development

Authors: Merin Lawrence 1, Gerhard Schlosser 1

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Neural crest (NC) cells and cranial placodes share evolutionary and embryological origins and are of fundamental importance to vertebrate development, evolution, and disease. NC cells are multipotent and form most of the bone, cartilage, and connective tissue of the head. They also form the face, peripheral autonomic and enteric neurons, and most peripheral somatosensory neurons [1, 2]. Placodes form the paired peripheral sense organs including the eye lenses, olfactory, otic, and lateral line, as well as neurons associated with taste, fertility, and homeostasis [3]. Defects in NC and cranial placode development underlie a broad range of birth defects and disorders including cancer, sensory deficits, and abnormal physiological function in juveniles and adults. The SOX E proteins SOX9 and SOX10 are transcription factors acting as neural crest specifiers in vertebrates. They are also expressed in the otic placode, which gives rise to the inner ear, but very little is known about their functions there [4, 5, 6]. My Ph.D. project aims to analyse the role of Sox9 and Sox10 in neural crest and cranial placode development. I study this during two developmental stages in X.laevis namely neurulation and organogenesis. I have already generated RNA sequencing data from X.laevis explants taken during neurulation and organogenesis allowing me to select candidate targets of SOX9 and SOX10. These genes will now be validated using CUT&RUN sequencing. Validation and functional studies will provide novel insights into the pathways regulated by Sox9 and Sox10, providing us with a broader understanding of how the gene regulatory networks function around NC and placodal development.

References:
[1] Schock EN, LaBonne C: Sorting Sox: Diverse Roles for Sox Transcription Factors During Neural Crest and Craniofacial Development. Frontiers in Physiology 2020 Dec;11.

[2] Simões-Costa M, Bronner ME. Insights into neural crest development and evolution from genomic analysis. Genome Research 2013 Jul;23(7):1069-80.

[3] Pieper M, Eagleson GW, et al.: Origin and segregation of cranial placodes in Xenopus laevis. Developmental biology 2011, 360(2):257-75.

[4] Spokony RF, Aoki Y, et al.: The transcription factor Sox9 is required for cranial neural crest development in Xenopus. Development 2002, 129(2):421-32.

[5] Saint-Germain N, Lee YH, et al: Specification of the otic placode depends on Sox9 function in Xenopus. Development 2004, 131(8):1755-63.

[6] Honoré SM, Aybar MJ, Mayor R: Sox10 is required for the early development of the prospective neural crest in Xenopus embryos. Developmental biology 2003, 260(1):79-96.

#36
Lee, Hongchan

Regulation of actin dynamics by actin depolymerizing factor, destrin, modulates ciliogenesis

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Abstract

The actin-based cytoskeleton is considered as a fundamental driving force for cell differentiation and development. Destrin (DSTN), a member of actin depolymerizing factor family, regulates actin dynamics by treadmilling actin filaments and increasing globular actin pools. However, the specific developmental roles of Destrin have not been fully elucidated. Here we investigated the function of DSTN in ciliogenesis using Xenopus laevis and human retinal pigmented epithelial (RPE1) cells. DSTN is expressed in anterior neural tissue and neural plate in Xenopus embryogenesis. The depletion of DSTN induced morphants having short body axis and a small head. Inhibition of dstn induced actin filament accumulation, resulting in impaired neural plate folding during neurulation. Notably, we discovered that DSTN is localized in Xenopus' multiciliated epithelia. The loss of DSTN increased the number of multiciliated cells in the Xenopus epithelium. In addition, DSTN deletion remarkably reduced the length of primary cilia in the Xenopus neural tube and RPE1 cells by affecting actin dynamics. Collectively, DSTN regulates ciliogenesis and our data suggest new insights for understanding the roles of actin dynamics in embryonic development, simultaneously presenting a new challenge for studying the complex networks governing the ciliogenesis involving actin dynamics.

#37
Levangie, Kaitlin

Assessing genes associated with heterotaxy phenotypes in X. tropicalis

Kaitlin Levangie, Cynthia Zerillo, Larisa Lozovatsky, Emily Mis, Mustafa Khokha

Yale University

Heterotaxy is a congenital condition in which the development of the left-right axis is disrupted, leading to internal organs which are abnormally formed, arranged, or absent. It is associated with physiological problems, like issues with the immune system, stomach, intestines, lungs, and cardiac abnormalities. Heterotaxy is estimated to affect 1 in 10,000 people worldwide, and accounts for ~3% of congenital heart disease (CHD). To study the genetic causes of heterotaxy, a list of candidate genes was compiled by identifying patients with heterotaxy phenotypes and novel mutations from studies performed by two programs, the Pediatric Cardiac Genomics Consortium (PCGC) and the Pediatric Genomics Discovery Program (PGDP). These genes were then targeted at multiple loci in X. tropicalis embryos using CRISPR-Cas9 microinjection, with embryos subsequently scored for abnormal heart-looping patterns. Thus far, 11% of the screened genes have exceeded our threshold for affecting left-right patterning (>10% affected), with an additional 16% of genes displaying moderate phenotypes (>6% affected). Embryos treated for genes that displayed a strong phenotype underwent whole-mount in situ hybridization to determine the expression of pitx2, a transcription factor involved in the genetic cascade controlling left-right patterning. This screen indicates approximately 27% of genes from the candidate list are worth further investigation as to how their roles affect left-right patterning. Further characterization of these genes will allow for a more complete understanding of the mechanisms that drive left-right patterning. This work is supported by the National Institutes of Health (1R01HD102186).

#38
Lopes L. Empke, Stéfany

The Role of CAD and CDX1 Genes in Heterotaxy Syndrome and Cardiac Malformation

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Heterotaxy (Htx) refers to a group of malformations characterized by an abnormal right-left (LR) axial determination. In individuals with Htx, LR development is defective, and organs are mispatterned relative to the LR axis, resulting in compromised LR architecture of the heart and clinically severe cardiac dysfunction. Even though Htx is primarily a genetic disease, the causal genes remain mainly unknown. Exome sequencing from two programs, the Pediatric Cardiac Genomics Consortium (PCGC) and the Pediatric Genomics Discovery Program (PGDP) identified three individuals with Htx and heart malformation who had a de novo mutation in either CAD (Carbamoyl-phosphate synthetase 2, Aspartate transcarbamoylase, and Dihydroorotase) or CDX1 (Caudal Type Homeobox 1) genes. CAD is a multifunctional protein involved in the first three rate-limiting stages of pyrimidine nucleotide synthesis. This metabolic system, which is maintained in all living species, is required for cell development and proliferation. CDX proteins are crucial in determining the posterior identity of different tissues. Loss of function studies, for example, reveal that CDX family members act redundantly as upstream regulators for HOX genes in different developmental processes. We used CRISPR/Cas-9 genome editing to knock out CAD and CDX1 in Xenopus tropicalis tadpoles to attempt to replicate Htx and found abnormal left-right patterning. These findings suggest that the genes CAD and CDX1 are significantly associated with cardiac malformation and Htx.

#39
Lyu, Cooper

Adheren Junction Dynamics during Epithelial-Mesenchymal Transition

Epithelial-Mesenchymal Transition (EMT) is a mechanism crucial for cell migration in development and cancer metastasis. It allows epithelial cells to undergo biological changes to assume a mesenchymal phenotype, conveying an enhanced migratory capacity. Such drastic cellular changes require extensive interactions among cell population. The goal of this project is to shed light on cell-cell interaction during EMT through the lens of adheren junctions (AJ) during Xenopus laevis cranial neural crest (CNC) migration. To analyze AJ-based cell-cell interactions, we focused on E-cadherin expressed during Xenopus CNC migration. We differentially labeled CNC cells via embryonic injection to elucidate the spatial relationship between E-cadherin-expressing cell protrusions and cell membrane. We found that E-cadherin- expressing cell protrusions are intermembrane structures that change the membrane conformation of neighboring cells during collective cell migration. Time-lapse imaging and endocytosis assay implicated a role of intercellular trafficking in the observed protrusions, echoing a functional significance of the intermembrane characteristics. Potential proteins of interests were selected from proteomics analysis using CNC explants from different migratory stages to corroborate findings with E-cadherin. Together, we theorize that E-cadherin is required in collective CNC migration to maintain tissue cohesion through forming extended actin-based protrusions that interdigitate with neighboring cells' membrane.

#40
McCluskey, Kate

Autism risk genes in enteric nervous system development

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People with autism spectrum disorder (ASD) often have gastrointestinal (GI) distress, but the reason for this comorbidity is unknown. Many large-effect risk genes have been identified for ASD, providing an opportunity to understand this comorbidity. Here we develop Xenopus tropicalis as a powerful in vivo model organism to study the development of the nervous system in the gut, the enteric nervous system (ENS), which is completely neural crest-derived. We used this system to perturb multiple autism risk genes in parallel to determine their role in ENS development. Specifically, we targeted 3 high-confidence, large-effect ASD risk genes of differing cellular annotations with CRISPR/Cas9, and assayed development of the neural crest and ENS progenitors. We show that disruptions in any of the 3 (DYRK1A, kinase; SYNGAP1, synaptic protein; and CHD2, chromatin modifier) cause defects in the development of neural crest cells and their migration into the gut. We determined that the migration defects can be independent of crest specification defects by inhibiting DYRK1A pharmacologically after crest specification was complete. Then we profiled GI symptoms in databases of patient clinical records for all 3 genes and observed high incidence of reported GI issues. Finally, we describe our plans for drug screening to rescue these defects by treating tadpoles fed with fluorescent beads and assaying gut motility to uncover mechanisms that will be clinically translatable. This work lays the foundation for using Xenopus as a higher-throughput in vivo model organism to study vertebrate ENS development and disorders of gut motility.

#41
Mckeown, Rachel

Interactions of mechanical and long-range chemical signalling in the developing Xenopus brain

Contributors: Rachel Mckeown, Sarah Foster, Kristian Franze

Presenter: Rachel Mckeown

Affiliation: University of Cambridge

Developing neurons extend axons which must navigate potentially long distances to connect with their correct downstream targets. Previous experiments supported chemical guidance models of axon pathfinding, whereby gradients of diffusive attractive or repulsive proteins determine axon trajectories. However, this fails to explain all in vivo axon guidance phenotypes. Recent studies have revealed that axons also sense and respond to the mechanical properties of their environment, which vary in space and time in the developing brain. Our work explores how chemical and mechanical signals interact during axon guidance, using the developing visual system of the African clawed frog Xenopus laevis as model system. Our data suggest that the response of retinal ganglion axons to Semaphorin3A, a classical repulsive guidance cue expressed in the Xenopus brain, is regulated by tissue stiffness. This mechanical regulation is mediated by the mechanosensitive ion channel, Piezo1. In vivo, long-range gradients of diffusing proteins are likely to be noisy and shallow; therefore, the integration of mechanical and chemical signals may increase the signal-to-noise ratio, ensuring that axons are guided accurately and reproducibly.

#42
Mis, Emily

A novel SHH variant associated with Holoprosencephaly-3 modeled in Xenopus

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Holoprosencephaly-3 (HPE-3) is caused by loss-of-function variants in SHH and has wide clinical features. Even within families, abnormalities range from normal brain structure to the presence of a single ventricle with no interhemispheric fissure and other midline birth defects. However, incomplete penetrance and the inaccessibility of variant-specific functional assays often result in inconclusive genetic diagnoses, especially in the case of inherited missense variants. We used whole exome sequencing to identify a novel variant, R310P, in a fetus with alobar HPE and the unaffected mother, whose mother was determined also to have the variant via Sanger sequencing. To assess the functional effects of R310P of SHH protein, we performed in vivo overexpression of WT SHH in Xenopus laevis and compared this to overexpression of SHH harboring the R310P variant. We found no changes to Ptch1 and Gli1 expression upon overexpression of the R310P variant, indicating a loss of normal SHH function. Due to incomplete penetrance seen in HPE-3 cases, we also tested previously reported SHH variants to assess the variant-specific effects on SHH function. We aim to provide evidence that a SHH variant identified in a single family is causative for HPE-spectrum findings over three generations. We also test our hypothesis that Xenopus is an effective model for functional characterization of SHH variants.

#43
Moreira, Sofia

CRISPR-CAS13 knockdown in Xenopus laevis

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CAS13 is a class 2 type VI CRISPR-Cas RNA endonuclease produced by prokaryotic cells as a defense mechanism to induce cleavage and degradation of nucleic acids derived from bacteriophages. Recent advances have demonstrated that CRISPR-CAS13 can be effectively used as a knockdown tool in fission yeast, mammalian cell lines and also in multicellular organisms such as plants, zebrafish and mouse embryos. While several knockdown tools have been successfully applied in Xenopus, the applicability of CRISPR-CAS13 in Xenopus laevis remains to be determined. In our lab, we have successfully employed this cutting-edge strategy to downregulate both exogenous and endogenous transcripts in Xenopus laevis. Remarkably, our results recapitulate described loss-of-function phenotypes of known developmental markers and have been useful in uncovering new roles for Sox8 during embryonic development. Here, we aim to provide a comprehensive workflow outlining the use CRISPR-CAS13 for targeted mRNA downregulation in a comparatively efficient, specific, cost-effective and reliable manner. We anticipate that this tool will be used within our Xenopus community to generate well controlled and in turn reproducible data.

Key-words: CRISPR-CAS13, RNA knockdown, Xenopus laevis

#44
Murray, Kenan

The role of primary cilium in modulating Dishevelled functions during RPE maturation in the developing eye

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The Retinal Pigment Epithelium (RPE) plays a critical role in the development of photoreceptors in the eye, and defects in its maturation can lead to photoreceptor degeneration and eventual blindness. The primary cilium has been shown to have vital roles in RPE maturation by regulating PKCδ activation and suppressing the Wnt-β-catenin signaling pathway. In a previous study, we identified that the conformational change of Dishevelled (Dvl2) switches its canonical and non-canonical Wnt signaling. We also discovered that proteins binding to the C-terminus of Dvl2 can activate non-canonical Wnt signaling. In this study, we aimed to identify novel Dvl2 C- terminal binding partners that can switch its canonical and non-canonical Wnt signaling and elucidate the mechanisms by which this occurs. To achieve this, we performed mass spectrometry analysis and identified Kizuna as a potential binding partner of the C-terminus of Dsh2, known as a ciliary gene. Interestingly, mutations in the Kizuna gene in human patients have been implicated in deformities of the RPE and retinal dystrophy caused by retinal degeneration. Our results showed that knockdown of Kizuna in developing Xenopus embryos also showed loss of RPE layers. This study provides insights into the role of the primary cilia and Dvl2 C-terminal binding partner, Kizuna, in RPE maturation and may have implications for understanding retinal degeneration diseases caused by defects in primary cilium and Wnt signaling.

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#45
Niazi, Bilal

TBC1d24 is potentially involved in endosomal membrane trafficking through its TBC domain and oxidative stress resistance through its Tldc domain. In human patients, mutations in TBC1d24 lead to various rare genetic disease states, including DOORS (Deafness, Onychodystrophy, Osteodystrophy, Retardation, Seizures) Syndrome. Deafness is a common phenotype associated with TBC1d24-related diseases; however, the role of TBC1d24 in the ear remains unclear. A previous study demonstrated that TBC1d24 regulates cranial neural crest migration by forming complexes with EphrinB2 and Dishevelled to inactivate Rab35 in developing Xenopus embryos. The objective of our study is to determine the molecular function of TBC1d24 in otic vesicle formation. In our preliminary analysis, we injected TBC1d24 morpholino into Xenopus embryos at the 2-cell stage and observed defects in otic vesicle development. We plan to replicate the TBC1d24 MO injection experiment in otic fated early embryonic cells and examine changes in the otic vesicle using Whole Mount In-Situ Hybridization. Additionally, we are developing transgenic lines to visualize the otic vesicle through pTransgenesis. By elucidating the role of TBC1d24 in otic development, we aim to identify potential therapeutic targets for future treatments of individuals affected by DOORS or other deafness causing TBC1d24 disease states.

Affiliated with National Cancer Institute

#46
Ogar, Paulina

Transcriptional control of early nephrogenesis in Xenopus tropicalis

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In mammalian kidney specification, interaction between the ureteric bud epithelium (UB) and the metanephric mesenchyme (MM) is an essential driver of nephron formation. In Xenopus pronephros (early kidney) development, the tubule anlage condenses, mesenchymal to epithelial transition occurs and functional tubules subsequently form, but the available analysis has yet to uncover distinct tissue domains. Moreover, transcription factors Pax8, Hnf1b, and Sall1 that underpin early kidney development, and are associated with Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) are localised in distinct regions within the UB and MM in mammals. Curiously, however, these factors are roughly co-expressed within the same pronephric region in Xenopus. Here, we outline an imaging strategy combining immunostaining and confocal microscopy to examine pronephros development in molecular detail and to correlate these morphogenetic events to pax8, hnf1b and sall1. We show that KO of pax8, hnf1b and sall1 by CRISPR/Cas9 disrupts size, cell organisation and the basement membrane of the pronephros. Lastly, to investigate transcriptional circuitry, we performed targeted CRISPR/Cas9 KO of pax8, hnf1b and sall1 and conducted RNA-seq on individual embryos (single embryo RNA-seq). Several renal and morphogenetic genes were identified after intersection with a pronephros-enriched RNA-seq dataset (e.g., slc12a1, fgf8, lama1). Further transcriptomic analysis, in tandem with our imaging strategy, will evaluate key differentially regulated genes, to understand the roles and hierarchies of pax8, hnf1b, sall1 within the transcriptional network underlying early kidney development. Identifying these will improve our understanding of inherited kidney malformations and disease.

#47
Pineros, Liliana

Temporal coordination of cell cycle in artificial cells.

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Whether a cell grows and divides is a highly regulated decision controlled by a large and complex network of proteins. The healthy development and tissue homeostasis of organisms depend critically on this cell cycle regulatory network. DNA and nuclei have been shown to play an essential role in this process, though the exact mechanism remains unclear. Moreover, it is still not fully understood how the dynamic interactions between different regulatory proteins and subcellular structures control the biochemical oscillations that drive the cell cycle forward.

To answer such questions, we aim to characterize the biochemical oscillations in vitro using cell-free extracts prepared from Xenopus eggs. This extract has been used extensively as a versatile and powerful biological model system to study various aspects of cellular and developmental biology. We test an artificial cell system consisting of droplets of Xenopus frog extracts of varying sizes, which allows mimicking cell cycle oscillations, periodic nuclear assembly and destruction, aspects such as actin and microtubule organization and size scaling of nuclei. Different fluorescent reporters are used to visualize these periodic cell cycle processes in various setups in combination with time-lapse fluorescence microscopy. This approach allows for controlling the spatial and chemical environment accurately.

We analyze how different biochemical interactions, DNA amounts, nuclear-to-cytoplasmic ratio, nuclear size, and cell size contribute to the coordination of the cell cycle in time. This experimental work is complemented by computational modeling to gain insight into how having a nuclear compartment regulates cell cycle timing.

#48
Prabha, Haritha

Role of VRK1 in Nuclear Morphology

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ABSTRACT. Acquisition of the characteristic size of intracellular organelle is a fundamental question in cell biology. It is important to gain a better understanding of the mechanisms that control nuclear size in humans because nuclear size and shape are known to change in cancer. Vaccinia-Related Kinase (VRK1) is a serine- threonine protein kinase belonging to the casein kinase-1 family. Its expression is increased in actively dividing cells in testis, fetal liver, and carcinomas with poor prognosis. VRK1 regulates entry into the cell cycle, chromatin condensation and Cajal body dynamics. VRK1 also plays important roles in the assembly and disassembly of the nuclear envelope, and a recent screen for nuclear morphology effectors identified VRK1 as a putative regulator of nuclear size. We are investigating the function of VRK1 in nuclear size regulation using Xenopus laevis egg extracts that support de novo nuclear assembly and growth. Immunofluorescence confirmed the presence of VRK1 in egg extracts, revealing a punctate intranuclear staining pattern. In order to understand the role of VRK1 in regulating nuclear size we supplemented extract with a small molecule inhibitor of VRK1 either during or after nuclear assembly. We find that VRK1 inhibition results in a significant reduction in nuclear size concomitant with the appearance of nuclear membrane invaginations and nuclei remain import- competent and intact. This effect was more prominent when VRK1 was inhibited post-nuclear assembly. We also observed a redistribution of chromatin upon VRK1 inhibition. Future work will focus on identifying the VRK1 substrate relevant to the regulation of nuclear size.

#49
Qian, Wenchao

Spatial Patterning of Genome Activation is Required for Vertebrate Early Development

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During early embryogenesis, cells divide without growing, triggering the zygotic genome activation (ZGA) in animal species. In Xenopus embryos, the onset of ZGA is linked to the cells reaching a critical size threshold, with smaller animal pole cells activating their genome before larger vegetal pole cells. However, the significance of this conserved embryonic cell-size gradient remains unclear. In this study, we investigated the role of the gradient by reversing it in Xenopus embryos and evaluating the developmental consequences. We used a temperature controller to slow down cell division in the animal pole, leading to an inverted cell size gradient and delayed genome activation in this region. Strikingly, embryos with extreme delays in animal pole cell division underwent early death before the gastrula stage, similar to the embryonic quality control pathway where apoptosis is activated following exposure to early stress. To determine if delayed animal pole transcription triggers the apoptosis pathway, we selectively injected transcription inhibitors into animal pole cells and found that it caused similar spreading of lysing cells. Furthermore, staining for the hallmark of apoptosis, active Caspase 3, revealed an accumulation of apoptotic cells inside the blastocoel of embryos. However, administering caspase inhibitors eliminated this population of apoptotic cells and rescued the embryos from rapid death. From literature, we learned that the zygotic protein PDGF is necessary for the migration of mesoderm, and a lack of PDGF leads to mesoderm cell disassociation and apoptosis. Intriguingly, injecting PDGF protein into the blastocoel of embryos with inhibited animal-pole transcription reduced apoptosis activity and enabled further embryonic development. Our findings underscore the vital role of early genome activation in the animal pole in embryonic quality control and preventing embryo death.

#50
Rankin, Scott

RFX6 (Regulatory Factor binding to the X-box member 6) is an evolutionarily conserved DNA-binding transcription factor. Mutations in human RFX6 cause Mitchell-Riley Syndrome, a rare congenital gastrointestinal (GI) organ disorder with a wide phenotypic spectrum including pancreatic hypoplasia/agenesis, neonatal diabetes, intestinal atresias, intestinal malrotation, biliary atresia / extrahepatic biliary and/or gall bladder hypoplasia/agenesis. The exact genotype-phenotype correlations of most RFX6 mutations are poorly understood. Despite RFX6 having such a critical function in GI organogenesis, two major questions regarding RFX6 exist: 1) what are the developmental signaling pathways that function upstream of RFX6 to regulate its expression in the developing GI tract? 2) what are the downstream gene regulatory networks that RFX6 controls during GI organogenesis? We have begun to investigate these questions using the amphibian Xenopus, an excellent vertebrate model organism for GI birth defect research, and during the invitro directed differentiation of human pluripotent stem cells (hPSCs) into GI tract epithelium. We find Retinoic Acid (RA) signaling is sufficient to activate RFX6 expression in Xenopus endoderm and during directed differentiation of hPSCs into GI tract endoderm cells. We identify Hedgehog (Hh) ligands as transcriptional targets induced by RFX6 and find Hh signaling is necessary downstream of RFX6 to promote development of adjacent GI tract mesoderm and intestinal rotation in Xenopus. Our data suggest 1) RFX6 plays an evolutionarily conserved, essential role in proper patterning of GI endoderm and mesoderm during embryogenesis; and 2) disruptions to RFX6-dependent Hh signaling could partially explain many phenotypes observed in human Mitchell-Riley Syndrome patients.

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#51
Rao, Venkatramanan

Vertebrate model to study cilia regeneration

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Motile cilia beating is critical for muco-ciliary barrier to function as the first line of defense in the lungs. Due to exposure to several factors (such as acid reflux, infection, and inflammation), these cilia can undergo damage, that can be repaired by regeneration. Though cilia regeneration is well understood in unicellular models, but not in vertebrates. We used Xenopus tropicalis embryos to study motile cilia regeneration and found that multiciliated cells regenerate their cilia rather than undergo stem cell-based renewal. Precursor pools of ciliary proteins in the cells drive initial regeneration, but in the absence of protein synthesis, ciliated cells prioritize fewer but longer cilia by concentrating the protein pool around the basal bodies. We further studied the site of deciliation and observed that the deciliation does not affect the apical actin lattice and the basal body number, organization, or polarity in the MCCs. We observe that the transition zone (TZ) is lost during deciliation. We studied the TZ assembly during cilia regeneration using the electron tomography and by observing the dynamics of known TZ protein B9D1 during regeneration. While all ciliogenesis models predict that TZ is assembled first followed by axoneme assembly, we observed that motile cilia in Xenopus embryos begin to regenerate in the absence of TZ. Thus, Xenopus tropicalis could be answer potential questions such as transition zone assembly and ciliogenesis.

#52
Reynolds, Zoë

Human disease modeling in Xenopus laevis and Xenopus tropicalis at the Xenopus Mutant Resource

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At the Xenopus Mutant Resource (XMR), we have closely collaborated with the Xenopus community to prioritize the generation of null knockout mutants in both Xenopus tropicalis and Xenopus laevis genes of interest. Generating these lines at the XMR allows the community to take advantage of our efficient CRISPR pipeline, in addition to the husbandry and lab resources available to help expedite your research project. To date we have generated over 250 mutant lines spanning many research disciplines. My work consists of managing and establishing over 40 mutant gene lines. Specific genes I have targeted have resulted in striking phenotypes. In F0 X. tropicalis edn3 mutants present we find loss of pigment. Pax7 null X. laevis froglets feature severe bloating around stage 54 of development. We have also created several mutants in genes involved in sex determination, including dm-w and dmrt1, both of which display a striking phenotype. We have also created X. tropicalis mutants naglu and lamp2 are actively being examined for a phenotype due to their role in lysosomal storage and regulation. In this poster, I will present our methodology and progress in generating these various CRISPR knockouts and how the resources at the XMR can help streamline research on these human disease models.

#53
Romero Mora, Adrian

A novel role for Dyrk1a in kidney development

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ABSTRACT

Objective: DYRK1A is a protein kinase that belongs to an evolutionarily conserved family known as DYRKs (dual-specificity tyrosine-phosphorylation-regulated kinase). It phosphorylates diverse substrate proteins that are relevant in transcription, splicing and signaling. In humans, the DYRK1A gene is located in Down syndrome critical region on chromosome 21 indicating its fundamental role in Down syndrome patient phenotypes. DYRK1a haploinsufficiency causes intellectual disability, developmental delay, microcephaly and dysmorphic facial features. In these patients, there is an increased prevalence of renal abnormalities. Here we evaluated early kidney development upon Dyrk1a depletion to understand the role of Dyrk1a in Xenopus kidney formation.
Experimental Methods: Immunostaining, in vivo labeling of fluorescently tagged Dyrk1a protein and in situ hybridization chain reaction (HCR) were used to determine the subcellular localization within the nephron progenitors. The effects of loss-of-function upon Dyrk1a depletion with a morpholino was characterized using kidney, proliferation and apoptosis markers. The nephron structure upon CRISPR editing of the dyrk1a gene within the developing kidney of X. tropicalis confirmed the phenotype. Every experiment was made in around 50 embryos/condition, with three replicates. The imaris software was used for quantitative evaluation.

Results: Fluorescent in situ hybridization confirmed the expression of Dyrk1a in kidney. Confocal imaging showed that Dyrk1a localizes to the membrane, cytoplasm and the nucleus of mesenchyme and kidney progenitors. The fluorescently tagged Dyrk1a confirmed the localization in the nephron progenitor nuclei. The knockdown resulted in abnormal kidney morphology with a significant reduction in the number of nephron progenitor cells as well as increased apoptosis. The specific Dyrk1a inhibitor (proINDI) confirmed the nephron progenitors phenotype.

Conclusion: Our data suggest that Dyrk1a is expressed in nephron progenitors and influences the number of cells that give rise to the kidney. Preliminary data indicate that Dyrk1a may modulate apoptosis to play a significant role in the morphogenesis of the kidney. This study will significantly impact our understanding of congenital kidney anomalies observed in DYRK1A-related intellectual disability patients and will determine the pathways downstream of Dyrk1a in nephrogenesis with goal of identifying targetable pathways to treat this condition. This work is expected to expand our understanding DYRK1A-related intellectual disability syndrome, potentially altering clinical diagnosis and treatment of this disease

#54
Rushing, Amy

TNRC18 May Contribute to Congenital Heart Disease Through Disruption of Cilia Function

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Congenital heart disease (CHD) affects 1:100 children born, and it is the leading cause of birth-defect related mortality. While genetics are predicted to contribute to 90% of CHD, the underlying mechanisms remain poorly understood. Programs such as the Pediatric Genomics Discovery Program aim to identify candidate genes from patients with CHD, which can then be studied in model organisms. One such candidate gene is TNRC18. The function of the encoded TNRC18 protein is not well established, but it may be involved in chromatin binding to control gene expression. Preliminary studies using CRISPR/Cas9 sgRNA directed to tnrc18 in Xenopus tropicalis show embryos with decreased cilia flow. Because proper cilia flow is critical in establishing the left-right organizer allowing for normal heart development, disruption of cilia via TNRC18 may contribute to CHD.

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#55
Salonna, Marika

“C-clamp, or not C-clamp, that is the question”: evolution and function of TCF isoforms in vertebrates

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The evolutionary conserved Wnt/β-catenin pathway is a signalling cascade that plays crucial roles in metazoan development. TCF proteins are the major mediators of Wnt/β-catenin signalling, which recognize DNA through their HMG domain. Invertebrate TCFs and some vertebrate TCF isoforms contain an additional DNA binding domain known as the C-clamp, whose specific function has not yet been characterized. We are investigating the evolution, expression, and function of the TCF C-clamp-containing/lacking isoforms to explore whether they can differentially regulate Wnt target genes during vertebrate development.

Comparing the genomes of chordates, we confirmed that the four vertebrate TCF genes (TCF7, LEF1, TCF7L1 and TCF7L2) likely derive from two rounds of whole genome duplications from a single invertebrate TCF gene. Invertebrate TCF proteins always contain a C-clamp; TCF7 and TCF7L2 are the only vertebrate TCF genes that encode C-clamp-containing isoforms. Our analysis suggests that the ancestral vertebrate TCF gene was most similar to TCF7L2, and that C-clamp-lacking variants may be an early vertebrate-specific evolutionary innovation.

We are currently focusing our investigations on the expression and function of TCF7 isoforms during Xenopus tropicalis embryonic development. Expression analyses has not revealed any stage-specific isoform distribution, suggesting we need to explore possible tissue-specific expression and activity. We successfully knocked down/out TCF7 and over-expressed TCF7 isoforms with and without a C-clamp. Preliminary data indicate that TCF7 C-clamp-lacking isoforms may differently regulate Wnt target genes at the mid-gastrula stage compared to C-clamp-containing variants. Further experiments are needed to clarify their functional diversity in vertebrate development.

#56
Seal, Subham

Characterizing the functions of Prdm12 during neural crest EMT and migration

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The neural crest (NC), an important, multipotent embryonic population, undergoes epithelial-to-mesenchymal transition at the end of neurulation. The NC cells then delaminate, migrate to different parts of the vertebrate body and give rise to a host of different tissues. Although the mechanics of NC migration are well studied, the transcriptional regulation of EMT is not well understood. We study the role of Prdm12, a transcriptional repressor, during neural crest EMT and migration in Xenopus laevis embryos. Prdm12 is a PR domain-containing protein and is a direct target of Pax3, a major transcription factor regulating the neural border, from which the neural crest derives. A previous study has shown that at mid-neurula stages, Prdm12 restricts the NC domain, and contributes to establishing its boundary. However, we observe that Prdm12 is important for NC EMT: Prdm12 depletion leads to a decrease of expression of late EMT markers such as Sox10 and N-cadherin, but not Twist1. This leads to a concomitant defect in NC migration. Loss of Prdm12 also decreases the levels of canonical Wnt signaling, a pathway essential for NC specification: Prdm12 depletion leads to an increase in Dkk1, a canonical Wnt antagonist. This gives us a possible mechanism of Prdm12 function. Additionally, since the Wnt pathway also affects the cytoskeleton, we are currently studying the actomyosin dynamics during NC migration. Our preliminary results show that Prdm12 affects the morphology of the migrating cells. Our study will thus explain the relationship between transcriptional regulation of EMT and cytoskeletal dynamics of NC migration.

#57
Sepaniac, Leslie

Synthetic control of dynamic pattern formation at the cell cortex

Leslie A. Sepaniac, Ani Michaud, William M. Bement

The cell cortex, including the plasma membrane, cytoskeleton, and various cytoskeletal regulators, undergoes dramatic remodeling to drive cell shape changes necessary for mitosis, polarization, motility, and morphogenesis. Cortical dynamics are regulated by Rho family GTPases, such as Rho, Rac, and Cdc42. Rho family proteins cycle between an active, GTP- bound state via Guanine nucleotide exchange factors, or GEFs, and an inactive, GDP-bound state via GTPase activating proteins, or GAPs.
Rho family GTPases generate discrete patterns at the cortex such as patches, rings, or stripes, in addition to propagating, complementary waves of Rho GTPase activity and F-actin disassembly – referred to as cortical excitability. We have previously shown that cortical excitability can be experimentally induced in Xenopus oocytes (which are not normally excitable) via co-expression of two Rho regulators essential for cytokinesis: Ect2 (a GEF) and RGA-3/4 (a GAP). To further our understanding of this excitable circuit, I produced synthetic GEF and GAP constructs to provide positive and negative feedback of Rho. The synthetic activator, Rho Positive Feedback 1 (RhoP1), comprises the Rho GEF domain of LARG fused with an rGBD, a domain that binds active Rho. A synthetic inhibitor, Rho Negative Feedback 1 (RhoN1), contains a GAP domain and tropomyosin (to bind F-actin). Both RhoP1 and RhoN1 can support excitability, and when co-expressed together, produce fully synthetic, semi-stable patterns of extremely high amplitude. Development of additional synthetic constructs to interact with both Rho and F-actin or their regulators will reveal information about the regulation and propagation of excitable dynamics of this circuit.

#58
Softley, Charlotte

Tra2b – a new paradigm in coordinated post-transcriptional control of ciliogenesis

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Transformer 2b (Tra2b) is an alternative splicing factor that is essential for normal development in mice, flies and Xenopus. Our work addresses a novel function of Tra2b in regulating cilia. RNAseq in Tra2b-depleted Xenopus embryos identified hundreds of transcripts with altered splicing, with an enrichment in cilia-related terms. A selection involved in systemic ciliopathies and kidney disease were chosen for further analysis.

These were studied using morpholinos to alter splicing or knock them down. Splice and knockdown manipulations show different effects, confirming the differing roles of splice variants and the coordinated effects on cilia of the Tra2b targets. They particularly affected cilia formation, basal body formation and F-actin organisational defects. The combined effect of this is an alternative splicing factor that steers splicing in MCCs to allow a multitude of processes to occur correctly, resulting in successful fluid flow within the embryo. When splicing is manipulated, a reduced or more chaotic flow is seen. This represents a new paradigm in cilia regulation and function: Tra2b regulates coordinated alternative splicing in a functionally coherent set of transcripts, all contributing to a common cilia-related function.

Gaining insights on this novel mode of regulation in ciliated cells could lead to identification of a variety of therapeutic targets for genetic cilia-related diseases, and elucidate how splicing is functionally coordinated to regulate cell type- and tissue-specific cell biology. It is critical that we learn more about these mechanisms, and how defects are induced at the molecular level during basal body assembly, ciliogenesis and cell function.

#59
Sutton, Patrick

Palmitoylated ImporIn a Regulates MitoIc Spindle Orientation Through Interaction with Nuclear Mitotic Apparatus, NuMA

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Abstract:
Proper control of cell division orientation is a fundamental biological process critical to differentiation and tissue homeostasis. Developing neuroblasts require tight control of division orientation to properly segregate cell fate determinants. Misorientation of the mitotic spindle and thus misorientation of the plane of division can lead to depletion of neuroblasts resulting in a lack of differentiated tissue in the brain, a hallmark of microcephaly. Microtubules emanating from the mitotic spindle bind a conserved complex of proteins tethered to the cell cortex which orients the spindle. It remains unclear, however, how this complex is localized and maintained at the cortex.

Using cell culture and Xenopus laevis embryos we demonstrate that the nuclear transport protein importin a, when tethered to the membrane via palmitoylation, plays a critical role in mitotic spindle orientation through localizing key factors to the cell cortex. We demonstrate that in mitotic cells importin a partitions to the cell cortex where it binds NuMa, thereby facilitating anchoring of astral microtubules. Disruption of palmitoylation results in mislocalization of importin a and NuMa, thereby abolishing control of spindle orientation. We also observe developmental defects in Xenopus laevis when importin a palmitoylation is disrupted, including smaller head and brains (microcephaly), presumably resulting from decreased neuroprogenitor populations, a hallmark of spindle orientation deregulation. These findings not only characterize a novel role for importin a in spindle orientation, but also characterize a novel role for importin a palmitoylation which may have broader significance for other cellular processes.

#60
Tarannum, Nawseen

Mechanical regulation of cell division orientation: investigating the role of nuclear mitotic apparatus protein

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Cells within tissues experience a variety of mechanical forces from their environment and must respond appropriately to these forces to avoid failures in embryogenesis and prevent diseases such as cancer. One process regulated by mechanical force is cell division orientation, which is determined by the mitotic spindle. For example, stretching a tissue causes divisions to align with the stretch axis and spindle-associated cortical proteins may be important in this process. Nuclear mitotic apparatus protein (NuMA), which is key to spindle positioning, has been implicated in mechanosensitive spindle orientation. However, the mechanistic details remain uncharacterised, especially in a tissue context. Therefore, we utilised the Xenopus laevis animal cap tissue, to which tensile forces can be applied externally, to understand the role of NuMA in mechanosensitive spindle orientation. We show that cortical localisation of GFP-NuMA is dynamic and sensitive to mechanical stretch. Furthermore, simulated spindles, where microtubule pulling forces are amplified at sites of cortical NuMA, orient in a manner that most closely matches experimental data. Additionally, knockdown of NuMA disrupts cell division orientation according to stretch and cell shape. Interestingly, our data suggest that mechanosensitive spindle orientation through NuMA is an effect of direct force sensing rather than sensing changes in cell shape. Furthermore, using two different tissue stretch regimes, we demonstrate that NuMA responds specifically to anisotropic tension to orient cell divisions in stretched Xenopus tissues. Overall, using live tissue imaging and mathematical modelling, our results indicate that NuMA is vital to orient the mitotic spindle according to external force.

#61
Torrejon, Marcela

Gαi-2 acts as a mechanotransducer controlling cell tension during cranial NC migration

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The neural crest (NC) is a transient embryonic cell population that migrates extensively during development. We have found that, in cranial NC cells from Xenopus, Gαi-2, a subunit from heterotrimeric G protein families, is controlling migration acting as a master regulator of cell polarity, morphology and cell adhesion dynamics, possible by regulating MT dynamics and cytoskeleton crosstalk. The migration along gradients of extracellular mechanical stiffness requires the cells to trigger mechanosensitive pathways, mainly promoted by mechanical tension, which regulates adhesion and tension forces of the cells. Here, we aim to study changes at the cytoskeleton and cell tension on the substrate regulated by Gαi-2 during cranial NC cells migration. In this work, we have found that loss of function of Gαi2 subunit impairs cranial NC cell migration in vivo and in vitro and cells show longer protrusions and an increased acetylated tubulin towards the leading edge in comparison to control cells. In addition, loss of function of Gαi-2 increased the traction force of cranial NC cells and provoked changes at the localization of p-myosin. We observed interaction between Gαi2 and EB1, EB3 and tubulin. Finally, loss of function of the Gαi2 subunit inhibits Xenopus cranial NC cells migration, evidencing an abnormal cortical actin phenotype and an abnormal subcellular localization of EB1, however further studies are needed to reinforce these claims. These results together, suggest that Gαi-2 acts as a mechanotransducer controlling cytoskeleton organization to regulate cell tension during cranial NC migration.

#62
Traversari, Julia

In vitro spermatogenesis for germ cell gene editing

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The objective of this research is to develop methods to expedite the generation of genetically modified Xenopus tropicalis lines by germline gene editing, thus bypassing a generation and eliminating the mosaic effects commonly seen with embryo gene editing and enabling targeting of essential genes. These methods are also intended to reduce the number of animals required for the creation of mutant and transgenic lines.
Presently, in vitro spermatogenesis has been achieved in various aquatic species, but has remained a challenge in Xenopus frogs. In this study, germ cells were obtained from enzymatically dissociated Xenopus tropicalis testes and maintained in culture. Long-term cell survival was observed and the spermatogenic process successfully replicated in vitro. The cultured sperm cells retained fertilization potential. Furthermore, nucleofection experiments showed that spermatogonia can be transfected without excessive cell loss.
Other methods, such as spermatogonial transplantation, will also be explored in the near future. By combining gene editing with innovative techniques, streamlining the generation of genetically modified Xenopus tropicalis lines is conceivable, as underpinned by the encouraging preliminary results obtained so far.

#63
Truchado, Marta

The Rho GEF Plekhg3 is selectively required for involution movements during Xenopus Gastrulation

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The coordination of cytoskeletal activity with cell surface and adhesion behaviors underlies tissue shape changes that occur in embryonic development. To study the regulation of individual processes in Xenopus gastrulation, we focus on regulators of Rho, the Rho guanine nucleotide exchange factors (Rho GEFs). Precedents for local regulation of Rho signaling in morphogenesis include Shroom3 in neurulation and the Rho GEF Plekhg5 in bottle cell formation. Like plekhg5, the Rho GEF plekhg3 peaks in expression during gastrulation, is elevated in the marginal zone, and plekhg3 crispants showed gastrulation defects in the strength and persistence of early involution movements. Frequently, despite aberrant early gastrulation, the hoop stresses driven by normal convergence of the neural plate cause a separate buckling of the marginal zone and snap the blastopore closed. This illustrates how different force generating machines function separately and can to some extent compensate for each other. Interestingly, over expression of Plekhg3, but not Plekhg5, led to failure of cytokinesis, producing multinucleated giant cells where F-actin and Plekhg3 colocalize at abortive cleavage furrows. Plekhg3 colocalizes with Anillin, a primary component of the cleavage furrow; both Anillin and Plekhg3 also localize to the subcortical cytoskeleton, which is required globally for tissue stiffness. WeproposethatPlekhg3 regulates actin polymerization through Anillin and Rho and stiffens the tissues that support fibronectin assembly and proper involution movements. Because we observe no defects in bottle cell formation or convergence and extension, the results emphasize independent regulation of different gastrulation movements by spatially overlapping regulators. We acknowledge funding from NIH and the critical contributions of D. Shook, R. Keller, D. DeSimone, A. Straight, A. Miller, L. Davidson, C. Field and M. Kirschner groups andfunding from MIRA R35GM127069, 1R21HD107363

#64
Viviano, Stephen

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Modeling congenital hydrocephalus gene SMARCC1 in Xenopus tropicalis

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Congenital Hydrocephalus (CH), the pathological expansion of the cerebral ventricles due to cerebrospinal fluid (CSF) accumulation, is a common birth defect affecting 1 in every 770 births, with high mortality and morbidity. Current human genetics studies identified SMARCC1, required for neural stem cell (NSC) proliferation during forebrain development, as a high-confidence candidate gene in CH. The role of SMARRC1 in hydrocephalus pathogenesis is unknown. In this work, first, we depleted SMARCC1 using morpholino oligos and the CRISPR/Cas9 system and showed that Xenopus recapitulated the patient's hydrocephalus phenotype. Then, via rescue experiments, we determined that the patient variant was pathogenic. Finally, based on the bulk RNAseq analysis of the patient's fetal brain tissue, we identified altered developmental pathways linked to NSC proliferation which we further analyzed and verified in our Xenopus model, suggesting that increased pathological proliferation around the cerebral aqueduct is the initial mechanism of the obstructive hydrocephalus seen in our patients. In summary, we have developed Xenopus as a rapid, inexpensive disease model to investigate the role of CH genes in development and examine the mechanisms of CH pathogenesis caused by the candidate genes.

#65
Walker, Brandy

Dnmbp-associated vesicle transport facilitates assembly of cadherin-mediated junctions in epithelializing nephric tubules

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Disruption of nephron development is one of the many congenital anomalies that cause CAKUT, often resulting in chronic or end-stage renal disease which requires transplant. During nephron epithelialization, the formation of stable cadherin-mediated adhesion junctions is essential for maintaining cell-cell contacts. To understand the cell behaviors underlying abnormalities in renal morphology and cyst formation and to facilitate the application of novel treatments for congenital birth defects, a better understanding of the cellular mechanisms driving cell junction formation during nephron formation is needed. Given that adhesion complex components are known to be transported via membrane vesicles, we examine the role of the exocyst-associated scaffolding protein, Dynamin binding protein (Dnmbp), during junction assembly in epithelializing nephric tubules. We show that disruption of Dnmbp affects adhesion and junctional integrity of nephron progenitor cells by significantly reducing junctional E-cadherin localization when compared with standard controls. Additionally, Dnmbp-depleted nephron progenitor cells appear to have disordered membrane borders, further indicating a reduction in junctional integrity. This thesis enhances our understanding of adhesion and junctional integrity of nephron progenitor cells during epithelialization.

#66
Yamamoto, Taiyo

Investigating cyst formation in a Xenopus model for autosomal dominant polycystic kidney disease (ADPKD)

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monoallelic disorders, affecting over 0.1% of the population worldwide. It is typically caused by mutations in the PKD1 or PKD2 genes. The symptoms encompass progressively growing renal cysts leading to kidney enlargement, fibrosis, and eventually end-stage renal disease, requiring dialysis or a kidney transplant in patients.

Though one drug (Tolvaptan) has been FDA approved for treatment of ADPKD in 2018, it only marginally slows down cyst progression while further burdening patients with side effects. A reason for this lack of suitable treatment options is the incomplete understanding of the PKD gene products and the molecular events leading up to cyst development.

The unique advantages of the Xenopus model have allowed us to intravitally observe renal cyst development. Interestingly, initial kidney development is unimpeded by knockout of pkd1. It is only with the onset of flow through the pronephric ducts that cysts rapidly start forming. This cyst formation is segment independent and equally affects the whole length of the kidney tubule. Having a strictly temporally defined model of cystic onset in ADPKD allows us to perform targeted interventions and provides us with clear phenotypic readouts.

Using mutant lines, CISPR-Cas9, morpholino, and drug-based interventions, we currently aim to understand ciliary and cytoskeletal involvement in initial cyst development. On a broader scale, the developed model will allow us to efficiently screen candidate disease mechanisms, as well as potential interventions for ameliorating patient outcomes.

#67
Yartasi Tik, Elif

Modelling human microcephaly in the Xenopus model system

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Background: Microcephaly is defined as a cranium significantly smaller than the average population where the head circumference is reduced more than two standard deviations below the mean. The incidence of microcephaly ranges between 1.3-150 / 100,000 live births and can be acquired or inherited. The inherited form is primary, non-syndromal, autosomal recessive primary microcephaly and affects 1:30,000-250,000 live births worldwide. Only 28 genes have been identified to lead to microcephaly, yet the candidate gene list is growing. However, these genetic variants' screening, functional validation, and characterization have been extremely limited.

Methods: We used the frog Xenopus as our model system to evaluate the candidate genes derived from our patients with microcephaly. We used CRISPR/CAS9 system for loss of function essays and imaging we utilized optical coherence tomography (OCT) to examine the tadpole brain for microcephaly phenotype.

Results: We first analyzed a known primary microcephaly gene, ASPM, in our Xenopus model system. We showed that when ASPM was depleted, tadpoles developed microcephaly recapitulating the human microcephaly phenotype. We used two-cell injections for these experiments to deplete ASPM on one hemisphere and use the adjacent hemisphere as the internal control. Indeed, brain size measurements by OCT imaging of the mutants showed reduced brain size on the mutant hemisphere. We then analyzed our candidate gene MAG identified in a patient with primary microcephaly. MAG knockdown demonstrated a significant brain size reduction in both one-cell and two-cell loss-of-function assays, recapitulating the patient's phenotype.

Conclusions: Microcephaly remains one of the most debilitating childhood diseases, extending to adulthood with no medical treatments and clear preventative strategies. To identify medical targets, we need to understand pathogenesis better. In this work, we show that using our frog model system, we can model human microcephaly and use our model as a functional screening tool for the identified variants. As the next step, we plan to analyze the specific patient variant via rescue experiments and identify the earliest defects in neurogenesis to improve our understanding of microcephaly pathogenesis.

#68
Yoder, Mlck

Development of the vertebrate embryo requires strict coordination of a highly complex series of signaling cascades, that drive cell proliferation, differentiation, migration, and the general morphogenetic program. Members of the Map kinase signaling pathway are repeatedly required throughout development to activate the downstream effectors, ERK, p38, and JNK. Regulation of these pathways occurs at many levels in the signaling cascade, with the Map3Ks playing an essential role in target selection. The thousand and one amino acid kinases (Taoks) are Map3Ks that have been shown to activate both p38 and JNK and are linked to neurodevelopment in both invertebrate and vertebrate organisms. In vertebrates, there are three Taok paralogs (Taok1, Taok2, and Taok3) which have not yet been ascribed a role in early development. Here we describe the spatiotemporal expression of Taok1, Taok2, and Taok3 in the model organism Xenopus laevis. The X. laevis Tao kinases share roughly 80% identity to each other, with the bulk of the conservation in the kinase domain. Taok1 and Taok3 are highly expressed in pre-gastrula and gastrula stage embryos, with initial expression localized to the animal pole and later expression in the ectoderm and mesoderm. All three Taoks are expressed in the neural and tailbud stages, with overlapping expression in the neural tube, notochord, and many anterior structures (including branchial arches, brain, otic vesicles, and eye). The expression patterns described here provide evidence that the Tao kinases may play a central role in early development, in addition to their function during neural development, and establish a framework to better understand the developmental roles of Tao kinase signaling.

#69
Yoshida, Hitoshi

Efficient knock-in using a combination of Cas9-Halo tag and Halo-ligand donor in Xenopus cell lines

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Genome editing techniques have become more reliable and efficient in Xenopus. Several groups have been working to optimize efficient knock-in (KI) methods in Xenopus via homology-directed repair and non-homologous end joining. We tested whether HDR would work efficiently in our new Xenopus cell lines using several different methods. We first tested knock-in of mEGFP into the actb locus using well-known efficient donors, 5'-phosphorothionated single strand DNA (5'-PT ssDNA) and Biotinylated double strand DNA (Biotin dsDNA) in a novel Xenopus tropicalis cell line XTN-10. 5'-PT ssDNA and Biotin dsDNA provided 5-320 and 2-186 GFP-positive colonies, respectively. Targeting a second locus, tuba1b with these methods proved much less efficient with under 30 GFP+ cells. The low efficiency at tuba1b locus was also observed in two other X. tropicalis cell lines, XTN-8 and XTN-12. To improve KI efficiency we used a Cas9-Halo tag with chloroalkane modified primers in the XTN-10 cell line. At the actb locus, chloroalkane-dsDNA donor increased the number of GFP-positive colonies (519-547 colonies) by 103.8-547 and 8.8-14.7 times compared to 5'-PT ssDNA (1-5 colonies) and Biotin dsDNA (37-59 colonies), respectively. Similar results were obtained in the XTN-8 and XTN-12 cell lines. These findings suggest that novel Xenopus tropicalis cell lines are useful for optimizing knock-in experiments and that use of Cas9-Halo tag and appropriate donor DNA was much more efficient than other approaches.

#70
Yourston, Liam

Mitotic Oscillator Precision is Governed by ATP-Dependent Free Energy Dissipation: Insights from a Cell-Free Microfluidic System

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Cell cycles in early Xenopus Laevis embryo exhibit remarkable precision. This consistent periodic behavior is a result of a tightly regulated Cdk1/cyclin B1 network called mitotic oscillator, which orchestrates the progression of many downstream mitotic events. The molecular interactions involved in this network are therefore responsible for maintaining the precision of cell cycles despite intrinsic and environmental noises. However, sustaining such a precise oscillating biochemical system outside of equilibrium necessitates a cost in free energy. While previous theoretical work has shed light into the cost- performance tradeoff for noisy oscillations, experimental evidence remains limited. To this end, we utilized microfluidics to make droplets of Xenopus egg cytosol extract capable of mitotic oscillations, and quantified cell cycle signals with a Cdk1 FRET biosensor. By performing high throughput studies, we present quantitative information regarding cell-cycle dynamics and oscillation signal precision in variable metabolic landscapes and under signal network modifications.

#71
Yun, Seongmin

NF1 inactivation in tetraploid Xenopus laevis for modeling neurofibromatosis type I with low-temperature-active engineered Cas12a

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Gene knockout using the CRISPR/Cas system revolutionized forward-genetic studies in model organisms due to its designability targeting almost all genetic elements with minimal limitation. Although SpCas9 (Cas9 derived from Streptococcus pyogenes) is still the most popular CRISPR/Cas system for genome editing, another CRISPR/Cas system with Cas12a (Cpf1) has expanded its application. However, it was hindered from being used in Xenopus, because of its low activity at the temperature Xenopus is normally raised (less than 25 °C). Recently an engineered Cas12a called Cas12a-Ultra was developed, which improves the in vivo endonuclease activity with less temperature dependency. Here we evaluated the performance of LbCpf1-Ultra in Xenopus laevis embryos. We first confirmed that they were more active in the low temperature that the X. laevis embryo is mostly raised (20~22 °C) based on disrupting the tyrosinase enzyme (tyr) that generates albino-like phenotypes.

We employed LbCpf1-Ultra to establish a model for a rare disease. Neurofibromatosis type I (NF1) is a rare single-gene disease that leads to complex tumors composed of axonal processes in humans. Embryos with inactivated Nf1 gene by LbCpf1-Ultra commonly displayed a reduction in head size and iris abnormalities. Around stage 48-49, we observed the formation of irregular lumps along the spinal cord at regular intervals, starting from the periphery of the brain. Our findings demonstrate the remarkable efficacy of LbCpf1-Ultra in achieving efficient gene inactivation at low temperatures, thereby enabling the development of an X. laevis model for NF1 targeting the Nf1 gene. These results hold significant implications for both the advancement of genome editing techniques in Xenopus and the study of Neurofibromatosis.

#72
Okumura, Akinori

Molecular definition of the stepwise model in amphibian limb regeneration: distinct transitions of transcriptome between frog and newt

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Frog limb regeneration is incomplete and only forms a cone-shaped cartilage structure called a "spike" after limb amputation, whereas salamanders completely regenerate their limbs. It remains unknown what causes the differences in regenerative capacity between frogs and salamanders. From previous reports, two important steps in the limb regeneration process are described. One is the nerve-dependent formation of blastema which consists of dedifferentiated cells after wound healing, and the other is morphogenetic (growth and pattern formation) induction by the developmental cues from the surrounding tissues. This process of limb regeneration is proposed as “the stepwise model” (Endo et al., 2004). Molecular characterization of the branching points in the model is essential for understanding amphibian regenerative ability. Therefore, to elucidate the continuous gene expression dynamics in limb regeneration, we performed large-scale RNA-seq analysis in Xenopus laevis (frog) and Pleurodeles waltl (newt) up to 15 days after amputation. Pseudotime analysis revealed molecular transition during regeneration process and identified clusters corresponding to the distinct steps in the stepwise model. Additionally, through a comparative analysis, steps that potentially contribute to the bifurcation point of regenerative capabilities, to regenerate or not regenerate, were discovered. These findings provide us with valuable insights into the molecular mechanisms underlying limb regeneration in amphibians. Based on the finding that differences between frogs and newts, we would like to discuss whether limb regeneration ability in anurans is an intermediate state between mammals and urodeles.

#73
Chu, Chih-Wen

Mechanisms setting up two planar cell polarity axes in the vertebrate neural plate

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Vertebrate neural plate exhibits clear planar cell polarity (PCP) necessary to coordinate tissue remodeling during neural tube closure. We have previously shown that Rab11, a small GTPase required for protein recycling, is planar polarized toward the neural plate midline (ML-PCP) in a way distinct from the anterior-posterior polarization of core PCP proteins such as Vangl2 (AP-PCP), suggesting the existence of two orthogonal PCP axes in the neural plate. We now show that Ssx2ip, a microtubule anchoring factor, is also planar polarized along the mediolateral axis like Rab11. Ssx2ip and Rab11 were colocalized as cytosolic puncta and pulled down together by immunoprecipitation. While Rab11 exhibits both ML-PCP and AP-PCP in the neural plate, Ssx2ip is specifically required for the ML-PCP. Surprisingly, Ssx2ip depletion also disrupted Vangl2 anterior polarization in the anterior but not posterior neural plate. Importantly, Ssx2ip became polarized in response to actomyosin-dependent tensions from the neighboring cells. These observations point to spatially and molecularly distinct PCP signaling mechanisms in the neural plate. We propose that while Wnt signals from the posterior end instruct the AP-PCP of the posterior neural plate, the ML-PCP is driven by actomyosin-dependent mechanical forces and involves Ssx2ip. Our findings also highlight potential cross-talks between polarized microtubule arrays and vesicular trafficking during neural tube closure.

#74
Robert, Jaques

Microplastic Water Contaminants Impact on Amphibian Xenopus Development and Immunity

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Abstract:
Small plastic debris (0.1 μm to 5 mm) or Microplastics (MPs) have become major pollutants of aquatic ecosystems worldwide and studies suggest that MPs exposure can pose serious threats to human health. We are leveraging the amphibian Xenopus laevis to define the biodistribution of representative cryomilled virgin plastics and experimentally aged MPs; and determine whether postembryonic exposure to these MPs induce perturbations of development, fitness, immune homeostasis, and antimicrobial immunity. Our preliminary study indicates that short-term exposure to 2 to 100 μm fluorescently labeled polystyrene (PS) and polyethylene terephthalate (PET) result in detectable MP biodistribution in intestine, gills, liver and kidney as determined by by fluorescence microscopy on whole mount tissues. MP accumulation rate in tissues is further evaluated via a novel in situ enzymatic digestion and subsequent filtration using silicon nanomembranes. Longer exposure (1 month) of tadpoles to these MPs do not result in marked developmental and morphological alterations. However, exposure to PET (2.5 mg/L) significantly increase tadpole susceptiblity to ranavirus infection and alters antiviral immune response. In vitro studies also indicate that both PS and PET MPs are readily phagocytosed by adult frog peritoneal macrophages. Effects of MPs on macrophage biology (survival, polarization, and resistance to viral infection) are currently under investigation.

#75

Godwin, Annie

Functional genomic studies in Xenopus tropicalis can be used to inform clinical interventions in rare genetic diseases.

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High throughput, next generation DNA sequencing has revolutionised understanding of human genetic diseases by enabling clinical research to identify causal variants within a gene. For many patients, the link between the gene identified by sequence comparisons and their disease is insufficiently robust for clinical decision making. With the backing of the European Xenopus Resource Centre, we have established a UK government funded multidisciplinary team of clinicians, geneticists, bioinformaticians and developmental biologists focused on discovering the genetic and molecular basis of rare diseases in the South of England.

Here we report some recent findings arising from this collaboration using the model organism Xenopus tropicalis to test genotype-phenotype correlations in syndromic models of dysmorphology, intellectual disability and developmental syndromes. We show how F0 CRISPR screens in Xenopus tropicalis can test the genotype-phenotype correlations of candidate variants of unknown significance and prioritise those suitable for precision modelling. We also compare findings between the F0 and F1 CRISPR generations in autosomal dominant disease presentation, demonstrating the creation of stable lines for elucidating the developmental origin of disease, studying cellular or molecular mechanisms in greater detail and screening novel or re-purposed therapeutics. This work includes developing a pipeline to screen phenotypes in these disease models including high-resolution imaging techniques and novel behavioural parameters of working memory, anxiety, and motility in tadpoles, that are comparable to higher vertebrates. Collectively, these results not only strengthen disease modelling in Xenopus tadpoles but also advance knowledge of human gene function.

#76
Guille, Matt

European Xenopus Resource Centre (EXRC) Poster

#77
Ahsan, Arifa

Optimization of the BONCAT method to investigate Neuronal Membrane Proteasomes (NMPs)- mediated degradation of nascent proteins in response to enhanced visual experience in the optic tectum of Xenopus laevis tadpoles

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Proteostasis plays critical roles in cellular functions. In neurons, plasticity-inducing neuronal activity is known to upregulate protein synthesis. However, our understanding of how these activity-induced nascent proteins are regulated remains incomplete. Neuronal membrane proteasome (NMP) is a recently discovered neuronal proteasome found to specifically degrade activity-induced nascent proteins both in vitro and in vivo under pharmacologically stimulated conditions. To investigate how NMPs function under physiologically relevant conditions, we took advantage of the developing retinotectal circuit in Xenopus laevis tadpoles, which is a well-established, robust experimental system for studying visual experience- dependent plasticity mechanisms. The unique features of the tadpole model allow direct intraventricular injections and the injected reagents can diffuse within minutes to targeted brain regions for fast spatial and temporal control. We optimized the BioOrthogonal Noncanonical Amino Acid Tagging (BONCAT) method to effectively label nascent proteins synthesized in live tadpole brains within a period as short as 30 minutes for quantitative analysis. This allows us to delineate the in vivo dynamics with unprecedented temporal resolution of activity-induced protein synthesis in response to a brief period of enhanced visual experience (VE). With an NMP-specific inhibitor, we were also able to pinpoint distinct time periods when NMP- mediated nascent protein degradation is recruited by VE. Additionally, by combining BONCAT with protein synthesis inhibitors, we implemented a time-stamping protocol to distinguish between pre-existing and nascent proteins, and demonstrated that NMPs preferentially degrade nascent proteins in vivo. This protocol offers a powerful new tool to study activity-dependent proteostasis in whole animals.

#78
Bestman, Jennifer

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Connecting mitochondrial dynamics to the fate of radial glial neural progenitor cells in the Xenopus laevis optic tectum

Radial glial neural progenitor cells (NPCs), slender cells that contact both the ventricle and the outer pial surface, are the neural stem cells in the tadpole optic tectum. Early on, these highly polarized cells divide symmetrically to expand this progenitor pool, but by stage 46, NPCs have begun to divide asymmetrically to produce neurons. Levels of neurogenesis are plastic in Xenopus, and enhancing the visual experience of the tadpoles promotes the shift to neurogenesis. We hypothesize that NPC mitochondria serve as a signaling hub between extrinsic signals and the cell's irreversible commitment to asymmetric cell division. A feature of stem cells is their reliance on non-mitochondrial ATP production, yet they contain ample, morphologically complex and motile mitochondria. We are interested in whether NPCs, like other types of stem cells, compartmentalize mitochondria leading up to cell division and through their distribution, influence the fate of the cells' progeny. We use in vivo time-lapse confocal microscopy to capture fluorescent protein-labeled NPCs, their mitochondria, and their progeny. This type of longitudinal time lapse data revealed that mitochondria are not equally inherited as the NPCs divide. Using photo-activatable GFP to label subpopulations of mitochondria in NPCs reveals high levels of mitochondria fusion and organelles that migrate >100 μms to reach the site of cytokinesis. Our manipulations of key transcription factors to increase or decrease mitochondrial biogenesis result in changes in the expected levels of asymmetric, neurogenic cell division. Together our data suggest that mitochondria contribute to the fate of NPCs.

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