THE ART AND DESIGN OF GENETIC SCREENS: *CAENORHABDITIS ELEGANS*

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The nematode *Caenorhabditis elegans* was chosen as a model genetic organism because its attributes, chiefly its hermaphroditic lifestyle and rapid generation time, make it suitable for the isolation and characterization of genetic mutants. The most important challenge for the geneticist is to design a genetic screen that will identify mutations that specifically disrupt the biological process of interest. Since 1974, when Sydney Brenner published his pioneering genetic screen, researchers have developed increasingly powerful methods for identifying genes and genetic pathways in *C. elegans*.

In 1963, Sydney Brenner realized that the central dogma of molecular biology — that DNA makes RNA makes protein — had been outlined in form, and was casting about for the next big problem to tackle. Brenner concluded that the organizing principles of multicellular organisms were the mysteries that scientists must confront in the future. Specifically, he believed that the nervous system and embryonic development were the most exciting areas to study in biology. Brenner decided that a new experimental organism was needed for a genetic approach to these problems (reviewed in REF. 1). He chose the nematode *Caenorhabditis elegans* (FIG. 1).

One criterion for selecting a genetic organism is that it should be the simplest organism that has the traits of interest. Brenner thought that the simplicity of *C. elegans* (BOX 1) would make the worm the T4 phage of metazoan studies. A second guideline in the selection of model organisms is ease of manipulation. *C. elegans* is small, and can be grown either in small Petri dishes or in liquid culture if large numbers are required. It has a three-day generation time at room temperature, and strains can be kept as frozen stocks. In 1967, Brenner carried out his first mutagenesis and, in 1974, he published the characterization of ~100 genes that generated viable, visible phenotypes[2]. The screening approaches that are described in this landmark paper remain a guide for the design of simple genetic screens, as well as for the more elaborate ones that have since been developed.

In this review, we first discuss simple screens that are used routinely in worm laboratories. These simple screens can be conducted in a mutant strain to identify suppressor or enhancer mutations and, thereby, genes that are involved in a specific process. Second, we discuss screens that are designed to identify mutations in a particular biological process. Some of these have used selection procedures to accelerate the identification of mutants, whereas others have required a great deal of labour to identify the relevant mutants. Finally, we discuss screens that are likely to be used more often in the future; specifically, sensitized screens and screens for redundant genes.

Simple screens

Most of the mutations that were identified in Brenner's paper were visible, recessive mutations. Such mutations can be identified using a simple F_2 screen (FIG. 2). In these screens, a mutagen, such as ethyl methane sulphonate (EMS), is used to induce mutations in the sperm and oocytes of wild-type hermaphrodites (how this is done in practice is outlined in BOX 2). The mutagenized worms are placed on Petri dishes and grown for two generations to produce homozygous mutants. Worms that show a mutant phenotype are then transferred

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Figure 1 | **Scanning electron micrograph of a *Caenorhabditis elegans* hermaphrodite.** (Photo courtesy of Juergen Berger and Ralf Sommer, Max Planck Institute, Tuebingen, Germany.)

individually to new plates to determine whether their phenotype is transmitted to the next generation. In a typical screen that lasts about two weeks, 12,000 copies of any particular gene can be assayed. Using standard concentrations of mutagen, the frequency at which mutations at any particular locus are recovered is about one null mutation for every 2,000 copies of the gene that is analysed in the screen. So, in a typical screen of 12,000 haploid genomes, we would expect to recover six mutations in a particular gene.

Using this approach, Brenner identified 619 mutants with visible phenotypes. Of particular interest was the uncoordinated class, which failed to move normally. Movement is largely dispensable for the worm under laboratory conditions because food is plentiful and sex is superfluous. Therefore, this kind of screen is perfectly suited for identifying proteins, such as the homeodomain transcription factor UNC-30 (uncoordinated 30) that regulate neuronal fate[3]. UNC-30 is required for several aspects of the differentiation of certain GABA NEURONS; the muscles of these mutants can contract, but have difficulty relaxing owing to the lack of inhibitory neurotransmission. Brenner also identified worms with defects in neuronal pathfinding — for example, those caused by loss of the graded extracellular signal UNC-6/netrin[4,5]. As a result, these mutants have severe defects in the axon outgrowth of the motor neurons and move poorly.

Brenner's screen was designed primarily to show that visible mutants could be obtained in the nematode. In addition to the uncoordinated class, Brenner identified mutants with small bodies, blistered cuticles, twitching muscles or rolling locomotion. He also identified mutants with long bodies, dumpy bodies, forked heads or bent heads. The variety of phenotypes that was observed by Brenner answered the criticism that it would be impossible to obtain enough useful genetic markers in an organism that was shaped like a pipe. One decade later, molecular tools were added to the geneticist's toolbox for the characterization of genes. Specifically, the genome was subcloned and sequenced, and straightforward transgenic techniques were developed (BOX 3).

GABA NEURON
A neuron that releases the inhibitory neurotransmitter GABA (γ-aminobutyric acid).

Identifying genetic pathways

A hunt for a mutant is, by nature, a random process. Although geneticists have preconceived ideas of what phenotypes are expected to be recovered in a screen, they are usually surprised at the actual phenotypic landscape of the organism. However, the presence of a particular mutant phenotype provides a geneticist with a point of entry into a biological process. Once the phenotype that is associated with a particular biological process is known, more genes that are involved in that process can be identified in two ways: using additional simple screens and using modifier screens.

Simple screens. The most straightforward method for identifying more genes that function in a given process is to screen for more mutants with the same phenotype. For example, John Sulston and H. Robert Horvitz[6] identified mutants with defects in the differentiation of the vulva from epidermal cells on the ventral surface of the animal. These mutants were identified as strains that either lacked a vulva ('vulvaless') or had ectopic vulvae (that is, showed the 'multivulva' phenotype; FIG. 3b). Chip Ferguson, who was working with Horvitz, subsequently isolated 95 mutations that caused these phenotypes[7], comprising a total of 22 genes. Subsequent molecular studies revealed that mutants with vulvaless phenotypes defined two signalling pathways — the epidermal growth factor (EGF)/RAS pathway and the Notch signalling pathway[8].

Modifier screens. Another way to identify components that function in a genetic pathway is to use modifier screens — that is, enhancer or suppressor screens. The starting material in such a screen is a strain whose genetic composition causes a defined phenotypic defect. Second-site mutations that either enhance (worsen) or suppress (ameliorate) that phenotype can then be screened. These second-site mutations frequently identify proteins that are involved in the same process as that disrupted in the starting strain. Historically, suppressor screens are more important than enhancer screens in *C. elegans* research. However, enhancer screens are likely to become more important in the future (as discussed below) because they are more generally applicable and because they are inherently more powerful. Typically, many genes can enhance a mutant phenotype, whereas only mutations in a few key regulators can suppress a mutant phenotype.

In some cases, mutations in a protein constitutively activate a signalling cascade. For example, activating mutations in the RAS GTPase cause a multivulva phenotype[9,10]. Second-site mutations that disrupt proteins in the signalling cascade that is initiated by activated RAS suppressed the formation of the ectopic vulvae and identified proteins in the MAP (mitogen-activated protein)

Box 1 | **Morphology and life cycle of *Caenorhabditis elegans***

Adult worms

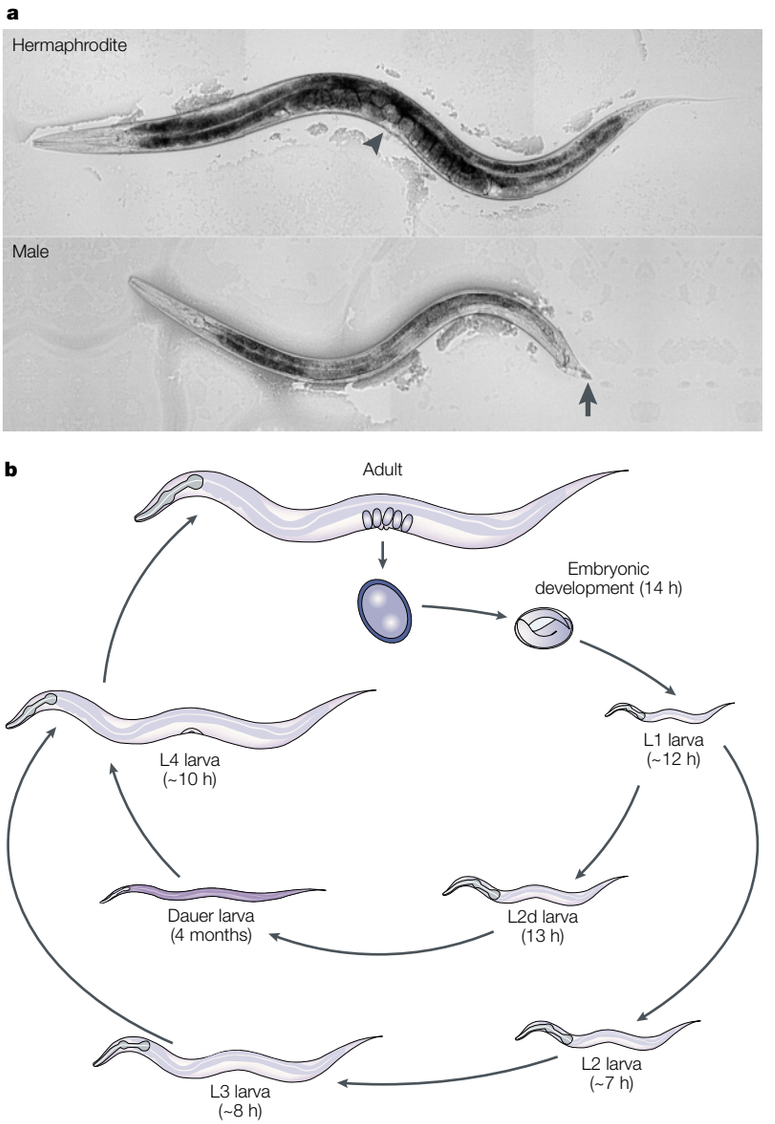
The *Caenorhabditis elegans* hermaphrodite comprises 959 somatic nuclei. The worm has many of the tissues and organs of more complex animals (such as muscles, nervous system, gonad, epidermis and gastrointestinal tract), but each is radically simplified. For example, the worm has only 302 neurons and 95 body-wall muscles[102]. This simplicity, coupled with the stereotyped development of *C. elegans*, allows researchers to track biological processes at the resolution of single cells. However, this streamlined anatomy also limits its use for studying tissues that are absent from the worm — for example, bones or the immune system.

An unusual feature of this nematode is that it can be maintained as a hermaphrodite (**a**, top panel), which means that an interesting mutant can be transferred to a fresh plate and, in three days, the self-progeny can be inspected to see if the phenotype breeds true. However, males (**a**, bottom panel) are also produced and are essential for moving mutations between strains. Hermaphrodites are determined by the presence of two X chromosomes. Males, which develop from an XO karyotype, are occasionally generated spontaneously by non-disjunction of X chromosomes during meiosis. Hermaphrodites can be distinguished by the presence of a vulva (arrowhead); males can be distinguished by the fan-like tail (arrow).

Life cycle

The embryo develops through a series of invariant cell divisions that occur during the first 5 h of embryonic development at 25 °C (**b**). John Sulston documented these cell divisions and determined the fate of all the cells that were generated from these divisions[103] — an analysis that led to a description of the cell lineage and FATE MAP of the worm.

After about 14 h of development in the egg case, the larva hatches from the eggshell. The animal then passes through four larval stages (L1–L4) that are separated by a period of lethargus, during which the animal sheds its old cuticle. Under crowded conditions and with limited food, the L1 larvae can enter an alternative developmental programme called the dauer stage, in which the animal can survive for months under harsh conditions. The genetic analysis of dauer development has been a rich area of study[104].



kinase signalling pathway[11–18]. Suppressor screens for loss-of-function alleles have also been used to analyse a pathway. Mutations in the negative regulators of a pathway cause constitutive activation of the signalling cascade. For example, the *lin-15* (*lineage 15*) OPERON negatively regulates the RAS pathway (FIG. 3a). Loss-of-function mutations of this operon result in a multivulval phenotype by allowing constitutive activation of EGF-receptor signalling. Screens for second-site mutations that suppressed the multivulval phenotype of *lin-15* mutations identified components upstream and downstream of RAS in this signalling cascade, including mutations in the EGF tyrosine-kinase receptor LET-23 (lethal 23), the adaptor protein SEM-5 (sex muscle abnormal 5), the guanylyl-nucleotide-releasing factor LET-341 (also known as SOS-1) and RAS itself[19].

Negative regulatory hierarchies are particularly amenable to suppressor screens because simple loss-of-function mutations at several steps can activate the pathway, and mutations in alternating steps can suppress the activated pathway. Moreover, epistatic relationships — that is, the phenotypes of double mutants — can be used to order the genes in the pathway. The sex-determination pathway in *C. elegans* is an inhibitory cascade that results in the determination of either a hermaphrodite or a male (FIG. 3b). Mutations in genes in this pathway lead to the absence of males or the absence of hermaphrodites; for example, mutations in the *her-1* (*hermaphrodization 1*) gene transform XO males into XO hermaphrodites. But how can we spot the rare, transformed hermaphrodite on a plate that is covered with hermaphrodites? Jonathan Hodgkin conducted a

FATE MAP
The description of the cell divisions from fertilized egg to adult, linked to the eventual anatomical position of the cell in the animal and the differentiated state, or fate, of the cell.

OPERON
A locus consisting of two or more genes that are transcribed as a unit and are expressed in a coordinated manner.

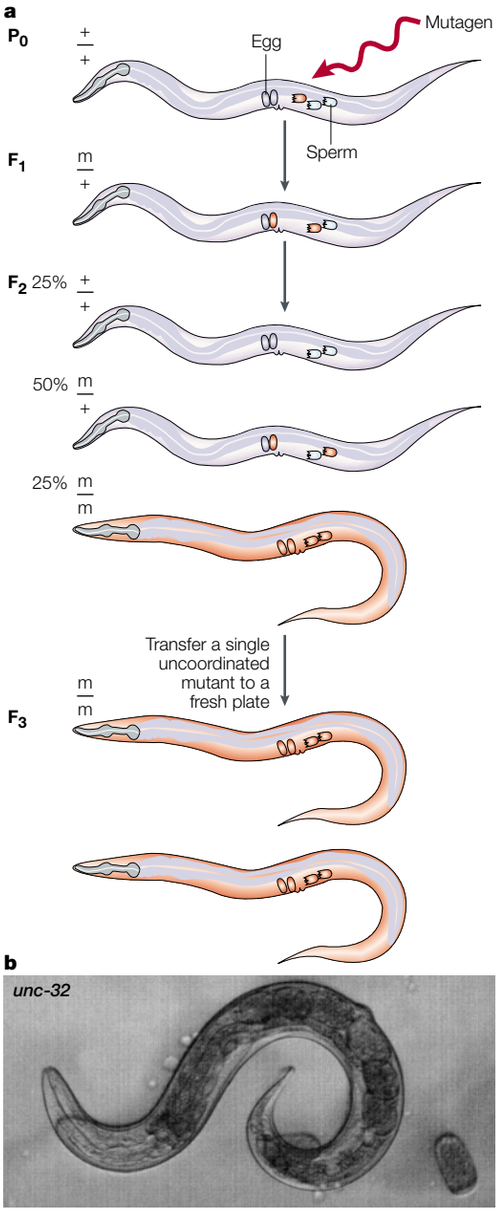


Figure 2 | **A simple F_2 screen. a** | A population of wild-type hermaphrodites is exposed to a mutagen and genes are randomly mutated in the germ cells (mutated germ cells are indicated in red). For example, one sperm could be mutated for the gene *unc-32*, which is required for the correct functioning of the nervous system. Fertilization of an egg by this sperm will result in a heterozygous F_1 individual. Because this animal is a self-fertilizing hermaphrodite, it will produce eggs and sperm that bear this mutated gene; one-quarter of its F_2 progeny will be homozygous for the mutation and result in a coiled phenotype (shown in **b**). Such an animal can be transferred to a plate, and in three days, its F_3 progeny can be inspected to determine whether the mutant phenotype breeds true. **b** | An *unc-32(f131)* mutant. *unc-32* encodes the *a* subunit of the V0 complex of the vacuolar ATPase[119] — a proton pump that generates the energy that is required for transporting neurotransmitter into synaptic vesicles. Animals that lack the proton pump do not release normal levels of neurotransmitter at the synapse and therefore have uncoordinated movement.

screen for genes that are required for male development in a *him-5* (*high incidence of males 5*) *dpy-21* (*dumpy 21*) background[20]. *him-5* causes a high frequency of XO males to be segregated due to non-disjunction of the X chromosome. *dpy-21* causes all XX, but not XO, animals to be dumpy (short and fat). Mutations in *her-1* will cause XO animals to appear as non-dumpy hermaphrodites and are easily spotted among the many dumpy hermaphrodites on the plate. The identification of mutations that transform one sex into the other can be used in subsequent screens to identify more components of the pathway. Specifically, second-site mutations can be observed that restore the presence of males or hermaphrodites. The XOL-1 protein (encoded by *xol-1*; *XO lethal 1*) specifies male development and enhances X-linked gene expression in males to compensate for their lower X-chromosomal dosage compared with hermaphrodites (FIG. 3b). *him-5 xol-1* strains produce numerous XO animals owing to the *him-5* mutation; however, these animals die because the *xol-1* mutation results in under-expression of X-linked genes. By screening for mutations that restored male viability in the *him-5 xol-1* strain — that is, for mutations that would allow the initiation of male development in XO animals and correct the levels of dosage compensation — Chad Nusbaum, Barbara Meyer and colleagues identified 21 alleles of *sdc-2* (*sex and dosage compensation 2*), which is directly downstream of, and repressed by, *xol-1* (REFS 21,22) (FIG. 3).

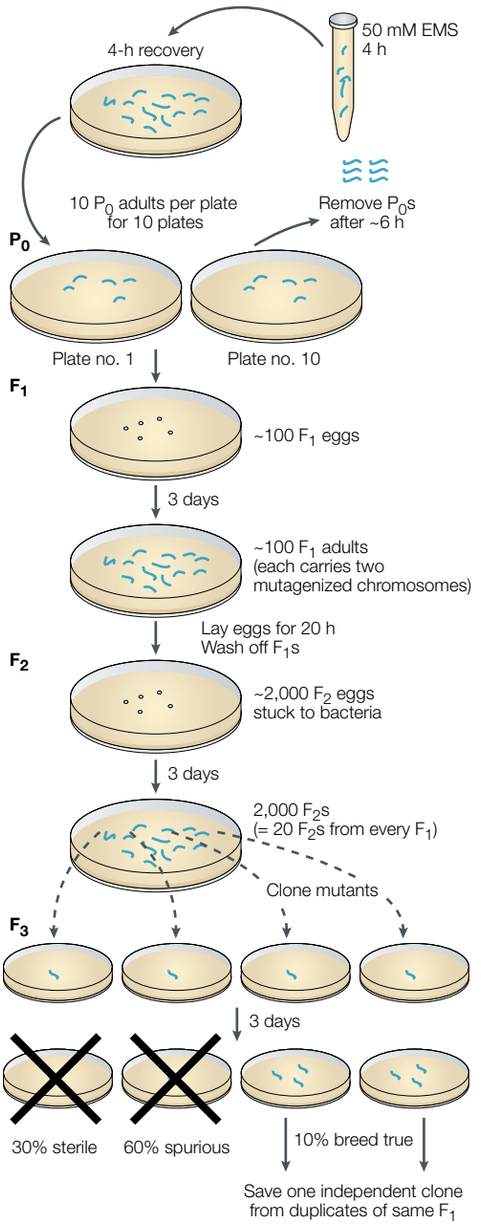
Screens from heaven

There is a painting from Pieter Brueghel that portrays a magical land in which everything is edible — houses are made of pies, chickens cook themselves and throw themselves onto the plate, and pigs conveniently carry knives tucked into them so that a piece of bacon can be cut off. Geneticists dream of this land, a land in which mutants throw themselves onto Petri dishes and require minimal effort to isolate. Such a land exists in the form of selection screens. In contrast to the simple screens described above, which require every animal to be inspected carefully for the presence or absence of a particular phenotype, selection screens facilitate the identification of the desired mutant by eliminating individuals of irrelevant genotypes.

Selections. The easiest of such screens are drug selections. For example, Brenner screened for mutants that were resistant to inhibitors of acetylcholinesterase[2]. Acetylcholine is normally removed from neuromuscular junctions by the enzyme acetylcholinesterase. Drugs such as the nerve gas Sarin or the pesticide Aldicarb kill an organism by blocking the degradative enzyme. In wild-type animals that are treated with Aldicarb, acetylcholine builds up in the synaptic cleft and eventually kills the animal by chronic excitation. Mutants that cannot release acetylcholine into the synaptic cleft cannot accumulate the neurotransmitter in the presence of the drug, and so these animals survive. Using this screen for Aldicarb-resistant mutants, Brenner identified mutations in a gene, *unc-17*, that is required to transport

Box 2 | **Protocol for EMS mutagenesis in worms**

Worms are manipulated in the following manner for a typical F_2 screen. After mutagenesis, about ten worms (P_0 generation) are transferred to large Petri plates and allowed to lay ~100 F_1 progeny. The P_0s are then removed and the F_1s grow to adulthood. After one day of egg laying, the F_1 parents and any hatched F_2 progeny are washed off. However, the F_2 eggs stick on the plate, leaving a 14-h cohort of F_2 progeny to survey. So, a fraction (~20–30 individuals) of the 250 potential progeny from any particular F_1 are sampled. One-quarter of these 20 progeny will be homozygous for the mutant chromosome, and so about five individuals that are homozygous for any mutation that occurred in the germ line of the P_0 are expected. Because each plate of worms descends from 100 F_1s, 200 copies of a particular locus can be assayed for mutations. Using typical concentrations of a mutagen (for EMS (ethyl methane sulphate) this is 50 mM), 2,000 copies of a gene need to be screened to find a mutation. So, about ten plates of worms need to be inspected to find a mutation in the gene of interest. See also REF. 105. The F_2 progeny are inspected for the phenotype of interest and candidate mutants are removed to a fresh plate to evaluate if the character breeds true.



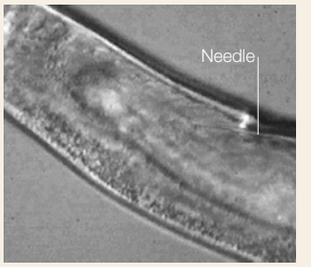
acetylcholine into the synaptic vesicle[23]. Many other Aldicarb-resistant mutants have been isolated, including mutants for the kinesin that transports synaptic vesicles to the synapse[24], proteins that are involved in synaptic vesicle exocytosis[25–27] and proteins that are involved in endocytosis[28,29].

Selections for suppressors. Screens that are suppressor screens as well as selections are particularly powerful. In some cases, the initial genotype can be engineered into the strain by expressing proteins that constitutively activate a pathway. For example, the trimeric G protein $G_s\alpha$ can be locked in the active form by mutating the GTPase domain. The expression of activated G_s under the control of a heat-shock promoter[30] or a neuron-specific promoter[31] causes neuronal degeneration, which induces death or paralysis in the animal. In theory, mutations that suppress this phenotype, by restoring viability or movement to these animals, could be found. Specifically, mutations in downstream components in the pathway — that is, in genes that encode proteins required for neuronal death — were identified as second-site mutations that restored cell viability. Suppressors of neuronal degeneration showed that necrotic cell death acts through cyclic-AMP signals because adenylyl cyclase is required for normal levels of G_s-mediated cell death.

Benefits of large-scale screens. In the best cases, suppressor and selection screens in *C. elegans* can be designed to survey millions of animals. Screens of this magnitude are a powerful means to define genetic pathways for three reasons. First, saturation for genes that can be mutated to give a specific phenotype can identify many or all of the components in a particular pathway. The presence of many alleles in all complementation groups shows that the screen is saturated and suggests most of the components of a genetic pathway have been identified. Importantly, however, even saturation screens can miss some components. For example, a gene that functions in more than one pathway might be missed because it is associated with a different phenotype than the specific phenotype that is being selected. Alternatively, certain genes might be small targets for mutagenesis. *lin-4* is a 22-nucleotide functional RNA that regulates the timing and order of larval stages during development[32]. Only two alleles of *lin-4* have been isolated, despite extensive screening. Finally, the function of some proteins might be redundant with other proteins that are encoded elsewhere in the genome; null mutations in a single gene would therefore have a wild-type phenotype.

A second advantage of large-scale screens is that they can identify unusual mutations that arise less frequently than null mutations. For example, null mutations for most components of the RAS signalling pathway are lethal. However, partial loss-of-function alleles are associated with viable, vulvaless phenotypes that allowed researchers to define components of the RAS pathway[7]. Large-scale screens can also identify gain-of-function alleles. Such alleles might be very rare as they might require changes in specific amino acids; for example, five gain-of-function alleles of RAS have been isolated that activate the protein, but all five cause an identical amino-acid change[18]. Large-scale suppressor screens can also be carried out to obtain allele-specific suppressors. Such suppressors can identify proteins that interact physically with each other because altered conformations of a protein can be suppressed by compensating mutations in its partner. Normally, the size of screens that is necessary to identify such specific changes precludes their use in higher eukaryotes, unless the suppressor screen is designed as a selection. David Miller and co-workers[33] identified a protein that interacts

Box 3 | **Cloning in *Caenorhabditis elegans*: moving from mutation to gene**



Identifying the molecular nature of mutated genes relies on three criteria: a consistent map position, sequenced mutations and, most importantly, rescue of the mutant phenotype by microinjection. The cosmid library of the entire worm genome, which was created by Alan Coulson and John Sulston in 1986 (REF. 106), was enormously useful for identifying mutated genes by microinjection rescue. DNA can be injected into an oocyte to form large, stable concatamers called extrachromosal arrays[107]. So, a gene can be identified by its ability to rescue (complement) the mutant phenotype when it is introduced into the oocyte of a mutant animal (see photo). Image courtesy of Elizabeth B. Davis, Paul J. Muhlrad and Samuel Ward, University of Arizona, USA.

The choice of mutagen can have an effect on how rapidly a gene can be cloned. Brenner mutagenized the worm genome with every conceivable mutagen, but researchers converged on ethyl methane sulphonate (EMS) because of its relative efficiency and low toxicity. However, mapping an EMS-induced mutation can be difficult if the phenotype of the gene or the neighbouring markers is difficult to score. Mapping point mutations has been made easier by taking advantage of the many single-nucleotide polymorphisms between the Bristol and Hawaiian strains of *Caenorhabditis elegans*[108]. Gene cloning can also be made easier by tagging genes during mutagenesis with transposons. Some of the first gene-cloning methods used the endogenous *Tc* transposons[109]. However, the background number of endogenous transposons makes it complicated to identify the transposon of interest. *mariner* elements from *Drosophila melanogaster* have been successfully mobilized in *C. elegans*[110], so that the relevant transposon insertion is unique in the mutant strain. However, *mariner* elements are not as flexible as *P*-elements in *Drosophila* and are not useful for introducing large DNA fragments into the worm genome.

The discovery of RNA INTERFERENCE[111–113] has been very useful for analysing the worm genome, particularly now that the whole genome sequence is available. Although such studies do not rigorously determine gene function, the method can be used to rapidly estimate the function of a gene and identify candidates for more complete studies[114].

Gene-targeting methods now exist for yeast, flies and mice, but worm geneticists have no such method for selectively mutating a gene. However, researchers have overcome this limitation by using brute force. Worms can be randomly mutated with a mutagen that causes deletions and can then be maintained as a library of small pools. DNA from a fraction of each pool is prepared and populations of worms with deletions in the gene of interest can be identified using specific PCR primers for that gene[115,116]. Under standard conditions, a deletion in a gene of interest is generated at a frequency of ~10^{-6}, which, in practice, means that a deletion allele will typically be found by screening ~200 96-well plates, with each well holding DNA from 40 haploid genomes[117,118].

physically with the homeodomain protein UNC-4. *unc-4* mutants cannot crawl backwards because of defects in synaptic connectivity. By searching for mutants that could suppress a conditional allele of *unc-4*, Miller identified gain-of-function mutations in *unc-37*. As expected, these unusual mutations in *unc-37* arose at a very low frequency: Miller found only four suppressor mutations after screening 10^6 haploid genomes, which is one thousandfold lower than the frequency of obtaining typical loss-of-function alleles. To make it feasible to survey such a large number of animals, Miller began with a strain carrying two mutations that together induced a 'stuck' phenotype — *unc-4* (cannot move backwards) and *unc-24* (cannot move forwards) — and searched for worms that could move backwards towards a distant source of food. Importantly, the gain-of-function *unc-37* alleles that were isolated in this screen could not suppress a null allele of *unc-4*, which shows that suppression was not due to a bypass of *unc-4* activity. Moreover, the phenotype of loss-of-function mutations in *unc-37* resembled those associated with mutations in *unc-4*. Subsequent studies have shown that UNC-37 is a homologue of the *Drosophila melanogaster* gene known as *groucho*, and is a co-factor for UNC-4; together these two proteins regulate transcription in neuronal cells[34].

Third, large-scale screens can be used for the structure–function analysis of a protein. For example, the analysis of the 16 mutations of SDC-3 revealed that this protein mediates dual functions[35]. The zinc-finger domain is required for downregulating the expression of genes on the two X chromosomes in hermaphrodites to match the gene dosage provided by the single X chromosome in males (FIG. 3b). A second domain in SDC-3 is similar to the ATP-binding domain of myosin and is required in hermaphrodites for sex determination. A second example of structure–function analysis comes from Andrew Chisholm's lab, which isolated and characterized many alleles of the ephrin receptor *vab-1* (*variable 1*), and its ligand *vab-2*. This work indicated that VAB-1 might be both a receptor and a ligand[36,37]. Eliminating the kinase domain of VAB-1 resulted in a weak phenotype compared with the null allele, which reveals that VAB-1 has functions that are independent of its kinase activity. Eliminating VAB-2 ephrin, the ligand for VAB-1, also produced a weak phenotype. Only by removing both VAB-1 kinase activity and VAB-2 ephrin were these researchers able to regenerate the VAB-1-null phenotype. Chisholm concluded that the VAB-1-null phenotype reflected a loss of intracellular signalling through the VAB-1 kinase domain and a loss of intercellular signalling that uses the ephrin ligand as a receptor. Structure–function studies of mutations in endogenous genes have an added benefit of

RNA INTERFERENCE (RNAi). A process by which double-stranded RNA silences specifically the expression of homologous genes through degradation of their cognate mRNA. In worms, a gene can be selectively disabled and its phenotype determined simply by feeding wild-type animals double-stranded RNA.

ANCHOR CELL
A somatic cell in the gonad that induces vulval development in the underlying epidermal cells.

being expressed appropriately. By contrast, exogenous constructs are often expressed at a non-physiological level, time or place.

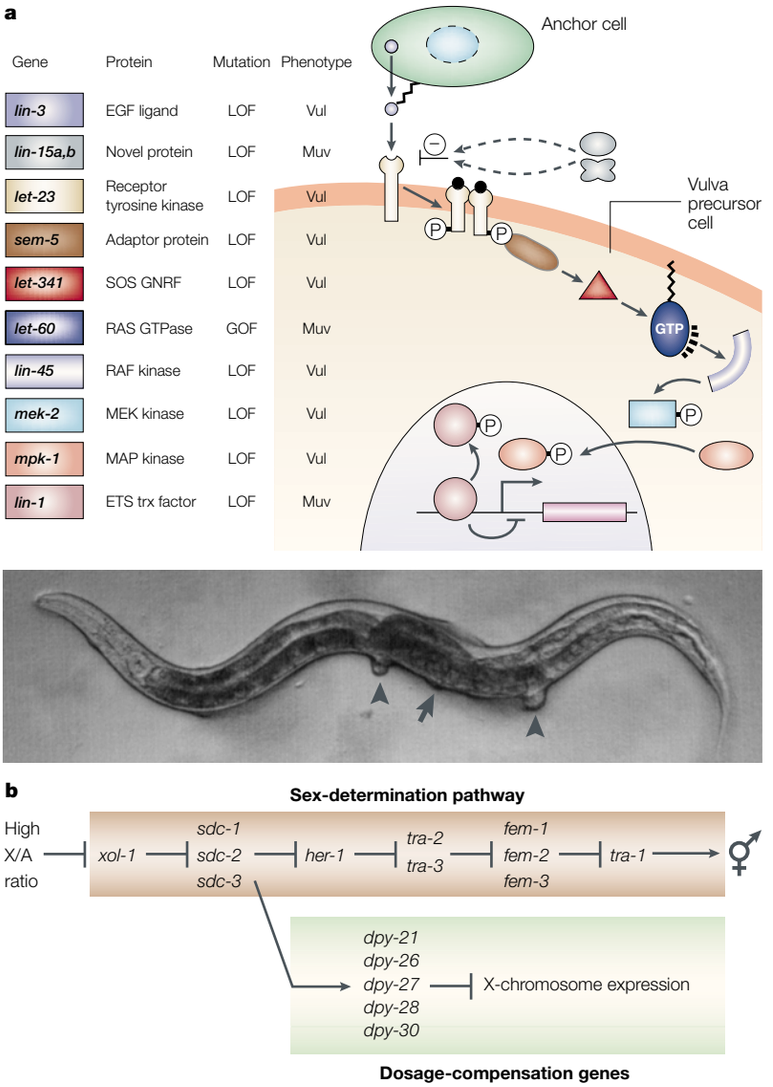


Figure 3 | **Signalling cascades. a** | The Ras signalling pathway is required for the formation of the vulva (the absence of the vulva is known as the vulvaless phenotype, Vul). The epidermal growth factor (EGF) ligand produced by the ANCHOR CELL binds the receptor tyrosine kinase on one or a few of the five vulval precursor cells, and initiates a signal-transduction cascade that culminates in the inactivation of the LIN-1 transcriptional inhibitor. Constitutive formation of the vulva by all of the vulval precursor cells can be caused by a gain-of-function (GOF) mutation in Ras or by loss-of-function (LOF) mutations in, for example, both *lin-15a* and *lin-15b* (the two *lin-15* isoforms act redundantly). Constitutive activation of the Ras pathway causes the formation of pseudovulvae along the ventral surface (arrowheads; the arrow points to the true vulva). This multivulval (Muv) phenotype can be suppressed by lof mutations in downstream components of the pathway. **b** | The sex-determination pathway. The ratio of X chromosomes to autosomes (A) regulates the expression of the *xol-1* gene. In hermaphrodites, which have two X chromosomes, a high X/A ratio inhibits *xol-1* expression. In the absence of XOL-1 protein, the *sdc* genes are expressed. These genes inhibit the male-determining signal HER-1 and simultaneously activate the dosage-compensation genes. *dpy*, *dumpy*; ETS, E26 transformation defective; *fem*, *feminization*; GNRF, guanylyl-nucleotide-release factor; *her*, *hermaphrodization*; *let*, *lethal*; *lin*, *abnormal cell lineage*; *map*, *mitogen-activated protein*; *mek*, *MAPK/ERK kinase*; *mpk*, *MAP kinase*; *raf*, *Ras-associated factor*; *sdc*, *sex determination and dosage compensation*; *sem*, *sex muscle abnormal*; *SOS*, *son of sevenless*; *tra*, *transformer*; trx, transcription; *xol*, *XO lethal*.

Screens from hell

Pieter Brueghel was also famous for his depiction of hell — a place where one toils forever with marginal progress. Such a place exists for geneticists as well. Not all biological processes are amenable to visible screens or selections. Some pathways have to be approached using brute force. A good example of this is the study of worms that are defective in mechanosensation. Lack of mechanosensation is not a visible phenotype. These screens, conducted by Marty Chalfie and colleagues[38], required that every worm be touched by an eyebrow hair to determine whether it failed to respond to touch. These methods identified an ion channel that is likely to be activated by mechanical stress, as well as the associated proteins that are required for transduction of the mechanical signal.

Multigenerational screens. Some phenotypes cannot be observed in the first generation of homozygotes and can be evaluated only in subsequent generations. Germ lines are immortal: these cells do not senesce like somatic cells, as they are renewed every generation. So, germ-line cells have factors that prevent the ageing that occurs in somatic cells. To identify such factors, Shawn Ahmed and Jonathan Hodgkin screened for mutants with mortal germ lines by establishing clonal lines from 400 F_2s and then picking single worms for more than ten generations[39]. They then looked for strains that became sterile after the fourth generation. This screen identified *mrt-2* (*mortal germline 2*), which encodes a DNA-damage checkpoint protein that is homologous to the *Schizosaccharomyces pombe* protein RAD1. This protein is required to prevent telomere shortening. Because the phenotype of these mutants does not appear for many generations, the identification of these genes was laborious.

Microscope screens. Many phenotypes cannot be observed using a dissecting microscope but can be assayed only under high magnification. Visual screening using differential-interference contrast optics was pioneered by Ed Hedgecock. He examined living F_2 worms that were mounted *en masse* on a slide under a siliconized coverslip. The precise location of a mutant on the slide was recorded, and the animal recovered by sliding the coverslip off and transferring the worm back onto a culture dish. These screens were the first to identify mutants that are defective in the phagocytosis of dead cells, because the presence of persistent cell corpses was visible at high magnification[40]. An adaptation of this screen was used by Ronald Ellis and H. Robert Horvitz to identify mutants that are defective for the execution of apoptosis[41,42]. The only phenotype of such mutants is the presence of extra cells in the animal. Ellis mounted mutagenized F_2 worms on slides and counted cells in the pharynx — worms with extra pharyngeal cells were recovered from the slides for propagation. This screen was arduous but fruitful; for example, it identified an allele of a Bcl2 (B-cell leukaemia 2) homologue, *ced-9*, which established the function of this oncogene in normal programmed cell death[43,44].

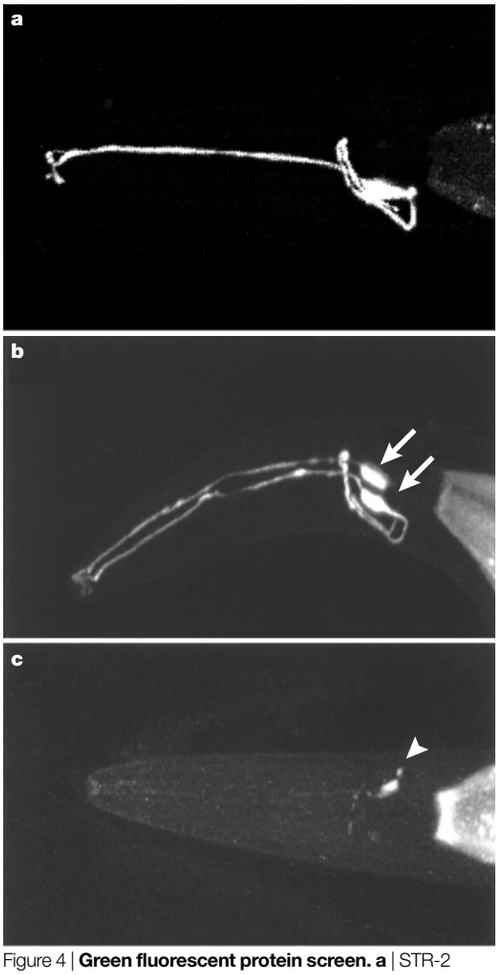


Figure 4 | **Green fluorescent protein screen. a** | STR-2 (seven trasmembrane receptor 2) is a candidate odorant receptor that is expressed in only one of the bilaterally symmetric AWC olfactory neurons in the wild type. Screens for mutants that are defective in left–right asymmetry identified mutants that expressed *str-2::GFP* in either **b** | both cells (arrows) or **c** | neither cell, although there is still faint staining in the cell body (arrowhead)[57]. Photos reproduced with permission from REF. 57 © (2001) the Genetics Society of America, courtesy of C. Bargmann. In all panels, dorsal is top and ventral is bottom. Anterior is to the left.

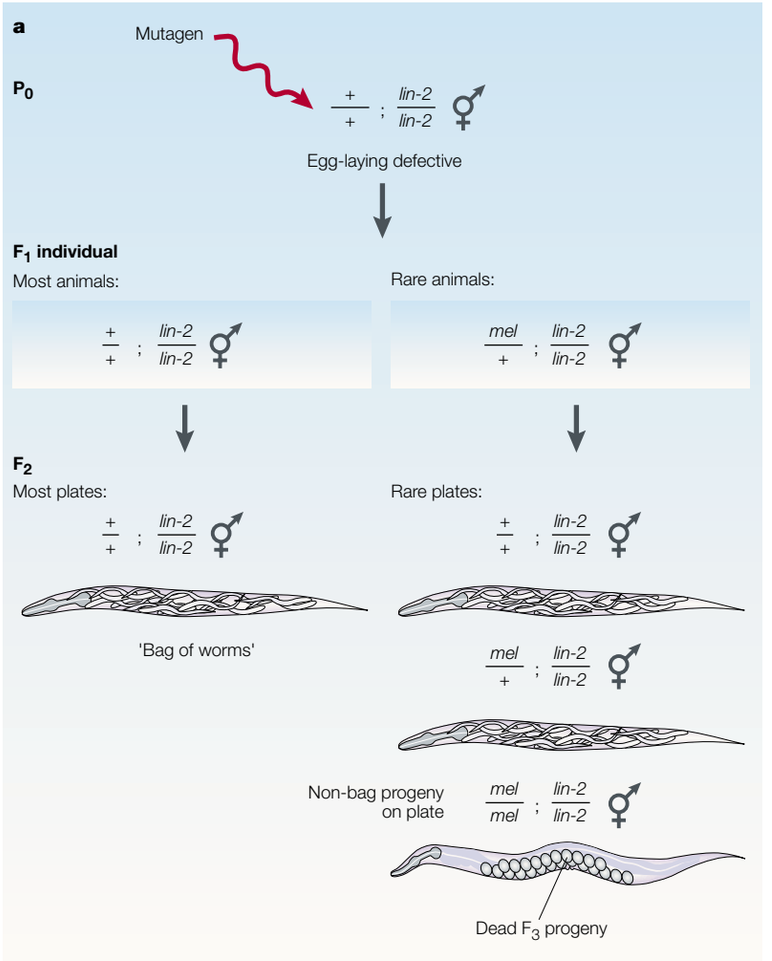
Green fluorescent protein screens. The screening of microscopic phenotypes has been greatly aided by the introduction into worms of green fluorescent protein (GFP) from the jellyfish *Aequoria victoria*[45]. These screens are particularly useful when the only phenotype of the mutant is the repositioning of a cell or protein in the mutant animal. GFP can be used to mark a protein, cell or subcellular compartment, and changes in GFP expression can be screened. For example, Mike Nonet[46] tagged synaptic vesicles by fusing GFP to the synaptic-vesicle-protein synaptobrevin. Synaptic vesicles cluster at synapses, and synaptic number and position can then be assayed in transgenic animals using fluorescence microscopy. Three groups have used tagged synaptobrevin to screen for mutants that affect synaptic positioning and number[47–49]. Although they analysed synaptic distribution in different neurons, these screens identified similar sets of genes, including *rpm-1* (*regulator of presynaptic morphology 1*) and *sad-1* (*synapses of the amphid defective 1*). RPM-1 and SAD-1 are required in sensory neurons and GABA motor neurons for the placement and distribution of synapses along axons. *rpm-1* and *sad-1* mutants move well and were therefore not recovered from screens for visibly uncoordinated animals. Other researchers have used GFP screens to identify mutants in various cellular processes, such as axonal pathfinding (for example, *sax-3*/*Robo*[50]), cell-fate specification (*ttx-1*/*otd*[51]), epigenetic silencing (*tam-1* (REF. 52)), endocytosis[53–55] and developmental timing (*lin-58* (REF. 56)). Emily Troemel and Cori Bargmann made particularly elegant use of GFP in a screen for defects in left–right asymmetry. They placed GFP under the control of a promoter that is expressed in either the left or the right olfactory neuron, but not both cells[57], then screened for mutants that either expressed the marker in both cells or in neither cell (FIG. 4). This screen revealed that signalling between the bilaterally symmetric neurons induces one of the neurons to adopt the 'ON' state from the basal 'OFF' state.

Laser ablation screens. The grand prizewinner for difficult screens was conducted by Leon Avery, a geneticist who is known for clever and unusual screens. Avery was studying peristaltic pumping of the worm pharynx. If he ablated the M4 MOTOR NEURON with a laser microbeam, the pharynx lacked peristalsis owing to a lack of muscle contraction, and the animals that had been operated on arrested development[58,59]. Avery reasoned that mutations in genes that are responsible for muscle repolarization would restore contractions to the pharyngeal muscle. He mutagenized worms and then ablated the M4 motor neuron in hundreds of F_2 individuals and looked for mutants that continued to develop beyond the arrest caused by the surgery. In this screen, he identified an allele of a gene (*exp-2*) that encodes a sodium–potassium ATPase[60]. Mutations in this gene cause the resting membrane potential of the muscle to be close to threshold and thereby restore muscle contractions. Although this screen is technically a selection, the physical manipulations that were required to screen each F_2 worm place it firmly in hell.

Screens from purgatory

Lethal mutations. Unbiased screens for lethal mutations indicate that there might be 4,000 essential genes in the *C. elegans* genome[61,62] (reviewed in REF. 63). Among these are genes that are involved in the developmental processes being examined in many laboratories; however, studying lethal mutations introduces two special problems, which makes this class of mutants a geneticist's purgatory. One difficulty is isolating and maintaining lethal mutations as heterozygotes. Second, because many genes can be mutated to a lethal phenotype, a researcher is faced with the daunting prospect of sorting through many lethal mutants to identify those that are defective for a particular biological process. We discuss each of these problems below.

M4 MOTOR NEURON
A motor neuron in the pharynx that is required for the peristaltic movements of the muscle that move food into the grinder.



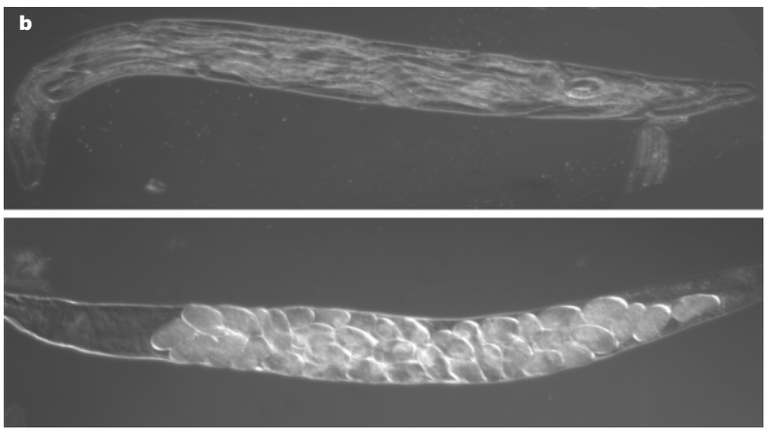


Figure 5 | **Maternal-effect screen. a** | Scheme for a maternal-effect screen. F_1 mothers (*mel*/+) that are derived from mutagenized parents are placed individually on plates and allowed to self-fertilize, producing +/+, *mel*/+ and *mel*/*mel* F_2 worms. *lin-2* animals are egg-laying defective so that self-progeny from the mother are not laid (*lin-2* animals are otherwise viable and fertile). The progeny eventually hatch and consume the mother to form a 'bag of worms'. So, at adulthood, the +/+ and *mel*/+ worms are lysed by their viable progeny. Animals that are homozygous for a maternal-effect mutation (*mel*/*mel*) will be viable adults filled with inviable progeny. The mutation cannot be recovered from these animals, but can be recovered from the *mel*/+ siblings on the plate. **b** | A *lin-2* homozygote is consumed by her progeny and the cuticle is filled with L1 larvae (top). The progeny from a double mutant of *lin-2* and an unidentified maternal-effect mutant (bottom) die as embryos and fill the mother with dead eggs. *lin*, *abnormal cell lineage*; *mel*, *maternal-effect lethal*.

Standard F_2 screens are inadequate for the isolation of lethal mutations, because such screens rely on breeding a homozygous mutant animal (FIG. 1). However, lethal mutations can be recovered from their heterozygous siblings using a clonal F_1 screen[2]. In this method, F_1 individuals from mutagenized P_0 animals are placed onto small Petri plates. If one-quarter of the F_2 progeny show the lethal phenotype of interest, the mutation is retrieved from heterozygous siblings. For this screen, a hermaphrodite is a tremendous advantage, because a single heterozygous mother is used to produce both homozygous-lethal mutants, which can be analysed, and heterozygous siblings, which are crucial to propagate the strain. Heterozygous strains can be maintained by inspecting the progeny from individual worms every generation: worms that no longer segregate arrested progeny are discarded. Lethal mutations are more conveniently maintained for long periods of time as heterozygotes by using a BALANCER CHROMOSOME[64,65].

An adaptation of the clonal F_1 screen can be carried out on large plates by simply preventing the F_2 progeny from being laid by using egg-laying-defective mutant mothers[66]. Such animals retain developing embryos in their uteruses; because the worm is transparent, individuals that segregate 25% arrested progeny can be identified and their phenotypes examined under the light microscope. The F_1 individual can then be transferred to a fresh plate. Although the F_1 mother is eventually consumed by her progeny to form 'a bag of worms' (FIG. 5b), the mutation can be recovered from the heterozygous F_2 progeny. The advantage of this approach is that it bypasses the need to clone individual F_1 animals onto plates.

Maternal-effect lethal screens. The very earliest divisions of an embryo are directed by RNAs and proteins that are deposited by the mother. So, a homozygous embryo that lacks the gene for these components can still develop normally owing to normal RNAs and proteins that the heterozygous mother has deposited into the egg. However, F_3 individuals that are born from these homozygous progeny die because they lack this maternal contribution. Genes that are involved in these early developmental decisions can be identified in maternal-effect lethal (*mel*) screens[67,68]. In such a screen, an egg-laying-defective mutant is used to retain the eggs in the F_2 mother[69] (FIG. 5a). Most animals die as a bag of worms (FIG. 5b, top). The presence of viable adult worms that are filled with inviable progeny (FIG. 5b, bottom) is evidence of a maternal-effect mutation, which can then be recovered from the progeny of the lysed *mel*/+ worms (siblings of the F_2 mothers). These screens have identified many components that are required to establish the early embryo, such as *skn-1* (*skinhead 1*), which specifies blastomere fate, and the *par* (*embryonic partitioning abnormal*) genes, which are required for embryonic asymmetry[70,71]. Alternatively, a screen can be carried out for maternal-effect-lethal mutations that are temperature sensitive, and the progeny from the homozygous

BALANCER CHROMOSOME
Balancer chromosomes are used *in trans* to a chromosome that carries a lethal mutation. Such chromosomes carry deleterious mutations, so that heterozygotes have a selective advantage and are easily maintained. They are used as genetic tools because they allow lethal mutations to be propagated indefinitely. In addition, balancer chromosomes frequently contain rearrangements or translocations that disrupt recombination between the homologues.

MRNA SURVEILLANCE PATHWAY
A pathway that recognizes and degrades mRNA molecules that bear nonsense mutations.

mother can be recovered. Kevin O'Connell and John White also used egg-laying-defective animals to detect maternal-effect mutants[72]; however, they carried out their screens at 25 °C so that temperature-sensitive mutations would be exposed to the non-permissive temperature. Animals that were filled with dead embryos were recovered and placed at the permissive temperature of 15 °C to allow development of live progeny and the recovery of the temperature-sensitive mutations. These screens yielded conditional lethal mutations in 19 genes that are required for early embryonic divisions in *C. elegans*.

Finding the right lethal mutant. Hundreds of lethal mutations have been identified. Many are involved in 'housekeeping' functions (for example, *dif-1* (*differentiation defective 1*), a probable mitochondrial solute carrier[73]), whereas others encode developmental regulators that are needed to pattern the embryo[74,75]. One problem posed by lethal mutant screens is how to distinguish between these two classes of mutant. As lethality is not a specific phenotype, a secondary screen is essential to recognize interesting mutants. For example, Pierre Gönczy and colleagues screened chromosome III for all maternal-effect-lethal mutations, by using an inverted chromosome to balance and maintain the induced mutations. By re-screening these strains under high magnification for defects in cell division in the one-cell-stage embryo[76], these researchers identified 34 loci that are required for mitosis and cytokinesis in the first cell division.

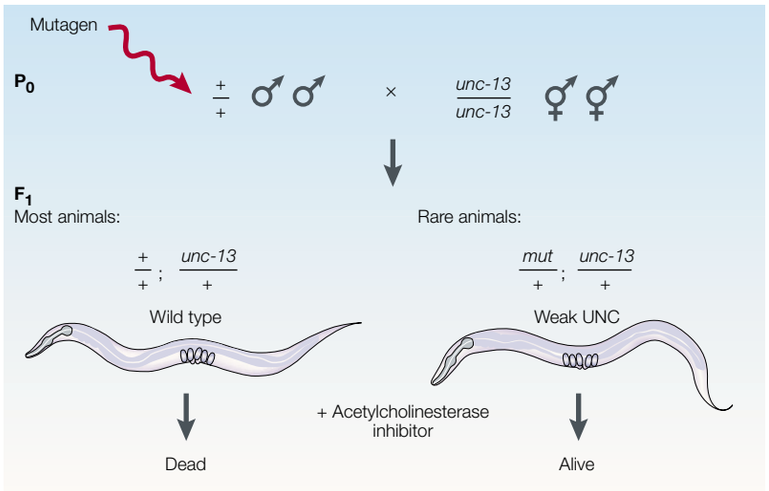


Figure 6 | **Non-allelic non-complementation screen.** These screens involve the search for recessive mutations that show a phenotype as a double heterozygote with an initial mutation. The design of this screen resembles that of a non-complementation screen that is intended to find more alleles of a particular gene; however, it can also identify mutations at a second site. Mutagenized males are crossed to hermaphrodites that carry a homozygous mutation for one gene (*unc-13* in this example) to generate heterozygous animals that can be screened for the desired phenotype. In cases of non-allelic non-complementation, the observed phenotype reflects a new mutation in a second gene (*mut*). In the screen illustrated here, the double heterozygotes are selected using an acetylcholinesterase inhibitor such as Aldicarb (see main text), because UNC-13 is required for the release of neurotransmitter. Even if this new mutation is homozygous lethal it can still be recovered from the heterozygous F_1 mutant animal. *mut*, *mutation*; *unc*, *uncoordinated*.

Another successful strategy for identifying interesting lethal-mutant phenotypes has been to manipulate worms physically and thereby predict the relevant phenotype for which to screen. For example, because it has an invariant lineage, *C. elegans* was originally characterized as a simple developmental system that depended on the segregation of determinants into specific lineages — quite different from the development of higher organisms. However, Jim Priess removed a single cell in the four-cell-stage embryo and discovered that the development of a neighbouring cell, called ABa, was disrupted[77]. ABa made epidermis and neurons, as it did usually, but failed to produce pharyngeal muscles. By screening for mutants in which ABa failed to produce pharyngeal cells, Priess and colleagues identified three genes that are involved in the Notch signalling pathway[69,78,79]. These studies and others revealed that the stereoptypical lineage of *C. elegans* relies on invariant cell–cell interactions more than it does on the segregation of determinants into specific lineages.

The future of *Caenorhabditis elegans* genetics

Sensitized screens. A promising scheme for the future is the sensitized screen, in which recessive mutations that are involved in a specific process produce dominant phenotypes. Sensitized screens for dominant enhancers are routine in *Drosophila*. In flies, such screens often use a temperature-sensitive mutation; one strategy would be to maintain the F_1 progeny from mutagenized animals at a marginally permissive temperature so that the pathway of interest is compromised but not blocked completely. Mutations in a second locus disrupt the process so that a phenotype is visible even though this second locus is heterozygous. For example, a screen for dominant enhancers of temperature-sensitive mutations in the *sevenless* tyrosine kinase identified loss-of-function mutations in the guanylyl-nucleotide-release factor for RAS[80]. These screens are particularly powerful because they can be conducted in the F_1 generation. Because *C. elegans* hermaphrodites produce homozygous offspring, screens for recessive mutations are fairly easy to carry out, and so a search for dominant enhancers is usually unnecessary. However, in some circumstances, dominant F_1 screens can save a great deal of labour as every mutagenized genome can be scored once and only once (unlike in most F_2 screens, in which a mutagenized genome can be sampled several times). For example, in a screen for dominant enhancers, Chalfie and colleagues[81] used a temperature-sensitive mutation in the gene that encodes the MEC-4 mechanosensory ion channel. They screened for dominant mutations that conferred a Mec mutant phenotype at the permissive temperature. Mutations in four genes enhanced the phenotype in a dominant manner. These protein products are believed to interact directly with the MEC-4 protein, which indicates that these types of screen might identify proteins that are members of protein complexes.

In another example, Brian Cali and Phil Anderson screened for alleles that were dominant when the MRNA SURVEILLANCE PATHWAY was inactivated[82]. They reasoned that, in the wild type, mRNAs produced from genes

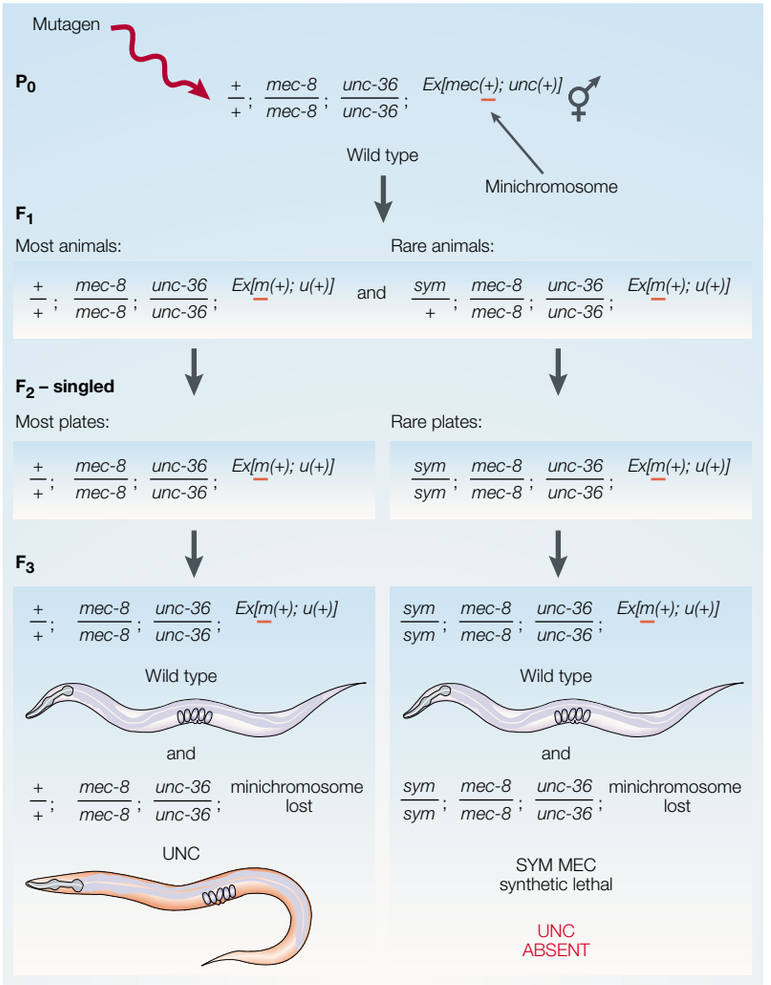


Figure 7 | **Synthetic lethal screens.** Robert Herman and co-workers[99] screened for mutants that were viable as a single mutant but were synthetically lethal with a *mec-8* null allele. They generated a transgenic strain with homozygous-null mutations at the endogenous *mec-8* and *unc-36* loci and an exogenous copy of the *mec-8* and *unc-36* genes carried as an extrachromosomal array (*Ex*[*mec*(+);*unc*(+)]. A green fluorescent protein marker can be added as an extra marker for the presence of the array. Because extrachromosomal arrays are not transmitted to all progeny, this strain normally segregated some worms that had lost the exogenous DNA and were phenotypically Mec, which is difficult to score, and Unc, which is easy to score. After mutagenesis, Herman and colleagues identified mutants that no longer segregated Unc progeny. Because the double mutant between *mec-8* and the new allele (*sym*; synthetically lethal with *mec-8*) were synthetically lethal, only animals that retained the *mec-8(+)* array were viable. See main text for details.

with nonsense mutations were rapidly degraded, precluding synthesis of mutant protein. However, if mRNA surveillance were inactivated, mutant alleles would produce stabilized mRNAs that could code for truncated proteins with dominant phenotypes. Their approach yielded dominant alleles in 15 genes, 7 of which depended, to some extent, on loss of mRNA surveillance[82]. Many of these mutations caused uncoordinated movement as heterozygotes but were lethal as homozygotes. Presumably, these genes have a role in nervous-system function but are also required for viability. This pleiotropy would make such genes difficult to identify in conventional screens.

A special case of a sensitized screen is a non-allelic non-complementation screen (also called second-site non-complementation or extragenic non-complementation). This involves the isolation of recessive mutations that show a phenotype as a double heterozygote. Such mutations can be identified in a standard non-complementation screen, which is normally used to identify more alleles of a locus. In circumstances of non-allelic non-complementation, second-site mutations will seem to be dominant and can be observed in the F_1 progeny (FIG. 6). Even if these mutations are recessive lethal, they can be isolated and maintained as heterozygotes. In studies of genes that function at the synapse, this interaction required at least one partially functional protein[83]. The strongest interactions were observed between proteins that formed a complex; presumably, the partially functional protein 'poisoned' the complex to some degree. However, interactions were also observed between distant components of the exocytosis pathway. So, non-allelic non-complementation screens can identify genes that act in a pathway that functions in adults even if the mutations are recessive lethals.

Redundancy. It has been estimated that perhaps only 6,000 of the 20,000 genes in *C. elegans* can be mutated to a visible, lethal or sterile phenotype[84–88]. How will we determine the functions of the other 14,000 genes? Numerous genes might show apparently wild-type null phenotypes because they are redundant under laboratory conditions, and only show a phenotype if mutations eliminate the activity of two or more loci[89]. These estimates of redundant genes argue that devising strategies to reveal the function of 'silent' genes is crucial if we are to decipher the *C. elegans* genome.

In the simplest situation, functional redundancy arises because a gene has been duplicated and two or more closely related genes exist, any of which can carry out a particular task. This phenomenon is called homologous redundancy. For example, *end-1* (*endoderm 1*) and *end-3* encode tightly linked GATA-transcription-factor homologues[90] (J. Rothman, personal communication). Loss of both genes leads to an absence of endoderm; however, mutants for either gene alone have no phenotype. The synergism between *end-1* and *end-3* was discovered because a deletion exists that removes both genes and produces arrested embryos that lack endoderm[90]. Interestingly, the *C. elegans* genome contains many small duplications that carry pairs of potentially redundant genes[85]. Mutagens that generate deletions might therefore be useful for the design of screens that circumvent redundancy.

In other cases, homologous genes might have diverged in function such that the genes are only partially redundant. Although the phenotype of each single mutant might be deceptive or uninformative, double mutants could direct researchers to new phenotypes that can be screened to isolate other components of a pathway. For example, a double mutant of the Notch-receptor homologues *glp-1* (*germline proliferation 1*) and *lin-12* has a distinctive lethal phenotype that differs

from either single mutant[91]. Kimble and colleagues used an F_1 clonal screen to search for new mutations with the same phenotype as the *glp-1 lin-12* double mutant and identified two new genes, *lag-1* (*lin-12* and *glp-1*) and *lag-2*. *lag-1* and *lag-2* encode a downstream effector and an upstream ligand in the Notch pathway, respectively. LAG-1 and LAG-2 presumably function with both GLP-1 and LIN-12 (REFS 92–94).

A more complex form of redundancy is non-homologous redundancy**.** In this case, proteins that do not resemble one another can nevertheless still provide the same cellular function. The most famous example of non-homologous redundancy is the synthetic multivulval, or SynMuv, pathways in vulval development. In this case, either of two separate molecular pathways can inhibit the unregulated formation of the vulva by the epidermal precursor cells. Only mutants that lack at least one component in both pathways result in a multivulval phenotype[95] (FIG. 3). The initial identification of the SynMuv pathways required a gambler's luck, as the simultaneous mutation in two unlinked genes, *lin-8* and *lin-9*, in the genome occurs at a frequency of 10^{-7}. However, once such a combination had been identified, Ferguson separated the loci and re-screened the genome for second-site mutations that generated the synthetic phenotype; this was in fact a sensitized screen designed for the purpose of revealing redundant genes. Because one pathway had already been inactivated, isolation of the second-site mutation occurred at a frequency typical for single loss-of-function mutations (10^{-3}). For example, Ferguson used *lin-8* class A homozygotes to screen for second mutations in the class B pathway and found six loci that showed a synthetic interaction[95]. Molecular analysis of this pathway showed that the B-pathway components are homologous to proteins in the retinoblastoma (Rb) pathway and regulate the NuRD (nucleosome remodelling and histone deacetylase) complex[96]. Many other components of the SynMuv pathway are new proteins, and it will be exciting to learn whether homologues in other organisms also interface with chromatin-remodelling machinery.

The identification of non-homologous redundant pathways almost always depends on blind luck. Another example of this is to be found in the genetic interaction observed between *mec-8* and *unc-52*. Bob Herman discovered that mutations in these two genes were synthetically lethal when he found he could not construct a chromosome marked with both mutations. MEC-8 is a protein that is required for exon skipping in certain alternatively spliced mRNAs, whereas *unc-52* encodes perlecan, an essential component of the extracellular matrix. Transcripts from the *unc-52* locus are alternatively spliced, and the MEC-8 protein is required to skip exons 17 and 18. Nonsense mutations in exons 17 or 18 of *unc-52* are viable because MEC-8 can direct the synthesis of functional transcripts that lack these exons. However, double mutants between the viable alleles of *unc-52* and *mec-8* are lethal and resemble null alleles of *unc-52* (REF. 97). This synthetic interaction indicated a way to identify splicing factors that promote alternative splice forms or to identify other targets of MEC-8 splicing. Andrew Davies and Robert Herman carried out a screen that was similar to synthetic-lethal screens carried out in yeast to look for the presence of lethal sectors in a colony[98]. They screened for mutants that were viable as simple homozygotes but were synthetically lethal with a *mec-8* mutation[99] (FIG. 7). They found mutations in five *sym* (*synthetically lethal with mec-8*) genes, which probably encode targets of MEC-8 or genes that are involved in alternatively splicing reactions with MEC-8. The design of the *mec-8* screen has been adapted to other contexts. For example, a similar approach was used to identify alleles of *fzr-1*/*fizzy related*, which show a genetic interaction with *lin-35*/Rb[100]. Mutations in each of these genes alone show a wild-type phenotype, but together they cause hyperproliferation of cells derived from all germ layers, which leads to double mutants that are very sick or are dead.

The long perspective

The nematode *C. elegans* contains ~20,000 genes[63], fewer than 10% of which have been defined by mutation. Of these, 575 have been cloned and characterized at a molecular level. A goal for the future is to elucidate the role of the remaining genes. About one-third of these have a mammalian counterpart, and understanding their function in *C. elegans* will help us to determine their function in more complex animals. Many of the remainder could define genes that are specific to nematodes and will not be found in other phyla. Such genes are interesting from a macroevolutionary perspective in that they describe which proteins are required by an organism for the peculiarities of its lifestyle. However, the function of many of these proteins might not be clear in worms that are removed from their natural ecology and might be difficult to observe in the simple environment of the laboratory. So, studies of the ecology of natural populations of *C. elegans* will be essential for an in-depth understanding of the *C. elegans* genome. Microevolutionary processes can also be studied. Such processes define why populations of *C. elegans* differ from each other. For example, variations at the *npr-1* (*neuropeptide receptor 1*) locus are responsible for differences in social behaviour between worm populations[101]. Some of these behavioural differences might not be due to changes at single loci but rather are the result of concerted configurations of several genes. *C. elegans* is well suited for such studies, as the genome is well covered by single-nucleotide polymorphisms. Crosses between natural isolates can then be used to map multilocus or QUANTITATIVE contributions to differences between natural isolates.

It is clear that the genome of *C. elegans* is guarding secrets about the organization of life that will be pursued for many years to come. Forward genetic screens will continue to be an important component of those studies, particularly when integrated with emerging technologies of whole-genome analysis, such as expression studies using microarray chips or gene reporters, protein-interaction maps and RNA-interference studies.

QUANTITATIVE TRAIT
A measurable trait that typically depends on the cumulative action of many genes and the environment. **[ok?]**

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