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Reference C

Caenorhabditis elegans: Genetic Portrait of a Simple Multicellular Animal

The nematode *Caenorhabditis elegans,* one of the simplest multicellular organisms, lives in soils worldwide and feeds on soil bacteria. Adults are about 1 mm in length and contain an invariant number of somatic cells (**Fig. C.1**). The mature "female," which is actually a hermaphrodite able to produce both eggs and sperm, has precisely 959 somatic cells that arose from progenitor cells by a reproducible pattern of cell division. The mature male, which produces sperm and has genitalia that enable it to mate with the hermaphrodite, includes precisely 1031 somatic cells that also arose by a reproducible pattern of cell division. *C. elegans* has a short life cycle and an enormous reproductive capacity, progressing in just three days from the fertilized egg of one generation to between 250 and 1000 fertilized eggs of the next generation. It is transparent at all stages, so that investigators can use the light microscope to track development at the cellular level throughout the life cycle. Its small size and small cell number, precisely reproducible and viewable cellular composition, short life cycle, and capacity for prolific reproduction make *C. elegans* an ideal subject for the genetic analysis of development. The fact that the genome for *C. elegans* was sequenced in 1998 makes it an even more appealing organism to study.

An adult C. elegans *hermaphrodite surrounded by larvae of various stages.*

Although *C. elegans* and most other free-living species of nematodes are generally beneficial, they are related to nematodes that parasitize animals and plants, causing human disease and agricultural damage. Knowledge gained from the study of *C. elegans* will help combat these problems.

Three unifying themes surface in our discussion of *C. elegans.* First, the invariance of cell number and fates forms the basis of many experimental protocols used to study nematode development. Second, the invariant specification of cellular divisions and fates depends on a varied palette of developmental strategies. These include the segregation of particular molecules to particular daughter cells at division, inductive signals sent from one cell to influence the development of an adjacent cell, signal transduction pathways within each cell that respond to the arrival of an inductive signal, and a genetically determined program that causes the death of specific cells. Third, genetic studies on the development of *C. elegans* reveal the simultaneous conservation and innovation of evolution. Because the nematode exhibits many features of development, physiology, and behavior found in other complex animals such as *Drosophila* and humans, studies of *C. elegans* can help elucidate developmental pathways and genes conserved throughout animal evolution. But because other features of *C. elegans* development, such as the invariant spatial and temporal pattern of cell positions, divisions, and fates, are quite different from those found in more complex animals, studies of *C. elegans* provide a comparative counterpoint that deepens our understanding of the full range of genetic controls over development in multicellular eukaryotes.

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Figure C.1 Photomicrograph of adult *C. elegans* hermaphrodite (*top*) and male (*bottom*) caught *in flagrante delicto*. The animals are oriented in opposite directions during their mating: the symbol denoting the male is placed near the head, while that for the hermaphrodite is near the tail. Note that the body is transparent, enabling visualization of internal structures. In this photo, it is easy to see the eggs in the hermaphrodite.

Our genetic portrait of *C. elegans* presents:

- An overview of *C. elegans* as an experimental organism, including descriptions of its genome, its life cycle and anatomy, the precise patterns of its cell lineages throughout development, and techniques of genetic and molecular analysis that biologists use to study its development.
- The genetic dissection of several developmental processes in *C. elegans,* including the specification of early embryonic blastomeres, the role of programmed cell death, and the timing of decision making during larval development.
- A comprehensive example on the use of genetics to probe a signaling pathway that helps control development of the hermaphrodite vulva.

C.1 An Overview of *C. elegans* As an Experimental Organism

C. elegans was selected for genome analysis under the Human Genome Project because the structure and function of this worm had already been extensively studied using classical genetics.

The Nuclear Genome of *C. elegans*

The completely sequenced *C. elegans* genome is small for a multicellular animal, only 97 Mb in size. While this is about 10 times the size of the yeast genome, it is only two-thirds the size of the *Drosophila* genome. The *C. elegans* genome is packaged into six chromosomes, designated I, II, III, IV, V, and X. These chromosomes are small and of approximately the same size, which makes it difficult to obtain much genetic information from cytogenetic and karyotypic analyses. **Figure C.2** shows simplified genetic maps of all six chromosomes.

Note that the maps lack centromeres, an important feature found on all other eukaryotic chromosomes described in this book. The *C. elegans* maps lack this feature because the chromosomes do not have a defined centromere. Instead, like the chromosomes of some insects and ciliates, *C. elegans* chromosomes are **holocentric;** that is, they have a diffuse centromere. The distribution of centromeric activity along the length of the chromosome allows many points of spindle attachment during both meiosis and mitosis. This unusual centromere structure does not in general affect genetic analysis because the end result of chromosome segregation and recombination in *C. elegans* is similar to that of chromosomes in organisms with defined centromeres. As we see later, however, the behavior of holocentric chromosomes provides the basis of a technique important in the construction of *C. elegans* genetic mosaics.

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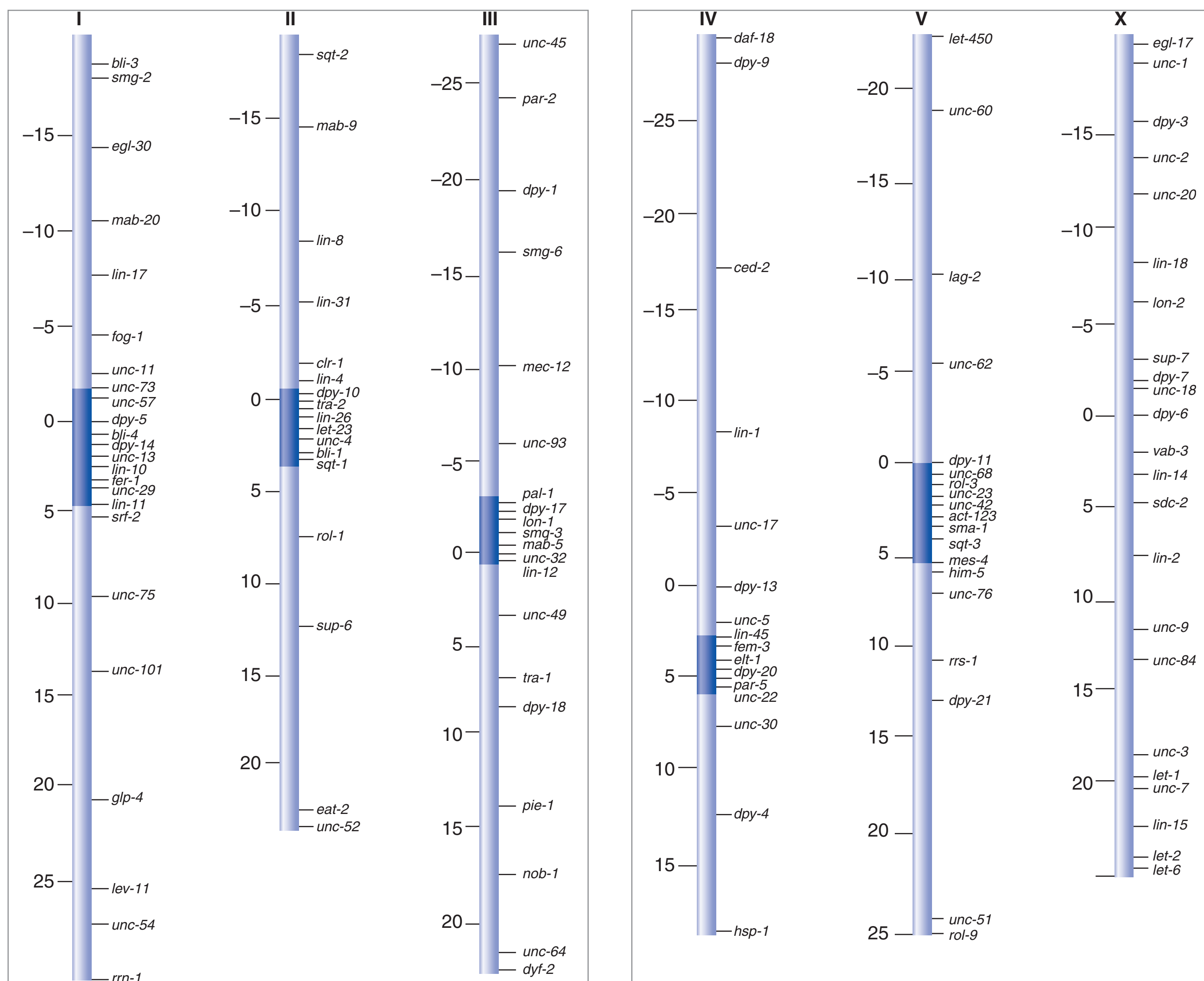

Figure C.2 **Skeleton genetic maps of the six *C. elegans* chromosomes.** Only a small subset of the approximately 19,000 *C. elegans* genes is shown. Distances on these maps represent recombination frequencies in centimorgans. The zero position on each chromosome was chosen arbitrarily, as these chromosomes do not have defined centromeres. Each of the five autosomes has a central cluster (*darker blue*) in which the gene density appears to be unusually high, reflecting a lower rate of recombination per kilobase of DNA.

Aligning the Physical and Genetic Maps

Researchers have constructed a detailed physical map of the *C. elegans* genome showing the order of cosmid and YAC clones covering each of the six chromosomes. The physical map has been useful not only for genomic sequencing but also for the positional cloning of genes important in development.

Ongoing mapping and molecular identification of genes and DNA markers are providing an increasingly accurate alignment of the genetic and physical maps. Interestingly, the relationship between recombination frequency and physical distance varies in different parts of the *C. elegans* genome. Recombination on the arms in 3-10 X higher than in the central region of the chromosomes. The depression of recombination in the central region means that the genetic map is not an accurate reflection of the physical distribution of *C. elegans* genes. One symptom of this anomaly is that genetic maps based on linkage analysis show a marked clustering of genes near the centers of the autosomes (Fig. C.2), although the genomic sequence has shown that the average gene density is no greater in these regions.

Genome Sequence and Organization

The sequencing of the *C. elegans* genome, completed in 1998, provided researchers with the first complete DNA sequence of a multicellular organism. The results established a genome size of 97 Mb, and computer analysis predicted

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that there were about 19,000 genes in the genome, making the average gene density about 1 gene per 5 kb of DNA. Compared with the gene densities seen in larger animals, *C. elegans* genes are closely packed, in part because its introns are smaller on average than those of most other animals.

Annotation of the complete genome sequence, that is, the confirmation and cataloging of all the predicted genes, will take many years. Already, however, the completed sequence has enabled researchers to make some generalizations about the *C. elegans* **proteome:** the complete set of proteins encoded by the 19,000 known and predicted *C. elegans* genes. The majority of these proteins match homologous proteins in current databases derived from sequencing the genomes of other organisms. Based on the known functions of these homologs, most of the *C. elegans* proteins can be assigned to a functional class such as transcription factor, protein kinase, membrane-bound receptor, and so on.

About 20% of the predicted *C. elegans* proteins carry out "core" biological functions that are common to all living cells. The enzymes of intermediary metabolism; the machinery for DNA, RNA, and protein synthesis; and components of the cytoskeleton are all in this category. These proteins have functional homologs, in about the same proportions, in yeast as well as in other metazoans. Interestingly, genes for the core-function proteins are found preferentially in the central regions of the chromosomes, where recombination frequencies are lower.

The remainder of the *C. elegans* proteins whose functions can be surmised are involved in processes required only in multicellular organisms; such processes include specialized signal transduction pathways and programmed cell death. These multicellular-based proteins have homologs in other animals, but not in yeast. Almost all the signaling pathways found in other animals are represented in *C. elegans.*

In addition to the 19,000 known and predicted genes, there are several kinds of repetitive DNA sequences dispersed throughout the *C. elegans* genome. The best characterized of these sequences are seven kinds of transposable elements, named Tc1 through Tc7. Different strains of *C. elegans* carry different numbers of these elements. In most strains, the elements are stable, but in some strains, they actively transpose, allowing transposon mutagenesis.

Gene Expression

Two aspects of *C. elegans* gene expression are unique among multicellular animals. First, more than 70% of mRNAs have had one of two splice-leader sequences trans-spliced onto the 5′ end of the message (**Fig. C.3**). Pre-mRNAs do not contain these splice-leader sequences, but during processing in the nucleus, they are trans-spliced to acceptor sites near the 5′ ends of the corresponding primary transcripts. The function of splice leaders is not known, but it is striking that these 22-nucleotide sequences have been completely conserved among all nematode genera examined, suggesting functional significance. The second aspect of *C. elegans* gene expression unique among mulitcellular animals is that about 25% of adjacent genes are transcribed as operons to produce a polycistronic primary transcript (Fig. C.3). During the subsequent processing of this transcript, trans-splicing with splice-leader sequences produces constituent single-gene mRNAs. Before the discovery of these unique aspects of *C. elegans* gene expression, polycistronic transcripts had been reported only in prokaryotic cells and trans-splicing had been reported only in trypanosomes.

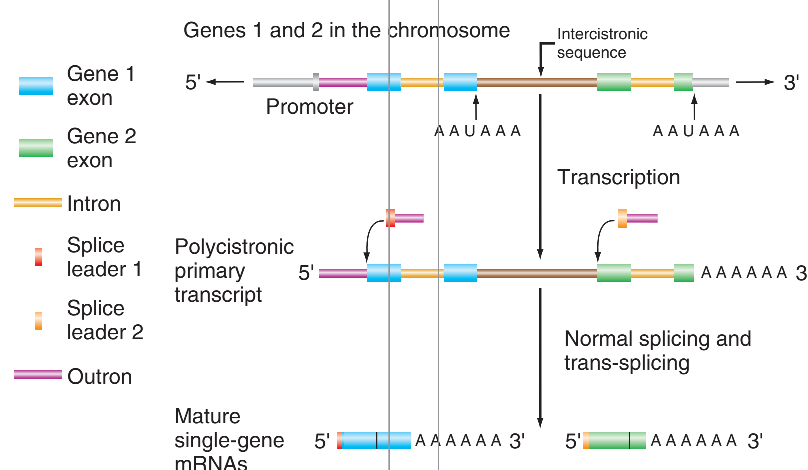

Figure C.3 Polycistronic transcription and trans-splicing in *C. elegans.* A process of trans-splicing and cleavage of multigenic transcripts results in the addition of splice leaders (encoded elsewhere in the genome) to the 5′ ends and poly-A tracts to the 3′ ends of what will become the individual mature mRNAs for each gene. The splice leader SL1 trans-splices to the most 5′ gene of the operon, while SL2 trans-splices to the internal genes. The sequence found at the 5′ end of the primary transcript that is removed by trans-splicing is technically not an intron (because it is not flanked on both sides by exons); it is sometimes called an *outron.*

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Life Cycle, Development, and Anatomy

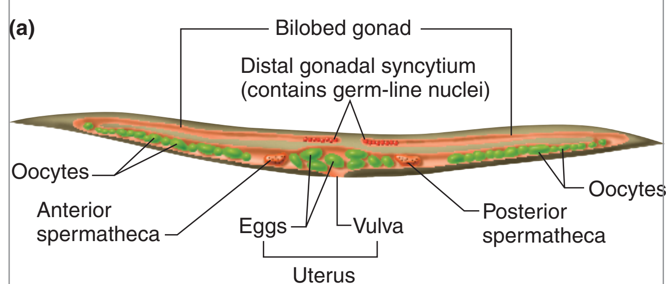
We saw in the introduction to this chapter that *C. elegans* females are actually hermaphrodites that produce both oocytes and sperm. In the absence of males, they can reproduce by self-fertilization. Mating with males, however, produces cross-fertilized progeny. In the wild, mating promotes mixing of the gene pool; in the laboratory, it makes experimental genetics possible. Since a typical hermaphrodite produces 200-300 sperm and a larger number of oocytes, it can generate 200-300 progeny by self-fertilization and over 1000 progeny when fertilized by a male. One external feature that distinguishes the sexes is the tail, which is simple and tapered in the hermaphrodite but fan-shaped and specialized for mating in the male (see Fig. C.1).

Gamete Production and Fertilization

The *C. elegans* hermaphrodite manufactures both oocytes and sperm in its bilobed gonad (**Fig. C.4a**). The far (distal) end of each lobe contains germ-line nuclei, not separated by cell membranes, which divide mitotically in a common core of cytoplasm (forming a syncytium). The nuclei move toward the other (proximal) end of the lobe, which terminates in the uterus. As they move toward the bend between the distal and proximal arms of the lobe, the nuclei enter into meiosis. At the bend, individual nuclei are pinched off as cells, first to form sperm during a brief period just before adulthood, and then to form oocytes once the hermaphrodite has become an adult. The oocytes move through the oviducts toward the central uterus.

At the end of each oviduct lies a spermatheca, the storage site for sperm (from either the hermaphrodite or the male). These sperm are unusual in that they are amoeboid rather than flagellated like the sperm in most animals (Fig. C.4b). Each spermatheca of a young, unmated adult hermaphrodite contains about 150 sperm. Fertilization occurs as oocytes pass through the spermatheca into the uterus, where the fertilized eggs begin to develop. Eggs exit the uterus through the vulva, which forms a characteristic protrusion on the ventral side of the hermaphrodite.

To effect cross-fertilization, the male lies next to the hermaphrodite and slides its fan-shaped tail along the hermaphrodite's body surface until it contacts the vulva. The male then inserts specialized structures at the base of its tail into the hermaphrodite's vulva (see Fig. C.1). Sperm move from the male's tail through the vulva into the hermaphrodite's uterus. From the uterus, the sperm migrate to the spermathecae. There the male sperm somehow gain an advantage over the resident hermaphrodite sperm so that for several hours after mating, the hermaphrodite produces almost exclusively outcross progeny.



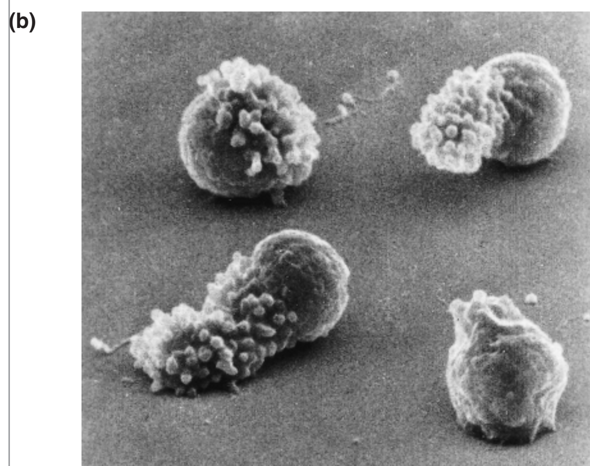
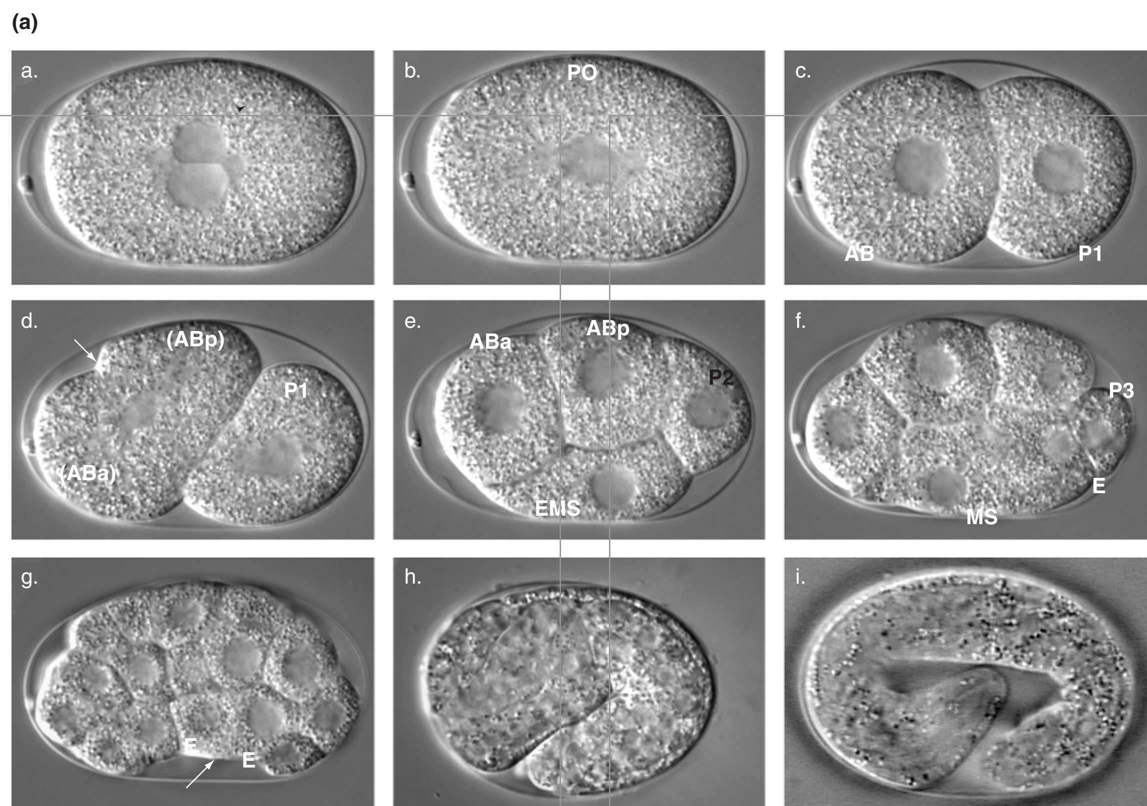


Figure C.4 **Reproduction in *C. elegans.*** **(a)** The hermaphrodite reproductive system. During the life of a hermaphrodite, the first 40 or so germ cells that enter meiosis in each arm of the gonad develop into approximately 150 sperm that are deposited into the spermatheca. Thereafter, the sexual fate switches so that all subsequent germ cells differentiate as oocytes. **(b)** Scanning electron micrograph of *C. elegans* sperm. These amoeboid cells do not swim; instead they crawl along surfaces by extending and retracting *pseudopods*.

Development from Zygote to Adult

At room temperature, *C. elegans* progresses from a fertilized egg to the fertilized eggs of the next generation in about three days. Embryonic development begins in the hermaphrodite's uterus with secretion of a tough chitinous shell around the fertilized egg and continues after the hermaphrodite lays her eggs about 2 hours later. **Figure C.5a** shows several stages of embryonic development. The large cells produced by early embryonic cleavages are called **blastomeres.** Many of the early cleavages are unequal, producing daughter blastomeres of different sizes. Until the 28-cell stage, all blastomeres contact the surface of the embryo. At the 28-cell stage, two gut precursor cells begin to move to the embryo's interior, initiating gastrulation. Continued cell proliferation and cell movements during gastrulation produce the three embryonic germ layers (endoderm, mesoderm, and ectoderm) that give rise to the basic body plan of the larval and adult stages. Later in embryogenesis, cell divisions cease while the various tissues differentiate and become further organized. Contractile proteins arranged into filaments around the circumference of the animal literally squeeze the embryo into its final worm-like shape. Roughly 14 hours after fertilization, the first-stage

(a)



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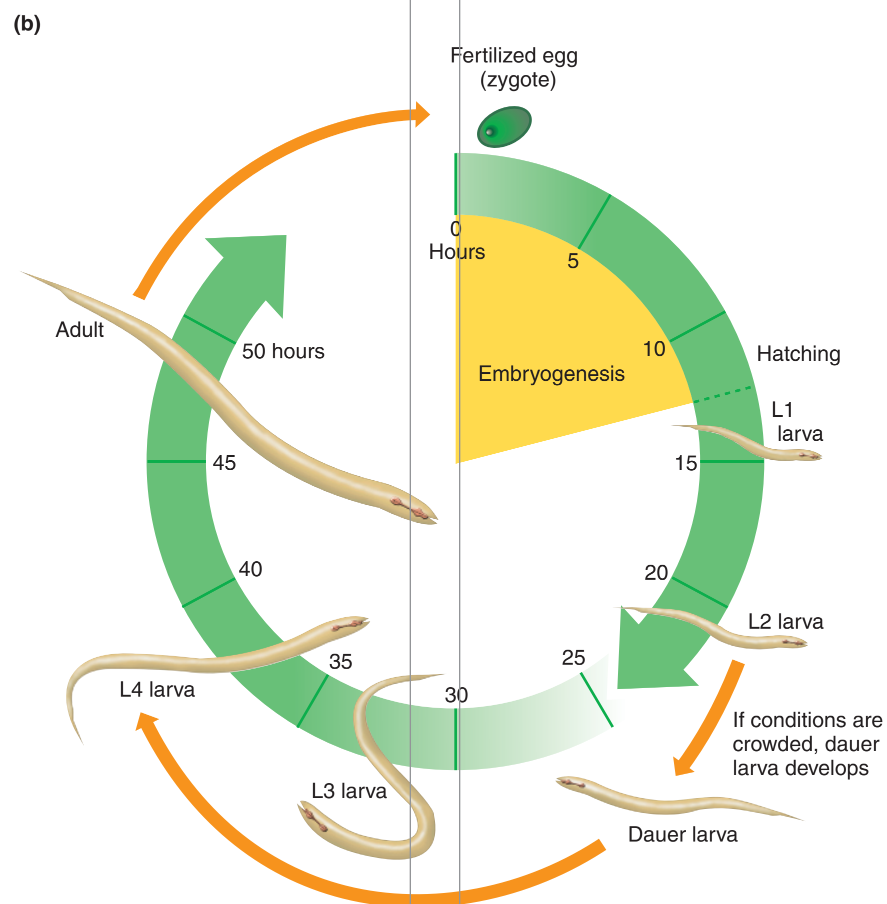

Figure C.5 **Stages of *C. elegans* development.** **(a)** Embryonic development of *C. elegans*. Several of the earliest cell cleavages in the embryo are unequal; for example, the first mitotic division produces a larger AB daughter cell and a smaller P_1 daughter cell (*panel c*). Gastrulation begins at the 28-cell stage as the two cells marked with a small *E* move into the embryo's interior (*panel g*). Later on, the embryo adopts a wormlike shape prior to hatching (*panel i*). **(b)** The *C. elegans* life cycle. Embryogenesis, the time period illustrated in part (a), is highlighted in *yellow*. Under unfavorable environmental conditions, L2 larvae can molt into dormant, long-lived dauer larvae.

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juvenile, known as the L1 larva, hatches from the egg shell. It is 250 mm long and consists of 558 cells.

Over the next 50 hours, development proceeds through three additional larval stages—L2, L3, and L4—separated from each other by molts (Fig. C.5b). During each molt, *C. elegans* synthesizes a new cuticle under the old cuticle, and then it sheds the old. The structures of these cuticles differ from one stage to the next. In parasitic nematodes, the cuticles of different stages help the animal adapt to existence in a particular plant or animal host.

During larval development, most of the cells present at the hatching of the L1 larva do not undergo further cell divisions, although they may increase in size as the animal grows to adulthood. A few blast cells, however, do divide further during larval development to produce additional neurons, muscles, and the structures involved in mating and reproduction. Some of these blast cells are referred to by one-letter names, for example, the laterally situated P cells that give rise to ventral cord neurons and the vulva. Sperm production begins in both hermaphrodites and males during the L4 stage. After the final L4-to-adult molt, the hermaphrodite germ line switches to oocyte production only. At this molt in the male, the development of specialized mating structures in the tail reaches completion, and the animal becomes competent to mate.

In the laboratory, investigators usually grow *C. elegans* on agar plates covered with a lawn of *Escherichia coli* bacteria, which serve as food. They transfer individual animals from one plate to another with a flattened platinum wire. Under laboratory conditions, adult nematodes continue to reproduce for three to four days. However, they live up to two more weeks.

The short life span makes *C. elegans* an excellent model system for the study of aging and its genetic control. One curious aspect of the life cycle adds interest to the question of how life span is determined. If conditions are crowded during early larval development, the L2 larva can molt to an alternative L3 form known as the **dauer larva** (German for long-lasting larva; Fig. C.5b). Dauer larvae do not feed and have a specialized cuticle that resists desiccation. They move around rapidly at first and then become more dormant, although they are still able to respond to stimuli. Dauer larvae that do not experience excessive dehydration can survive for at least six months. Whenever food becomes available within that time, they molt to L4 larvae and resume normal development. Apparently *C. elegans* aging can stop and then restart in response to environmental signals.

Activation of the alternative dauer-larva pathway in response to crowded conditions allows *C. elegans* to survive when conditions for reproduction are unfavorable and resume development later when conditions for reproduction improve. Nearly all *C. elegans* isolated from the wild are dauers. Interestingly, initiation of dauer-larva formation is controlled by proteins homologous to components of the insulin-signaling pathway in mammals, which also controls responses to changes in the abundance of available food.

Anatomy of the Adult

At 1 mm in length, *C. elegans* adults are just visible to the naked eye. As we learned earlier, hermaphrodites have 959 somatic cells, while males have 1031. The *C. elegans* body plan, typical of nematodes in general, is in essence two concentric tubes (**Fig. C.6a**). The outer tube is made up of a tough cuticle covering the *hypodermis* (the "skin" of the

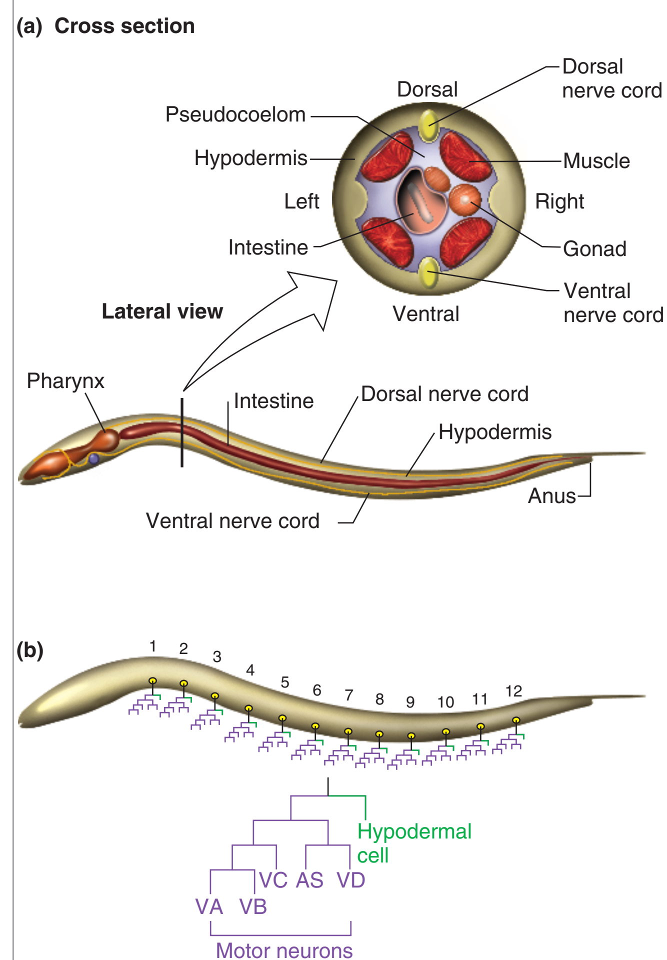

Figure C.6 The body plan of *C. elegans*. (a) The basic features of *C. elegans* adult anatomy in cross-section and lateral view. **(b)** Development along the anterior-posterior axis includes repeating sublineages. In the L1 larva, 12 precursor cells in the ventral nerve cord (the P cells) each initiate a similar series of divisions that will create a group of cells related by ancestry (a sublineage). Within each of these sublineages, one of the cells usually becomes a hypodermal cell (*green lines*), while the remaining cells become motor neurons (*purple lines*). The ultimate fate of corresponding cells in the 12 sublineages is not as invariant as suggested by this diagram. For example, one or more of the motor neurons undergoes programmed cell death in some sublineages, while in certain others the hypodermal cell undergoes additional rounds of cell division later in development (*not shown*).

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worm), under which are neurons and muscles. The inner tube consists of the intestine, which runs from the anterior *pharynx* to the posterior *anus.* The space between the two tubes is called the *pseudocoelom;* in adults, it contains the gonad.

Lineage Diagrams Show the Life History of Every Somatic Cell

Because *C. elegans* is transparent at all stages of its life cycle, it is possible to use light microscopy to observe and analyze development at the cellular level, following living animals from fertilization to adulthood. A microscope equipped with differential interference contrast (Nomarski) optics provides a shallow depth of field with clear imaging of any plane in the specimen and little disturbance from out-of-focus information. With such a microscope, nematode investigators were able to observe and record the positions, movements, and divisions of every cell throughout the life cycle (review Fig. C.5a). In this way, they learned that both embryonic and larval development proceed by an invariant temporal sequence of cell divisions and spatial patterns of cell positions. From their observations, they succeeded in piecing together the entire **cell lineage** of *C. elegans,* establishing the ancestry of every cell in the adult animal all the way back to the fertilized zygote. Among animals of the same sex, the cell lineage is identical for every individual with one exception: The germ-line cell divisions that occur during larval development and into adulthood are variant, because different animals of either sex produce slightly different numbers of gametes. In the somatic tissues, the invariant lineage differs somewhat between hermaphrodites and males, reflecting their anatomical differences.

To study particular developmental processes in detail, *C. elegans* researchers focus on a small part of the cell lineage, such as the one diagrammed in Fig. C.6b. Although *C. elegans* is not segmented, its body plan is partially *metameric,* that is, composed of repeating patterns of cell groups with similar functions. For example, along the ventral nerve cord, a set of 12 precursor cells located along the body of the L1 larva undergo similar patterns of division to generate similar groups of cells known as *sublineages.* The cells produced by each of these 12 sublineages generally include five types of motor neurons and a hypodermal cell. There are, however, variations in cell fate specific to particular sublineages that can be likened to the differences in segment identity that arise during the embryogenesis of truly segmented animals such as *Drosophila* (described in Genetic Portrait D on our website).

The diagram in **Fig. C.7a** shows the complete lineage for all 959 somatic cells in the hermaphrodite. Such diagrams provide an unusual view of the developmental process. The vertical axis represents time, each horizontal line represents a cell division, and the end of each branch represents a cell in the adult. Because the history and fate of each of these cells are reproducible from one individual to another, every somatic cell present at every stage of development can be given its own unique name, using a nomenclature we describe next.

Another important part of the *C. elegans* cell lineage is diagrammed in Fig. C.7b. This higher-resolution view depicts the early divisions after fertilization that give rise to the six so-called *founder cells,* the progenitors of the major embryonic lineages. These cells arise during the first 100 minutes of cleavage, between fertilization and the 28-cell stage when gastrulation begins (corresponding to the stages seen earlier in panels a–g of Fig. C.5a). Daughters of the founder cells (not shown in Fig. C.7b but seen in Fig. C.5a) and their descendants are given lineage names according to their relative positions after division; for example, the anterior and posterior daughters of the AB founder cell are named ABa and ABp, respectively.

Three of the founder cells—E (gut), D (muscle), and P_4 (germ line)—give rise to only single tissues, while the other three—AB, MS, and C—contribute to more than one of the three embryonic germ layers and thus to more than one tissue. Conversely, several tissues include cells from more than one founder cell lineage. For example, in different parts of the body, the body-wall muscle derives from progeny of the MS, C, D, and AB founder cells.

The cell divisions that produce the founder cells are asynchronous (Fig. C.7b). For example, though EMS and P_2 are both the daughters of P_1, EMS divides a few minutes before P_2. In contrast, the later cell divisions within a founder lineage tend to be synchronous. The timing of cell division in *C. elegans* is under genetic control, and considerable progress has been made in identifying the genes responsible for this kind of developmental regulation.

Laser Ablation: An Experimental Tool for Investigating Development and Cell Lineage

Because *C. elegans* is transparent, researchers can deploy a laser beam to kill, or *ablate,* specific cells and then analyze the consequences to determine the functions of the ablated cells in development, physiology, or behavior. Used in conjunction with an understanding of the genetics and molecular biology of the animal, and based on the invariance of cell lineages, this laser technology is a powerful tool. In ablation experiments, a beam of laser light is directed into the microscope through the optical system so that it is focused on the same point and focal plane that the viewer sees (there is no danger to the viewer). When the laser is fired, the beam can fatally damage a single cell without harming the cells around it. If appropriate for the experiment, an investigator can individually kill as many as 20 or 30 cells in a single animal. Much of the knowledge of development and neurobiology presented in this chapter depends on laser ablation technology.

The central finding of cell ablation experiments in which researchers destroy a single cell is that every cell in

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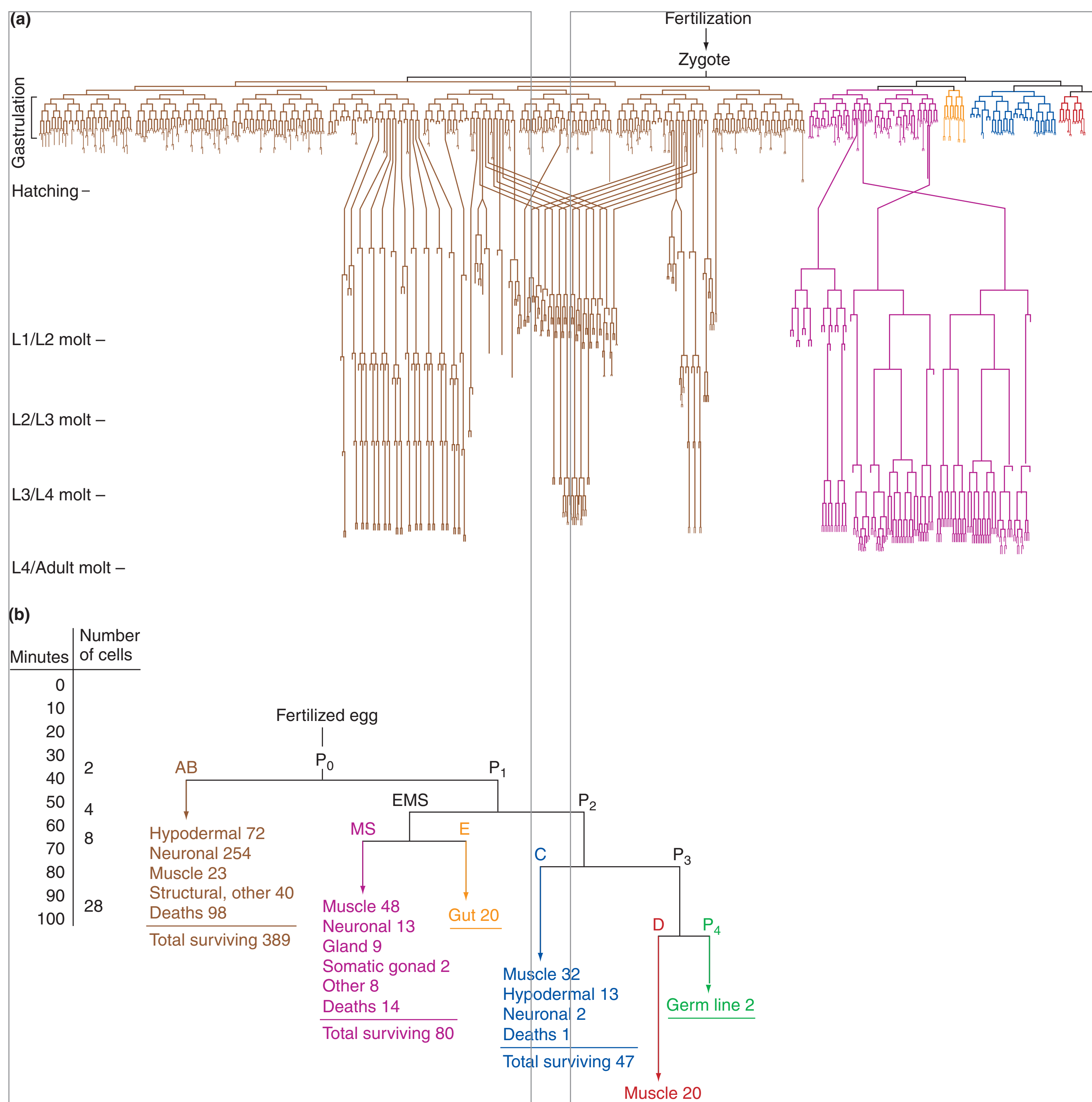

Figure C.7 Cell lineage diagrams. (a) The complete somatic cell lineage for *C. elegans* hermaphrodites. Development begins at the top with the zygote and progresses to adulthood at the bottom of the diagram. Each horizontal line represents a cell division. The timing and pattern of these divisions are invariant from animal to animal. Colors highlight the lineages established by the six founder cells. **(b)** Lineage diagram of the early cleavages in the *C. elegans* embryo that give rise to the six founder cells, which are indicated by different colors. Cells labeled in *black*—P_0 (the original zygote), P_1, P_2, P_3, and EMS—are each precursors of two or more founder cells. Below each founder cell is a list of the kinds and numbers of cells in its lineage at the time of hatching, color-coded as in part (a).

the early embryo is essential for normal development, and the organism cannot replace destroyed cells. Thus the developmental potential of these cells is already restricted to a limited set of fates at the time each cell is born. This suggests that each cell has received a specific set of molecular instructions that differ from those received by its neighbors, instructions that autonomously define its subsequent development. However, this is not the whole story. Ablation of a particular cell can also affect the subsequent fates of adjacent cells in the embryo, indicating that early

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development depends not only on specific internal cell-autonomous signals but also on inductive signaling, in which one cell transmits instructions to others in its neighborhood. Examples of both these mechanisms for specifying cell fates during *C. elegans* development have been studied using genetic analysis and are now understood at the molecular level, as we see later in this chapter.

It is interesting to note that the results of cell ablation experiments in *C. elegans* are very different from the results obtained in embryos of mammals, including humans. For example, on pp. ••–•• of Chapter 11, where we describe how to determine the genotype of an early human embryo by removing and examining a blastomere from the 6-10-cell stage, we mention that the remaining five to nine blastomeres are still able to develop into an adult. Identical twins, formed when early blastomeres separate from each other, are another case in point. Cells in the early human embryo are still **totipotent:** They retain the complete developmental potential of the fertilized egg. This property is responsible for the enormous potential utility of cell lines derived from such early embryos, **embryonic stem cells,** which theoretically can be made to differentiate into any human tissue type in the laboratory. Only later in development do the cells in mammalian embryos become restricted to specific fates.

The Use of Genetic Analysis and Recombinant DNA Technology to Study Development

C. elegans is a diploid organism, except for the sex chromosome in males. Males have only a single X chromosome and are described as XO; hermaphrodites are XX. Males arise spontaneously as the result of a low frequency of X-chromosome nondisjunction during hermaphrodite gametogenesis, which produces 0.1% to 0.2% XO embryos. Once males are present in a population, mating generates more of them in the following manner. When male sperm fertilize a hermaphrodite, half the progeny result from sperm that carry no X chromosome; these progeny are XO males.

Mutant Isolation

Chemical mutagens such as ethylmethane sulfonate (EMS) induce a high forward mutation rate in *C. elegans,* and mutants are simple to isolate. Hermaphrodite genetics takes some getting used to, because a mated hermaphrodite can produce both self-fertilization and outcross progeny, but it has several advantages. It is easy to recover recessive alleles following mutagenesis because a hermaphrodite heterozygous for a new mutation will produce 1/4 homozygous mutant progeny by self-fertilization. As a further convenience, because hermaphrodites can reproduce without having to mate, it is possible to recover and propagate mutations causing very severe defects, for example, complete paralysis of the body-wall muscles. Any mutant hermaphrodite that can feed well enough to survive and has a functional reproductive system can produce progeny.

By mutagenesis, screening, and genetic analysis, researchers have identified and placed on the genetic map more than 1600 genes defined by mutation. Easily recognized mutant phenotypes often used as markers in mapping experiments include animals with abnormal body shape, such as Dumpy (Dpy) or Long (Lon), and animals that move abnormally, designated Unc for "Uncoordinated." By convention, *C. elegans* phenotypes are capitalized and not italicized, while genes and alleles are in lowercase, italic type. An example is *dpy-10(e128),* which designates the *e128* mutant allele of the *dpy-10* gene; this allele results in the Dpy phenotype. Protein gene products are indicated by all uppercase letters; for example, the protein encoded by the *dpy-10* gene is designated DPY-10.

DNA Transformation

Many aspects of *C. elegans* genetic and molecular analysis depend on DNA transformation, the ability to create transgenic animals by introducing cloned DNA sequences into the germ line. To transform *C. elegans,* experimenters generally microinject DNA through the cuticle into the syncytial distal gonad of the hermaphrodite (**Fig. C.8a**). The injected DNA transgenes recombine with each other to form large extrachromosomal arrays that are incorporated into oocytes and retained in most cells during embryogenesis. The presence of centromere-like activity all along the chromosomes of *C. elegans* makes the maintenance of these linear fragments feasible. It is possible to demonstrate gene expression from such transgenes by the phenotypic rescue of a mutant phenotype, if the corresponding wild-type gene is present in the injected DNA. It is also possible to demonstrate expression visually if a suitable reporter gene is present in the injected DNA. One reporter gene is the *E. coli* β-galactosidase gene *lacZ,* whose expression can be detected by X-Gal staining of fixed preparations (Fig. C.8b). In *C. elegans,* the most widely used reporter gene is the jellyfish **green fluorescent protein (GFP)** gene, which has the advantage of being visible in living animals. An animal expressing a GFP reporter is shown in Fig. C.8c. Researchers use reporter genes to study the regulation of gene expression in *C. elegans* as they do in other organisms (see p. •• in Chapter 18).

If an embryo from an injected hermaphrodite retains the transgenic array in its germ line, it can give rise to a *transmitting line* in which the array passes from one generation to the next. However, at a low frequency that seems to depend on the individual array, it will occasionally be lost at cell division. Loss can occur during meiosis, resulting in progeny that lack the array. Loss can also occur at a mitosis during development to produce a clone of somatic cells

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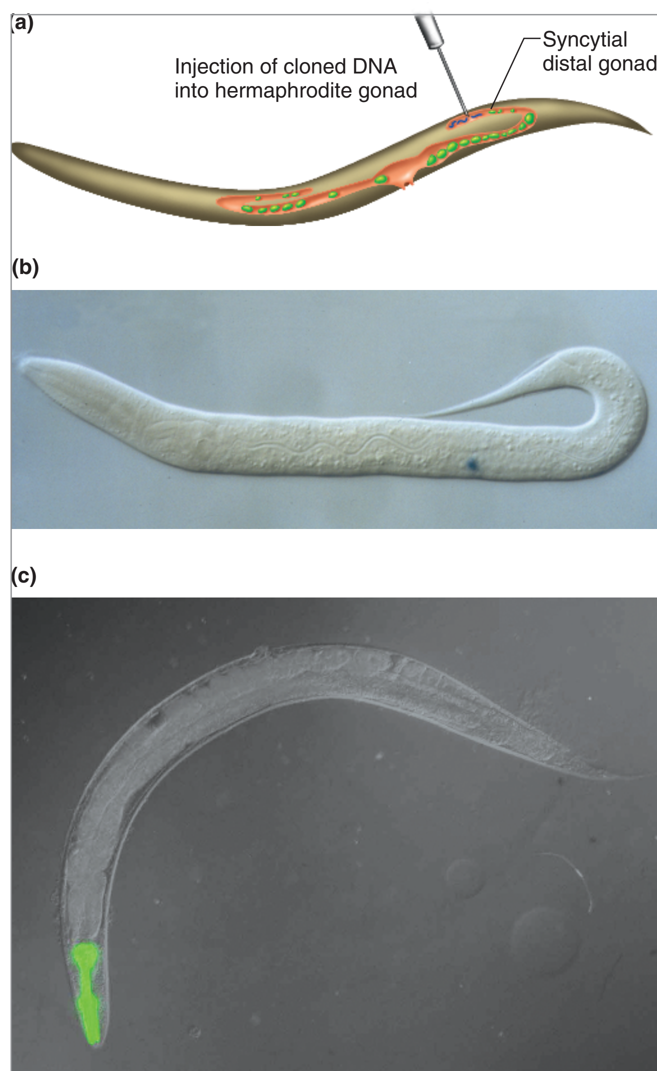

Figure C.8 DNA transformation in *C. elegans*.
(a) Researchers inject DNA into the distal syncytial gonad of hermaphrodites, where it can be taken up by any of the germ cell nuclei in the common syncytial cytoplasm. Irradiation promotes the integration of transgenes into random positions in the genome, stabilizing their transmission. **(b)** Reporter constructs show the expression of transgenes. Here, the *lin-3* gene (including its regulatory region and part of its coding sequence) was fused in frame to the gene for β-galactosidase, which can be detected by X-Gal staining (blue color). Expression of this reporter gene in transformed animals is specific to the anchor cell. **(c)** A commonly used reporter gene is green fluorescent protein (GFP), which can serve as a tag in living animals.

that do not carry the array. This property can be useful for mosaic analysis of gene function.

Irradiation of transmitting lines with X or γ rays can induce integration of a portion of the array at random into one of the chromosomes. Such integrated arrays are inherited in a completely stable fashion like any other chromosomal marker. However, integration of an injected transgene by homologous recombination at the site of the identical *endogenous gene* occurs only at extremely low frequencies. (An endogenous gene is a copy of a gene at its normal place in the genome.)

RNA-Mediated Interference (RNAi)

Another powerful method for analyzing gene function became available recently, when researchers discovered that injection of double-stranded copies of a specific *C. elegans* mRNA (dsRNA) into adult hermaphrodites could cause silencing of the corresponding gene in the injected animal and its progeny. This surprising phenomenon has been called **RNA-mediated interference (RNAi)** (see the Tools of Genetics box in Chapter 18). When an injected dsRNA enters a worm cell, an enzyme complex, named "dicer," cleaves the incoming dsRNA into small segments of about 22 bp, called *short interfering RNAs (siRNAs),* which appear to be the active agents conferring specificity. These siRNAs are transported from one cell to another, apparently through specialized membrane channels, so that the siRNAs can spread from the initial site of entry to all cells in the body except possibly neurons. Because of this transport, the initial entry site of the dsRNA is not important, and researchers have even found strong specific inhibitory effects on gene expression when a dsRNA is fed to the worms. Feeding can be conveniently accomplished using bacteria that express the dsRNA as the worms' food. A second group of proteins promotes unwinding of the siRNAs and complexing of their antisense strands with complementary sequences of the corresponding endogenous mRNA. This sense-antisense complex can meet either of two fates. In one, destruction of the mRNA accomplishes gene silencing. In the second, the bound antisense strand acts as a primer for reverse transcription to form a new dsRNA, which is then cleaved by the dicer complex to produce a new generation of siRNAs, still specific for the same gene (**Fig. C.9**). This second alternative leads to amplification of the siRNA population. One consequence of amplification is that an RNAi effect can persist through two or even three generations after initial exposure to a dsRNA.

Cloning of *C. elegans* Genes

Genes defined by transposon insertion mutations can be cloned directly by transposon tagging, as in other organisms. However, to clone a gene defined only genetically by point mutations, the gene must be isolated by **positional cloning,** that is, cloning based only on the gene's position in the genome (see pp. ••–•• of Chapter 11). Positional cloning begins by precise genetic mapping relative to known markers and extrapolation to an approximate position on the physical map. Next comes fine mapping using not only phenotypic markers but also DNA markers such as polymorphisms resulting from transposon insertion or **single-nucleotide polymorphisms** (see Chapter 11, p. ••), of which thousands have now been identified in *C. elegans.*

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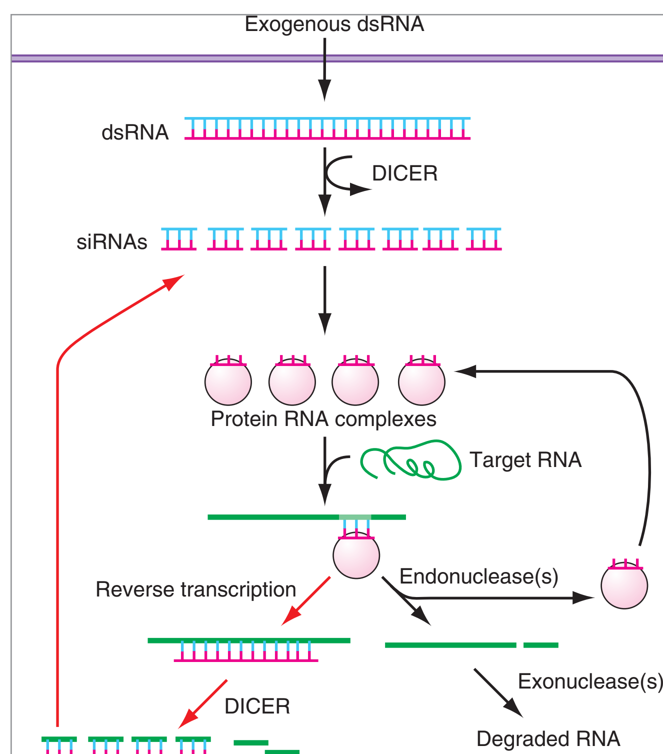

Figure C.9 **Mechanism of RNA interference (RNAi).** RNA interference can be mediated by RNA that enters the cell from outside (*shown here*) or it can be generated by viruses, transposons, or endogenous genes within the cell. Once siRNAs (small interfering RNAs) are produced, they can exert their effects within the same cell and/or be transported between cells.

DNA markers have a significant advantage: Because their precise physical locations are known from the genomic sequence, positioning a new mutation between two DNA markers precisely defines the physical interval in which the new gene must lie.

Once the smallest possible physical interval has been established, there are several approaches by which the gene can be identified. In the classical approach, still often the method of choice, researchers inject into mutant hermaphrodites each of the cosmids corresponding to the defined interval. This allows them to identify first the cosmid and then smaller subclones of the cosmid that complement ("rescue") the mutant phenotype. From the annotated genomic sequence, researchers can then look up the predicted gene present in the smallest rescuing clone.

Availability of the complete genomic sequence makes possible a sometimes simpler *candidate gene* approach. If the physical interval is small, containing only a few candidate genes, researchers can determine the sequences of these genes in DNA from the mutant, and they can identify which of the genes contains the mutation by comparison to the known wild-type sequence in the database. A variation of this approach is made possible by RNAi. If the gene being identified is defined by a loss-of-function mutation, then candidate genes can be tested by silencing them one at a time using RNAi and determining which RNAi-silenced candidate gene produces the mutant phenotype.

Defining the Biological Function of a Gene Identified Only by Its DNA Sequence

The isolation of a mutant on the basis of phenotype and subsequent identification of the gene producing the aberrant developmental phenotype through positional cloning have driven the field of developmental genetics until recently. Because this methodology is an extension of the original approach taken by geneticists since Mendel, it is sometimes called **forward genetics.** One begins with a mutant phenotype, which defines the normal functions of the mutant gene, and the task is to isolate the gene itself for molecular analysis. With the advent of complete genome sequencing, an opposite approach called **reverse genetics** is becoming more important. Here, one begins with a gene of possible developmental interest that has been predicted from the genomic DNA sequence, and perhaps assigned to a functional class based on its protein product's homology with other known proteins. The task is then to determine its function in the animal. To assign functions to the thousands of genes identified by genome sequencing projects, efficient methods for determining the function of genes identified through sequence analysis have become a high priority for all model organisms.

The obvious way to determine whether a gene identified from genomic sequence data is essential for normal development or physiological function in *C. elegans* is to somehow create mutations of the gene in the animal and then analyze the resulting phenotype. But while yeast and mouse geneticists have long been able to obtain knockout mutations by homologous recombination of suitably modified transgenes with their corresponding endogenous genes, researchers have not yet devised a reliable method for targeted homologous recombination in *C. elegans.* Nevertheless, because of the worm's small genome size, suitability for large genetic screens, and ability to survive freezing, it is possible to obtain mutations in a predicted *C. elegans* gene through PCR methods (described on pp. ••–•• of Chapter 9). In this approach, suitable primers are designed to detect novel amplification products from the gene of interest after random mutagenesis. Mutants are often generated using a transposon, which generates mutations upon insertion into a gene, or using mutagens such as the DNA cross-linker trimethylpsoralen, which causes a high frequency of small deletions. **Figure C.10** shows the basis of these techniques.

An alternative approach to determining function has become available with the development of RNAi. Researchers can make dsRNA *in vitro* from a cDNA corresponding to the gene of interest, inject it into or feed it to wild-type worms, and observe phenotypes among the progeny. For many genes, RNAi results in the null phenotype, as well as in less severe hypomorphic phenotypes as the RNAi is taking effect or wearing off. Nevertheless, since

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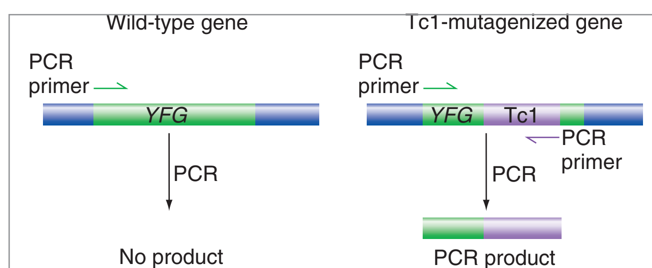

Figure C.10 Identification of mutations caused by insertion of a transposable element. Researchers first construct many independent worm strains, starting with worms in which the Tc1 transposon is highly mobile. They isolate genomic DNA from a small sample of each line and freeze the remaining worms (*C. elegans* can survive indefinitely in the frozen state). Using one primer specific to Tc1 and a second primer specific to their gene of interest (*YFG* for "your favorite gene"), a PCR product will be formed only if Tc1 has inserted into the gene. The worms in lines identified in this way can be thawed and grown for study.

some genes are not efficiently silenced by RNAi, it is preferable to check RNAi results by obtaining and characterizing a deletion predicted to be a null mutation.

The availability of procedures for defining the biological function of genes known only by their DNA sequence substantially increases the usefulness of *C. elegans* as an experimental system. For example, researchers can mutate *C. elegans* genes that are homologous to human genes involved in genetic diseases and analyze the nematode genes genetically and phenotypically. It may even be possible to develop an animal model in *C. elegans* for some human genetic diseases far more quickly and at much less expense than in a mammalian species such as the mouse.

Genetic Mosaic Analysis

Genetically constructed animals composed of both wild-type and mutant cells are called **genetic mosaics.** The analysis of such mosaics is one way to determine the *focus of action* for a gene of interest, that is, the cells in which the gene must be active to allow the animal to develop and function normally. For example, if a mutant *Unc* gene causes paralysis as the result of a muscle defect, then mosaics whose muscle cells are all wild type will not have the uncoordinated Unc phenotype even if the rest of the cells in the animal are mutant. By contrast, if a mutant gene causes paralysis as the result of a neuronal defect, only mosaics in which the neurons that control the body-wall muscle cells have the mutant gene will be uncoordinated (Unc), and the genotype of the muscle cells themselves will be irrelevant. This result would demonstrate that the focus of action for this gene is in the neurons.

To construct genetic mosaics in *C. elegans,* one can use chromosomal fragments generated by X or γ irradiation. Because *C. elegans* chromosomes are holocentric, even small chromosomal fragments have sufficient centromeric activity to segregate fairly normally during meiosis and mitosis. These fragments can thus be maintained as extrachromosomal, *free duplications* in a genetic stock. Even so, cells lose them at relatively high frequencies, ranging from one loss per 200 cell divisions for small fragments to much lower frequencies for larger fragments.

To carry out a mosaic analysis, a researcher constructs a *C. elegans* strain that is chromosomally homozygous for a recessive mutation in a gene of interest and introduces into that strain a free duplication including the wild-type allele of the same gene. If the free duplication is lost during mitosis at some point during somatic development, a clone of mutant cells will arise. The inclusion of appropriate marker mutations in the experimental strain makes it possible to identify in adult mosaic animals those cells that have lost the free duplication. And since the entire cell lineage is known, it is usually possible to establish the precise division at which the fragment was lost and thereby ascertain the genotype of every cell in the animal. Phenotypic analysis of a large number of mosaic animals can then reveal which cells must express the wild-type gene for an animal to develop and function normally.

Figure C.11 diagrams a genetic mosaic experiment using a free duplication. In the developing somatic gonad, the cells Z1.ppp and Z4.aaa make cell-fate decisions requiring the function of the *lin-12* gene. In half of all wild-type animals, Z1.ppp becomes the anchor cell (AC) and Z4.aaa becomes a ventral uterine precursor cell (VU). The other half of the time, Z1.ppp becomes a VU and Z4.aaa becomes the AC. In *lin-12* loss-of-function (null) mutants, both cell types become ACs. In *lin-12* gain-of-function (constitutively active) mutants, both cell types become VUs. Using mosaic analysis of *lin-12,* researchers showed that Z1.ppp and Z4.aaa cells must express the *lin-12* gene product to become a VU. If only one of these two cells lacked *lin-12* function, that cell always became the AC, and the other became a VU cell. If neither cell could manufacture LIN-12 protein, then both became AC. This is consistent with the anticipated role of the *lin-12* gene product, which is similar in amino-acid sequence to the membrane-bound Notch growth-factor receptor in *Drosophila.* The LIN-12 protein presumably receives the signal that induces Z1.ppp or Z4.aaa to become a VU cell.

This procedure depends on obtaining a free duplication that includes the gene of interest, which is sometimes difficult. If the gene of interest has been cloned, instead of using a free duplication, it is possible to use a transgenic array that includes a wild-type copy of the gene as well as a GFP reporter made with a gene that is expressed in most cells throughout development. Mutant animals carrying such an array should be phenotypically normal, because the array rescues the mutant defect, and almost all their cells will fluoresce. Mosaic animals arising in the population that have lost the array in some of their cells can be analyzed under the microscope, and losses in particular cells can be correlated with appearance of the mutant phenotype

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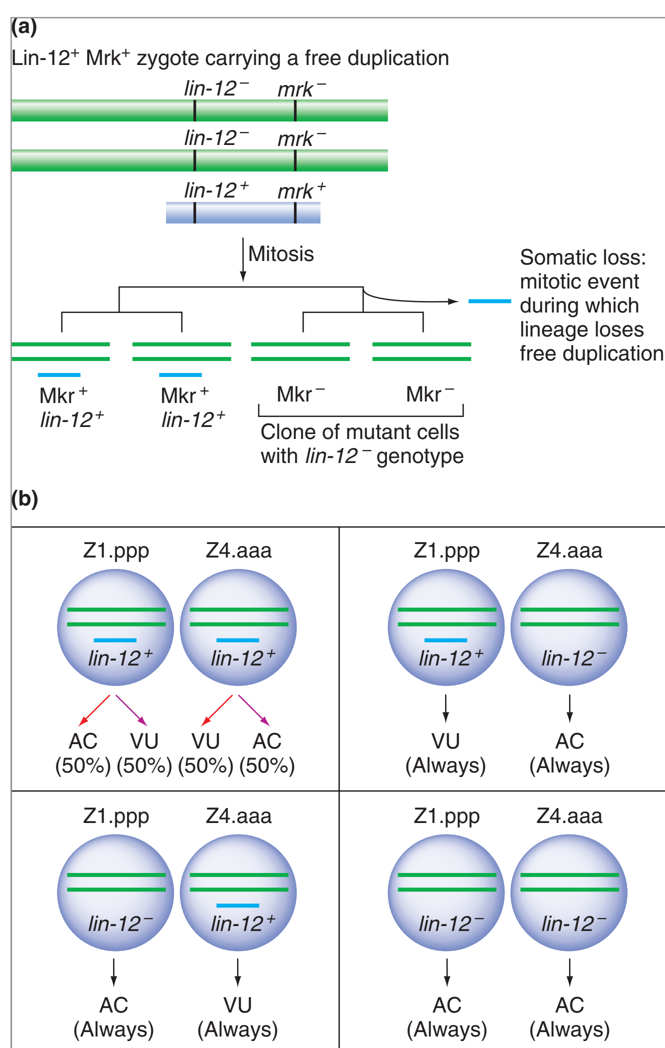

Figure C.11 **Mosaic analysis in *C. elegans*.** **(a)** Mosaic analysis is set up so that a free duplication carries a wild-type allele of a gene of interest (*lin-12*$^+$ in this example) in a mutant (*lin-12*$^-$) worm. Mitotic loss of the duplication creates a clone of cells that lack *lin-12* function. An additional gene (*mrk*$^+$ on the duplication, *mrk*$^-$ in the background) provides a phenotypic marker that indicates which cells have lost the *lin-12*$^+$ free duplication. By examining many mosaic animals, researchers can determine which cells must have the wild-type allele for the animal to develop normally. **(b)** Results of mosaic analysis showing that a Z1.ppp or Z4.aaa cell must have *lin-12*$^+$ activity to assume a VU fate. The 50/50 distribution of fates when both cells are *lin-12*$^+$ occurs as a consequence of cross-talk between these cells that randomly turns up expression of *lin-12* in one of the cells (which will become VU) and down in the other (the presumptive AC).

to determine which cells must express the wild-type gene for development to be normal.

C. elegans Research in the Postgenomic Era

The complete genomic sequence of *C. elegans* has given researchers an overall picture of the genes and provided the data with which to explore not only individual gene functions but also the entire genome as a functional system. This pursuit, sometimes called **functional genomics,** includes the identification of *all* the genes that are expressed and *all* the genes that are required during various stages of development or in response to a physiological stimulus.

Global analysis of gene expression at specific times in development is possible using DNA chips that contain ordered microarrays of DNA sequences representing all of the 19,000 predicted genes in the genome, each at a known location on the chip. If investigators take populations of mRNAs from, for example, two different stages of development, label them with fluorescent tags, and hybridize them to the chip, they can analyze the fluorescence intensities at each location to determine which transcripts are present as well as their relative abundance for each developmental stage (see Figs. 1.•• and 10.••). By making many such comparisons, the investigators can create a temporal expression profile for the entire genome at each stage of development. Analysis of spatial expression is more difficult, but projects are also underway to determine *in situ* hybridization patterns at various stages of development using probes for every predicted gene.

C.2 The Genetic Dissection of Developmental Processes in *C. elegans*

The techniques of functional genomics described previously hold great promise for the future, but most current knowledge of *C. elegans* developmental and physiological mechanisms has come from the application of forward genetics (isolation of mutants by phenotype followed by identification of the responsible genes), often supplemented with laser ablation experiments. Through mutagenesis and a variety of clever screening strategies, geneticists have identified genes that control a range of developmental and physiological processes in *C. elegans.* To understand how the protein products of these genes work together at the molecular level, these scientists have carried out more refined genetic analyses (for example, epistasis tests and mosaic analysis), and they have cloned the genes to allow molecular investigations into cellular functions of the corresponding encoded proteins.

In addition to confirming that many developmental processes in *C. elegans* have been conserved in more complex animals, these studies have revealed that genes controlling aspects of development that seem to be unique in the nematode often have counterparts in other animals. This finding demonstrates that closely related proteins can play substantially different roles in different organisms.

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We now describe genetic studies of several aspects of development in *C. elegans.* These examples illustrate the power of genetic analysis in the dissection of development.

Specification of Early Embryonic Blastomeres

The lineage diagrams in Fig. C.7 show that successive cleavages of embryonic cells produce blastomeres that give rise to different sets of tissues and structures during embryogenesis. Genetic screens for mutations that cause abnormal early cleavage patterns have identified many genes that control early cell fates. Analyses of the functions of these genes suggest that the determination of these fates depends on two factors: the segregation of cell-autonomous determinants, which act independently in single cells; and the transmission of inductive signals between cells.

Early Cleavages Depend on Maternally Supplied Components

As in most animals, the transcription of all but a few genes in the embryonic nuclei of *C. elegans* begins only after several rounds of cell division have occurred. Therefore, the fates of the earliest blastomeres must be guided by mRNAs or proteins that were present in the egg before fertilization. These molecules—the *maternal components* that the mother supplied to the egg during oogenesis—are the products of *maternal-effect genes.* Mutations in maternal-effect genes (known as **maternal-effect mutations**) disrupt the early events of embryogenesis in *C. elegans.*

A genetic screen devised to identify the maternal-effect genes transcribed during oogenesis is based on the fact that fertilization and early embryonic development occur in the hermaphrodite's uterus. If, for example, a hermaphrodite is unable to lay her fertilized eggs because of mutations that block development of the vulva, the eggs will grow into larvae within the uterus. These larvae will eventually eat the mother up from the inside and cause her to burst open. However, if the mother is homozygous for a mutation in a gene encoding a maternal component needed for early development (a maternal-effect lethal mutation), the embryos in her uterus will be unable to grow into larvae, and she will survive. Investigators can then look in the microscope at the defective embryos to determine how the mutation has disrupted their development (**Fig. C.12**).

Cell-Autonomous Determinants Must Be Distributed to the Cells in Which They Act

The unfertilized *C. elegans* oocyte has no predetermined anterior or posterior end. Instead, the polarity of the embryo depends on the site of sperm entry, which becomes the posterior end. The sperm entry point somehow directs flowing movements of the cytoplasm within the fertilized egg, and these cytoplasmic movements cause some molecules

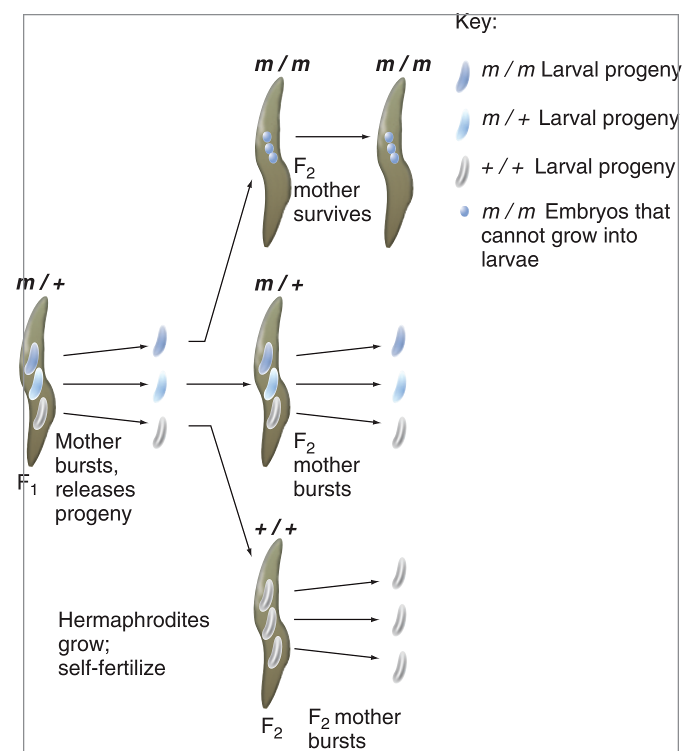

Figure C.12 Identification of maternal-effect lethal mutations in *C. elegans*. Mutagenized parental animals (*not shown*) produce F_1 hermaphrodites heterozygous for a maternal-effect lethal mutation (*m*). One-quarter of the self-fertilization progeny of such an *m* / + F_1 mother will be *m* / *m* F_2 hermaphrodites. Embryos made within these *m* / *m* mothers cannot hatch into larvae when they lack a maternally supplied component needed for embryogenesis. The hermaphrodites also have a mutation that prevents formation of the vulva, and therefore they will be eaten from the inside if their self-fertilized progeny hatch into larvae. In a screen for maternal-effect lethal mutations, researchers look for cultures derived from individual F_1 hermaphrodites in which some F_2 hermaphrodites (those that are *m* / *m*) survive because their progeny arrest development before hatching. The mutation can be maintained in *m* / + heterozygotes from the same population.

and molecular complexes to localize in an asymmetric pattern. An example of this phenomenon are the so-called P granules: ribonucleoprotein particles that are necessary for the development of the germ line. Before fertilization, P granules are uniformly distributed in the egg. After sperm entry, the granules become localized to the posterior part of the one-cell zygote (**Fig. C.13a**). After the first cleavage, they are found in the posterior P_1 daughter cell (from which the germ line descends), but not in the anterior AB daughter cell. Another example of the polarity of the first division is the fact that the P_1 and AB cells are not the same size (see panel c in Fig. C.5). How does an embryo that starts its life without polarity develop anterior and posterior ends by the first cleavage?

To answer this question, researchers looked through collections of hermaphrodites for maternal-effect lethal mutants that produce two-cell embryos with no evidence of polarity.

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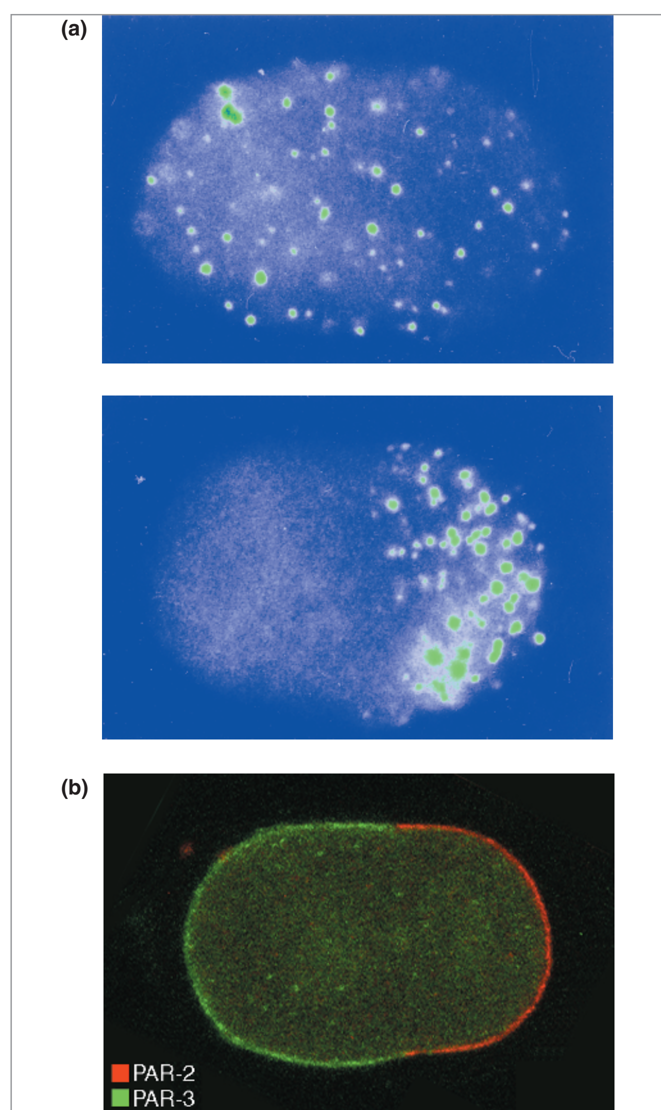

Figure C.13 The PAR proteins help direct embryonic polarity. (a) In wild-type zygotes, P granules (*green*) are initially uniformly distributed (*top*), but then they become localized to the posterior part of the zygote (*bottom*). **(b)** Shortly after fertilization, PAR-2 protein is found in the posterior cortex and PAR-3 protein in the anterior cortex.

The investigators identified mutations in six *par* (for "partitioning") genes. Mothers bearing these mutations produce embryos that have P granules in both the P_1 and AB cells and/or P_1 and AB cells that are the same size. Because this lack of polarity disrupts the patterning of the embryo, the embryos from mutant mothers eventually arrest their growth as amorphous masses of partially differentiated cells.

How the proteins encoded by the *par* genes respond to the cue given by entry of the sperm is not yet known, but molecular analysis of these genes is providing clues. Many of the PAR proteins become localized to the embryonic cortex (the part of the cytoplasm just under the cell membrane) at one or the other end of the embryo. For example, PAR-2 accumulates in the cortex at the posterior end, while PAR-3 is restricted to the cortex at the anterior end (Fig. C.13b). Localization of PAR proteins occurs after fertilization but before the P granules move to the posterior part of the zygote, as would be expected if the PAR proteins regulate the positioning of the P granules. Further experiments show that interactions between PAR proteins are required for the establishment of polarity. For example, in eggs produced by a *par-2* mutant mother, the PAR-3 protein is distributed uniformly around the cortex; the same is true of the PAR-2 protein in eggs from a *par-3* mutant hermaphrodite. Thus, the PAR-2 protein excludes PAR-3 from its half of the embryo's cortex, and vice versa. Current research is focusing on the possibility that the PAR proteins influence microfilaments in the cortex of the one-cell embryo that are responsible for cytoplasmic flows.

Interestingly, even animals such as *Drosophila* and humans, whose early embryonic development contrasts sharply with that of *C. elegans,* have genes that are closely related to and perform functions homologous with many of the *par* genes. The *Drosophila* and human PAR-like proteins are also involved in establishing polarity in developing cells.

Inductive Signals Control Cell Fates in Early Embryogenesis

The PAR proteins exert their effects only within the individual cells that contain them. Because of this cellular localization, researchers consider them *cell autonomous.* Early *C. elegans* development also depends on other asymmetrically partitioned factors that control signals sent via cell contact between adjacent blastomeres. Because the patterns of early cleavage and later gastrulation cell movements are invariant, blastomere contacts and cell-to-cell interactions are reproducible from embryo to embryo.

An example of signaling via cell contact is the process by which different fates are established for the ABa and ABp daughter cells produced by cleavage of the AB founder cell. The two daughter cells are developmentally equivalent when they arise at the second cleavage. They become different as a result of interactions between ABp and the P_2 cell. Normally P_2 contacts ABp but not ABa (**Fig. C.14a**). If P_2 is removed by laser ablation, ABp adopts an ABa pattern of differentiation. Alternatively, when the embryo is manipulated so that ABa contacts P_2, the ABa lineage develops in an ABp-like fashion. These observations suggest that the P_2 cell provides an inductive signal receivable by AB cells that contact it.

To identify molecules involved in this signaling, researchers searched collections of maternal-effect lethal mutations for mutations that disrupt the fate of the ABp lineage. They found that the receptor for the inductive signal is encoded by the *glp-1* gene. Like *lin-12, glp-1* is closely related to the *Drosophila Notch* gene. *Notch* encodes a membrane-bound receptor for a membrane-bound **ligand** (signaling molecule) encoded by the *Delta* gene, which is expressed in adjacent cells. *glp-1* mRNA is present in all cells at the four-cell stage, but asymmetrically partitioned factors repress its translation in P_2 and EMS, so that it is expressed only in AB cells and their descendants. The signaling ligand for ABp determination is encoded by the *apx-1* gene. Asymmetrically

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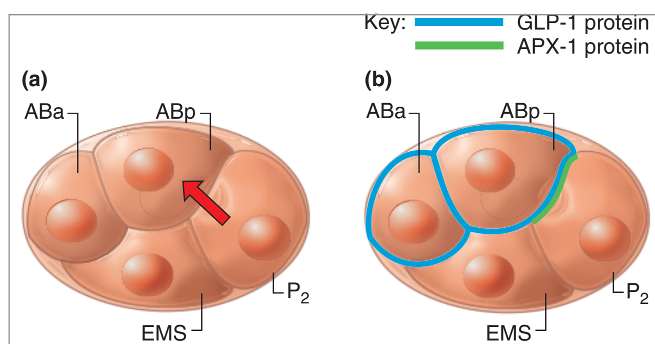

Figure C.14 **Intercellular signaling determines the fates of the ABa and ABp cells. (a)** Contact between P_2 and ABp enables P_2 to send a signal to ABp that is required for the proper fate of the ABp lineage. **(b)** Molecular basis of the intercellular signal. The P_2 cell expresses APX-1 protein on its surface, while ABa and ABp both have GLP-1 protein, the receptor for the APX-1 ligand, on their surfaces. Binding of APX-1 to GLP-1 in the membrane junction between P_2 and ABp initiates the proper developmental program within ABp. It is not clear why the APX-1 ligand localizes only to the surface of P_2 in contact with ABp; perhaps it is stabilized there by interaction with GLP-1.

segregated factors allow its expression only in P_2 cells, where the APX-1 protein localizes to the membrane junction between P_2 and ABp and contacts the GLP-1 protein (Fig. C.14b). *apx-1* is a homolog of *Drosophila Delta.* Thus, very similar ligands and receptors mediate signaling through cell contact in both *C. elegans* and *Drosophila.*

Programmed Cell Death

About one-sixth of the cells that arise during *C. elegans* embryogenesis are eliminated by a process of cellular suicide called *programmed cell death* or **apoptosis.** During apoptosis, the affected cell shrinks, degrades its DNA, and dies, after which it is engulfed and digested by a neighboring cell. In *C. elegans,* the pattern of apoptosis, like most other aspects of development, is invariant: The identity of the cells that die and the timing of their deaths is the same from animal to animal. The function of programmed cell death seems to be the elimination of unneeded cells that could be detrimental to the organism.

Mutations that cause the failure of all programmed cell deaths during development have identified the *ced* (for cell death) genes that control apoptosis. For example, loss-of-function mutations in the *ced-3* and *ced-4* genes and gain-of-function mutations in the *ced-9* gene cause the complete failure of programmed cell death. Surprisingly, although animals bearing such mutations have 12% more somatic cells than normal, they show only very subtle phenotypic defects in their growth rate and fertility. By contrast, loss-of-function *ced-9* mutations cause the death of many cells that would normally have lived, and this extensive cell death is lethal to the embryo.

Epistasis tests and phenotypic analysis of *ced* mutations led researchers to formulate the developmental pathway shown in **Fig. C.15.** Cells make a decision to die based on the action of genes upstream from the *ced* genes. The upstream genes control cell fates and activate or repress *ced-9.* Since most if not all cells require the function of the *ced-9* gene to prevent activation of the cell-death process, repression of *ced-9* allows activation of the events leading to apoptosis. Next, the *ced-3* and *ced-4* genes function to carry out the cell-death program. CED-3 is a member of the caspase family of proteases, with homologs in both *Drosophila* and humans that are also essential for apoptosis. The CED-4 protein is required for activation of CED-3. Targets of the CED-3 protease, whose cleavage must trigger the subsequent events of apoptosis, are still being identified. Other genes, such as *ced-1* and *ced-2,* act in the last stages of the cell-death process. These genes do not directly contribute to cell death itself. Instead, they are required for the engulfment and digestion of dead cells by neighboring cells.

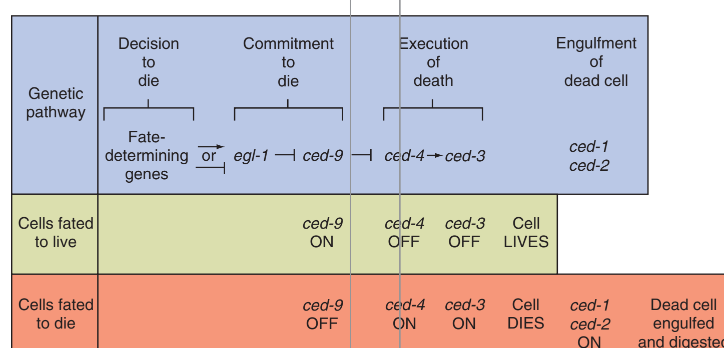

Figure C.15 **Control of programmed cell death in *C. elegans*.** The *blue-shaded area* describes four steps in the cell-death program and some of the genes involved in each of these steps. In the genetic pathway, → indicates positive regulation (the protein encoded by the gene to the left activates the gene to the right or its encoded protein), while ⊣ indicates negative regulation (the protein encoded by the gene on the left represses the gene to the right or inhibits its encoded protein). Inputs from several pathways can activate or deactivate a sensor encoded by the *egl-1* gene, which in its active state represses *ced-9.* All these genes act within the cell that is fated to die except for *ced-1* and *ced-2,* which function in the engulfing cell. *Green-* and *orange-shaded areas* detail gene activity and subsequent events within cells that are fated to live or undergo apoptosis.

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Mutations in *ced-1* and *ced-2* cause a "persistent corpse" phenotype in which cells die as usual but remain in the worm for several days.

Programmed cell death in *C. elegans* appears very similar to apoptosis in mammals. In both types of organisms, the process helps control the size of cell populations by eliminating excess cells. As we saw in Chapter 19 (pp. ••–••), the investigation of genes that control apoptosis is an important aspect of current cancer research. Although tumor formation often involves the release of normal controls on cell proliferation, it can also result from the failure of apoptosis. Sequence similarity between the membrane-associated protein product of the *C. elegans ced-9* gene and the mutant protein product of the *bcl-2* oncogene, which helps cause tumors by blocking apoptosis in humans, underscores the close relation between the control of programmed cell death in *C. elegans* and mammals. In fact, although nematodes and humans are separated by almost a billion years of evolution, their apoptosis-controlling genes are still functionally interchangeable: The normal human allele of the *bcl-2* gene can restore the normal process of cell death when injected into a *C. elegans ced-9* mutant.

Control of Timing During Larval Development

Throughout development, control over the timing of a cellular event is as important as control over where it occurs. Researchers have used *heterochronic mutations,* that result in the inappropriate timing of cell divisions and cell-fate decisions during larval development to identify *C. elegans* genes involved in temporal controls.

The genes identified by heterochronic mutations often behave as *switch genes* in which loss-of-function and gain-of-function mutations have opposite temporal effects. For example, loss-of-function mutations in the *lin-14* gene cause precocious development: Cell lineages that normally emerge late in development appear too early. Conversely, a gain-of-function mutation in the *lin-14* gene causes retarded development, in which lineage changes that normally appear only in early larval stages continue to appear at later larval stages (**Fig. C.16a**). Mutations in *lin-14* not only change the timing of certain developmental programs, but they can also alter the cell cycle. Cells that normally divide at a specific time in larval development will divide earlier than normal in *lin-14* null mutants and later than normal in *lin-14* gain-of-function mutants (Fig. C.16b). In addition, heterochronic mutations can affect the formation of dauer larvae in crowded animals. Instead of occurring normally at the L2 molt, the dauer larvae can form at the L1 or L3 molts. Investigators have identified mutations in several other genes that produce effects either similar or opposite to those associated with *lin-14.* Interactions between these genes control the timing mechanisms that specify many aspects of larval development.

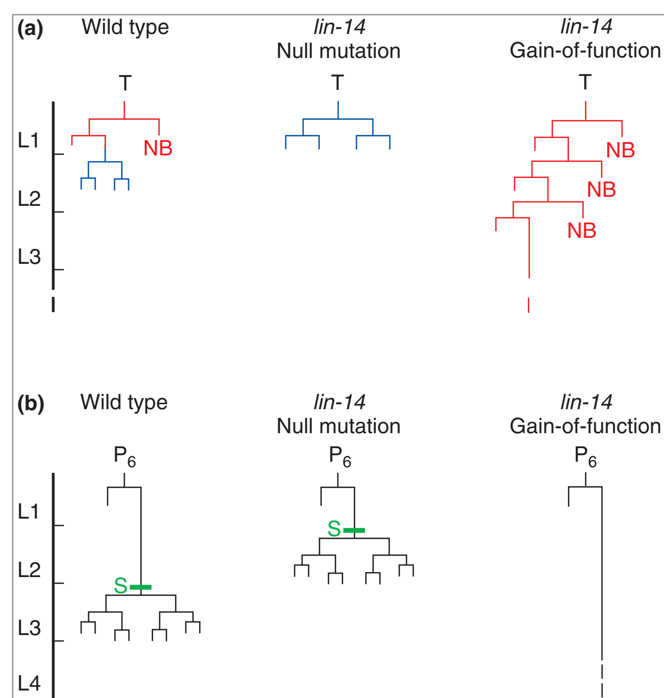

Figure C.16 **Effects of mutations in the heterochronic gene *lin-14.*** **(a)** Transformations of cell fates in the T lineage. In wild type, the T cell undergoes a pattern of division specific for the L1 larval stage (*red; NB* signifies a neuroblast), and one of the granddaughters of T undergoes an L2-specific cleavage pattern (*blue*). In *lin-14* null mutants, the T cell precociously initiates the L2 pattern in the L1 stage. In *lin-14* gain-of-function mutants, the lineage inappropriately reiterates the L1 pattern at later larval stages. **(b)** Effects on the timing of cell divisions in the hypodermal P6 lineage. Loss of *lin-14* function causes one of the daughters of P6 to start cell division too early (*S* indicates the DNA synthesis phase of this division). Gain of *lin-14* function can delay this division indefinitely.

The LIN-14 protein, which shows no similarity to other proteins of known function, behaves as a central clock component whose concentration descends from a high in L1 animals to a low in L4 animals. During larval development, cells somehow use the relative abundance of LIN-14 to "tell time." The level of *lin-14* transcript, however, remains constant during larval development, which suggests that control of LIN-14 protein concentration is at the level of translation or protein stability. The pathway defined by the heterochronic genes appears to act ultimately through regulation of the *lin-14* gene.

Epistasis tests have shown that one of the pathway's temporal control genes, *lin-4,* negatively regulates expression of *lin-14* by a novel regulatory mechanism. The *lin-4* gene does not encode a protein. The *lin*-4 RNA transcript includes self-complementary sequences that form a double-stranded hairpin structure. The RNA is processed to produce a 22-nucleotide micro-RNA (miRNA) whose sequence is complementary to sequences in the 3′ untranslated region of the *lin*-14 mRNA. (see pp. ••–•• of Chapter 18 for a detailed

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description of miRNAs.) Protein components of the RNAi machinery appear to promote the complexing of the mature *lin-4* RNA with the 3′ untranslated region of *lin-14* mRNA, rendering this mRNA untranslatable (rather than degrading it). This inhibition, coupled to a constant rate of LIN-14 protein degradation, results in decreasing levels of LIN-14 during development.

Small regulatory RNAs like *lin-4* RNA play a much larger role in development than originally suspected, and not only in nematodes. A second such RNA, derived from the transcript of the genetically identified *let-7* gene, plays a role similar to that of *lin-4* RNA in controlling the expression of additional *C. elegans* timing proteins that mediate the molt from L4 to adult. Recently, researchers have identified more than 50 other noncoding RNAs of about 22 nucleotides in length that could also play regulatory roles in *C. elegans*. The genes for these RNAs, identified by comparison of their sequences with the genomic DNA sequence, are predicted to encode somewhat larger, self-complementary RNAs that must be processed to produce the 22-nucleotide products. Moreover, database searches have yielded the exciting result that many of these sequences are highly conserved in the *Drosophila* and human genomes, where they also perform regulatory functions.

C.3 Using Genetics to Probe the Development of the Hermaphrodite Vulva: A Comprehensive Example

A major contribution of developmental genetic studies in *C. elegans* has been the demonstration that a signaling pathway for cell-fate determination includes a gene similar to the *ras* oncogene in humans, which is responsible for a substantial fraction of malignant tumors arising from connective tissue. The *C. elegans* signal transduction pathway operates during larval development in the formation of the hermaphrodite vulva, the passageway through which sperm from the male enter and fertilized eggs exit the gonad.

Mutant Screens Identify Genes Involved in Vulva Formation

Embedded in the developing hypodermis just below the somatic gonad on the ventral side of the L3 larva, there are six cells capable of giving rise to the vulva. These cells are numbered P3p through P8p and are known as vulval precursor cells, or VPCs. Only three of the VPCs (P5p, P6p, and P7p) normally form the vulva in response to an inductive signal from the anchor cell (AC) in the gonad, as shown in **Fig. C.17a.** Researchers inferred the

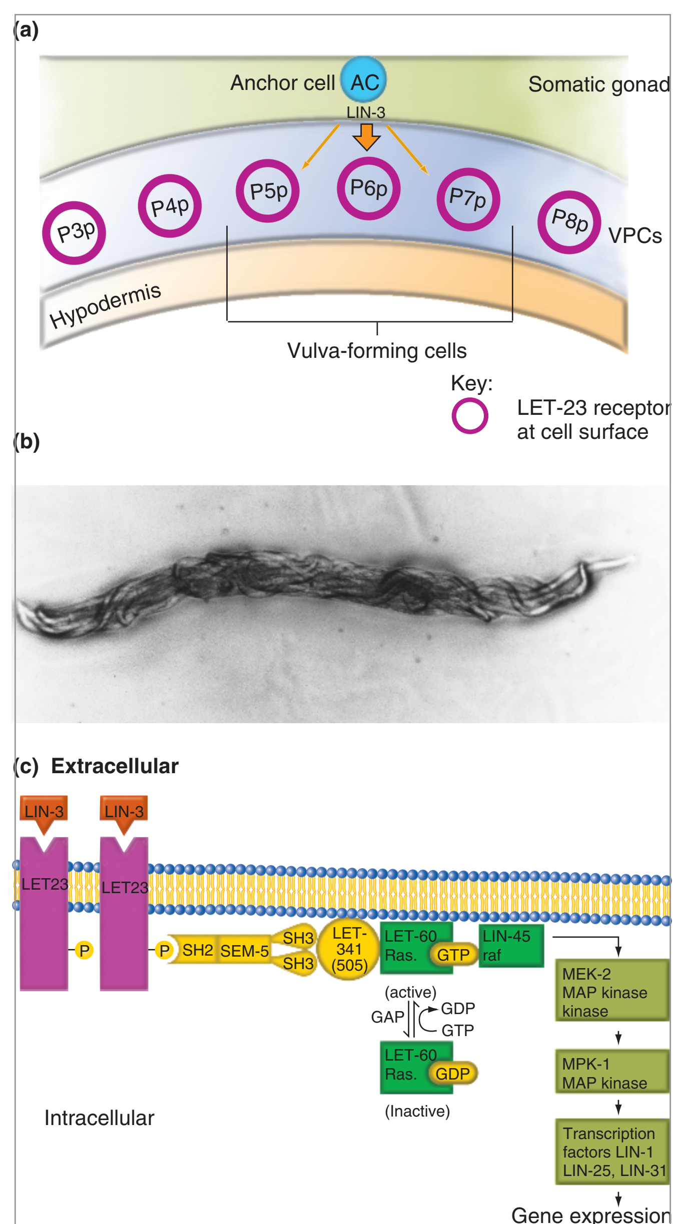

Figure C.17 Cell signaling and signal transduction cascades in vulval development. (a) The anchor cell (AC) of the gonad induces vulval fates in the vulval precursor cells (VPCs) P5p, P6p, and P7p adjacent to the underlying hypodermis. The signaling molecule produced by the AC is the LIN-3 protein; the receptor for this ligand on the VPCs is the LET-23 protein. **(b)** A "bag of worms" in which a Vulvaless mother is consumed by her progeny. **(c)** The signal transduction pathway activated by binding of the LIN-3 ligand to the LET-23 receptor. Earliest events are to the *left,* subsequent events to the *right.* The signal-bound receptor activates the adaptor proteins SEM-5 and LET-341 (a homolog of Drosophila Sos), which in turn help catalyze the conversion of LET-60 into an active GTP-bound form. Activated LET-60 initiates a cascade of protein kinases; the phosphorylated form of the last of these kinases (MPK-1) regulates the activity of several transcription factors in the nucleus including LIN-1 and LIN-31. These transcription factors in turn regulate the expression of the ultimate targets of the pathway: those genes whose products are required to make vulval structures.

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existence of this signal from experiments showing that even though the AC does not become part of the vulva, elimination of this cell by laser ablation prevents vulva formation. The remaining three VPCs (P3p, P4p, and P8p) normally produce only additional hypodermal cells, but they can adopt vulval fates under certain experimental conditions.

Worm geneticists have devised several powerful genetic screens to identify the genes involved in the AC induction of P5p, P6p, and P7p to produce vulval cell lineages. The screens all take advantage of the fact that a hermaphrodite can survive and propagate without a functional vulva.

In one procedure, investigators search for Vulvaless mutants. Although these hermaphrodites cannot lay their eggs, they can self-fertilize them normally. The resulting zygotes, which develop and hatch within the uterus, grow by devouring the mother's organs to produce a "bag of worms" that is easy to spot in a mutant hunt (Fig. C.17b). Loss-of-function mutations that prevent formation of the vulva define genes required for vulval development.

In another type of genetic screen, researchers look for mutants with a Multivulva phenotype. These animals form additional, ectopic vulvalike structures from descendants of the P3p, P4p, and P8p cells, which do not normally participate in the process. Loss-of-function mutations that cause ectopic vulva formation define genes whose products act as negative regulators of vulval development.

From these screens, researchers identified various genes that participate in the inductive signaling pathway that specifies the fates of vulval precursor cells. Interestingly, many of these genes are essential for viability. The discovery of their role in vulval determination was possible only because the screens were so powerful that investigators could recognize animals with rare hypomorphic (partial loss-of-function) or hypermorphic (gain-of-function) mutations.

Organizing the Genes Contributing to Vulva Formation into a Signaling Pathway

By combining epistasis tests, mosaic analysis, and phenotypic characterization, researchers constructed a genetic pathway that clarifies the relationship between many of the genes involved in vulva formation (Fig. C.17c). Subsequent cloning of the genes elucidated many of the pathway's protein components and led to an understanding of the pathway at the molecular level.

The Binding of a Signal Molecule to a Cell Surface Receptor Initiates Events Leading to Vulva Formation

In this first step of the signaling pathway, the anchor cell emits a signal that must be received by the P5p, P6p, and P7p vulva precursor cells (Fig. C.17). This AC signal is a small protein encoded by the *lin-3* gene. The LIN-3 protein is similar in structure to mammalian epidermal growth factor (EGF). The receptor for the LIN-3 ligand on the VPC cells is encoded by the *let-23* gene, and the LET-23 protein it encodes is similar to mammalian EGF receptors. As expected for genes whose products are needed for vulval specification, partial loss-of-function mutations in both *lin-3* and *let-23* confer Vulvaless phenotypes. However, overproduction of the LIN-3 protein causes a Multivulva phenotype. As required by the pathway model, LIN-3 (the ligand) is expressed in the AC cell, while LET-23 (the receptor) is expressed in the VPCs.

A Signal Transduction System Conveys the Signal Received at the Cell Surface to the Cell's Nucleus

After the LIN-3 signal binds to the LET-23 receptor, subsequent steps in the vulva-induction pathway occur within the P5p, P6p, and P7p cells (Fig. C.17c). These intracellular steps together constitute a signal transduction pathway that converts the signal received at the cell surface into changes in the transcription of particular genes in the cell nucleus. It is these changes in gene activity that cause the cells to adopt vulval fates.

A key player in the signal transduction system for vulva induction is the *let-60* gene, which encodes a *C. elegans* RAS protein. Gain-of-function mutations in *let-60* produce alterations in the LET-60 protein identical to those that convert mammalian *ras* products to their oncogenic form. The resulting mutant LET-60 becomes active even without a signal, and the ensuing inappropriate induction of vulval cell fates in P3p, P4p, and P8p leads to a Multivulva phenotype. By contrast, partial loss-of-function mutations in *let-60* cause a Vulvaless phenotype.

Like other RAS proteins, LET-60 is a guanine nucleotide-binding protein whose activity is indirectly controlled by an EGF receptor. Binding of the ligand signal activates the receptor, which in turn activates certain adaptor proteins. These adaptor proteins help transform the inactive GDP-bound form of LET-60 into an active GTP-bound form.

Also genetically identified in *C. elegans* are the downstream genes through which *ras* activity eventually influences events in the nucleus. These genes include *lin-45,* which encodes a protein kinase similar to one encoded by the mammalian protooncogene *raf,* as well as *mek-2* and *mpk-1,* which encode a MAP-kinase kinase and a MAP kinase, respectively. These proteins form a protein kinase cascade in which each member catalyzes the phosphorylation of the next member. Although the details are still unclear, epistasis tests and biochemical evidence suggest strongly that this cascade modifies the nuclear transcription factors encoded by *lin-1, lin-25,* and *lin-31* to control their activities.

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This Type of Signaling Pathway Has Been Conserved in Evolution

Signaling pathways including a ligand, its receptor, and a signal transduction system activated by the binding of ligand to receptor are a feature of intercellular signaling in many organisms. In *Drosophila,* for example, a system similar to the one we have described here operates in the development of the eyes; in humans, defects in such signaling systems contribute to cancer formation.

Geneticists are thus very interested in extending the genetic dissection of vulval development to identify additional genes that influence this ubiquitous signaling pathway. They have recently begun two new types of searches. First, they are looking for mutations that suppress the Multivulva phenotype caused by *let-60* gain-of-function mutations. Second, they are screening for mutations that enhance the Vulvaless phenotype caused by *let-60* partial loss-of-function mutations. Through these mutant screens, they have already identified the kinase-encoding *ksr-1* gene, a component of the signal transduction system that eluded detection by other means (such as biochemical analysis). The results of these investigations may be of value in understanding and eventually controlling the production of tumors caused by the *ras* and *raf* oncogenes in humans.

Connections

The special features of *C. elegans,* including its small, invariant number of cells and transparency at all stages of the short life cycle, make it an excellent model for the genetic analysis of development in multicellular animals. With knowledge of the progenitors and position of every cell in the embryo, larvae, and adult, researchers have been able to identify many of the genes that specify cell fates during development. The completion of the genomic DNA sequence and the development of high-throughput methods for determining gene-expression patterns and functions should make it possible in the foreseeable future to understand the roles of most of the 19,000 predicted *C. elegans* genes in development and physiology.

The next genetic portrait is of another multicellular animal: *Drosophila melanogaster.* In *Drosophila,* the developmental fates of individual cells are determined by a much more flexible mechanism than the invariant cell lineage seen in *C. elegans.* Through the analysis of these two model systems for animal development, biologists can compare the ways in which evolution has generated various structures and functions. Sometimes the strategies different organisms use to perform the same basic task are similar, but as the fundamental dichotomy in the mechanisms of cell determination suggests, sometimes they are very different.

Essential Concepts

1. *C. elegans* is an ideal subject for genetic analysis because of its transparency, invariant cell lineage, short life cycle, and capacity for prolific reproduction.
2. The small genome of 97 Mb has been completely sequenced. It is packaged into six small chromosomes. The 19,000 genes contain small introns. *Trans-splicing* and the production of *polycistronic transcripts* are two unusual aspects of *C. elegans* gene expression.
3. RNA-mediated interference (RNAi), discovered in *C. elegans,* also functions in many other organisms. It provides researchers with a powerful method for silencing genes predicted from genomic sequencing, in order to determine their functions.
4. The life cycle from the fertilized egg of one generation to the fertilized eggs of the next takes about three days. Mating results in cross-fertilization. Hermaphrodites can self-fertilize.
5. Because the cell lineage of *C. elegans* is almost completely invariant, developmental biologists have been able to construct complete *cell lineage diagrams* that show the routes by which all the somatic cells in the adult arise from the fertilized zygote. Six *founder cells* give rise to the major embryonic lineages. The study of mutants with abnormal cell lineage patterns is a major focus of *C. elegans* research.
6. Determination of blastomere fate depends on inductive signaling between cells as well as on the segregation of cell-autonomous factors acting only in the cells that contain them.

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7. Mutations that cause the failure of all programmed cell deaths during development helped researchers identify the genes that control *apoptosis*. Although nematodes and humans are separated by almost a billion years of evolution, some of their apoptosis-controlling genes are functionally interchangeable.
8. The interactions of various *heterochronic "switch" genes* set the timing of different stages of larval development. Novel RNAs involved in control of this process are representative of a widespread class of small regulatory RNA molecules.
9. Induction of the hermaphrodite vulva depends on a *signal transduction pathway* that includes a gene similar to the *ras* oncogene in humans. Identification of the genes active in this pathway in *C. elegans* may help researchers understand tumor formation in humans.
10. Despite some unusual features, *C. elegans* shares many of its regulatory pathways and developmental mechanisms with other multicellular animals.

Internet Resources for *C. elegans*

Wormbase (genome database):
www.wormbase.org/

Wormbook (online book of current research and reviews of topics in *C. elegans*, including some movies):
www.wormbook.org/

Solved Problems

I. You need to construct a strain of *C. elegans* that is homozygous for the *dpy-10* and *unc-54* mutations. The $dpy\text{-}10^-$ mutation is a defect in the collagen gene that results in a short, stubby-shaped worm (Dumpy). The $unc\text{-}54^-$ mutation, a defect in a myosin heavy-chain gene, affects muscle control so severely that the worm is paralyzed (Uncoordinated). *unc-54* and *dpy-10* are unlinked, autosomal genes and both mutations are recessive. Stock hermaphrodite worms that are homozygous for $dpy\text{-}10^-$ and another stock that is homozygous for $unc\text{-}54^-$ are available for your use. The first step is to cross wild-type males to homozygous $dpy\text{-}10^-$ hermaphrodites.
   a. What genotypes and phenotypes (including sex phenotype) do you predict in the progeny?
   b. The wild-type male progeny from the cross-fertilization are crossed to virgin hermaphrodites that are homozygous for the $unc\text{-}54^-$ mutation. (To isolate virgins, L4 larval-stage worms are transferred individually to petri plates, ensuring that mating has not occurred yet with any available males in the population.) What are the genotypes and phenotypes that result from this cross?
   c. What worms will you use from the second cross to get your double recessive $dpy\text{-}10^-$ $unc\text{-}54^-$ strain? Will you be able to identify worms with the specific genotype you need for the next step?
   d. What is the phenotype of worms with the desired double recessive genotype? What proportion of the worms emerging from the last step of the crossing scheme will have the double recessive genotype?

Answer

This problem requires an understanding of basic Mendelian ratios and of *C. elegans* matings.

a. The cross can be represented:

$$\frac{dpy\text{-}10^+}{dpy\text{-}10^+}\ \text{males} \times \frac{dpy\text{-}10^-}{dpy\text{-}10^-}\ \text{hermaphrodites}$$

Either the hermaphrodite will self-fertilize or there will be cross-fertilization between males and hermaphrodites. *When the hermaphrodite self-fertilizes, all progeny will be Dumpy hermaphrodites with the homozygous* $dpy\text{-}10^-$ / $dpy\text{-}10^-$ *genotype.* (Males [XO] will also arise from self-fertilization due to nondisjunction of sex chromosomes during formation of gametes, but the frequency is low enough that we will generally ignore this group of males. These male worms would also have a Dumpy phenotype.) In a mating between the male and hermaphrodite, the male can produce only $dpy\text{-}10^+$ gametes. Half of these gametes contain an X chromosome and the other half contain no sex chromosome. *The outcross with*

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males results in heterozygous dpy-10$^+$ / dpy-10$^-$ *hermaphrodites and males that are normal in phenotype and heterozygous at the* dpy *locus.*

b. The second cross is:

$$\frac{dpy\text{-}10^+}{dpy\text{-}10^-}\ \frac{unc\text{-}54^+}{unc\text{-}54^+}\ \text{males}$$
$$\times\ \frac{dpy\text{-}10^+}{dpy\text{-}10^+}\ \frac{unc\text{-}54^-}{unc\text{-}54^-}\ \text{hermaphrodites}$$

The hermaphrodite will produce, by self-fertilization, hermaphrodites that are normal shaped (nondumpy) but paralyzed and have the genotype dpy-10$^+$ / dpy-10$^+$ unc-54$^-$ / unc-54$^-$. From the outcross to males, the male can contribute either the *dpy-10*$^+$ or *dpy-10*$^-$ allele with the *unc-54*$^+$ allele; whereas the hermaphrodite can produce only *dpy-10*$^+$ *unc-54*$^-$ alleles. Half of the sperm from males have an X chromosome and half do not have a sex chromosome. *Results of cross-fertilization:*

XX	*dpy-10*$^+$ / *dpy-10*$^+$ *unc-54*$^+$ / *unc-54*$^-$	wild-type hermaphrodites
XO	*dpy-10*$^+$ / *dpy-10*$^+$ *unc-54*$^+$ / *unc-54*$^-$	wild-type males
XX	*dpy-10*$^+$ / *dpy-10*$^-$ *unc-54*$^+$ / *unc-54*$^-$	wild-type hermaphrodites
XO	*dpy-10*$^+$ / *dpy-10*$^-$ *unc-54*$^+$ / *unc-54*$^-$	wild-type males

c. *To obtain a doubly homozygous recessive, a double heterozygote must be selfed. Notice that half of the wild-type hermaphrodites will have the genotype desired for this cross, but you cannot distinguish between the two genotypes.* Pick several wild-type hermaphrodites and allow them to self-fertilize.

d. *The phenotype of the desired doubly recessive worm is short, stubby (Dpy), and paralyzed (Unc). The proportion of worms with a double recessive phenotype from selfing heterozygotes is 1/4 for each gene. But only half of the worms picked were heterozygous. (1/4)(1/4)(1/2) = 1/32 worms are expected to have the double homozygous recessive genotype.*

II. In *C. elegans,* the cells at the tip of the gonad go through several mitotic divisions before switching to meiotic divisions and differentiating into germ cells. As a result of the mitotic and meiotic divisions, up to 1500 germ cells are produced. When the somatic cell at the very tip of the developing gonad (distal tip cell) is killed by laser ablation, the few germ cell precursors surrounding it will immediately go into meiosis instead of mitosis.

a. What effect would this ablation have on the number of germ cells produced?
b. The mutation *glp-1*$^-$ mimics the laser ablation results. In the *glp-1*$^-$ mutant, cells immediately go into meiosis, without mitotic divisions, and only a few germ cells are produced. The hypothesis is that the distal tip cell produces a molecule (inducer) that binds to a receptor to control mitotic and meiotic divisions. In what cells would you expect to see *glp-1* expression if it encodes a receptor?
c. The GLP-1 protein functions in other processes in addition to germ cell development. For example, GLP-1 is required in the earliest cell divisions in the embryo to establish the fate of cells that develop into the pharynx. In addition, some mutations in the *glp-1* gene affect only a subset of the *glp-1* activities. Speculate on how the GLP-1 can act as a receptor in processes that have a very different outcome.

Answer

The question involves thinking through cell-to-cell interactions and their consequences.

a. Without mitotic divisions, only a few cells are available to go through meiosis and produce gametes. *Many fewer gametes are produced.*
b. If *glp-1* encodes a receptor, it should be expressed in cells that receive and interpret the signal. Those cells are the *germ cell precursors.*
c. GLP-1 protein, because of its role in various cell-to-cell interactions, could be considered a general responding protein. This general protein could be *interacting with different cell-type-specific proteins to give an appropriate, specific response.* Alternatively, or perhaps in addition, the GLP-1 receptor *may have different regions that respond to different inducing signals* and send specific messages into the cell based on which region of the receptor has been contacted.

Problems

C-1 Choose the best matching phrase in the right column for each of the terms in the left column.

a. ablation	1. having centromere activity along the length of the chromosome
b. apoptosis	2. cell death caused by outside forces such as lasers
c. heterochronic	3. extrachromosomal fragments of DNA
d. polycistronic	4. change in the time at which an event occurs
e. trans-splicing	5. programmed cell death caused by signals inside the cell
f. holocentric	6. sections of different RNAs are joined together
g. free duplication	7. containing several genes

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C-2 To construct a library for which there is a 99% probability that any one genomic sequence will be represented, the amount of DNA represented in the independent clones should be approximately five times the genome size. Assuming an average cosmid size of 50 kb and a genome size of 100 Mb, how many cosmids would you need to have in your library to ensure that each sequence in *C. elegans* was represented at least once?

C-3 What is (are) the advantage(s) in using a hermaphrodite when screening for mutants?

C-4 Vulval development in the *C. elegans* hermaphrodite has provided an excellent model system for studying the molecular basis of cell interaction and signaling during development. Genetic analysis and cell ablation studies have been critical in identifying genes and determining the role of their gene products in vulval development. For which of the following purpose(s) would you want to

a. combine different vulval mutations into one strain?
b. introduce RNAi specific for a gene expressed in vulval precursor cells?
c. isolate suppressors of a vulval mutant phenotype?
d. ablate a cell?
   i. to identify mutations that bypass a normal mechanism
   ii. to identify genes that work in the same pathway
   iii. to determine the function of a cell during development
   iv. to determine if mutations are in the same gene
   v. to determine if a gene is needed for proper vulval development

C-5 You isolated a cDNA for an actin gene. Which of the following results would suggest that trans-splicing occurs during processing?

a. the 5′ end of the cDNA hybridized with many DNA clones from the genome
b. the 5′ end of the cDNA hybridized with many RNAs in the worm
c. the 3′ end of the cDNA hybridized with many DNA clones from the genome
d. the 3′ end of the cDNA hybridized with many RNAs in the worm
e. the 5′ end of the cDNA hybridized with one DNA clone from the genome
f. the 5′ end of the cDNA hybridized with one RNA in the worm
g. the 3′ end of the cDNA hybridized with one DNA clone from the genome
h. the 3′ end of the cDNA hybridized with one RNA in the worm

C-6 Laser ablation of cells can be done during embryonic development, during larval stages, or in the adult. To determine if the anchor cell is involved in establishing the identity of surrounding vulval precursor cells, describe (in general) the time at which you would ablate the anchor cell.

C-7 The concentration of LIN-14 protein, high during the L1 larval stages and decreasing in later stages, regulates the timing of cell divisions in the T-cell lineage in *C. elegans.* According to the hypothesis presented in this chapter, *lin-4* RNA regulates the level of LIN-14 protein by binding to the *lin-14* mRNA and preventing translation. If you could introduce extra copies of *lin-4* into the cells and more *lin-4* RNA was produced, what effect do you think the extra copies of *lin-4* would have on translation of LIN-14? What effect could this have on cell lineages during development?

C-8 The *C. elegans* strain Bergerac has a mutation rate significantly higher than that seen in other strains. For example, spontaneous mutations in the *unc-22* gene arise at a frequency 100 times higher than the frequency of mutation in the Bristol strain. You think the mutations may be caused by insertion of transposons. How could you test whether the transposition of the Tc1 element caused some or all of the mutations in the *unc-22* gene? (Assume that the *unc-22* gene and Tc1 DNA have been cloned.)

C-9 How would you construct a hermaphrodite with the genotype *dpy-10*$^-$ / *dpy-10*$^-$ *daf-1*$^-$ / *daf-1*$^-$? You are given stocks of wild-type males, *dpy-10*$^-$ / *dpy-10*$^-$ hermaphrodites and *daf-1*$^-$ / *daf-1*$^-$ hermaphrodites. (*dpy-10* and *daf-1* are unlinked autosomal genes.)

a. What is the first cross you would do?
b. Which worms would you use in the second cross?
c. What is the final cross you do to get the doubly homozygous worms? What proportion of the hermaphrodites do you expect to have the doubly homozygous genotype?

C-10 The *unc-54* (uncoordinated) and *daf-8* (dauer constitutive) genes are 18 m.u. apart on chromosome I of *C. elegans.* You want to construct a worm strain homozygous for mutations in both genes. Stocks of wild-type males and *unc-54*$^-$ / *unc-54*$^-$ hermaphrodites and *daf-8*$^-$ / *daf-8*$^-$ hermaphrodites are available for your use. The first cross is wild-type males X *unc-54*$^-$ / *unc-54*$^-$ hermaphrodites.

a. What progeny of this first cross will you use for the second cross?
b. What progeny arise from your second cross?
c. What worms will you use for the final cross to get the desired double homozygote?

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d. What event has to happen to get a double homozygote?
e. What proportion of the progeny will have the desired genotype?

C-11 Muscles in the worm can be classified in four major groups: body muscles involved in locomotion, enteric muscles involved in defecation, pharynx muscles involved in feeding, and egg-laying muscles in the hermaphrodite. Uncoordinated (Unc) worms have been isolated that are defective in neuromuscular control, resulting in a variety of phenotypes from slow-moving to completely paralyzed.

a. *unc-103*$^{-}$ is a mutation that causes defects in all muscle groups except pharyngeal muscles and has the phenotype of complete paralysis. The approximate location of the gene was determined by genetic mapping. One gene already identified in the region encodes a potassium (K^+) channel protein. Because ion channels and membrane depolarization are involved in muscle response, it was hypothesized that the *unc-103* mutation might be in the channel gene. Taking a candidate gene approach, how would you proceed to determine if the *unc-103* mutation was in fact a mutation in the K^+ channel gene?
b. Some of the *unc-103* alleles were constructed by insertion of the Tc1 transposon. How could you use this type of mutation and the physical map information on the location of the K^+ channel gene to determine if *unc-103* is in fact the K^+ channel gene?
c. Depolarization of the membrane (increase in the positive charge inside the cell) is necessary for muscle contraction. Opening of a K^+ channel is involved in the repolarization event, allowing K^+ to flow out of the cell and reestablish the negative charge on the inside of the cell. *unc-103* gain-of-function alleles lead to an inappropriately open K^+ channel so potassium continues to flow out of the cell. Do you think the gain-of-function *unc-103* allele is dominant or recessive and why?
d. Another gene identified by mutations that cause defects in locomotion, defecation, and egg-laying is the *egl-2* gene. This gene also encodes a K^+ channel. When a fusion was made between the 5′ end of the *egl-2* gene and a reporter gene, the expression of the fusion gene could be traced in specific cells. It was found to be expressed in muscles and nerves required for egg-laying and defecation but also in a subset of the chemosensory neurons—the AWC but not the AWA cells. Each of these cell types is involved in sensing different chemoattractants. The AWC neurons sense volatile substances such as isoamyl alcohol, and AWA neurons sense soluble substances such as diacetyl alcohol. How could you determine if the *egl-2* mutation disrupts chemosensory function in these cells?

C-12 Guidance nerve cell axons growing toward their ultimate targets are models for cellular movement during development. Because the location of all neurons in the *C. elegans* adult and the ancestry of all cells is known, much can be learned about the process of axonal growth using mutants defective in the final function of the nerve cells. Molecules involved in axon guidance have been identified by isolation of *unc* (Uncoordinated) mutants that are defective in locomotion. Proteins called netrins that act as guidance molecules along the path of growth have been identified in flies, worms, and humans. The UNC-6 gene product in *C. elegans* is a netrin, and the UNC-5 protein is a receptor protein on some nerve cells that interacts with UNC-6 protein in the basal lamina (extracellular matrix) and receives guidance for growth.

a. Mouse Netrin-1 has similar properties to *C. elegans* UNC-6. How could you determine if mouse Netrin-1 could substitute for UNC-6 in *C. elegans?*
b. Other cells migrate during development in *C. elegans.* How would you know if these migrations require *unc-5* or *unc-6* function?
c. UNC-5 protein is present on the surface of the growing neuron and receives guidance from the UNC-6 protein for axon growth. Starting with an *unc-5* defective mutation (but not one completely lacking the protein), how could you isolate genes encoding proteins that interact with (and perhaps relay messages from) the UNC-5 receptor to the inside of the cell?
d. The appropriate growth of axons is hypothesized to involve a series of attractions and repulsions that may depend on the type of receptor expressed at a particular time by a nerve cell. If the time of expression of receptor/sensing genes such as *unc-5* is altered, there may be an effect on guidance. Describe a general approach you could take to express *unc-5* constitutively in the cells of *C. elegans.*