17

Cellular Mechanisms of Development

Concept Outline

17.1 Development is a regulated process.

Overview of Development.   Studies of cellular mechanisms have focused on mice, fruit flies, nematodes, and flowering plants.

Vertebrate Development.   Vertebrates develop in a highly orchestrated fashion.

Insect Development.   Insect development is highly specialized, many key events occurring in a fused mass of cells.

Plant Development.   Unlike animal development, which is buffered from the environment, plant development is sensitive to environmental influences.

17.2 Multicellular organisms employ the same basic mechanisms of development.

Cell Movement and Induction.   Animal cells move by extending protein cables that they use to pull themselves past surrounding cells. Transcription within cells is influenced by signal molecules from other cells.

Determination.   Cells become reversibly committed to particular developmental paths.

Pattern Formation.   Diffusion of chemical inducers governs pattern formation in fly embryos.

Expression of Homeotic Genes.   Master genes determine the form body segments will take.

Programmed Cell Death.   Some genes, when activated, kill their cells.

17.3 Four model developmental systems have been extensively researched.

The Mouse.   *Mus musculus.*
The Fruit Fly.   *Drosophila melanogaster.*
The Nematode.   *Caenorhabditis elegans.*
The Flowering Plant.   *Arabidopsis thaliana.*

17.4 Aging can be considered a developmental process.

Theories of Aging.   While there are many ideas about why cells age, no one theory of aging is widely accepted.

FIGURE 17.1
A collection of future fish undergo embryonic development. Inside a transparent fish egg, a single cell becomes millions of cells that form eyes, fins, gills, and other body parts.

In the previous chapter, we explored gene expression from the perspective of an individual cell, examining the diverse mechanisms that may be employed by a cell to control the transcription of particular genes. Now we will broaden our perspective and look at the unique challenge posed by the development of a cell into a multicellular organism (figure 17.1). In the course of this developmental journey, a pattern of decisions about transcription are made that cause particular lines of cells to proceed along different paths, spinning an incredibly complex web of cause and effect. Yet, for all its complexity, this developmental program works with impressive precision. In this chapter, we will explore the mechanisms used by multicellular organisms to control their development and achieve this precision.

17.1 Development is a regulated process.

Overview of Development

Organisms in all three multicellular kingdoms—fungi, plants, and animals—realize cell specialization by orchestrating gene expression. That is, different cells express different genes at different times. To understand development, we need to focus on how cells determine which genes to activate, and when.

Among the fungi, the specialized cells are largely limited to reproductive cells. In basidiomycetes and ascomycetes (the so-called higher fungi), certain cells produce hormones that influence other cells, but the basic design of all fungi is quite simple. For most of its life, a fungus has a two-dimensional body, consisting of long filaments of cells that are only imperfectly separated from each other. Fungal maturation is primarily a process of growth rather than specialization.

Development is far more complex in plants, where the adult individuals contain a variety of specialized cells organized into tissues and organs. A hallmark of plant development is flexibility; as a plant develops, the precise array of tissues it achieves is greatly influenced by its environment.

In animals, development is complex and rigidly controlled, producing a bewildering array of specialized cell types through mechanisms that are much less sensitive to the environment. The subject of intensive study, animal development has in the last decades become relatively well understood.

Here we will focus our attention on four developmental systems which researchers have studied intensively: (1) an animal with a very complexly arranged body, a mammal; (2) a less complex animal with an intricate developmental cycle, an insect; (3) a very simple animal, a nematode; and (4) a flowering plant (figure 17.2).

To begin our investigation of development, we will first examine the overall process of development in three quite different organisms, so we can sort through differences in the gross process to uncover basic similarities in underlying mechanisms. We will start by describing the overall process in vertebrates, because it is the best understood among the animals. Then we will examine the very different developmental process carried out by insects, in which genetics has allowed us to gain detailed knowledge of many aspects of the process. Finally we will look at development in a third very different organism, a flowering plant.

Almost all multicellular organisms undergo development. The process has been well studied in animals, especially in mammals, insects, nematodes, and flowering plants.

Mammal

Insect

Nematode

Flowering plant
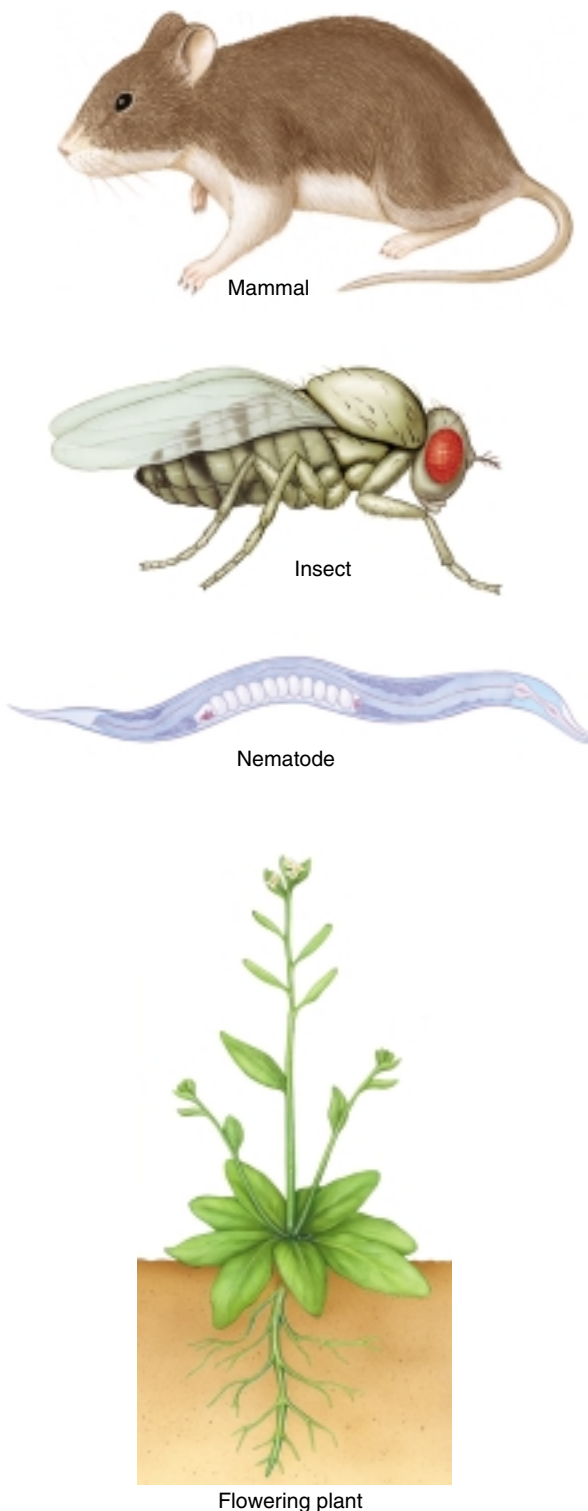
FIGURE 17.2
Four developmental systems. Researchers studying the cellular mechanisms of development have focused on these four organisms.
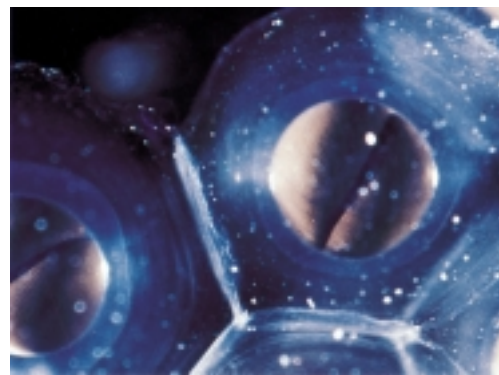
Vertebrate Development

Vertebrate development is a dynamic process in which cells divide rapidly and move over each other as they first establish the basic geometry of the body (figure 17.3). At different sites, particular cells then proceed to form the body's organs, and then the body grows to a size and shape that will allow it to survive after birth. The entire process, described more fully in chapter 60, is traditionally divided into phases. As in mitosis, however, the boundaries between phases are somewhat artificial, and the phases, in fact, grade into one another.

Cleavage

Vertebrates begin development as a single fertilized egg, the zygote. Within an hour after fertilization, the zygote begins to divide rapidly into a larger and larger number of smaller and smaller cells called **blastomeres,** until a solid ball of cells is produced (figure 17.4). This initial period of cell division, termed **cleavage,** is not accompanied by any increase in the overall size of the embryo; rather, the contents of the zygote are simply partitioned into the daughter cells. The two ends of the zygote are traditionally referred to as the **animal** and **vegetal poles.** In general, the blastomeres of the animal pole will go on to form the external tissues of the body, while those of the vegetal pole will form the internal tissues. The initial top-bottom (dorsal-ventral) orientation of the embryo is determined at fertilization by the location where the sperm nucleus enters the egg, a point that corresponds roughly to the future belly. After about 12 divisions, the burst of cleavage divisions slows, and transcription of key genes begins within the embryo cells.
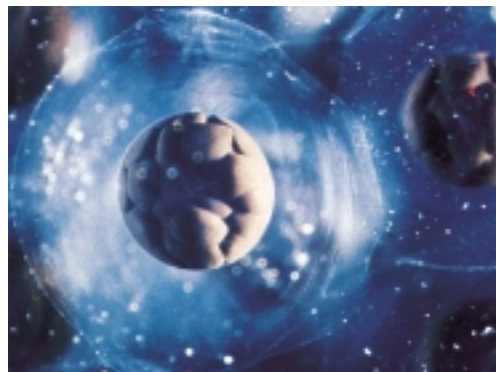

FIGURE 17.3
The miracle of development. This nine-week-old human fetus started out as a single cell: a fertilized egg, or zygote. The zygote's daughter cells have been repeatedly dividing and specializing to produce the distinguishable features of a fetus.

(a)

(b)

FIGURE 17.4
Cleavage divisions producing a frog embryo. (*a*) The initial divisions are, in this case, on the side of the embryo facing you, producing (*b*) a cluster of cells on this side of the embryo, which soon expands to become a (*c*) compact mass of cells. (*d*) This mass eventually invaginates into the interior of the embryo, forming a gastrula, then a neurula.
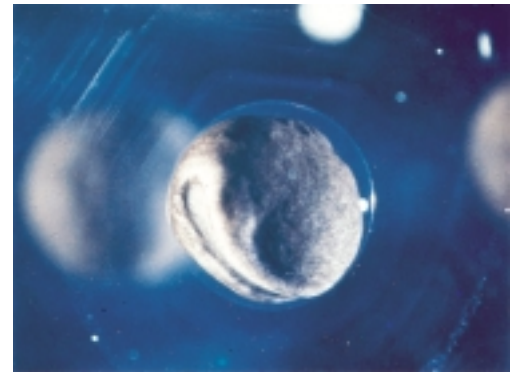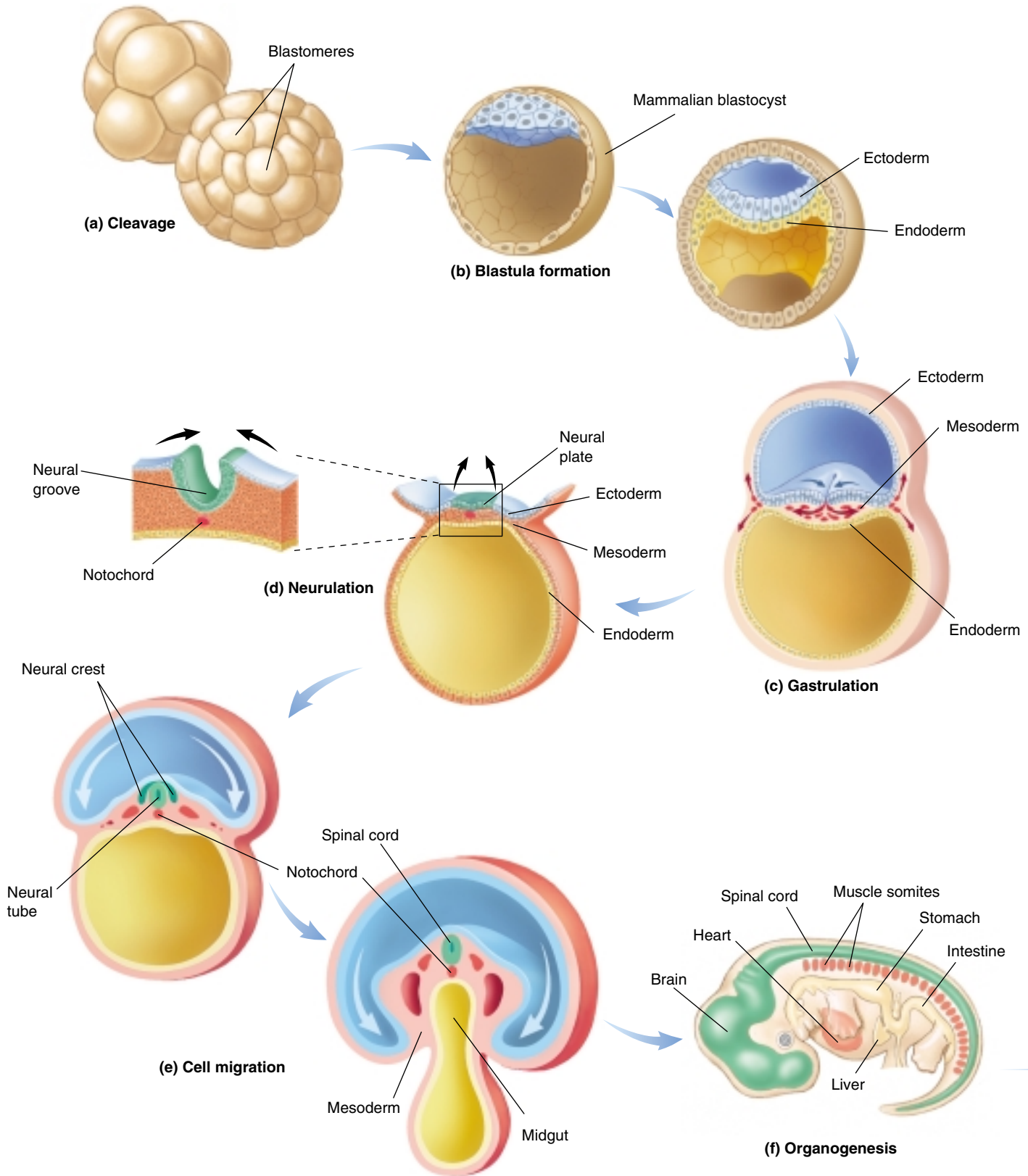

(c)

(d)

FIGURE 17.5
The path of vertebrate development. An illustration of the major events in the development of *Mus musculus*, the house mouse.
(*a*) Cleavage. (*b*) Formation of blastula. (*c*) Gastrulation. (*d*) Neurulation. (*e*) Cell migration. (*f*) Organogenesis. (*g*) Growth.

Formation of the Blastula

The outermost blastomeres (figure 17.5*a*) in the ball of cells produced during cleavage are joined to one another by tight junctions, which, as you may recall from chapter 7, are belts of protein that encircle a cell and weld it firmly to its neighbors. These tight junctions create a seal that isolates the interior of the cell mass from the surrounding medium. At about the 16-cell stage, the cells in the interior of the mass begin to pump Na$^+$ from their cytoplasm into the spaces between cells. The resulting osmotic gradient causes water to be drawn into the center of the cell mass, enlarging the intercellular spaces. Eventually, the spaces coalesce to form a single large cavity within the cell mass. The resulting hollow ball of cells is called a **blastula,** or **blastocyst** in mammals (figure 17.5*b*).

Gastrulation

Some cells of the blastula then push inward, forming a **gastrula** that is invaginated. Cells move by using extensions called lamellipodia to crawl over neighboring cells, which respond by forming lamellipodia of their own. Soon a sheet of cells contracts on itself and shoves inward, starting the invagination. Called **gastrulation** (figure 17.5*c*), this process creates the main axis of the vertebrate body, converting the blastula into a bilaterally symmetrical embryo with a central gut. From this point on, the embryo has three **germ layers** whose organization foreshadows the future organization of the adult body. The cells that invaginate and form the tube of the primitive gut are endoderm; they give rise to the stomach, lungs, liver, and most of the other internal organs. The cells that remain on the exterior are ectoderm, and their derivatives include the skin on the outside of the body and the nervous system. The cells that break away from the invaginating cells and invade the space between the gut and the exterior wall are mesoderm; they eventually form the notochord, bones, blood vessels, connective tissues, and muscles.

Neurulation

Soon after gastrulation is complete, a broad zone of ectoderm begins to thicken on the dorsal surface of the embryo, an event triggered by the presence of the notochord beneath it. The thickening is produced by the elongation of certain ectodermal cells. Those cells then assume a wedge shape by contracting bundles of actin filaments at one end. This change in shape causes the neural tissue to roll up into a tube, which eventually pinches off from the rest of the ectoderm and gives rise to the brain and spinal cord. This tube is called the **neural tube,** and the process by which it forms is termed **neurulation** (figure 17.5*d*).

Cell Migration

During the next stage of vertebrate development, a variety of cells migrate to form distant tissues, following specific paths through the embryo to particular locations (figure 17.5*e*). These migrating cells include those of the **neural crest,** which pinch off from the neural tube and form a number of structures, including some of the body's sense organs; cells that migrate from central blocks of muscle tissue called **somites** and form the skeletal muscles of the body; and the precursors of blood cells and gametes. When a migrating cell reaches its destination, receptor proteins on its surface interact with proteins on the surfaces of cells in the destination tissue, triggering changes in the cytoskeleton of the migrating cell that cause it to cease moving.

Organogenesis and Growth

At the end of this wave of cell migration and colonization, the basic vertebrate body plan has been established, although the embryo is only a few millimeters long and has only about 10^5 cells. Over the course of subsequent development, tissues will develop into organs (figure 17.5*f*), and the embryo will grow to be a hundred times larger, with a million times as many cells (figure 17.5*g*).

(g) Growth

Vertebrates develop in a highly orchestrated fashion. The zygote divides rapidly, forming a hollow ball of cells that then pushes inward, forming the main axis of an embryo that goes on to develop tissues, and after a process of cell migration, organs.

Insect Development

Like all animals, insects develop through an orchestrated series of cell changes, but the path of development is quite different from that of a vertebrate. Many insects produce two different kinds of bodies during their development, the first a tubular eating machine called a **larva,** and the second a flying machine with legs and wings. The passage from one body form to the other is called **metamorphosis** and involves a radical shift in development. Here we will describe development in the fruit fly *Drosophila* (figure 17.6), which is the subject of much genetic research.

FIGURE 17.6
The fruit fly, *Drosophila melanogaster*. A dorsal view of *Drosophila*, one of the most intensively studied animals in development.

Maternal Genes

The development of an insect like *Drosophila* begins before fertilization, with the construction of the egg. Specialized nurse cells that help the egg to grow move some of their own mRNA into the end of the egg nearest them (figure 17.7*a*). As a result, mRNAs produced by maternal genes are positioned in particular locations in the egg, so that after repeated divisions subdivide the fertilized egg, different daughter cells will contain different maternal products. Thus, the action of maternal (rather than zygotic) genes determines the initial course of development.

Syncytial Blastoderm

After fertilization, 12 rounds of nuclear division without cytokinesis produce about 6000 nuclei, all within a single cytoplasm. All of the nuclei within this **syncytial blastoderm** (figure 17.7*b*) can freely communicate with one another, but nuclei located in different sectors of the egg experience different maternal products. The nuclei then space themselves evenly along the surface of the blastoderm, and membranes grow between them. Folding of the embryo and primary tissue development soon follow, in a process fundamentally similar to that seen in vertebrate development. The tubular body that results within a day of fertilization is a larva.

Larval Instars

The larva begins to feed immediately, and as it does so, it grows. Its chitinous exoskeleton cannot stretch much, however, and within a day it sheds the exoskeleton. Before the new exoskeleton has had a chance to harden, the larva expands in size. A total of three larval stages, or **instars,** are produced over a period of four days (figure 17.7*c*).

Imaginal Discs

During embryonic growth, about a dozen groups of cells called **imaginal discs** are set aside in the body of the larva (figure 17.7*d*). Imaginal discs play no role in the life of the larva, but are committed to form key parts of the adult fly's body.
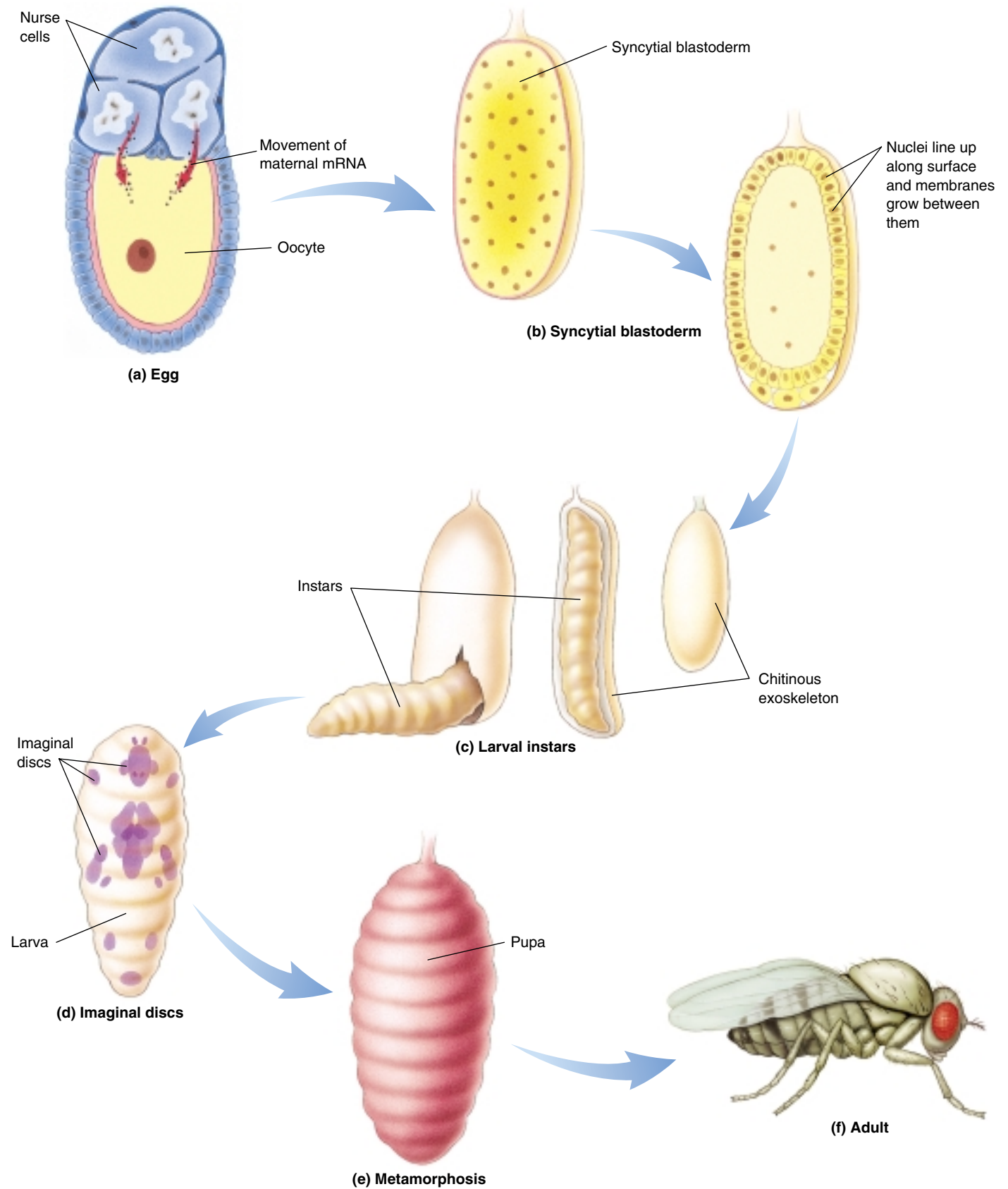
Metamorphosis

After the last larval stage, a hard outer shell forms, and the larva is transformed into a **pupa** (figure 17.7*e*). Within the pupa, the larval cells break down and release their nutrients, which are used in the growth and development of the various imaginal discs (eye discs, wing discs, leg discs, and so on). The imaginal discs then associate with one another, assembling themselves into the body of the adult fly (figure 17.7*f*). The metamorphosis of a *Drosophila* larva into a pupa and then into adult fly takes about four days, after which the pupal shell splits and the fly emerges.

Drosophila **development proceeds through two discrete phases, the first a larval phase that gathers food, then an adult phase that is capable of flight and reproduction.**

FIGURE 17.7
The path of insect development. An illustration of the major events in the development of *Drosophila melanogaster*.
(*a*) Egg. (*b*) Syncytial blastoderm. (*c*) Larval instars. (*d*) Imaginal discs. (*e*) Metamorphosis. (*f*) Adult.

Plant Development

At the most basic level, the developmental paths of plants and animals share many key elements. However, the mechanisms used to achieve body form are quite different. While animal cells follow an orchestrated series of movements during development, plant cells are encased within stiff cellulose walls, and, therefore, cannot move. Each cell in a plant is fixed into position when it is created. Instead of using cell migration, plants develop by building their bodies outward, creating new parts from special groups of self-renewing cells called **meristems.** As meristem cells continually divide, they produce cells that can differentiate into the tissues of the plant.

Another major difference between animals and plants is that most animals are mobile and can move away from unfavorable circumstances, while plants are anchored in position and must simply endure whatever environment they experience. Plants compensate for this restriction by relaxing the rules of development to accommodate local circumstances. Instead of creating a body in which every part is specified to have a fixed size and location, a plant assembles its body from a few types of modules, such as leaves, roots, branch nodes, and flowers. Each module has a rigidly controlled structure and organization, but how the modules are utilized is quite flexible. As a plant develops, it simply adds more modules, with the environment having a major influence on the type, number, size, and location of what is added. In this way the plant is able to adjust the path of its development to local circumstances.

Early Cell Division

The first division of the fertilized egg in a flowering plant is off-center, so that one of the daughter cells is small, with dense cytoplasm (figure 17.8*a*). That cell, the future embryo, begins to divide repeatedly, forming a ball of cells. The other daughter cell also divides repeatedly, forming an elongated structure called a **suspensor,** which links the embryo to the nutrient tissue of the seed. The suspensor also provides a route for nutrients to reach the developing embryo. Just as the animal embryo acquires its initial axis as a cell mass formed during cleavage divisions, so the plant embryo forms its root-shoot axis at this time. Cells near the suspensor are destined to form a root, while those at the other end of the axis ultimately become a shoot.

Tissue Formation

Three basic tissues differentiate while the plant embryo is still a ball of cells (figure 17.8*b*), analogous to the formation of the three germ layers in animal embryos, although in plants, no cell movements are involved. The outermost cells in a plant embryo become **epidermal cells.** The bulk of the embryonic interior consists of **ground tissue** cells that eventually function in food and water storage. Lastly, cells at the core of the embryo are destined to form the future **vascular tissue.**

Seed Formation

Soon after the three basic tissues form, a flowering plant embryo develops one or two seed leaves called **cotyledons.** At this point, development is arrested, and the embryo is now either surrounded by nutritive tissue or has amassed stored food in its cotyledons (figure 17.8*c*). The resulting package, known as a *seed*, is resistant to drought and other unfavorable conditions; in its dormant state, it is a vehicle for dispersing the embryo to distant sites and allows a plant embryo to survive in environments that might kill a mature plant.

Germination

A seed germinates in response to changes in its environment brought about by water, temperature, or other factors. The embryo within the seed resumes development and grows rapidly, its roots extending downward and its leaf-bearing shoots extending upward (figure 17.8*d*).

Meristematic Development

Plants development exhibits its great flexibility during the assembly of the modules that make up a plant body. Apical meristems at the root and shoot tips generate the large numbers of cells needed to form leaves, flowers, and all other components of the mature plant (figure 17.8*e*). At the same time, meristems ensheathing the stems and roots produce the wood and other tissues that allow growth in circumference. A variety of hormones produced by plant tissues influence meristem activity and, thus, the development of the plant body. Plant hormones (see chapter 41) are the tools that allow plant development to adjust to the environment.
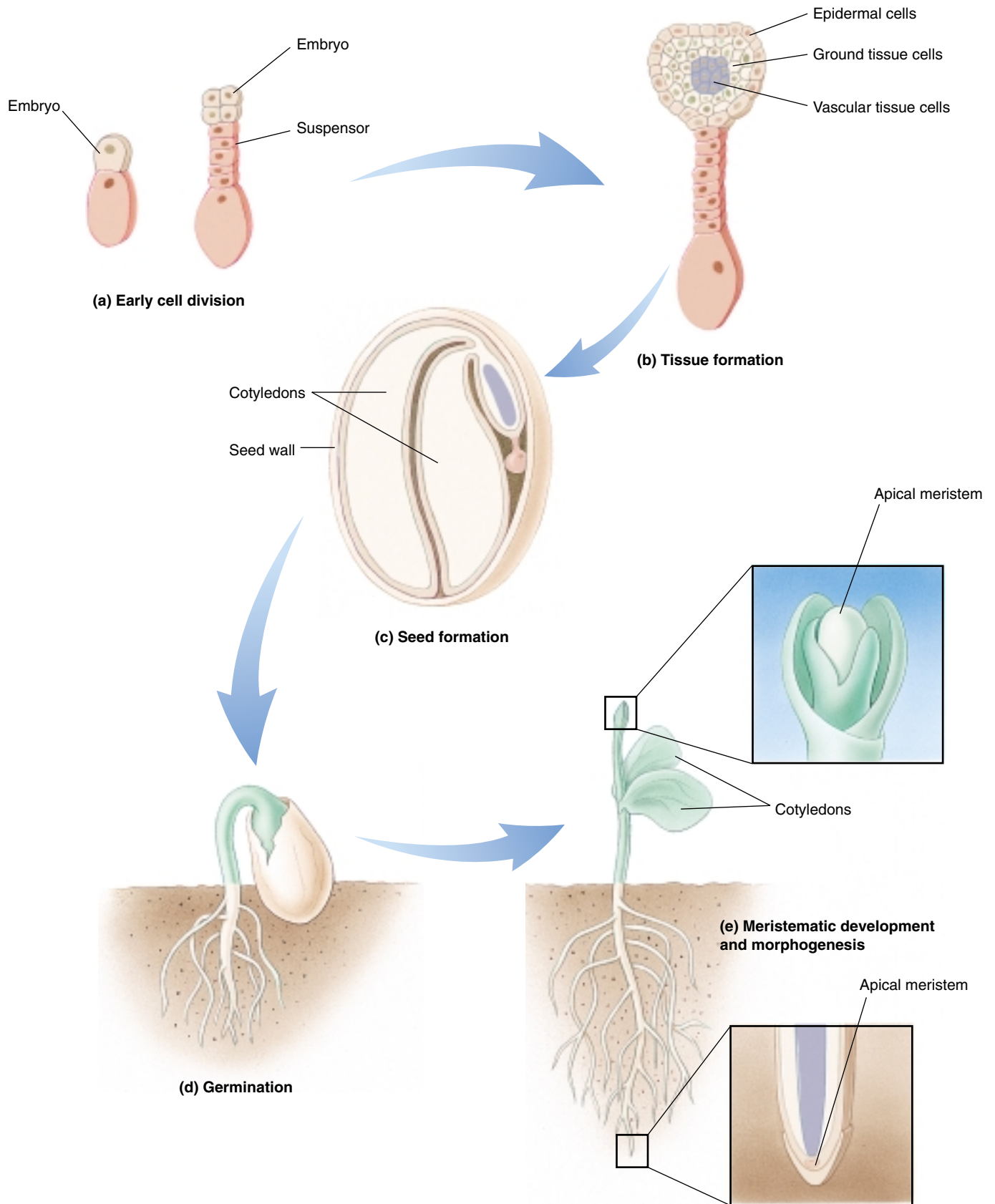
Morphogenesis

The form of a plant body is largely determined by controlled changes in cell shape as they expand osmotically after they form (see figure 17.8*e*). Plant growth-regulating hormones and other factors influence the orientation of bundles of microtubules on the interior of the plasma membrane. These microtubules seem to guide cellulose deposition as the cell wall forms around the outside of a new cell. The orientation of the cellulose fibers, in turn, determines how the cell will elongate as it increases in volume, and so determines the cell's final shape.

> In a developing plant, leaves, flowers, and branches are added to the growing body in ways that are strongly influenced by the environment.

FIGURE 17.8
The path of plant development. An illustration of the developmental stages of *Arabidopsis thaliana*. (*a*) Early cell division.
(*b*) Tissue formation. (*c*) Seed formation. (*d*) Germination. (*e*) Meristematic development and morphogenesis.

17.2 Multicellular organisms employ the same basic mechanisms of development.

Despite the many differences in the three developmental paths we have just discussed, it is becoming increasingly clear that most multicellular organisms develop according to molecular mechanisms that are fundamentally very similar. This observation suggests that these mechanisms evolved very early in the history of multicellular life. Here, we will focus on six mechanisms that seem to be of particular importance in the development of a wide variety of organisms. We will consider them in roughly the order in which they first become important during development.

Cell Movement and Induction

Cell Movement

Cells migrate during many stages in animal development, sometimes traveling great distances before reaching the site where they are destined to develop. By the time vertebrate development is complete, most tissues contain cells that originated from quite different parts of the early embryo. One way cells move is by pulling themselves along using cell adhesion molecules, such as the cadherin proteins you read about in chapter 7. Cadherins span the plasma membrane, protruding into the cytoplasm and extending out from the cell surface. The cytoplasmic portion of the molecule is attached to actin or intermediate filaments of the cytoskeleton, while the extracellular portion has five 100-amino acid segments linked end-to-end; three or more of these segments have Ca^{++} binding sites that play a critical role in the attachment of the cadherin to other cells. Over a dozen different cadherins have been discovered to date. Each type of cadherin attaches to others of its own type at its terminal segments, forming a two-cadherin link between the cytoskeletons of adjacent cells. As a cell migrates to a different tissue, the nature of the cadherin it expresses changes, and if cells expressing two different cadherins are mixed, they quickly sort themselves out, aggregating into two separate masses. This is how the different imaginal discs of a *Drosophila* larva assemble into an adult. Other calcium-independent cell adhesion molecules, such as the neural cell adhesion molecules (N-CAMs) expressed by migrating nerve cells, reinforce the associations made by cadherins, but cadherins play the major role in holding aggregating cells together.

In some tissues, such as connective tissue, much of the volume of the tissue is taken up by the spaces *between* cells. These spaces are not vacant, however. Rather, they are filled with a network of molecules secreted by surrounding cells, principally, a matrix of long polysaccharide chains covalently linked to proteins (proteoglycans), within which are embedded strands of fibrous protein (collagen, elastin, and fibronectin). Migrating cells traverse this matrix by binding to it with cell surface proteins called integrins, which was also described in chapter 7. Integrins are attached to actin filaments of the cytoskeleton and protrude out from the cell surface in pairs, like two hands. The "hands" grasp a specific component of the matrix such as collagen or fibronectin, thus linking the cytoskeleton to the fibers of the matrix. In addition to providing an anchor, this binding can initiate changes within the cell, alter the growth of the cytoskeleton, and change the way in which the cell secretes materials into the matrix.

Thus, cell migration is largely a matter of changing patterns of cell adhesion. As a migrating cell travels, it continually extends projections that probe the nature of its environment. Tugged this way and that by different tentative attachments, the cell literally feels its way toward its ultimate target site.
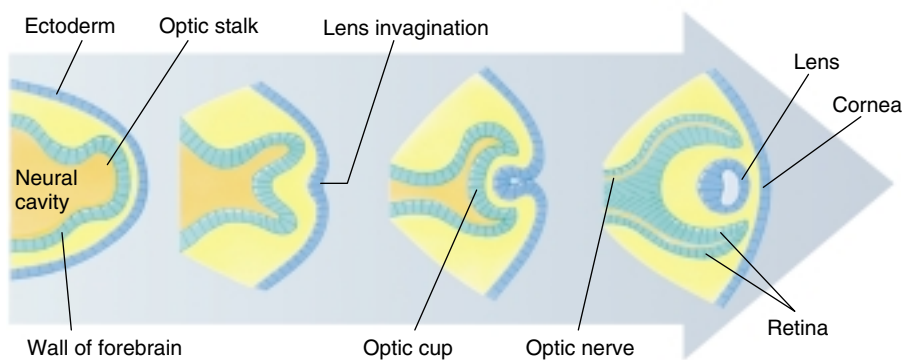
FIGURE 17.9
Development of the vertebrate eye proceeds by induction. The eye develops as an extension of the forebrain called the optic stalk that grows out until it contacts the ectoderm. This contact induces the formation of a lens from the ectoderm.

Induction

In *Drosophila* the initial cells created by cleavage divisions contain different developmental signals (called **determinants**) from the egg, setting individual cells off on different developmental paths. This pattern of development is called **mosaic development.** In mammals, by contrast, all of the blastomeres receive equivalent sets of determinants; body form is determined by cell-cell interactions, a pattern called **regulative development.**

We can demonstrate the importance of cell-cell interactions in development by separating the cells of an early blastula and allowing them to develop independently. Under these conditions, animal pole blastomeres develop features of ectoderm and vegetal pole blastomeres develop features of endoderm, but none of the cells ever develop features characteristic of mesoderm. However, if animal pole and vegetal pole cells are placed next to each other, some of the animal pole cells will develop as mesoderm. The interaction between the two cell types triggers a switch in the developmental path of the cells! When a cell switches from one path to another as a result of interaction with an adjacent cell, **induction** has taken place (figure 17.9).

How do cells induce developmental changes in neighboring cells? Apparently, the inducing cells secrete proteins that act as intercellular signals. Signal molecules, which we discussed in detail in chapter 7, are capable of producing abrupt changes in the patterns of gene transcription.

In some cases, particular groups of cells called **organizers** produce diffusible signal molecules that convey positional information to other cells. Organizers can have a profound influence on the development of surrounding tissues (see chapter 60). Working as signal beacons, they inform surrounding cells of their distance from the organizer. The closer a particular cell is to an organizer, the higher the concentration of the signal molecule, or **morphogen,** it experiences (figure 17.10). Although only a few morphogens have been isolated, they are thought to be part of a widespread mechanism for determining relative position during development.

A single morphogen can have different effects, depending upon how far away from the organizer the affected cell is located. Thus, low levels of the morphogen activin will cause cells of the animal pole of an early *Xenopus* embryo to develop into epidermis, while slightly higher levels will induce the cells to develop into muscles, and levels a little higher than that will induce them to form notochord (figure 17.11).

> Cells migrate by extending probes to neighboring cells which they use to pull themselves along. Interactions between cells strongly influence the developmental paths they take. Signal molecules from an inducing cell alter patterns of transcription in cells which come in contact with it.
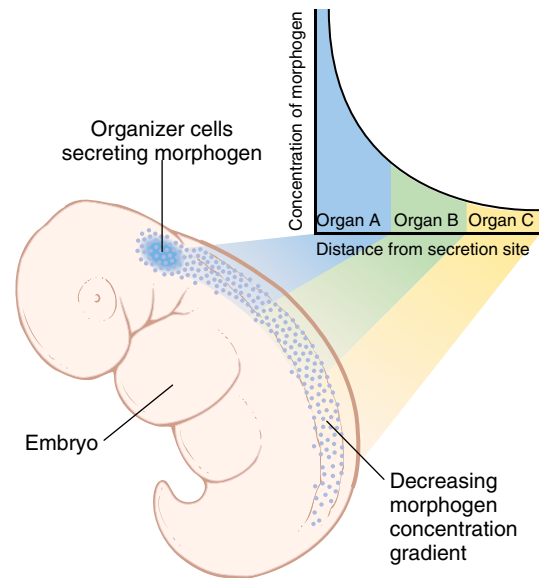
FIGURE 17.10
An organizer creates a morphogen gradient. As a morphogen diffuses from the organizer site, it becomes less concentrated. Different concentrations of the morphogen stimulate the development of different organs.
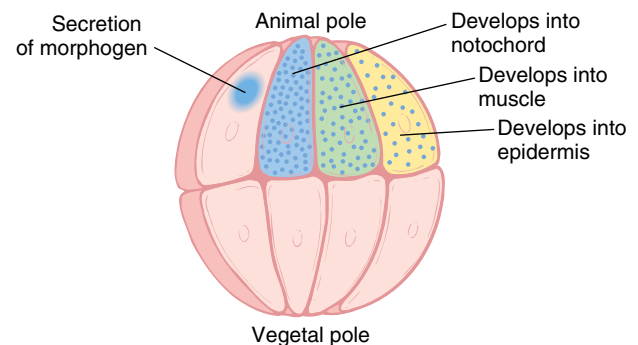
FIGURE 17.11
Fate of cells in an early *Xenopus* embryo. The fates of the individual cells are determined by the concentration of morphogen washing over them.

Determination

The mammalian egg is symmetrical in its contents as well as its shape, so that all of the cells of an early blastoderm are equivalent up to the eight-cell stage. The cells are said to be **totipotent,** meaning that they are potentially capable of expressing all of the genes of their genome. If they are separated from one another, any one of them can produce a completely normal individual. Indeed, just this sort of procedure has been used to produce sets of four or eight identical offspring in the commercial breeding of particularly valuable lines of cattle. The reverse process works, too; if cells from two different eight-cell-stage embryos are combined, a single normal individual results. Such an individual is called a **chimera,** because it contains cells from different genetic lines (figure 17.12).
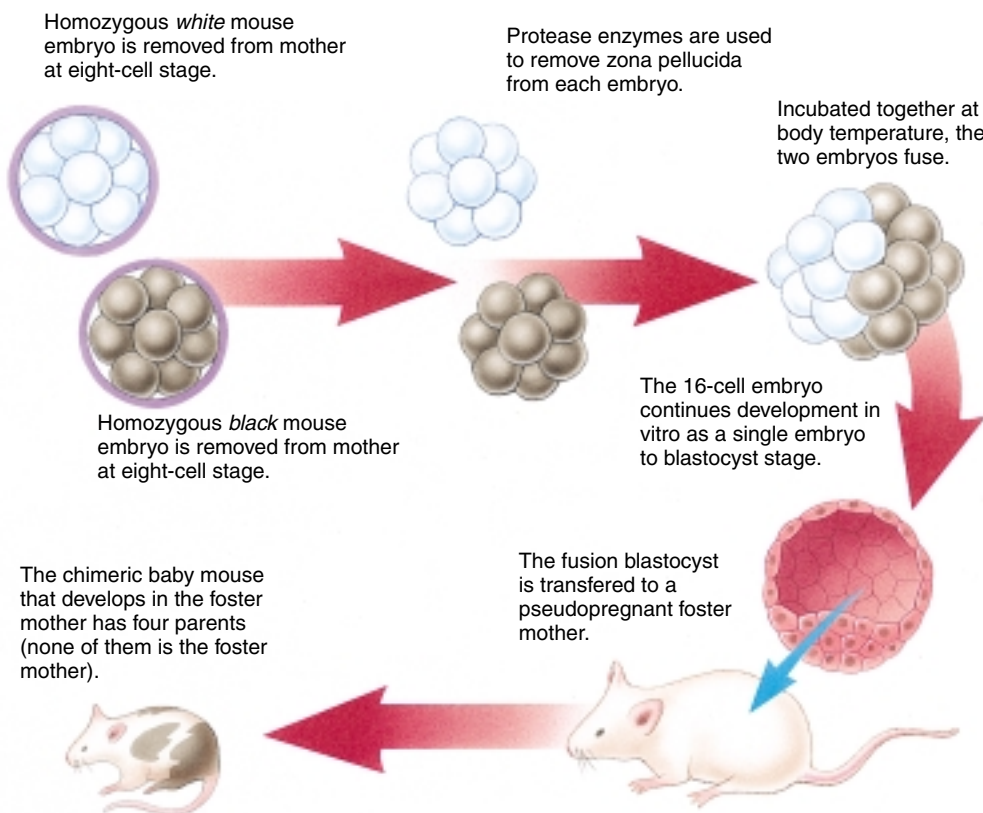
Mammalian cells start to become different after the eight-cell stage as a result of cell-cell interactions like those we just discussed. At this point, the pathway that will influence the future developmental fate of the cells is determined. The commitment of a particular cell to a specialized developmental path is called **determination.** A cell in the prospective brain region of an amphibian embryo at the early gastrula stage has not yet been determined; if transplanted elsewhere in the embryo, it will develop like its new neighbors (see chapter 60). By the late gastrula stage, however, determination has taken place, and the cell will develop as neural tissue no matter where it is transplanted. Determination must be carefully distinguished from **differentiation,** which is the cell specialization that occurs at the end of the developmental path. Cells may become *determined* to give rise to particular tissues long before they actually *differentiate* into those tissues. The cells of a *Drosophila* eye imaginal disc, for example, are fully determined to produce an eye, but they remain totally undifferentiated during most of the course of larval development.

The Mechanism of Determination

What is the molecular mechanism of determination? The gene regulatory proteins discussed in detail in chapter 16 are the tools used by cells to initiate developmental changes. When genes encoding these proteins are activated, one of their effects is to reinforce their own activation. This makes the developmental switch deterministic, initiating a chain of events that leads down a particular developmental pathway. Cells in which a set of regulatory genes have been activated may not actually undergo differentiation until some time later, when other factors interact with the regulatory protein and cause it to activate still other genes. Nevertheless, once the initial "switch" is thrown, the cell is fully committed to its future developmental path.

Often, before a cell becomes fully committed to a particular developmental path, it first becomes partially committed, acquiring **positional labels** that reflect its location in the embryo. These labels can have a great influence on how the pattern of the body subsequently develops. In a chicken embryo, if tissue at the base of the leg bud (which would normally give rise to the thigh) is transplanted to the tip of the identical-looking wing bud (which would normally give rise to the wing tip), that tissue will develop into a toe rather than a thigh! The tissue has already been determined as leg but is not yet committed to being a particular part of the leg. Therefore, it can be influenced by the positional signaling at the tip of the wing bud to form a tip (in this case a tip of leg).

Homozygous *white* mouse embryo is removed from mother at eight-cell stage.

Protease enzymes are used to remove zona pellucida from each embryo.

Incubated together at body temperature, the two embryos fuse.

Homozygous *black* mouse embryo is removed from mother at eight-cell stage.

The 16-cell embryo continues development in vitro as a single embryo to blastocyst stage.

The fusion blastocyst is transfered to a pseudopregnant foster mother.

The chimeric baby mouse that develops in the foster mother has four parents (none of them is the foster mother).

FIGURE 17.12
Constructing a chimeric mouse.
Cells from two eight-cell individuals fuse to form a single individual.
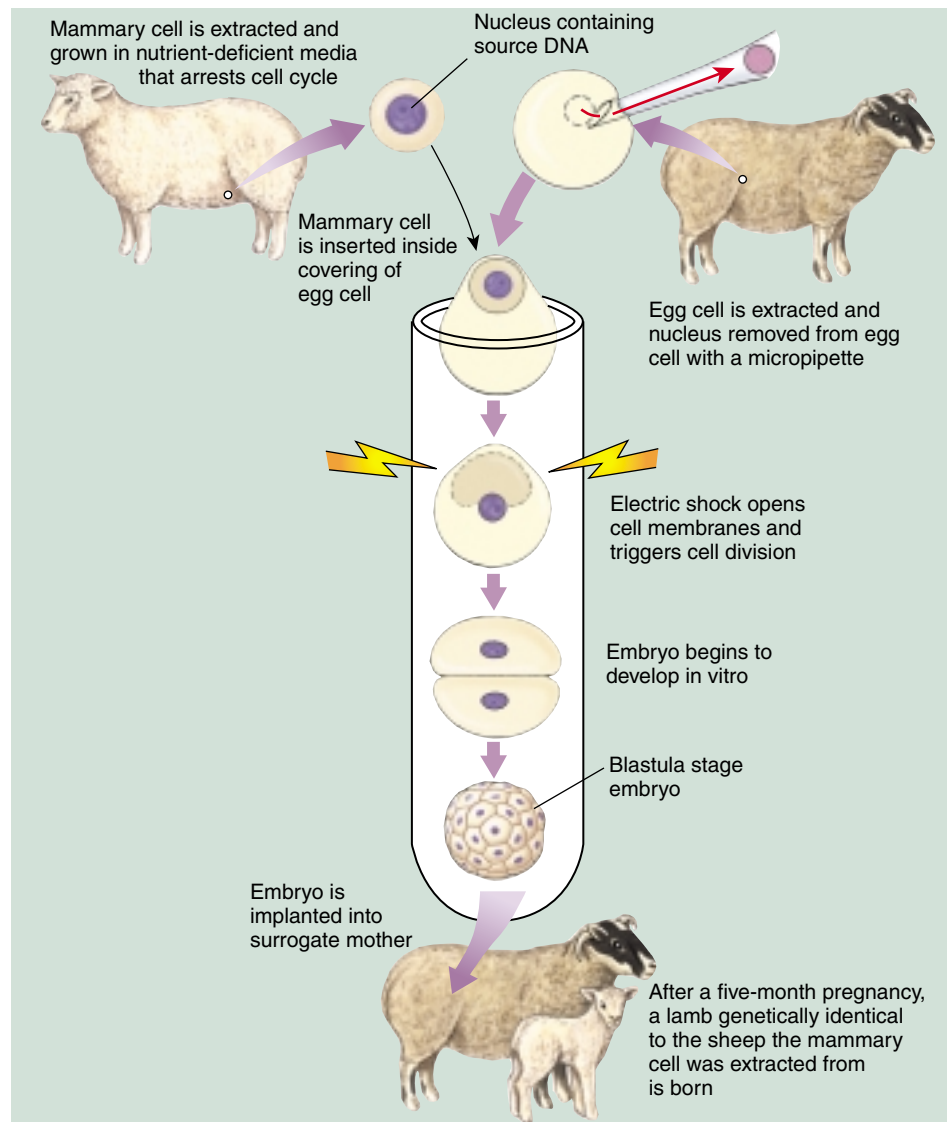
Is Determination Irreversible?

Until very recently, biologists thought determination was irreversible. Experiments carried out in the 1950s and 1960s by John Gurdon and others made what seemed a convincing case: using very fine pipettes (hollow glass tubes) to suck the nucleus out of a frog or toad egg, these researchers replaced the egg nucleus with a nucleus sucked out of a body cell taken from another individual (see figure 14.3). If the transplanted nucleus was obtained from an advanced embryo, the egg went on to develop into a tadpole, but died before becoming an adult.

Nuclear transplant experiments were attempted without success by many investigators, until finally, in 1984, Steen Willadsen, a Danish embryologist working in Texas, succeeded in cloning a sheep using the nucleus from a cell of an early embryo. The key to his success was in picking a cell very early in development. This exciting result was soon replicated by others in a host of other organisms, including pigs and monkeys.

Only early embryo cells seemed to work, however. Researchers became convinced, after many attempts to transfer older nuclei, that animal cells become irreversibly committed after the first few cell divisions of the developing embryo.

We now know this conclusion to have been unwarranted. The key advance unraveling this puzzle was made in Scotland by geneticists Keith Campbell and Ian Wilmut, who reasoned that perhaps the egg and the donated nucleus needed to be at the same stage in the cell cycle. They removed mammary cells from the udder of a six-year-old sheep. The origin of these cells gave the clone its name, "Dolly," after the country singer Dolly Parton. The cells were grown in tissue culture; then, in preparation for cloning, the researchers substantially reduced for five days the concentration of serum nutrients on which the sheep mammary cells were subsisting. Starving the cells caused them to pause at the beginning of the cell cycle. In parallel preparation, eggs obtained from a ewe were enucleated (figure 17.13).

Mammary cells and egg cells were then surgically combined in January of 1996, inserting the mammary cells inside the covering around the egg cell. The researchers then applied a brief electrical shock. This caused the plasma membranes surrounding the two cells to become leaky, so

FIGURE 17.13
Proof that determination is reversible. This experiment by Campbell and Wilmut was the first successful cloning of an adult animal.

that the nucleus of the mammary cell passed into the egg cell—a neat trick. The shock also kick-started the cell cycle, causing the cell to begin to divide.

After six days, in 30 of 277 tries, the dividing embryo reached the hollow-ball "blastula" stage, and 29 of these were transplanted into surrogate mother sheep. A little over five months later, on July 5, 1996, one sheep gave birth to a lamb, Dolly, the first clone generated from a fully differentiated animal cell. Dolly established beyond all dispute that determination is reversible, that with the right techniques the fate of a fully differentiated cell *can* be altered.

The commitment of particular cells to certain developmental fates is fully reversible.

Pattern Formation

All animals seem to use positional information to determine the basic pattern of body compartments and, thus, the overall architecture of the adult body. How is positional information encoded in labels and read by cells? To answer this question, let us consider how positional labels are used in pattern formation in *Drosophila*. The Nobel Prize in Physiology or Medicine was awarded in 1995 for the unraveling of this puzzle.

As we noted previously, a *Drosophila* egg acquires an initial asymmetry long before fertilization as a result of maternal mRNA molecules that are deposited in one end of the egg by nurse cells. Part of this maternal mRNA, from a gene called *bicoid*, remains near its point of entry, marking what will become the embryo's front end. Fertilization causes this mRNA to be translated into bicoid protein, which diffuses throughout the syncytial blastoderm, forming a morphogen gradient. Mothers unable to make bicoid protein produce embryos without a head or thorax (in effect, these embryos are two-tailed, or bicaudal—hence the name "bicoid"). Bicoid protein establishes the anterior (front) end of the embryo. If bicoid protein is injected into the anterior end of mutant embryos unable to make it, the
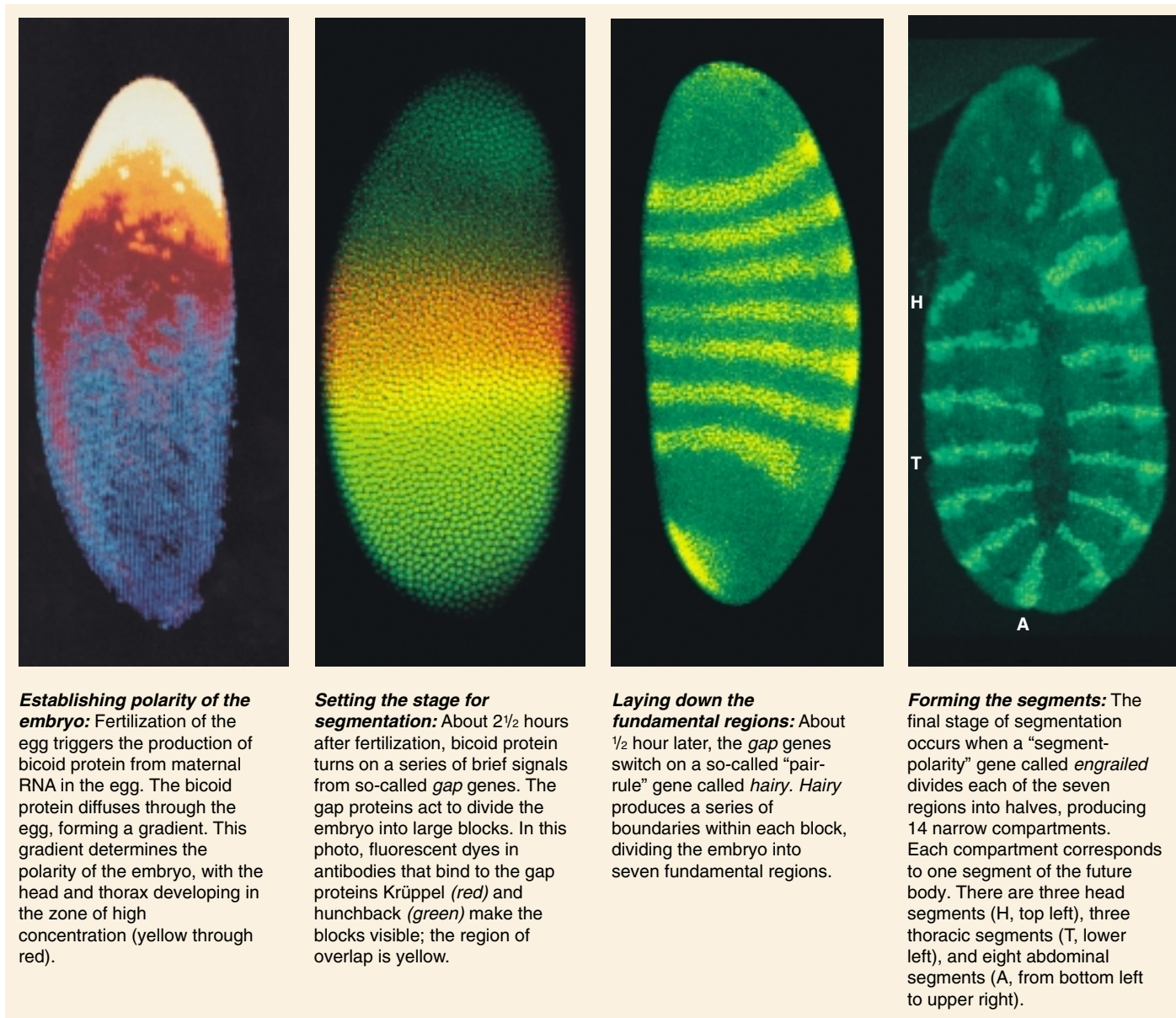

Establishing polarity of the embryo: Fertilization of the egg triggers the production of bicoid protein from maternal RNA in the egg. The bicoid protein diffuses through the egg, forming a gradient. This gradient determines the polarity of the embryo, with the head and thorax developing in the zone of high concentration (yellow through red).

Setting the stage for segmentation: About 2½ hours after fertilization, bicoid protein turns on a series of brief signals from so-called *gap* genes. The gap proteins act to divide the embryo into large blocks. In this photo, fluorescent dyes in antibodies that bind to the gap proteins Krüppel *(red)* and hunchback *(green)* make the blocks visible; the region of overlap is yellow.

Laying down the fundamental regions: About ½ hour later, the *gap* genes switch on a so-called "pair-rule" gene called *hairy*. Hairy produces a series of boundaries within each block, dividing the embryo into seven fundamental regions.

Forming the segments: The final stage of segmentation occurs when a "segment-polarity" gene called *engrailed* divides each of the seven regions into halves, producing 14 narrow compartments. Each compartment corresponds to one segment of the future body. There are three head segments (H, top left), three thoracic segments (T, lower left), and eight abdominal segments (A, from bottom left to upper right).

FIGURE 17.14
Body organization in an early *Drosophila* embryo. In these images by 1995 Nobel laureate, Christiane Nüsslein-Volhard, and Sean Carroll, we watch a *Drosophila* egg pass through the early stages of development, in which the basic segmentation pattern of the embryo is established.

embryos will develop normally. If it is injected into the opposite (posterior) end of normal embryos, a head and thorax will develop at that end.

Bicoid protein exerts this profound effect on the organization of the embryo by activating genes that encode the first mRNAs to be transcribed after fertilization. Within the first two hours, before cellularization of the syncytial blastoderm, a group of six genes called the **gap genes** begins to be transcribed. These genes map out the coarsest subdivision of the embryo (figure 17.14). One of them is a gene called *hunchback* (because an embryo without *hunchback* lacks a thorax and so, takes on a hunched shape). Although *hunchback* mRNA is distributed throughout the embryo, its translation is controlled by the protein product of another maternal mRNA called *nanos* (named after the Greek word for "dwarf," as mutants without *nanos* genes lack abdominal segments and hence, are small). The **nanos** protein binds to *hunchback* mRNA, preventing it from being translated. The only place in the embryo where there is too little nanos protein to block translation of *hunchback* mRNA is the far anterior end. Consequently, hunchback protein is made primarily at the anterior end of the embryo. As it diffuses back toward the posterior end, it sets up a second morphogen gradient responsible for establishing the thoracic and abdominal segments.

Other gap genes act in more posterior regions of the embryo. They, in turn, activate 11 or more **pair-rule genes.** (When mutated, each of these genes alters every other body segment.) One of the pair-rule genes, named *hairy*, produces seven bands of protein, which look like stripes when visualized with fluorescent markers. These bands establish boundaries that divide the embryo into seven zones. Finally, a group of 16 or more **segment polarity genes** subdivide these zones. The *engrailed* gene, for example, divides each of the seven zones established by *hairy* into anterior and posterior compartments. The 14 compartments that result correspond to the three head segments, three thoracic segments, and eight abdominal segments of the embryo.

Thus, within three hours after fertilization, a highly orchestrated cascade of segmentation gene activity produces the fly embryo's basic body plan. The activation of these and other developmentally important genes (figure 17.15) depends upon the free diffusion of morphogens that is possible within a syncytial blastoderm. In mammalian embryos with cell partitions, other mechanisms must operate.

In *Drosophila* diffusion of chemical inducers produces the embryo's basic body plan, a cascade of genes dividing it into 14 compartments.

(a)

(d)

(b)

(e)

(c)

(f)
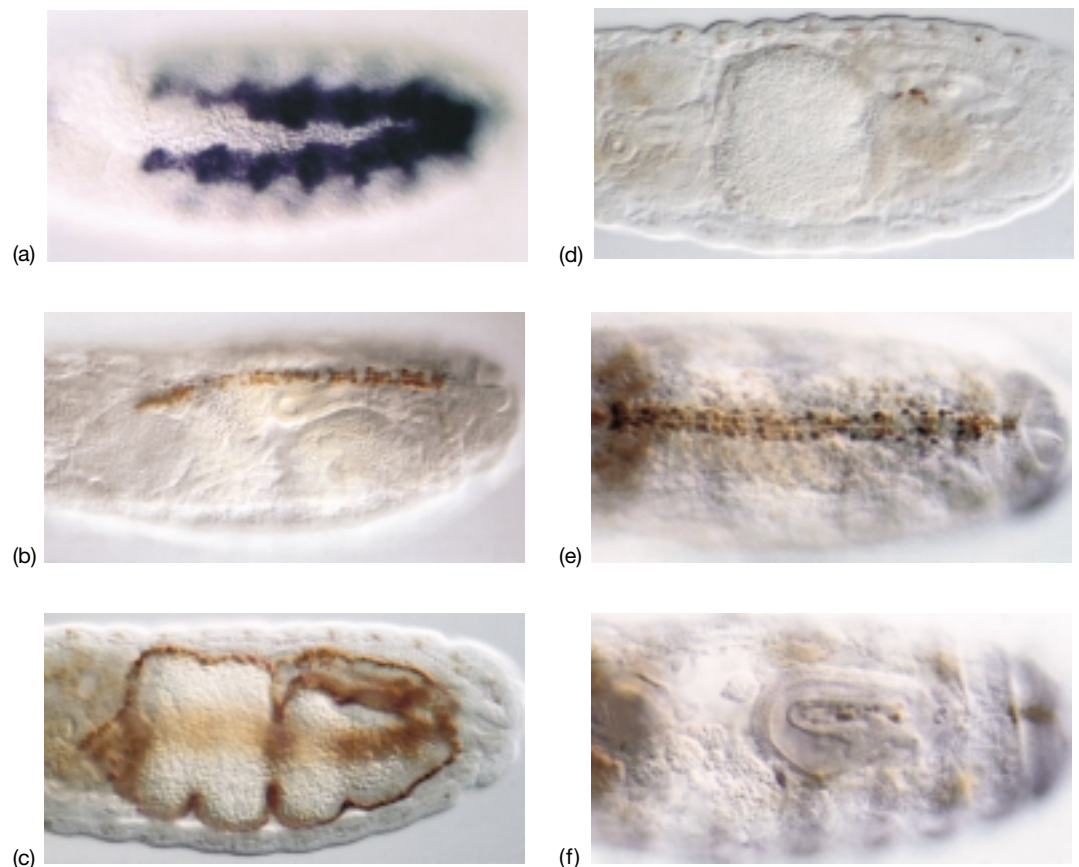
FIGURE 17.15
A gene controlling organ formation in *Drosophila*. Called *tinman*, this gene is responsible for the formation of gut musculature and the heart. The dye shows expression of the *tinman* in five-hour (*a*) and seventeen-hour (*b*) *Drosophila* embryos. The gut musculature then appears along the edges of normal embryos (*c*) but is not present in embryos in which the gene has been mutated (*d*). The heart tissue develops along the center of normal embryos (*e*) but is missing in *tinman* mutant embryos (*f*).

Expression of Homeotic Genes

After pattern formation has successfully established the number of body segments in *Drosophila*, a series of **homeotic genes** act as master switches to determine the forms these segments will assume. Homeotic genes code for proteins that function as transcription factors. Each homeotic gene activates a particular module of the genetic program, initiating the production of specific body parts within each of the 14 compartments.

Homeotic Mutations

Mutations in homeotic genes lead to the appearance of perfectly normal body parts in unusual places. Mutations in *bithorax* (figure 17.16), for example, cause a fly to grow an extra pair of wings, as if it had a double thoracic segment, and mutations in *Antennapedia* cause legs to grow out of the head in place of antennae! In the early 1950s, geneticist Edward Lewis discovered that several homeotic genes, including *bithorax*, map together on the third chromosome of *Drosophila*, in a tight cluster called the **bithorax complex.**

FIGURE 17.16
Mutations in homeotic genes. Three separate mutations in the *bithorax* gene caused this fruit fly to develop an extra thoracic segment, with accompanying wings. Compare this photograph with that of the normal fruit fly in figure 17.6.

FIGURE 17.17
FIGURE 17.17
***Drosophila* homeotic genes.** Called the homeotic gene complex, or HOM complex, the genes are grouped into two clusters, the Antennapedia complex (anterior) and the bithorax complex (posterior).

Drosophila embryo

Drosophila HOM genes
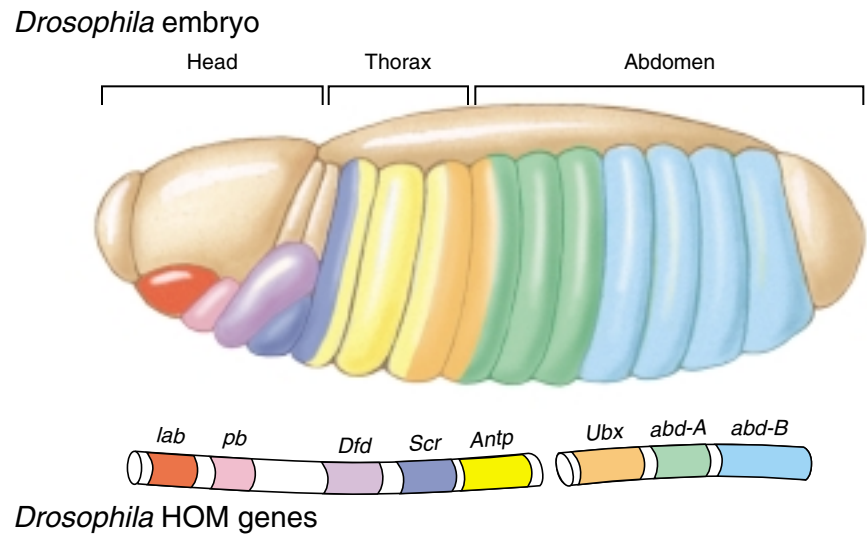
Mutations in these genes all affect body parts of the thoracic and abdominal segments, and Lewis concluded that the genes of the bithorax complex control the development of body parts in the rear half of the thorax and all of the abdomen. Most interestingly, the order of the genes in the bithorax complex mirrors the order of the body parts they control, as if the genes are activated serially! Genes at the beginning of the cluster switch on development of the thorax, those in the middle control the anterior part of the abdomen, and those at the end affect the tip of the abdomen. A second cluster of homeotic genes, the **Antennapedia complex,** was discovered in 1980 by Thomas Kaufmann. The Antennapedia complex governs the anterior end of the fly, and the order of genes in it also corresponds to the order of segments they control (figure 17.17).

The Homeobox

Drosophila homeotic genes typically contain the **homeobox,** a sequence of 180 nucleotides that codes for a 60-amino acid DNA-binding peptide domain called the homeodomain (figure 17.18). As we saw in chapter 16, proteins that contain the homeodomain function as transcription factors, ensuring that developmentally related genes are transcribed at the appropriate time. Segmentation genes such as *bicoid* and *engrailed* also contain the homeobox sequence. Clearly, the homeobox distinguishes those portions of the genome devoted to pattern formation.
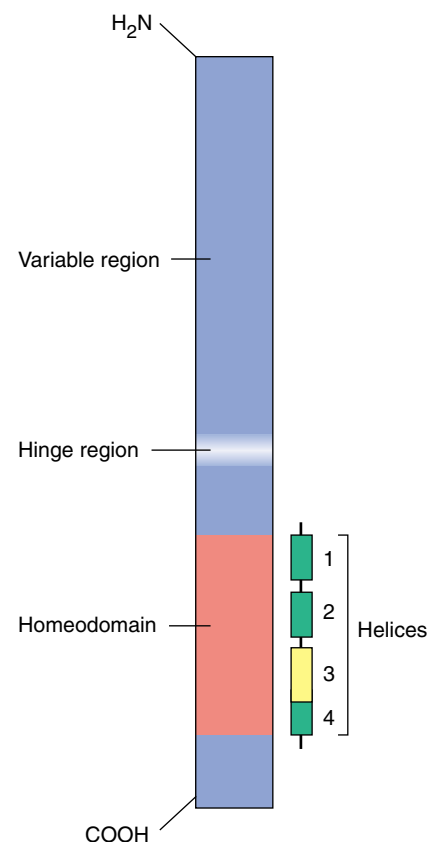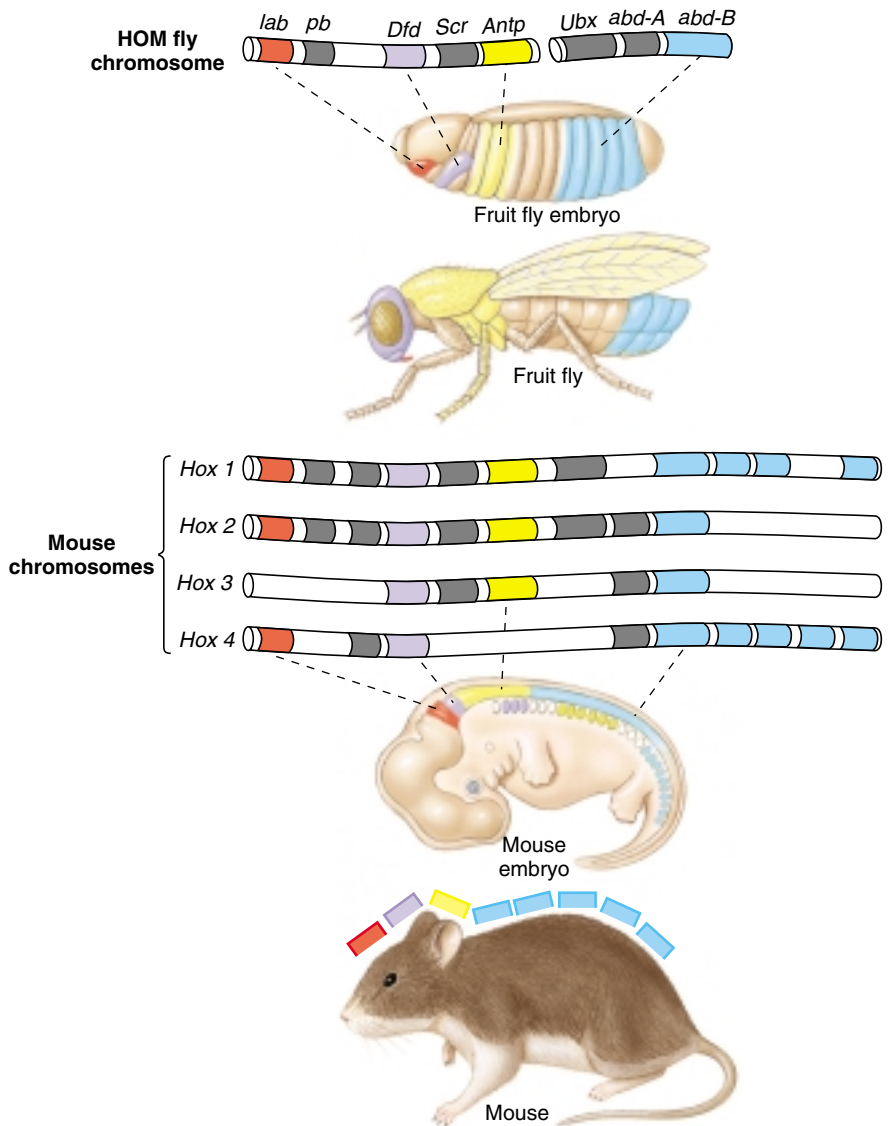

FIGURE 17.18
Homeodomain protein. This protein plays an important regulatory role when it binds to DNA and regulates expression of specific genes. The variable region of the protein determines the specific activity of the protein. Also included in this protein is a small hinge region and the homeodomain, a 60-amino-acid sequence common to all proteins of this type. The homeodomain region of the protein is coded for by the homeobox region of genes and is composed of four α helices. One of the helices recognizes and binds to a specific DNA sequence in target genes.

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Evolution of Homeobox Genes

Since their initial discovery in *Drosophila*, homeotic genes have also been found in mice and humans, which are separated from insects by over 600 million years of evolution. Their presence in mammals and insects indicates that homeotic genes governing the positioning of body parts must have arisen very early in the evolutionary history of animals. Similar genes also appear to operate in flowering plants. Gene probes made using the homeobox sequence of *Drosophila* have been used to identify very similar sequences in a wide variety of other organisms, including frogs, mice, humans, cows, chickens, beetles, and even earthworms. Mice and humans have four clusters of homeobox-containing genes, called *Hox* genes in mice. Just as in flies, the homeotic genes of mammals appear to be lined up in the same order as the segments they control (figure 17.19). Thus, the ordered nature of homeotic gene clusters is highly conserved in evolution (figure 17.20). There is a total of 38 *Hox* genes in the four homeotic clusters of a mouse, and we are only beginning to understand how they interact.

> **Homeotic genes encode transcription factors that activate blocks of genes specifying particular body parts.**

FIGURE 17.19
A comparison of homeotic gene clusters in the fruit fly *Drosophila melanogaster* and the mouse *Mus musculus*. Similar genes, the *Drosophila* HOM genes and the mouse *Hox* genes, control the development of front and back parts of the body. These genes are located on a single chromosome in the fly, and on four separate chromosomes in mammals. The genes are color-coded to match the parts of the body in which they are expressed.

FIGURE 17.20
The remarkably conserved homeobox series. By inserting a mouse homeobox-containing gene into a fruit fly, a mutant fly (*right*) can be manufactured with a leg (*arrow*) growing from where its antenna would be in a normal fly (*left*).
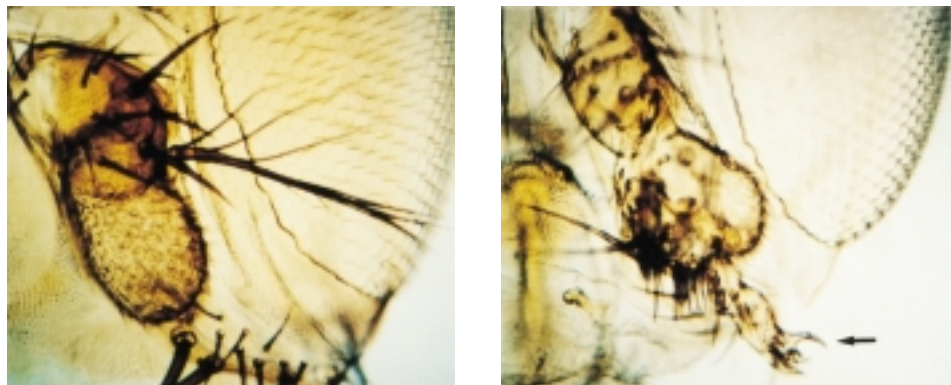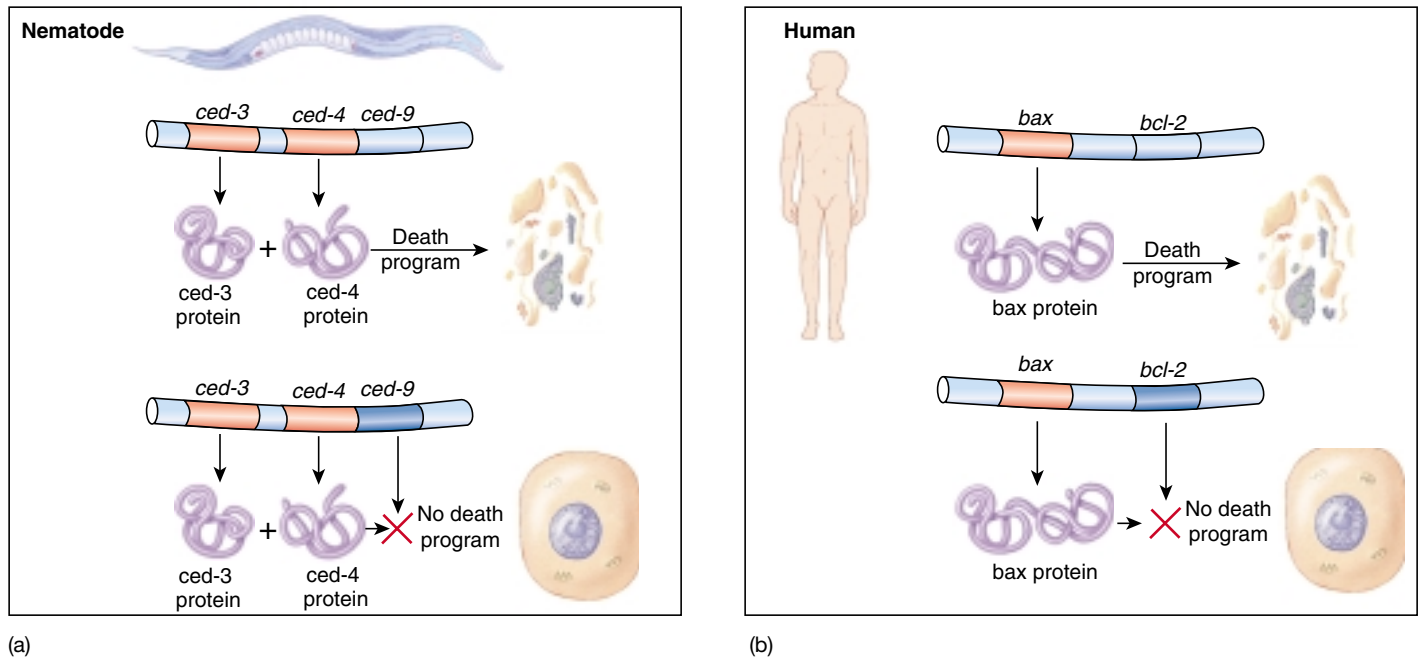
FIGURE 17.21
Programmed cell death. Apoptosis, or programmed cell death, is necessary for normal development of all animals. (*a*) In the developing nematode, for example, two genes, *ced-3* and *ced-4*, code for proteins that cause the programmed cell death of 131 specific cells. In the other cells of the developing nematode, the product of a third gene, *ced-9*, represses the death program encoded by *ced-3* and *ced-4*. (*b*) In developing humans, the product of a gene called *bax* causes a cell death program in some cells and is blocked by the *bcl-2* gene in other cells.

Programmed Cell Death

Not every cell that is produced during development is destined to survive. For example, the cells between your fingers and toes die; if they did not, you would have paddles rather than digits. Vertebrate embryos produce a very large number of neurons, ensuring that there are enough neurons to make all of the necessary synaptic connections, but over half of these neurons never make connections and die in an orderly way as the nervous system develops. Unlike accidental cell deaths due to injury, these cell deaths are planned for and indeed required for proper development. Cells that die due to injury typically swell and burst, releasing their contents into the extracellular fluid. This form of cell death is called **necrosis.** In contrast, cells programmed to die shrivel and shrink in a process called **apoptosis** (from the Greek word meaning shedding of leaves in autumn), and their remains are taken up by surrounding cells.

Gene Control of Apoptosis

This sort of developmentally regulated cell suicide occurs when a "death program" is activated. All animal cells appear to possess such programs. In the nematode worm, for example, the same 131 cells always die during development in a predictable and reproducible pattern of apoptosis. Three genes govern this process. Two (*ced-3* and *ced-4*) constitute the death program itself; if either is mutant, those 131 cells do not die, and go on instead to form nervous and other tissue. The third gene (*ced-9*) represses the death program encoded by the other two (figure 17.21*a*). The same sorts of apoptosis programs occur in human cells: the *bax* gene encodes the cell death program, and another, an oncogene called *bcl-2*, represses it (figure 17.21*b*). The mechanism of apoptosis appears to have been highly conserved during the course of animal evolution. The protein made by *bcl-2* is 25% identical in amino acid sequence to that made by *ced-9*. If a copy of the human *bcl-2* gene is transferred into a nematode with a defective *ced-9* gene, *bcl-2* suppresses the cell death program of *ced-3* and *ced-4!*

How does *bax* kill a cell? The bax protein seems to induce apoptosis by binding to the permeability pore of the cell's mitochondria, increasing its permeability and in doing so triggering cell death. How does *bcl-2* prevent cell death? One suggestion is that it prevents damage from free radicals, highly reactive fragments of atoms that can damage cells severely. Proteins or other molecules that destroy free radicals are called **antioxidants.** Antioxidants are almost as effective as *bcl-2* in blocking apoptosis.

> Animal development involves programmed cell death (apoptosis), in which particular genes, when activated, kill their cells.
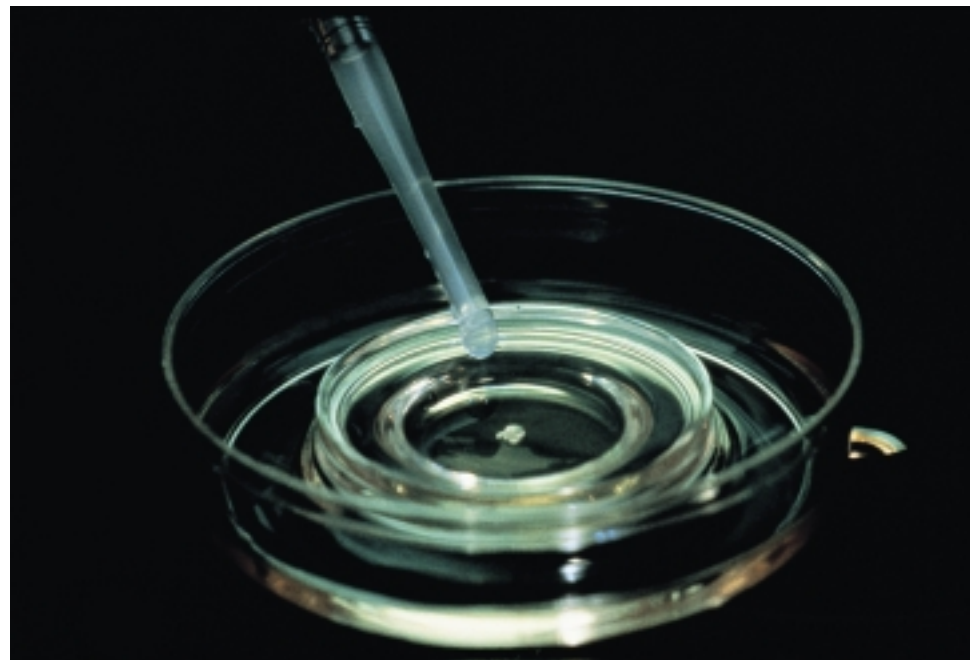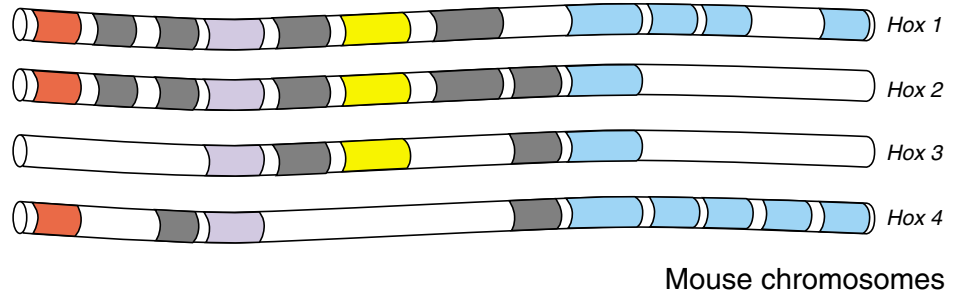
The Mouse

Some of the most elegant investigations of the cellular mechanisms of development are being done with mammals, particularly the mouse *Mus musculus*. Mice have a battery of homeotic genes, the *Hox* genes (figure 17.22), which seem to be closely related to the homeotic genes of *Drosophila*. Very interestingly, not only do the same genes occur, but they also seem to operate in the same order! Clearly, the homeotic gene system has been highly conserved during the course of animal evolution.

What lends great power to this developmental model system is the ability to create chimeric mice containing cells from two different genetic lines. Mammalian embryos are unusual among vertebrates in that they arise from symmetrical eggs; there are no chemical gradients, and during the initial cleavage divisions, all of the daughter cells are identical. Up to the eight-cell stage, any one of the cells, if isolated, will form a normal adult. Moreover, two different eight-cell-stage embryos can be fused to form a single embryo that will go on to form a normal adult. The resulting adult is a chimera, containing cells from both embryos. In a very real sense, these chimeric mice each have four parents!
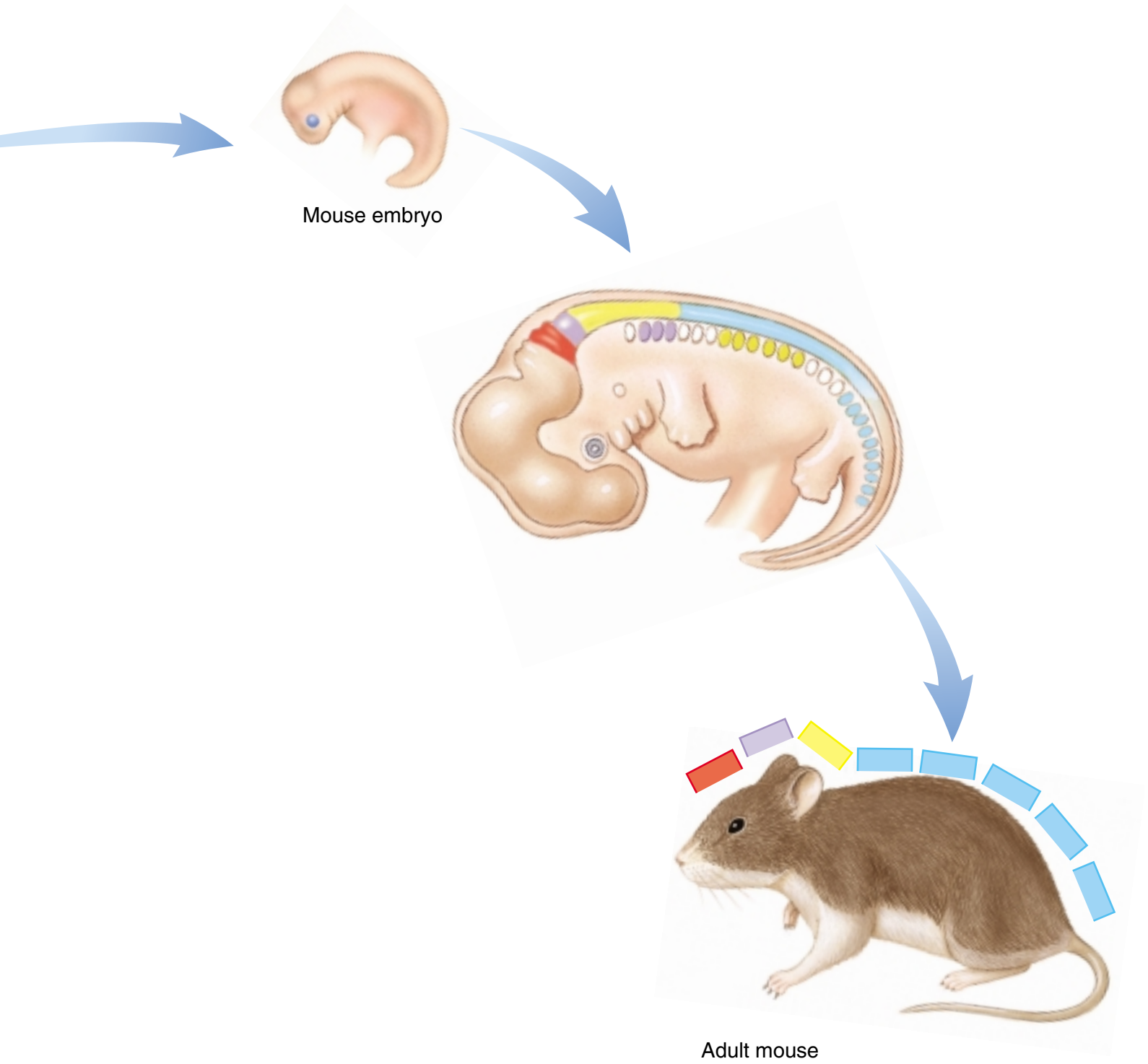
The *Hox* genes control body part development in mice.

Mouse chromosomes

FIGURE 17.22
Studying development in the mouse.

Mouse embryo

Adult mouse

The Fruit Fly

The tiny fruit fly *Drosophila melanogaster* has been a favorite of geneticists for over 90 years and is now playing a key role in our growing understanding of the cellular mechanisms of development. Over the last 10 years, researchers have pieced together a fairly complete picture of how genes expressed early in fruit fly development determine the pattern of the adult body (figure 17.23). The major parts of the adult body are determined as patches of tissue called imaginal discs that float within the body of the larva; during the pupal stage, these discs grow, develop, and associate to form the adult body.

The adult *Drosophila* body is divided into 17 segments, some bearing jointed appendages such as wings or legs. These segments are established during very early development, before the many nuclei of the blastoderm are fully separated from one another. Chemical gradients, established within the egg by material from the mother, create a polarity that directs embryonic development. Reacting to this gradient, a series of segmentation genes progressively subdivide the embryo, first into four broad stripes, and then into 7, 14, and finally 17 segments.

Within each segment, the development of key body parts is under the control of homeotic genes that determine where the body part will form. As we have seen, there are two clusters of homeotic genes, one called Antennapedia that governs the front (anterior) end of the body, and another called bithorax that governs the rear (posterior) end. The organization of genes within each cluster corresponds nicely with the order of the segments they affect. A very similar set of homeotic genes governs body architecture in mice and humans.
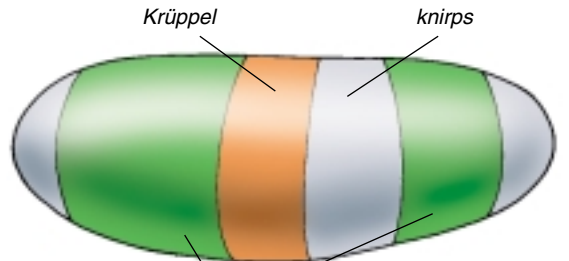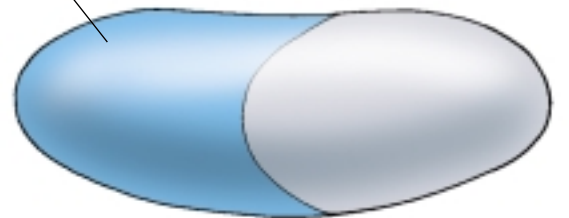
> **A series of segmentation genes divides a *Drosophila* embryo into parts; Antennapedia genes control anterior development, and bithorax genes control the development of the posterior.**
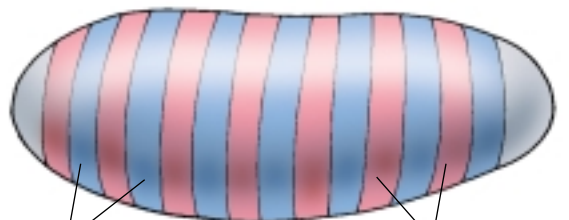
FIGURE 17.23
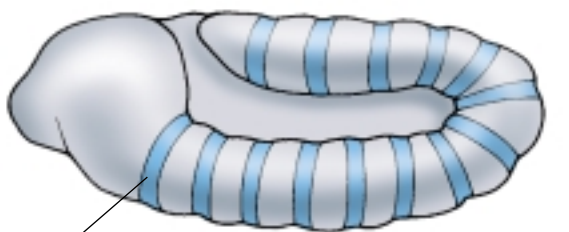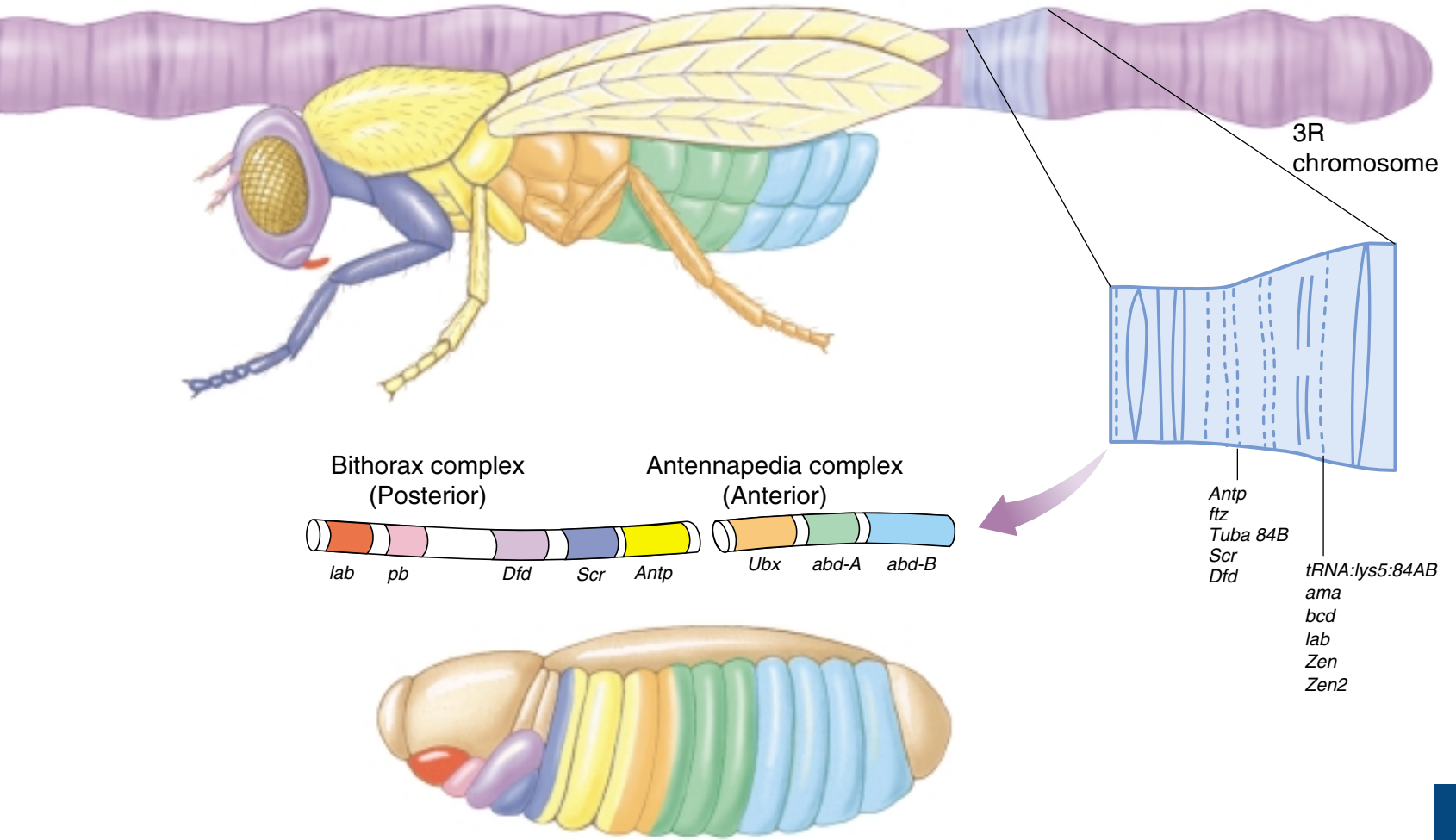**Studying development in the fruit fly.**

Drosophila egg

bicoid

Krüppel   knirps

hunchback

even-skipped   fushi-tarazu

engrailed

3R chromosome

Bithorax complex
(Posterior)

Antennapedia complex
(Anterior)

lab *pb* *Dfd* *Scr* *Antp* *Ubx* *abd-A* *abd-B*

Antp
ftz
Tuba 84B
Scr
Dfd

tRNA:lys5:84AB
ama
bcd
lab
Zen
Zen2

Drosophila embryo

Larva with imaginal discs

Lip

Mouthparts

Prothorax   Antenna   Eye   Leg (3)   Wing   Rudimentary
wing   Genital

Mouthparts

Lip

Adult fly

Nervous system   Pharynx

The Nematode

One of the most powerful models of animal development is the tiny nematode *Caenorhabditis elegans*. Only about 1 mm long, it consists of 959 somatic cells and has about the same amount of DNA as *Drosophila*. The entire genome has been mapped as a series of overlapping fragments, and a serious effort is underway to determine the complete DNA sequence of the genome.

Because *C. elegans* is transparent, individual cells can be followed as they divide. By observing them, researchers have learned how each of the cells that make up the adult worm is derived from the fertilized egg. As shown on this lineage map (figure 17.24), the egg divides into two, and then its daughter cells continue to divide. Each horizontal line on the map represents one round of cell division. The length of each vertical line represents the time between cell divisions, and the end of each vertical line represents one fully differentiated cell. In figure 17.24, the major organs of the worm are color-coded to match the colors of the corresponding groups of cells on the lineage map.
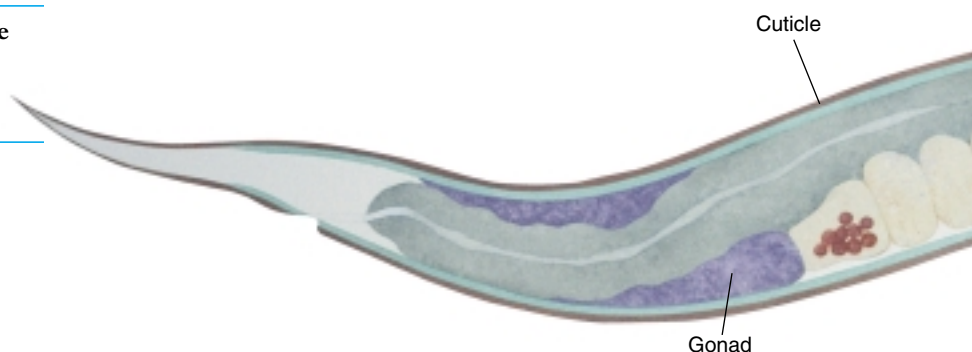
Some of these differentiated cells, such as some of the cells that generate the worm's external cuticle, are "born" after only 8 rounds of cell division; other cuticle cells require as many as 14 rounds. The cells that make up the worm's pharynx, or feeding organ, are born after 9 to 11 rounds of division, while cells in the gonads require up to 17 divisions.

Exactly 302 nerve cells are destined for the worm's nervous system. Exactly 131 cells are programmed to die, mostly within minutes of their birth. The fate of each cell is the same in every *C. elegans* individual, except for the cells that will become eggs and sperm.
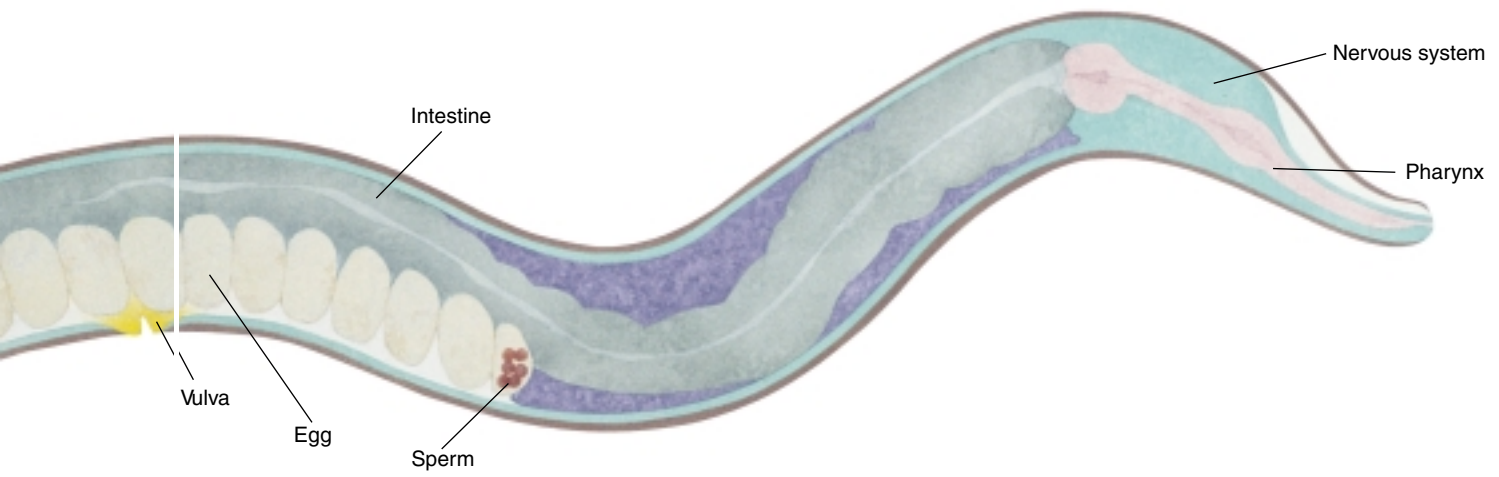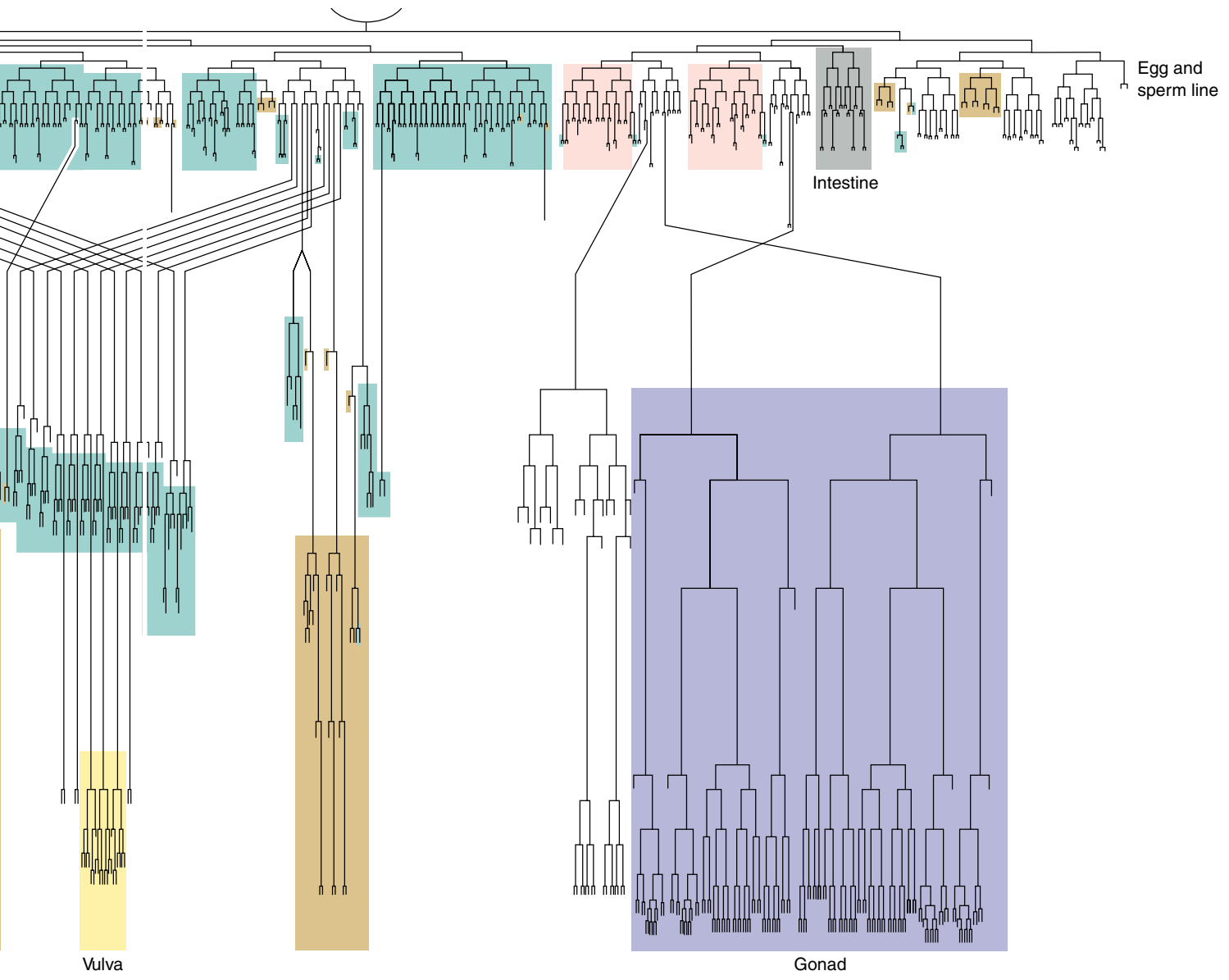
> The nematode develops 959 somatic cells from a single fertilized egg in a carefully orchestrated series of cell divisions which have been carefully mapped by researchers.

Cuticle-making cells

Cuticle

Gonad

FIGURE 17.24
Studying development in the nematode.

Egg and
sperm line

Intestine

Vulva

Gonad

Nervous system

Intestine

Pharynx

Vulva

Egg

Sperm

The Flowering Plant

Scientists are only beginning to unravel the molecular biology of plant development, largely through intensive recent study of a small weedy relative of the mustard plant, the wall cress *Arabidopsis thaliana*. Easy to grow and cross, and with a short generation time, *Arabidopsis* makes an ideal model for investigating plant development. It is able to self-fertilize, like Mendel's pea plants, making genetic analysis convenient. *Arabidopsis* can be grown indoors in test tubes, a single plant producing thousands of offspring after only two months. Its genome is approximately the same size as those of the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. An ordered library of *Arabidopsis* gene clones was made available to researchers in 1997, and the full genome sequence was completed in 1999.

Pattern Formation

Much of the current work investigating *Arabidopsis* development has centered on obtaining and studying mutations that alter the plant's development. Many different sorts of mutations have been identified. Some of the most interesting of them alter the basic architecture of the embryo, the pattern of tissues laid down as the embryo first forms. Mutations in over 50 different genes that alter pattern formation in *Arabidopsis* embryos are now known, affecting every stage of development. While work in this area is still very preliminary, it appears that the mechanisms that establish patterns in the early *Arabidopsis* embryo are broadly similar to those known to function in animal development.
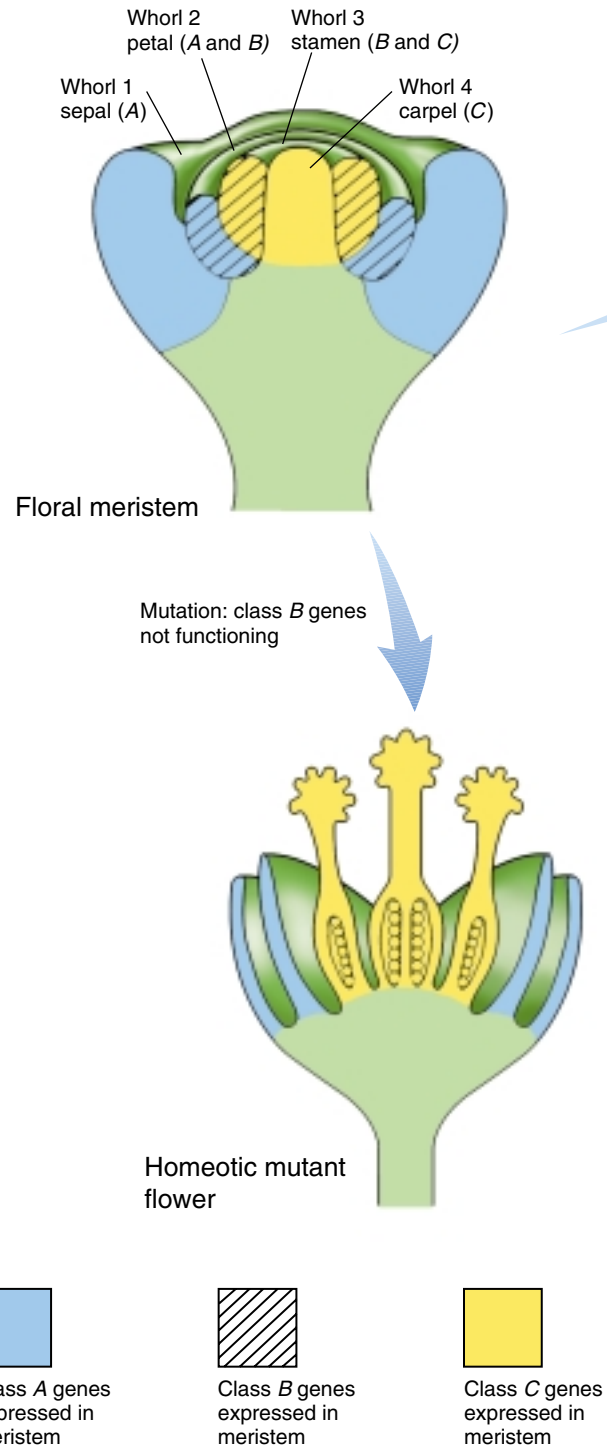
Organ Formation

Importantly, the subsequent development of organs in *Arabidopsis* also seems to parallel organ development in animals, and a similar set of regulatory genes control development in *Arabidopsis*, *Drosophila*, and mice. *Arabidopsis* flowers, for example, are modified leaves formed as four whorls in a specific order, and homeotic mutations have been identified that convert one part of the pattern to another, just as they do in the body segments of a fly (figure 17.25).
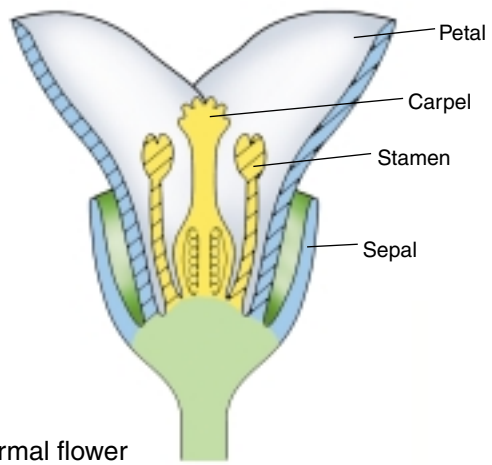
Scientists are only beginning to understand the molecular biology of plant development. In broad outline, it appears quite similar to the development in animals. The genes that determine pattern formation and organ development, for example, operate in the same way in plants and animals.
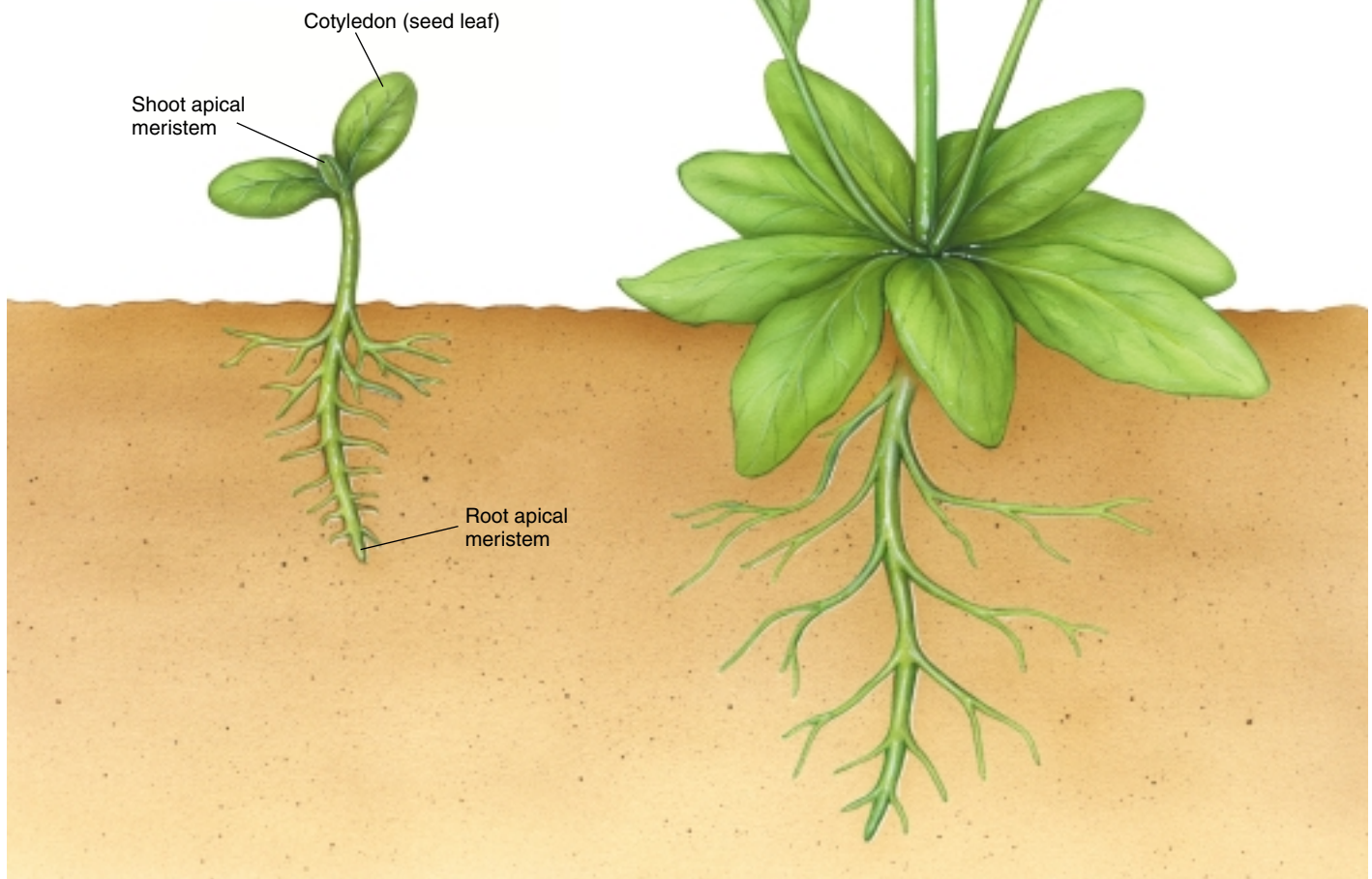

FIGURE 17.25
Studying development in a flowering plant.

Petal

Carpel

Stamen

Sepal

Normal flower

Cotyledon (seed leaf)

Shoot apical
meristem

Root apical
meristem

17.4 Aging can be considered a developmental process.

Theories of Aging

All humans die. The oldest documented person, Jeanne Louise Calment of Arles, France, reached the age of 122 years before her death in 1997. The "safest" age is around puberty. As you can see in figure 17.26, 10- to 15-year-olds have the lowest risk of dying. The death rate begins to increase rapidly after puberty; the rate of mortality then begins to increase as an exponential function of increasing age. Plotted on a log scale as in figure 17.26 (in a so-called Gompertz plot), the mortality rate increases as a straight line from about 15 to 90 years, doubling about every eight years (the "Gompertz number"). By the time we reach 100, age has taken such a toll that the risk of dying reaches 50% per year.

A wide variety of theories have been advanced to explain why humans and other animals age. No one theory has gained general acceptance, but the following four are being intensively investigated:

Accumulated Mutation Hypothesis

The oldest general theory of aging is that cells accumulate mutations as they age, leading eventually to lethal damage. Careful studies have shown that somatic mutations do indeed accumulate during aging. As cells age, for example, they tend to accumulate the modified base 8-hydroxyguanine, in which an —OH group is added to the base guanine. There is little direct evidence, however, that these mutations *cause* aging. No acceleration in aging occurred among survivors of Hiroshima and Nagasaki despite their enormous added mutation load, arguing against any general relationship between mutation and aging.

Telomere Depletion Hypothesis

In a seminal experiment carried out in 1961, Leonard Hayflick demonstrated that fibroblast cells growing in tissue culture will divide only a certain number of times (figure 17.27). After about 50 population doublings, cell division stops, the cell cycle blocked just before DNA replication. If a cell sample is taken after 20 doublings and frozen, when thawed it resumes growth for 30 more doublings, then stops.

An explanation of the "Hayflick limit" was suggested in 1986 when Howard Cooke first glimpsed an extra length of DNA at the ends of chromosomes. These **telomeric regions,** repeats of the sequence TTAGGG, were found to be substantially shorter in older somatic tissue, and Cooke speculated that a 100 base-pair portion of the telomere cap was lost by a chromosome during each cycle of DNA replication. Eventually, after some 50 replication cycles, the protective telomeric cap would be used up, and
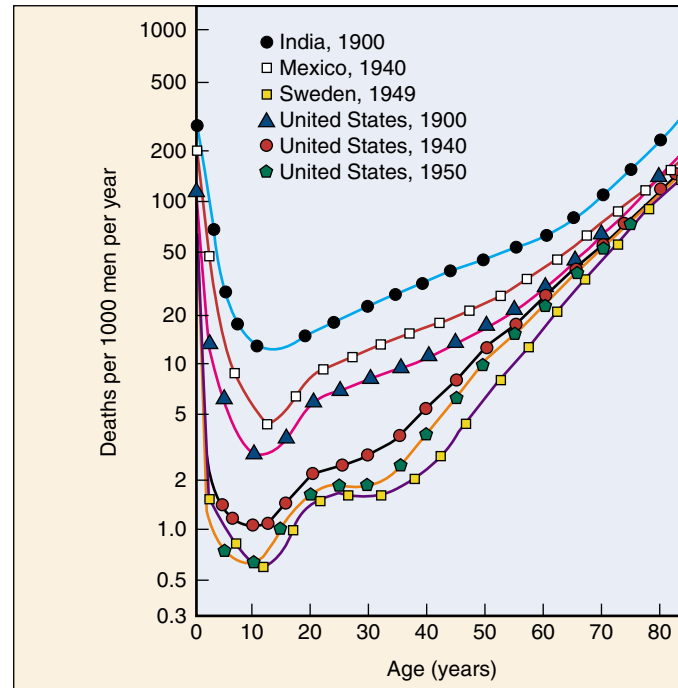
FIGURE 17.26
Gompertz curves. While human populations may differ 25-fold in their mortality rates before puberty, the slopes of their Gompertz curves are about the same in later years.

the cell line would then enter senescence, no longer able to proliferate. Cancer cells appear to avoid telomeric shortening.

Research reported in 1998 has confirmed Cooke's hypothesis, providing direct evidence for a causal relation between telomeric shortening and cell senescence. Using genetic engineering, researchers transferred into human primary cell cultures a gene that leads to expression of telomerase, an enzyme that builds TTAGGG telomeric caps. The result was unequivocal. New telomeric caps were added to the chromosomes of the cells, and the cells with the artificially elongated telomeres did not senesce at the Hayflick limit, continuing to divide in a healthy and vigorous manner for more than 20 additional generations.

Wear-and-Tear Hypothesis

Numerous theories of aging focus in one way or another on the general idea that cells wear out over time, accumulating damage until they are no longer able to function. Loosely dubbed the "wear-and-tear" hypothesis, this idea implies that there is no inherent designed-in limit to aging, just a statistical one—over time, disruption, wear, and damage eventually erode a cell's ability to function properly.

There is considerable evidence that aging cells do accumulate damage. Some of the most interesting evidence concerns free radicals, fragments of molecules or atoms that contain an unpaired electron. Free radicals are very reactive chemically and can be quite destructive in a cell. Free radicals are produced as natural by-products of oxidative metabolism, but most are mopped up by special enzymes that function to sweep the cell interior free of their destructive effects.

One of the most damaging free radical reactions that occurs in cells causes glucose to become linked to proteins, a nonenzymatic process called glycation. Two of the most commonly glycated proteins are collagen and elastin, key components of the connective tissues in our joints. Glycated collagen and elastin are not replaced, and individual molecules may be as old as the individual.

Glycation of collagen, elastin, and a diverse collection of other proteins within the cell produces a complex mixture of glucose-linked proteins called advanced glycosylation end products (AGEs). AGEs can cross-link to one another, reducing the flexibility of connective tissues in the joints and producing many of the other characteristic symptoms of aging.

Gene Clock Hypothesis

There is very little doubt that at least some aspects of aging are under the direct control of genes. Just as genes regulate the body's development, so they appear to regulate its rate of aging. Mutations in these genes can produce premature aging in the young. In the very rare recessive Hutchinson-Gilford syndrome, growth, sexual maturation, and skeletal development are retarded; atherosclerosis and strokes usually lead to death by age 12 years. Only some 20 cases have ever been described.

The similar Werner's syndrome is not as rare, affecting some 10 people per million worldwide. The syndrome is named after Otto Werner, who in Germany in 1904 reported a family affected by premature aging and said a genetic component was at work. Werner's syndrome makes its appearance in adolescence, usually producing death before age 50 of heart attack or one of a variety of rare connective tissue cancers. The gene responsible for Werner's syndrome was identified in 1996. Located on the short arm of chromosome 8, it seems to affect a helicase enzyme involved in the repair of DNA. The gene, which codes for a 1432-amino-acid protein, has been fully sequenced, and four mutant alleles identified. Helicase enzymes are needed to unwind the DNA double helix whenever DNA has to be replicated, repaired, or transcribed. The high incidence of certain cancers among Werner's syndrome patients leads investigators to speculate that the mutant helicase may fail to activate critical tumor suppressor genes. The potential role of helicases in aging is the subject of heated research.
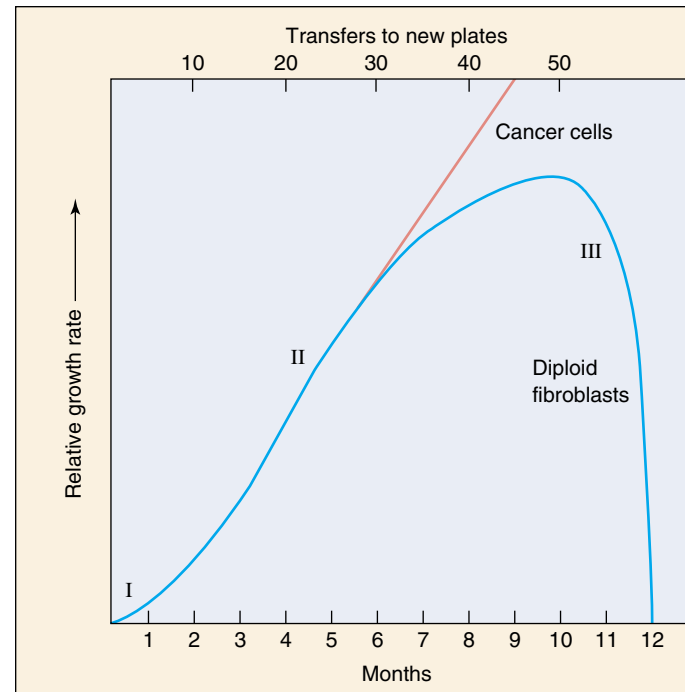
FIGURE 17.27
Hayflick's experiment. Fibroblast cells stop growing after about 50 doublings. Growth is rapid in phases I and II, but slows in phase III, as the culture becomes senescent, until the final doubling. Cancer cells, by contrast, do not "age."

Research on aging in other animals strongly supports the hypothesis that genes regulate the rate of aging. Particularly impressive results have been obtained in the nematode *Caenorhabditis elegans*, where genes discovered in 1996 seem to affect an intrinsic genetic clock. A combination of mutations can increase the worm's lifespan fivefold, the largest increase in lifespan seen in any organism! Mutations in the clock gene *clk-1* cause individual cells to divide more slowly, and the animal spends more time in each phase of its life cycle. Mutations in two other clock genes, *clk-2* and *clk-3*, have similar effects. Nematodes with mutations in two of the clock genes lived three to four times longer than normal. It seems that slowing life down in nematodes extends it. Perhaps, as the "wear-and-tear" theory suggests, aging results from damage to cells and their DNA by highly reactive oxidative by-products of metabolism. Living more slowly, destructive by-products may be produced less frequently, accumulate more slowly, and their damage be repaired more efficiently. Similar genes have been reported in yeasts, and attempts are now underway to isolate and clone these genes.

Among the many theories advanced to explain aging, many involve the progressive accumulation of damage to DNA. When genes affecting aging have been isolated, they affect DNA repair processes.

Summary	*Questions*	*Media Resources*

17.1   Development is a regulated process.

- Vertebrate development is initiated by a rapid cleavage of the fertilized egg into a hollow ball of cells, the blastula. Cell movements then form primary germ layers and organize the structure of the embryo.
- Cells determined in the insect embryo are carried within the body of larvae as imaginal discs, which are assembled into the adult body during pupation.
- Plant meristems continuously produce new tissues, which then differentiate into body parts. This differentiation is significantly influenced by the environment.

1. What is cleavage? How does the type of cleavage influence subsequent embryonic development?

2. What is a blastula? How does it form and what does it turn into?

3. What is a gastrula? Where are the germ layers in a gastrula?

4. What is neurulation? How and when does it occur?

- **ESP**
- Introduction to Development

- Vertebrate Limb Formation

17.2   Multicellular organisms employ the same basic mechanisms of development.

- Cell movement in animal development is carried out by altering a cell's complement of surface adhesion molecules, which it uses to pull itself over other cells.
- A key to animal development is the ability of cells to alter the developmental paths of adjacent cells, a process called induction. Induction is achieved by diffusible chemicals called morphogens.
- Determination of a cell's ultimate developmental fate often involves the addition to it of positional labels that reflect its location in the embryo.
- The location of structures within body segments is dictated by a spatially organized assembly of homeotic genes, first discovered in *Drosophila* but now known to occur in all animals.
- Many cells are genetically programmed to die, usually soon after they are formed during development, in a process called apoptosis.

5. What role do cadherins and integrins play in cell movement?

6. What is the difference between mosaic development and regulative development?

7. How do organizers and morphogens participate in induction?

8. How is determination distinguished from differentiation?

9. What role does maternal mRNA play in the development of a *Drosophila* embryo?

10. What are homeotic genes and what do they do?

- **ESP**
- Induction
- Pattern Formation

17.3   Four model developmental systems have been extensively researched.

- The four most intensively studied model systems of development are the mouse *Mus musculus*, the fruit fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, and the flowering plant *Arabidopsis thaliana*.

11. What are the major differences between vertebrate, insect, and plant developmental pathways? What are the similarities?

17.4   Aging can be considered a developmental process.

- Aging is not well understood, although not for want of theories, most of which involve progressive damage to DNA.

12. Cancer cell cultures never seem to grow old, dividing ceaselessly. What can you deduce about the state of their telomerase gene?