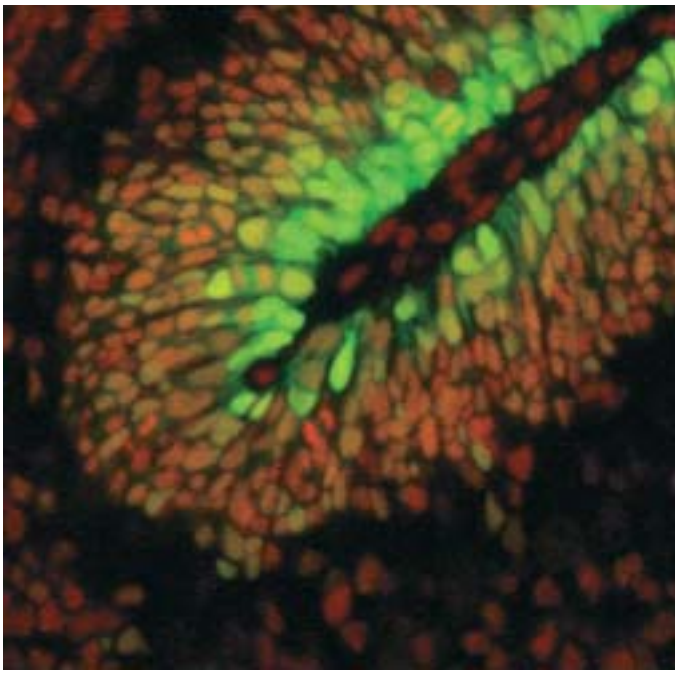
22

CELL BIRTH, LINEAGE, AND DEATH



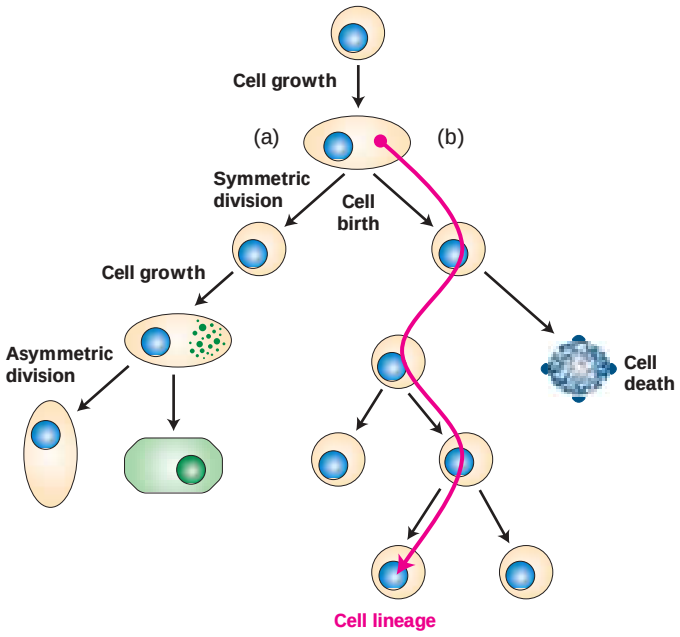
Cells being born in the developing cerebellum. All nuclei are labeled in red; the green cells are dividing and migrating into internal layers of the neural tissue. [Courtesy of Tal Raveh, Matthew Scott, and Jane Johnson.]

During the evolution of multicellular organisms, new mechanisms arose to diversify cell types, to coordinate their production, to regulate their size and number, to organize them into functioning tissues, and to eliminate extraneous or aged cells. Signaling between cells became even more important than it is for single-celled organisms. The mode of reproduction also changed, with some cells becoming specialized as **germ cells** (e.g., eggs, sperm), which give rise to new organisms, as distinct from all other body cells, called **somatic cells.** Under normal conditions somatic cells will never be part of a new individual.

The formation of working tissues and organs during **development** of multicellular organisms depends in part on specific patterns of mitotic cell division. A series of such cell divisions akin to a family tree is called a *cell lineage*, which traces the progressive determination of cells, restricting their developmental potential and their *differentiation* into specialized cell types. Cell lineages are controlled by intrinsic (internal) factors—cells acting according to their history and internal regulators—as well as by extrinsic (external) factors such as cell-cell signals and environmental inputs (Figure 22-1). A cell lineage begins with **stem cells,** unspecialized cells that can potentially reproduce themselves and generate more-specialized cells indefinitely. Their name comes from the image of a plant stem, which grows upward, continuing to form more stem, while sending off leaves and branches to the side. A cell lineage ultimately culminates in formation of terminally differentiated cells such as skin cells, neurons, or muscle cells. Terminal differentiation generally is irreversible, and the resulting highly specialized cells often cannot divide; they survive, carry out their functions for varying lengths of time, and then die.

Many cell lineages contain intermediate cells, referred to as *precursor cells* or *progenitor cells*, whose potential to form different kinds of differentiated cells is more limited than that of the stem cells from which they arise. (Although some researchers distinguish between precursor and progenitor cells, we will use these terms interchangeably.) Once a new precursor cell type is created, it often produces transcription factors characteristic of its fate. These transcription factors

OUTLINE



▶ FIGURE 22-1 **Overview of the birth, lineage, and death of cells.** Following growth, cells are "born" as the result of symmetric or asymmetric cell division. (a) The two daughter cells resulting from symmetric division are essentially identical to each other and to the parental cell. Such daughter cells subsequently can have different fates if they are exposed to different signals. The two daughter cells resulting from asymmetric division differ from birth and consequently have different fates. Asymmetric division commonly is preceded by the localization of regulatory molecules (green) in one part of the parent cell. (b) A series of symmetric and/or asymmetric cell divisions, called a cell lineage, gives birth to each of the specialized cell types found in a multicellular organism. The pattern of cell lineage can be under tight genetic control. Programmed cell death occurs during normal development (e.g., in the webbing that initially develops when fingers grow) and also in response to infection or poison. A series of specific programmed events, called apoptosis, is activated is these situations.

coordinately activate, or repress, batteries of genes that direct the differentiation process. For instance, a few key regulatory transcription factors create the different mating types of budding yeast and coordinate the numerous genes necessary to turn a precursor cell into a muscle cell, two examples that we discuss in this chapter.

Typically we think of cell fates in terms of the differentiated cell types that are formed. A quite different cell fate, **programmed cell death,** also is absolutely crucial in the formation and maintenance of many tissues. A precise genetic regulatory system, with checks and balances, controls cell death just as other genetic programs control cell differentiation. In this chapter, then, we consider the life cycle of cells—their birth, their patterns of division (lineage), and their death. These aspects of cell biology converge with developmental biology and are among the most important processes regulated by various signaling pathways discussed in earlier chapters.

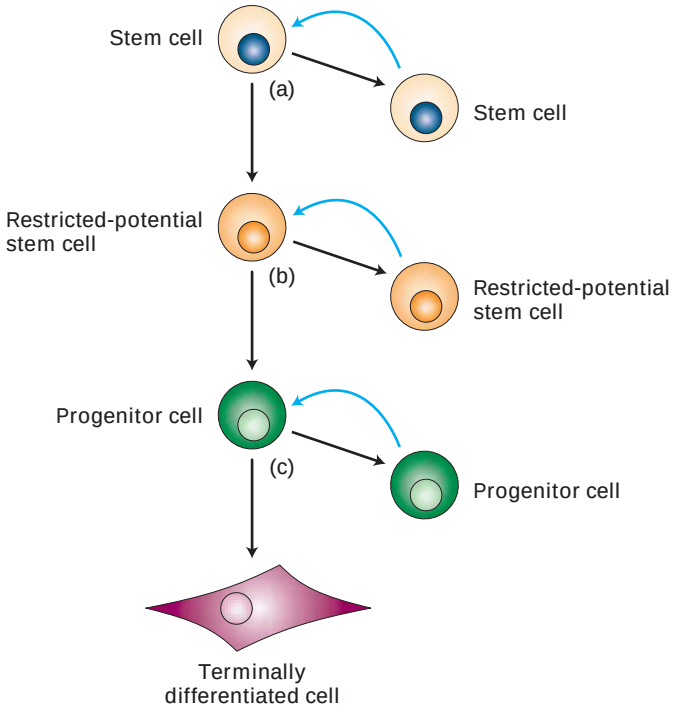
22.1 The Birth of Cells

Many descriptions of cell division imply that the parental cell gives rise to two daughter cells that look and behave exactly like the parental cell, that is, cell division is *symmetric*, and the progeny do not change their properties. But if this were always the case, none of the hundreds of differentiated cell types would ever be formed. Differences among cells can arise when two initially identical daughter cells diverge by receiving distinct developmental or environmental signals. Alternatively, the two daughter cells may differ from "birth," with each inheriting different parts of the parental cell (see Figure 22-1). Daughter cells produced by such **asymmetric cell division** may differ in size, shape, and/or composition, or their genes may be in different states of activity or potential activity. The differences in these internal signals confer different fates on the two cells.

Here we discuss some general features of how different cell types are generated, culminating with the best-understood complex cell lineage, that of the nematode *Caenorhabditis elegans*. In later sections, we focus on examples of the molecular mechanisms that determine particular cell types in yeast, *Drosophila*, and mammals.

Stem Cells Give Rise to Stem Cells and to Differentiating Cells

Stem cells, which give rise to the specialized cells composing the tissues of the body, exhibit several patterns of cell division. A stem cell may divide symmetrically to yield two daughter stem cells identical to itself (Figure 22-2a). Alternatively, a stem cell may divide asymmetrically to generate a copy of itself and a derivative stem cell that has more-restricted capabilities, such as dividing for a limited period of time or giving rise to fewer types of progeny compared with the parental stem cell (Figure 22-2b). A *pluripotent* (or multipotent) stem cell has the capability of generating a number of different cell types, but not all. For instance, a pluripotent blood stem cell will form more of itself plus multiple types of blood cells, but never a skin cell. In contrast, a *unipotent* stem cell divides to form a copy of itself plus a cell that can form only one cell type. In many cases, asymmetric division of a stem cell generates a progenitor cell, which embarks on a path of differentiation, or even a terminally differentiating cell (Figure 22-2c, d).



▲ FIGURE 22-2 **Patterns of stem-cell division.** (a) Division of a stem cell produces two cells, one of which is a stem cell like the mother cell. In this way the population of stem cells is maintained. (b) The other daughter cell—a stem cell of more-restricted potential—starts on a pathway toward producing more differentiated cells. When it divides, one of the daughters will be the same sort of restricted-potential stem cell as the mother and the other will be a progenitor cell for a certain type of differentiated cell. Progenitor cells can divide to reproduce themselves and, in response to appropriate signals, can differentiate into a terminally differentiated, nondividing cell.

The two critical properties of stem cells that together distinguish them from all other cells are the ability to reproduce themselves indefinitely, often called *self-renewal*, and the ability to divide asymmetrically to form one daughter stem cell identical to itself and one daughter cell that is different and usually of more restricted potential. In this way, mitotic division of stem cells preserves a population of undifferentiated cells while steadily producing a stream of differentiating cells. Although some types of precursor cells can divide symmetrically to form more of themselves, they do so only for limited periods of time. Moreover, in contrast to stem cells, if a precursor cell divides asymmetrically, it generates two distinct daughter cells, neither of which is identical to the parental precursor cell.

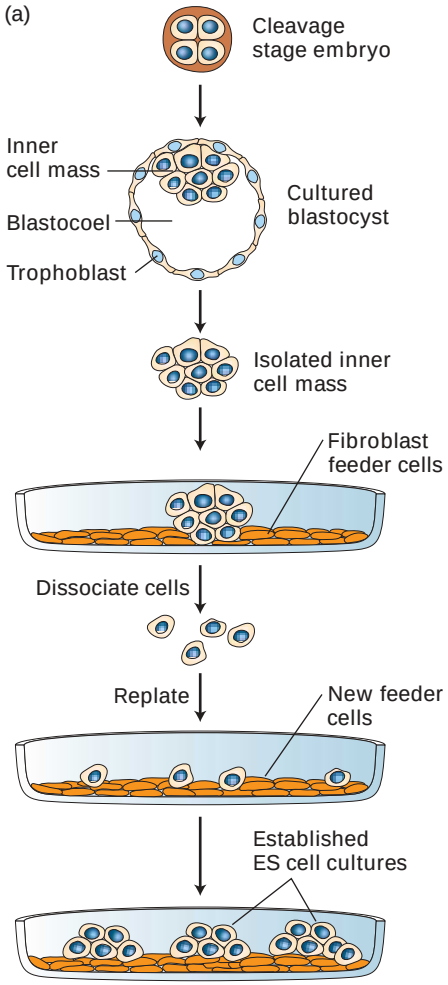
The fertilized egg, or **zygote,** is the ultimate totipotent cell because it has the capability to generate all the cell types of the body. Although not technically a stem cell because it is not self-renewing, the zygote does give rise to cells with stem-cell properties. For example, the early mouse embryo passes through an eight-cell stage in which each cell can give rise to any cell type of the embryo. If the eight cells are experimentally separated and individually implanted into a suitable foster mother, each can form a whole mouse with no parts missing. This experiment shows that the eight cells are all able to form every tissue; that is, they are *totipotent*. Thus the subdivision of body parts and tissue fates among the early embryonic cells has not irreversibly occurred at the eight-cell stage. At the 16-cell stage, this is no longer true; some of the cells are committed to particular differentiation paths.

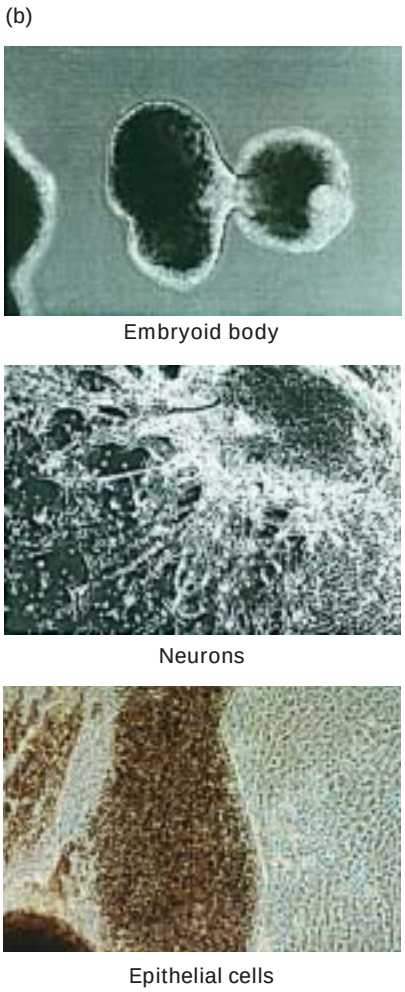
Quite different specialized cell types can arise from a common precursor cell. A hematopoietic stem cell can give rise to all the multifarious types of blood cell. However, a whole series of cell divisions is not required; a single division of a pluripotent precursor cell can yield distinct progeny. For instance, lineage studies in which cells are marked by stable infection with a detectable retrovirus have shown that neurons and glial cells can arise from a single division of a particular precursor cell. These cell types are quite different: neurons propagating and transmitting electrical signals and glial cells providing electrical insulation and support. The precursor that generates neurons and glial cells is not a stem cell, since it is incapable of self-renewal; presumably the neuron–glial cell precursor arises from a stem cell further back in the lineage. Recent observations have raised the interesting possibility that stem cells for one tissue may be induced under certain conditions to act as stem cells for a rather different tissue. As we discuss below, postnatal animals contain stem cells for many tissues including the blood, intestine, skin, ovaries and testes, muscle, and liver. Even some parts of the adult brain, where little cell division normally occurs, has a population of stem cells. In muscle and liver, stem cells are most important in healing, as relatively little cell division occurs in the adult tissues otherwise.

Cultured Embryonic Stem Cells Can Differentiate into Various Cell Types

Embryonic stem (ES) cells can be isolated from early mammalian embryos and grown in culture (Figure 22-3a). Cultured ES cells can differentiate into a wide range of cell types, either in vitro or after reinsertion into a host embryo. When grown in suspension culture, human ES cells first differentiate into multicellular aggregates, called embryoid bodies, that resemble early embryos in the variety of tissues they form. When these are subsequently transferred to a solid medium, they grow into confluent cell sheets containing a variety of differentiated cell types including neural cells and pigmented and nonpigmented epithelial cells (Figure 22-3b). Under other conditions, ES cells have been induced to differentiate into precursors for various types of blood cells.

▶ EXPERIMENTAL FIGURE 22-3
Embryonic stem (ES) cells can be maintained in culture and form differentiated cell types. (a) Human blastocysts are grown from cleavage-stage embryos produced by in vitro fertilization. The inner cell mass is separated from the surrounding extra-embryonic tissues and plated onto a layer of fibroblast cells that help to nourish the embryonic cells. Individual cells are replated and form colonies of ES cells, which can be maintained for many generations and can be stored frozen. (b) In suspension culture, human ES cells differentiate into multicellular aggregates (embryoid bodies) (*top*). After embryoid bodies are transferred to a gelatinized solid medium, they differentiate further into confluent cell sheets containing a variety of differentiated cell types including neural cells (*middle*), and pigmented and nonpigmented epithelial cells (*bottom*). [Parts (a) and (b) adapted from J. S. Odorico et al., 2001, *Stem Cells* **19**:193–204.]





MEDICINE

The possibility of using stem cells therapeutically to restore or replace damaged tissue is fueling much research on how to recognize and culture these remarkable cells from embryos and from various tissues in postnatal (adult) animals. For example, if neurons that produce the neurotransmitter dopamine could be generated from stem cells grown in culture, it might be possible to treat people with Parkinson's disease who have lost such neurons. For such an approach to succeed, a way must be found to direct a population of embryonic or other stem cells to form the right type of dopamine-producing neurons, and rejection by the immune system nust be prevented. In one ongoing study in which embryonic neurons were transplanted into more than 300 Parkinson's patients, some of the inserted cells have survived for more than 12 years and have provided significant clinical improvement. However, the fetal tissue used in this study is scarce and its use is controversial. Stem cells grown from very early embryos are another option for treating Parkinson's disease and perhaps other neurodegenerative conditions such as Alzheimer's disease. Similar possibilities exist for generating blood, pancreas, and other cell types. Many important questions must be answered before the feasibility of using stem cells for such purposes can be assessed adequately. ▮

Apart from their possible benefit in treating disease, ES cells have already proven invaluable for producing mouse mutants useful in studying a wide range of diseases, developmental mechanisms, behavior, and physiology. By techniques described in Chapter 9, it is possible to eliminate or modify the function of a specific gene in ES cells (see Figure 9-38). Then the mutated ES cells can be employed to produce mice with a **gene knockout** (see Figure 9-39). Analysis of the effects caused by deleting or modifying a gene in this way often provides clues about the normal function of the gene and its encoded protein.

Tissues Are Maintained by Associated Populations of Stem Cells

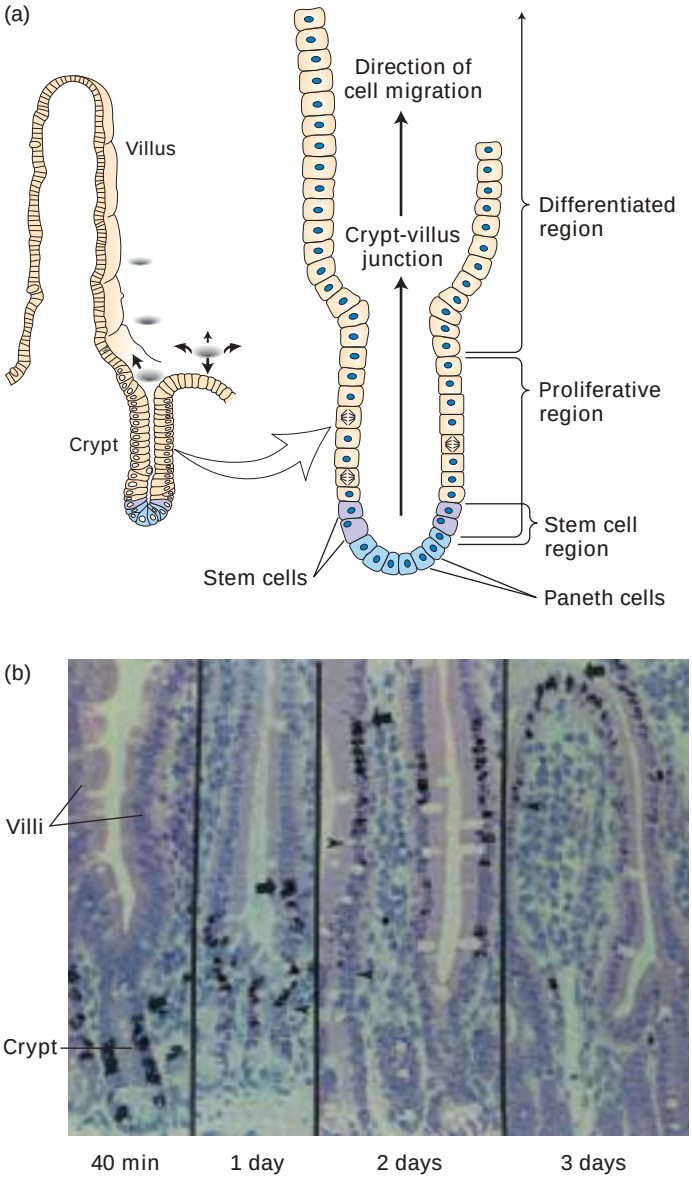
Many differentiated cell types are sloughed from the body or have life spans that are shorter than that of the organism. Disease and trauma also can lead to loss of differentiated

cells. Since these cells generally do not divide, they must be replenished from nearby stem-cell populations.

Our skin, for instance, is a multilayered **epithelium** (the epidermis) underlain by a layer of stem cells that give rise both to more of themselves and to *keratinocytes*, the major cell type in skin. Keratinocytes then move toward the outer surface, becoming increasingly flattened and filled with keratin intermediate filaments. It normally takes about 15–30 days for a newly "born" keratinocyte in the lowest layer to differentiate and move to the topmost layer. The "cells" forming the topmost layer are actually dead and are continually shed from the surface.

In contrast to epidermis, the epithelium lining the small intestine is a single cell thick (Figure 6-4). This thin layer keeps toxins and pathogens from entering our bodies and also transports nutrients essential for survival from the intestinal lumen into the body (Chapter 7). The cells of the intestinal epithelium continuously regenerate from a stem-cell population located deep in the intestinal wall in pits called *crypts* (Figure 22-4a). The stem cells produce precursor cells that proliferate and differentiate as they ascend the sides of crypts to form the surface layer of the finger-like gut projections called *villi*, across which intestinal absorption occurs. Pulse-chase labeling experiments have shown that the time from cell birth in the crypts to the loss of cells at the tip of the villi is only about 2 to 3 days (Figure 22-4b). Thus enormous numbers of cells must be produced continually to keep the epithelium intact. The production of new cells is precisely controlled: too little division would eliminate villi and lead to breakdown of the intestinal surface; too much division would create an excessively large epithelium and also might be a step toward cancer.

Specific signals are required for creating and maintaining stem-cell populations. In both the skin and the intestinal epithelium, stem-cell growth is regulated in part by *β-catenin*, a protein that helps link certain cell-cell junctions to the cytoskeleton (see Figure 6-7) and also functions as a signal transducer in the Wnt pathway (see Figure 15-32). Activation of β-catenin moves cells from epidermis to hair cell fates. In contrast, removal of β-catenin specifically from the skin of engineered mice eliminates hair cell fates. Skin stem cells then form only epidermis, not hair cells. β-catenin thus acts as a switch between alternative cell fates. Overproduction of active β-catenin leads to excess proliferation of the intestinal epithelium. Blocking the function of β-catenin by interfering with the TCF transcription factor that it activates abolishes the stem cells in the intestine. It seems likely that Wnt signaling, or at least components of the intracellular pathway, is critical for forming, maintaining, or activating stem cells in a variety of tissues.



◀ EXPERIMENTAL FIGURE 22-4 **Regeneration of the intestinal epithelium from stem cells can be demonstrated in pulse-chase experiments.** (a) Schematic drawing of the lining of the small intestine, which contains numerous villi formed from a column of cells. These epithelial cells are born near the base of pits (crypts) located between the villi. Located at the very bottom of crypts are Paneth cells, a type of support cell; just above these are four to six stem cells, which divide about once a day, forming precursor cells that also actively divide. As the differentiated cells enter the epithelium of a villus, they stop dividing and begin taking up nutrients from the gut. (b) Results from a pulse-chase experiment in which radioactively labeled thymidine (the pulse) was added to a tissue culture of the intestinal epithelium. Dividing cells incorporated the labeled thymidine into their newly synthesized DNA. The labeled thymidine was washed away and replaced with nonlabeled thymidine (the chase) after a brief period; cells that divided after the chase did not become labeled. These micrographs show that 40 minutes after labeling, all the label is in cells near the base of the crypt. At later times, the labeled cells are seen progressively farther away from their point of birth in the crypt. Cells at the top are shed. This process ensures contant replenishment of the gut epithelium with new cells. [Part (a) adapted from C. S. Potten, 1998, *Philos. Trans. R. Soc. London, Ser. B* **353**:821. Part (b) courtesy of C. S. Potten, from P. Kaur and C. S. Potten, 1986, *Cell Tiss. Kinet.* **19**:601]

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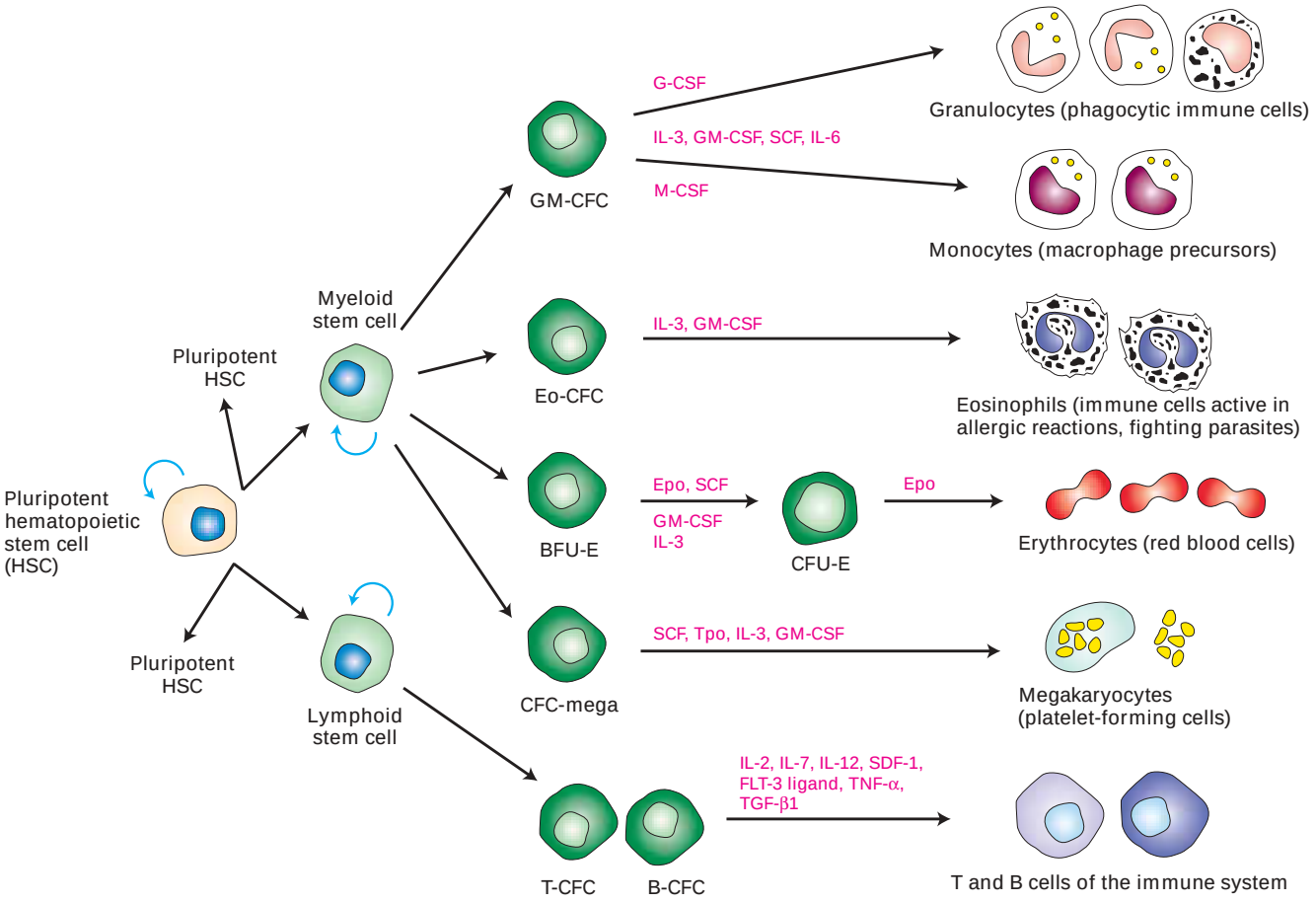
Skin also contains dendritic epidermal T cells, an immune-system cell that produces a certain form of the T-cell receptor (see Table 14-1). When dendritic epidermal T cells are genetically modified so they do not produce T-cell receptors, wound healing is slow and less complete than in normal skin. Normal healing is restored by addition of keratinocyte growth factor. The current hypothesis is that when dendritic epidermal T cells recognize antigens on cells in damaged tissue, they respond by producing stimulating proteins, such as keratinocyte growth factor, that promote keratinocyte growth and wound healing. Many other signals also control the growth of skin cells, including Wnt/β-catenin, Hedgehog, calcium, transforming growth factor α (TGFα), and TGFβ. Discovering how all these signals work together to control growth and stimulate healing is a substantial challenge that will advance our understanding of diseases such as psoriasis and skin cancer and perhaps pave the way for effective treatments. ▮

Another continuously replenished tissue is the blood, whose stem cells are located in the bone marrow in adult animals. The various types of blood cells all derive from a single type of *pluripotent hematopoietic stem cell*, which gives rise to the more-restricted myeloid and lymphoid stem cells (Figure 22-5). The frequency of hematopoietic stem cells is about 1 cell per 10^4 bone marrow cells, even lower than the frequency of intes-



▲ FIGURE 22-5 **Formation of differentiated blood cells from hematopoietic stem cells in the bone marrow.** Pluripotent stem cells may divide symmetrically to self-renew (curved arrow) or divide asymmetrically to form a myeloid or lymphoid stem cell (light green) and a daughter cell that is pluripotent like the parental cell. Although these stem cells are capable of self-renewal, they are committed to one of the two major hematopoietic lineages. Depending on the types and amounts of cytokines present, the myeloid and lymphoid stem cells generate different types of precursor cells (dark green), which are incapable of self-renewal. Precursor cells are detected by their ability to form colonies containing the differentiated cell types shown at right, measured as "colony-forming cells (CFCs)." The colonies are detected on the spleen of animals that have had their own cells eliminated and the precursor cells introduced. Further cytokine-induced proliferation, commitment, and differentiation of the precursor cells give rise to the various types of blood cells. Some of the cytokines that support this process are indicated (red labels). GM = granulocyte-macrophage; Eo = eosinophil; E = erythrocyte; mega = megakaryocyte; T = T-cell; B = B-cell; CFU = colony-forming unit; CSF = colony-stimulating factor; IL = interleukin; SCF = stem cell factor; Epo = erythropoietin; Tpo = thrombopoietin; TNF = tumor necrosis factor; TGF = transforming growth factor; SDF = stromal cell–derived factor; FLT-3 ligand = ligand for fms-like tyrosine kinase receptor 3. [Adapted from M. Socolovsky et al., 1998, *Proc. Nat'l. Acad. Sci. USA* **95**:6573.]

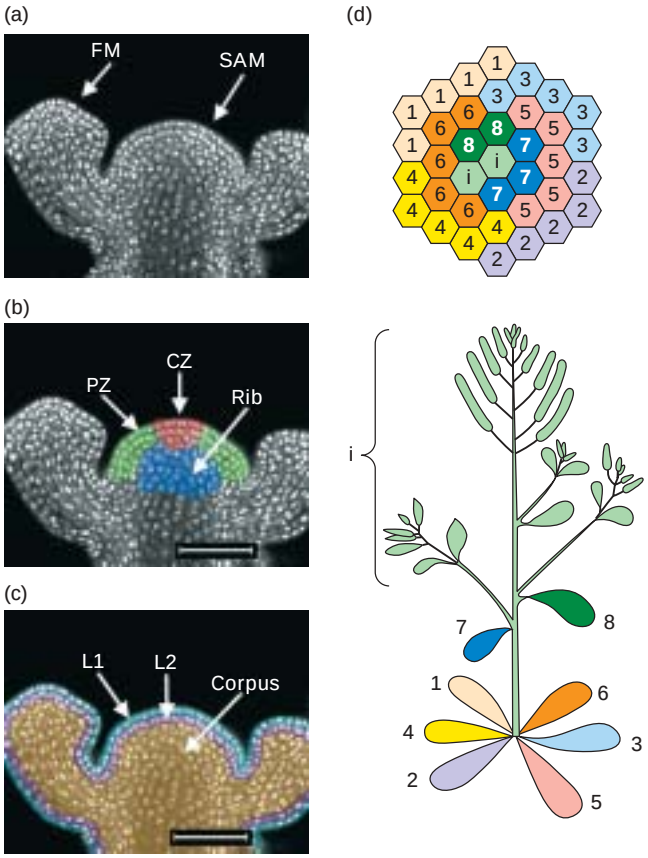
tinal stem cells in crypts. Numerous extracellular growth factors called **cytokines** regulate proliferation and differentiation of the precursor cells for various blood-cell lineages. For example, **erythropoietin** can activate several different intracellular signal-transduction pathways, leading to changes in gene expression that promote formation of erythrocytes (see Figure 14-7).

The hematopoietic lineage originally was worked out by injecting the various types of precursor cells into mice whose precursor cells had been wiped out by irradiation. By observing which blood cells were restored in these transplant experiments, researchers could infer which precursors or terminally differentiated cells (e.g., erythrocytes, monocytes) arise from a particular type of precursor. The first step in these experiments was separation of the different types of hematopoietic precursors. This is possible because each type produces unique combinations of cell-surface proteins that can serve as type-specific markers. If bone marrow extracts are treated with fluorochrome-labeled antibodies for these markers, cells with different surface markers can be separated in a fluorescence-activated cell sorter (see Figure 5-34).

To date, bone marrow transplants represent the most successful and widespread use of stem cells in medicine. The stem cells in the transplanted marrow can generate new, functional blood cells in patients with certain hereditary blood diseases and in cancer patients who have received irradiation and/or chemotherapy, both of which destroy the bone marrow cells as well as cancer cells. Recent work is directed at exploring whether embryonic stem cells can be induced to differentiate into cells types that would be useful therapeutically. For example, mouse stem cells treated with inhibitors of phosphatidylinositol-3 kinase, a regulator in one of the phosphoinositide signaling pathways (Chapter 14), turn into cells that resemble pancreatic β cells in their production of insulin, their sensitivity to glucose levels, and their aggregation into structures reminiscent of pancreas structures. Implantation of these cells into diabetic mice restored their growth, weight, glucose levels, and survival rates to normal. ▮

Stem cells in plants are located in **meristems,** populations of undifferentiated cells found at the tips of growing shoots. Shoot apical meristems (SAMs) produce leaves and shoots, and of course more stem cells that constitute the nearly immortal meristems. Meristems can persist for thousands of years in long-lived species such as redwood trees and bristlecone pines. As a plant grows, the cells "left behind" the meristems are encased in rigid cell walls and can no longer grow. SAMs can split to form branches, each branch with its own SAM, or be converted into floral meristems (Figure 22-6). Floral meristems give rise to the four floral organs—sepals, stamens, carpels, and petals—that form flowers. Unlike SAMs, floral meristems are gradually depleted as they give rise to the floral organs.

Numerous genes have been found to regulate the formation, maintenance, and properties of meristems. Many of these genes encode transcription factors that direct progeny of stem cells down different paths of differentiation. For instance, a hierarchy of regulators, particularly transcription factors, controls the separation of differentiating cells from SAMs as leaves form; similarly, three types of regulators control formation of the floral organs from floral meristems (Chapter 15). In both cases, a cascade of gene interactions occurs, with earlier transcription factors causing production of later ones. At the same time, cells are dividing and the differentiating ones are spreading away from their original birth sites. ▮



▲ FIGURE 22-6 **Cell fates in meristems of *Arabidopsis.*** In these longitudinal sections through a shoot apical meristem, cell nuclei are revealed by staining with propidium iodide, which binds to DNA. (a) The shoot apical meristem (SAM) produces shoots, leaves, and more meristem. Flower production occurs when the meristem switches from leaf/shoot production to flower production, concomitant with an increase in the number of meristem cells to form floral meristems (FMs), as shown here. (b) Cells in a SAM exhibit different fates and behaviors. Cells divide rapidly in the peripheral zone (PZ) to produce leaves and in the rib zone (Rib) to produce central shoot structures. Cells in the central zone (CZ) divide more slowly, producing an ongoing source of meristem and contributing cells to the PZ and Rib. (c) The layers of the meristem, colored here, are each derived (cloned) from the same precursor cell. The fates of cells in different positions in the L2 layer are shown color-coded in part (d). Scale bars, 50 μm. [Parts (a)–(c) from E. Meyerowitz, 1997, *Cell* **88**:299; micrographs courtesy of Elliot Meyerowitz. Part (d) after C. Wolpert et al., 2002, *Principles of Development,* 2d ed. (Oxford: Oxford University Press).]

Cell Fates Are Progressively Restricted During Development

The eight cells resulting from the first three divisions of a mammalian zygote (fertilized egg) all look the same. As demonstrated experimentally in sheep, each of the cells has the potential to give rise to a complete animal. Additional divisions produce a mass, composed of ≈64 cells, that separates into two cell types: trophectoderm, which will form extraembryonic tissues like the placenta, and the *inner cell mass*, which gives rise to the embryo proper. The inner cell mass eventually forms three germ layers, each with distinct fates. One layer, the **ectoderm,** will make neural and epidermal cells; another, the **mesoderm,** will make muscle and connective tissue; the third layer, the **endoderm,** will make gut epithelia (Figure 22-7). This conclusion is based on experiments with chimeric animals composed of chicken and quail cells. Embryos composed of cells from both bird species develop fairly normally, yet the cells derived from each donor are distinguishable under the microscope. Thus the contributions of the different donor cells to the final bird can be ascertained. If cells from one germ layer are transplanted into one of the other layers, they do not give rise to cells appropriate to their new location.

Once the three germ layers are established, they subsequently divide into cell populations with different fates. For instance, the ectoderm becomes divided into those cells that are precursors to the skin epithelium and those that are precursors to the nervous system. There appears to be a progressive restriction in the range of cell types that can be formed from stem cells and precursor cells as development proceeds. An early embryonic stem cell, as we've seen, can form every type of cell, an ectodermal cell has a choice between neural and epidermal fates, while a keratinocyte precursor can form skin but not neurons.

Another restriction that occurs early in animal development is the setting aside of cells that will form the **germ line,** that is, the stem cells and precursor cells that eventually will give rise to eggs in a female and sperm in a male. Only the genome of the germ line will ever be passed on to progeny. The setting aside of germ-line cells early in development has been hypothesized to protect chromosomes from damage by reducing the number of rounds of replication they undergo or by allowing special protection of the cells that are critical to heredity. Whatever the reason, the early segregation of the germ line is widespread (though not universal) among animals. In contrast, plants do nothing of the sort; most meristems can give rise to germ-line cells.

One consequence of the early segregation of germ-line cells is that the loss or rearrangement of genes in somatic cells would not affect the inherited genome of a future zygote. Although segments of the genome are rearranged and lost during development of lymphocytes from hematopoietic precursors, most somatic cells seem to have an intact genome, equivalent to that in the germ line. Evidence that at least some somatic cells have a complete and functional genome comes from the successful production of cloned animals by nuclear-transfer cloning. In this procedure, the nucleus of an adult (somatic) cell is introduced into an egg that lacks its nucleus; the manipulated egg, which contains the diploid number of chromosomes and is equivalent to a zygote, then is implanted into a foster mother. The only source of genetic information to guide development of the embryo is the nuclear genome of the donor somatic cell. The frequent failure of such cloning experiments, however, raises

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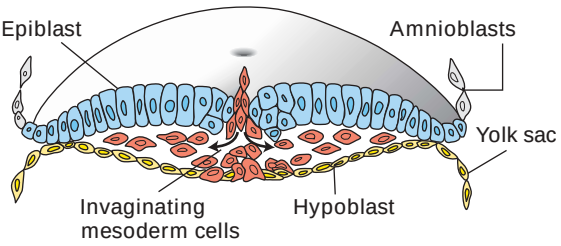

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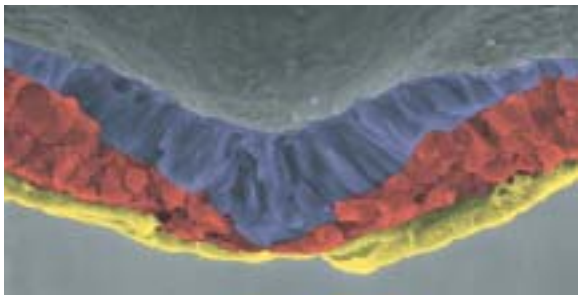


(c)

ECTODERM	MESODERM	ENDODERM
Central nervous system	Skull	Stomach
Retina and lens	Head, skeletal muscle	Colon
Cranial and sensory	Skeleton	Liver
Ganglia and nerves	Dermis of skin	Pancreas
Pigment cells	Connective tissue	Urinary bladder
Head connective tissue	Urogenital system	Epithelial parts of
Epidermis	Heart	trachea
Hair	Blood, lymph cells	lungs
Mammary glands	Spleen	pharynx
		thyroid
		intestine

▲ FIGURE 22-7 **Fates of the germ layers in animals.** During the early period of mammalian development, cells migrate to create the three germ layers: ectoderm, mesoderm, and endoderm. This process is called gastrulation. Cells in the different layers have largely distinct fates and therefore represent distinct cell lineages. The detailed cell lineage, however, is not exactly the same in different individuals. (a) Shown here is a sketch of a human embryo about 16 days after fertilization of an egg. The first cells to move from the epiblast into the interior form endoderm, and they are followed by invaginating cells that become mesoderm. The remaining epiblast cells become ectoderm. Hypoblast = covering layer. Amnioblasts = cells that will line amniotic cavity. Yolk sac = reminder of our egg-bound ancestors. (b) Scanning electron micrograph of a cross section of a similar-stage embryo. (c) Some of the tissue derivatives of the three germ layers are listed. [Part (a) after T. W. Sadler, 2000, *Langman's Medical Embryology,* 8th ed. (Baltimore: Lippincott Williams and Wilkins), pp. 64–65, Figure 4.3). Part (b) courtesy of Kathy Sulik, University of North Carolina–Chapel Hill.]

questions about how many adult somatic cells do in fact have a complete functional genome. Even the successes, like the famous cloned sheep "Dolly," appear to have some medical problems. Even if differentiated cells have a physically complete genome, clearly only portions of it are transcriptionally active (Chapter 10). Whether the genome of a differentiated cell can revert to having the full developmental potential characteristic of an embryonic cell is a matter of considerable debate. A cell could, for example, have an intact genome, but be unable to properly reactivate it due to inherited chromatin states.

These observations raise two important questions: How are cell fates progressively restricted during development? Are these restrictions irreversible? In addressing these questions, it is important to remember that what a cell in its normal in vivo location will do may differ from what a cell is capable of doing if it is manipulated experimentally. Thus the observed limits to what a cell can do may result from natural regulatory mechanisms or may reflect a failure to find conditions that reveal the cell's full potential.

Although our focus in this chapter is on how cells become different, their ability to remain the same also is critical to the functioning of tissues and the whole organism. Nondividing differentiated cells with particular characteristics must retain them, sometimes for many decades. Stem cells that divide regularly, such as a skin stem cell, must produce one daughter cell with the properties of the parental cell, retaining its characteristic composition, shape, behavior, and responses to specific external signals. Meanwhile, the other daughter cell with its own distinct inheritance, as the result of asymmetric cell division, embarks on a particular differentiation pathway, which may be determined both by the signals the cell receives and by intrinsic bias in the cell's potential, such as the previous activation of certain genes.

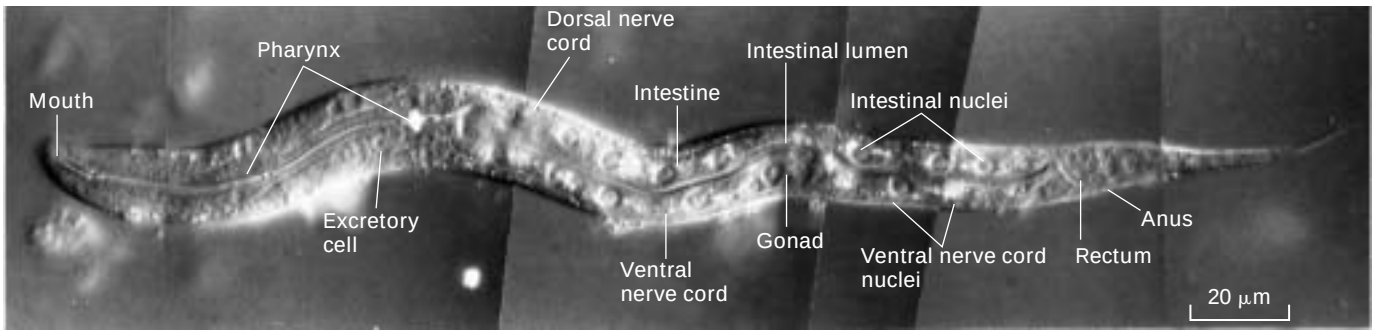
The Complete Cell Lineage of *C. elegans* Is Known

In the development of some organisms, cell lineages are under tight genetic control and thus are identical in all individuals of a species. In other organisms the exact number and arrangement of cells vary substantially among different individuals. The best-documented example of a reproducible pattern of cell divisions comes from the nematode *C. elegans*. Scientists have traced the lineage of all the somatic cells in *C. elegans* from the fertilized egg to the mature worm by following the development of live worms using Nomarski interference microscopy (Figure 22-8).

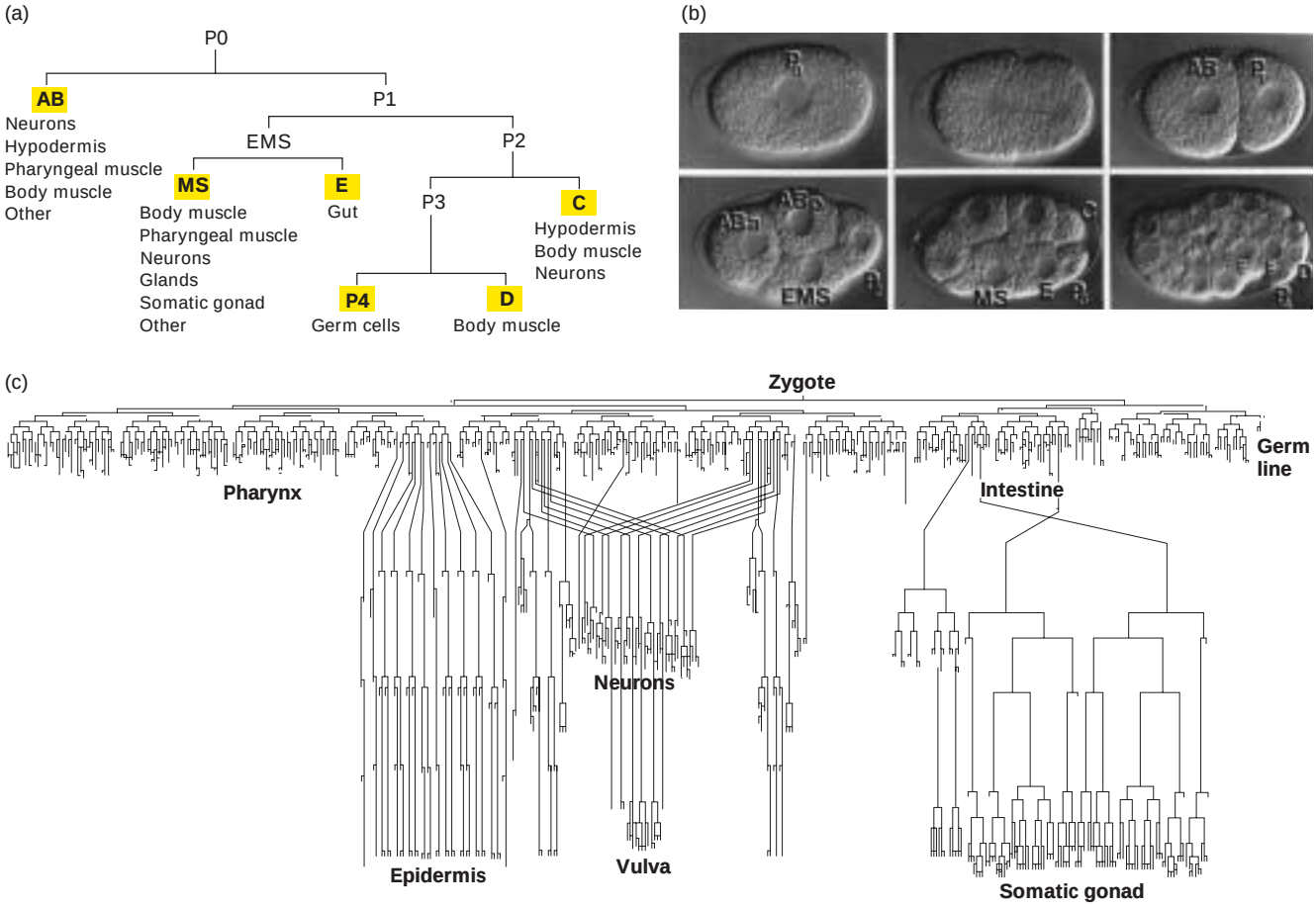
About 10 rounds of cell division, or fewer, create the adult worm, which is about 1 mm long and 70 μm in diameter. The adult worm has 959 somatic cell nuclei (hermaphrodite form) or 1031 (male). The number of somatic cells is somewhat fewer than the number of nuclei because some cells contain multiple nuclei (i.e., they are syncytia). Remarkably, the pattern of cell divisions starting from a *C. elegans* fertilized egg is nearly always the same. As we discuss later in the chapter, many cells that are generated during development undergo programmed cell death and are missing in the adult worm. The consistency of the *C. elegans* cell lineage does not result entirely from each newly born cell inheriting specific information about its destiny. That is, at their birth cells are not necessarily "hard wired" by their own internal inherited instructions to follow a particular path of differentiation. In some cases, various signals direct initially identical cells to different fates, and the outcomes of these signals are consistent from one animal to the next.

The first few cell divisions in *C. elegans* produce six different *founder cells*, each with a separate fate as shown in Figure 22-9a, b. The initial division is asymmetric, giving rise to P1 and the AB founder cell. Further divisions in the P lineage form the other five founder cells. Some of the signals controlling division and fate asymmetry are known. For example, Wnt signals from the P2 precursor control the asymmetric division of the EMS cell into E and MS founder cells. Wnt signaling (see Figure 15-32) is also used in other asymmetric divisions in worms.

The bilateral symmetry of the worm implies duplication of lineages on the two sides; curiously, though, functionally equivalent cells on each side can arise from a pattern of division that is different on the two sides. Some of the embryonic cells function as stem cells, dividing repeatedly to form more of themselves or another type of stem cell, while



▲ FIGURE 22-8 **Newly hatched larva of *C. elegans*.** Many of the 959 somatic cell nuclei in this hermaphrodite form are visualized in this micrograph obtained by Nomarski interference microscopy. [From J. E. Sulston and H. R. Horvitz, 1977, *Devel. Biol.* **56**:110.]



▲ FIGURE 22-9 ***C. elegans* lineage.** (a) Pattern of the first few divisions starting with P0 (the zygote) and leading to formation of the six founder cells (yellow highlights). The first division, into P1 and AB, is asymmetric. Further divisions in the P lineage generates the other founder cells. Note that more than one lineage can lead to the same tissue type (e.g., muscle or neurons). The EMS cell is so named because it is the precursor to most of the endoderm and mesoderm. The lineage beginning with the P4 cell gives rise to all of the germ-line cells, which are set aside very early, as in most animals. All the other lineages give rise to somatic cells. (b) Light micrographs of the first few divisions of the embryo that generate the founder cells with cells labeled as in part (a). The texture of the cells shows the presence of organelles and is revealed by differential interference contrast microscopy, sometimes called Nomarski microscopy. (c) Full lineage of the entire body of the worm, showing some of the tissues formed. Note that any particular cell undergoes relatively few divisions, typically fewer than 15. [Part (b) from Einhard Schierenberg Zoologisches Institut, Universität Köln.]

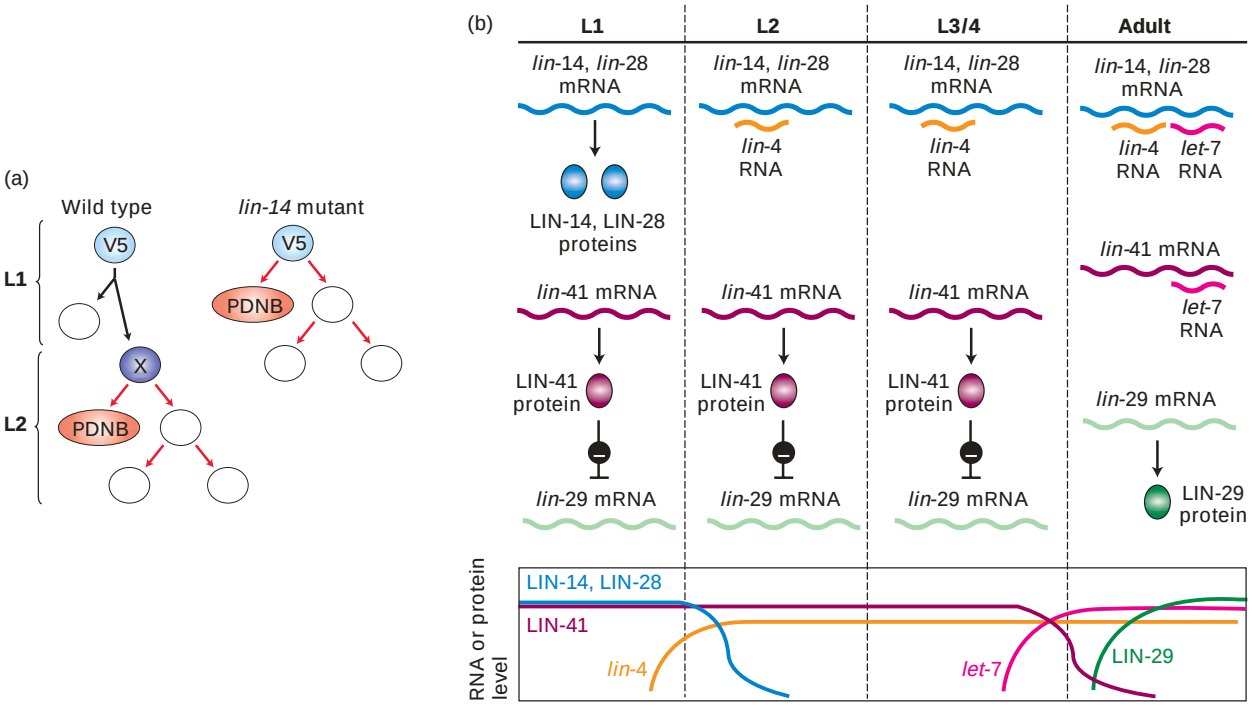
MEDIA CONNECTIONS
Video: Time-Lapse Imaging of *C. elegans* Embryogenesis

spinning off differentiating cells that give rise to a particular tissue. In one pattern of division, one of the daughter cells from a division initiates a repeat of the lineage pattern of the previous few divisions. The complete lineage of *C. elegans* is shown in Figure 22-9c. This organism has been a powerful model system for genetic studies to identify the regulators that control cell lineages in time and space.

Heterochronic Mutants Provide Clues About Control of Cell Lineage

Intriguing evidence for the genetic control of cell lineage has come from isolation and analysis of *heterochronic mutants*. In these mutants, a developmental event typical of one stage of development occurs too early (precocious development) or too late (retarded development). An example of the former is premature occurrence of a cell division that yields a cell that differentiates and a cell that dies; as a result, the lineage that should have followed from the dead cell never happens. In the latter case, the delayed occurrence of a lineage causes juvenile structures to be produced, incorrectly, in more mature animals. In both cases, the character of a parental cell is, in essence, changed to the character of a cell at a different stage of development.

One example of precocious development in *C. elegans* comes from loss-of-function mutations in the *lin-14* gene, which cause premature formation of the PDNB neuroblast (Figure 22-10a). The *lin-14* gene and several others found to



▲ FIGURE 22-10 **Timing of specific types of cell division during development of *C. elegans*.** (a) The pattern of cell division for the V5 cell of *C. elegans* is shown for normal worms and for a heterochronic mutant called *lin-14.* In the *lin-14* mutant, the pattern of cell division (red arrows) that normally occurs only in the second larval stage (L2) occurs in the first larval stage (L1), causing the PDNB neuroblast to be generated prematurely. In the mutant, the V5 cell behaves during L1 like cell "X" (purple) normally does in L2. The inference is that the LIN-14 protein prevents L2-type cell divisions, although precisely how it does so is unknown. (b) Two small regulatory RNAs, *lin-4* and *let-7,* serve as coordinating timers of gene expression. Binding of the *lin-4* RNA to the 3′ untranslated regions (UTRs) of *lin-14* and *lin-28* mRNAs prevents translation of these mRNAs into protein. This occurs following the first larval (L1) stage, permitting development to proceed to the later larval stages. Starting in the fourth larval stage (L4), production of *let-7* RNA begins. It hybridizes to *lin-14, lin-28*, and *lin-41* mRNAs, preventing their translation. LIN-41 protein is an inhibitor of translation of the *lin-29* mRNA, so the appearance of *let-7* RNA allows production of LIN-29 protein, which is needed for generation of adult cell lineages. LIN-4 may also bind to *lin-41* RNA at later stages. Only the 3′ UTRs of the mRNAs are depicted. [Adapted from B. J. Reinhart et al., 2000, *Nature* **403**:901]

be defective in heterochronic worm mutants encode RNA-binding or DNA-binding proteins, which presumably coordinate expression of other genes. However, two other genes (*lin-4* and *let-7*) involved in regulating the timing of cell divisions were initially extremely puzzling, as they appeared to encode small RNAs that do not encode any protein. To discover the products of these genes, scientists first determined which pieces of genomic DNA could restore gene function, and therefore proper cell lineage, to mutants defective in each gene. They then did the same thing with genomic DNA from the corresponding genomic regions of different species of worm. Comparison of the "rescuing" fragments from the different species revealed that they shared common short sequences with little protein-coding potential.

The short RNA molecules encoded by *lin-4* and *let-7* were subsequently shown to inhibit translation of the mRNAs encoded by *lin-14* and other heterochronic genes (Figure 22-10b). These small RNAs, or **micro RNAs (miRNAs),** are complementary to sequences in the 3′ untranslated parts of target mRNAs and are believed to control translation of the mRNAs by hybridizing them. Temporal changes in the production of these and other miRNAs during the life cycle of *C. elegans* serve as a regulatory clock for cell lineage. Molecules related to *let-7* RNA have been identified in many other animals including vertebrates and insects; since their production is temporally regulated in these animals as well, they may serve a similar function in their development as in *C. elegans*. How production of these regulatory miRNAs is temporally controlled is not yet known, but they have turned out to play many roles in regulating gene expression (Chapter 12).

KEY CONCEPTS OF SECTION 22.1

The Birth of Cells

■ In asymmetric cell division, two different types of daughter cells are formed from one mother cell. In contrast, both daughter cells formed in symmetric divisions are identical but may have different fates if they are exposed to different external signals (see Figure 22-1).

- Pluripotent stem cells can produce more than one type of descendant cell, including in some cases a stem cell with a more-restricted potential to produce differentiated cell types (see Figure 22-2).
- Cultured embryonic stem cells (ES cells) are capable of giving rise to many kinds of differentiated cell types. They are useful in production of genetically altered mice and offer potential for therapeutic uses.
- Populations of stem cells associated with most tissues (e.g., skin, intestinal epithelium, blood) regenerate differentiated tissue cells that are damaged or sloughed or become aged (see Figures 22-4 and 22-5).
- Stem cells are prevented from differentiating by specific controls. A high level of β-catenin, a component of the Wnt signaling pathway, has been implicated in preserving stem cells in the skin and intestine by directing cells toward division rather than differentiation states.
- Plant stem cells persist for the life of the plant in the meristem. Meristem cells can give rise to a broad spectrum of cell types and structures.
- During development, precursor cells generally lose potential; that is, they become progressively restricted in the number of different cell types they can form.
- Early in animal development, the three germ layers—ectoderm, mesoderm, and endoderm—form. Each gives rise to specific tissues and organs (see Figure 22-7).
- Germ-line cells give rise to eggs or sperm. By definition, all other cells are somatic cells.
- Embryonic development of *C. elegans* begins with asymmetric division of the fertilized egg (zygote). The lineage of all the cells in adult worms is known and is highly reproducible (see Figure 22-9).
- Short regulatory RNAs control the timing of developmental cell divisions by preventing translation of mRNAs whose encoded proteins control cell lineages (see Figure 22-10).

22.2 Cell-Type Specification in Yeast

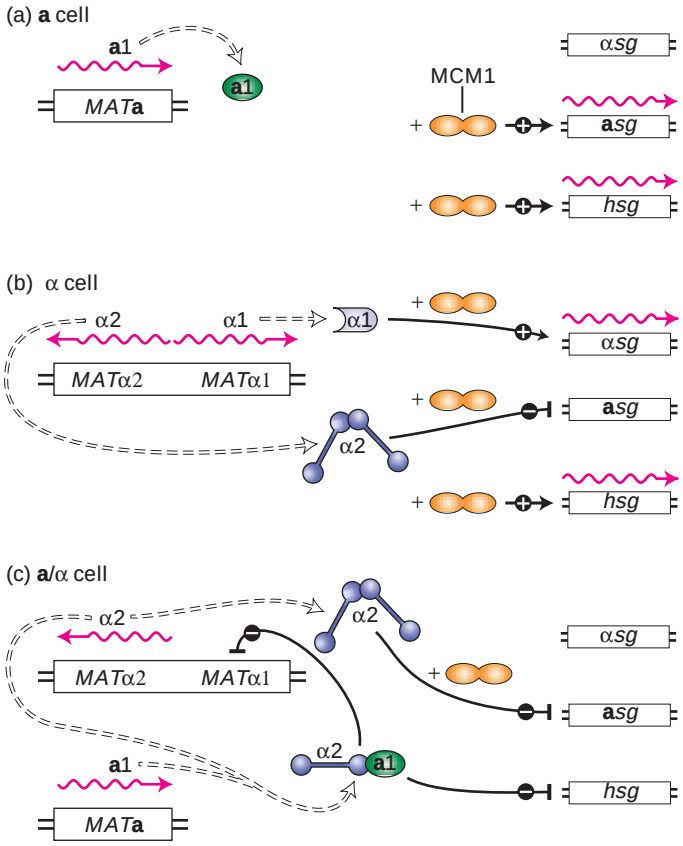
In the previous section, we saw that stem cells and precursor/progenitor cells produce progeny that embark upon specific differentiation paths. The elegant regulatory mechanisms whereby cells become different is referred to as *cell-type specification*. Specification usually involves a combination of external signals with internal signal-transduction mechanisms like those described in Chapters 14 and 15. The transition from an undifferentiated cell to a differentiating one often involves the production of one or a small number of **transcription factors.** The newly produced transcription factors are powerful switches that trigger the activation (and sometimes repression) of large batteries of subservient genes. Thus an initially modest change can cause massive changes in gene expression that confer a new character on the cell.

Our first example of cell-type specification comes from the budding yeast, *S. cerevisiae*. We introduced this useful unicellular eukaryote way back in Chapter 1 and have encountered it in several other chapters. *S. cerevisiae* forms three cell types: haploid **a** and α cells and diploid **a**/α cells. Each type has its own distinctive set of active genes; many other genes are active in all three cell types. In a pattern common to many organisms and tissues, cell-type specification in yeast is controlled by a small number of transcription factors that coordinate the activities of many other genes. Similar regulatory features are found in the responses of higher eukaryotic cells to environmental signals and in the specification and patterning of cells and tissues during development.

Recent **DNA microarray** studies of transcription patterns of ~ 6000 genes in *S. cerevisiae* have provided a genome-wide picture of the fluctuations of gene expression in the different cell types and different stages of the yeast life cycle (see Figure 9-35 for an explanation of the DNA microarray technique). A powerful advantage of the microarray approach is that it systematically identifies a large fraction of the relevant genes controlling various processes, allowing scientists to concentrate on the most important players that regulate differences in cell types. Among other things, the yeast studies identified 32 genes that are transcribed at more than twofold higher levels in α cells than in **a** cells. Another 50 genes are transcribed at more than twofold higher levels in **a** cells than in α cells. The products of these 82 genes, which initially are activated by cell-type specification transcription regulators, convey many of the critical differences between the two cell types. The results clearly demonstrate that changes in expression of only a small fraction of the genome can significantly alter the behavior and properties of cells. Transcription of a much larger number of genes, about 25 percent of the total assayed, differed substantially in diploid cells compared with haploid cells. **a** and α cells are very similar, and haploid and diploid cells are quite different, so the array results make sense.

Transcription Factors Encoded at the *MAT* Locus Act in Concert with MCM1 to Specify Cell Type

Each of the three *S. cerevisiae* cell types expresses a unique set of regulatory genes that is responsible for all the differences among the three cell types. All haploid cells express certain haploid-specific genes; in addition, **a** cells express **a**-specific genes, and α cells express α-specific genes. In **a**/α diploid cells, diploid-specific genes are expressed, whereas haploid-specific, **a**-specific, and α-specific genes are not. As illustrated in Figure 22-11, three cell type–specific transcription factors (α1, α2, and **a**1) encoded at the *MAT locus* in combination with a general transcription factor called *MCM1*, which is expressed in all three cell types, mediate cell type–specific gene expression in *S. cerevisiae*. Thus the actions of just three transcription factors can set the yeast cell on a specific differentiation pathway culminating in a particular cell type. From the DNA microarray experiments we know one effect of these key players: the activation or repression of many dozens of genes that control cell characteristics.



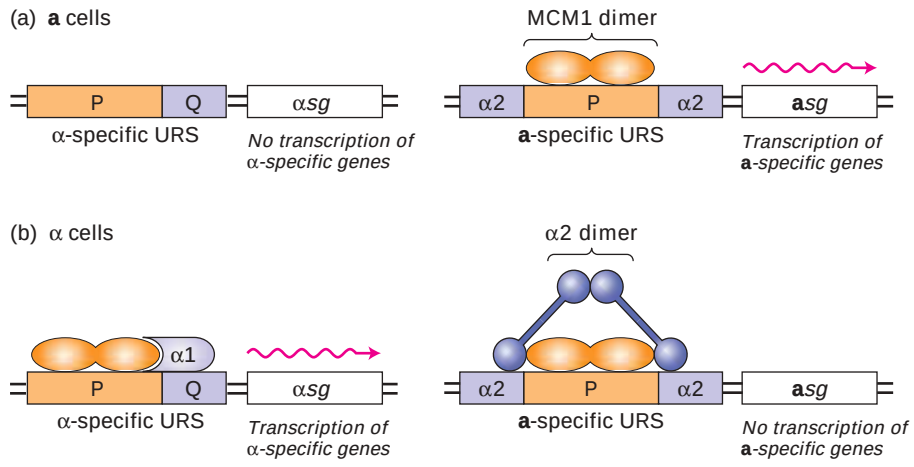
▲ FIGURE 22-11 **Transcriptional control of cell type–specific genes in *S. cerevisiae*.** The coding sequences carried at the *MAT* locus differ in haploid α and **a** cells and in diploid cells. Three type-specific transcription factors (α1, α2, and **a**1) encoded at the *MAT* locus act with MCM1, a constitutive transcription factor produced by all three cell types, to produce a distinctive pattern of gene expression in each of the three cell types. **a***sg* = **a**-specific genes/mRNAs; α*sg* = α-specific genes/mRNAs; *hsg* = haploid-specific genes/mRNAs.

MCM1 was the first member of the *MADS family* of transcription factors to be discovered. (MADS is an acronym for the initial four factors identified in this family.) The DNA-binding proteins composing this family dimerize and contain a similar N-terminal MADS domain. In Section 22.3 we will encounter other MADS transcription factors that participate in development of skeletal muscle. MADS transcription factors also specify cell types in floral organs (see Figure 15-28). MCM1 exhibits different activity in haploid **a** and α cells due to its association with α1 or α2 protein in α cells. Acting alone, MCM1 activates transcription of **a**-specific genes in **a** cells and of haploid-specific genes in both α and **a** cells (see Figure 22-11a, b). As a result of this combinatorial action, MCM1 promotes transcription of α-specific genes and represses transcription of **a**-specific genes in α cells. Now let's take a closer look at how MCM1 and the *MAT*-encoded proteins exert their effects.

MCM1 and α1-MCM1 Complexes Activate Gene Transcription

In **a** cells, homodimeric MCM1 binds to the so-called P box sequence in the upstream regulatory sequences (URSs) of **a**-specific genes, stimulating their transcription (Figure 22-12a). Transcription of α-specific genes is controlled by two adjacent sequences—the P box and the Q box—located in the URSs associated with these genes. Although MCM1 alone binds to the P box in **a**-specific URSs, it does not bind to the P box in α-specific URSs. Thus **a** cells do not transcribe α-specific genes.

In α cells, which produce the α1 transcription factor encoded by *MAT*α, the simultaneous binding of MCM1 and α1 to PQ sites occurs with high affinity (Figure 22-12b). This binding turns on transcription of α-specific genes. Therefore **a**-specific transcription is a simple matter of a single transcription factor binding to its target genes, while α-specific



◀ FIGURE 22-12 **Activity of MCM1 in a and α yeast cells.** MCM1 binds as a dimer to the P site in α-specific and **a**-specific upstream regulatory sequences (URSs), which control transcription of α-specific genes and **a**-specific genes, respectively. (a) In **a** cells, MCM1 stimulates transcription of **a**-specific genes. MCM1 does not bind efficiently to the P site in α-specific URSs in the absence of α1 protein. (b) In α cells, the activity of MCM1 is modified by its association with α1 or α2. The α1-MCM1 complex stimulates transcription of α-specific genes, whereas the α2-MCM1 complex blocks transcription of **a**-specific genes. The α2-MCM1 complex also is produced in diploid cells, where it has the same blocking effect on transcription of **a**-specific genes (see Figure 22-11c).

transcription requires a combination of two factors—neither of which can activate target genes alone.

α2-MCM1 and α2-a1 Complexes Repress Transcription

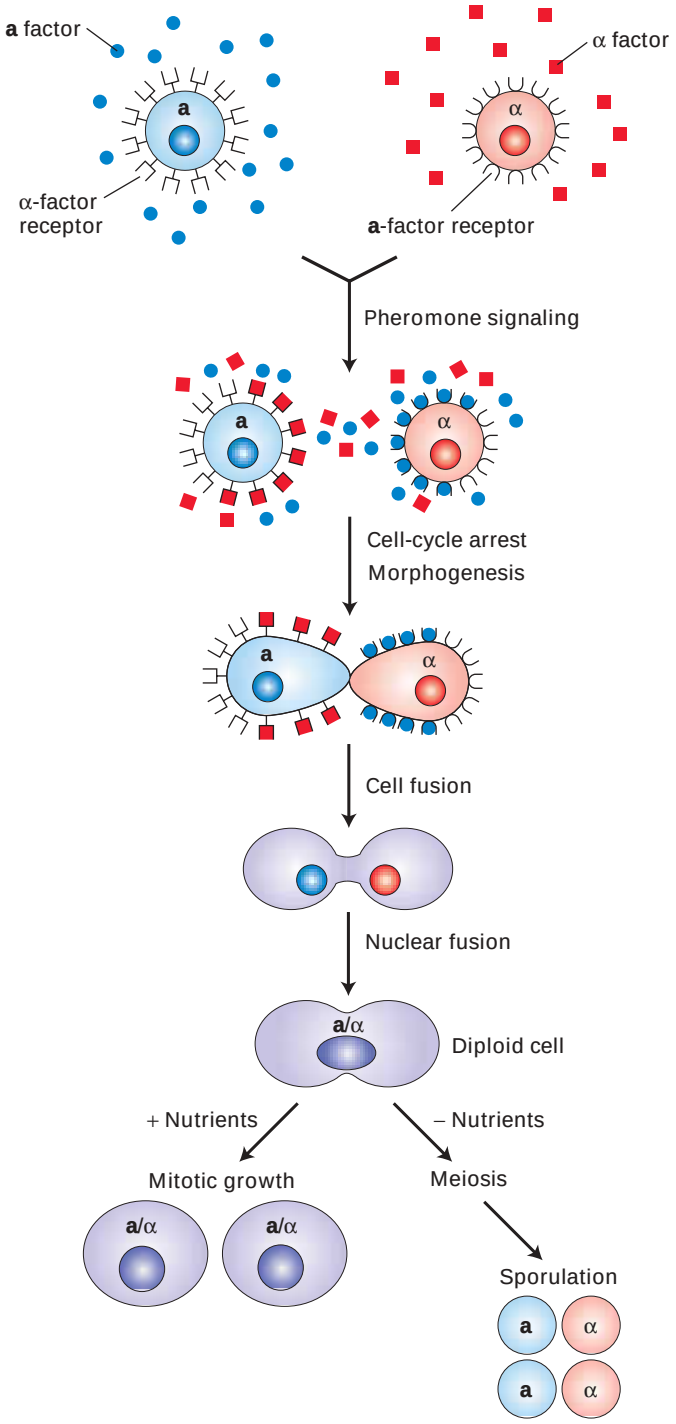
Highly specific binding occurs as a consequence of the interaction of α2 with other transcription factors at different sites in DNA. Flanking the P box in each **a**-specific URS are two α2-binding sites. Both MCM1 and α2 can bind independently to an **a**-specific URS with relatively low affinity. However, in α cells highly cooperative, simultaneous binding of both α2 and MCM1 proteins to these sites occurs with high affinity. This high-affinity binding represses transcription of **a**-specific genes, ensuring that they are not expressed in α cells and diploid cells (see Figure 22-12b, *right*). MCM1 promotes binding of α2 to an **a**-specific URS by orienting the two DNA-binding domains of the α2 dimer to the α2-binding sequences in this URS. Since a dimeric α2 molecule binds to both sites in an α-specific URS, each DNA site is referred to as a half-site. The relative positions of both half-sites and their orientation are highly conserved among different **a**-specific URSs.

Combinations of transcription factors create additional specificity in gene regulation. The presence of numerous α2-binding sites in the genome and the "relaxed" specificity of α2 protein may expand the range of genes that it can regulate. For instance, in **a**/α diploid cells, α2 forms a heterodimer with **a**1 that represses both haploid-specific genes and the gene encoding α1 (see Figure 22-11c). The example of α2 suggests that relaxed specificity may be a general strategy for increasing the regulatory range of a single transcription factor.

Pheromones Induce Mating of α and a Cells to Generate a Third Cell Type

An important feature of the yeast life cycle is the ability of haploid **a** and α cells to mate, that is, attach and fuse giving rise to a diploid **a**/α cell (see Figure 1-5). Each haploid cell type secretes a different *mating factor,* a small polypeptide **pheromone,** and expresses a cell-surface G protein–coupled receptor that recognizes the pheromone secreted by cells of the other type. Thus **a** and α cells both secrete and respond to pheromones (Figure 22-13). Binding of the mating factors to their receptors induces expression of a set of genes encoding proteins that direct arrest of the cell cycle in G_1 and promote attachment/fusion of haploid cells to form diploid cells. In the presence of sufficient nutrients, the diploid cells will continue to grow. Starvation, however, induces diploid cells to progress through meiosis, each yielding four haploid spores. If the environmental conditions become conducive to vegetative growth, the spores will germinate and undergo mitotic division.

Studies with yeast mutants have provided insights into how the **a** and α pheromones induce mating. For instance, haploid yeast cells carrying mutations in the *sterile 12 (STE12)* locus cannot respond to pheromones and do not



▲ FIGURE 22-13 **Pheromone-induced mating of haploid yeast cells.** The α cells produce α mating factor and **a** receptor; the **a** cells produce **a** factor and α receptor. Binding of the mating factors to their cognate receptors on cells of the opposite type leads to gene activation, resulting in mating and production of diploid cells. In the presence of sufficient nutrients, these cells will grow as diploids. Without sufficient nutrients, cells will undergo meiosis and form four haploid spores.

mate. The *STE12* gene encodes a transcription factor that binds to a DNA sequence referred to as the pheromone-responsive element, which is present in many different **a**- and α-specific URSs. Binding of mating factors to cell-surface receptors induces a cascade of signaling events, resulting in phosphorylation of various proteins including the Ste12 protein (see Figure 14-24). This rapid phosphorylation is correlated with an increase in the ability of Ste12 to stimulate transcription. It is not yet known, however, whether Ste12 must be phosphorylated to stimulate transcription in response to pheromone.

Interaction of Ste12 protein with DNA has been studied most extensively at the URS controlling transcription of *STE2*, an **a**-specific gene encoding the receptor for the α pheromone. Pheromone-induced production of the α receptor encoded by *STE2* increases the efficiency of the mating process. Adjacent to the **a**-specific URS in the *STE2* gene is a pheromone-responsive element that binds Ste12. When **a** cells are treated with α pheromone, transcription of the *STE2* gene increases in a process that requires Ste12 protein. Ste12 protein has been found to bind most efficiently to the pheromone-responsive element in the *STE2* URS when MCM1 is simultaneously bound to the adjacent P site. We saw previously that MCM1 can act as an activator or a repressor at different URSs depending on whether it complexes with α1 or α2. In this case, the function of MCM1 as an activator is stimulated by the binding of yet another transcription factor, Ste12, whose activity is modified by extracellular signals.

KEY CONCEPTS OF SECTION 22.2

Cell-Type Specification in Yeast

- Specification of each of the three yeast cell types—the **a** and α haploid cells and the diploid **a**/α cells—is determined by a unique set of transcription factors acting in different combinations at specific regulatory sites in the yeast genome (see Figure 22-11).
- Some transcription factors can act as repressors or activators depending on the specific regulatory sites they bind and the presence or absence of other transcription factors bound to neighboring sites.
- Binding of mating-type pheromones by haploid yeast cells activates expression of genes encoding proteins that mediate mating, thereby generating the third yeast cell type (see Figure 22-13).

22.3 Specification and Differentiation of Muscle

As indicated by global expression patterns, yeast cells of different mating types are still rather similar. Developmental biologists do not yet know the complete set of molecules that distinguishes any one cell type (e.g., muscle) from all the other cell types in a multicellular organism. The extensive cell specification and differentiation that occur during development of animals and plants depend on both quantitative and qualitative differences in gene expression, controlled at the level of transcription, as well as on cell structures and protein activity states.

An impressive array of molecular strategies, some analogous to those found in yeast cell-type specification, have evolved to carry out the complex developmental pathways that characterize multicellular organisms. Muscle cells have been the focus of many such studies because their development can be studied in cultured cells as well as in intact animals. Early advances in understanding the formation of muscle cells (myogenesis) came from discovery of regulatory genes that could convert cultured cells into muscle cells. Then mouse mutations affecting those genes were created and studied to learn the functions of the proteins encoded by these genes, following which scientists have investigated how the muscle regulatory genes control other genes.

Twist protein is a transcription factor necessary to create muscle cells in flies and other animals. Twist turns on production of other transcription factors that in turn activate genes encoding myosin, actin, and other muscle-specific proteins. DNA microarray analysis has been applied to understand the function of *twist* and other regulatory genes in muscle development. For example, the expression pattern of normal *Drosophila* embryos recently has been compared with that of mutant embryos in which the *twist* gene is defective. To assess how many genes are needed to specify muscle, researchers determined the expression of about 4000 fly genes (about 30 percent of the total) in normal fly embryos and in *twist* mutants. Of the genes included in the microarray, about 130 (3.3 percent), including many known muscle differentiation genes, were transcribed at lower levels (or not at all) in the *twist* mutants. These results suggest that transcriptional changes in at least several hundred genes are associated with differentiation of a highly specialized cell type such as muscle.

Other recent microarray studies have looked for genes whose transcription differs in various subtypes of muscle in mice. These have identified 49 genes out of 3000 genes examined that are transcribed at substantially different levels in red (endurance) muscle and white (fast response) muscle. Clues to the molecular basis of the functional differences between red and white muscle are likely to come from studying those 49 genes and their products.

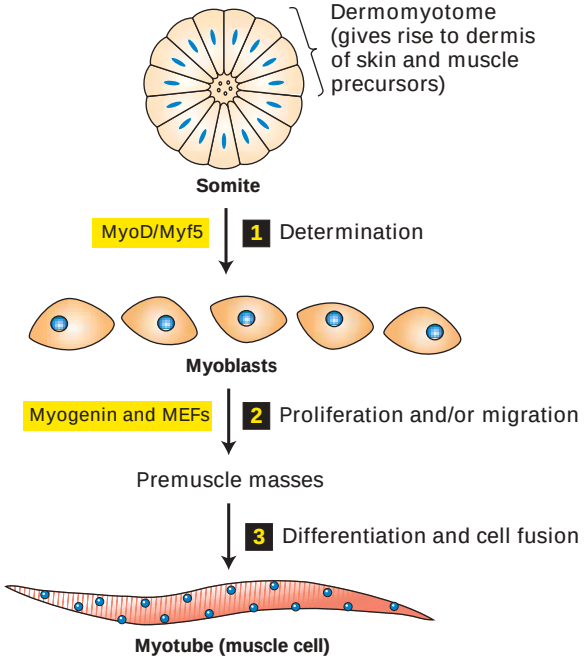
Here we examine the role of certain transcription factors in creating skeletal muscle in vertebrates. These muscle regulators illustrate how coordinated transcription of sets of target genes can produce differentiated cell types and how a cascade of transcriptional events and signals is necessary to coordinate cell behaviors and functions.

Embryonic Somites Give Rise to Myoblasts, the Precursors of Skeletal Muscle Cells

Vertebrate skeletal myogenesis proceeds through three stages: **determination** of the precursor muscle cells, called *myoblasts;* proliferation and in some cases migration of myoblasts; and their terminal differentiation into mature muscle (Figure 22-14). In the first stage, myoblasts arise from blocks of mesoderm cells, called *somites,* that are located next to the neural tube in the embryo. Specific signals from surrounding tissue play an important role in determining where myoblasts will form in the developing somite. At the molecular level, the decision of a mesoderm cell to adopt a muscle cell fate reflects the activation of genes encoding particular transcription factors.

As myoblasts proliferate and migrate, say, to a developing limb bud, they become aligned, stop dividing, and fuse to form a **syncytium** (a cell containing many nuclei but sharing a common cytoplasm). We refer to this multinucleate cell as a *myotube.* Concomitant with cell fusion is a dramatic rise in the expression of genes necessary for further muscle development and function.

The specific extracellular signals that induce determination of each group of myoblasts are expressed only transiently. These signals trigger production of intracellular factors that maintain the myogenic program after the inducing signals are gone. We discuss the identification and functions of these myogenic proteins, and their interactions, in the next several sections.



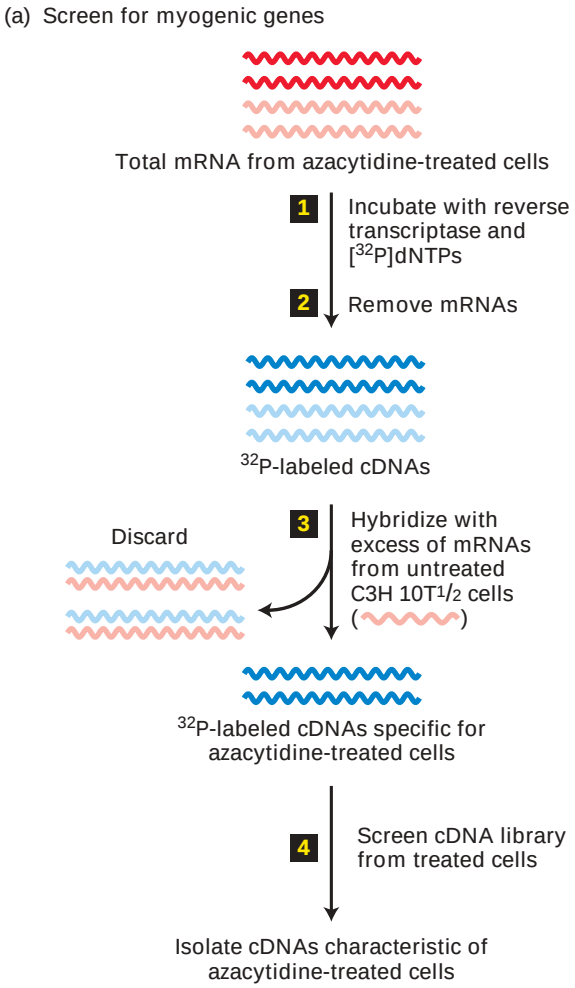
▲ FIGURE 22-14 **Three stages in development of vertebrate skeletal muscle.** Somites are epithelial spheres of embryonic mesoderm cells, some of which (the myotome) become determined as myoblasts after receiving signals from other tissues (1). After the myoblasts proliferate and migrate to the limb buds and elsewhere (2), they undergo terminal differentiation into multinucleate skeletal muscle cells, called myotubes (3). Key transcription factors that help drive the myogenic program are highlighted in yellow. See also Figure 22-17.

Myogenic Genes Were First Identified in Studies with Cultured Fibroblasts

Myogenic genes are a fine example of how transcription factors control the progressive differentiation that occurs in a cell lineage. In vitro studies with the fibroblast cell line designated C3H 10T½ have played a central role in dissecting the transcription control mechanisms regulating skeletal myogenesis. When these cells are incubated in the presence of 5-azacytidine, a cytidine derivative that cannot be methylated and therefore alters transcription, they differentiate into myotubes. Upon entry into cells, 5-azacytidine is converted to 5-azadeoxycytidine triphosphate and then is incorporated into DNA in place of deoxycytidine. Because methylated deoxycytidine residues commonly are present in transcriptionally inactive DNA regions, replacement of cytidine residues with a derivative that cannot be methylated may permit activation of genes previously repressed by methylation.

The high frequency at which azacytidine-treated C3H 10T½ cells are converted into myotubes suggested to early workers that reactivation of one or a small number of closely linked genes is sufficient to drive a myogenic program. To test

▶ EXPERIMENTAL FIGURE 22-15 **Myogenic genes isolated from azacytidine-treated cells can drive myogenesis when transfected into other cells.** (a) When C3H 10T½ cells (a fibroblast cell line) are treated with azacytidine, they develop into myotubes at high frequency. To isolate the genes responsible for converting azacytidine-treated cells into myotubes, all the mRNAs from treated cells first were isolated from cell extracts on an oligo-dT column. Because of their poly(A) tails, mRNAs are selectively retained on this column. Steps 1 and 2: The isolated mRNAs were converted to radiolabeled cDNAs. Step 3: When the cDNAs were mixed with mRNAs from *untreated* C3H 10T½ cells, only cDNAs derived from mRNAs (light red) produced by both azacytidine-treated cells and untreated cells hybridized. The resulting double-stranded DNA was separated from the unhybridized cDNAs (dark blue) produced only by azacytidine-treated cells. Step 4: The cDNAs specific for azacytidine-treated cells then were used as probes to screen a cDNA library from azacytidine-treated cells (Chapter 9). At least some of the clones identified with these probes correspond to genes required for myogenesis. (b) Each of the cDNA clones identified in part (a) was incorporated into a plasmid carrying a strong promoter. Steps 1 and 2: C3H 10T½ cells were cotransfected with each recombinant plasmid plus a second plasmid carrying a gene conferring resistance to an antibiotic called G418; only cells that have incorporated the plasmids will grow on a medium containing G418. One of the selected clones, designated *myoD,* was shown to drive conversion of C3H 10T½ cells into muscle cells, identified by their binding of labeled antibodies against myosin, a muscle-specific protein (step 3). [See R. L. Davis et al., 1987, *Cell* **51**:987.]

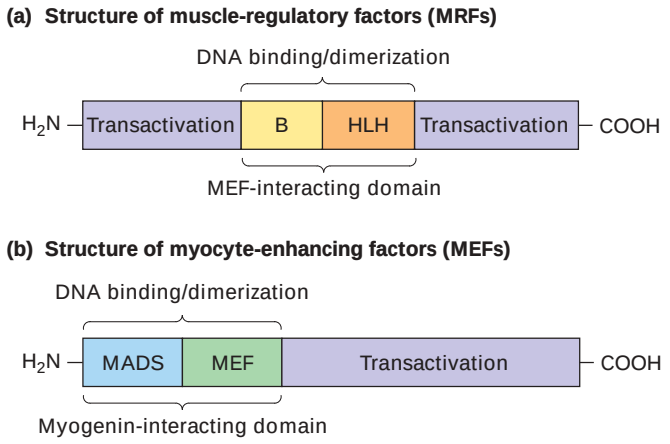


this hypothesis, researchers isolated DNA from C3H 10T½ cells grown in the presence of 5-azacytidine and transfected it into untreated cells. The observation that 1 in 10^4 cells transfected with this DNA was converted into a myotube is consistent with the hypothesis that one or a small set of closely linked genes is responsible for converting fibroblasts into myotubes.

Subsequent studies led to the isolation and characterization of four different but related genes that can convert C3H 10T½ cells into muscle. Figure 22-15 outlines the experimental protocol for identifying and assaying one of these genes, called the *myogenic determination (myoD)* gene. C3H 10T½ cells transfected with *myoD* cDNA and those treated with 5-azacytidine both formed myotubes. The *myoD* cDNA also was able to convert a number of other cultured cell lines into muscle. Based on these findings, the *myoD* gene was proposed to play a key role in muscle development. A similar approach identified three other genes—*myogenin*, *myf5*, and *mrf4*—that also function in muscle development.

Muscle-Regulatory Factors (MRFs) and Myocyte-Enhancing Factors (MEFs) Act in Concert to Confer Myogenic Specificity

The four myogenic proteins—MyoD, Myf5, myogenin, and MRF4—are all members of the **basic helix-loop-helix** (bHLH) family of DNA-binding transcription factors (see Figure 11-22b). Near the center of these proteins is a DNA-binding basic (B) region adjacent to the HLH domain, which mediates dimer formation. Flanking this central DNA-binding/dimerization region are two activation domains. We refer to the four myogenic bHLH proteins collectively as *muscle regulatory factors*, or *MRFs* (Figure 22-16a).



▲ FIGURE 22-16 **General structures of two classes of transcription factors that participate in myogenesis.** MRFs (muscle regulatory factors) are bHLH (basic helix-loop-helix) proteins produced only in developing muscle. MEFs (myocyte-enhancing factors), which are produced in several tissues in addition to developing muscle, belong to the MADS family. The myogenic activity of MRFs is enhanced by their interaction with MEFs.

bHLH proteins form homo- and heterodimers that bind to a 6-bp DNA site with the consensus sequence CANNTG (N = any nucleotide). Referred to as the E box, this sequence is present in many different locations within the genome (on a purely random basis the E box will be found every 256 nucleotides). Thus some mechanism(s) must ensure that MRFs specifically regulate muscle-specific genes and not other genes containing E boxes in their transcription control regions. One clue to how this myogenic specificity is achieved was the finding that the DNA-binding affinity of MyoD is tenfold greater when it binds as a heterodimer complexed with E2A, another bHLH protein, than when it binds as a homodimer. Moreover, in azacytidine-treated C3H 10T½ cells, MyoD is found as a heterodimer complexed with E2A, and both proteins are required for myogenesis in these cells. The DNA-binding domains of E2A and MyoD have similar but not identical amino acid sequences, and both proteins recognize E box sequences. The other MRFs also form heterodimers with E2A that have properties similar to MyoD-E2A complexes. This heterodimerization restricts activity of the myogenic transcription factors to genes with closely linked E boxes.

Since E2A is expressed in many tissues, the requirement for E2A is not sufficient to confer myogenic specificity. Subsequent studies suggested that specific amino acids in the bHLH domain of all the MRFs confer myogenic specificity by allowing MRF-E2A complexes to bind specifically to another family of DNA-binding proteins called *myocyte enhancing factors,* or *MEFs.* MEFs were considered excellent candidates for interaction with MRFs for two reasons. First, many muscle-specific genes contain recognition sites for both MEFs and MRFs. Second, although MEFs cannot induce myogenic conversion of azacytidine-treated C3H 10T½ cells by themselves, they enhance the ability of MRFs to do so. This enhancement requires physical interaction between a MEF and MRF-E2A heterodimer. MEFs belong to the MADS family of transcription factors and to contain a MEF domain, adjacent to the MADS domain, that mediates interaction with myogenin (Figure 22-16b). The synergistic action of the MEF homodimer and MRF-E2A heterodimer is thought to drive high-level expression of muscle-specific genes.

Knockout mice and *Drosophila* mutants have been used to explore the roles of MRF and MEF proteins in conferring myogenic specificity in intact animals, extending the work in cell culture. These experiments demonstrated the importance of three of the MRF proteins and of MEF proteins for distinct steps in muscle development (see Figure 22-14). The function of the fourth myogenic protein, Mrf4, is not entirely clear; it may be expressed later and help maintain differentiated muscle cells and by combinatorial control to ensure that only muscle-specific genes are activated.

Terminal Differentiation of Myoblasts Is Under Positive and Negative Control

Powerful developmental regulators like the MRFs cannot be allowed to run rampant. In fact, their actions are circumscribed at several levels. First, production of the muscle regulators is activated only in mesoderm cells, at the right place and time in the embryo, in response to spatial regulators of the sorts that are described in Chapter 15. Other proteins mediate additional mechanisms for assuring tight control over myogenesis: chromatin-remodeling proteins are needed to make target genes accessible to MRFs; inhibitory proteins can restrict when MRFs act; and antagonistic relations between cell-cycle regulators and differentiation factors like MRFs ensure that differentiating cells will not divide, and vice versa. All these factors control when and where muscles form.

Activating Chromatin-Remodeling Proteins MRF proteins control batteries of muscle-specific genes, but can do so only if chromatin factors allow access. Remodeling of chromatin, which usually is necessary for gene activation, is carried out by large protein complexes (e.g., Swi/Snf complex) that have ATPase and perhaps helicase activity. These complexes are thought to recruit histone acetylases that modify chromatin to make genes accessible to transcription factors (Chapter 11). The hypothesis that remodeling complexes help myogenic factors was tested using dominant-negative versions of the ATPase proteins that form the cores of these complexes. (Recall from Chapter 9 that a dominant-negative mutation produces a mutant phenotype even when a normal allele of the gene also is present.) When genes carrying these dominant-negative mutations were transfected into C3H 10T½ cells, the subsequent introduction of myogenic genes no longer converted the cells into myotubes. In addition, a muscle-specific gene that is normally activated did not exhibit its usual pattern of chromatin changes in the doubly transfected C3H 10T½ cells. These results indicate that transcription activation by myogenic proteins depends on a suitable chromatin structure in the regions of muscle-specific genes.

Inhibitory Proteins Screens for genes related to *myoD* led to identification of a related protein that retains the HLH dimerization region but lacks the basic DNA-binding region and hence is unable to bind to E box sequences in DNA. By binding to MyoD or E2A, this protein inhibits formation of MyoD-E2A heterodimers and hence their high-affinity binding to DNA. Accordingly, this protein is referred to as Id, for inhibitor of DNA binding. Id prevents cells that produce MyoD and E2A from activating transcription of the muscle-specific gene encoding creatine kinase. As a result, the cells remain in a proliferative growth state. When these cells are induced to differentiate into muscle (for instance, by the removal of serum, which contains the growth factors required for proliferative growth), the Id concentration falls. MyoD-E2A dimers now can form and bind to the regulatory regions of target genes, driving differentiation of C3H 10T½ cells into myoblast-like cells.

Recent work shows that histone acetylases and deacetylases are also crucial for regulating muscle-specific genes. As

explained in Chapter 11, acetylation of histones in chromatin is necessary to activate many genes; in contrast, histone deacetylases cause transcriptional repression (see Figure 11-32). MEF2 recruits histone acetylases such as p300/CBP, through another protein that serves as a mediator, thus activating transcription of target genes. Chromatin immunoprecipitation experiments with antibodies against acetylated histone H4 show that the acetylated histone level associated with MEF2-regulated genes is higher in differentiated myotubes than in myoblasts (see Figure 11-31). The role of histone deacetylases in muscle development was revealed in experiments in which scientists first introduced extra *myoD* genes into cultured C3H 10T½ cells to raise the level of MyoD. This resulted in increased activation of target genes and more rapid differentiation of the cells into myotubes. However, when genes encoding histone deacetylases also were introduced into the C3H 10T½ cells, the muscle-inducing effect of MyoD was blocked and the cells did not differentiate into myotubes.

The explanation for how histone deacetylases inhibit MyoD-induced muscle differentiation came from the surprising finding that MEF2 can bind, through its MADS domain, to a histone deacetylase. This interaction, which can prevent MEF2 function and muscle differentiation, is normally blocked during differentiation because the histone deacetylase is phosphorylated by a calcium/calmodulin-dependent protein kinase; the phosphorylated deacetylase then is moved from the nucleus to the cytoplasm. Taken together, these results indicate that activation of muscle genes by MyoD and MEF2 is in competition with inactivation of muscle genes by repressive chromatin structures and that nuclear versus cytoplasmic localization of chromatin factors is a key regulatory step.

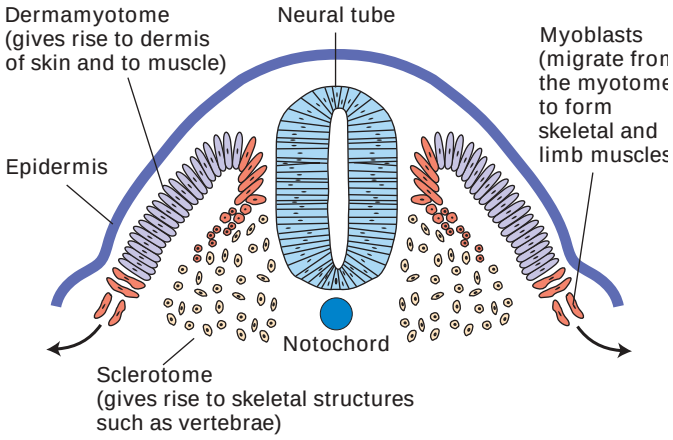
Cell-Cycle Proteins

The onset of terminal differentiation in many cell types is associated with arrest of the cell cycle, most commonly in G_1, suggesting that the transition from the determined to differentiated state may be influenced by cell-cycle proteins including **cyclins** and **cyclin-dependent kinases** (Chapter 21). For instance, certain inhibitors of cyclin-dependent kinases can induce muscle differentiation in cell culture, and the amounts of these inhibitors are markedly higher in differentiating muscle cells than in nondifferentiating ones in vivo. Conversely, differentiation of cultured myoblasts can be inhibited by transfecting the cells with DNA encoding cyclin D1 under the control of a constitutively active promoter. Expression of cyclin D1, which normally occurs only during G_1, is induced by mitogenic factors in many cell types and drives the cell cycle (see Figure 21-28). The ability of cyclin D1 to prevent myoblast differentiation in vitro may mimic aspects of the in vivo signals that antagonize the differentiation pathway. The antagonism between negative and positive regulators of G_1 progression is likely to play an important role in controlling myogenesis in vivo.

Cell-Cell Signals Are Crucial for Muscle Cell-Fate Determination and Myoblast Migration

As noted already, after myoblasts arise from somites, they must not only proliferate but also move to their proper locations and form the correct attachments as they differentiate into muscle cells (Figure 22-17). Myogenic gene expression often follows elaborate events that tell certain somite cells to delaminate from the somite epithelium and which way to move. A transcription factor, Pax3, is produced in the subset of somite cells that will form muscle. Pax3 appears to be at the top of the regulatory hierarchy controlling muscle formation in the body wall and limbs. Myoblasts that will migrate, but not cells that remain behind, also produce a transcription factor called Lbx1. If Pax3 is not functional, Lbx1 transcripts are not seen and myoblasts do not migrate. Both Pax3 and Lbx1 can affect expression of *myoD*.

The departure of myoblasts from somites depends upon reception of a secreted protein signal appropriately called *scatter factor*, or *hepatocyte growth factor (SF/HGF)*. This signal is produced by embryonic connective tissue cells (mesenchyme) in the limb buds to which myoblasts migrate. Production of SF/HGF is previously induced by other secreted signals such as fibroblast growth factor and Sonic hedgehog, which are critical to limb development (Chapter 15). The cell-surface receptor for SF/HGF, which is expressed by myoblasts, belongs to the receptor tyrosine kinase (RTK) class of receptors (Chapter 14). Cells migrate from the somites at the regions along the head-to-tail body axis where limbs will form, and not



▲ FIGURE 22-17 **Embryonic determination and migration of myoblasts in mammals.** After formation of the neural tube, each somite forms sclerotome, which develops into skeletal structures, and dermomyotome. The dermomyotome gives rise to the dermis of the skin and to the muscles. Lateral myoblasts migrate to the limb bud; medial myoblasts develop into the trunk muscles. The remainder of a dermomyotome gives rise to the connective tissue of the skin. [Adapted from M. Buckingham, 1992, *Trends Genet.* **8**:144.]

elsewhere, due to the presence of SF/HGF at limb locations and not elsewhere. If the SF/HGF signal or its receptor is not functional, somite cells will produce Lbx1 but not go on to migrate; thus no muscles will form in the limbs. Expression of the *myogenin* gene, which is necessary for myotube formation, and of *mrf4*, which is necessary for muscle fiber differentiation, does not begin until migrating myoblasts approach their limb-bud destination; these steps in terminal muscle differentiation are presumably not compatible with migration.

We have touched on just a few of the many external signals and transcription factors that participate in development of a properly patterned muscle. The function of all these regulatory molecules must be coordinated both in space and in time during myogenesis.

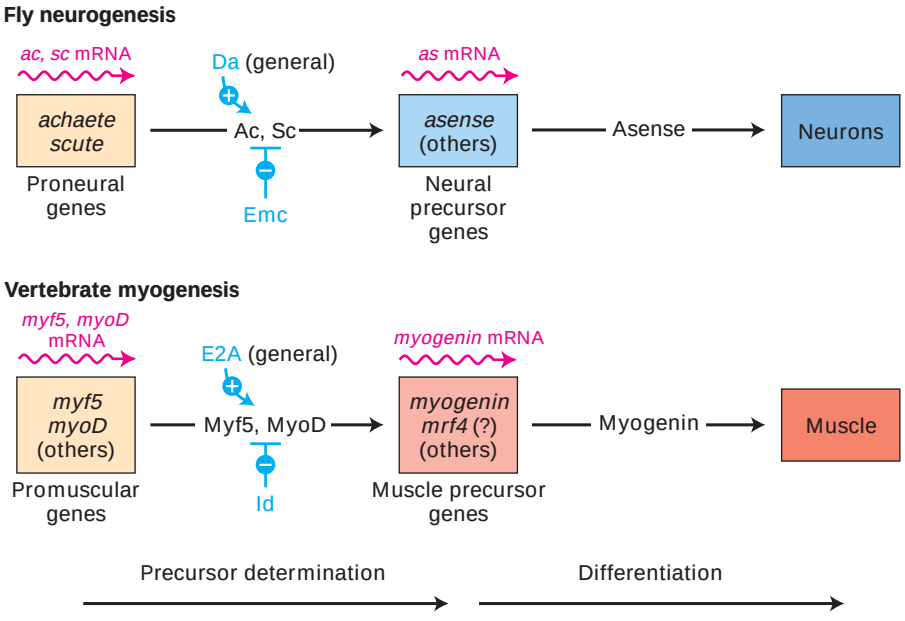
bHLH Regulatory Proteins Function in Creation of Other Tissues

Four bHLH transcription factors that are remarkably similar to the myogenic bHLH proteins control neurogenesis in *Drosophila*. Similar proteins appear to function in neurogenesis in vertebrates and perhaps in the determination and differentiation of hematopoietic cells.

The neurogenic *Drosophila* bHLH proteins are encoded by an ≈100-kb stretch of genomic DNA, termed the *achaete-scute complex* (AS-C), containing four genes designated *achaete (ac), scute (sc), lethal of scute (l'sc),* and *asense (a).* Analysis of the effects of loss-of-function mutations indicate that the Achaete (Ac) and Scute (Sc) proteins participate in determination of neuronal stem cells, called *neuroblasts*, while the Asense (As) protein is required for differentiation of the progeny of these cells into neurons. These functions are analogous to the roles of MyoD and Myf5 in muscle determination and of myogenin in differentiation. Two other *Drosophila* proteins, designated Da and Emc, are analogous in structure and function to vertebrate E2A and Id, respectively. For example, heterodimeric complexes of Da with Ac or Sc bind to DNA better than the homodimeric forms of Ac and Sc. Emc, like Id, lacks a DNA-binding basic domain; it binds to Ac and Sc proteins, thus inhibiting their association with Da and binding to DNA. The similar functions of these myogenic and neurogenic proteins are depicted in Figure 22-18.

A family of bHLH proteins related to the *Drosophila* Achaete and Scute proteins has been identified in vertebrates. One of these, called neurogenin, which has been identified in the rat, mouse, and frog, controls the formation of neuroblasts. In situ hybridization experiments showed that neurogenin is produced at an early stage in the developing nervous system and induces production of NeuroD, another bHLH protein that acts later (Figure 22-19a). Injection of large amounts of *neurogenin* mRNA into *Xenopus* embryos further demonstrated the ability of neurogenin to induce neurogenesis (Figure 22-19b). These studies suggest that the function of neurogenin is analogous to that of the Achaete and Scute in *Drosophila;* likewise, NeuroD and Asense may have analogous functions in vertebrates and *Drosophila,* respectively.

In addition to neurons, the nervous system contains a large number of glial cells, which also arise from the neuroectoderm. Glial cells support and insulate neurons; they also provide guidance and contact surfaces for migrating neurons during development and send a signal to neurons that promotes formation of synapses. Neurogenins control the fates of precursor cells that are capable of making either neurons or glial cells by promoting neural development and repressing glial development. Neurogenins are therefore switches that control the decision between two alternative cell fates, just as the yeast mating-type regulators select among three cell types.



▶ FIGURE 22-18 **Comparison of genes that regulate *Drosophila* neurogenesis and mammalian myogenesis.** bHLH transcription factors have analogous functions in determination of precursor cells (i.e., neuroblasts and myoblasts) and their subsequent differentiation into mature neurons and muscle cells. In both cases, the proteins encoded by the earliest-acting genes (*left*) are under both positive and negative control by other related proteins (blue type). [Adapted from Y. N. Jan and L. Y. Jan, 1993, *Cell* **75**:827.]

neurogenin mRNA | *neuroD* mRNA | β-*tubulin* mRNA | *neurogenin* mRNA

▲ EXPERIMENTAL FIGURE 22-19 **In situ hybridization and injection experiments demonstrate that neurogenin acts before NeuroD in vertebrate neurogenesis.** (a) Sections of rat neural tube were treated with a probe specific for *neurogenin* mRNA (*left*) or *neuroD* mRNA (*right*). The open space in the center is the ventricle, and the cells lining this cavity are in the ventricular layer. All the neural cells are born in the ventricular layer and then migrate outward. As illustrated in these micrographs, *neurogenin* mRNA is produced in proliferating neuroblasts in the ventricular layer (*arrow*), whereas *neuroD* mRNA is present in migrating neuroblasts that have left the ventricular zone (*other arrow*). (b) One of the two cells in early *Xenopus* embryos was injected with *neurogenin* mRNA (inj) and then stained with a probe specific for neuron-specific mRNAs encoding β-tubulin (*left*) or NeuroD (*right*). The region of the embryo derived from the uninjected cell served as a control (con). The *neurogenin* mRNA induced a massive increase in the number of neuroblasts expressing *neuroD* mRNA and neurons expressing *β-tubulin* mRNA in the region of the neural tube derived from the injected cell. [From Q. Ma et al., 1996, *Cell* **87**:43; courtesy of D. J. Anderson.]

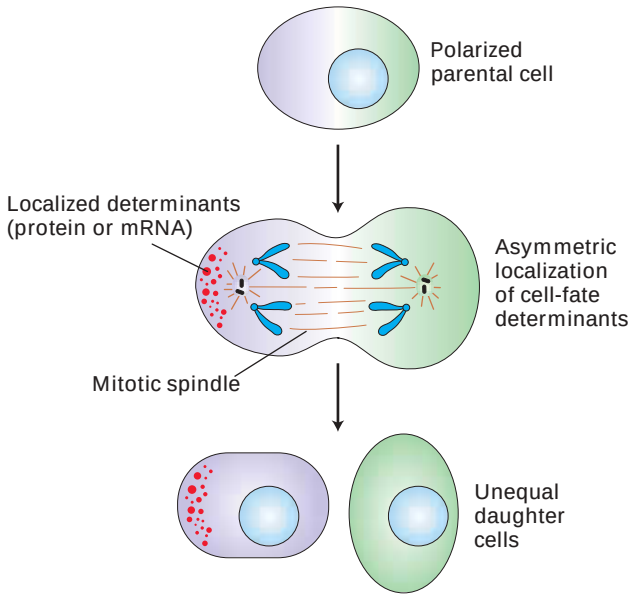
KEY CONCEPTS OF SECTION 22.3

Specification and Differentiation of Muscle

- Development of skeletal muscle begins with the signal-induced determination of certain mesoderm cells in somites as myoblasts. Following their proliferation and migration, myoblasts stop dividing and differentiate into multinucleate muscle cells (myotubes) that express muscle-specific proteins (see Figure 22-14).
- Four myogenic bHLH transcription factors—MyoD, myogenin, Myf5, and MRF4, called muscle-regulatory factors (MRFs)—associate with E2A and MEFs to form large transcriptional complexes that drive myogenesis and expression of muscle-specific genes.
- Dimerization of bHLH transcription factors with different partners modulates the specificity or affinity of their binding to specific DNA regulatory sites, and also may prevent their binding entirely.
- The myogenic program driven by MRFs depends on the Swi/Snf chromatin-remodeling complex, which makes target genes accessible.
- The myogenic program is inhibited by binding of Id protein to MyoD, thereby blocking binding of MyoD to DNA, and by histone deacetylases, which repress activation of target genes by MRFs.
- Migration of myoblasts to the limb buds is induced by scatter factor/hepatocyte growth factor (SF/HGF), a protein signal secreted by mesenchymal cells (see Figure 22-17). Myoblasts must express both the Pax3 and Lbx1 transcription factors to migrate.
- Terminal differentiation of myoblasts and induction of muscle-specific proteins do not occur until myoblasts stop dividing and begin migrating.
- Neurogenesis in *Drosophila* depends upon a set of four neurogenic bHLH proteins that are conceptually and structurally similar to the vertebrate myogenic proteins (see Figure 22-18).
- A related vertebrate protein, neurogenin, is required for formation of neural precursors and also controls their division into neurons or glial cells.

22.4 Regulation of Asymmetric Cell Division

During **embryogenesis,** the earliest stage in animal development, asymmetric cell division often creates the initial diversity that ultimately culminates in formation of specific differentiated cell types. Even in bacteria, cell division may yield unequal daughter cells, for example, one that remains attached to a stalk and one that develops flagella used for swimming. Essential to asymmetric cell division is *polarization* of the parental cell and then differential incorporation



▲ FIGURE 22-20 **General features of asymmetric cell division.** Various mechanisms can lead to asymmetric distribution of cytoplasmic components, such as particular proteins or mRNAs (red dots) to form a polarized parental cell. Division of a polarized cell will be asymmetric if the mitotic spindle is oriented so that the localized cytoplasmic components are distributed unequally to the two daughter cells, as shown here. However, if the spindle is positioned differently relative to the localized cytoplasmic components, division of a polarized cell may produce equivalent daughter cells.

of parts of the parental cell into the two daughters (Figure 22-20). A variety of molecular mechanisms are employed to create and propagate the initial asymmetry that polarizes the parental cell. In addition to being different, the daughter cells must often be placed in a specific orientation with respect to surrounding structures.

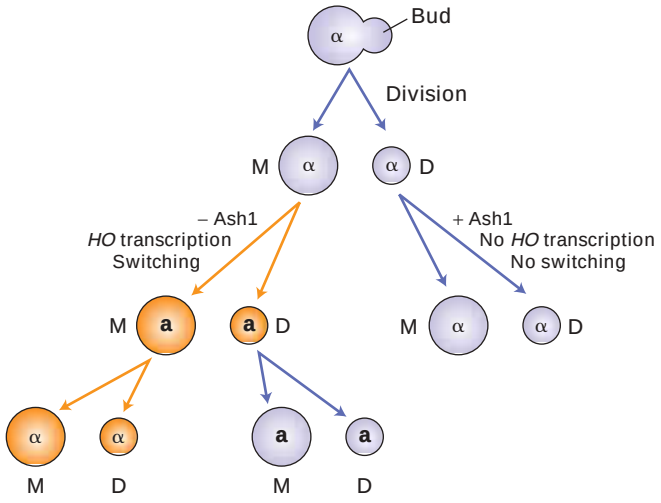
We begin with an especially well-understood example of asymmetric cell division, the budding of yeast cells, and move on to recently discovered protein complexes important for asymmetric cell divisions in multicellular organisms. We see in this example an elegant system that links asymmetric division to the process of controlling cell type.

Yeast Mating-Type Switching Depends upon Asymmetric Cell Division

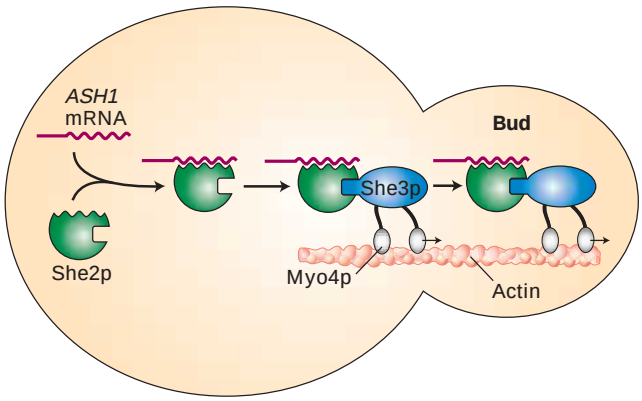
S. cerevisiae cells use a remarkable mechanism to control the differentiation of the cells as the cell lineage progresses. Whether a haploid yeast cell exhibits the α or **a** mating type is determined by which genes are present at the *MAT* locus (see Figure 22-11). As described in Chapter 11, the *MAT* locus in the *S. cerevisiae* genome is flanked by two "silent," transcriptionally inactive loci containing the alternative α or **a** sequences (see Figure 11-28). A specific DNA rearrangement brings the genes that encode the α-specific or **a**-specific transcription factors from these silent loci to the active *MAT* locus where they can be transcribed.

Interestingly, some haploid yeast cells can switch repeatedly between the α and **a** types. *Mating-type switching* occurs when the α allele occupying the *MAT* locus is replaced by the **a** allele, or vice versa. The first step in this process is catalyzed by HO endonuclease, which is expressed in mother cells but not in daughter cells. Thus mating-type switching occurs only in mother cells (Figure 22-21). Transcription of the *HO* gene is dependent on the *Swi/Snf chromatin-remodeling complex* (see Figure 11-37), the same complex that we encountered earlier in our discussion of myogenesis. Daughter yeast cells arising by budding from mother cells contain a protein called Ash1 that prevents recruitment of the Swi/Snf complex to the *HO* gene, thereby preventing its transcription. The absence of Ash1 from mother cells allows them to transcribe the *HO* gene.

Recent experiments have revealed how the asymmetry in the distribution of Ash1 between mother and daughter cells is established. *ASH1* mRNA accumulates in the growing bud that will form a daughter cell due to the action of a myosin motor protein (Chapter 19). This motor protein, called Myo4p, moves the *ASH1* mRNA, as a ribonucleoprotein complex, along actin filaments in one direction only, toward the bud (Figure 22-22). Two connector proteins tether *ASH1* mRNA to the motor protein. By the time the bud separates from the mother cell, the mother cell is largely depleted of *ASH1* mRNA and thus can switch mating type in the fol-



▲ FIGURE 22-21 **Specificity of mating-type switching in haploid yeast cells.** Division by budding forms a larger mother cell (M) and smaller daughter cell (D), both of which have the same mating type as the original cell (α in this example). The mother can switch mating type during G_1 of the next cell cycle and then divide again, producing two cells of the opposite **a** type. Switching depends on transcription of the *HO* gene, which occurs only in the absence of Ash1 protein. The smaller daughter cells, which produce Ash1 protein, cannot switch; after growing in size through interphase, they divide to form a mother cell and daughter cell. Orange cells and arrows indicate switch events.



▶ FIGURE 22-22 **Model for restriction of mating-type switching to mother cells in *S. cerevisiae*.** Ash1 protein prevents a cell from transcribing the *HO* gene whose encoded protein initiates the DNA rearrangement that results in mating-type switching from **a** to α or α to **a**. Switching occurs only in the mother cell, after it separates from a newly budded daughter cell, because Ash1 protein is present in the daughter cell but not in the mother cell. The molecular basis for this differential localization of Ash1 is the one-way transport of *ASH1* mRNA into the bud. A linking protein, She2p, binds to specific 3′ untranslated sequences in the *ASH1* mRNA and also binds to She3p protein. This protein in turn binds to a myosin motor, Myo4p, which moves along actin filaments into the bud. [See S. Koon and B. J. Schnapp, 2001, *Curr. Biology* **11**:R166–R168.]

MEDIA CONNECTIONS
Video: ASH1 mRNA Localization

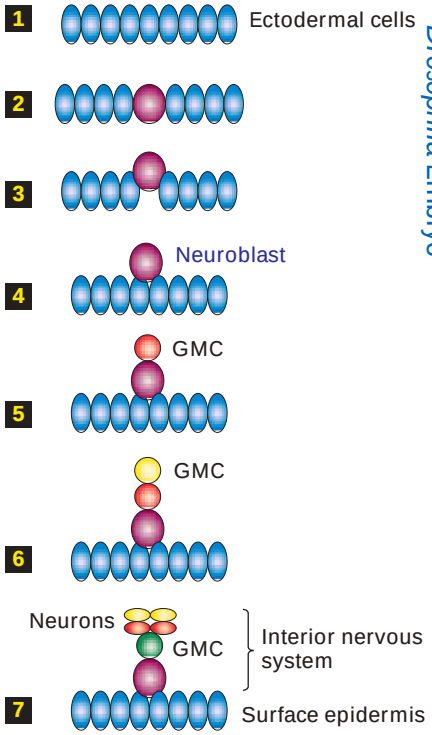
lowing G_1 before additional *ASH1* mRNA is produced and before DNA replication in the S phase.

Budding yeasts use a relatively simple mechanism to create molecular differences between the two cells formed by division. In higher organisms, polarization of the parental cell involves many more participants, and in addition, as in yeast, the mitotic spindle must be oriented in such a way that each daughter cell receives its own set of cytoplasmic components. To illustrate these complexities, we focus on asymmetric division of neuroblasts in *Drosophila*. Genetic studies in *C. elegans* and *Drosophila* have revealed the key participants, a first step in understanding at the molecular level how asymmetric cell division is regulated in multicellular organisms.

Critical Asymmetry-Regulatory Proteins Are Localized at Opposite Ends of Dividing Neuroblasts in *Drosophila*

Fly neuroblasts, which are stem cells, arise from a sheet of ectoderm cells that is one cell thick. As in vertebrates, the *Drosophila* ectoderm forms both epidermis and the nervous system, and many ectoderm cells have the potential to assume either a neural or epidermal fate. Under the control of genes that become active only in certain cells, some of the cells increase in size and begin to loosen from the ectodermal layer. At this point, the delaminating cells use Notch signaling to mediate **lateral inhibition** of their neighbors, causing them to retain the epidermal fate (see Figures 14-29 and 15-36). The delaminating cells move inside and become spherical neuroblasts, while the prospective epidermal cells remain behind and close up to form a tight sheet.

Once formed, the neuroblasts undergo asymmetric divisions, at each division recreating themselves and producing a *ganglion mother cell (GMC)* at the basal side of the neuroblast (Figure 22-23). A single neuroblast will produce several GMCs; each GMC in turn forms two neurons. Depending on where they form in the embryo and consequent regulatory events, neuroblasts may form more or fewer GMCs.

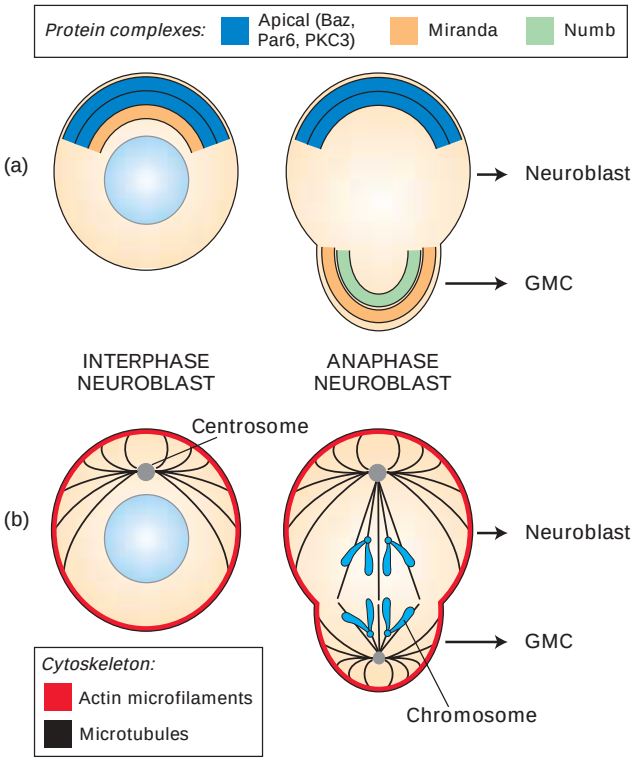


▶ FIGURE 22-23 **Asymmetric cell division during *Drosophila* neurogenesis.** The ectodermal sheet (1) of the early embryo gives rise to both epidermal cells and neural cells. Neuroblasts, the stem cells for the nervous system, are formed when ectoderm cells enlarge, separate from the ectodermal epithelium, and move into the interior of the embryo (2–4). Each neuroblast that arises divides asymmetrically to recreate itself and produce a ganglion mother cell (GMC) (5). Subsequent divisions of a neuroblast produce more GMCs, creating a stack of these precursor cells (6). Each GMC divides once to give rise to two neurons (7). Neuroblasts and their GMC descendants can have different fates depending on their location.

MEDIA CONNECTIONS
Video: Ectodermal Cell Divisions in the *Drosophila* Embryo

Neuroblasts and GMCs in different locations exhibit different patterns of gene expression, an indicator of their fates. Analysis of fly mutants led to the discovery of key proteins that participate in creating specific neuroblasts, inhibiting epidermal cells from becoming neuroblasts, and directing neuroblasts to divide asymmetrically.

Apical Baz/Par6/aPKC Complex A ternary complex of proteins, referred to as the *apical complex*, is located at the end of a fly neuroblast that will become the "new" neuroblast in the process of division. This complex is already localized at one end of all ectoderm cells. It stays at the apical end as a prospective neuroblast delaminates from the ectodermal sheet (Figure 22-24a, *left*). Three proteins compose the apical complex: Baz, Par6, and aPKC, the last being an isoform of protein kinase C. The Baz/Par6/aPKC complex persists at the apical end from late interphase (G_2 stage) until late anaphase in mitosis and then disperses or is destroyed (see Figure 20-29 for mitotic stages). After each neuroblast division, the complex re-forms at the apical end of the progeny neuroblast in the next interphase. It remains unclear how polarity information is preserved through telophase, since no known proteins are localized during that time. As noted previously, the very first *C. elegans* cell division after fertilization is asymmetric, forming an AB cell and P1 cell, which have quite different fates (see Figure 22-9). Remarkably, a protein complex like the fly Baz/Par6/aPKC complex controls the asymmetry of the P1-AB division; similar protein complexes exist in mammals as well.



▲ FIGURE 22-24 **Localized proteins that control asymmetric cell division in the *Drosophila* neuroblast.** (a) The apical complex (blue), which consists of three proteins (Baz, Par6, and aPKC), is localized in ectoderm cells and in delaminating neuroblasts. As a neuroblast begins dividing, Miranda protein (orange) accumulates at the apical side and then moves to the basal side, where it will be incorporated into the ganglion mother cell (GMC). The second basal component, the Numb protein (green), is detected only basally. Mutations in genes that encode polarized proteins disrupt asymmetric cell division. (b) Motor protein–mediated transport along cytoskeletal filaments is thought to localize the apical and basal complexes. Actin microfilaments (red) lie just under the cell surface at all times. Microtubules (blue) radiate from the centrosome during interphase and then assemble into the mitotic spindle, attached to the duplicated centrosomes, during cell division. Note that in the neuroblast-GMC division the centrosome is skewed to the neuroblast end of the cell, and the chromosomes (light gray) are diagrammed lined up on the spindle. [Adapted from C. Q. Doe and B. Bowerman, 2001, *Curr. Opin. Cell Biol.* **13**:68.]

Two mechanisms control the apical localization of the *Drosophila* Baz/Par6/aPKC complex: the first operates in the ectoderm prior to neuroblast delamination; the second is active during the repeating divisions of delaminated neuroblasts. The first mechanism involves at least three proteins, Scribble (Scrib), Discs-large (Dlg), and Lethal giant larvae (Lgl). These proteins are located in the cortical region of ectoderm cells (i.e., the region just below the plasma membrane), but are not polarized along the apical-basal axis of these cells. Thus, though necessary for the apical localization of the Baz/Par6/aPKC complex, the Scrib/Dlg/Lgl proteins probably are not sufficient. Once a neuroblast has separated from the ectoderm, the G_α subunit of the heterotrimeric G protein and two other proteins called Inscuteable and Partner of Inscuteable join the Baz/Par6/aPKC complex, forming a six-protein complex. These two additional proteins probably stabilize the Baz/Par6/aPKC complex. Reassembly of the entire complex at the apical end following each neuroblast division requires actin microfilaments (Figure 22-24b). Generally, asymmetric protein segregation requires actin but not tubulin, suggesting that motor protein–mediated transport of these proteins along microfilaments plays a role in their apical localization.

Basal Proteins In a dividing neuroblast two basal proteins are found at the end that will form a ganglion mother cell (GMC) at division (see Figure 22-24a, *right*). Localization of these proteins, including Miranda and Numb, at the basal end of neuroblasts requires the apical Baz/Par6/aPKC complex. The apical proteins set up polarity during interphase, and the polarity is read and interpreted by the machinery that transports Numb and Miranda at mitosis. The Numb protein is located throughout the cell during interphase; at prophase it becomes basally located. In contrast, Miranda initially assembles at the apical end; it then moves to the basal end at prophase. The apical Baz/Par6/aPKC complex forms even when either of the basal proteins is missing due to

mutations. Mutations in any of the components of the apical complex, however, prevent the Miranda and Numb proteins from moving to the basal region. Instead they accumulate all around the cortical region or in clumps throughout the cell. The simplest idea, that the basal complexes form wherever the apical complex is absent, is probably wrong, since parts of the cell contain neither of these complexes.

Basally located molecules include Prospero protein, a transcription factor that contains a DNA-binding homeodomain; Miranda protein, which has a coiled-coil structure; Staufen, an RNA-binding protein; and *prospero* mRNA. During each round of neuroblast division, new Prospero protein is made during interphase and incorporated into the basal region, which is distributed to the GMC at the time of cleavage. In the absence of Prospero, GMCs do not transcribe the appropriate genes and do not develop into normal neurons. The localization of a transcription factor in the cytoplasm provides a link between the asymmetry of the cytoplasm and subsequent cell-fate determination, potentially explaining how a transcription factor is preferentially distributed to one of two daughter cells.

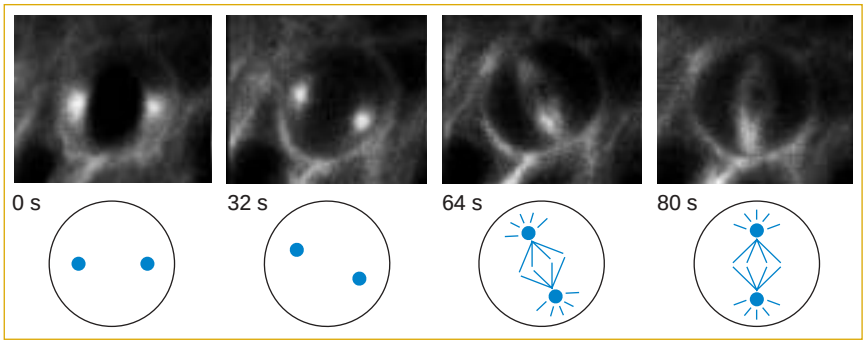
Orientation of the Mitotic Spindle Is Linked to Cytoplasmic Cell-Asymmetry Factors

For localized protein complexes to be differentially incorporated into two daughter cells requires that the plane of cell division be appropriately oriented. In dividing fly neuroblasts, the **mitotic spindle** first aligns perpendicular to the apical-basal axis and then turns 90 degrees to align with it at the same time that the basal complexes become localized to the basal side (Figure 22-25). The apical complex, which is already in place before spindle rotation, controls the final orientation of the spindle. This is supported by the finding that mutations in any of the components of the apical complex eliminate the coordination of the spindle with apical-basal polarity, causing the spindle orientation to become random.

Spindle orientation is regulated by actin and myosin-related proteins. Mammalian Par6, a component of the apical complex, can bind two small GTPase proteins, Cdc42 and Rac1, that control the arrangement of actin microfilaments in the cytoskeleton (see Figure 19-29). The protein Lethal giant larvae (Lgl), a component of the Scrib/Dlg/Lgl asymmetry pathway, helps to localize Miranda basally in fly neuroblasts.

Lgl binds to myosin II, which functions in cytokinesis (see Figure 19-20). Lgl itself is uniformly localized around the cortex. Lgl is phosphorylated by the apical complex and may be inactivated on the apical side to allow basal Miranda accumulation. Two yeast proteins related to Lgl also bind to myosin II; they have been implicated in exocytosis and secretion, specifically in the docking of post-Golgi vesicles with the plasma membrane (Chapter 17). Mutations in the two yeast proteins suppress mutations in the *myosin II* gene. This observation is interpreted to mean that the function of myosin II in spindle orientation is opposite to that of the Lgl-like proteins: reduction of one protein's function is ameliorated by reduction of the other, restoring a semblance of the normal balance. In this case, therefore, myosin and Lgl probably act in opposite directions: myosin II moving Miranda or other materials to control spindle orientation and Lgl restraining it.

Further evidence for the importance of myosin in spindle orientation comes the finding that another myosin relative,



▲ EXPERIMENTAL FIGURE 22-25 **Time-lapse fluorescence imaging reveals rotation of the mitotic spindle in asymmetrically dividing neuroblasts.** Early *Drosophila* embryos were injected with a hybrid gene composed of the gene encoding green fluorescent protein (GFP) fused to the gene encoding Tau, a protein that binds to microtubules. At the top are time-lapse images of a single dividing neuroblast in a live embryo. The basal side is at the top, and the apical side at the bottom. At time 0, equivalent to prophase, the two centrosomes are visible on opposite sides of the cell. These function as the spindle poles; as mitosis proceeds the microtubules forming the mitotic spindle are assembled from the poles (see Figure 20-29). In successive images (at 32, 64, and 80 seconds), the bipolar spindle can be seem to form and rotate 90 degrees to align with the apical-basal axis, as schematically depicted at the bottom. [From J. A. Kaltschmidt et al., 2000, *Nature Cell Biol.* **2**:7–12; courtesy of J. Kaltschmidt and A. H. Brand, Wellcome/CRC Institute, Cambridge University.]

MEDIA CONNECTIONS
Video: Mitotic Spindle Assymetry in *Drosophila* Neuroblast Cell Division

myosin VI, binds directly to Miranda. In addition, mutations in myosin VI in flies prevent basal targeting of Miranda and simultaneously block proper spindle orientation. These various findings give us the beginning of a picture of how localized protein complexes are coordinated with cell division, so that each daughter cell receives the appropriate amounts of the various complexes.

At least some of the asymmetric cell division regulators discovered in flies and worms are present in vertebrates and have similar roles there. For example, mammalian Numb is required to maintain the neural stem-cell population. Evolutionary conservation of proteins and mechanisms facilitates rapid progress in research.

KEY CONCEPTS OF SECTION 22.4

Regulation of Asymmetric Cell Division

- Asymmetric cell division requires polarization of the dividing cell, which usually entails localization of some cytoplasmic components, and then the unequal distribution of these components to the daughter cells (see Figure 22-20).
- In the asymmetric division of budding yeasts, a myosin-dependent transport system carries *ASH1* mRNA into the bud (see Figure 22-22).
- Ash1 protein is produced in the daughter cell soon after division and prevents expression of HO endonuclease, which is necessary for mating-type switching. Thus a daughter cell cannot switch mating type, whereas the mother cell from which it arises can (see Figure 22-21).
- Asymmetric cell division in *Drosophila* neuroblasts depends on localization of the apical protein complex (Baz/Par6/PKC3) and two sets of basal proteins. The basal proteins are incorporated into the ganglion mother cell (GMC) and contain proteins that determine cell fate (see Figure 22-24).
- The apical Baz/Par6/aPKC complex also controls asymmetric cell division in *C. elegans* and perhaps mammals.
- Asymmetry factors exert their influence at least in part by controlling the orientation of the mitotic spindle, so that asymmetrically localized proteins and structures are differentially incorporated into the two daughter cells. Myosin proteins bind to proteins that control asymmetry factors of cells to control spindle orientation.

22.5 Cell Death and Its Regulation

Programmed cell death is a cell fate, an odd sort of cell fate but nonetheless one that is essential. Cell death keeps our hands from being webbed, our embryonic tails from persisting, our immune system from responding to our own proteins, and our brain from being filled with useless electrical connections. In fact, the majority of cells generated during brain development die during development.

Cellular interactions regulate cell death in two fundamentally different ways. First, most cells, if not all, in multicellular organisms require signals to stay alive. In the absence of such survival signals, frequently referred to as **trophic factors,** cells activate a "suicide" program. Second, in some developmental contexts, including the immune system, specific signals induce a "murder" program that kills cells. Whether cells commit suicide for lack of survival signals or are murdered by killing signals from other cells, recent studies suggest that death is mediated by a common molecular pathway. In this section, we first distinguish programmed cell death from death due to tissue injury, then consider the role of trophic factors in neuronal development, and finally describe the evolutionarily conserved effector pathway that leads to cell suicide or murder.

Programmed Cell Death Occurs Through Apoptosis

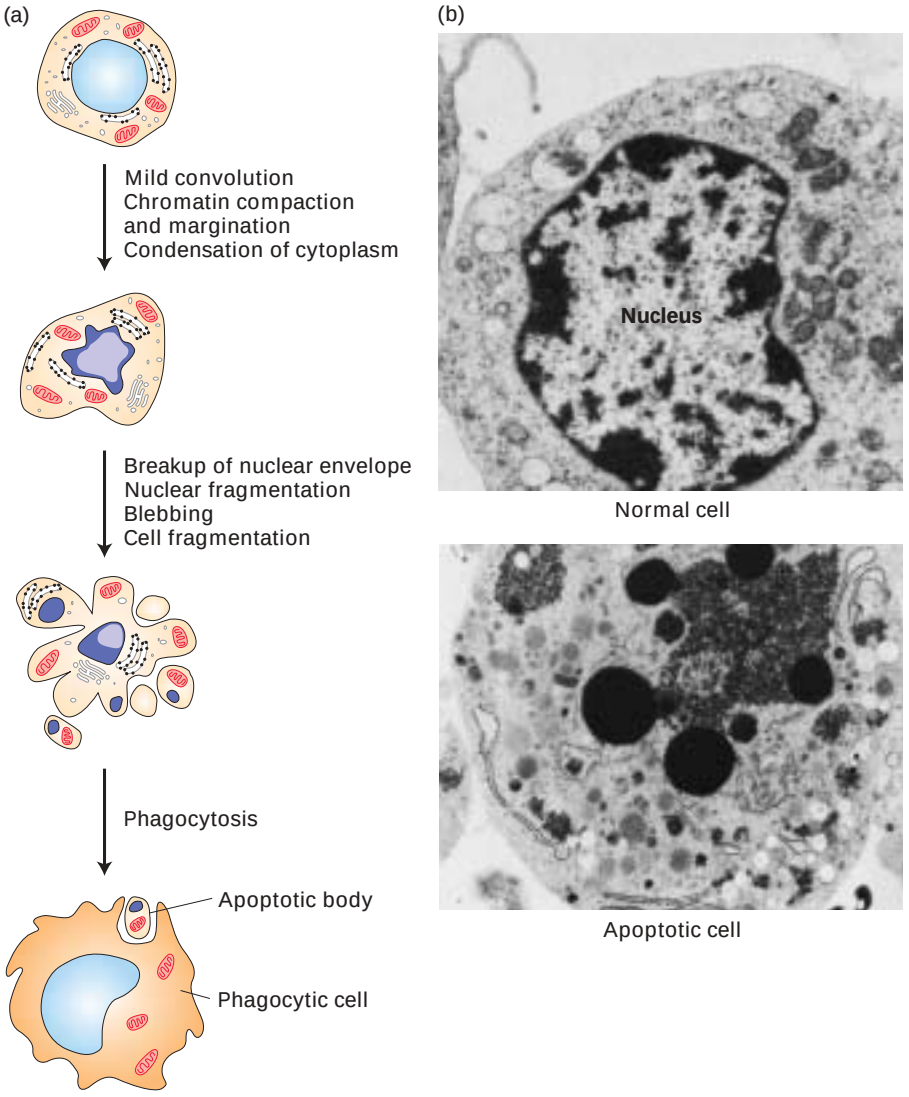
The demise of cells by programmed cell death is marked by a well-defined sequence of morphological changes, collectively referred to as **apoptosis,** a Greek word that means "dropping off" or "falling off," as leaves from a tree. Dying cells shrink and condense and then fragment, releasing small membrane-bound apoptotic bodies, which generally are engulfed by other cells (Figure 22-26; see also Figure 1-19). The nuclei condense and the DNA is fragmented. Importantly, the intracellular constituents are not released into the extracellular milieu where they might have deleterious effects on neighboring cells. The highly stereotyped changes accompanying apoptosis suggested to early workers that this type of cell death was under the control of a strict program. This program is critical during both embryonic and adult life to maintain normal cell number and composition.

The genes involved in controlling cell death encode proteins with three distinct functions:

- "Killer" proteins are required for a cell to begin the apoptotic process.
- "Destruction" proteins do things like digest DNA in a dying cell.
- "Engulfment" proteins are required for phagocytosis of the dying cell by another cell.

At first glance, engulfment seems to be simply an after-death cleanup process, but some evidence suggests that it is part of the final death decision. For example, mutations in killer genes always prevent cells from initiating apoptosis, whereas mutations that block engulfment sometimes allow cells to survive that would normally die. That is, cells with engulfment-gene mutations can initiate apoptosis but then sometimes recover.

In contrast to apoptosis, cells that die in response to tissue damage exhibit very different morphological changes, referred to as **necrosis.** Typically, cells that undergo this process swell and burst, releasing their intracellular contents, which can damage surrounding cells and frequently cause inflammation.



▶ FIGURE 22-26 **Ultrastructural features of cell death by apoptosis.** (a) Schematic drawings illustrating the progression of morphologic changes observed in apoptotic cells. Early in apoptosis, dense chromosome condensation occurs along the nuclear periphery. The cell body also shrinks, although most organelles remain intact. Later both the nucleus and cytoplasm fragment, forming apoptotic bodies, which are phagocytosed by surrounding cells. (b) Photomicrographs comparing a normal cell (*top*) and apoptotic cell (*bottom*). Clearly visible in the latter are dense spheres of compacted chromatin as the nucleus begins to fragment. [Part (a) adapted from J. Kuby, 1997, *Immunology,* 3d ed., W. H. Freeman & Co., p. 53. Part (b) from M. J. Arends and A. H. Wyllie, 1991, *Int'l. Rev. Exp. Pathol.* **32**:223.]

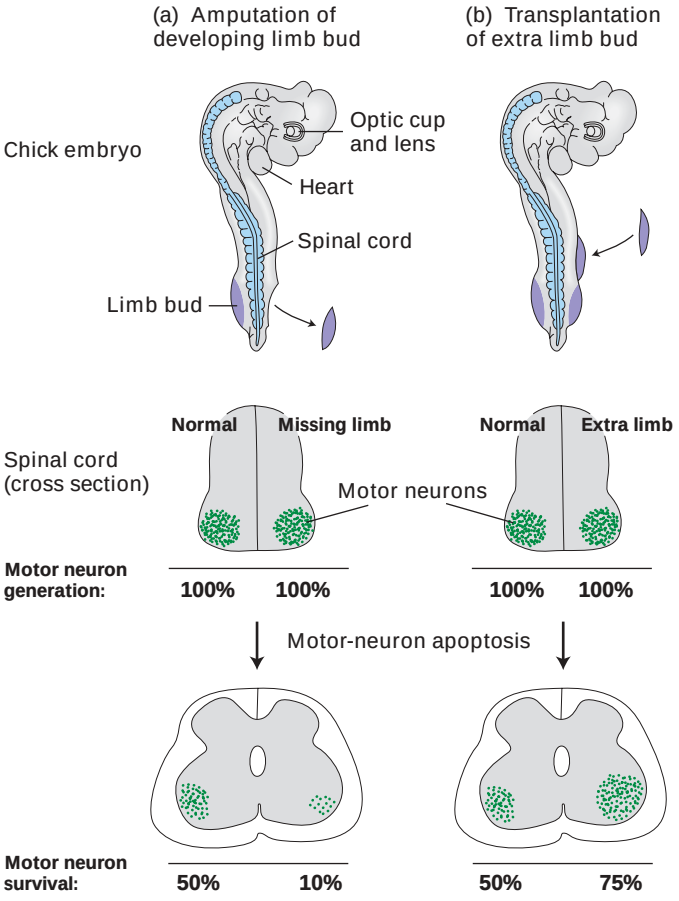
MEDIA CONNECTIONS
Focus Animation: Apoptosis

Neurotrophins Promote Survival of Neurons

The earliest studies demonstrating the importance of trophic factors in cellular development came from analyses of the developing nervous system. When neurons grow to make connections to other neurons or to muscles, sometimes over considerable distances, more cells grow than will eventually survive. Those that make connections prevail and survive; those that fail to connect die.

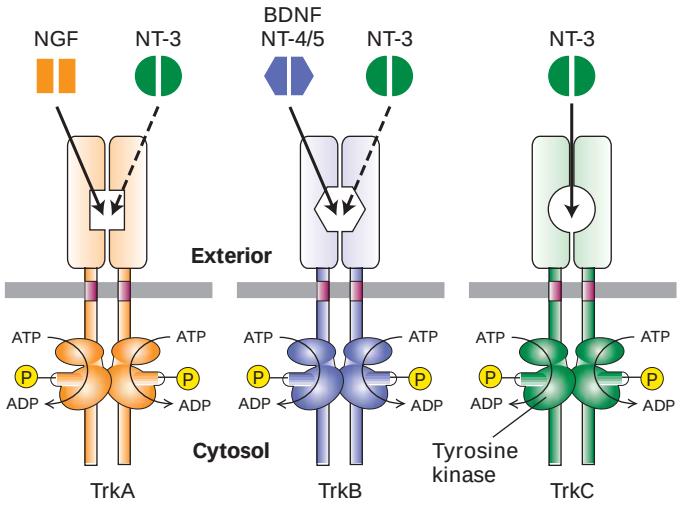
In the early 1900s the number of neurons innervating the periphery was shown to depend upon the size of the tissue to which they would connect, the so-called "target field." For instance, removal of limb buds from the developing chick embryo leads to a reduction in the number of sensory neurons and motoneurons innervating the bud (Figure 22-27). Conversely, grafting additional limb tissue to a limb bud leads to an increase in the number of neurons in corresponding regions of the spinal cord and sensory ganglia. Indeed, incremental increases in the target-field size are accompanied by commensurate incremental increases in the number of neurons innervating the target field. This relation was found to result from the selective survival of neurons rather than changes in their differentiation or proliferation. The observation that many sensory and motor neurons die after reaching their peripheral target field suggested that these neurons compete for survival factors produced by the target tissue.

Subsequent to these early observations, scientists discovered that transplantation of a mouse sarcoma tumor into a chick led to a marked increase in the numbers of certain types of neurons. This finding implicated the tumor as a rich source of the presumed trophic factor. To isolate and purify this factor, known simply as nerve growth factor (NGF), scientists used an in vitro assay in which outgrowth of neurites from sensory ganglia (nerves) was measured. Neurites are extensions of the cell cytoplasm that can grow to become the long wires of the nervous system, the **axons** and **dendrites**



▲ EXPERIMENTAL FIGURE 22-27 **The survival of motor neurons depends on the size of the muscle target field they innervate.** (a) Removal of a limb bud from one side of a chick embryo at about 2.5 days results in a marked decrease in the number of motor neurons on the affected side. In an amputated embryo, normal numbers of motor neurons are generated on both sides (*middle*). Later in development, many fewer motor neurons remain on the side of the spinal cord with the missing limb than on the normal side (*bottom*). Note that only about 50 percent of the motor neurons that originally are generated normally survive. (b) Transplantation of an extra limb bud into an early chick embryo produces the opposite effect, more motor neurons on the side with additional target tissue than on the normal side. [Adapted from D. Purves, 1988, *Body and Brain: A Trophic Theory of Neural Connections* (Cambridge, MA: Harvard University Press), and E. R. Kandel, J. H. Schwartz, and T. M. Jessell, 2000, *Principles of Neural Science,* 4th ed. (New York: McGraw-Hill), p. 1054, Figure 53-11.]

(see Figure 7-29). The later discovery that the submaxillary gland in the mouse also produces large quantities of NGF enabled biochemists to purify it to homogeneity and to sequence it. A homodimer of two 118-residue polypeptides, NGF belongs to a family of structurally and functionally related trophic factors collectively referred to as *neurotrophins*. Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) also are members of this protein family.



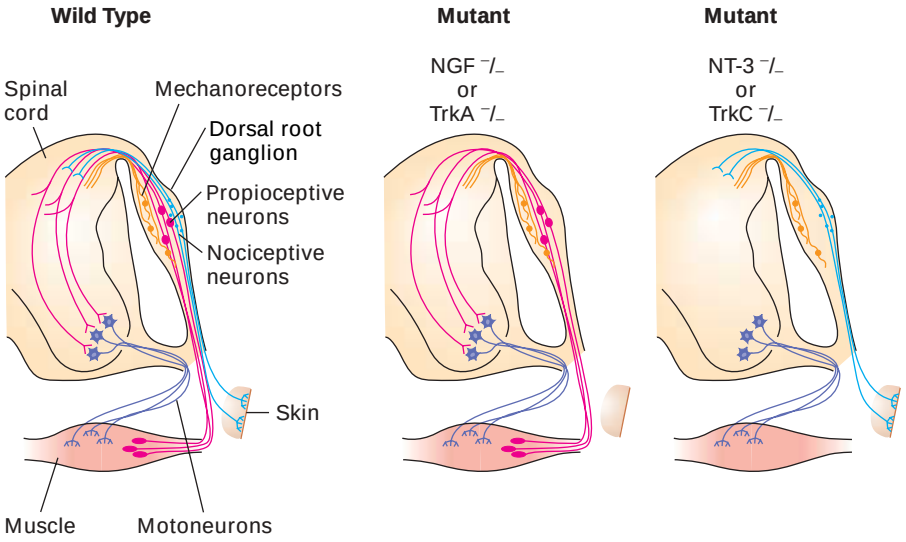
▲ FIGURE 22-28 **Specificity of neurotrophins for Trks, a family of receptor tyrosine kinases.** Each neurotrophin binds with high affinity to one Trk receptor indicated by the solid arrow from the ligand to the receptor. NT-3 also can bind with lower affinity to both TrkA and TrkB as indicated by the dashed arrow. In addition, neurotrophins bind to a distinct receptor called $p75^{NTR}$ either alone or in combination with Trks. NGF = nerve growth factor; BDNF = brain-derived neurotrophic factor; NT-3 = neurotrophin-3. [Adapted from W. D. Snider, 1994, *Cell* **77**:627.]

Neurotrophin Receptors

Neurotrophins bind to and activate a family of receptor tyrosine kinases called *Trks* (pronounced "tracks"). (The general structure of receptor tyrosine kinases and the intracellular signaling pathways they activate are covered in Chapter 14.) As shown in Figure 22-28, NGF binds to TrkA; BDNF, to TrkB; and NT-3, to TrkC. Binding of these factors to their receptors provides a survival signal for different classes of neurons. A second type of receptor called $p75^{NTR}$ (NTR = neurotrophin receptor) also binds to neurotrophins, but with lower affinity. However, $p75^{NTR}$ forms heteromultimeric complexes with the different Trk receptors; this association increases the affinity of Trks for their ligands. Some studies indicate that the binding of NGF to $p75^{NTR}$ in the absence of TrkA may promote cell death rather than prevent it.

Knockouts of Neurotrophins and Their Receptors

To critically address the role of the neurotrophins in development, scientists produced mice with knockout mutations in each of the neurotrophins and their receptors. These studies revealed that different neurotrophins and their corresponding receptors are required for the survival of different classes of sensory neurons, which carry signals from peripheral sensory systems to the brain (Figure 22-29). For instance, pain-sensitive (nociceptive) neurons, which express TrkA, are selectively lost from the dorsal root ganglion of knockout mice lacking NGF or TrkA, whereas TrkB- and TrkC-expressing neurons are unaffected in such knockouts. In contrast, TrkC-expressing proprioceptive neurons, which detect the position of the limbs, are missing from the dorsal root ganglion in *TrkC* and *NT-3* mutants.



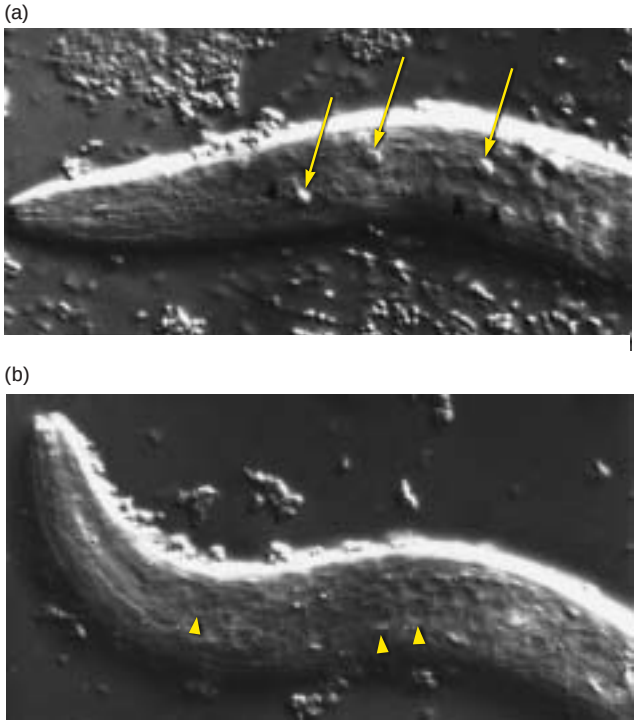
◀ EXPERIMENTAL FIGURE 22-29 **Different classes of sensory neurons are lost in knockout mice lacking different trophic factors or their receptors.** In animals lacking nerve growth factor (NGF) or its receptor TrkA, small nociceptive (pain-sensing) neurons (blue) that innervate the skin are missing. These neurons express TrkA receptor and innervate NGF-producing targets. In animals lacking either neurotrophin-3 (NT-3) or its receptor TrkC, large propioceptive neurons (red) innervating muscle spindles are missing. Muscle produces NT-3 and the propioceptive neurons express TrkC. Mechanoreceptors (brown), another class of sensory neurons in the dorsal root ganglion, are unaffected in these mutants. [Adapted from W. D. Snider, 1994, *Cell* **77**:627.]

A Cascade of Caspase Proteins Functions in One Apoptotic Pathway

Key insights into the molecular mechanisms regulating cell death came from genetic studies in *C. elegans*. As we noted in Section 22.1, scientists have traced the lineage of all the somatic cells in *C. elegans* from the fertilized egg to the mature worm (see Figure 22-9). Of the 947 nongonadal cells generated during development of the adult hermaphrodite form, 131 cells undergo programmed cell death.

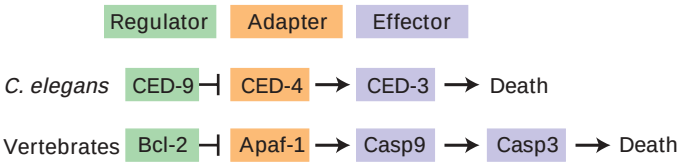
Specific mutations have identified a variety of genes whose encoded proteins play an essential role in controlling programmed cell death during *C. elegans* development. For instance, programmed cell death does not occur in worms carrying loss-of-function mutations in the *ced-3* gene or the *ced-4* gene; as a result, the 131 "doomed" cells survive (Figure 22-30). CED-4 is a protease-activating factor that causes autocleavage of the CED-3 precursor protein, creating an active CED-3 protease that initiates cell death (Figure 22-31). In contrast, in *ced-9* mutants, all cells die during embryonic life, so the adult form never develops. These genetic studies indicate that the CED-3 and CED-4 proteins are required for cell death, that CED-9 suppresses apoptosis, and that the apoptotic pathway can be activated in all cells. Moreover, the finding that cell death does not occur in *ced-9/ced-3* double mutants suggests that CED-9 acts "upstream" of CED-3 to suppress the apoptotic pathway.

The confluence of genetic studies in worms and studies on human cancer cells first suggested that an evolutionarily conserved pathway mediates apoptosis. The first apoptotic gene to be cloned, *bcl-2*, was isolated from human follicular lymphomas. A mutant form of this gene, created in lymphoma cells by a chromosomal rearrangement, was shown to act as an **oncogene** that promoted cell survival rather than cell death (Chapter 23). The human Bcl-2 protein and worm CED-9 protein are homologous, and a *bcl-2* transgene can



▲ EXPERIMENTAL FIGURE 22-30 **Mutations in the *ced-3* gene block programmed cell death in *C. elegans*.** (a) Newly hatched larva carrying a mutation in the *ced-1* gene. Because mutations in this gene prevent engulfment of dead cells, highly refractile dead cells accumulate (arrows), facilitating their visualization. (b) Newly hatched larva with mutations in both the *ced-1* and *ced-3* genes. The absence of refractile dead cells in these double mutants indicates that no cell deaths occurred. Thus CED-3 protein is required for programmed cell death. [From H. M. Ellis and H. R. Horvitz, 1986, *Cell* **91**:818; courtesy of Hilary Ellis.]

MEDIA CONNECTIONS
Video: Programmed Cell Death in *C. elegans* Embryonic Development



▲ FIGURE 22-31 **Overview of the evolutionarily conserved apoptotis pathway in *C. elegans* and vertebrates.** Three general types of proteins are critical in this conserved pathway. Regulators either promote or suppress apoptosis; two orthologous regulators shown here, CED-9 and Bcl-2, suppress apoptosis in the presence of trophic factors. Adapters interact with both regulators and effectors; in the absence of trophic factors, they promote activation of effectors. Proteases called caspases serve as effector proteins; their activation leads to degradation of various intracellular substrates and eventually cell death. [Adapted from D. L. Vaux and S. J. Korsemeyer, 1999, *Cell* **96**:245.]

block the extensive cell death found in *ced-9* mutant worms. Thus both proteins act as regulators that suppress the apoptotic pathway see (Figure 22-31). In addition, both proteins contain a single transmembrane domain and are localized to the outer mitochondrial, nuclear, and endoplasmic reticulum membranes, where they serve as sensors that control the apoptotic pathway in response to external stimuli. As we discuss below, other regulators promote apoptosis.

The *effector proteins* in the apoptotic pathway are enzymes called **caspases** in vertebrates, so named because they contain a key **c**ysteine residue in the catalytic site and selectively cleave proteins at sites just C-terminal to **as**partate residues. The principal effector protease in *C. elegans* is the CED-3 caspase. Humans have 15 different caspases, all of which are initially made as procaspases that must be cleaved to become active. Such proteolytic processing of proproteins is used repeatedly in blood clotting, generation of digestive enzymes, and generation of hormones (Chapter 17). Initiator caspases (e.g., caspase-9) are activated by autoproteolysis induced by other types of proteins (CED-4 in worms, for example), which help the initiators to aggregate. Activated initiator caspases cleave effector caspases (e.g., caspase-3) and thus quickly amplify the total caspase activity level in the dying cell. The various effector caspases recognize and cleave short amino acid sequences in many different target proteins. They differ in their preferred target sequences. Their specific intracellular targets include proteins of the nuclear lamina and cytoskeleton whose cleavage leads to the demise of a cell. Seven lines of knockout mice have been made, each lacking a particular caspase function. Some homozygotes die as embryos; others are viable but with subtle defects. Strikingly, some exhibit an excessive number of neurons (i.e., neuronal hyperplasia), reaffirming the importance of cell death in development of the nervous system; other mutants have cells that are resistant to apoptosis.

While caspases are critical in many apoptosis events, caspase-independent apoptosis also occurs. Apoptosis-inducing factor (AIF; a flavoprotein) and endonuclease G are two proteins that kill cells on their release from mitochondria.

Cell-culture studies have yielded important insights about a group of *adapter proteins* that coordinate the action of regulators and effectors to control apoptosis (see Figure 22-31). For instance, expression of *C. elegans* CED-4 in a human kidney cell line induces rapid apoptosis. This can be blocked by co-expression of the negative regulator CED-9 (or mammalian Bcl-2), indicating that CED-9 opposes CED-4 action. In addition, CED-9 has been shown to directly bind to CED-4 and move it from the cytosol to intracellular membranes. Thus the pro-apoptotic function of CED-4 is directly suppressed by the anti-apoptotic function of CED-9. CED-4 also binds directly to the CED-3 caspase (and related mammalian caspases) and promotes activation of its protease activity, as noted previously. Biochemical studies have shown that CED-4 can simultaneously bind to both CED-9 and CED-3. These results fit with the genetics, which shows that the absence of CED-9 has no effect if CED-3 is also missing (*ced-3/ced-9* double mutants have no cell death, like *ced-3* mutants).

Pro-Apoptotic Regulators Permit Caspase Activation in the Absence of Trophic Factors

Having introduced the major participants in the apoptotic pathway, we now take a closer look at how the effector caspases are activated in mammalian cells. Although the normal function of CED-9 or Bcl-2 is to suppress the cell-death pathway, other intracellular regulatory proteins promote apoptosis. The first pro-apoptotic regulator to be identified, named Bax, was found associated with Bcl-2 in extracts of cells expressing high levels of Bcl-2. Sequence analysis demonstrated that Bax is related in sequence to CED-9 and Bcl-2, but overproduction of Bax induces cell death rather than protecting cells from apoptosis, as CED-9 and Bcl-2 do. Thus this family of regulatory proteins comprises both *anti-apoptotic* members (e.g., CED-9, Bcl-2) and *pro-apoptotic* members (e.g., Bax). All members of this family, which we refer to as the **Bcl-2 family,** are single-pass transmembrane proteins and can participate in oligomeric interactions. In mammals, six Bcl-2 family members prevent apoptosis and nine promote it. Thus the fate of a given cell—survival or death—may reflect the particular spectrum of Bcl-2 family members made by that cell and the intracellular signaling pathways regulating them.

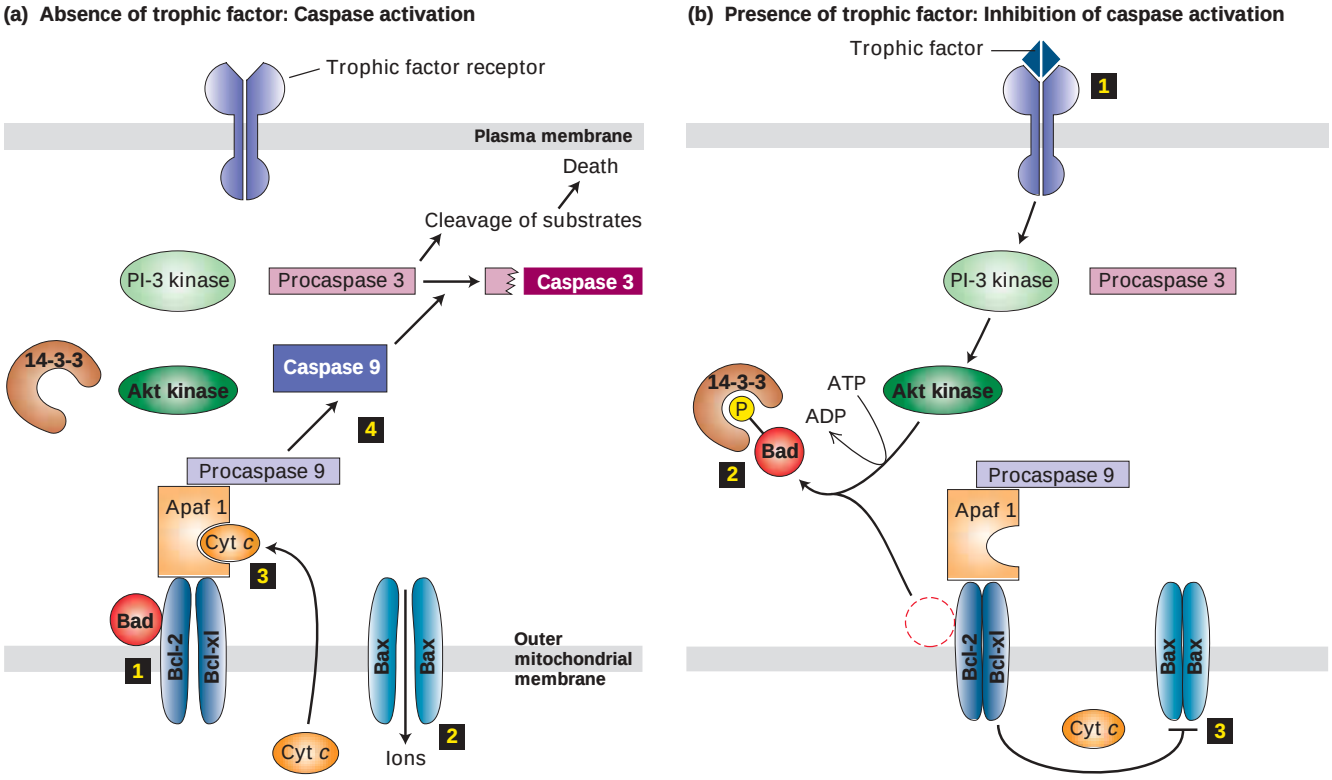
Some Bcl-2 family members preserve or disrupt the integrity of mitochondria, thereby controlling release of mitochondrial proteins such as cytochrome *c*. In normal healthy cells, cytochrome *c* is localized between the inner and outer mitochondrial membrane, but in cells undergoing apoptosis, cytochrome *c* is released into the cytosol. This release can be blocked by overproduction of Bcl-2; conversely, overproduction of Bax promotes release of cytochrome *c* into the cytosol and apoptosis. Moreover, injection of cytochrome *c* into the cytosol of cells induces apoptosis. A variety of death-inducing stimuli cause Bax monomers to move from the cytosol to the outer mitochondrial membrane where they oligomerize. Bax

homodimers, but not Bcl-2 homodimers or Bcl-2/Bax heterodimers, permit influx of ions through the mitochondrial membrane. It remains unclear how this ion influx triggers the release of cytochrome *c*. Once cytochrome *c* is released into the cytosol, it binds to the adapter protein Apaf-1 (the mammalian homolog of CED-4) and promotes activation of a caspase cascade leading to cell death (Figure 22-32a). Bax can cause cell death by a second pathway that triggers mitochondrial dysfunction independently of any caspase action, at least in part by stimulating mitochondrial depolarization.

Gene knockout experiments have dramatically confirmed the importance of both pro-apoptotic and anti-apoptotic Bcl-2 family members in neuronal development. Mice lacking the *bcl-xl* gene, which encodes an anti-apoptotic protein, have massive defects in nervous system development with widespread cell death in the spinal cord, dorsal root ganglion, and brain of developing embryos. In contrast, *bax* knockouts exhibit a marked increase in neurons in some regions of the nervous system. These knockouts also demonstrate that the effects of Bcl-2 family members generally are tissue specific.

Some Trophic Factors Induce Inactivation of a Pro-Apoptotic Regulator

We saw earlier that neurotrophins such as nerve growth factor (NGF) protect neurons from cell death. The intracellular signaling pathways linking such survival factors to inactivation of the cell-death machinery are quite elaborate. The finding that trophic factors appear to work largely independent of protein synthesis suggested that these external signals lead to changes in the activities of preexisting proteins rather than to activation of gene expression. Scientists demonstrated that in the absence of trophic factors, the nonphosphorylated form of Bad is associated with Bcl-2/Bcl-xl at the mitochondrial membrane (see Figure 22-32a). Binding of Bad inhibits the anti-apoptotic function of Bcl-2/Bcl-xl, thereby promoting



▲ FIGURE 22-32 **Proposed intracellular pathways leading to cell death by apoptosis or to trophic factor–mediated cell survival in mammalian cells.** (a) In the absence of a trophic factor, Bad, a soluble pro-apoptotic protein, binds to the anti-apoptotic proteins Bcl-2 and Bcl-xl, which are inserted into the mitochondrial membrane (1). Bad binding prevents the anti-apoptotic proteins from interacting with Bax, a membrane-bound pro-apoptotic protein. As a consequence, Bax forms homo-oligomeric channels in the membrane that mediate ion flux (2). Through an as-yet-unknown mechanism, this leads to the release of cytochrome *c* into the cytosol, where it binds to the adapter protein Apaf-1 (3), promoting a caspase cascade that leads to cell death (4). (b) In some cells, binding of a trophic factor (e.g., NGF) stimulates PI-3 kinase activity, leading to activation of the downstream kinase Akt, which phosphorylates Bad (1). Phosphorylated Bad then forms a complex with the 14-3-3 protein (2). With Bad sequestered in the cytosol, the anti-apoptotic Bcl-2/Bcl-xl proteins can inhibit the activity of Bax (3), thereby preventing the release of cytochrome *c* and activation of the caspase cascade. [Adapted from B. Pettman and C. E. Henderson, 1998, *Neuron* **20**:633.]

MEDIA CONNECTIONS
Focus Animation: Apoptosis

cell death. Phosphorylated Bad, however, cannot bind to Bcl-2/Bcl-xl and is found in the cytosol complexed to the phosphoserine-binding protein 14-3-3. Hence, signaling pathways leading to Bad phosphorylation would be particularly attractive candidates for transmitting survival signals.

A number of trophic factors including NGF have been shown to trigger the PI-3 kinase signaling pathway, leading to activation of a downstream kinase called PKB (see Figure 14-27). Activated PKB phosphorylates Bad at sites known to inhibit its pro-apoptotic activity. Moreover, a constitutively active form of PKB can rescue cultured neurotrophin-deprived neurons, which otherwise would undergo apoptosis and die. These findings support the mechanism for the survival action of trophic factors depicted in Figure 22-32b. In other cell types, different trophic factors may promote cell survival through post-translational modification of other components of the cell-death machinery.

Tumor Necrosis Factor and Related Death Signals Promote Cell Murder by Activating Caspases

Although cell death can arise as a default in the absence of survival factors, apoptosis can also be stimulated by positively acting *"death" signals*. For instance, *tumor necrosis factor (TNF),* which is released by macrophages, triggers the cell death and tissue destruction seen in certain chronic inflammatory diseases. Another important death-inducing signal, the *Fas ligand*, is a cell-surface protein expressed by activated natural killer cells and cytotoxic T lymphocytes. This signal can trigger death of virus-infected cells, some tumor cells, and foreign graft cells.

Both TNF and Fas ligand act through cell-surface "death" receptors that have a single transmembrane domain and are activated when ligand binding brings three receptor molecules into close proximity. The trimeric receptor complex attracts a protein called FADD (Fas-associated death domain), which serves as an adapter to recruit and in some way activate caspase-8, an initiator caspase, in cells receiving a death signal. The death domain found in FADD is a sequence that is present in a number of proteins involved in apoptosis. Once activated, caspase-8 activates other caspases and the amplification cascade begins. To test the ability of the Fas receptor to induce cell death, researchers incubated cells with antibodies against the receptor. These antibodies, which bind and cross-link Fas receptors, were found to stimulate cell death, indicating that activation of the Fas receptor is sufficient to trigger apoptosis.

KEY CONCEPTS OF SECTION 22.5

Cell Death and Its Regulation

- All cells require trophic factors to prevent apoptosis and thus survive. In the absence of these factors, cells commit suicide.
- Genetic studies in *C. elegans* defined an evolutionarily conserved apoptotic pathway with three major components: regulatory proteins, adapter proteins, and effector proteases called caspases in vertebrates (see Figure 22-31).
- Once activated, apoptotic proteases cleave specific intracellular substrates leading to the demise of a cell. Adapter proteins (e.g., Apaf-1), which bind both regulatory proteins and caspases, are required for caspase activation.
- Pro-apoptotic regulator proteins (e.g., Bax, Bad) promote caspase activation, and anti-apoptotic regulators (e.g., Bcl-2) suppress activation. Direct interactions between pro-apoptotic and anti-apoptotic proteins lead to cell death in the absence of trophic factors. Binding of extracellular trophic factors can trigger changes in these interactions, resulting in cell survival (see Figure 22-32).
- The Bcl-2 family contains both pro-apoptotic and anti-apoptotic proteins; all are single-pass transmembrane proteins and engage in protein-protein interactions. Bcl-2 molecules can control the release of cytochrome *c* from mitochondria, triggering cell death.
- Binding of extracellular death signals, such as tumor necrosis factor and Fas ligand, to their receptors activates an associated protein (FADD) that in turn triggers the caspase cascade leading to cell murder.

PERSPECTIVES FOR THE FUTURE

Cell birth, lineage, and death lie at the heart of the growth of an organism and are also central to disease processes, most notably cancer. Few transformations seem more remarkable than the blooming of cell types during development. A lineage beginning with a fertilized egg, a "plain vanilla" 200-μm sphere, produces neurons a yard long, pulsating multinucleate muscle cells, exquisitely light-sensitive retina cells, ravenous macrophages that recognize and engulf germs, and all the hundreds of other cell types. Regulators of cell lineage produce this rich variety by controlling two critical decisions: (1) when and where to activate the cell division cycle (Chapter 21) and (2) whether the two daughter cells will be the same or different. A cell may be just like its parent, or it may embark on a new path.

Cell birth is normally carefully restricted to specific locales and times, such as the basal layer of the skin or the root meristem. Liver regenerates when there is injury, but liver cancer is prevented by restricting unnecessary growth at other times. Cell lineage is patterned by the asymmetric distribution of key regulators to the daughter cells of a division. Some of these regulators are intrinsic to the parent cell, becoming asymmetrically distributed during polarization of the cell; other regulators are external signals that differentially reach the daughter cells. Asymmetry of cells becomes asymmetry of tissues and whole organisms. Our left and right hands differ only as a result of cell asymmetry.

Some cells persist for the life of the organism, but others such as blood and intestinal cells turn over rapidly. Many cells live for awhile and are then programmed to die and be replaced by others arising from a stem-cell population. Programmed cell death is also the basis for the meticulous elimination of potentially harmful cells, such as autoreactive immune cells, which attack the body's own cells, or neurons that have failed to properly connect. Cell-death programs have also evolved as a defense against infection, and virus-infected cells are selectively murdered in response to death signals. Viruses, in turn, devote much of their effort to evading host defenses. For example, p53, a transcription factor that senses cell stresses and damage and activates transcription of pro-apoptotic members of the *bcl-2* gene family, is inhibited by the adenovirus E1B protein. It has been estimated that about a third of the adenovirus genome is directed at evading host defenses. Cell death is relevant to toxic chemicals as well as viral infections; malformations due to poisons often originate from excess apoptosis.

Failures of programmed cell death can lead to uncontrolled cancerous growth (Chapter 23). The proteins that prevent the death of cancer cells therefore become possible targets for drugs. A tumor may contain a mixture of cells, some capable of seeding new tumors or continued uncontrolled growth, and some capable only of growing in place or for a limited time. In this sense the tumor has its own stem cells, and they must be found and studied, so they become vulnerable to our medicine. One option is to manipulate the cell-death pathway to our own advantage, to send the signals that will make cancer cells destroy themselves.

Much attention is now being given to the regulation of stem cells in an effort to understand how dividing populations of cells are created and maintained. This has clear implications for repair of tissue, for example, to help damaged eyes, torn cartilage, degenerating brain tissue, or failing organs. One interesting possibility is that some populations of stem cells with the potential to generate or regenerate tissue are normally eliminated by cell death during later development. If so, finding ways to selectively block the death of the cells could make regeneration more likely. Could the elimination of such cells during mammalian development be the difference between an amphibian capable of limb regeneration and a mammal that is not?

KEY TERMS

apical complex *922*
apoptosis *924*
asymmetric cell division *900*
β-catenin *903*
Bcl-2 family *928*
caspases *928*
cell lineage *899*
"death" signals *930*
determination *914*
differentiation *899*
ectoderm *906*
embryonic stem (ES) cells *901*
endoderm *906*
ganglion mother cell (GMC) *921*
germ line *906*
heterochronic mutants *908*
MAT locus *910*
mating factor *912*
mating-type switching *920*
meristems *905*
mesoderm *906*
micro RNAs (miRNAs) *909*
muscle regulatory factors (MRFs) *915*
neurotrophins *926*
pluripotent *900*
precursor/progenitor cells *899*
somatic cells *899*
stem cells *899*
trophic factors *924*

REVIEW THE CONCEPTS

1. What two properties define a stem cell? Distinguish between a totipotential stem cell, a pluripotent stem cell, and a progenitor cell.

2. Where are stem cells located in plants? Where are stem cells located in adult animals? How does the concept of stem cell differ between animal and plant systems?

3. In 1997, Dolly the sheep was cloned by a technique called somatic cell nuclear transfer. A nucleus from an adult mammary cell was transferred into an egg from which the nucleus had been removed. The egg was allowed to divide several times in culture, then the embryo was transferred to a surrogate mother who gave birth to Dolly. Dolly died in 2003 after mating and giving birth herself to viable offspring. What does the creation of Dolly tell us about the potential of nuclear material derived from a fully differentiated adult cell? Does the creation of Dolly tell us anything about the potential of an intact, fully differentiated adult cell? Name three types of information that function to preserve cell type. Which of these types of information was shown to be reversible by the Dolly experiment?

4. The roundworm *C. elegans* has proven to be a valuable model organism for studies of cell birth, cell lineage, and cell death. What properties of *C. elegans* render it so well suited for these studies? Why is so much information from *C. elegans* experiments of use to investigators interested in mammalian development?

5. In the budding yeast *S. cerevisiae*, what is the role of the MCM1 protein in the following?

a. transcription of **a**-specific genes in **a** cells

b. blocking transcription of α-specific genes in **a** cells

c. transcription of α-specific genes in α cells

d. blocking transcription of **a**-specific genes in α cells

6. In *S. cerevisiae*, what ensures that **a** and α cells mate with one another rather than with cells of the same mating type (i.e., **a** with **a** or α with α)?

7. Exposure of C3H 10T1/2 cells to 5-azacytidine, a nucleotide analog, is a model system for muscle differentiation. How was 5-azacytidine treatment used to isolate the genes involved in muscle differentiation?

8. Through the experiments on C3H 10T1/2 cells treated with 5-azacytidine, MyoD was identified as a key transcription factor in regulating the differentiation of muscle. To what general class of DNA-binding proteins does MyoD belong? How do the interactions of MyoD with the following proteins affect its function? (a) E2A, (b) MEFs, (c) Id

9. The mechanisms that regulate muscle differentiation in mammals and neural differentiation in *Drosophila* (and probably mammals as well) bear remarkable similarities. What proteins function analogous to MyoD, myogenin, Id, and E2A in neural cell differentiation in *Drosophila*? Based on these analogies, predict the effect of microinjection of MyoD mRNA on the development of *Xenopus* embryos.

10. Predict the effect of the following mutations on the ability of mother and daughter cells of *S. cerevisae* to undergo mating type switching following cell division:

a. loss-of-function mutation in the HO endonuclease

b. gain-of-function mutation that renders HO endonuclease gene constitutively expressed independent of SWI/SNF

c. gain-of-function mutation in SWI/SNF that renders it insensitive to Ash1

11. Asymmetric cell division often relies on cytoskeletal elements to generate or maintain asymmetric distribution of cellular factors. In *S. cerevisiae*, what factor is localized to the bud by myosin motors? In *Drosophila* neuroblasts, what factors are localized apically by microtubules?

12. How do studies of brain development in knockout mice support the statement that apoptosis is a default pathway in neuronal cells?

13. What morphologic features distinguish programmed cell death and necrotic cell death? TNF and Fas ligand bind cell surface receptors to trigger cell death. Although the death signal is generated external to the cell, why do we consider the death induced by these molecules to be apoptotic rather than necrotic?

14. Predict the effects of the following mutations on the ability of a cell to undergo apoptosis:

a. mutation in Bad such that it cannot be phosphorylated by Akt

b. overexpression of Bcl-2

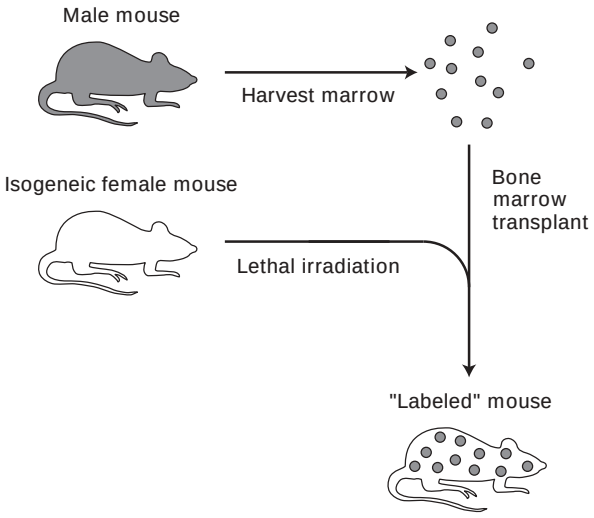
c. mutation in Bax such that it cannot form homodimers

One common characteristic of cancer cells is a loss of function in the apoptotic pathway. Which of the mutations listed might you expect to find in some cancer cells?

ANALYZE THE DATA

To better understand the potential of adult stem cells to differentiate into various cell types, the following studies were performed.

Bone marrow was harvested from adult male mice and then transplanted into isogeneic female recipient mice that had been treated with a level of irradiation sufficient to destroy their own hematopoetic stem cells (see the figure). Although the dose of irradiation given was lethal to mice that did not receive a transplant, the majority of the recipient mice receiving the transplant survived. After 4 weeks, peripheral blood from the recipient mice was analyzed. The composition of the blood was normal with respect to all blood cell types. Every blood cell examined was determined to be positive for the presence of the Y chromosome.



a. What was the purpose of using male mice as the bone marrow donors and female mice as the recipients? Would the converse experiment (female donors, male recipients) have worked?

b. What was the purpose of irradiating the recipient mice prior to transplantation? What outcome might you expect if bone marrow cells were transplanted to nonirradiated mice?

c. What method could have been used to purify hematopoetic stem cells from bone marrow before transplanting these cells into the recipient mouse? How might a purification step have affected the outcome of the experiment?

After 8 weeks, some of the recipient mice were sacrificed and histochemical analysis was performed on tissue sections of

various organs. Occasional Y chromosome–positive cells were found in the liver, skeletal muscle, and brain. Furthermore, the Y chromosome–positive cells were positive for markers of liver hepatocytes, skeletal muscle cells, and neurons (albumin, dystrophin, and NeuN, respectively).

d. What does the appearance of Y chromosome–positive cells in multiple organs and the expression of organ-specific markers indicate about the localization of cells derived from bone marrow? About their differentiation potential? About their function?

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