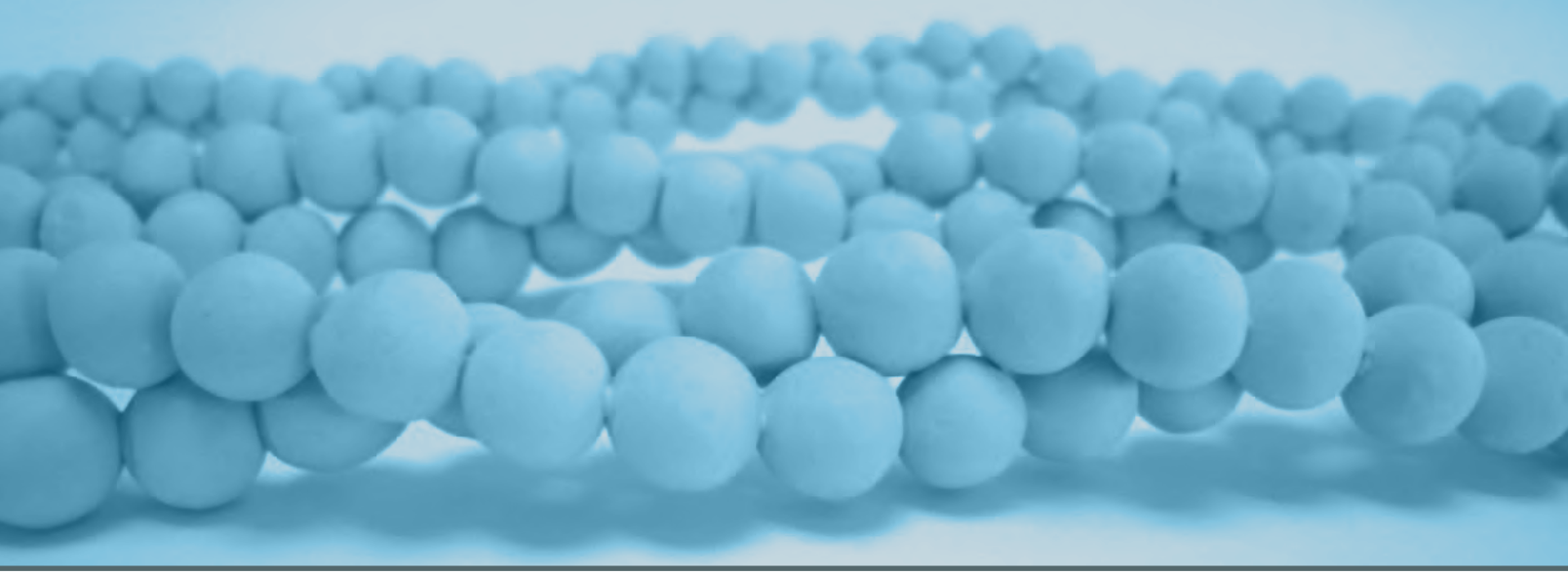


CHAPTER **SEVENTEEN** 17

Cytoskeleton

The ability of eukaryotic cells to adopt a variety of shapes, organize the many components in their interior, interact mechanically with the environment, and carry out coordinated movements depends on the **cytoskeleton**—an intricate network of protein filaments that extends throughout the cytoplasm (**Figure 17–1**). This filamentous architecture helps to support the large volume of cytoplasm, a function that is particularly important in animal cells, which have no cell walls. Although some cytoskeletal components are present in bacteria, the cytoskeleton is most prominent in the large and structurally complex eukaryotic cell.

Unlike our own bony skeleton, however, the cytoskeleton is a highly dynamic structure that is continuously reorganized as a cell changes shape, divides, and responds to its environment. The cytoskeleton is not only the "bones" of a cell but its "muscles" too, and it is directly responsible for large-scale movements, including the crawling of cells along a surface, the contraction of muscle cells, and the changes in cell shape that take place as an embryo develops. Without the cytoskeleton, wounds would never heal, muscles would not contract, and sperm would never reach the egg.

Like any factory making a complex product, the eukaryotic cell has a highly organized interior in which organelles that carry out specialized functions are concentrated in different areas and linked by transport systems (discussed in Chapter 15). The cytoskeleton controls the location of the organelles and provides the machinery for transport between them. It is also responsible for the segregation of chromosomes into two daughter cells at cell division and for pinching apart those two new cells, as we discuss in Chapter 18.

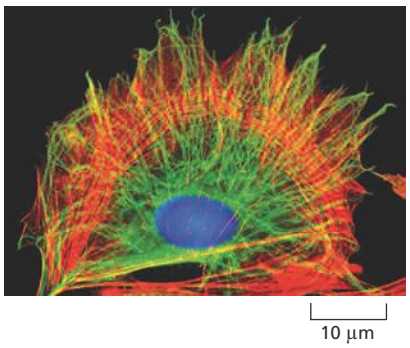
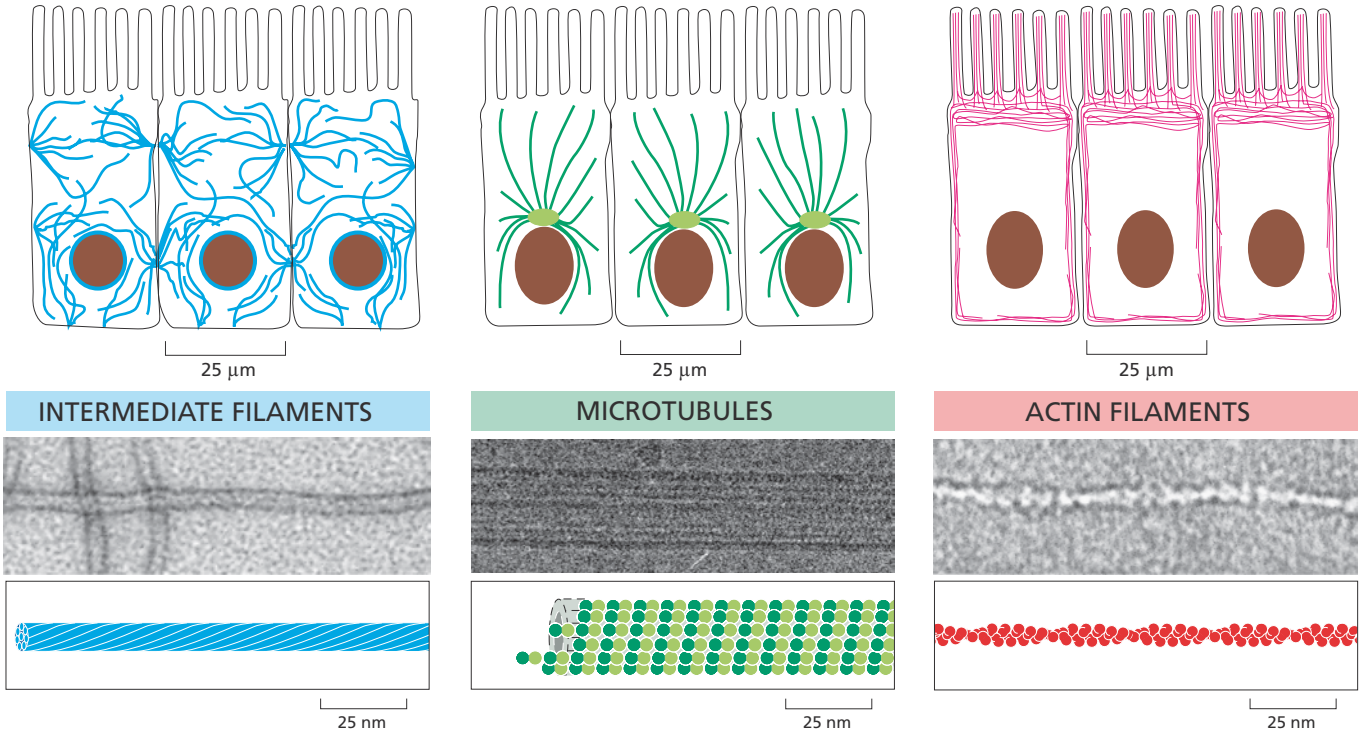


Figure 17–1 The cytoskeleton gives a cell its shape and allows the cell to organize its internal components and to move. An animal cell in culture has been labeled to show two of its major cytoskeletal systems, the microtubules (*green*) and the actin filaments (*red*). Where the two filaments overlap, they appear *yellow*. The DNA in the nucleus is labeled in *blue*. (Courtesy of Albert Tousson.)

The cytoskeleton is built on a framework of three types of protein filaments: *intermediate filaments*, microtubules, and *actin filaments*. Each type of filament has distinct mechanical properties and is formed from a different protein subunit. A family of fibrous proteins forms the intermediate filaments; globular *tubulin* subunits form microtubules; and globular *actin* subunits form actin filaments (**Figure 17–2**). In each case, thousands of subunits assemble into fine threads that sometimes extend across the entire cell.

In this chapter, we consider the structure and function of each of these protein filament networks. We begin with intermediate filaments, which provide cells with mechanical strength. We then see how microtubules organize the cytoplasm of eukaryotic cells and form the hairlike motile appendages that enable cells like protozoa and sperm to swim. We next consider how the actin cytoskeleton supports the cell surface and allows fibroblasts and other cells to crawl. Finally, we discuss how the actin cytoskeleton enables our muscles to contract.

Figure 17–2 The three types of protein filaments that form the cytoskeleton differ in their composition, mechanical properties, and roles inside the cell. They are shown here in epithelial cells, but they are all found in almost all animal cells.



Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of fibrous intermediate filament proteins. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength and distributing the mechanical stresses in an epithelial tissue by spanning the cytoplasm from one cell–cell junction to another. Intermediate filaments are very flexible and have great tensile strength. They deform under stress but do not rupture. (Micrograph courtesy of Roy Quinlan.)

Microtubules are hollow cylinders made of the protein tubulin. They are long and straight and typically have one end attached to a single microtubule-organizing center called a *centrosome*. With an outer diameter of 25 nm, microtubules are more rigid than actin filaments or intermediate filaments, and they rupture when stretched. (Micrograph courtesy of Richard Wade.)

Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures, with a diameter of about 7 nm, that are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, the layer of cytoplasm just beneath the plasma membrane. (Micrograph courtesy of Roger Craig.)

INTERMEDIATE FILAMENTS

Intermediate filaments have great tensile strength, and their main function is to enable cells to withstand the mechanical stress that occurs when cells are stretched. The filaments are called "intermediate" because, in the smooth muscle cells where they were first discovered, their diameter (about 10 nm) is between that of the thinner actin filaments and the thicker *myosin filaments*. Intermediate filaments are the toughest and most durable of the cytoskeletal filaments: when cells are treated with concentrated salt solutions and nonionic detergents, the intermediate filaments survive, while most of the rest of the cytoskeleton is destroyed.

Intermediate filaments are found in the cytoplasm of most animal cells. They typically form a network throughout the cytoplasm, surrounding the nucleus and extending out to the cell periphery. There they are often anchored to the plasma membrane at cell–cell junctions called *desmosomes* (discussed in Chapter 20), where the plasma membrane is connected to that of another cell (**Figure 17–3**). Intermediate filaments are also found within the nucleus of all eukaryotic cells. There they form a meshwork called the *nuclear lamina*, which underlies and strengthens the nuclear envelope. In this section, we see how the structure and assembly of intermediate filaments makes them particularly suited to strengthening cells and protecting them from tearing.

Intermediate Filaments Are Strong and Ropelike

An intermediate filament is like a rope in which many long strands are twisted together to provide tensile strength (**Movie 17.1**). The strands of this cable are made of intermediate filament proteins, fibrous subunits each containing a central elongated rod domain with distinct unstructured domains at either end (**Figure 17–4A**). The rod domain consists of an extended α-helical region that enables pairs of intermediate filament

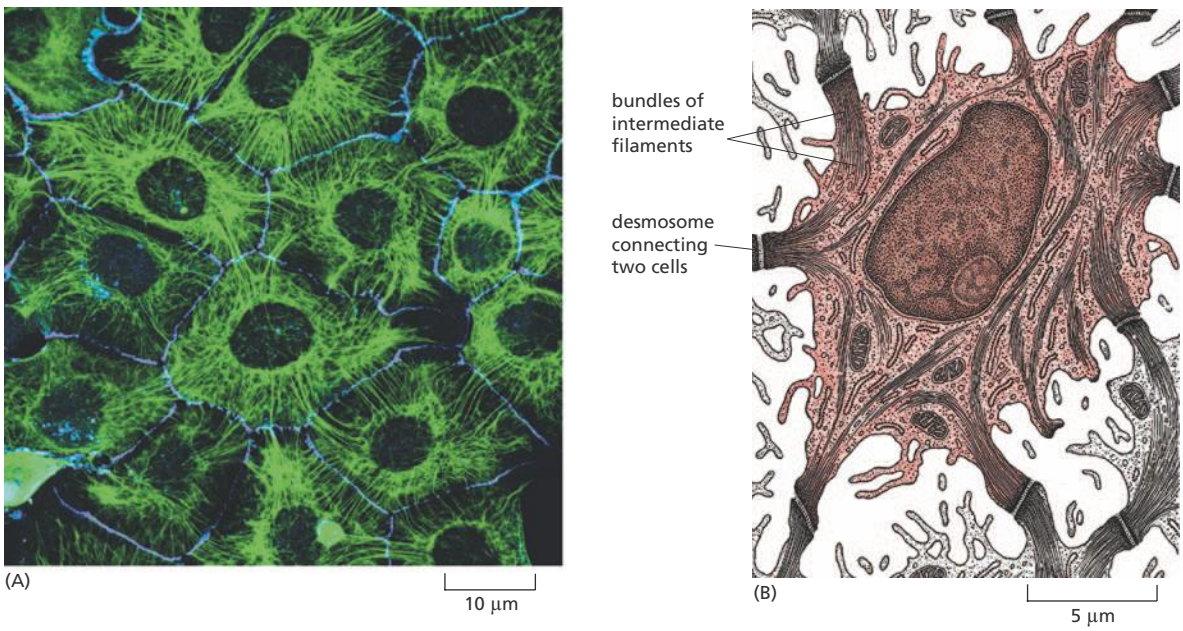


Figure 17–3 Intermediate filaments form a strong, durable network in the cytoplasm of the cell. (A) Immuno-fluorescence micrograph of a sheet of epithelial cells in culture stained to show the lacelike network of intermediate keratin filaments (*green*), which surround the nuclei and extend through the cytoplasm of the cells. The filaments in each cell are indirectly connected to those of neighboring cells through the desmosomes, establishing a continuous mechanical link from cell to cell throughout the epithelial sheet. A second protein (*blue*) has been stained to show the locations of the cell boundaries. (B) Drawing from an electron micrograph of a section of a skin cell showing the bundles of intermediate filaments that traverse the cytoplasm and are inserted at desmosomes. (A, courtesy of Kathleen Green and Evangeline Amargo; B, from R.V. Krstić, Ultrastructure of the Mammalian Cell: An Atlas. Berlin: Springer, 1979. With permission from Springer-Verlag.)

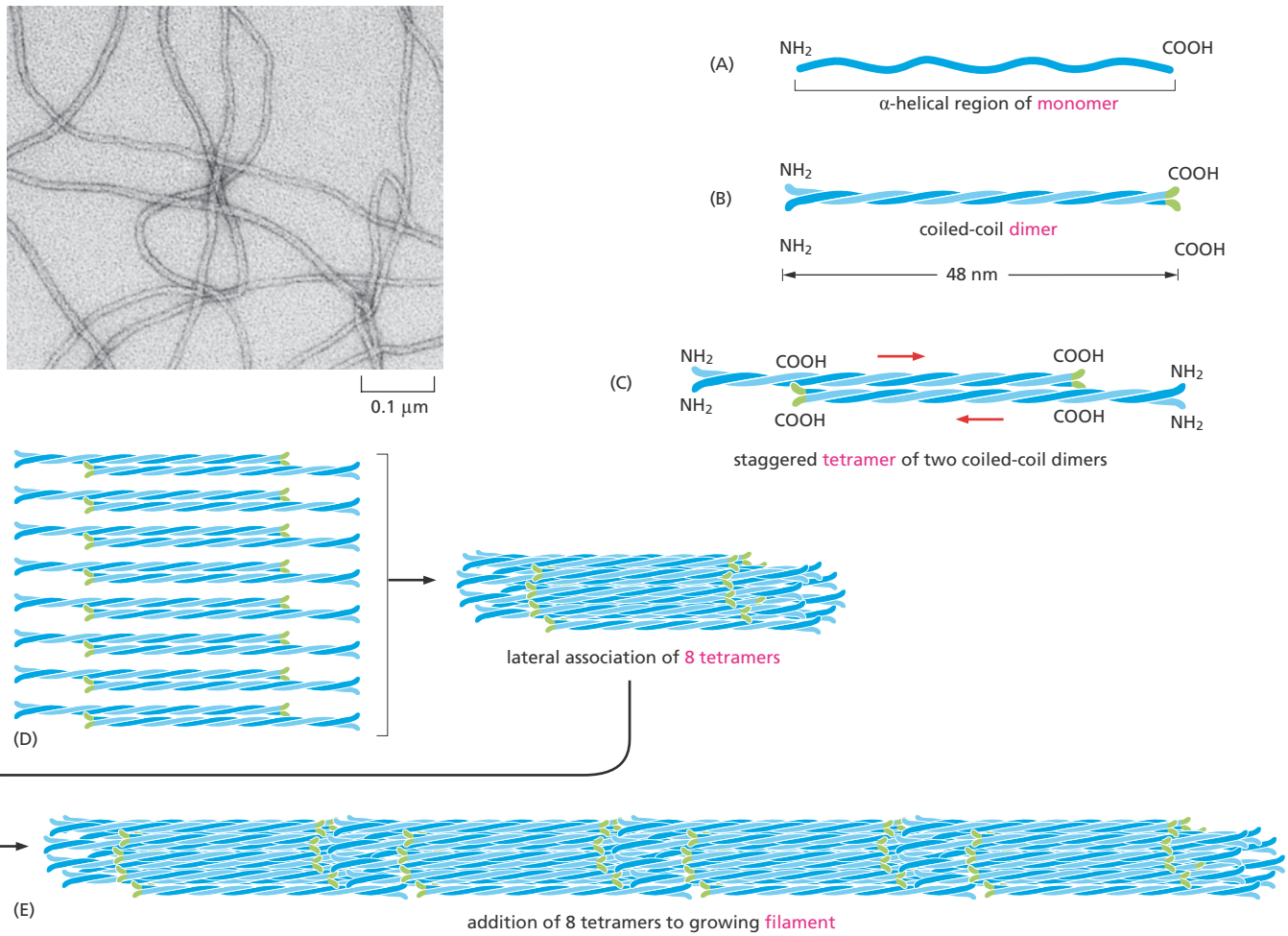


Figure 17–4 Intermediate filaments are like ropes made of long, twisted strands of protein. The intermediate filament monomer consists of an α-helical central rod domain (A) with unstructured regions at either end (not shown). Pairs of monomers associate to form a dimer (B), and two dimers then line up to form a staggered, antiparallel tetramer (C). Tetramers can pack together into a helical array containing eight tetramer strands (D), which in turn assemble into the final ropelike intermediate filament (E). An electron micrograph of intermediate filaments is shown on the upper left. (Micrograph courtesy of Roy Quinlan.)

proteins to form stable dimers by wrapping around each other in a coiled-coil configuration (**Figure 17–4B**), as described in Chapter 4. Two of these coiled-coil dimers, running in opposite directions, associate to form a staggered tetramer (**Figure 17–4C**). These dimers and tetramers are the soluble subunits of intermediate filaments. The tetramers associate with each other side-by-side (**Figure 17–4D**) and then assemble to generate the final ropelike intermediate filament (**Figure 17–4E**).

Because the two dimers point in opposite directions, the two ends of the tetramer are the same, as are the two ends of assembled intermediate filaments; as we will see, this distinguishes these filaments from microtubules and actin filaments, whose structural polarity is crucial for their function. All the interactions between the intermediate filament proteins depend solely on noncovalent bonding; it is the combined strength of the overlapping lateral interactions along the length of the proteins that gives intermediate filaments their great tensile strength.

The central rod domains of different intermediate filament proteins are all similar in size and amino acid sequence, so that when they pack together they always form filaments of similar diameter and internal structure. By contrast, the terminal domains vary greatly in both size and amino acid sequence from one type of intermediate filament protein to another.

These unstructured domains are exposed on the surface of the filament, where they allow it to interact with specific components in the cytoplasm.

Intermediate Filaments Strengthen Cells Against Mechanical Stress

Intermediate filaments are particularly prominent in the cytoplasm of cells that are subject to mechanical stress. They are present in large numbers, for example, along the length of nerve cell axons, providing essential internal reinforcement to these extremely long and fine cell extensions. They are also abundant in muscle cells and in epithelial cells such as those of the skin. In all these cells, intermediate filaments distribute the effect of locally applied forces, thereby keeping cells and their membranes from tearing in response to mechanical shear. A similar principle is used to strengthen composite materials such as fiberglass or reinforced concrete, in which tension-bearing linear elements such as carbon fibers (in fiberglass) or steel bars (in concrete) are embedded in a space-filling matrix to give the material strength.

Intermediate filaments can be grouped into four classes: (1) *keratin filaments* in epithelial cells; (2) *vimentin* and *vimentin-related filaments* in connective-tissue cells, muscle cells, and supporting cells of the nervous system (glial cells); (3) *neurofilaments* in nerve cells; and (4) *nuclear lamins*, which strengthen the nuclear envelope. The first three filament types are found in the cytoplasm, whereas the fourth is found in the nucleus (**Figure 17–5**). Filaments of each class are formed by polymerization of their corresponding intermediate filament subunits.

The **keratin filaments** are the most diverse class of intermediate filament. Every kind of epithelium in the vertebrate body—whether in the tongue, the cornea, or the lining of the gut—has its own distinctive mixture of keratin proteins. Specialized keratins also occur in hair, feathers, and claws. In each case, the keratin filaments are formed from a mixture of different keratin subunits. Keratin filaments typically span the interiors of epithelial cells from one side of the cell to the other, and filaments in adjacent epithelial cells are indirectly connected through desmosomes (see Figure 17–3B). The ends of the keratin filaments are anchored to the desmosomes, and the filaments associate laterally with other cell components through the globular head and tail domains that project from their surface. This cabling of high tensile strength, formed by the filaments throughout the epithelial sheet, distributes the stress that occurs when the skin is stretched. The importance of this function is illustrated by the rare human genetic disease *epidermolysis bullosa simplex*, in which mutations in the keratin genes interfere with the formation of keratin filaments in the epidermis. As a result, the skin is highly vulnerable to mechanical injury, and even a gentle pressure can rupture its cells, causing the skin

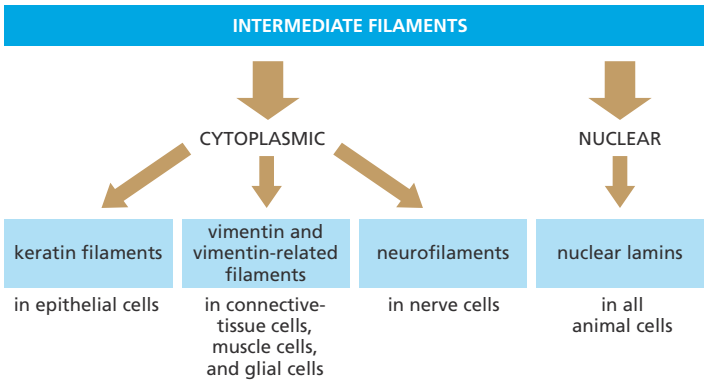


Figure 17–5 Intermediate filaments are divided into four major classes. These classes can include numerous subtypes. Humans, for example, have more than 50 keratin genes.

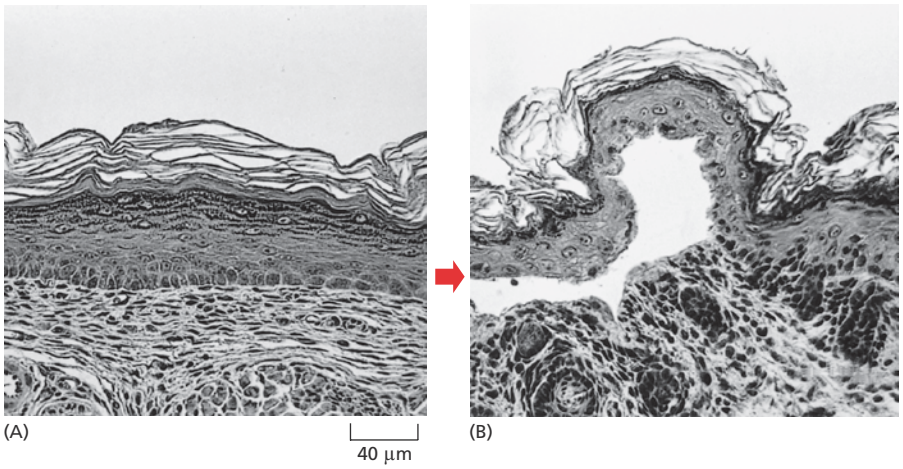


Figure 17–6 A mutant form of keratin makes skin more prone to blistering. A mutant gene encoding a truncated keratin protein was introduced into a mouse. The defective protein assembles with the normal keratins and thereby disrupts the keratin filament network in the skin. (A) Light micrograph of a cross section of normal skin, which is resistant to mechanical pressure. (B) Cross section of skin from mutant mouse shows the formation of a blister, which results from the rupturing of cells in the basal layer of the mutant epidermis (short *red* arrow). (From P.A. Coulombe et al., *J. Cell Biol.* 115:1661–1674, 1991. With permission from The Rockefeller University Press.)

to blister. The disease can be reproduced in transgenic mice expressing a mutant *keratin* gene in their skin (**Figure 17–6**).

Many of the intermediate filaments are further stabilized and reinforced by accessory proteins, such as *plectin*, that cross-link the filaments into bundles and link them to microtubules, to actin filaments, and to adhesive structures in the desmosomes (**Figure 17–7**). Mutations in the gene for plectin cause a devastating human disease that combines features of epidermolysis bullosa simplex (caused by disruption of skin keratin), muscular dystrophy (caused by disruption of intermediate filaments in muscle), and neurodegeneration (caused by disruption of neurofilaments). Mice lacking a functional plectin gene die within a few days of birth, with blistered skin and abnormal skeletal and heart muscle. Thus although plectin may not be necessary for the initial formation of intermediate filaments, its cross-linking action is required to provide cells with the strength they need to withstand mechanical stress.

QUESTION 17–1

Which of the following types of cells would you expect to contain a high density of intermediate filaments in their cytoplasm? Explain your answers.

A. *Amoeba proteus* (a free-living amoeba)
B. Skin epithelial cell
C. Smooth muscle cell in the digestive tract
D. *Escherichia coli*
E. Nerve cell in the spinal cord
F. Sperm cell
G. Plant cell

The Nuclear Envelope Is Supported by a Meshwork of Intermediate Filaments

Whereas cytoplasmic intermediate filaments form ropelike structures, the intermediate filaments lining and strengthening the inside surface of the inner nuclear membrane are organized as a two-dimensional meshwork (**Figure 17–8**). As mentioned earlier, the intermediate filaments that form this tough **nuclear lamina** are constructed from a class of intermediate filament proteins called *lamins* (not to be confused with laminin, which is an extracellular matrix protein). The nuclear lamina disassembles and re-forms at each cell division, when the nuclear envelope breaks down during mitosis and then re-forms in each daughter cell (discussed in Chapter 18). Cytoplasmic intermediate filaments also disassemble in mitosis.

The disassembly and reassembly of the nuclear lamina are controlled by the phosphorylation and dephosphorylation of the lamins. When the

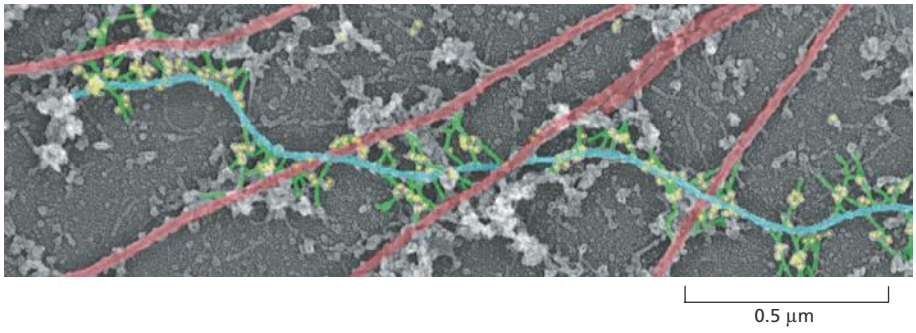


Figure 17–7 Plectin aids in the bundling of intermediate filaments and links these filaments to other cytoskeletal protein networks. In this scanning electron micrograph of the cytoskeletal protein network from cultured fibroblasts, the actin filaments have been removed, and the plectin, intermediate filaments, and microtubules have been artificially colored. Note how the plectin (*green*) links an intermediate filament (*blue*) to three microtubules (*red*). The *yellow dots* are gold particles linked to antibodies that recognize plectin. (From T.M. Svitkina and G.G. Borisy, *J. Cell Biol.* 135:991–1007, 1996. With permission from The Rockefeller University Press.)

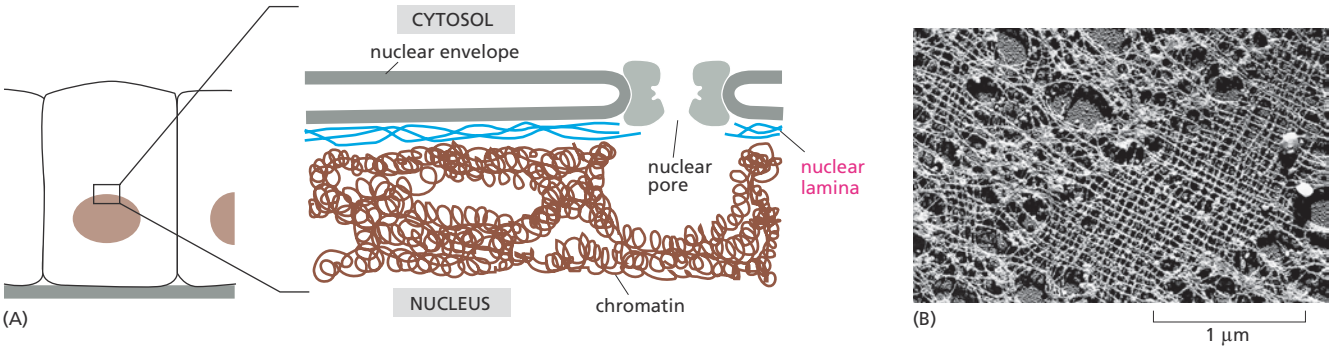


Figure 17–8 Intermediate filaments support and strengthen the nuclear envelope. (A) Schematic cross section through the nuclear envelope. The intermediate filaments of the nuclear lamina line the inner face of the nuclear envelope and are thought to provide attachment sites for the chromosomes. (B) Electron micrograph of a portion of the nuclear lamina from a frog egg. The lamina is formed from a square lattice of intermediate filaments composed of lamins. (The nuclear lamina in other cell types is not always as regularly organized as the one shown here.) (B, courtesy of Ueli Aebi.)

lamins are phosphorylated by protein kinases (discussed in Chapter 4), the consequent conformational change weakens the binding between the lamin tetramers and causes the filaments to fall apart. Dephosphorylation by protein phosphatases at the end of mitosis causes the lamins to reassemble (see Figure 18–30).

Defects in a particular nuclear lamin are associated with certain types of *progeria*—rare disorders that cause affected individuals to age prematurely. Children with progeria have wrinkled skin, lose their teeth and hair, and often develop severe cardiovascular disease by the time they reach their teens (**Figure 17–9**). How the loss of a nuclear lamin could lead to this devastating condition is not yet known, but it may be that the resulting nuclear instability leads to impaired cell division, increased cell death, a diminished capacity for tissue repair, or some combination of these.

MICROTUBULES

Microtubules have a crucial organizing role in all eukaryotic cells. These long and relatively stiff hollow tubes of protein can rapidly disassemble in one location and reassemble in another. In a typical animal cell, microtubules grow out from a small structure near the center of the cell called the *centrosome* (**Figure 17–10A and B**). Extending out toward the cell periphery, they create a system of tracks within the cell, along which vesicles, organelles, and other cell components can be transported. These cytoplasmic microtubules are the part of the cytoskeleton mainly responsible for transporting and positioning membrane-enclosed organelles within the cell and for guiding the intracellular transport of various cytosolic macromolecules.

When a cell enters mitosis, the cytoplasmic microtubules disassemble and then reassemble into an intricate structure called the *mitotic spindle*. As we discuss in Chapter 18, the mitotic spindle provides the machinery that will segregate the chromosomes equally into the two daughter cells just before a cell divides (**Figure 17–10C**). Microtubules can also form stable structures, such as rhythmically beating *cilia* and *flagella* (**Figure 17–10D**). These hairlike structures extend from the surface of many eukaryotic cells, which use them either to swim or to sweep fluid over their surface. The core of a eukaryotic cilium or flagellum consists of a highly organized and stable bundle of microtubules. (Bacterial flagella have an entirely different structure and allow the cells to swim by a very different mechanism.)

Figure 17–9 Defects in a nuclear lamin can cause a rare class of premature aging disorders called progeria. Children with progeria begin to show advanced features of aging around 18 to 24 months of age. (Courtesy of Progeria Research Foundation.)

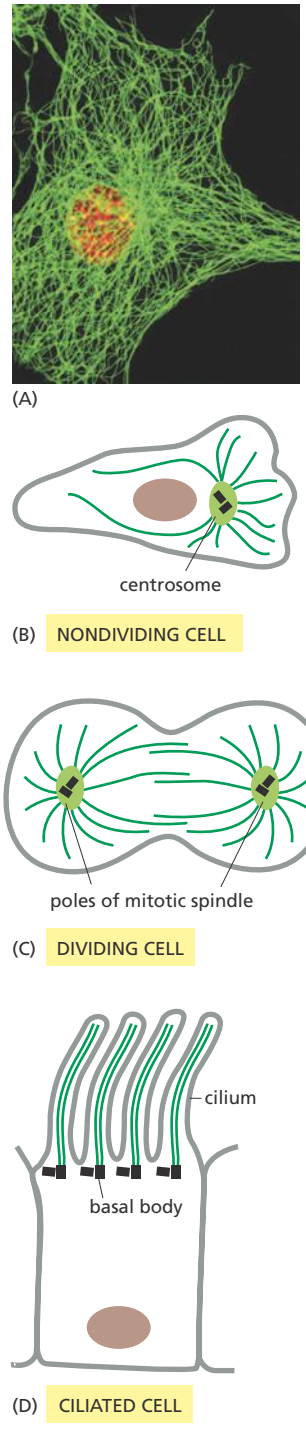


Figure 17–10 Microtubules usually grow out from an organizing center. (A) Fluorescence micrograph of a cytoplasmic array of microtubules in a cultured fibroblast. Unlike intermediate filaments, microtubules (*dark green*) extend from organizing centers such as (B) a centrosome, (C) the two poles of a mitotic spindle, or (D) the basal body of a cilium. They can also grow from fragments of existing microtubules (not shown). (A, courtesy of Michael Davidson and The Florida State University Research Foundation.)

In this section, we first consider the structure and assembly of microtubules. We then discuss their role in organizing the cytoplasm—an ability that depends on their association with accessory proteins, especially the *motor proteins* that propel organelles along cytoskeletal tracks. Finally, we discuss the structure and function of cilia and flagella, in which microtubules are stably associated with motor proteins that power the beating of these mobile appendages.

Microtubules Are Hollow Tubes with Structurally Distinct Ends

Microtubules are built from subunits—molecules of **tubulin**—each of which is itself a dimer composed of two very similar globular proteins called α-tubulin and β-tubulin, bound tightly together by noncovalent interactions. The tubulin dimers stack together, again by noncovalent bonding, to form the wall of the hollow cylindrical microtubule. This tubelike structure is made of 13 parallel protofilaments, each a linear chain of tubulin dimers with α- and β-tubulin alternating along its length (**Figure 17–11**). Each protofilament has a structural polarity,

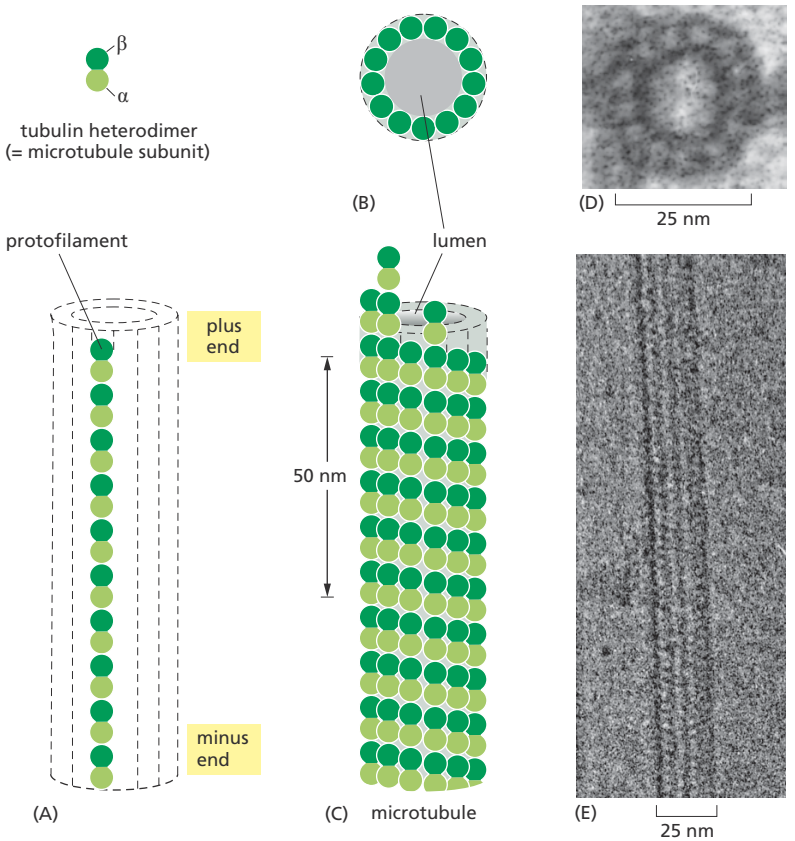


Figure 17–11 Microtubules are hollow tubes made of globular tubulin subunits. (A) One tubulin subunit (an αβ dimer) and one protofilament are shown schematically, together with their location in the microtubule wall. Note that the tubulin dimers are all arranged in the protofilament with the same orientation. (B and C) Schematic diagrams of a microtubule, showing how tubulin dimers pack together in the microtubule wall. At the top, 13 β-tubulin molecules are shown in cross section. Below this, a side view of a short section of a microtubule shows how the dimers are aligned in the same orientation in all the protofilaments; thus, the microtubule has a definite structural polarity—with a designated plus and a minus end. (D) Electron micrograph of a cross section of a microtubule with its ring of 13 distinct subunits, each of which corresponds to a separate tubulin dimer. (E) Electron micrograph of a microtubule viewed lengthwise. (D, courtesy of Richard Linck; E, courtesy of Richard Wade.)

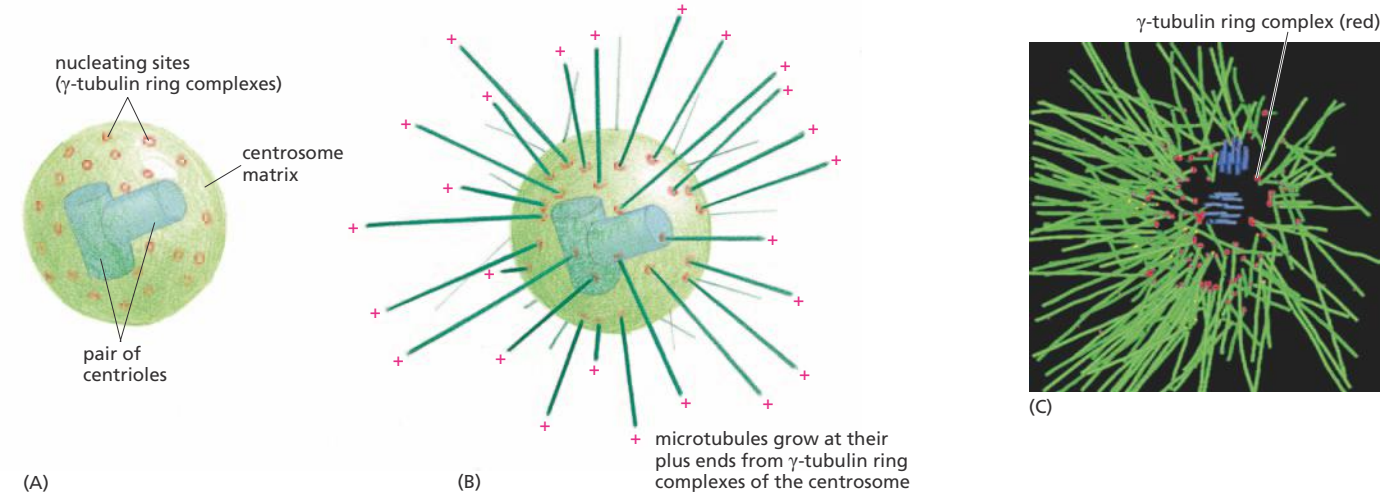


Figure 17–12 Tubulin polymerizes from nucleation sites on a centrosome. (A) Schematic drawing showing that an animal cell centrosome consists of an amorphous matrix of various proteins, including the γ-tubulin rings (*red*) that nucleate microtubule growth, surrounding a pair of centrioles, oriented at right angles to each other. Each member of the centriole pair is made up of a cylindrical array of short microtubules. (B) Diagram of a centrosome with attached microtubules. The minus end of each microtubule is embedded in the centrosome, having grown from a γ-tubulin ring complex, whereas the plus end of each microtubule extends into the cytoplasm. (C) A reconstructed image of a centrosome of a *C. elegans* cell showing a dense thicket of microtubules emanating from γ-tubulin ring complexes. A pair of centrioles (*blue*) can be seen at the center. (C, from E.T. O'Toole et al., *J. Cell Biol.* 163:451–456, 2003. With permission from The Rockefeller University Press.)

with α-tubulin exposed at one end and β-tubulin at the other, and this **polarity**—the directional arrow embodied in the structure—is the same for all the protofilaments, giving a structural polarity to the microtubule as a whole. One end of the microtubule, thought to be the β-tubulin end, is called its *plus end*, and the other, the α-tubulin end, its *minus end*.

In a concentrated solution of pure tubulin in a test tube, tubulin dimers will add to either end of a growing microtubule. However, they add more rapidly to the plus end than to the minus end, which is why the ends were originally named this way—not because they are electrically charged. The polarity of the microtubule—the fact that its structure has a definite direction, with the two ends being chemically and functionally distinct—is crucial, both for the assembly of microtubules and for their role once they are formed. If microtubules had no polarity, they could not guide intracellular transport, for example.

The Centrosome Is the Major Microtubule-organizing Center in Animal Cells

Inside cells, microtubules grow from specialized organizing centers that control the location, number, and orientation of the microtubules. In animal cells, for example, the **centrosome**—which is typically close to the cell nucleus when the cell is not in mitosis—organizes an array of microtubules that radiates outward through the cytoplasm (see Figure 17–10B). The centrosome consists of a pair of **centrioles**, surrounded by a matrix of proteins. The centrosome matrix includes hundreds of ring-shaped structures formed from a special type of tubulin, called *γ-tubulin*, and each *γ-tubulin ring complex* serves as the starting point, or *nucleation site*, for the growth of one microtubule (**Figure 17–12A**). The αβ-tubulin dimers add to each γ-tubulin ring complex in a specific orientation, with the result that the minus end of each microtubule is embedded in the centrosome, and growth occurs only at the plus end that extends into the cytoplasm (**Figure 17–12B and C**).

The paired centrioles at the center of an animal cell centrosome are curious structures; each centriole, sitting perpendicular to its partner, is made of a cylindrical array of short microtubules. Yet centrioles have no role in the nucleation of microtubules from the centrosome (the γ-tubulin ring complex alone is sufficient), and their function remains something of a mystery, especially as plant cells lack them. Centrioles do, however, act as the organizing centers for the microtubules in cilia and flagella, where they are called *basal bodies* (see Figure 17–10D), as we discuss later.

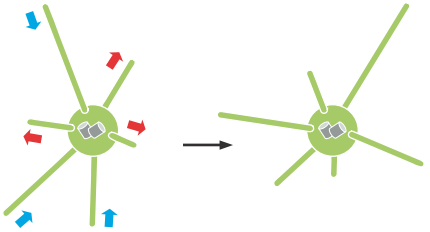


Figure 17–13 Each microtubule grows and shrinks independently of its neighbors. The array of microtubules anchored in a centrosome is continually changing, as new microtubules grow (*red arrows*) and old microtubules shrink (*blue arrows*).

Why do microtubules need nucleating sites such as those provided by the γ-tubulin rings in the centrosome? The answer is that it is much harder to start a new microtubule from scratch, by first assembling a ring of αβ-tubulin dimers, than it is to add such dimers to a preexisting γ-tubulin ring complex. Although purified αβ-tubulin dimers at a high concentration can polymerize into microtubules spontaneously *in vitro*, in a living cell, the concentration of free αβ-tubulin is too low to drive the difficult first step of assembling the initial ring of a new microtubule. By providing organizing centers at specific sites, and keeping the concentration of free αβ-tubulin dimers low, cells can control where microtubules form.

Growing Microtubules Display Dynamic Instability

Once a microtubule has been nucleated, it typically grows outward from the organizing center for many minutes by the addition of αβ-tubulin dimers to its plus end. Then, without warning, the microtubule can suddenly undergo a transition that causes it to shrink rapidly inward by losing tubulin dimers from its free plus end (**Movie 17.2**). It may shrink partially and then, no less suddenly, start growing again, or it may disappear completely, to be replaced by a new microtubule that grows from the same γ-tubulin ring complex (**Figure 17–13**).

This remarkable behavior—switching back and forth between polymerization and depolymerization—is known as **dynamic instability**. It allows microtubules to undergo rapid remodeling, and is crucial for their function. In a normal cell, the centrosome (or other organizing center) is continually shooting out new microtubules in different directions in an exploratory fashion, many of which then retract. A microtubule growing out from the centrosome can, however, be prevented from disassembling if its plus end is stabilized by attachment to another molecule or cell structure so as to prevent its depolymerization. If stabilized by attachment to a structure in a more distant region of the cell, the microtubule will establish a relatively stable link between that structure and the centrosome (**Figure 17–14**). The centrosome can be compared to a fisherman casting a line: if there is no bite at the end of the line, the line is quickly withdrawn, and a new cast is made; but, if a fish bites, the line remains in place, tethering the fish to the fisherman. This simple strategy of random exploration and selective stabilization enables the centrosome and other nucleating centers to set up a highly organized system of microtubules in selected parts of the cell. The same strategy is used to position organelles relative to one another.

Dynamic Instability is Driven by GTP Hydrolysis

The dynamic instability of microtubules stems from the intrinsic capacity of tubulin dimers to hydrolyze GTP. Each free tubulin dimer contains one GTP molecule tightly bound to β-tubulin, which hydrolyzes the GTP to GDP shortly after the dimer is added to a growing microtubule. This GDP remains tightly bound to the β-tubulin. When polymerization

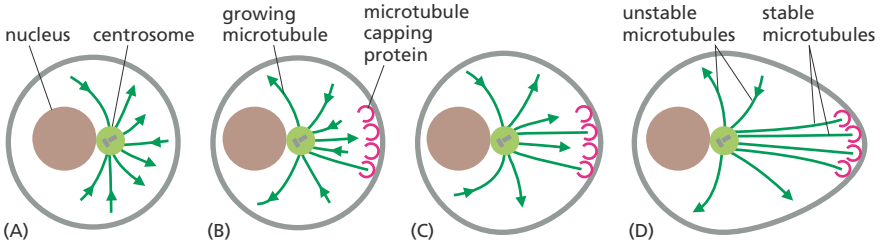


Figure 17–14 The selective stabilization of microtubules can polarize a cell. A newly formed microtubule will persist only if both its ends are protected from depolymerization. In cells, the minus ends of microtubules are generally protected by the organizing centers from which the microtubules grow. The plus ends are initially free but can be stabilized by binding to specific proteins. Here, for example, a nonpolarized cell is depicted in (A), with new microtubules growing from a centrosome in many directions before shrinking back randomly. If a plus end happens to encounter a protein (capping protein) in a specific region of the cell cortex, it will be stabilized (B). Selective stabilization at one end of the cell will bias the orientation of the microtubule array (C) and, ultimately, will convert the cell to a strongly polarized form (D).

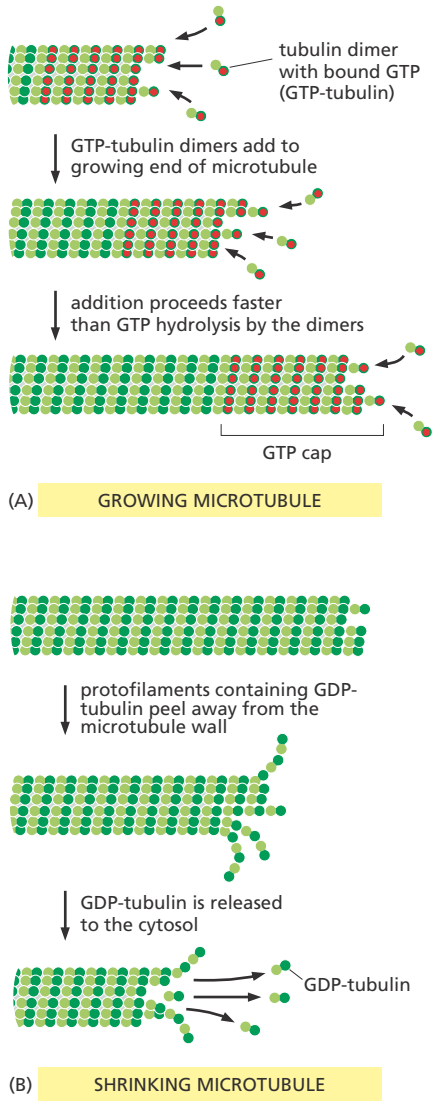


Figure 17–15 GTP hydrolysis controls the dynamic instability of microtubules. (A) Tubulin dimers carrying GTP (*red*) bind more tightly to one another than do tubulin dimers carrying GDP (*dark green*). Therefore, rapidly growing plus ends of microtubules, which have freshly added tubulin dimers with GTP bound, tend to keep growing. (B) From time to time, however, especially if microtubule growth is slow, the dimers in this GTP cap will hydrolyze their GTP to GDP before fresh dimers loaded with GTP have time to bind. The GTP cap is thereby lost. Because the GDP-carrying dimers are less tightly bound in the polymer, the protofilaments peel away from the plus end, and the dimers are released, causing the microtubule to shrink (Movie 17.3).

is proceeding rapidly, tubulin dimers add to the end of the microtubule faster than the GTP they carry is hydrolyzed. As a result, the end of a rapidly growing microtubule is composed entirely of GTP-tubulin dimers, which form a "GTP cap." GTP-associated dimers bind more strongly to their neighbors in the microtubule than do dimers that bear GDP, and they pack together more efficiently. Thus the microtubule will continue to grow (**Figure 17–15A**).

Because of the randomness of chemical processes, however, it will occasionally happen that the tubulin dimers at the free end of the microtubule will hydrolyze their GTP before the next dimers are added, so that the free ends of protofilaments are now composed of GDP-tubulin. These GDP-bearing dimers associate less tightly, tipping the balance in favor of disassembly (**Figure 17–15B**). Because the rest of the microtubule is composed of GDP-tubulin, once depolymerization has started, it will tend to continue; the microtubule starts to shrink rapidly and continuously and may even disappear.

The GDP-tubulin that is freed as the microtubule depolymerizes joins the pool of unpolymerized tubulin already in the cytosol. In a typical fibroblast, for example, at any one time about half of the tubulin in the cell is in microtubules, while the remainder is free in the cytosol, forming a pool of tubulin dimers available for microtubule growth. The tubulin dimers joining the pool rapidly exchange their bound GDP for GTP, thereby becoming competent again to add to another microtubule that is in a growth phase.

Microtubule Dynamics Can be Modified by Drugs

Drugs that prevent the polymerization or depolymerization of tubulin dimers can have a rapid and profound effect on the organization of microtubules—and thereby on the behavior of the cell. Consider the mitotic spindle, the microtubule-based apparatus that guides the chromosomes during mitosis (see Figure 17–10C). If a cell in mitosis is exposed to the drug *colchicine*, which binds tightly to free tubulin dimers and prevents their polymerization into microtubules, the mitotic spindle rapidly disappears, and the cell stalls in the middle of mitosis, unable to partition the chromosomes into two groups. This finding, and others like it, demonstrates that the mitotic spindle is normally maintained by a continuous balanced addition and loss of tubulin subunits: when tubulin addition is blocked by colchicine, tubulin loss continues until the spindle disappears.

The drug *Taxol* has the opposite effect. It binds tightly to microtubules and prevents them from losing subunits. Because new subunits can still be added, the microtubules can grow but cannot shrink. However, despite this difference in their mechanism of action, Taxol has the same overall effect as colchicine—arresting dividing cells in mitosis. These experiments show that for the mitotic spindle to function, microtubules must be able to assemble and disassemble. We discuss the behavior of the spindle in more detail in Chapter 18, when we consider mitosis.

QUESTION 17–2

Why do you suppose it is much easier to add tubulin to existing microtubules than to start a new microtubule from scratch? Explain how γ-tubulin in the centrosome helps to overcome this hurdle.

TABLE 17–1 DRUGS THAT AFFECT MICROTUBULES

Microtubule-specific drugs	Action
Taxol	binds and stabilizes microtubules
Colchicine, colcemid	binds tubulin dimers and prevents their polymerization
Vinblastine, vincristine	binds tubulin dimers and prevents their polymerization

The inactivation or destruction of the mitotic spindle eventually kills dividing cells. Because cancer cells divide in a less controlled way than do normal cells of the body, they can sometimes be killed preferentially by microtubule-stabilizing or microtubule-destabilizing *antimitotic drugs*. These drugs include colchicine, Taxol, vincristine, and vinblastine—all of which are used in the treatment of human cancer (**Table 17–1**). As we discuss shortly, there are also drugs that stabilize or destabilize actin filaments.

Microtubules Organize the Cell Interior

Cells are able to modify the dynamic instability of their microtubules for particular purposes. As cells enter mitosis, for example, microtubules become initially more dynamic, switching between growing and shrinking much more frequently than cytoplasmic microtubules normally do. This change enables microtubules to disassemble rapidly and then reassemble into the mitotic spindle. On the other hand, when a cell has differentiated into a specialized cell type, the dynamic instability of its microtubules is often suppressed by proteins that bind to either the ends or the sides of the microtubules and stabilize them against disassembly. The stabilized microtubules then serve to maintain the organization of the differentiated cell.

Most differentiated animal cells are polarized; that is, one end of the cell is structurally or functionally different from the other. Nerve cells, for example, put out an axon from one end of the cell and dendrites from the other (see Figure 12–29). Cells specialized for secretion have their Golgi apparatus positioned toward the site of secretion, and so on. The cell's polarity is a reflection of the polarized systems of microtubules in its interior, which help to position organelles in their required location within the cell and to guide the streams of vesicular and macromolecular traffic moving between one part of the cell and another. In the nerve cell, for example, all the microtubules in the axon point in the same direction, with their plus ends toward the axon terminals; along these oriented tracks, the cell is able to transport organelles, membrane vesicles, and macromolecules—either from the cell body to the axon terminals or in the opposite direction (**Figure 17–16**).

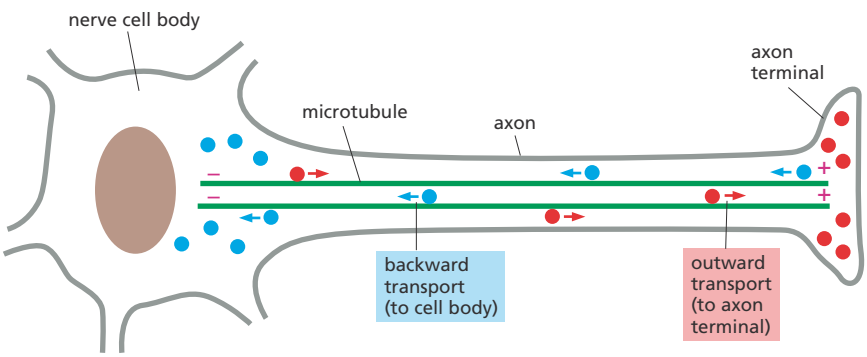


Figure 17–16 Microtubules guide the transport of organelles, vesicles, and macromolecules in both directions along a nerve cell axon. All of the microtubules in the axon point in the same direction, with their plus ends toward the axon terminal. The oriented microtubules serve as tracks for the directional transport of materials synthesized in the cell body but required at the axon terminal. For an axon passing from your spinal cord to a muscle in your shoulder, say, the journey takes about two days. In addition to this outward traffic (*red* circles), which is driven by one set of motor proteins, there is traffic in the reverse direction (*blue* circles), which is driven by another set of motor proteins. The backward traffic includes worn-out mitochondria and materials ingested by the axon terminals.

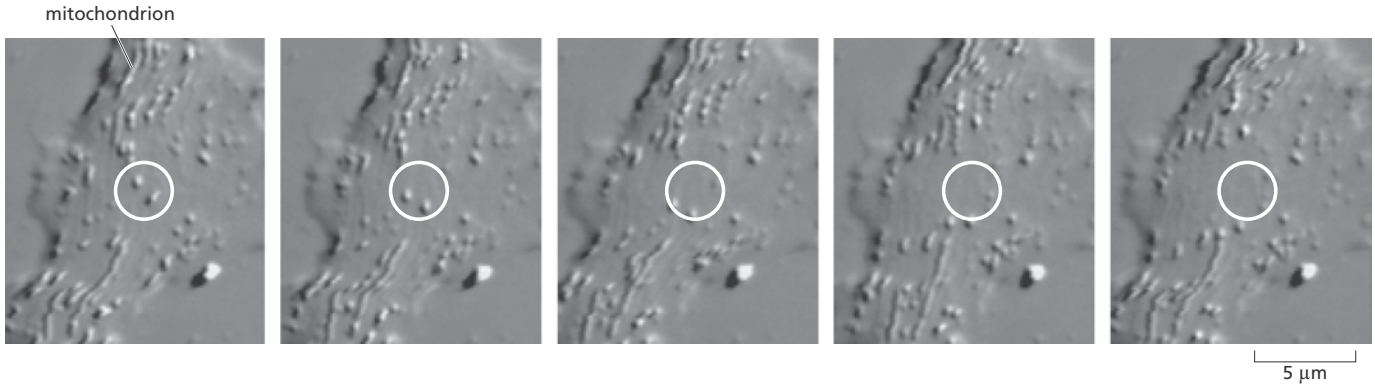


Figure 17–17 Organelles can move rapidly and unidirectionally in a nerve cell axon. In this series of video-enhanced images of a flattened area of an invertebrate nerve axon, numerous membrane vesicles and mitochondria are present, many of which can be seen to move. The *white circle* provides a fixed frame of reference. These images were recorded at intervals of 400 milliseconds. The two vesicles in the circle are moving outward along microtubules, toward the axon terminal. (Courtesy of P. Forscher.)

Some of the traffic along axons travels at speeds in excess of 10 cm a day (**Figure 17–17**), which means that it could still take a week or more for materials to reach the end of a long axon in larger animals. Nonetheless, movement guided by microtubules is immeasurably faster and more efficient than movement driven by free diffusion. A protein molecule traveling by free diffusion could take years to reach the end of a long axon—if it arrived at all (see Question 17–12).

The microtubules in living cells do not act alone. Their activity, like those of other cytoskeletal filaments, depends on a large variety of accessory proteins that bind to them. Some of these **microtubule-associated proteins** stabilize microtubules against disassembly, for example, while others link microtubules to other cell components, including the other types of cytoskeletal filaments (see Figure 17–7). Still others are motor proteins that actively transport organelles, vesicles, and other macromolecules along microtubules.

Motor Proteins Drive Intracellular Transport

If a living cell is observed in a light microscope, its cytoplasm is seen to be in continual motion. Mitochondria and the smaller membrane-enclosed organelles and vesicles travel in small, jerky steps—moving for a short period, stopping, and then moving again. This *saltatory* movement is much more sustained and directional than the continual, small, Brownian movements caused by random thermal motions. Saltatory movements can occur along either microtubules or actin filaments. In both cases, the movements are driven by **motor proteins**, which use the energy derived from repeated cycles of ATP hydrolysis to travel steadily along the microtubule or actin filament in a single direction (see Figure 4–46). Because the motor proteins also attach to other cell components, they can transport this cargo along the filaments. There are dozens of different motor proteins; they differ in the type of filament they bind to, the direction in which they move along the filament, and the cargo they carry.

The motor proteins that move along cytoplasmic microtubules, such as those in the axon of a nerve cell, belong to two families: the **kinesins** generally move toward the plus end of a microtubule (outward from the cell body in Figure 17–16); the **dyneins** move toward the minus end (toward the cell body in Figure 17–16). Both kinesins and dyneins are generally

QUESTION 17–3

Dynamic instability causes microtubules either to grow or to shrink rapidly. Consider an individual microtubule that is in its shrinking phase.

A. What must happen at the end of the microtubule in order for it to stop shrinking and to start growing again?

B. How would a change in the tubulin concentration affect this switch?

C. What would happen if only GDP, but no GTP, were present in the solution?

D. What would happen if the solution contained an analog of GTP that cannot be hydrolyzed?

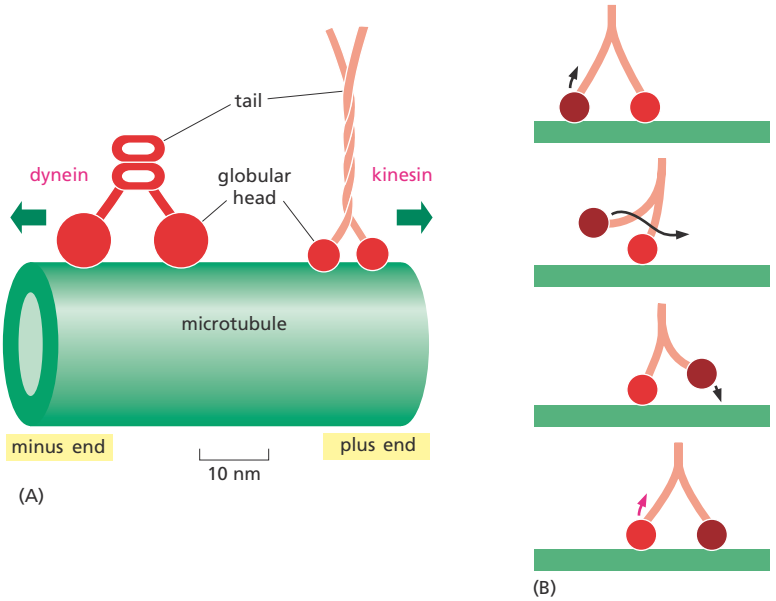


Figure 17–18 Both kinesins and dyneins move along microtubules using their globular heads. (A) Kinesins and cytoplasmic dyneins are microtubule motor proteins that generally move in opposite directions along a microtubule. Each of these proteins (drawn here roughly to scale) is a dimer composed of two identical subunits. Each dimer has two globular heads at one end, which bind and hydrolyze ATP and interact with microtubules, and a single tail at the other end, which interacts with cargo (not shown). (B) Schematic diagram of a generic motor protein "walking" along a filament; these proteins use the energy of ATP hydrolysis to move in one direction along the filament, as illustrated in Figure 4–46. (See also Figure 17–22B.)

dimers that have two globular ATP-binding heads and a single tail (**Figure 17–18A**). The heads interact with microtubules in a stereospecific manner, so that the motor protein will attach to a microtubule in only one direction. The tail of a motor protein generally binds stably to some cell component, such as a vesicle or an organelle, and thereby determines the type of cargo that the motor protein can transport (**Figure 17–19**). The globular heads of kinesin and dynein are enzymes with ATP-hydrolyzing (ATPase) activity. This reaction provides the energy for driving a directed series of conformational changes in the head that enable it to move along the microtubule by a cycle of binding, release, and rebinding to the microtubule (see Figure 17–18B and Figure 4–46). For a discussion of the discovery and study of motor proteins, see **How We Know**, pp. 580–581.

Microtubules and Motor Proteins Position Organelles in the Cytoplasm

Microtubules and motor proteins play an important part in positioning organelles within a eukaryotic cell. In most animal cells, for example, the tubules of the endoplasmic reticulum (ER) reach almost to the edge of the cell (**Movie 17.4**), whereas the Golgi apparatus is located in the cell interior, near the centrosome (**Figure 17–20A**). The ER extends out from its points of connection with the nuclear envelope along microtubules, which reach from the centrally located centrosome out to the plasma membrane. As a cell grows, kinesins attached to the outside of the ER membrane (via receptor proteins) pull the ER outward along microtubules, stretching it like a net (**Figure 17–20B**). Cytoplasmic *dyneins* attached to

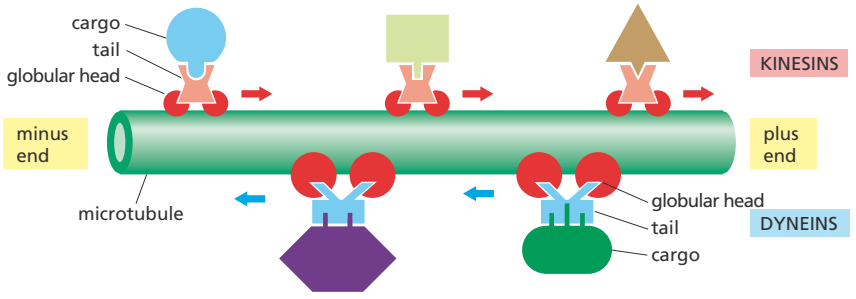


Figure 17–19 Different motor proteins transport different types of cargo along microtubules. Most kinesins move toward the plus end of a microtubule, whereas dyneins move toward the minus end (Movie 17.5). Both types of microtubule motor proteins exist in many forms, each of which is thought to transport a different type of cargo. The tail of the motor protein determines what cargo the protein transports.

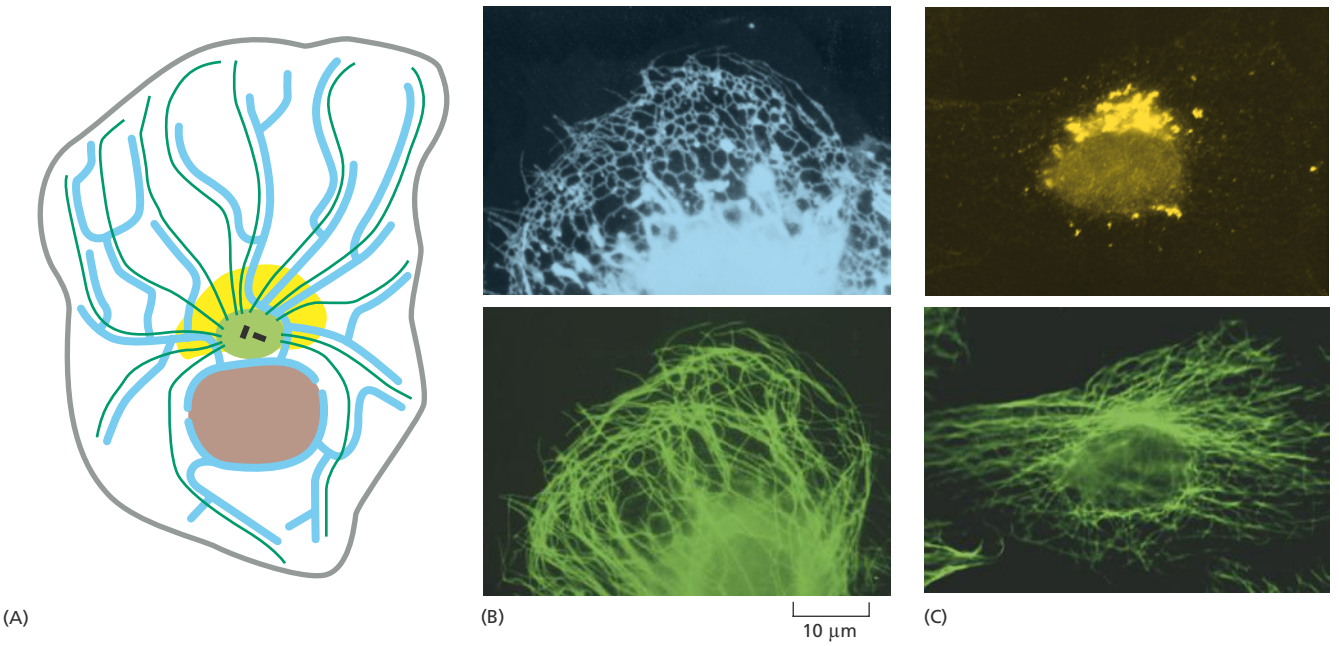


Figure 17–20 Microtubules help position organelles in a eukaryotic cell. (A) Schematic diagram of a cell showing the typical arrangement of cytoplasmic microtubules (*dark green*), endoplasmic reticulum (*blue*), and Golgi apparatus (*yellow*). The nucleus is shown in *brown*, and the centrosome in *light green*. (B) One part of a cell in culture stained with antibodies to the endoplasmic reticulum (*blue, upper panel*) and to microtubules (*green, lower panel*). Kinesin motor proteins pull the endoplasmic reticulum outward along the microtubules. (C) A different cell in culture stained with antibodies to the Golgi apparatus (*yellow, upper panel*) and to microtubules (*green, lower panel*). In this case, cytoplasmic dyneins pull the Golgi apparatus inward along the microtubules to its position near the centrosome, which is not visible but is located on the Golgi side of the nucleus. (B, courtesy of Mark Terasaki, Lan Bo Chen, and Keigi Fujiwara; C, courtesy of Viki Allan and Thomas Kreis.)

the Golgi membranes pull the Golgi apparatus along microtubules in the opposite direction, inward toward the nucleus (**Figure 17–20C**). In this way, the regional differences in these internal membranes—crucial for their respective functions—are created and maintained.

When cells are treated with colchicine—a drug that causes microtubules to disassemble—both the ER and the Golgi apparatus change their location dramatically. The ER, which is connected to the nuclear envelope, collapses around the nucleus; the Golgi apparatus, which is not attached to any other organelle, fragments into small vesicles, which then disperse throughout the cytoplasm. When the colchicine is removed, the organelles return to their original positions, dragged by motor proteins moving along the re-formed microtubules.

Cilia and Flagella Contain Stable Microtubules Moved by Dynein

We mentioned earlier that many microtubules in cells are stabilized through their association with other proteins and therefore do not show dynamic instability. Cells use such stable microtubules as stiff supports in the construction of a variety of polarized structures, including motile cilia and flagella. **Cilia** are hairlike structures about 0.25 μm in diameter, covered by plasma membrane, that extend from the surface of many kinds of eukaryotic cells; each cilium contains a core of stable microtubules, arranged in a bundle, that grow from a cytoplasmic *basal body*, which serves as an organizing center (see Figure 17–10D).

Cilia beat in a whiplike fashion, either to move fluid over the surface of a cell or to propel single cells through a fluid. Some protozoa, for example,

PURSUING MICROTUBULE-ASSOCIATED MOTOR PROTEINS

The movement of organelles throughout the cell cytoplasm has been observed, measured, and speculated about since the middle of the nineteenth century. But it was not until the mid-1980s that biologists identified the molecules that drive this movement of organelles and vesicles from one part of the cell to another.

Why the lag between observation and understanding? The problem was in the proteins—or, more precisely, in the difficulty of studying them in isolation outside the cell. To investigate the activity of an enzyme, for example, biochemists first purify the polypeptide: they break open cells or tissues and separate the protein of interest from other molecular components (see Panels 4–4 and 4–5, pp. 166–167). They can then study the protein in a test tube (*in vitro*), controlling its exposure to substrates, inhibitors, ATP, and so on. Unfortunately, this approach did not seem to work for studies of the motile machinery that underlies intracellular transport. It is not possible to break open a cell and pull out an intact, fully active transport system, free of extraneous material, that continues to carry mitochondria and vesicles from place to place.

That problem was solved by technical advances in two separate fields. First, improvements in microscopy allowed biologists to see that an operational transport system (with extraneous material still attached) could be squeezed from the right kind of living cell. At the same time, biochemists realized that they could assemble a working transport system from scratch—using purified cytoskeletal filaments, motors, and cargo—outside the cell. One such breakthrough started with a squid.

Teeming cytoplasm

Neuroscientists interested in the electrical properties of nerve cell membranes have long studied the giant axon from squid (see How We Know, pp. 406–407). Because of its large size, researchers found that they could squeeze the cytoplasm from the axon like toothpaste, and then study how ions move back and forth through various channels in the empty, tubelike plasma membrane (see Figure 12–33). The physiologists simply discarded the cytoplasmic jelly, as it appeared to be inert (and thus uninteresting) when examined under a standard light microscope.

Then along came video-enhanced microscopy. This type of microscopy, developed by Shinya Inoué, Robert Allen, and others, allows one to detect structures that are smaller than the resolving power of standard light microscopes, which is only about 0.2 μm, or 200 nm (see Panel 1–1, pp. 10–11). Sample images are captured by a video camera and then enhanced by computer processing to reduce the background and heighten contrast. When researchers in the early 1980s applied this new technique to preparations of squid axon cytoplasm (axoplasm), they observed, for the first time, the motion of vesicles and other organelles along cytoskeletal filaments.

Under the video-enhanced microscope, extruded axoplasm is seen to be teeming with tiny particles—from vesicles 30–50 nm in diameter to mitochondria some 5000 nm long, all moving to and fro along cytoskeletal filaments at speeds of up to 5 μm per second. If the axoplasm is spread thinly enough, individual filaments can be seen.

The movement continues for hours, allowing researchers to manipulate the preparation and study the effects. Ray Lasek and Scott Brady discovered, for example, that the organelle movement requires ATP. Substitution of ATP analogs, such as AMP-PNP, which resemble ATP but cannot be hydrolyzed (and thus provide no energy), inhibit the translocation.

Snaking tubes

More work was needed to identify the individual components that drive the transport system in squid axoplasm. What kind of filaments support this movement? What are the molecular motors that shuttle the vesicles and organelles along these filaments? Identifying the filaments was relatively easy: antibodies to tubulin revealed that they are microtubules. But what about the motor proteins? To find these, Ron Vale, Thomas Reese, and Michael Sheetz set up a system in which they could fish for proteins that power organelle movement.

Their strategy was simple yet elegant: add together microtubules and organelles and then look for molecules that induce motion. They used purified microtubules from squid brain, added organelles isolated from squid axons, and showed that organelle movement could be triggered by the addition of an extract from squid axoplasm. In this preparation, the researchers could either watch the organelles travel along the microtubules or watch the microtubules glide snakelike over the surface of a glass coverslip that had been coated with an axoplasm extract (see Question 17–18). Their challenge was to isolate the protein responsible for movement in this reconstituted system.

To do that, Vale and his colleagues took advantage of the earlier work with the ATP analog AMP-PNP. Although this analog inhibits the movement of vesicles along microtubules, it still allows organelles to attach to the microtubule filaments. So the researchers incubated the axoplasm extract with microtubules and organelles in the presence of AMP-PNP; they then pulled out the microtubules with what they hoped were the motor proteins still attached. Vale and his team then added ATP to

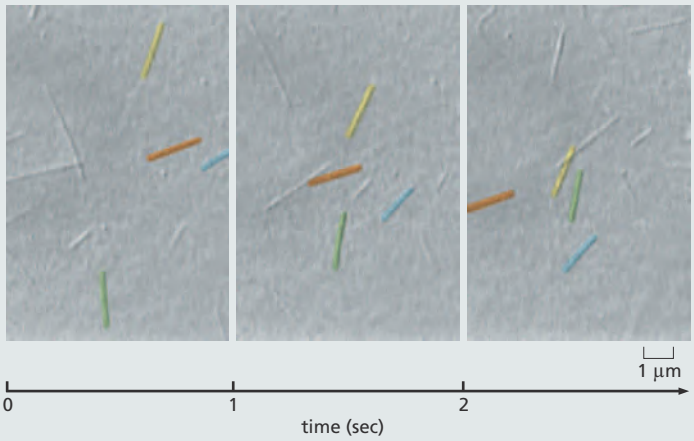


Figure 17–21 Kinesin causes microtubule gliding *in vitro*. In an *in vitro* motility assay, purified kinesin is mixed with microtubules in the presence of ATP. When a drop of the mixture is placed on a glass slide and examined by video-enhanced microscopy, individual microtubules can be seen gliding over the slide. They are driven by kinesin molecules, which attach to the glass slide by their tails. Images were recorded at 1-second intervals. The artificially colored microtubules moved at about 1–2 μm/sec. (Courtesy of Nick Carter and Rob Cross.)

release the attached proteins, and they found a 110-kilodalton polypeptide that could stimulate the gliding of microtubules along a glass coverslip (**Figure 17–21**). They dubbed the molecule kinesin (from the Greek *kinein*, "to move").

Similar *in vitro* motility assays have been instrumental in the study of other motor proteins—such as myosins, which move along actin filaments, as we discuss later. Subsequent studies showed that kinesin moves along microtubules from the minus end to the plus end; they also identified many other motor proteins of the kinesin family.

Lights, camera, action

Combining such assays with ever more refined microscopic techniques, researchers can now monitor the movement of individual motor proteins along single microtubules, even in living cells.

Observation of kinesin molecules coupled with green fluorescent protein (GFP) revealed that this motor protein marches along microtubules processively—that is, each molecule takes multiple "steps" along the filament (100 or so) before falling off. The length of each step is 8 nm, which corresponds to the spacing of individual tubulin dimers along the microtubule. Combining these observations with assays of ATP hydrolysis, researchers have confirmed that one molecule of ATP is hydrolyzed per step. Kinesin can move in a processive manner because it has two heads. This enables it to walk toward the plus end of the microtubule in a "hand-over-hand" fashion, each head repetitively binding and releasing the filament as it swings past the bound head in front (**Figure 17–22**). Such studies now allow us to follow the footsteps of these fascinating and industrious proteins—step by molecular step.

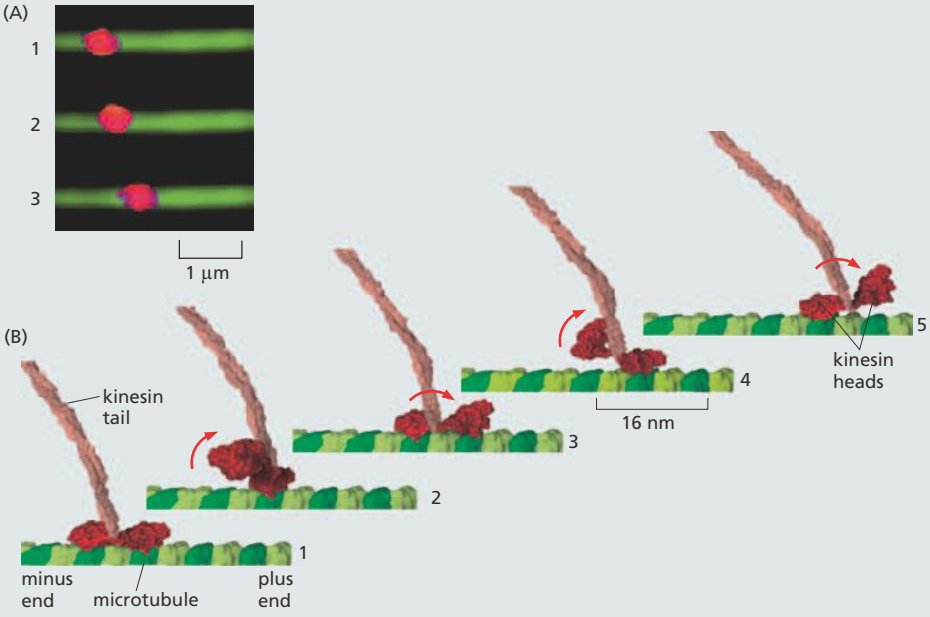


Figure 17–22 A single molecule of kinesin moves along a microtubule. (A) Three frames, separated by intervals of 1 second, record the movement of an individual kinesin-GFP molecule (*green*) along a microtubule (*red*); the labeled kinesin moves at a speed of 0.3 μm/sec. (B) A series of molecular models of the two heads of a kinesin molecule, showing how they are thought to walk processively along a microtubule in a series of 8-nm steps in which one head swings past the other (Movie 17.6). (A and B, courtesy of Ron Vale.)

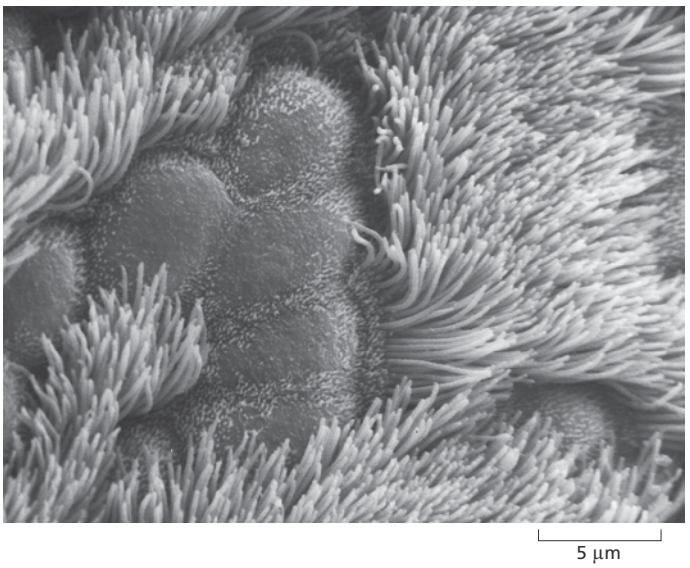


Figure 17–23 Many hairlike cilia project from the surface of the epithelial cells that line the human respiratory tract. In this scanning electron micrograph, thick tufts of cilia can be seen extended from these ciliated cells, which are interspersed with the dome-shaped surfaces of nonciliated epithelial cells. (Reproduced from R.G. Kessel and R.H. Karden, Tissues and Organs. San Francisco: W.H. Freeman & Co., 1979.)

use cilia to collect food particles, and others use them for locomotion. On the epithelial cells lining the human respiratory tract (**Figure 17–23**), huge numbers of beating cilia (more than a billion per square centimeter) sweep layers of mucus containing trapped dust particles and dead cells up toward the throat, to be swallowed and eventually eliminated from the body. Similarly, beating cilia on the cells of the oviduct wall create a current that helps to carry eggs along the oviduct. Each cilium acts as a small oar, moving in a repeated cycle that generates the movement of fluid over the cell surface (**Figure 17–24**).

The **flagella** (singular flagellum) that propel sperm and many protozoa are much like cilia in their internal structure but are usually very much longer. They are designed to move the entire cell, rather than moving fluid across the cell surface. Flagella propagate regular waves along their length, propelling the attached cell along (**Figure 17–25**).

The microtubules in cilia and flagella are slightly different from cytoplasmic microtubules; they are arranged in a curious and distinctive pattern, which was one of the most striking revelations of early electron microscopy. A cross section through a cilium shows nine doublet microtubules arranged in a ring around a pair of single microtubules (**Figure 17–26A**). This "9 + 2" array is characteristic of almost all eukaryotic cilia and flagella—from those of protozoa to those in humans.

The movement of a cilium or a flagellum is produced by the bending of its core as the microtubules slide against each other. The microtubules are associated with numerous accessory proteins (**Figure 17–26B**), which project at regular positions along the length of the microtubule bundle. Some of these proteins serve as cross-links to hold the bundle of microtubules together; others generate the force that causes the cilium to bend.

The most important of the accessory proteins is the motor protein *ciliary dynein*, which generates the bending motion of the core. It closely

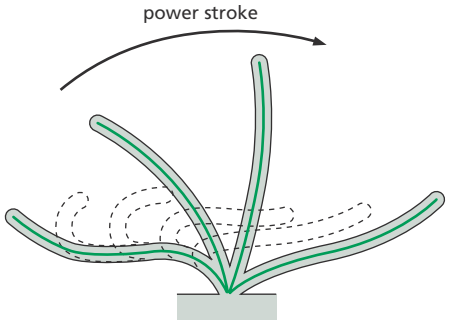
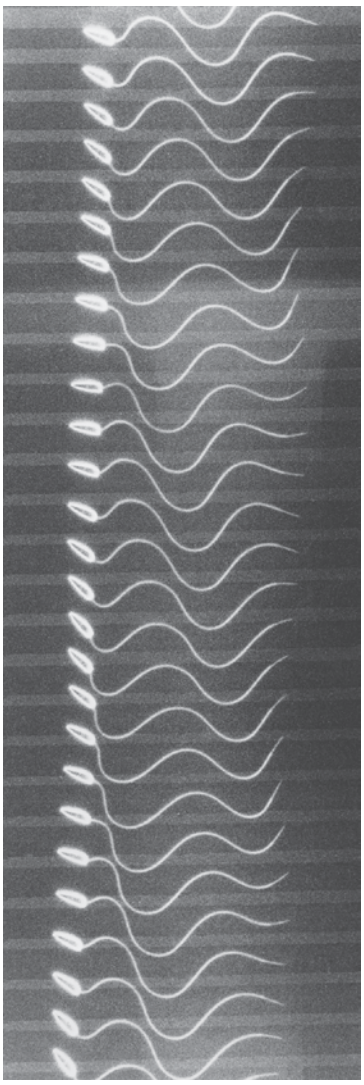


Figure 17–24 A cilium beats by performing a repetitive cycle of movements, consisting of a power stroke followed by a recovery stroke. In the fast power stroke, the cilium is fully extended and fluid is driven over the surface of the cell; in the slower recovery stroke, the cilium curls back into position with minimal disturbance to the surrounding fluid. Each cycle typically requires 0.1–0.2 second and generates a force parallel to the cell surface.



Figures 17–25 Flagella propel a cell through fluid using repetitive wavelike motion. The movement of a single flagellum on an invertebrate sperm is seen in a series of images captured by stroboscopic illumination at 400 flashes per second. (Courtesy of Charles J. Brokaw.)

resembles cytoplasmic dynein and functions in much the same way. Ciliary dynein is attached by its tail to one microtubule, while its two heads interact with an adjacent microtubule to generate a sliding force between the two microtubules. Because of the multiple links that hold the adjacent microtubule doublets together, the sliding force between adjacent microtubules is converted to a bending motion in the cilium (**Figure 17–27**). In humans, hereditary defects in ciliary dynein cause Kartagener's syndrome. Men with this disorder are infertile because their sperm are nonmotile, and they have an increased susceptibility to bronchial infections because the cilia that line their respiratory tract are paralyzed and thus unable to clear bacteria and debris from the lungs.

Many animal cells that lack beating cilia contain a single, nonmotile *primary cilium*. This appendage is much shorter than a beating cilium and functions as an antenna for sensing certain extracellular signal molecules.

ACTIN FILAMENTS

Actin filaments, polymers of the protein **actin**, are present in all eukaryotic cells and are essential for many of the cell's movements, especially those involving the cell surface. Without actin filaments, for example, an animal cell could not crawl along a surface, engulf a large particle by phagocytosis, or divide in two. Like microtubules, many actin filaments are unstable, but by associating with other proteins they can also form stable structures in cells, such as the contractile apparatus of muscle cells. Actin filaments interact with a large number of *actin-binding proteins* that enable the filaments to serve a variety of functions in cells. Depending on which of these proteins they associate with, actin filaments can form stiff and stable structures, such as the *microvilli* on the epithelial cells lining

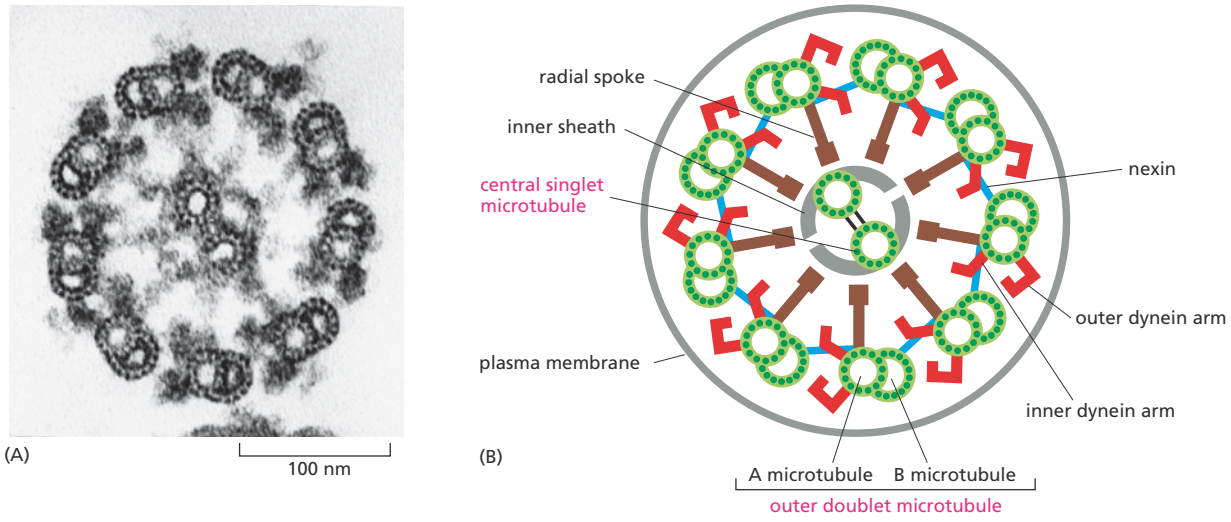


Figure 17–26 Microtubules in a cilium or flagellum are arranged in a "9 + 2" array. (A) Electron micrograph of a flagellum of the unicellular alga *Chlamydomonas* shown in cross section, illustrating the distinctive 9 + 2 arrangement of microtubules. (B) Diagram of the flagellum in cross section. The nine outer microtubules (each a special paired structure) carry two rows of dynein molecules. The heads of each dynein molecule appear in this view like arms reaching toward the adjacent doublet microtubule. In a living cilium, these dynein heads periodically make contact with the adjacent doublet microtubule and move along it, thereby producing the force for ciliary beating. The various other links and projections shown are proteins that serve to hold the bundle of microtubules together and to convert the sliding force produced by dyneins into bending, as illustrated in Figure 17–27. (A, courtesy of Lewis Tilney.)

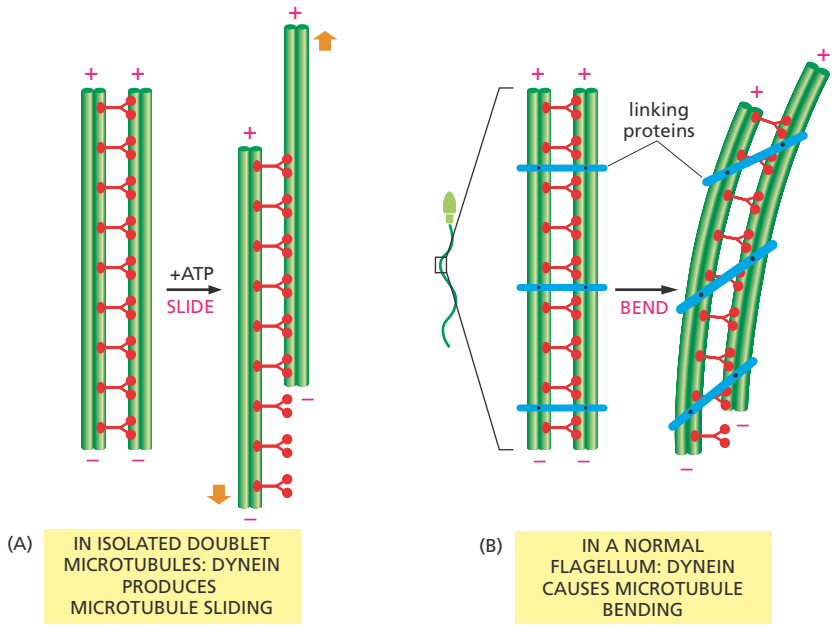


Figure 17–27 The movement of dynein causes the flagellum to bend. (A) If the outer doublet microtubules and their associated dynein molecules are freed from other components of a sperm flagellum and then exposed to ATP, the doublets slide against each other, telescope-fashion, due to the repetitive action of their associated dyneins. (B) In an intact flagellum, however, the doublets are tied to each other by flexible protein links so that the action of the system produces bending rather than sliding.

QUESTION 17–4

Dynein arms in a cilium are arranged so that, when activated, the heads push their neighboring outer doublet outward toward the tip of the cilium. Consider a cross section of a cilium (see Figure 17–26). Why would no bending motion of the cilium result if all dynein molecules were active at the same time? What pattern of dynein activity can account for the bending of a cilium in one direction?

the intestine (**Figure 17–28A**) or the small *contractile bundles* that can contract and act like tiny muscles in most animal cells (**Figure 17–28B**). They can also form temporary structures, such as the dynamic protrusions formed at the leading edge of a crawling cell (**Figure 17–28C**) or the *contractile ring* that pinches the cytoplasm in two when an animal cell divides (**Figure 17–28D**). Actin-dependent movements usually require actin's association with a motor protein called *myosin*.

In this section, we see how the arrangements of actin filaments in a cell depend on the types of actin-binding proteins present. Even though actin filaments and microtubules are formed from unrelated types of subunit proteins, we will see that the principles by which they assemble and disassemble, control cell structure, and work with motor proteins to bring about movement are strikingly similar.

Actin Filaments Are Thin and Flexible

Actin filaments appear in electron micrographs as threads about 7 nm in diameter. Each filament is a twisted chain of identical globular actin monomers, all of which "point" in the same direction along the axis of the chain. Like a microtubule, therefore, an actin filament has a structural polarity, with a plus end and a minus end (**Figure 17–29**).

Actin filaments are thinner, more flexible, and usually shorter than microtubules. There are, however, many more of them, so that the total length of all the actin filaments in a cell is generally many times greater than the total length of all of the microtubules. Unlike intermediate filaments and

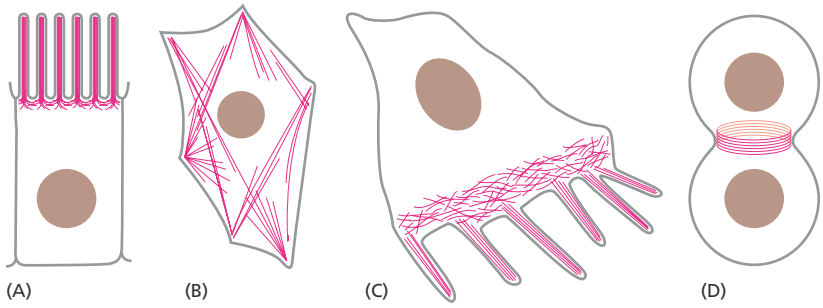


Figure 17–28 Actin filaments allow animal cells to adopt a variety of shapes and perform a variety of functions. The actin filaments in four different structures are shown here in *red*: (A) microvilli; (B) contractile bundles in the cytoplasm; (C) fingerlike *filopodia* protruding from the leading edge of a moving cell; (D) contractile ring during cell division.

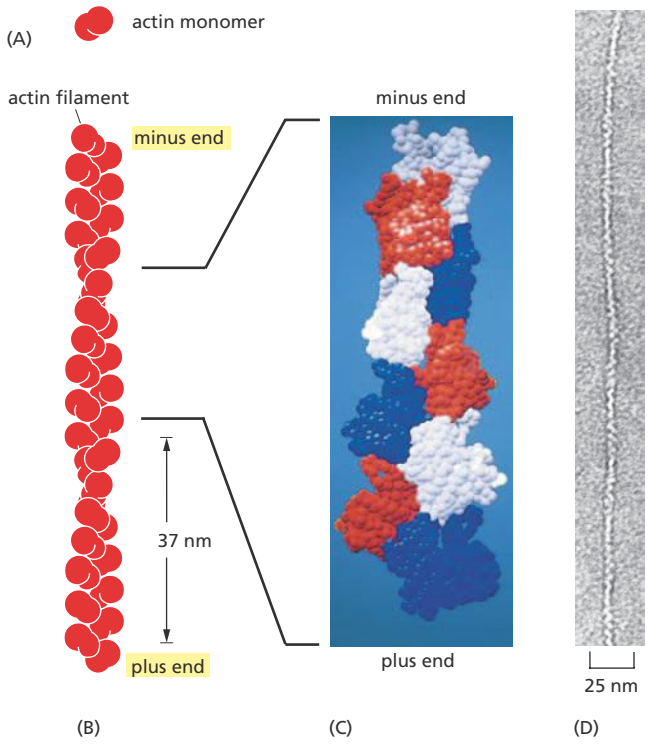


Figure 17–29 Actin filaments are thin, flexible protein threads. (A) The subunit of each actin filament is an actin monomer. A cleft in the monomer provides a binding site for ATP or ADP. (B) Arrangement of actin monomers in an actin filament. Each filament may be thought of as a two-stranded helix with a twist repeating every 37 nm. Multiple, lateral interactions between the two strands prevent the strands from separating. (C) Close-up view showing the identical subunits of an actin filament in different colors to emphasize the close interactions between each actin molecule and its four nearest neighbors. (D) Electron micrograph of a negatively stained actin filament. (C, from K.C. Holmes et al., *Nature* 347:44–49, 1990. With permission from Macmillan Publishers Ltd; D, courtesy of Roger Craig.)

microtubules, actin filaments rarely occur in isolation in the cell; they are generally found in cross-linked bundles and networks, which are much stronger than the individual filaments.

Actin and Tubulin Polymerize by Similar Mechanisms

Although actin filaments can grow by the addition of actin monomers at either end, like microtubules, their rate of growth is faster at the plus end than at the minus end. A naked actin filament, like a microtubule without associated proteins, is inherently unstable, and it can disassemble from both ends. In living cells, free actin monomers carry a tightly bound nucleoside triphosphate, in this case ATP. The actin monomer hydrolyzes its bound ATP to ADP soon after it is incorporated into the filament. As with the hydrolysis of GTP to GDP in a microtubule, hydrolysis of ATP to ADP in an actin filament reduces the strength of binding between the monomers, thereby decreasing the stability of the polymer. Thus in both cases, nucleotide hydrolysis promotes depolymerization, helping the cell to disassemble its microtubules and actin filaments after they have formed.

If the concentration of free actin monomers is very high, an actin filament will grow rapidly, adding monomers at both ends. At intermediate concentrations of free actin, however, something interesting takes place. Actin monomers add to the plus end at a rate faster than the bound ATP can be hydrolyzed, so the plus end grows. At the minus end, by contrast, ATP is hydrolyzed faster than new monomers can be added; because ADP-actin destabilizes the structure, the filament loses subunits from its minus end at the same time as it adds them to the plus end (**Figure 17–30**). Inasmuch as an individual monomer moves through the filament from the plus to the minus end, this behavior is called *treadmilling*.

Both the treadmilling of actin filaments and the dynamic instability of microtubules rely on the hydrolysis of a bound nucleoside triphosphate to regulate the length of the polymer. But the result is usually different.

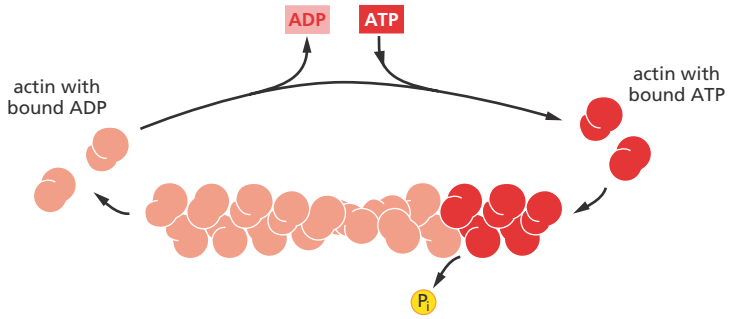


Figure 17–30 ATP hydrolysis decreases the stability of the actin polymer. Actin monomers in the cytosol carry ATP, which is hydrolyzed to ADP soon after assembly into a growing filament. The ADP molecules remain trapped within the actin filament, unable to exchange with ATP until the actin monomer that carries them dissociates from the filament.

Treadmilling involves a simultaneous gain of monomers at the plus end of an actin filament and loss at the minus end: when the rates of addition and loss are equal, the filament remains the same size (**Figure 17–31A**). Dynamic instability, on the other hand, involves a rapid switch from growth to shrinkage (or from shrinkage to growth) at only the plus end of the microtubule. As a result, microtubules tend to undergo more drastic changes in length than do actin filaments—either growing rapidly or collapsing rapidly (**Figure 17–31B**).

Actin filament function can be perturbed experimentally by certain toxins produced by fungi or marine sponges. Some, such as *cytochalasin* and *latrunculin*, prevent actin polymerization; others, such as *phalloidin*, stabilize actin filaments against depolymerization (**Table 17–2**). Addition of these toxins to the medium bathing cells or tissues, even in low concentrations, instantaneously freezes cell movements such as cell locomotion. Thus as with microtubules, many of the functions of actin filaments depend on the ability of the filament to assemble and disassemble, the rates of which depend on the dynamic equilibrium between the actin filaments, the pool of actin monomers, and various actin-binding proteins.

QUESTION 17–5

The formation of actin filaments in the cytosol is controlled by actin-binding proteins. Some actin-binding proteins significantly increase the rate at which the formation of an actin filament is initiated. Suggest a mechanism by which they might do this.

Many Proteins Bind to Actin and Modify Its Properties

About 5% of the total protein in a typical animal cell is actin; about half of this actin is assembled into filaments, and the other half remains as actin monomers in the cytosol. Thus unlike the situation for tubulin dimers, the concentration of actin monomers is high—much higher than the concentration required for purified actin monomers to polymerize spontaneously in a test tube. What, then, keeps the actin monomers in cells from polymerizing totally into filaments? The answer is that cells contain

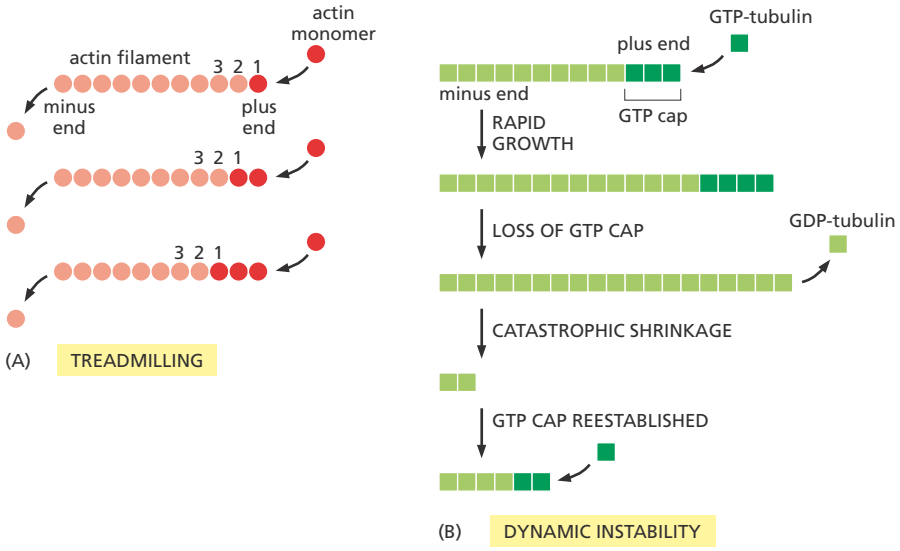


Figure 17–31 Treadmilling of actin filaments and dynamic instability of microtubules regulate polymer length in different ways. (A) Treadmilling occurs when ATP-actin adds to the plus end of an actin filament at the same time that ADP-actin is lost from the minus end. When the rates of addition and loss are equal, the filament stays the same length—although individual actin monomers (three of which are numbered) move through the filament from the plus to the minus end. (B) In dynamic instability, GTP-tubulin adds to the plus end of a growing microtubule. As discussed earlier, when GTP-tubulin addition is faster than GTP hydrolysis, a GTP cap forms at that end; when the rate of addition slows, the GTP cap is lost, and the filament experiences catastrophic shrinkage via the loss of GDP-tubulin from the same end. The microtubule will shrink until the GTP cap is regained—or until the microtubule disappears (see Figure 17–15).

TABLE 17–2 DRUGS THAT AFFECT ACTIN FILAMENTS	
Actin-specific drugs	
Phalloidin	binds and stabilizes filaments
Cytochalasin	caps filament plus ends, preventing polymerization there
Latrunculin	binds actin monomers and prevents their polymerization

small proteins, such as *thymosin* and *profilin*, that bind to actin monomers in the cytosol, preventing them from adding to the ends of actin filaments. By keeping actin monomers in reserve until they are required, these proteins play a crucial role in regulating actin polymerization. When actin filaments are needed, other actin-binding proteins such as *formins* and *actin-related proteins* (*ARPs*) promote actin polymerization.

There are a great many **actin-binding proteins** in cells. Most of these bind to assembled actin filaments rather than to actin monomers and control the behavior of the intact filaments (**Figure 17–32**). Actin-bundling proteins, for example, hold actin filaments together in parallel bundles in microvilli; others cross-link actin filaments together in a gel-like meshwork within the *cell cortex*—the specialized layer of actin-filament-rich

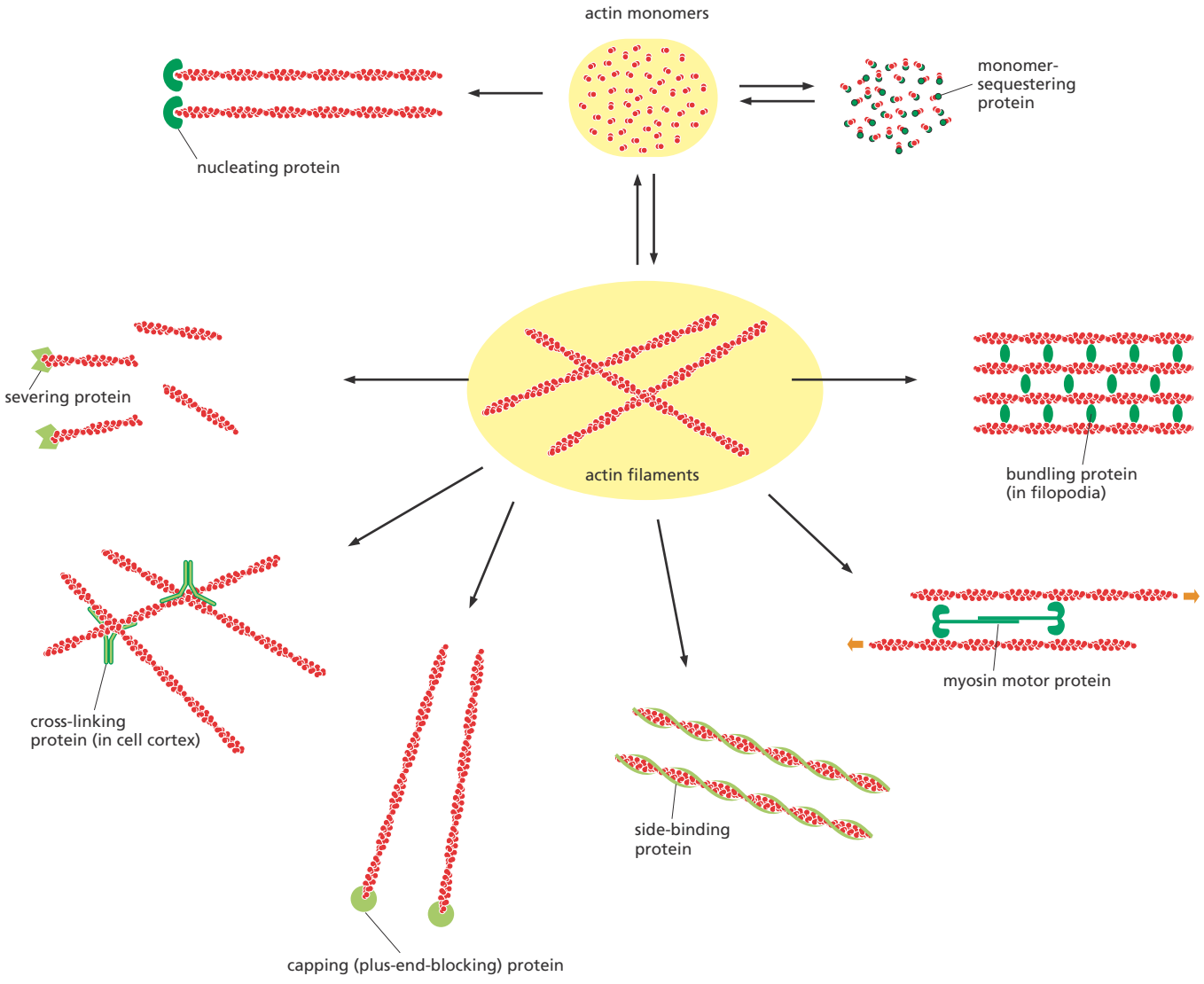


Figure 17–32 Actin-binding proteins control the behavior of actin filaments in vertebrate cells. Actin is shown in *red*, and the actin-binding proteins are shown in *green*.

cytoplasm just beneath the plasma membrane. Filament-severing proteins fragment actin filaments into shorter lengths and thus can convert an actin gel to a more fluid state. Actin filaments can also associate with myosin motor proteins to form contractile bundles, as in muscle cells. And they often form tracks along which myosin motor proteins transport organelles, a function that is especially conspicuous in plant cells.

In the remainder of this chapter, we consider some characteristic structures that actin filaments can form, and we discuss how different types of actin-binding proteins are involved in their assembly. We begin with the cell cortex and its role in cell locomotion, and we conclude with the contractile apparatus of muscle cells.

A Cortex Rich in Actin Filaments Underlies the Plasma Membrane of Most Eukaryotic Cells

Although actin is found throughout the cytoplasm of a eukaryotic cell, in most cells it is highly concentrated in a layer just beneath the plasma membrane. In this region, called the **cell cortex**, actin filaments are linked by actin-binding proteins into a meshwork that supports the plasma membrane and gives it mechanical strength. In human red blood cells, a simple and regular network of fibrous proteins—including actin and spectrin filaments—attaches to the plasma membrane, providing the support necessary for the cells to maintain their simple discoid shape (see Figure 11–29). The cell cortex of other animal cells, however, is thicker and more complex, and it supports a far richer repertoire of cell shapes and cell-surface movements. Like the cortex in the red blood cell, the cortex in other cells contains spectrin; however, it also includes a much denser network of actin filaments. These filaments become cross-linked into a three-dimensional meshwork, which governs cell shape and the mechanical properties of the plasma membrane: the rearrangements of actin filaments within the cortex provide much of the molecular basis for changes in both cell shape and cell locomotion.

Cell Crawling Depends on Cortical Actin

Many eukaryotic cells move by crawling over surfaces, rather than by swimming by means of beating cilia or flagella. Carnivorous amoebae crawl continually, in search of food. The advancing tip of a developing axon migrates in response to growth factors, following a path of chemical signals to its eventual synaptic target cells. White blood cells known as *neutrophils* migrate out of the blood into infected tissues when they "smell" small molecules released by bacteria, which the neutrophils seek out and destroy. For these hunters, such chemotactic molecules binding to receptors on the cell surface trigger changes in actin filament assembly that help direct the cells toward their prey (see **Movie 17.7**).

The molecular mechanisms of these and other forms of cell crawling entail coordinated changes of many molecules in different regions of the cell, and no single, easily identifiable locomotory organelle, such as a flagellum, is responsible. In broad terms, however, three interrelated processes are known to be essential: (1) the cell pushes out protrusions at its "front," or leading edge; (2) these protrusions adhere to the surface over which the cell is crawling; and (3) the rest of the cell drags itself forward by traction on these anchorage points (**Figure 17–33**).

All three processes involve actin, but in different ways. The first step, the pushing forward of the cell surface, is driven by actin polymerization. The leading edge of a crawling fibroblast in culture regularly extends thin, sheetlike **lamellipodia**, which contain a dense meshwork of actin filaments, oriented so that most of the filaments have their plus ends close

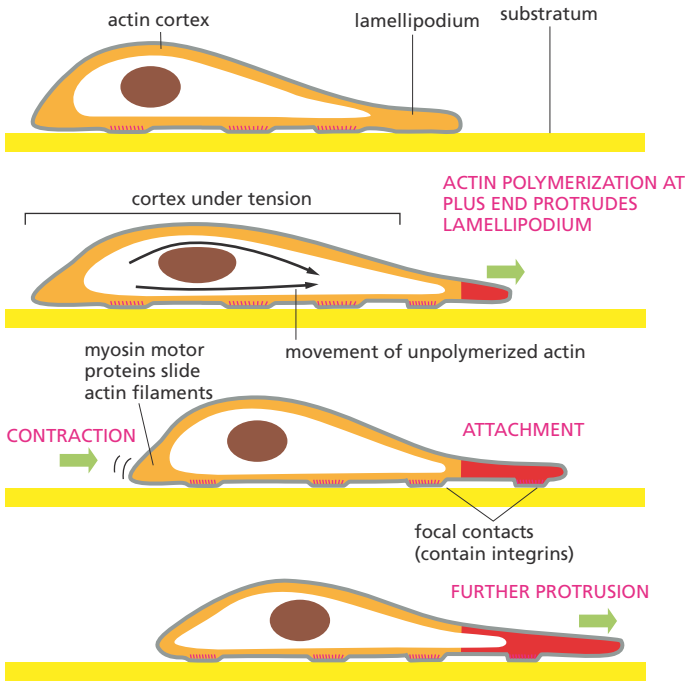


Figure 17–33 Forces generated in the actin-filament-rich cortex help move a cell forward. Actin polymerization at the leading edge of the cell *pushes* the plasma membrane forward (protrusion) and forms new regions of actin cortex, shown here in *red*. New points of anchorage are made between the bottom of the cell and the surface (substratum) on which the cell is crawling (attachment). Contraction at the rear of the cell—mediated by myosin motor proteins moving along actin filaments—then draws the body of the cell forward. New anchorage points are established at the front, and old ones are released at the back, as the cell crawls forward. The same cycle is repeated over and over again, moving the cell forward in a stepwise fashion.

to the plasma membrane. Many cells also extend thin, stiff protrusions called **filopodia**, both at the leading edge and elsewhere on their surface (**Figure 17–34**). These are about 0.1 μm wide and 5–10 μm long, and each contains a loose bundle of 10–20 actin filaments (see Figure 17–28C), again oriented with their plus ends pointing outward. The advancing tip (*growth cone*) of a developing nerve cell axon extends even longer filopodia, up to 50 μm long, which help it to probe its environment and find the correct path to its target cell. Both lamellipodia and filopodia are exploratory, motile structures that form and retract with great speed, moving at around 1 μm per second. Both are thought to be generated by the rapid local growth of actin filaments, which assemble close to the plasma membrane and elongate by the addition of actin monomers at their plus ends. In this way, the filaments push out the membrane without tearing it.

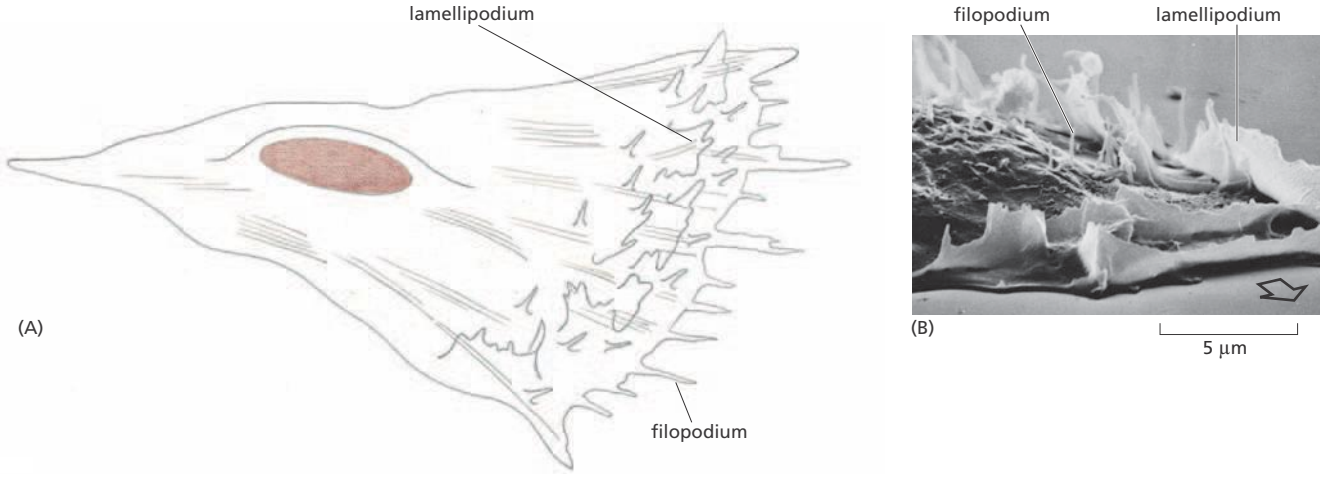


Figure 17–34 Actin filaments allow animal cells to migrate. (A) Schematic drawing of a fibroblast, showing flattened lamellipodia and fine filopodia projecting from its surface, especially in the regions of the leading edge. (B) Scanning electron micrograph showing lamellipodia and filopodia at the leading edge of a human fibroblast migrating in culture; the arrow shows the direction of cell movement. As the cell moves forward, the lamellipodia that fail to attach to the substratum are swept backward over the upper surface of the cell— a movement referred to as ruffling. (B, courtesy of Julian Heath.)

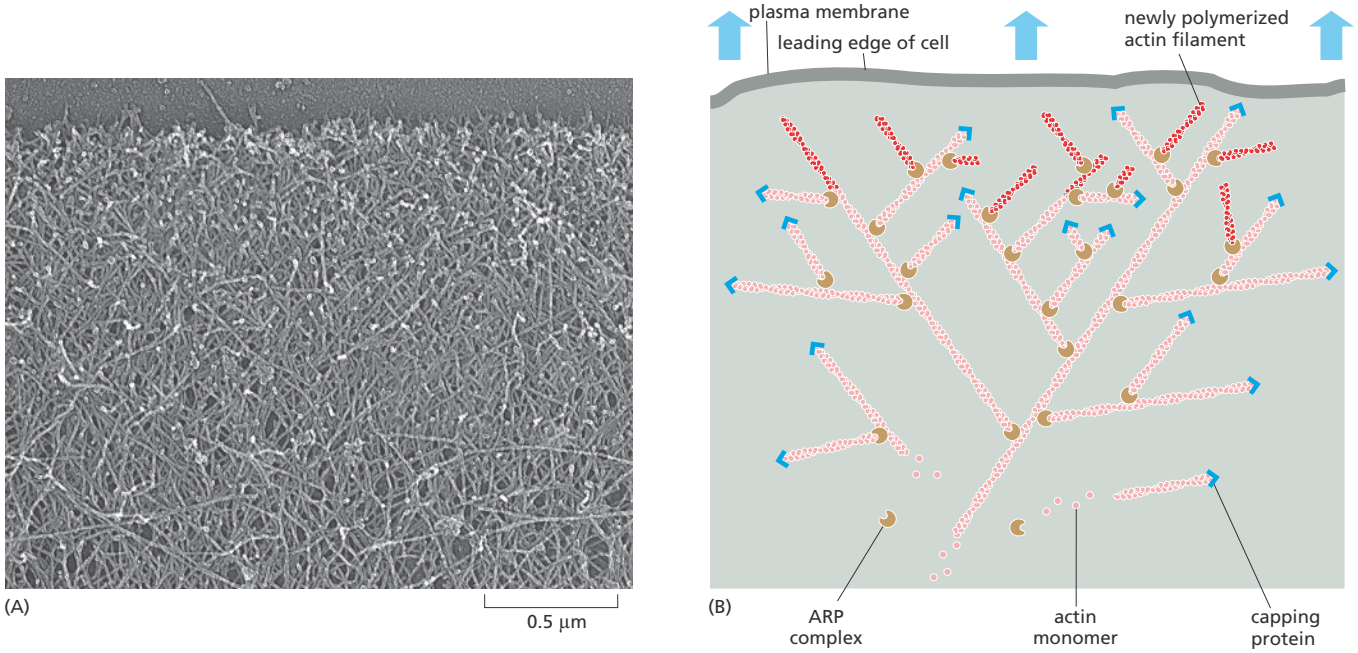


Figure 17–35 A web of polymerizing actin filaments pushes the leading edge of a lamellipodium forward. (A) A highly motile keratocyte from frog skin was fixed, dried, and shadowed with platinum, and examined in an electron microscope. Actin filaments form a dense network, with the fast-growing plus ends of the filaments terminating at the leading margin of the lamellipodium (top of figure). (B) Drawing showing how the nucleation of new actin filaments (*red*) is mediated by ARP complexes (*brown*) attached to the sides of preexisting actin filaments. The resulting branching structure pushes the plasma membrane forward. The plus ends of the actin filaments become protected from depolymerizing by capping proteins (*blue*), while the minus ends of actin filaments nearer the center of the cell continually disassemble through the action of depolymerizing proteins (not shown). The web of actin as a whole thereby undergoes a continual rearward movement due to the assembly of filaments at the front and their disassembly at the rear. This actin network is drawn to a different scale than the network shown in (A). (A, courtesy of Tatyana Svitkina and Gary Borisy.)

The formation and growth of actin filaments at the leading edge of a cell are assisted by various actin-binding proteins. The actin-related proteins—or ARPs—mentioned earlier promote the formation of a web of branched actin filaments in lamellipodia. ARPs form complexes that bind to the sides of existing actin filaments and nucleate the formation of new filaments, which grow out at an angle to produce side branches. With the aid of additional actin-binding proteins, this web undergoes continual assembly at the leading edge and disassembly further back, pushing the lamellipodium forward (**Figure 17–35**).

The other kind of cell protrusion, the filopodium, depends on *formins*, a nucleating protein that attaches to the growing plus ends of actin filaments and promotes the addition of new monomers to form straight, unbranched filaments. Formins are also used elsewhere to assemble unbranched filaments, as in the contractile ring that pinches a dividing animal cell in two.

When the lamellipodia and filopodia touch down on a favorable surface, they stick: transmembrane proteins in their plasma membrane, known as *integrins* (discussed in Chapter 20), adhere to molecules either in the extracellular matrix or on the surface of a neighboring cell over which the moving cell is crawling. Meanwhile, on the intracellular face of the crawling cell's plasma membrane, integrins capture actin filaments in the cortex, thereby creating a robust anchorage for the crawling cell (see Figures 17–33 and 20–15C). To use this anchorage to drag its body forward, the cell calls on the help of myosin motor proteins, as we now discuss.

QUESTION 17–6

Suppose that the actin molecules in a cultured skin cell have been randomly labeled in such a way that 1 in 10,000 molecules carries a fluorescent marker. What would you expect to see if you examined the lamellipodium (leading edge) of this cell through a fluorescence microscope? Assume that your microscope is sensitive enough to detect single fluorescent molecules.

Actin Associates with Myosin to Form Contractile Structures

All actin-dependent motor proteins belong to the **myosin** family. They bind to and hydrolyze ATP, which provides the energy for their movement along actin filaments toward the plus end. Myosin, along with actin, was first discovered in skeletal muscle, and much of what we know about the interaction of these two proteins was learned from studies of muscle. There are various types of myosins in cells, of which the myosin-I and myosin-II subfamilies are the most abundant. Myosin-I is present in all types of cells, whereas muscle cells make use of a specialized form of myosin-II. Because myosin-I is simpler in structure and mechanism of action, we discuss it first.

Myosin-I molecules have a head domain and a tail (**Figure 17–36A**). The head domain binds to an actin filament and has the ATP-hydrolyzing motor activity that enables it to move along the filament in a repetitive cycle of binding, detachment, and rebinding (**Movie 17.8**). The tail varies among the different types of myosin-I and determines what type of cargo the myosin drags along. For example, the tail may bind to a particular type of vesicle and propel it through the cell along actin filament tracks (**Figure 17–36B**), or it may bind to the plasma membrane and pull it into a different shape (**Figure 17–36C**).

Extracellular Signals Can Alter the Arrangement of Actin Filaments

We have seen that myosin and other actin-binding proteins can regulate the location, organization, and behavior of actin filaments. But the activities of these proteins are, in turn, controlled by extracellular signals, allowing the cell to rearrange its actin cytoskeleton in response to the environment.

The extracellular signal molecules that regulate the actin cytoskeleton activate a variety of cell-surface receptor proteins, which in turn activate various intracellular signaling pathways. These pathways often converge on a group of closely related *monomeric GTPase* proteins called the **Rho protein family**. As discussed in Chapter 16, monomeric GTPases behave as molecular switches that control intracellular processes by

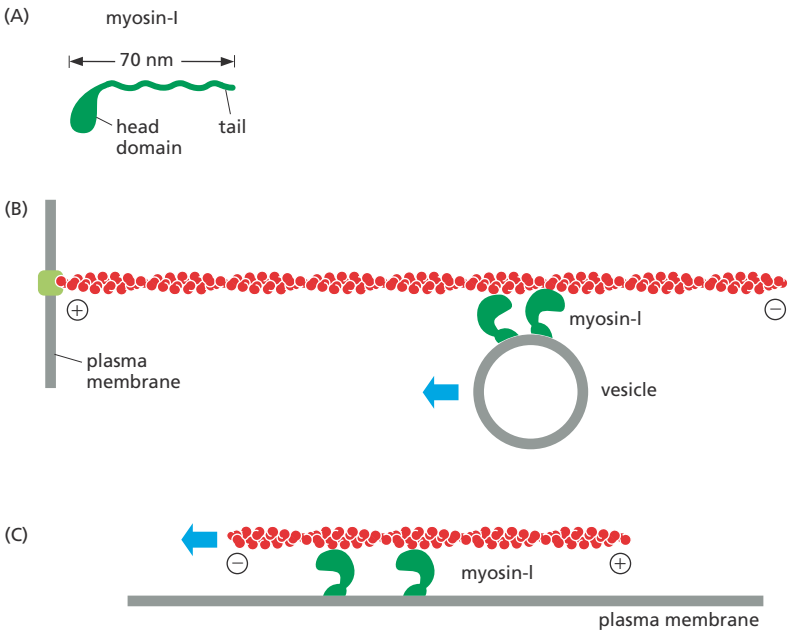


Figure 17–36 Myosin-I is the simplest myosin. (A) Myosin-I has a single globular head that attaches to an actin filament and a tail that attaches to another molecule or organelle in the cell. (B) This arrangement allows the head domain to move a vesicle relative to an actin filament, which in this case is anchored to the plasma membrane. (C) Myosin-I can also bind to an actin filament in the cell cortex, ultimately pulling the plasma membrane into a new shape. Note that the head group always walks toward the plus end of the actin filament.

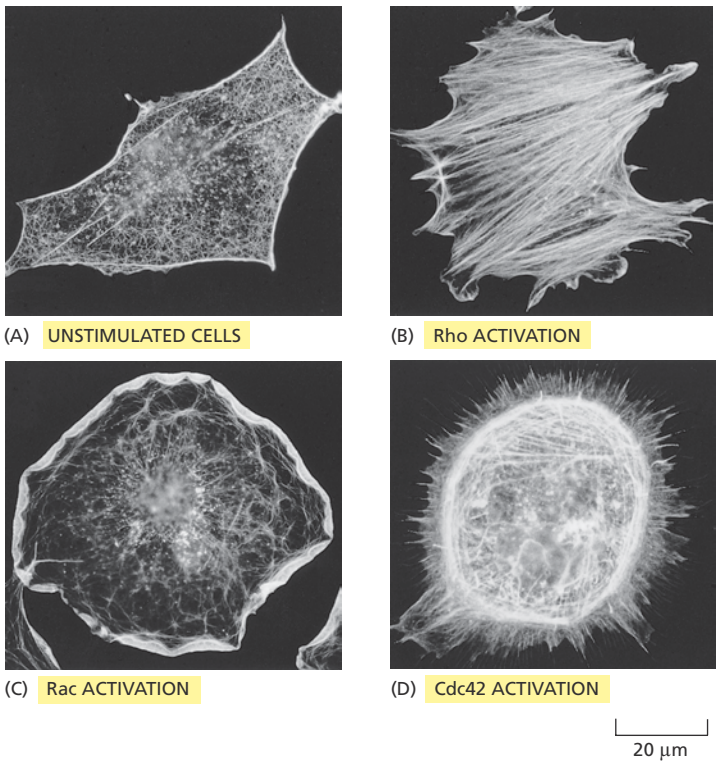


Figure 17–37 Activation of Rho family GTPases can have a dramatic effect on the organization of actin filaments in fibroblasts. In these micrographs, actin is stained with fluorescently labeled phalloidin, a molecule that binds specifically to actin filaments (see Table 17–2, p. 587). (A) Unstimulated fibroblasts have actin filaments primarily in the cortex. (B) Microinjection of an activated form of Rho promotes the rapid assembly of bundles of long, unbranched actin filaments; because myosin is associated with these bundles, they are contractile. (C) Microinjection of an activated form of Rac, a GTP-binding protein similar to Rho, causes the formation of an enormous lamellipodium that extends from the entire circumference of the cell. (D) Microinjection of an activated form of Cdc42, another Rho family member, stimulates the protrusion of many long filopodia at the cell periphery. (From A. Hall, *Science* 279:509–514, 1998. With permission from AAAS.)

QUESTION 17–7

At the leading edge of a crawling cell, the plus ends of actin filaments are located close to the plasma membrane, and actin monomers are added at these ends, pushing the membrane outward to form lamellipodia or filopodia. What do you suppose holds the filaments at their other ends to prevent them from just being pushed into the cell's interior?

cycling between an active GTP-bound state and an inactive GDP-bound state (see Figure 16–15B). In the case of the actin cytoskeleton, activation of different members of the Rho family affects the organization of actin filaments in different ways. For example, activation of one Rho family member triggers actin polymerization and filament bundling to form filopodia; activation of another promotes lamellipodia formation and ruffling; and activation of Rho itself drives the bundling of actin filaments with myosin-II and the clustering of cell-surface integrins, thus promoting cell crawling (**Figure 17–37**).

These dramatic and complex structural changes occur because the Rho family GTP-binding proteins, together with the protein kinases and accessory proteins with which they interact, act like a computational network to control actin organization and dynamics. This network receives external signals from nutrients, growth factors, and contacts with neighboring cells and the extracellular matrix, along with intracellular information about the cell's metabolic state and readiness for division. The Rho network then processes these inputs and activates intracellular signaling pathways that shape the actin cytoskeleton—for example, by activating the formin proteins that promote the formation of filopodia or by stimulating ARP complexes at the leading edge of the cell to generate large lamellipodia.

One of the most rapid rearrangements of cytoskeletal elements occurs when a muscle fiber contracts in response to a signal from a motor nerve, as we now discuss.

MUSCLE CONTRACTION

Muscle contraction is the most familiar and best understood of animal cell movements. In vertebrates, running, walking, swimming, and flying all depend on the ability of *skeletal muscle* to contract strongly and move various bones. Involuntary movements such as heart pumping and gut peristalsis depend on *cardiac muscle* and *smooth muscle*, respectively,

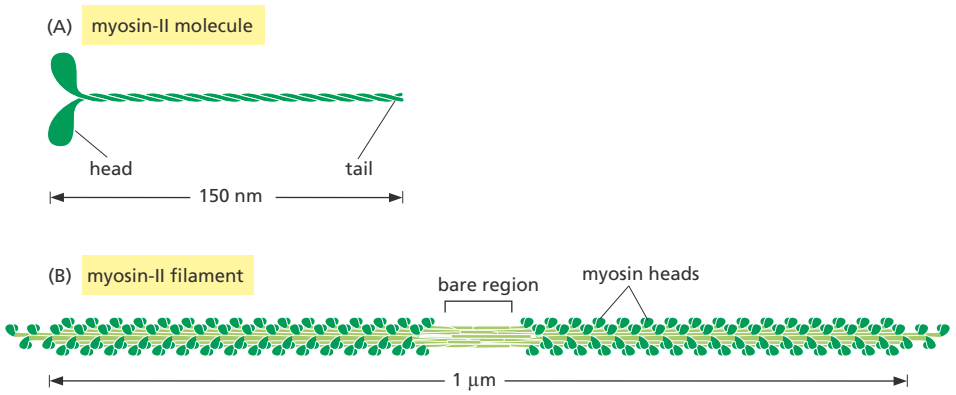


Figure 17–38 Myosin-II molecules can associate with one another to form myosin filaments. (A) A molecule of myosin-II contains two identical heavy chains, each with a globular head and an extended tail. (It also contains two light chains bound to each head, but these are not shown.) The tails of the two heavy chains form a single coiled-coil tail. (B) The coiled-coil tails of myosin-II molecules associate with one another to form a bipolar myosin filament in which the heads project outward from the middle in opposite directions. The bare region in the middle of the filament consists of tails only.

which are formed from muscle cells that differ in structure from skeletal muscle but use actin and myosin in a similar way to contract. Although muscle cells are highly specialized, many cell movements—from the locomotion of whole cells down to the motion of some components inside cells—also depend on the interaction of actin and myosin. Much of our understanding of the mechanisms of cell movement originated from studies of muscle cell contraction. In this section, we discuss how actin and myosin interact to produce this contraction.

Muscle Contraction Depends on Interacting Filaments of Actin and Myosin

Muscle myosin belongs to the **myosin-II** subfamily of myosins, all of which are dimers, with two globular ATPase heads at one end and a single coiled-coil tail at the other (**Figure 17–38A**). Clusters of myosin-II molecules bind to each other through their coiled-coil tails, forming a bipolar **myosin filament** from which the heads project (**Figure 17–38B**).

The myosin filament is like a double-headed arrow, with the two sets of myosin heads pointing in opposite directions, away from the middle. One set binds to actin filaments in one orientation and moves the filaments one way; the other set binds to other actin filaments in the opposite orientation and moves the filaments in the opposite direction. As a result, a myosin filament slides sets of oppositely oriented actin filaments past one another (**Figure 17–39**). We can see how, therefore, if actin filaments and myosin filaments are organized together in a bundle, the bundle can generate a strong contractile force. This is seen most clearly in muscle contraction, but it also occurs in the much smaller *contractile bundles* of actin filaments and myosin-II filaments (see Figure 17–28B) that assemble transiently in nonmuscle cells, and in the *contractile ring* that pinches a dividing cell in two by contracting and pulling inward on the plasma membrane (see Figure 17–28D).

QUESTION 17–8

If both the actin and myosin filaments of muscle are made up of subunits held together by weak noncovalent bonds, how is it possible for a human being to lift heavy objects?

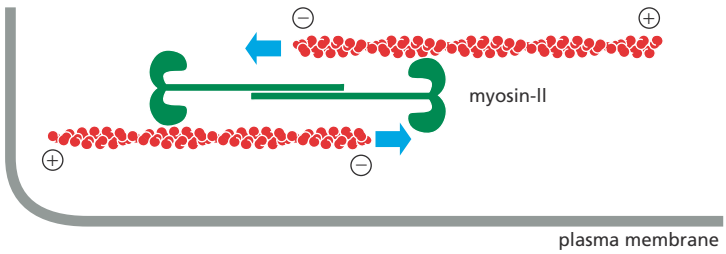


Figure 17–39 A small, bipolar myosin-II filament can slide two actin filaments of opposite orientation past each other. This sliding movement mediates the contraction of interacting actin and myosin-II filaments in both muscle and nonmuscle cells. As with myosin-I, a myosin-II head group walks toward the plus end of the actin filament with which it interacts.

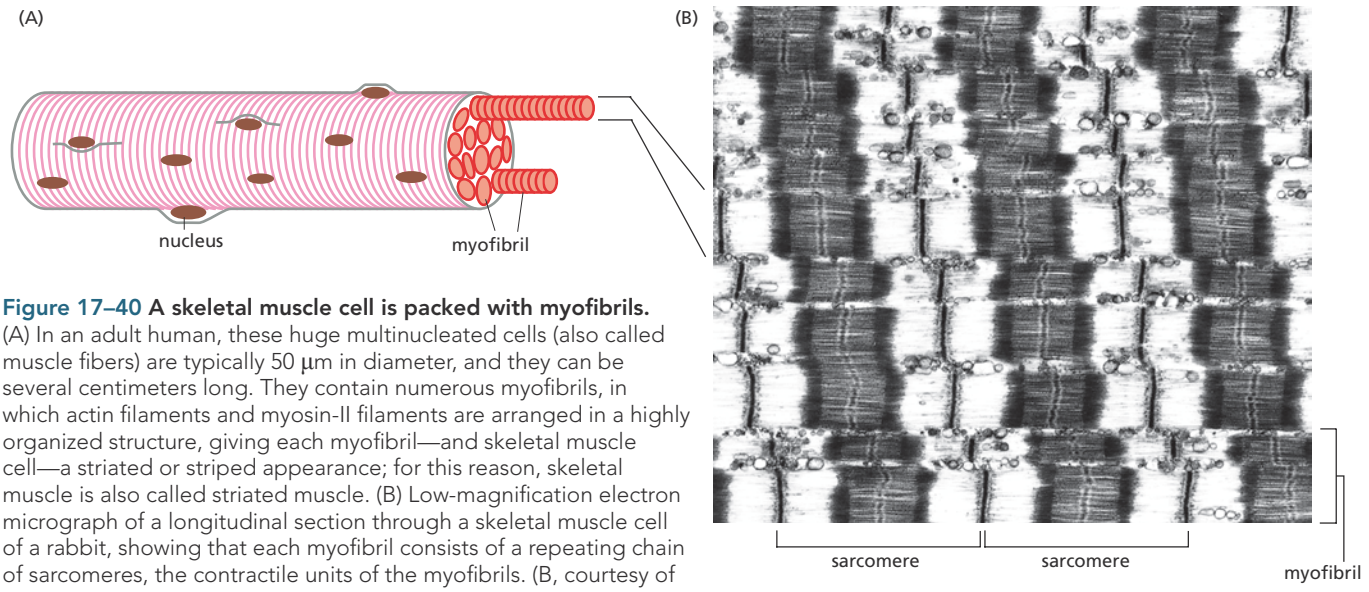


Figure 17–40 A skeletal muscle cell is packed with myofibrils. (A) In an adult human, these huge multinucleated cells (also called muscle fibers) are typically 50 μm in diameter, and they can be several centimeters long. They contain numerous myofibrils, in which actin filaments and myosin-II filaments are arranged in a highly organized structure, giving each myofibril—and skeletal muscle cell—a striated or striped appearance; for this reason, skeletal muscle is also called striated muscle. (B) Low-magnification electron micrograph of a longitudinal section through a skeletal muscle cell of a rabbit, showing that each myofibril consists of a repeating chain of sarcomeres, the contractile units of the myofibrils. (B, courtesy of Roger Craig.)

Actin Filaments Slide Against Myosin Filaments During Muscle Contraction

Skeletal muscle fibers are huge, multinucleated individual cells formed by the fusion of many separate smaller cells. The nuclei of the contributing cells are retained in the muscle fiber and lie just beneath the plasma membrane. The bulk of the cytoplasm is made up of **myofibrils**, the contractile elements of the muscle cell. These cylindrical structures are 1–2 μm in diameter and may be as long as the muscle cell itself (**Figure 17–40A**).

A myofibril consists of a chain of identical tiny contractile units, or **sarcomeres**. Each sarcomere is about 2.5 μm long, and the repeating pattern of sarcomeres gives the vertebrate myofibril a striped appearance (**Figure 17–40B**). Sarcomeres are highly organized assemblies of two types of filaments—actin filaments and myosin filaments composed of a muscle-specific form of myosin-II. The myosin filaments (the *thick filaments*) are centrally positioned in each sarcomere, whereas the more slender actin filaments (the *thin filaments*) extend inward from each end of the sarcomere, where they are anchored by their plus ends to a structure known as the *Z disc*. The minus ends of the actin filaments overlap the ends of the myosin filaments (**Figure 17–41**).

The contraction of a muscle cell is caused by a simultaneous shortening of all the cell's sarcomeres, which is caused by the actin filaments sliding past the myosin filaments, with no change in the length of either type of filament (**Figure 17–42**). The sliding motion is generated by myosin heads that project from the sides of the myosin filament and interact with adjacent actin filaments (see Figure 17–39). When a muscle is stimulated

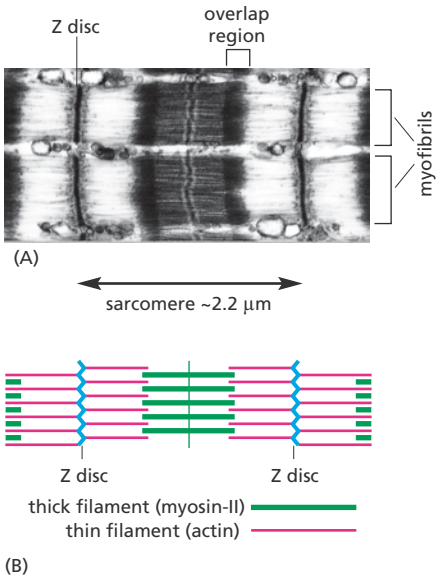


Figure 17–41 Sarcomeres are the contractile units of muscle. (A) Detail of the electron micrograph from Figure 17–40 showing two myofibrils; the length of one sarcomere and the region where the actin and myosin filaments overlap are indicated. (B) Schematic diagram of a single sarcomere showing the origin of the light and dark bands seen in the microscope. Z discs at either end of the sarcomere are attachment points for the plus ends of actin filaments. The centrally located thick filaments (*green*) are each composed of many myosin-II molecules. The thin vertical line running down the center of the thick filament bundle in (A) corresponds to the bare regions of the myosin filaments, as seen in Figure 17–38B. (A, courtesy of Roger Craig.)

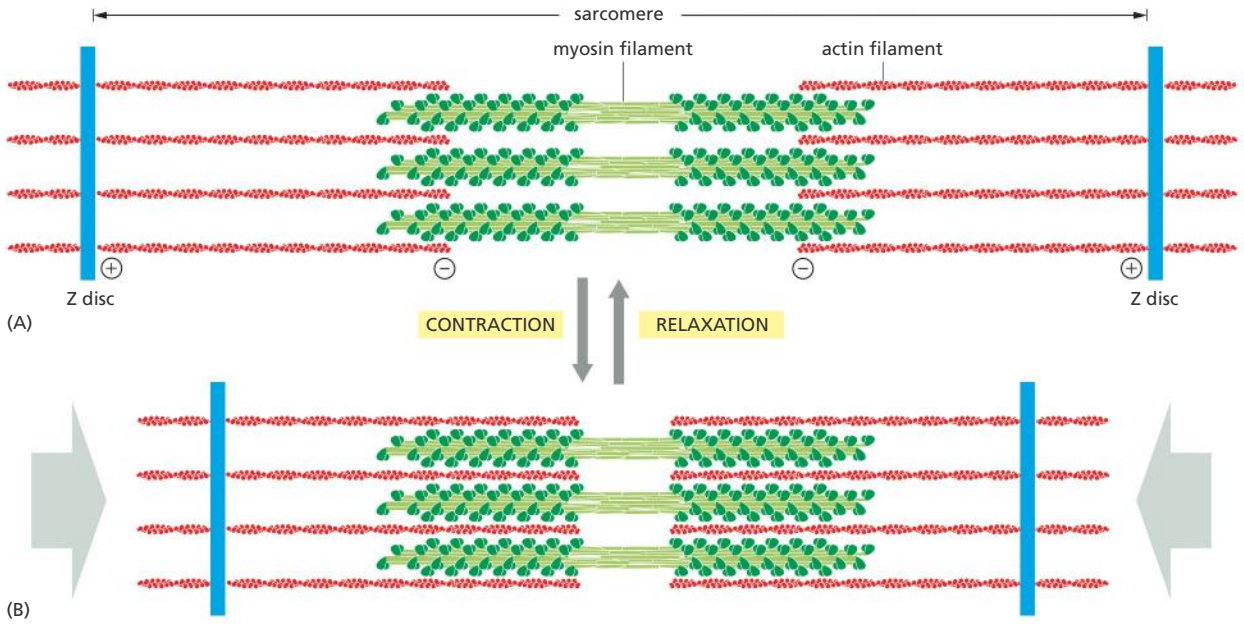


Figure 17–42 Muscles contract by a sliding-filament mechanism. (A) The myosin and actin filaments of a sarcomere overlap with the same relative polarity on either side of the midline. Recall that actin filaments are anchored by their plus ends to the Z disc and that myosin filaments are bipolar. (B) During contraction, the actin and myosin filaments slide past each other without shortening. The sliding motion is driven by the myosin heads walking toward the plus end of the adjacent actin filaments (Movie 17.9).

to contract, the myosin heads start to walk along the actin filament in repeated cycles of attachment and detachment. During each cycle, a myosin head binds and hydrolyzes one molecule of ATP. This causes a series of conformational changes that move the tip of the head by about 5 nm along the actin filament toward the plus end. This movement, repeated with each round of ATP hydrolysis, propels the myosin molecule unidirectionally along the actin filament (**Figure 17–43**). In so doing, the myosin heads pull against the actin filament, causing it to slide against the myosin filament. The concerted action of many myosin heads pulling the actin and myosin filaments past each other causes the sarcomere to contract. After a contraction is completed, the myosin heads all lose contact with the actin filaments, and the muscle relaxes.

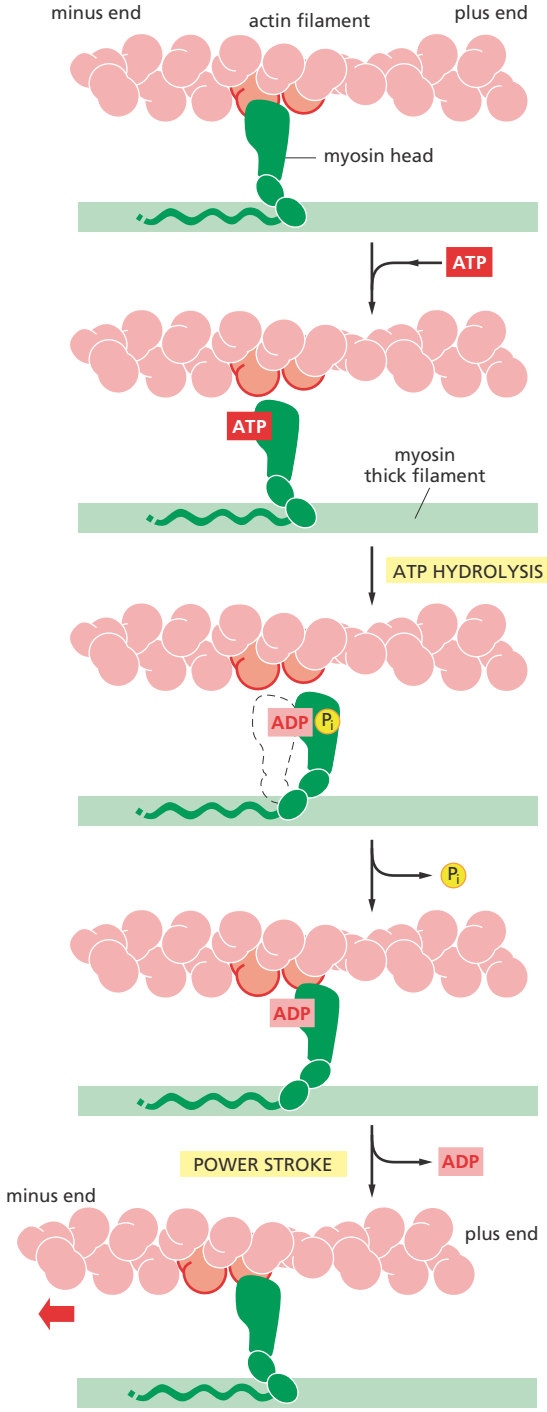
A myosin filament has about 300 myosin heads. Each myosin head can attach and detach from actin about five times per second, allowing the myosin and actin filaments to slide past one another at speeds of up to 15 μm per second. This speed is sufficient to take a sarcomere from a fully extended state (3 μm) to a fully contracted state (2 μm) in less than one-tenth of a second. All of the sarcomeres of a muscle are coupled together and are triggered simultaneously by the signaling system we describe next, so the entire muscle contracts almost instantaneously.

Muscle Contraction Is Triggered by a Sudden Rise in Cytosolic Ca^{2+}

The force-generating molecular interaction between myosin and actin filaments takes place only when the skeletal muscle receives a signal from a motor nerve. The neurotransmitter released from the nerve terminal triggers an action potential (discussed in Chapter 12) in the muscle cell plasma membrane. This electrical excitation spreads in a matter of milliseconds into a series of membranous tubes, called *transverse* (or *T*) *tubules*, that extend inward from the plasma membrane around

each myofibril. The electrical signal is then relayed to the *sarcoplasmic reticulum*, an adjacent sheath of interconnected flattened vesicles that surrounds each myofibril like a net stocking (**Figure 17–44**).

The sarcoplasmic reticulum is a specialized region of the endoplasmic reticulum in muscle cells. It contains a very high concentration of Ca^{2+}, and in response to the incoming electrical excitation, much of this Ca^{2+} is released into the cytosol through a specialized set of ion channels that



ATTACHED At the start of the cycle shown in this figure, a myosin head lacking a bound ATP or ADP is attached tightly to an actin filament in a *rigor* configuration (so named because it is responsible for *rigor mortis*, the rigidity of death). In an actively contracting muscle, this state is very short-lived, being rapidly terminated by the binding of a molecule of ATP to the myosin head.

RELEASED A molecule of ATP binds to the large cleft on the "back" of the myosin head (that is, on the side furthest from the actin filament) and immediately causes a slight change in the conformation of the domains that make up the actin-binding site. This reduces the affinity of the head for actin and allows it to move along the filament. (The space drawn here between the head and actin emphasizes this change, although in reality the head probably remains very close to the actin.)

COCKED The cleft closes like a clam shell around the ATP molecule, triggering a large shape change that causes the head to be displaced along the actin filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate (P_i) produced remain tightly bound to the myosin head.

FORCE-GENERATING A weak binding of the myosin head to a new site on the actin filament causes release of the inorganic phosphate produced by ATP hydrolysis, concomitantly with the tight binding of the head to actin. This release triggers the power stroke—the force-generating change in shape during which the head regains its original conformation. In the course of the power stroke, the head loses its bound ADP, thereby returning to the start of a new cycle.

ATTACHED At the end of the cycle, the myosin head is again bound tightly to the actin filament in a rigor configuration. Note that the head has moved to a new position on the actin filament, which has slid to the left along the myosin filament.

Figure 17–43 The head of a myosin-II molecule walks along an actin filament through an ATP-dependent cycle of conformational changes. Two actin monomers are highlighted to make the movement of the actin filament easier to see. Movie 17.10 shows actin and myosin in action. (Based on I. Rayment et al., *Science* 261:50–58, 1993. With permission from AAAS.)

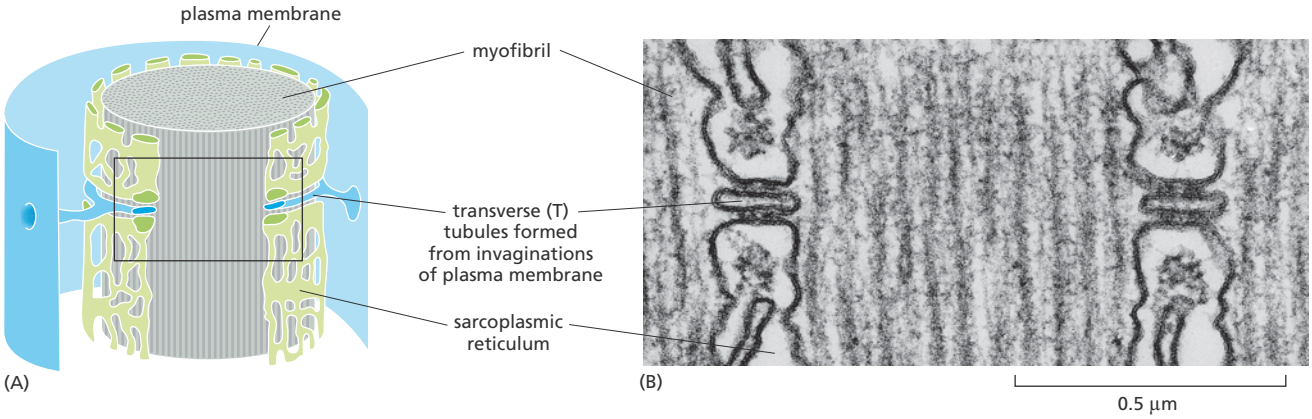


Figure 17–44 T tubules and the sarcoplasmic reticulum surround each myofibril. (A) Drawing of the two membrane systems that relay the signal to contract from the muscle cell plasma membrane to all of the myofibrils in the muscle cell. (B) Electron micrograph showing a cross section of two T tubules and their adjacent sarcoplasmic reticulum compartments. (B, courtesy of Clara Franzini-Armstrong.)

open in the sarcoplasmic reticulum membrane in response to the change in voltage across the plasma membrane and T tubules (**Figure 17–45**). As discussed in Chapter 16, Ca^{2+} is widely used as a small intracellular signal to relay a message from the exterior to the interior of cells. In muscle, the rise in cytosolic Ca^{2+} concentration activates a molecular switch made of specialized accessory proteins closely associated with the actin filaments (**Figure 17–46A**). One of these proteins is *tropomyosin*, a rigid, rod-shaped molecule that binds in the groove of the actin helix, where it prevents the myosin heads from associating with the actin filament. The other is *troponin*, a protein complex that includes a Ca^{2+}-sensitive protein associated with the end of a tropomyosin molecule. When the concentration of Ca^{2+} rises in the cytosol, Ca^{2+} binds to troponin and induces a change in the shape of the troponin complex. This in turn causes the tropomyosin molecules to shift their positions slightly, allowing myosin heads to bind to the actin filaments, initiating contraction (**Figure 17–46B**).

QUESTION 17–9

Compare the structure of intermediate filaments with that of the myosin-II filaments in skeletal muscle cells. What are the major similarities? What are the major differences? How do the differences in structure relate to their function?

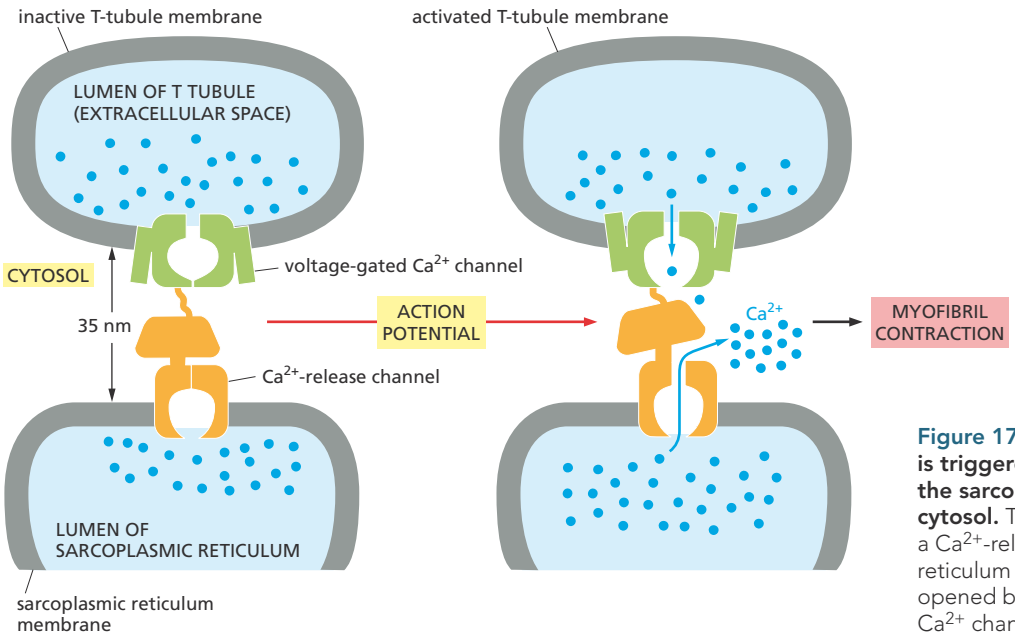


Figure 17–45 Skeletal muscle contraction is triggered by the release of Ca^{2+} from the sarcoplasmic reticulum into the cytosol. This schematic diagram shows how a Ca^{2+}-release channel in the sarcoplasmic reticulum membrane is thought to be opened by activation of a voltage-gated Ca^{2+} channel in the T-tubule membrane.

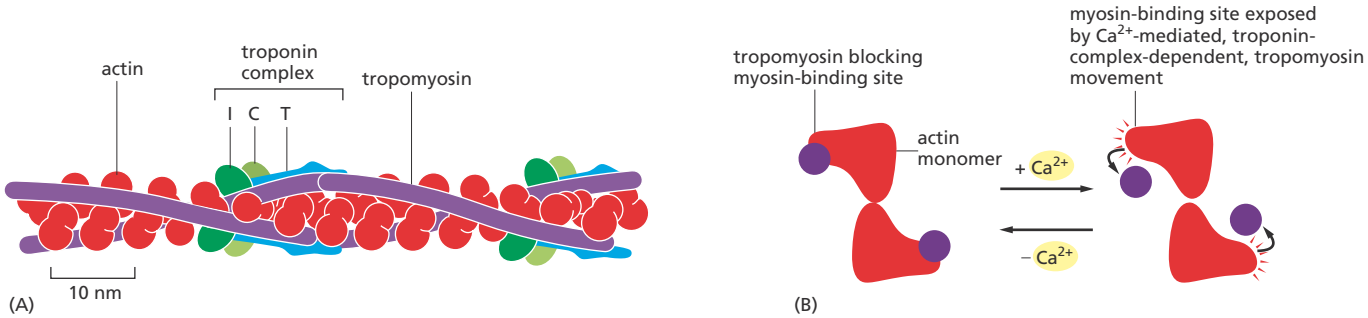


Figure 17–46 Skeletal muscle contraction is controlled by tropomyosin and troponin complexes. (A) An actin filament in muscle showing the positions of tropomyosin and troponin complexes along the filament. Every tropomyosin molecule has seven evenly spaced regions of similar amino acid sequence, each of which is thought to bind to an actin monomer in the filament. (B) When Ca^{2+} binds to a troponin complex, the complex moves the tropomyosin, which otherwise blocks the interaction of actin with the myosin heads. Here, the actin filament from (A) is shown end-on.

Because the signal from the plasma membrane is passed within milliseconds (via the transverse tubules and sarcoplasmic reticulum) to every sarcomere in the cell, all the myofibrils in the cell contract at the same time. The increase in Ca^{2+} in the cytosol is transient because, when the nerve signal terminates, the Ca^{2+} is rapidly pumped back into the sarcoplasmic reticulum by abundant Ca^{2+}-pumps in its membrane (discussed in Chapter 12). As soon as the Ca^{2+} concentration returns to the resting level, troponin and tropomyosin molecules move back to their original positions. This reconfiguration once again blocks myosin binding to actin filaments, thereby ending the contraction.

QUESTION 17–10

A. Note that in Figure 17–46, troponin molecules are evenly spaced along an actin filament, with one troponin found every seventh actin molecule. How do you suppose troponin molecules can be positioned this regularly? What does this tell you about the binding of troponin to actin filaments?
B. What do you suppose would happen if you mixed actin filaments with (i) troponin alone, (ii) tropomyosin alone, or (iii) troponin plus tropomyosin, and then added myosin? Would the effects be dependent on Ca^{2+}?

Different Types of Muscle Cells Perform Different Functions

The highly specialized contractile machinery in muscle cells is thought to have evolved from the simpler contractile bundles of myosin and actin filaments found in all eukaryotic cells. The myosin-II in nonmuscle cells is also activated by a rise in cytosolic Ca^{2+}, but the mechanism of activation is different from that of the muscle-specific myosin-II. An increase in Ca^{2+} leads to the phosphorylation of nonmuscle myosin-II, which alters the myosin conformation and enables it to interact with actin. A similar activation mechanism operates in *smooth muscle*, which is present in the walls of the stomach, intestine, uterus, and arteries, and in many other structures that undergo slow and sustained involuntary contractions. This mode of myosin activation is relatively slow, because time is needed for enzyme molecules to diffuse to the myosin heads and carry out the phosphorylation and subsequent dephosphorylation. However, this mechanism has the advantage that—unlike the mechanism used by skeletal muscle cells—it can be activated by a variety of extracellular signals: thus smooth muscle, for example, is triggered to contract by adrenaline, serotonin, prostaglandins, and several other signal molecules.

In addition to skeletal and smooth muscle, other forms of muscle each perform a specific mechanical function. Heart—or *cardiac*—muscle, for instance, drives the circulation of blood. The heart contracts autonomously for the entire life of the organism—some 3 billion (3×10^9) times in an average human lifetime. Even subtle abnormalities in the actin or myosin of heart muscle can lead to serious disease. For example, mutations in the genes that encode cardiac myosin-II or other proteins in the sarcomere cause familial hypertrophic cardiomyopathy, a hereditary disorder responsible for sudden death in young athletes.

The contraction of muscle cells represents a highly specialized use of the basic components of the eukaryotic cytoskeleton. In the following chapter, we discuss the crucial roles the cytoskeleton has in perhaps the most fundamental cell movements of all: the segregation of newly replicated chromosomes and the formation of two daughter cells during the process of cell division.

ESSENTIAL CONCEPTS

- The cytoplasm of a eukaryotic cell is supported and organized by a cytoskeleton of intermediate filaments, microtubules, and actin filaments.
- Intermediate filaments are stable, ropelike polymers—built from fibrous protein subunits—that give cells mechanical strength. Some intermediate filaments form the nuclear lamina that supports and strengthens the nuclear envelope; others are distributed throughout the cytoplasm.
- Microtubules are stiff, hollow tubes formed by globular tubulin dimers. They are polarized structures, with a slow-growing minus end and a fast-growing plus end.
- Microtubules grow out from organizing centers such as the centrosome, in which the minus ends remain embedded.
- Many microtubules display dynamic instability, alternating rapidly between growth and shrinkage. Shrinkage is promoted by the hydrolysis of the GTP that is tightly bound to tubulin dimers, reducing the affinity of the dimers for their neighbors and thereby promoting microtubule disassembly.
- Microtubules can be stabilized by localized proteins that capture the plus ends, thereby helping to position the microtubules and harness them for specific functions.
- Kinesins and dyneins are microtubule-associated motor proteins that use the energy of ATP hydrolysis to move unidirectionally along microtubules. They carry specific organelles, vesicles, and other types of cargo to particular locations in the cell.
- Eukaryotic cilia and flagella contain a bundle of stable microtubules. Their rhythmic beating is caused by bending of the microtubules, driven by the ciliary dynein motor protein.
- Actin filaments are helical polymers of globular actin monomers. They are more flexible than microtubules and are generally found in bundles or networks.
- Like microtubules, actin filaments are polarized, with a fast-growing plus end and a slow-growing minus end. Their assembly and disassembly are controlled by the hydrolysis of ATP tightly bound to each actin monomer and by various actin-binding proteins.
- The varied arrangements and functions of actin filaments in cells stem from the diversity of actin-binding proteins, which can control actin polymerization, cross-link actin filaments into loose networks or stiff bundles, attach actin filaments to membranes, or move two adjacent filaments relative to each other.
- A concentrated network of actin filaments underneath the plasma membrane forms the bulk of the cell cortex, which is responsible for the shape and movement of the cell surface, including the movements involved when a cell crawls along a surface.
- Myosins are motor proteins that use the energy of ATP hydrolysis to move along actin filaments. In nonmuscle cells, myosin-I can carry organelles or vesicles along actin-filament tracks, and myosin-II can cause adjacent actin filaments to slide past each other in contractile bundles.

- In skeletal muscle cells, repeating arrays of overlapping filaments of actin and myosin-II form highly ordered myofibrils, which contract as these filaments slide past each other.
- Muscle contraction is initiated by a sudden rise in cytosolic Ca^{2+}, which delivers a signal to the myofibrils via Ca^{2+}-binding proteins associated with the actin filaments.

KEY TERMS

actin-binding protein
actin filament
cell cortex
centriole
centrosome
cilium
cytoskeleton
dynamic instability
dynein
filopodium
flagellum
intermediate filament
keratin filament
kinesin
lamellipodium
microtubule
microtubule-associated protein
motor protein
myofibril
myosin
myosin filament
nuclear lamina
polarity
Rho protein family
sarcomere
tubulin

QUESTIONS

QUESTION 17–11

Which of the following statements are correct? Explain your answers.

A. Kinesin moves endoplasmic reticulum membranes along microtubules so that the network of ER tubules becomes stretched throughout the cell.

B. Without actin, cells can form a functional mitotic spindle and pull their chromosomes apart but cannot divide.

C. Lamellipodia and filopodia are "feelers" that a cell extends to find anchor points on the substratum that it will then crawl over.

D. GTP is hydrolyzed by tubulin to cause the bending of flagella.

E. Cells having an intermediate-filament network that cannot be depolymerized would die.

F. The plus ends of microtubules grow faster because they have a larger GTP cap.

G. The transverse tubules in muscle cells are an extension of the plasma membrane, with which they are continuous; similarly, the sarcoplasmic reticulum is an extension of the endoplasmic reticulum.

H. Activation of myosin movement on actin filaments is triggered by the phosphorylation of troponin in some situations and by Ca^{2+} binding to troponin in others.

QUESTION 17–12

The average time taken for a molecule or an organelle to diffuse a distance of x cm is given by the formul

$$t = x^2/2D$$

where t is the time in seconds and D is a constant called the diffusion coefficient for the molecule or particle. Using the above formula, calculate the time it would take for a small molecule, a protein, and a membrane vesicle to diffuse from one side to another of a cell 10 μm across. Typical diffusion coefficients in units of cm^2/sec are: small molecule, 5×10^{-6}; protein molecule, 5×10^{-7}; vesicle, 5×10^{-8}. How long would a membrane vesicle take to reach the end of an axon 10 cm long by free diffusion? How long would it take if it was transported along microtubules at 1 μm/sec?

QUESTION 17–13

Why do eukaryotic cells, and especially animal cells, have such large and complex cytoskeletons? List the differences between animal cells and bacteria that depend on the eukaryotic cytoskeleton.

QUESTION 17–14

Examine the structure of an intermediate filament shown in Figure 17–4. Does the filament have a unique polarity—that is, could you distinguish one end from the other by chemical or other means? Explain your answer.

QUESTION 17–15

There are no known motor proteins that move on intermediate filaments. Suggest an explanation for this.

QUESTION 17–16

When cells enter mitosis, their existing array of cytoplasmic microtubules has to be rapidly broken down and replaced with the mitotic spindle that forms to pull the chromosomes into the daughter cells. The enzyme katanin, named after Japanese samurai swords, is activated during the onset of mitosis, and chops microtubules into short pieces. What do you suppose is the fate of the microtubule fragments created by katanin? Explain your answer.

QUESTION 17–17

The drug Taxol, extracted from the bark of yew trees, has an opposite effect to the drug colchicine, an alkaloid from autumn crocus. Taxol binds tightly to microtubules and stabilizes them; when added to cells, it causes much of the free tubulin to assemble into microtubules. In contrast, colchicine prevents microtubule formation. Taxol is just as pernicious to dividing cells as colchicine, and both are used as anticancer drugs. Based on your knowledge of microtubule dynamics, suggest why both drugs are toxic to dividing cells despite their opposite actions.

QUESTION 17–18

A useful technique for studying microtubule motors is to attach them by their tails to a glass coverslip (which can be accomplished quite easily because the tails stick avidly to a clean glass surface) and then allow them to settle. The microtubules may then be viewed in a light microscope as they are propelled over the surface of the coverslip by the heads of the motor proteins. Because the motor proteins attach at random orientations to the coverslip, however, how can they generate coordinated movement of individual microtubules rather than engaging in a tug-of-war? In which direction will microtubules crawl on a 'bed' of kinesin molecules (i.e., will they move plus-end first, or minus-end first)?

QUESTION 17–19

A typical time course of polymerization of purified tubulin to form microtubules is shown in **Figure Q17–19**.

A. Explain the different parts of the curve (labeled A, B, and C). Draw a diagram that shows the behavior of tubulin molecules in each of the three phases.

B. How would the curve in the figure change if centrosomes were added at the outset?

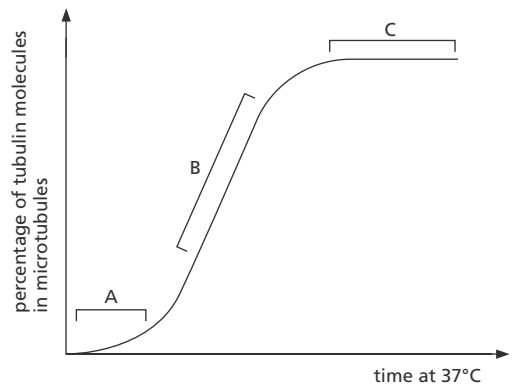


Figure Q17–19

QUESTION 17–20

The electron micrographs shown in **Figure Q17–20A** were obtained from a population of microtubules that were growing rapidly. **Figure Q17–20B** was obtained from microtubules undergoing "catastrophic" shrinking. Comment on any differences between A and B, and suggest likely explanations for the differences that you observe.

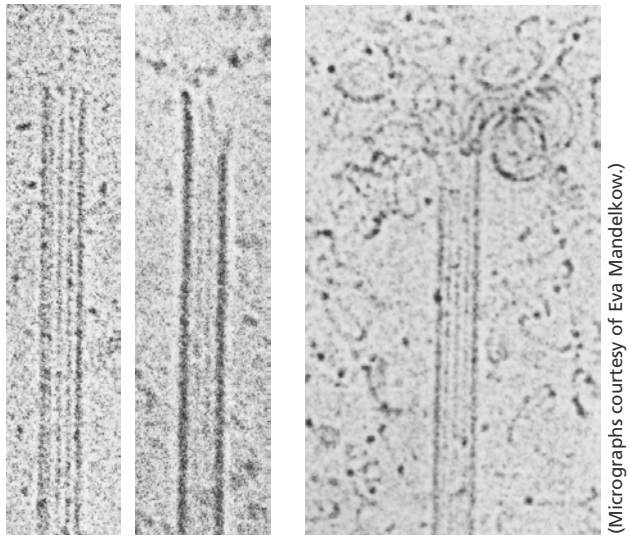


Figure Q17–20

QUESTION 17–21

The locomotion of fibroblasts in culture is immediately halted by the drug cytochalasin, whereas colchicine causes fibroblasts to cease to move directionally and to begin extending lamellipodia in seemingly random directions. Injection of fibroblasts with antibodies to vimentin has no discernible effect on their migration. What do these observations suggest to you about the involvement of the three different cytoskeletal filaments in fibroblast locomotion?

QUESTION 17–22

Complete the following sentence accurately, explaining your reason for accepting or rejecting each of the four phrases (more than one can be correct). The role of calcium in muscle contraction is:

A. To detach myosin heads from actin.

B. To spread the action potential from the plasma membrane to the contractile machinery.

C. To bind to troponin, cause it to move tropomyosin, and thereby expose actin filaments to myosin heads.

D. To maintain the structure of the myosin filament.

QUESTION 17–23

Which of the following changes takes place when a skeletal muscle contracts?

A. Z discs move farther apart.

B. Actin filaments contract.

C. Myosin filaments contract.

D. Sarcomeres become shorter.

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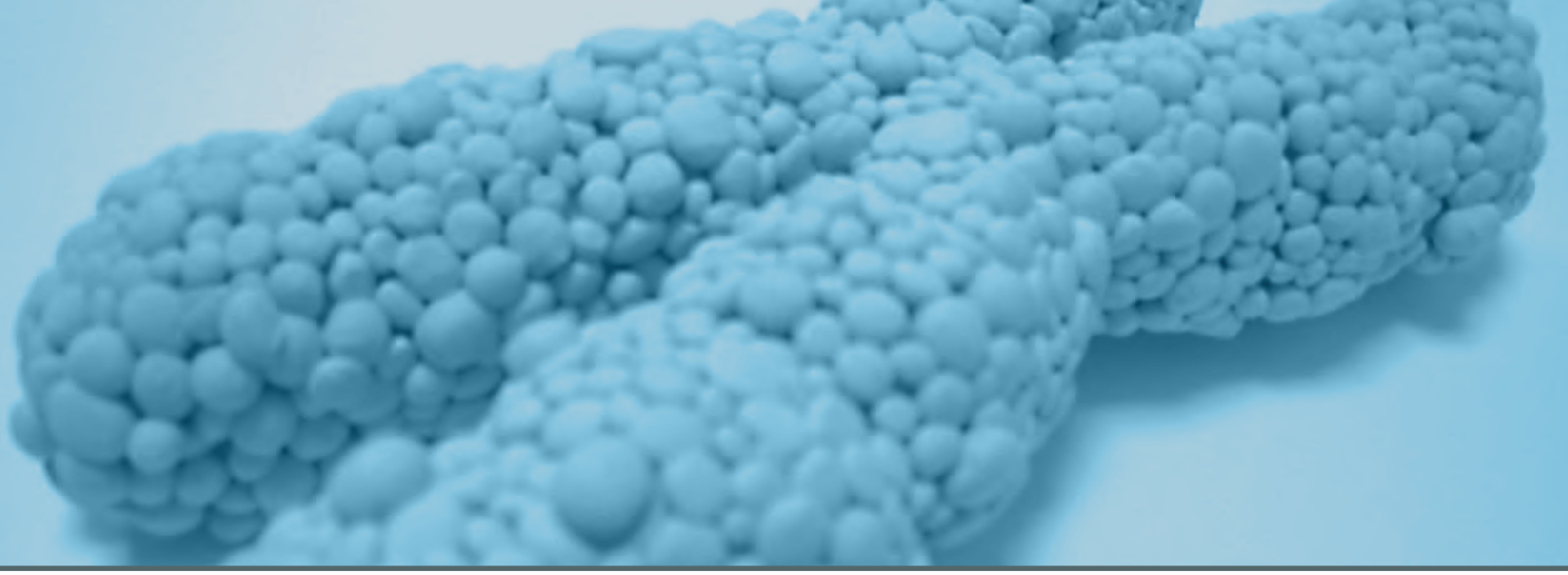


CHAPTER **EIGHTEEN**

18

The Cell-Division Cycle

"Where a cell arises, there must be a previous cell, just as animals can only arise from animals and plants from plants." This statement, which appears in a book written by German pathologist Rudolf Virchow in 1858, carries with it a profound message for the continuity of life. If every cell comes from a previous cell, all living organisms—from a unicellular bacterium to a multicellular mammal—are products of repeated rounds of cell growth and division that stretch back to the beginnings of life more than 3 billion years ago.

A cell reproduces by carrying out an orderly sequence of events in which it duplicates its contents and then divides in two. This cycle of duplication and division, known as the **cell cycle**, is the essential mechanism by which all living things reproduce. The details of the cell cycle vary from organism to organism and at different times in an individual organism's life. In unicellular organisms, such as bacteria and yeasts, each cell division produces a complete new organism, whereas many rounds of cell division are required to make a new multicellular organism from a fertilized egg. Certain features of the cell cycle, however, are universal, as they allow every cell to perform the fundamental task of copying and passing on its genetic information to the next generation of cells.

To explain how cells reproduce, we have to consider three major questions: (1) How do cells duplicate their contents—including the chromosomes, which carry the genetic information? (2) How do they partition the duplicated contents and split in two? (3) How do they coordinate all the steps and machinery required for these two processes? The first question is considered elsewhere in this book: in Chapter 6, we discuss how DNA is replicated, and in Chapters 7, 11, 15, and 17, we describe how the

eukaryotic cell manufactures other components, such as proteins, membranes, organelles, and cytoskeletal filaments. In this chapter, we tackle the second and third questions: how a eukaryotic cell distributes—or *segregates*—its duplicated contents to produce two genetically identical daughter cells, and how it coordinates the various steps of this reproductive cycle.

We begin with an overview of the events that take place during a typical cell cycle. We then describe the complex system of regulatory proteins called the *cell-cycle control system*, which orders and coordinates these events to ensure that they occur in the correct sequence. We next discuss in detail the major stages of the cell cycle, in which the chromosomes are duplicated and then segregated into the two daughter cells. At the end of the chapter, we consider how animals use extracellular signals to control the survival, growth, and division of their cells. These signaling systems allow an animal to regulate the size and number of its cells—and, ultimately, the size and form of the organism itself.

QUESTION 18–1

Consider the following statement: "All present-day cells have arisen by an uninterrupted series of cell divisions extending back in time to the first cell division." Is this strictly true?

OVERVIEW OF THE CELL CYCLE

The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then to segregate the DNA into genetically identical daughter cells such that each cell receives a complete copy of the entire genome (**Figure 18–1**). In most cases, a cell also duplicates its other macromolecules and organelles and doubles in size before it divides; otherwise, each time a cell split it would get smaller and smaller. Thus, to maintain their size, dividing cells coordinate their growth with their division. We return to the topic of cell-size control later in the chapter; here, we focus on cell division.

The duration of the cell cycle varies greatly from one cell type to another. In an early frog embryo, cells divide every 30 minutes, whereas a mammalian fibroblast in culture divides about once a day (**Table 18–1**). In this section, we describe briefly the sequence of events that occur in fairly rapidly dividing (proliferating) mammalian cells. We then introduce the cell-cycle control system that ensures that the various events of the cycle take place in the correct sequence and at the correct time.

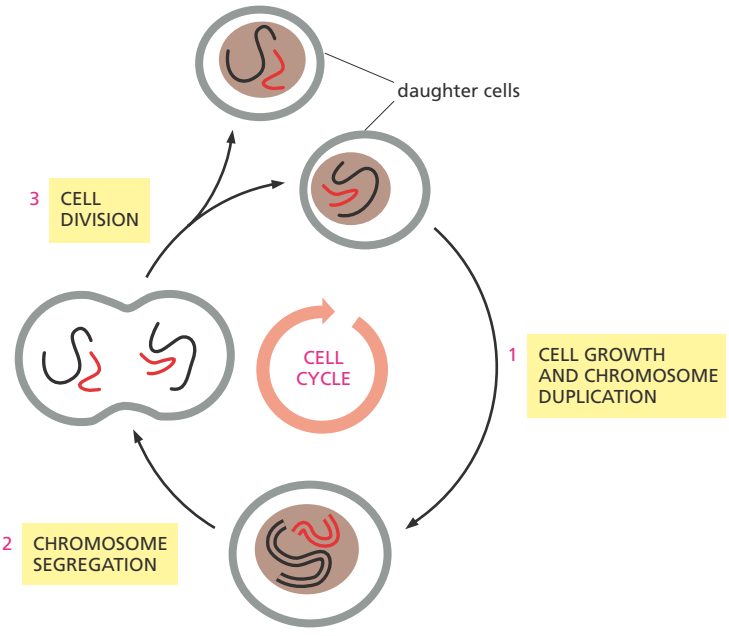


Figure 18–1 Cells reproduce by duplicating their contents and dividing in two, a process called the cell cycle. For simplicity, we use a hypothetical eukaryotic cell—with only one copy each of two different chromosomes—to illustrate how each cell cycle produces two genetically identical daughter cells. Each daughter cell can divide again by going through another cell cycle, and so on for generation after generation.

TABLE 18–1 SOME EUKARYOTIC CELL-CYCLE TIMES	
Cell Type	**Cell-Cycle Times**
Early frog embryo cells	30 minutes
Yeast cells	1.5 hours
Mammalian intestinal epithelial cells	~12 hours
Mammalian fibroblasts in culture	~20 hours

The Eukaryotic Cell Cycle Usually Includes Four Phases

Seen in a microscope, the two most dramatic events in the cell cycle are when the nucleus divides, a process called *mitosis*, and when the cell later splits in two, a process called *cytokinesis*. These two processes together constitute the **M phase** of the cycle. In a typical mammalian cell, the whole of M phase takes about an hour, which is only a small fraction of the total cell-cycle time (see Table 18–1).

The period between one M phase and the next is called **interphase**. Viewed with a microscope, it appears, deceptively, as an uneventful interlude during which the cell simply increases in size. Interphase, however, is a very busy time for a proliferating cell, and it encompasses the remaining three phases of the cycle. During **S phase** (S = synthesis), the cell replicates its DNA. S phase is flanked by two "gap" phases—called **G_1** and **G_2**—during which the cell continues to grow (**Figure 18–2**). During these gap phases, the cell monitors both its internal state and external environment. This monitoring ensures that conditions are suitable for reproduction and that preparations are complete before the cell commits to the major upheavals of S phase (which follows G_1) and mitosis (following G_2). At particular points in G_1 and G_2, the cell decides whether to proceed to the next phase or pause to allow more time to prepare.

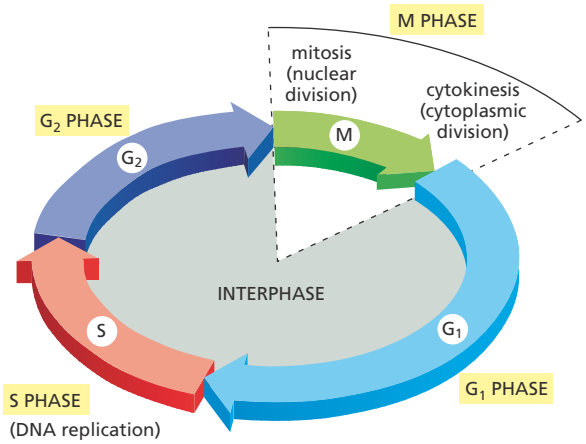
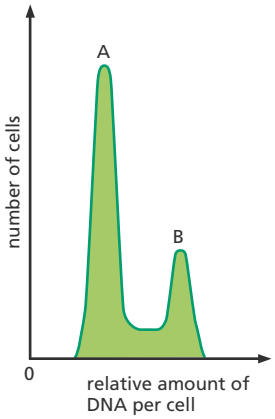


Figure 18–2 The eukaryotic cell cycle usually occurs in four phases. The cell grows continuously in interphase, which consists of three phases: G_1, S, and G_2. DNA replication is confined to S phase. G_1 is the gap between M phase and S phase, and G_2 is the gap between S phase and M phase. During M phase, the nucleus divides in a process called mitosis; then the cytoplasm divides, in a process called cytokinesis. In this figure—and in subsequent figures in the chapter—the lengths of the various phases are not drawn to scale: M phase, for example, is typically much shorter and G_1 much longer than shown.

QUESTION 18–2

A population of proliferating cells is stained with a dye that becomes fluorescent when it binds to DNA, so that the amount of fluorescence is directly proportional to the amount of DNA in each cell. To measure the amount of DNA in each cell, the cells are then passed through a flow cytometer, an instrument that measures the amount of fluorescence in individual cells. The number of cells with a given DNA content is plotted on the graph below.



Indicate on the graph where you would expect to find cells that are in G_1, S, G_2, and mitosis. Which is the longest phase of the cell cycle in this population of cells?

During all of interphase, a cell generally continues to transcribe genes, synthesize proteins, and grow in mass. Together with S phase, G_1 and G_2 provide the time needed for the cell to grow and duplicate its cytoplasmic organelles. If interphase lasted only long enough for DNA replication, the cell would not have time to double its mass before it divided and would consequently shrink with each division. Indeed, in some special circumstances that is just what happens. In an early frog embryo, for example, the first cell divisions after fertilization (called *cleavage divisions*) serve to subdivide the giant egg cell into many smaller cells as quickly as possible (see Table 18–1). In such embryonic cell cycles, the G_1 and G_2 phases are drastically shortened, and the cells do not grow before they divide.

A Cell-Cycle Control System Triggers the Major Processes of the Cell Cycle

To ensure that they replicate all their DNA and organelles, and divide in an orderly manner, eukaryotic cells possess a complex network of regulatory proteins known as the *cell-cycle control system*. This system guarantees that the events of the cell cycle—DNA replication, mitosis, and so on—occur in a set sequence and that each process has been completed before the next one begins. To accomplish this, the control system is itself regulated at certain critical points of the cycle by feedback from the process currently being performed. Without such feedback, an interruption or a delay in any of the processes could be disastrous. All of the nuclear DNA, for example, must be replicated before the nucleus begins to divide, which means that a complete S phase must precede M phase. If DNA synthesis is slowed down or stalled, mitosis and cell division must also be delayed. Similarly, if DNA is damaged, the cycle must arrest in G_1, S, or G_2 so that the cell can repair the damage, either before DNA replication is started or completed or before the cell enters M phase. The cell-cycle control system achieves all of this by employing molecular brakes, sometimes called *checkpoints*, to pause the cycle at certain transition points. In this way, the control system does not trigger the next step in the cycle unless the cell is properly prepared.

The cell-cycle control system regulates progression through the cell cycle at three main transition points (**Figure 18–3**). At the transition from G_1 to S phase, the control system confirms that the environment is favorable for proliferation before committing to DNA replication. Cell proliferation in animals requires both sufficient nutrients and specific signal molecules

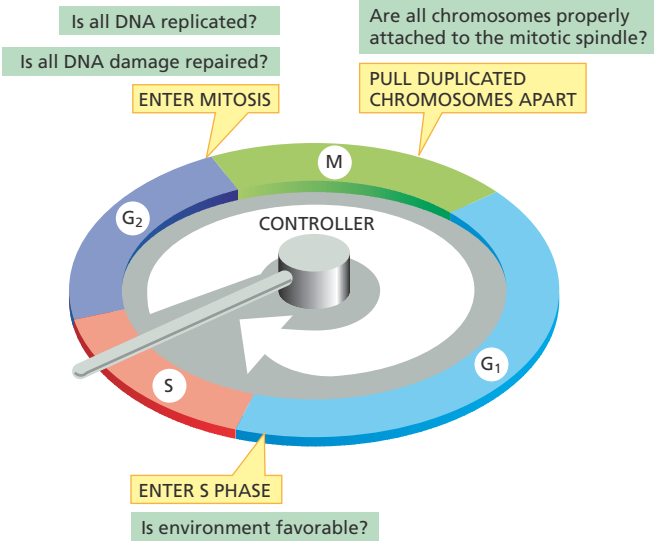


Figure 18–3 The cell-cycle control system ensures that key processes in the cycle occur in the proper sequence. The cell-cycle control system is shown as a controller arm that rotates clockwise, triggering essential processes when it reaches particular transition points on the outer dial. These processes include DNA replication in S phase and the segregation of duplicated chromosomes in mitosis. The control system can transiently halt the cycle at specific transition points—in G_1, G_2, and M phase—if extracellular or intracellular conditions are unfavorable.

in the extracellular environment; if these extracellular conditions are unfavorable, cells can delay progress through G_1 and may even enter a specialized resting state known as G_0 (G zero). At the transition from G_2 to M phase, the control system confirms that the DNA is undamaged and fully replicated, ensuring that the cell does not enter mitosis unless its DNA is intact. Finally, during mitosis, the cell-cycle control machinery ensures that the duplicated chromosomes are properly attached to a cytoskeletal machine, called the *mitotic spindle*, before the spindle pulls the chromosomes apart and segregates them into the two daughter cells.

In animals, the transition from G_1 to S phase is especially important as a point in the cell cycle where the control system is regulated. Signals from other cells stimulate cell proliferation when more cells are needed—and block it when they are not. The cell-cycle control system therefore plays a central part in the regulation of cell numbers in the tissues of the body; if the control system malfunctions such that cell division is excessive, cancer can result. We discuss later how extracellular signals influence the decisions made at the G_1 to S transition.

Cell-Cycle Control is Similar in All Eukaryotes

Some features of the cell cycle, including the time required to complete certain events, vary greatly from one cell type to another, even within the same organism. The basic organization of the cycle, however, is essentially the same in all eukaryotic cells, and all eukaryotes appear to use similar machinery and control mechanisms to drive and regulate cell-cycle events. The proteins of the cell-cycle control system first appeared more than a billion years ago, and they have been so well conserved over the course of evolution that many of them function perfectly when transferred from a human cell to a yeast (see How We Know, pp. 609–610).

Because of this similarity, biologists can study the cell cycle and its regulation in a variety of organisms and use the findings from all of them to assemble a unified picture of how the cycle works. Many discoveries about the cell cycle have come from a systematic search for mutations that inactivate essential components of the cell-cycle control system in yeasts. Likewise, studies of both cultured mammalian cells and the embryos of frogs and sea urchins have been critical for examining the molecular mechanisms that underlie the cycle and its control in multicellular organisms like ourselves.

THE CELL-CYCLE CONTROL SYSTEM

Two types of machinery are involved in cell division: one manufactures the new components of the growing cell, and another hauls the components into their correct places and partitions them appropriately when the cell divides in two. The **cell-cycle control system** switches all this machinery on and off at the correct times, thereby coordinating the various steps of the cycle. The core of the cell-cycle control system is a series of molecular switches that operate in a defined sequence and orchestrate the main events of the cycle, including DNA replication and the segregation of duplicated chromosomes. In this section, we review the protein components of the control system and discuss how they work together to trigger the different phases of the cycle.

The Cell-Cycle Control System Depends on Cyclically Activated Protein Kinases called Cdks

The cell-cycle control system governs the cell-cycle machinery by cyclically activating and then inactivating the key proteins and protein

Figure 18–4 Progression through the cell cycle depends on cyclin-dependent protein kinases (Cdks). A Cdk must bind a regulatory protein called a cyclin before it can become enzymatically active. This activation also requires an activating phosphorylation of the Cdk (not shown, but see Movie 18.1). Once activated, a cyclin–Cdk complex phosphorylates key proteins in the cell that are required to initiate particular steps in the cell cycle. The cyclin also helps direct the Cdk to the target proteins that the Cdk phosphorylates.

complexes that initiate or regulate DNA replication, mitosis, and cytokinesis. This regulation is carried out largely through the phosphorylation and dephosphorylation of proteins involved in these essential processes.

As discussed in Chapter 4, phosphorylation followed by dephosphorylation is one of the most common ways by which cells switch the activity of a protein on and off (see Figure 4–42), and the cell-cycle control system uses this mechanism extensively and repeatedly. The phosphorylation reactions that control the cell cycle are carried out by a specific set of protein kinases, while dephosphorylation is performed by a set of protein phosphatases.

The protein kinases at the core of the cell-cycle control system are present in proliferating cells throughout the cell cycle. They are activated, however, only at appropriate times in the cycle, after which they are quickly inactivated. Thus, the activity of each of these kinases rises and falls in a cyclical fashion. Some of these protein kinases, for example, become active toward the end of G_1 phase and are responsible for driving the cell into S phase; another kinase becomes active just before M phase and drives the cell into mitosis.

Switching these kinases on and off at the appropriate times is partly the responsibility of another set of proteins in the control system—the **cyclins**. Cyclins have no enzymatic activity themselves, but they need to bind to the cell-cycle kinases before the kinases can become enzymatically active. The kinases of the cell-cycle control system are therefore known as **cyclin-dependent protein kinases**, or **Cdks** (Figure 18–4). Cyclins are so-named because, unlike the Cdks, their concentrations vary in a cyclical fashion during the cell cycle. The cyclical changes in cyclin concentrations help drive the cyclic assembly and activation of the cyclin–Cdk complexes. Once activated, cyclin–Cdk complexes help trigger various cell-cycle events, such as entry into S phase or M phase (Figure 18–5). We discuss how the Cdks and cyclins were discovered in How We Know, pp. 609–610.

Different Cyclin–Cdk Complexes Trigger Different Steps in the Cell Cycle

There are several types of cyclins and, in most eukaryotes, several types of Cdks involved in cell-cycle control. Different cyclin–Cdk complexes trigger different steps of the cell cycle. As shown in Figure 18–5, the cyclin that acts in G_2 to trigger entry into M phase is called **M cyclin**, and the active complex it forms with its Cdk is called **M-Cdk**. Other cyclins, called **S cyclins** and **G_1/S cyclins**, bind to a distinct Cdk protein late in G_1 to form **S-Cdk** and **G_1/S-Cdk**, respectively; these cyclin-Cdk complexes help launch S phase. The actions of S-Cdk and M-Cdk are indicated in

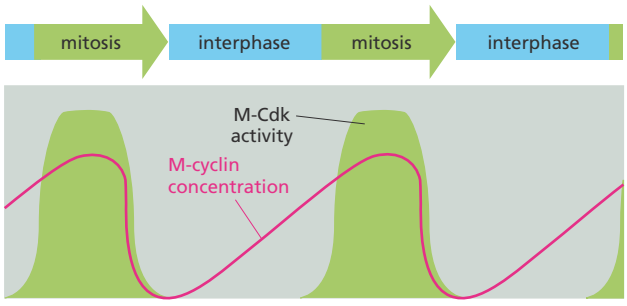


Figure 18–5 The accumulation of cyclins helps regulate the activity of Cdks. The formation of active cyclin–Cdk complexes drives various cell-cycle events, including entry into S phase or M phase. The figure shows the changes in cyclin concentration and Cdk protein kinase activity responsible for controlling entry into M phase. Increasing concentration of the relevant cyclin (called M cyclin) helps direct the formation of the active cyclin–Cdk complex (M–Cdk) that drives entry into M phase. Although the enzymatic activity of each type of cyclin-Cdk complex rises and falls during the course of the cell cycle, the concentration of the Cdk component does not (not shown).

DISCOVERY OF CYCLINS AND CDKS

For many years, cell biologists watched the "puppet show" of DNA synthesis, mitosis, and cytokinesis but had no idea what was behind the curtain, controlling these events. The cell-cycle control system was simply a "black box" inside the cell. It was not even clear whether there was a separate control system, or whether the cell-cycle machinery somehow controlled itself. A breakthrough came with the identification of the key proteins of the control system and the realization that they are distinct from the components of the cell-cycle machinery—the enzymes and other proteins that perform the essential processes of DNA replication, chromosome segregation, and so on.

The first components of the cell-cycle control system to be discovered were the cyclins and cyclin-dependent kinases (Cdks) that drive cells into M phase. They were found in studies of cell division conducted on animal eggs.

Back to the egg

The fertilized eggs of many animals are especially suitable for biochemical studies of the cell cycle because they are exceptionally large and divide rapidly. An egg of the frog *Xenopus*, for example, is just over 1 mm in diameter (**Figure 18–6**). After fertilization, it divides rapidly to partition the egg into many smaller cells. These rapid cell cycles consist mainly of repeated S and M phases, with very short or no G_1 or G_2 phases between them. There is no new gene transcription: all of the mRNAs and most of the proteins required for this early stage of embryonic development are already packed into the very large egg during its development as an oocyte in the ovary of the mother. In these early division cycles (*cleavage divisions*), no cell growth occurs, and all the cells of the embryo divide synchronously, growing smaller and smaller with each division (see **Movie 18.2**).

Because of the synchrony, it is possible to prepare an extract from frog eggs that is representative of the cell-cycle stage at which the extract is made. The biological activity of such an extract can then be tested by injecting it into a *Xenopus* oocyte (the immature precursor of the unfertilized egg) and observing, microscopically, its effects on cell-cycle behavior. The *Xenopus* oocyte is an especially convenient test system for detecting an activity that drives cells into M phase, because of its large size, and because it has completed DNA replication and is arrested at a stage in the meiotic cell cycle (discussed in Chapter 19) that is equivalent to the G_2 phase of a mitotic cell cycle.

Give us an M

In such experiments, Kazuo Matsui and colleagues found that an extract from an M-phase egg instantly drives the oocyte into M phase, whereas cytoplasm from a cleaving egg at other phases of the cycle does not. When they first made this discovery, they did not know the molecules or the mechanism responsible, so they referred to the unidentified agent as *maturation promoting factor*, or MPF (**Figure 18–7**). By testing cytoplasm from different stages of the cell cycle, Matsui and colleagues found that MPF activity oscillates dramatically during the course of each cell cycle: it increased rapidly just before the start of mitosis and fell rapidly to zero toward the end of mitosis (see Figure 18–5). This oscillation made MPF a strong candidate for a component involved in cell-cycle control.

When MPF was finally purified, it was found to contain a protein kinase that was required for its activity. But the kinase portion of MPF did not act alone. It had to have a specific protein (now known to be M cyclin) bound to it in order to function. M cyclin was discovered in a different type of experiment, involving clam eggs.

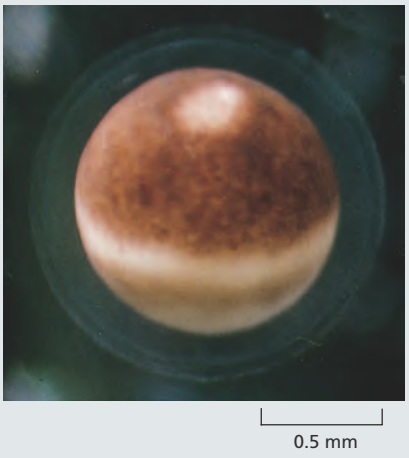


Figure 18–6 A mature *Xenopus* egg provides a convenient system for studying the cell cycle. (Courtesy of Tony Mills.)

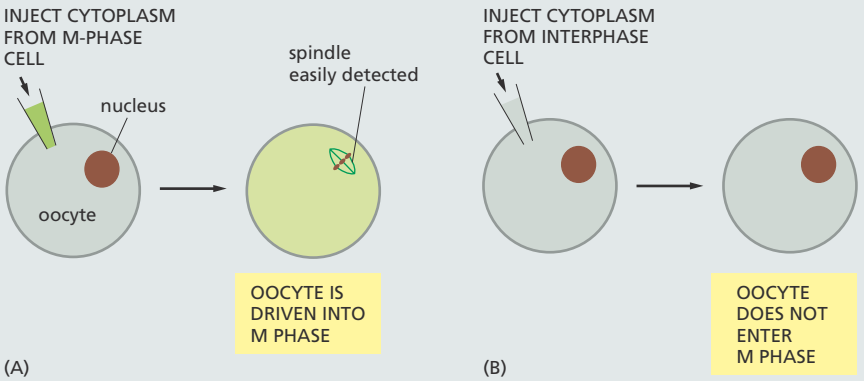


Figure 18–7 MPF activity was discovered by injecting *Xenopus* egg cytoplasm into *Xenopus* oocytes. (A) A *Xenopus* oocyte is injected with cytoplasm taken from a *Xenopus* egg in M phase. The cell extract drives the oocyte into M phase of the first meiotic division (a process called maturation), causing the large nucleus to break down and a spindle to form. (B) When the cytoplasm is instead taken from a cleaving egg in interphase, it does not cause the oocyte to enter M phase. Thus, the extract in (A) must contain some activity—a maturation promoting factor (MPF)—that triggers entry into M phase.

Fishing in clams

M cyclin was initially identified by Tim Hunt as a protein whose concentration rose gradually during interphase and then fell rapidly to zero as cleaving clam eggs went through M phase (see Figure 18–5). The protein repeated this performance in each cell cycle. Its role in cell-cycle control, however, was initially obscure. The breakthrough occurred when cyclin was found to be a component of MPF and to be required for MPF activity. Thus, MPF, which we now call M-Cdk, is a protein complex containing two subunits—a regulatory subunit, M cyclin, and a catalytic subunit, the mitotic Cdk. After the components of M-Cdk were identified, other types of cyclins and Cdks were isolated, whose concentrations or activities, respectively, rose and fell at other stages in the cell cycle.

All in the family

While biochemists were identifying the proteins that regulate the cell cycles of frog and clam embryos, yeast geneticists—led by Lee Hartwell, studying baker's yeast (*S. cerevisiae*), and Paul Nurse, studying fission yeast (*S. pombe*)—were taking a genetic approach to dissecting the cell-cycle control system. By studying mutants that get stuck or misbehave at specific points in the cell cycle, these researchers were able to identify many genes responsible for cell-cycle control. Some of these genes turned out to encode cyclin or Cdk proteins, which were unmistakably similar—in both amino acid sequence and function—to their counterparts in frogs and clams. Similar genes were soon identified in human cells.

Many of the cell-cycle control genes have changed so little during evolution that the human version of the gene will function perfectly well in a yeast cell. For example, Nurse and colleagues were the first to show that a yeast with a defective copy of the gene encoding its only Cdk fails to divide, but it divides normally if a copy of the appropriate human gene is artificially introduced into the defective cell. Surely, even Darwin would have been astonished at such clear evidence that humans and yeasts are cousins. Despite a billion years of divergent evolution, all eukaryotic cells—whether yeast, animal, or plant—use essentially the same molecules to control the events of their cell cycle.

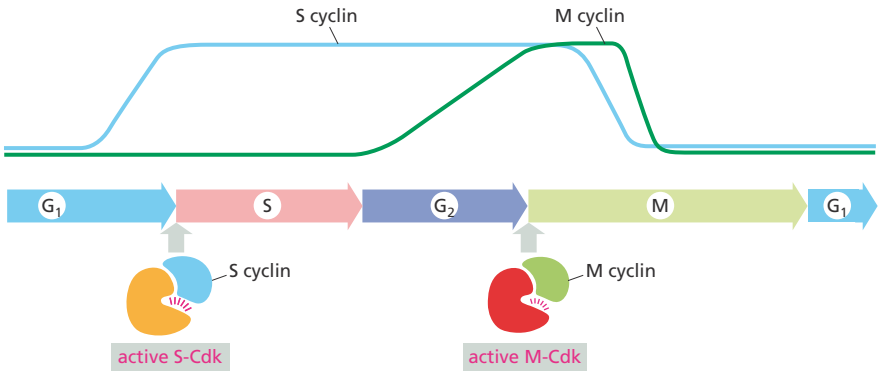


Figure 18–8 Distinct Cdks associate with different cyclins to trigger the different events of the cell cycle. For simplicity, only two types of cyclin–Cdk complexes are shown—one that triggers S phase and one that triggers M phase.

Figure 18–8. Another group of cyclins, called **G_1 cyclins**, act earlier in G_1 and bind to other Cdk proteins to form **G_1-Cdks**, which help drive the cell through G_1 toward S phase. We see later that the formation of these G_1-Cdks in animal cells usually depends on extracellular signal molecules that stimulate cells to divide. The names of the main cyclins and their Cdks are listed in Table 18–2.

Each of these cyclin–Cdk complexes phosphorylates a different set of target proteins in the cell. G_1-Cdks, for example, phosphorylate regulatory proteins that activate transcription of genes required for DNA replication. By activating different sets of target proteins, each type of complex triggers a different transition step in the cycle.

Cyclin Concentrations are Regulated by Transcription and by Proteolysis

As discussed in Chapter 7, the concentration of a given protein in the cell is determined by the rate at which the protein is synthesized and the rate at which it is degraded. Over the course of the cell cycle, the concentration of each type of cyclin rises gradually and then falls abruptly (see Figure 18–8). The gradual increase in cyclin concentration stems from increasing transcription of cyclin genes, whereas the rapid fall in cyclin concentration is precipitated by a full-scale targeted destruction of the protein.

The abrupt degradation of M and S cyclins part way through M phase depends on a large enzyme complex called—for reasons that will become clear later—**anaphase-promoting complex** (**APC**). This complex tags these cyclins with a chain of ubiquitin. As discussed in Chapter 7, proteins marked in this way are directed to proteasomes where they are rapidly degraded (see Figure 7–40). The ubiquitylation and degradation of the cyclin returns its Cdk to an inactive state (Figure 18–9).

TABLE 18–2 THE MAJOR CYCLINS AND CDKS OF VERTEBRATES

Cyclin–Cdk Complex	Cyclin	Cdk Partner
G_1-Cdk	cyclin D*	Cdk4, Cdk6
G_1/S-Cdk	cyclin E	Cdk2
S-Cdk	cyclin A	Cdk2
M-Cdk	cyclin B	Cdk1

*There are three D cyclins in mammals (cyclins D1, D2, and D3).

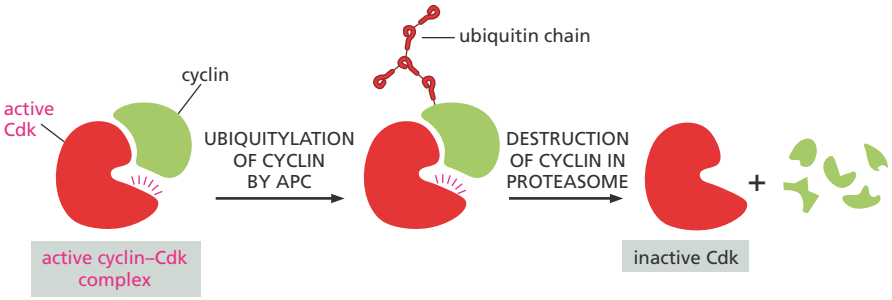


Figure 18–9 The activity of some Cdks is regulated by cyclin degradation. Ubiquitylation of S or M cyclin by APC marks the protein for destruction in proteasomes (as discussed in Chapter 7). The loss of cyclin renders its Cdk partner inactive.

Cyclin destruction can help drive the transition from one phase of the cell cycle to the next. For example, M-cyclin degradation and the resulting inactivation of M-Cdk leads to the molecular events that take the cell out of mitosis.

The Activity of Cyclin–Cdk Complexes Depends on Phosphorylation and Dephosphorylation

The appearance and disappearance of cyclin proteins play an important part in regulating Cdk activity during the cell cycle, but there must be more to the story: although cyclin concentrations increase gradually, the activity of the associated cyclin–Cdk complexes tends to switch on abruptly at the appropriate time in the cell cycle (see Figure 18–5). What could trigger the abrupt activation of these complexes? It turns out that the cyclin–Cdk complex contains inhibitory phosphates, and to become active, the Cdk must be dephosphorylated by a specific protein phosphatase (**Figure 18–10**). Thus protein kinases and phosphatases regulate the activity of specific cyclin–Cdk complexes and help control progression through the cell cycle.

Cdk Activity Can be Blocked by Cdk Inhibitor Proteins

In addition to phosphorylation and dephosphorylation, the activity of Cdks can also be modulated by the binding of **Cdk inhibitor proteins**. The cell-cycle control system uses these inhibitors to block the assembly or activity of certain cyclin–Cdk complexes. Some Cdk inhibitor proteins, for example, help maintain Cdks in an inactive state during the G_1 phase of the cycle, thus delaying progression into S phase (**Figure 18–11**). Pausing at this transition point in G_1 gives the cell more time to grow, or allows it to wait until extracellular conditions are favorable for division.

The Cell-Cycle Control System Can Pause the Cycle in Various Ways

As mentioned earlier, the cell-cycle control system can transiently delay progress through the cycle at various transition points to ensure that the major events of the cycle occur in a specific order. At these transitions,

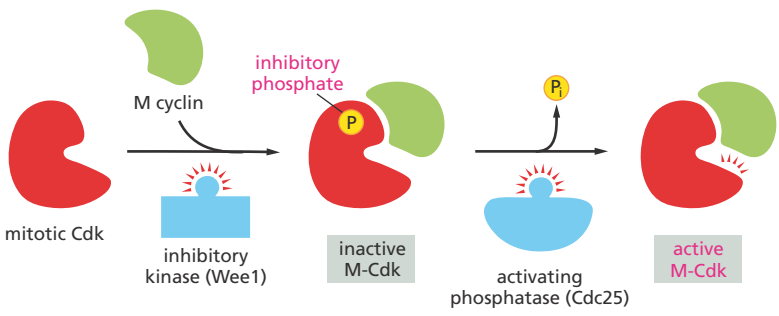


Figure 18–10 For M-Cdk to be active, inhibitory phosphates must be removed. As soon as the M cyclin–Cdk complex is formed, it is phosphorylated at two adjacent sites by an inhibitory protein kinase called Wee1. (For simplicity, only one inhibitory phosphate is shown.) This modification keeps M-Cdk in an inactive state until these phosphates are removed by an activating protein phosphatase called Cdc25. It is still not clear how the timing of the critical Cdc25 phosphatase triggering step shown here is controlled.

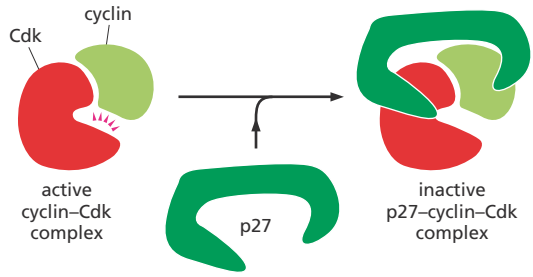


Figure 18–11 The activity of a Cdk can be blocked by the binding of a Cdk inhibitor. In this instance, the inhibitor protein (called p27) binds to an activated cyclin–Cdk complex. Its attachment prevents the Cdk from phosphorylating target proteins required for progress through G_1 into S phase.

the control system monitors the cell's internal state and the conditions in its environment, before allowing the cell to continue through the cycle. For example, it allows entry into S phase only if environmental conditions are appropriate; it triggers mitosis only after the DNA has been completely replicated; and it initiates chromosome segregation only after the duplicated chromosomes are correctly aligned on the mitotic spindle.

To accomplish these feats, the control system uses a combination of the mechanisms we have described. At the G_1-to-S transition, it uses Cdk inhibitors to keep cells from entering S phase and replicating their DNA (see Figure 18–11). At the G_2-to-M transition, it suppresses the activation of M-Cdk by inhibiting the phosphatase required to activate the Cdk (see Figure 18–10). And it can delay the exit from mitosis by inhibiting the activation of APC, thus preventing the degradation of M cyclin (see Figure 18–9).

These mechanisms, summarized in **Figure 18–12**, allow the cell to make "decisions" about whether to progress through the cell cycle. In the next section, we take a closer look at how the cell-cycle control system decides whether a cell in G_1 should commit to divide.

G_1 PHASE

In addition to being a bustling period of metabolic activity, cell growth, and repair, G_1 is an important point of decision-making for the cell. Based on intracellular signals that provide information about the size of the cell and extracellular signals reflecting the environment, the cell-cycle control machinery can either hold the cell transiently in G_1 (or in a more prolonged nonproliferative state, G_0), or allow it to prepare for entry into

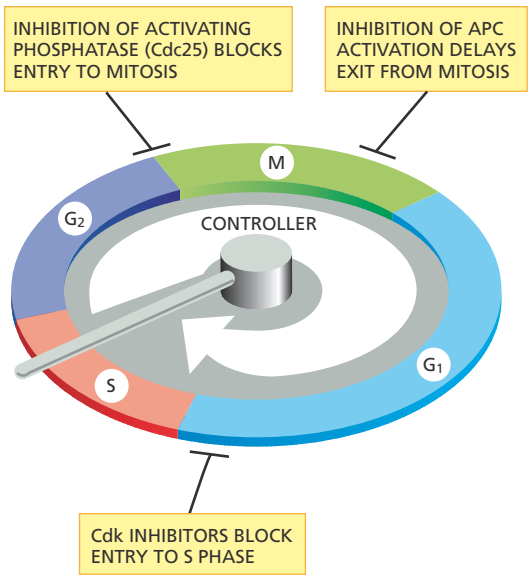


Figure 18–12 The cell-cycle control system uses various mechanisms to pause the cycle at specific transition points.

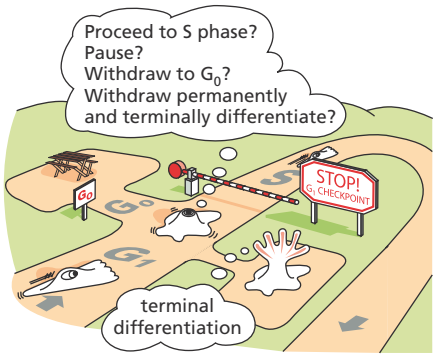


Figure 18–13 The transition from G_1 to S phase offers the cell a crossroad. The cell can commit to completing another cell cycle, pause temporarily until conditions are right, or withdraw from the cell cycle altogether—either temporarily in G_0, or permanently in the case of terminally differentiated cells.

the S phase of another cell cycle. Once past this critical G_1-to-S transition, a cell usually continues all the way through the rest of the cell cycle quickly—typically within 12–24 hours in mammals. In yeasts, the G_1-to-S transition is therefore sometimes called *Start*, because passing it represents a commitment to complete a full cell cycle (**Figure 18–13**).

In this section, we consider how the cell-cycle control system decides between these options—and what it does once the decision is made. The molecular mechanisms involved are especially important, as defects in them can lead to unrestrained cell proliferation and cancer.

Cdks are Stably Inactivated in G_1

During M phase, when cells are actively dividing, the cell is awash with active cyclin–Cdk complexes. If those S-Cdks and M-Cdks are not disabled by the end of M phase, the cell will immediately replicate its DNA and initiate another round of division, without spending any significant time in the G_1 or G_2 phases. Such rapid cycling is typically seen in some early embryos, where the cells get smaller with each division, having little time to grow in between.

To usher a cell from the upheaval of M phase to the relative tranquility of G_1, the cell-cycle control machinery must inactivate its inventory of S-Cdk and M-Cdk. It does so by eliminating all of the existing cyclins, by blocking the synthesis of new ones, and by deploying Cdk inhibitor proteins to muffle the activity of any remaining cyclin–Cdk complexes. The use of multiple mechanisms makes this system of suppression robust, ensuring that essentially all Cdk activity is shut down. This wholesale inactivation resets the cell-cycle control system and generates a stable G_1 phase, during which the cell can grow and monitor its environment before committing to a new round of division.

Mitogens Promote the Production of the Cyclins that Stimulate Cell Division

As a general rule, mammalian cells will multiply only if they are stimulated to do so by extracellular signals, called *mitogens*, produced by other cells. If deprived of such signals, the cell cycle arrests in G_1; if the cell is deprived of mitogens for long enough, it will withdraw from the cell cycle and enter a nonproliferating state, in which the cell can remain for days or weeks, months, or even for the lifetime of the organism, as we discuss shortly.

Escape from cell-cycle arrest—or from certain nonproliferating states—requires the accumulation of cyclins. Mitogens act by switching on cell signaling pathways that stimulate the synthesis of G_1 cyclins, G_1/S cyclins, and other proteins involved in DNA synthesis and chromosome duplication. The buildup of these cyclins triggers a wave of G_1/S-Cdk activity, which ultimately relieves the negative controls that otherwise block progression from G_1 to S phase.

A crucial negative control is mediated by the *Retinoblastoma (Rb) protein*. Rb was initially identified from studies of a rare childhood eye tumor called retinoblastoma, in which the Rb protein is missing or defective. Rb is abundant in the nuclei of all vertebrate cells, where it binds to particular transcription regulators and prevents them from turning on the genes required for cell proliferation. Mitogens release the Rb brake by triggering the activation of G_1-Cdks and G_1/S-Cdks. These complexes phosphorylate the Rb protein, altering its conformation so that it releases its bound transcription regulators, which are then free to activate the genes required for cell proliferation (**Figure 18–14**).

QUESTION 18–3

Why do you suppose cells have evolved a special G_0 phase to exit from the cell cycle, rather than just stopping in G_1 and not moving on to S phase?

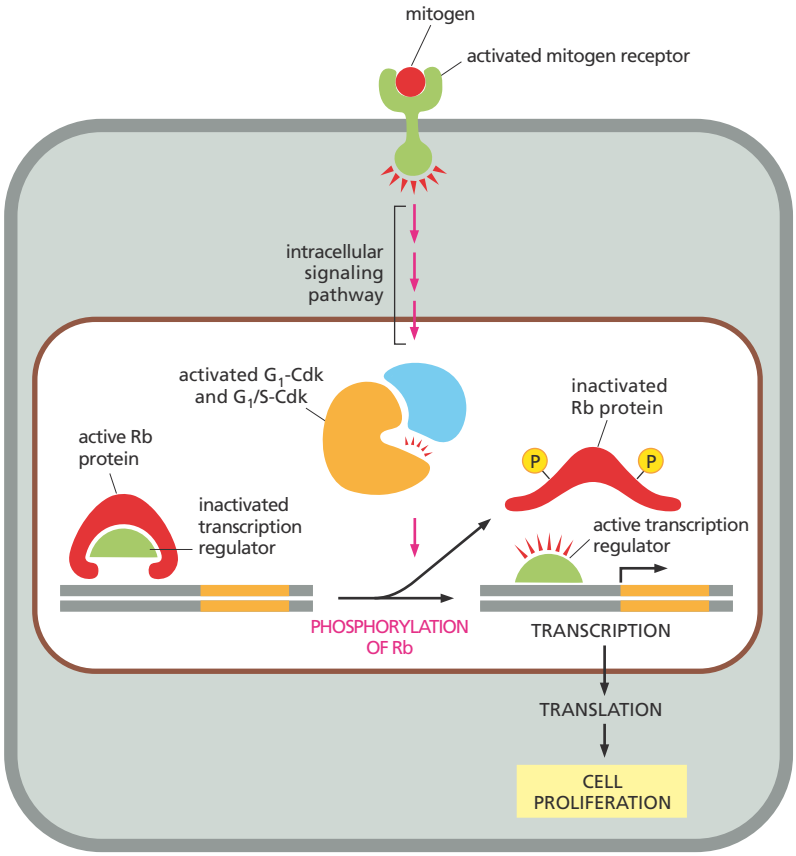


Figure 18–14 One way in which mitogens stimulate cell proliferation is by inhibiting the Rb protein. In the absence of mitogens, dephosphorylated Rb protein holds specific transcription regulators in an inactive state; these transcription regulators are required to stimulate the transcription of target genes that encode proteins needed for cell proliferation. Mitogens binding to cell-surface receptors activate intracellular signaling pathways that lead to the formation and activation of G_1-Cdk and G_1/S-Cdk complexes. These complexes phosphorylate, and thereby inactivate, the Rb protein, releasing the transcription regulators that activate the transcription of genes required for cell proliferation.

DNA Damage Can Temporarily Halt Progression Through G_1

The cell-cycle control system uses several distinct mechanisms to halt progress through the cell cycle if DNA is damaged. It can do so at various transition points from one phase of the cell cycle to the next. The mechanism that operates at the G_1-to-S transition, which prevents the cell from replicating damaged DNA, is especially well understood. DNA damage in G_1 causes an increase in both the concentration and activity of a protein called **p53**, which is a transcription regulator that activates the transcription of a gene encoding a Cdk inhibitor protein called p21. The p21 protein binds to G_1/S-Cdk and S-Cdk, preventing them from driving the cell into S phase (**Figure 18–15**). The arrest of the cell cycle in G_1 gives the cell time to repair the damaged DNA before replicating it. If the DNA damage is too severe to be repaired, p53 can induce the cell to kill itself by undergoing a form of programmed cell death called *apoptosis*, which we discuss later. If p53 is missing or defective, the unrestrained replication of damaged DNA leads to a high rate of mutation and the production of cells that tend to become cancerous. In fact, mutations in the *p53* gene are found in about half of all human cancers (**Movie 18.3**).

Cells Can Delay Division for Prolonged Periods by Entering Specialized Nondividing States

As mentioned earlier, cells can delay progress through the cell cycle at specific transition points, to wait for suitable conditions or to repair damaged DNA. They can also withdraw from the cell cycle for prolonged periods—either temporarily or permanently.

The most radical decision that the cell-cycle control system can make is to withdraw the cell from the cell cycle permanently. This decision has a special importance in multicellular organisms. Many cells in the

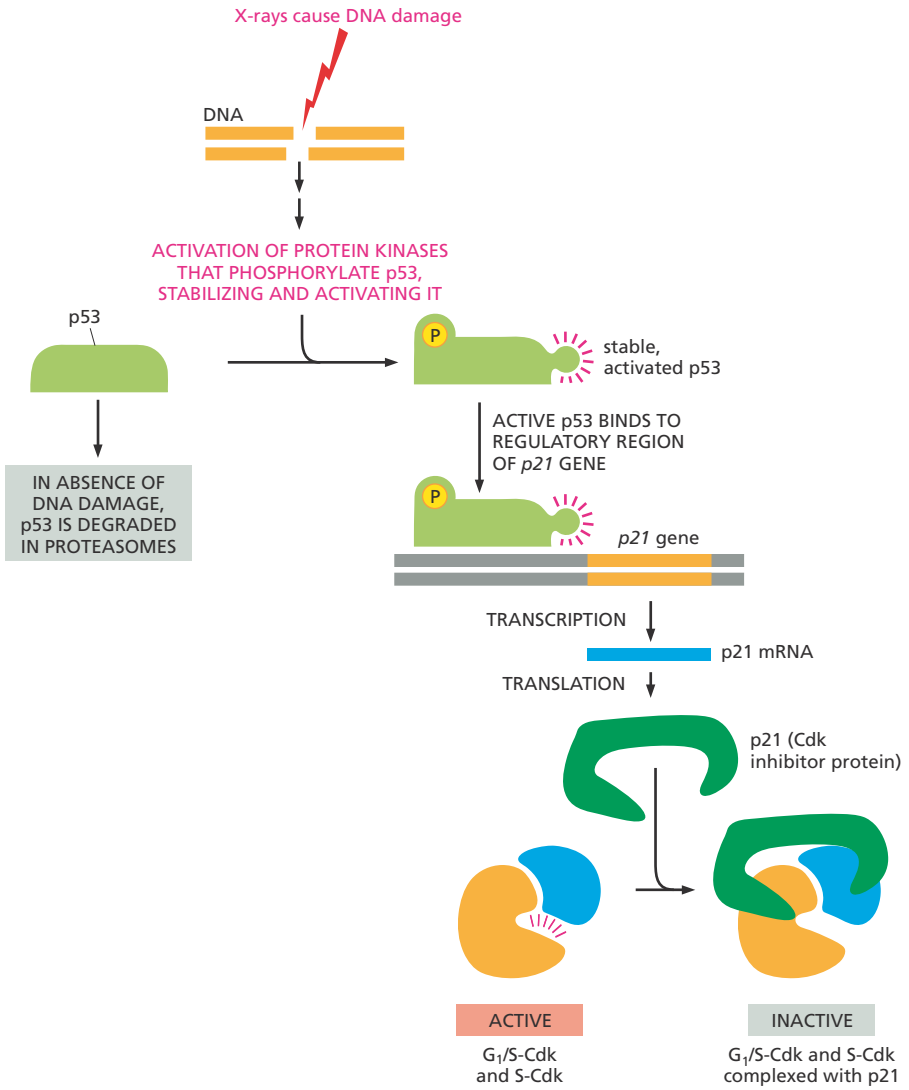


Figure 18–15 DNA damage can arrest the cell cycle in G_1. When DNA is damaged, specific protein kinases respond by both activating the p53 protein and halting its normal rapid degradation. Activated p53 protein thus accumulates and stimulates the transcription of the gene that encodes the Cdk inhibitor protein p21. The p21 protein binds to G_1/S-Cdk and S-Cdk and inactivates them, so that the cell cycle arrests in G_1.

human body permanently stop dividing when they differentiate. In such *terminally differentiated* cells, such as nerve or muscle cells, the cell-cycle control system is dismantled completely and genes encoding the relevant cyclins and Cdks are irreversibly shut down.

In the absence of appropriate signals, other cell types withdraw from the cell cycle only temporarily, entering an arrested state called G_0. They retain the ability to reassemble the cell-cycle control system quickly and to divide again. Most liver cells, for example, are in G_0, but they can be stimulated to proliferate if the liver is damaged.

Most of the diversity in cell-division rates in the adult body lies in the variation in the time that cells spend in G_0 or in G_1. Some cell types, including liver cells, normally divide only once every year or two, whereas certain epithelial cells in the gut divide more than twice a day to renew the lining of the gut continually. Many of our cells fall somewhere in between: they can divide if the need arises but normally do so infrequently.

QUESTION 18–4

What might be the consequences if a cell replicated damaged DNA before repairing it?

S PHASE

Before a cell divides, it must replicate its DNA. As we discuss in Chapter 6, this replication must occur with extreme accuracy to minimize the risk of mutations in the next cell generation. Of equal importance, every nucleotide in the genome must be copied once—and only once—to prevent the damaging effects of gene amplification. In this section, we consider the

elegant molecular mechanisms by which the cell-cycle control system initiates DNA replication and, at the same time, prevents replication from happening more than once per cell cycle.

S-Cdk Initiates DNA Replication and Blocks Re-Replication

Like any monumental task, configuring chromosomes for replication requires a certain amount of preparation. For eukaryotic cells, this preparation begins early in G_1, when DNA is made replication-ready by the recruitment of proteins to the sites along each chromosome where replication will begin. These nucleotide sequences, called *origins of replication*, serve as landing pads for the proteins and protein complexes that control and carry out DNA synthesis.

One of these protein complexes, called the *origin recognition complex* (*ORC*), remains perched atop the replication origins throughout the cell cycle. In the first step of replication initiation, the ORC recruits a protein called Cdc6, whose concentration rises early in G_1. Together these proteins load the DNA helicases that will open up the double helix and ready the origin of replication. Once this *prereplicative complex* is in place, the replication origin is loaded and ready to "fire."

The signal to commence replication comes from S-Cdk, the cyclin–Cdk complex that triggers S phase. S-Cdk is assembled and activated at the end of G_1. During S phase, it activates the DNA helicases in the prereplicative complex and promotes the assembly of the rest of the proteins that form the *replication fork* (see Figure 6–19). In doing so, S-Cdk essentially "pulls the trigger" that initiates DNA replication (**Figure 18–16**).

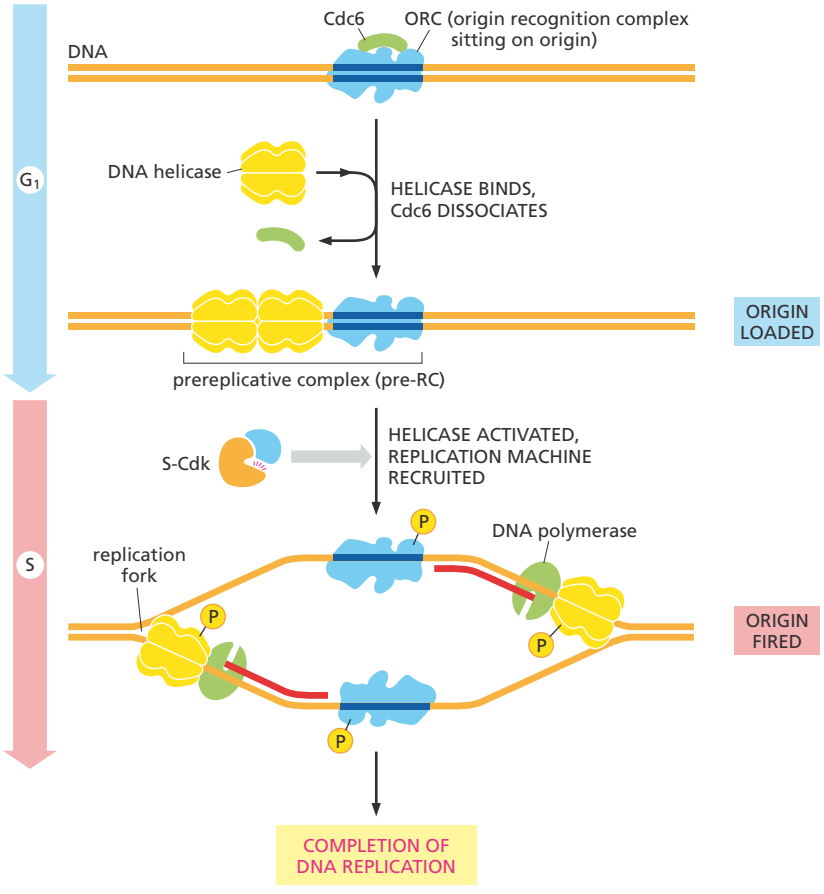


Figure 18–16 The initiation of DNA replication takes place in two steps. During G_1, Cdc6 binds to the ORC, and together these proteins load a pair of DNA helicases to form the prereplicative complex. At the start of S phase, S-Cdk triggers the firing of this loaded replication origin by guiding the assembly of the DNA polymerase (*green*) and other proteins (not shown) that initiate DNA synthesis at the replication fork (discussed in Chapter 6). S-Cdk also blocks re-replication by helping to phosphorylate Cdc6, which marks the protein for degradation (not shown).

S-Cdk not only triggers the initiation of DNA synthesis at a replication origin; it also helps prevent re-replication. It does so by helping phosphorylate Cdc6, which marks that protein for degradation. Eliminating Cdc6 helps ensure that DNA replication can not be reinitiated later in the same cell cycle.

Incomplete Replication Can Arrest the Cell Cycle in G_2

Earlier, we described how DNA damage can signal the cell-cycle control system to delay progress through the G_1-to-S transition, preventing the cell from replicating damaged DNA. But what if errors occur during DNA replication—or if replication is delayed? How does the cell keep from dividing with DNA that is incorrectly or incompletely replicated?

To address these issues, the cell-cycle control system uses a mechanism that can delay entry into M phase. As we saw in Figure 18–10, the activity of M-Cdk is inhibited by phosphorylation at particular sites. For the cell to progress into mitosis, these inhibitory phosphates must be removed by an activating protein phosphatase called Cdc25. When DNA is damaged or incompletely replicated, Cdc25 is itself inhibited, preventing the removal of the inhibitory phosphates. As a result, M-Cdk remains inactive and M phase is delayed until DNA replication is complete and any DNA damage is repaired.

Once a cell has successfully replicated its DNA in S phase, and progressed through G_2, it is ready to enter M phase. During this relatively brief period, the cell will divide its nucleus (mitosis) and then its cytoplasm (cytokinesis; see Figure 18–2). In the next three sections, we describe the events that occur during M phase. We first present a brief overview of M phase as a whole and then discuss, in sequence, the mechanics of mitosis and of cytokinesis, with a focus on animal cells.

M PHASE

Although M phase (mitosis plus cytokinesis) occurs over a relatively short amount of time—about one hour in a mammalian cell that divides once a day, or even once a year—it is by far the most dramatic phase of the cell cycle. During this brief period, the cell reorganizes virtually all of its components and distributes them equally into the two daughter cells. The earlier phases of the cell cycle, in effect, serve to set the stage for the drama of M phase.

The central problem for a cell in M phase is to accurately segregate the chromosomes that were duplicated in the preceding S phase, so that each new daughter cell receives an identical copy of the genome. With minor variations, all eukaryotes solve this problem in a similar way: they assemble two specialized cytoskeletal machines, one that pulls the duplicated chromosomes apart (during mitosis) and another that divides the cytoplasm into two halves (cytokinesis). We begin our discussion of M phase with an overview of how the cell sets the processes of M phase in motion.

M-Cdk Drives Entry Into M Phase and Mitosis

One of the most remarkable features of the cell-cycle control system is that a single protein complex, M-Cdk, brings about all the diverse and intricate rearrangements that occur in the early stages of mitosis. Among its many duties, M-Cdk helps prepare the duplicated chromosomes for segregation and induces the assembly of the mitotic spindle—the machinery that will pull the duplicated chromosomes apart.

M-Cdk complexes accumulate throughout G_2. But this stockpile is not activated until the end of G_2, when the activating phosphatase Cdc25 removes the inhibitory phosphates holding M-Cdk activity in check (see Figure 18–10). This activation is self-reinforcing: once activated, each M-Cdk complex can indirectly activate additional M-Cdk complexes—by phosphorylating and activating more Cdc25 (**Figure 18–17**). Activated M-Cdk also shuts down the inhibitory kinase Wee1 (see Figure 18–10), further promoting the production of activated M-Cdk. The overall consequence is that, once M-Cdk activation begins, it ignites an explosive increase in M-Cdk activity that drives the cell abruptly from G_2 into M phase.

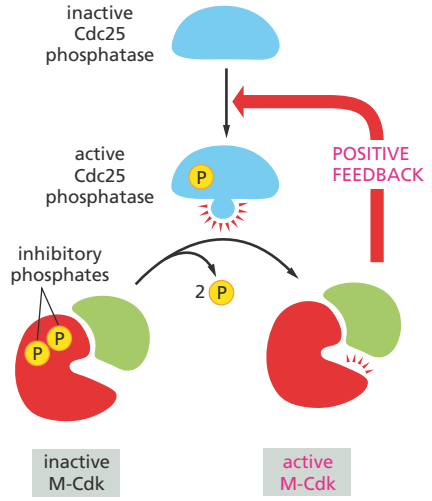


Figure 18–17 Activated M-Cdk indirectly activates more M-Cdk, creating a positive feedback loop. Once activated, M-Cdk phosphorylates, and thereby activates, more Cdk-activating phosphatase (Cdc25). This phosphatase can now activate more M-Cdk by removing the inhibitory phosphate groups from the Cdk subunit.

Cohesins and Condensins Help Configure Duplicated Chromosomes for Separation

When the cell enters M phase, the duplicated chromosomes condense, becoming visible under the microscope as threadlike structures. Protein complexes, called **condensins**, help carry out this **chromosome condensation**, which reduces mitotic chromosomes to compact bodies that can be more easily segregated within the crowded confines of the dividing cell. The assembly of condensin complexes onto the DNA is triggered by the phosphorylation of condensins by M-Cdk.

Even before mitotic chromosomes become condensed, the replicated DNA is handled in a way that allows cells to keep track of the two copies. Immediately after a chromosome is duplicated during S phase, the two copies remain tightly bound together. These identical copies—called **sister chromatids**—each contain a single, double-stranded molecule of DNA, along with its associated proteins. The sisters are held together by protein complexes called **cohesins**, which assemble along the length of each chromatid as the DNA is replicated. This cohesion between sister chromatids is crucial for proper chromosome segregation, and it is broken completely only in late mitosis to allow the sisters to be pulled apart by the mitotic spindle. Defects in sister-chromatid cohesion lead to major errors in chromosome segregation. In humans, such mis-segregation can lead to abnormal numbers of chromosomes, as in individuals with Down Syndrome, who have three copies of chromosome 21.

Cohesins and condensins are structurally related, and both are thought to form ring structures around chromosomal DNA. But, whereas cohesins tie the two sister chromatids together (**Figure 18–18A**), condensins assemble on each individual sister chromatid at the start of M phase and help each of these double helices to coil up into a more compact form (**Figure 18–18B and C**). Together, these proteins help configure replicated chromosomes for mitosis.

Different Cytoskeletal Assemblies Carry Out Mitosis and Cytokinesis

After the duplicated chromosomes have condensed, two complex cytoskeletal machines assemble in sequence to carry out the two mechanical processes that occur in M phase. The mitotic spindle carries out nuclear division (mitosis), and, in animal cells and many unicellular eukaryotes, the *contractile ring* carries out cytoplasmic division (cytokinesis) (**Figure 18–19**). Both structures disassemble rapidly after they have performed their tasks.

The mitotic spindle is composed of microtubules and the various proteins that interact with them, including microtubule-associated motor proteins (discussed in Chapter 17). In all eukaryotic cells, the mitotic spindle is responsible for separating the duplicated chromosomes and allocating one copy of each chromosome to each daughter cell.

QUESTION 18–5

A small amount of cytoplasm isolated from a mitotic cell is injected into an unfertilized frog oocyte, causing the oocyte to enter M phase (see Figure 18–7A). A sample of the injected oocyte's cytoplasm is then taken and injected into a second oocyte, causing this cell also to enter M phase. The process is repeated many times until, essentially, none of the original protein sample remains, and yet, cytoplasm taken from the last in the series of injected oocytes is still able to trigger entry into M phase with undiminished efficiency. Explain this remarkable observation.

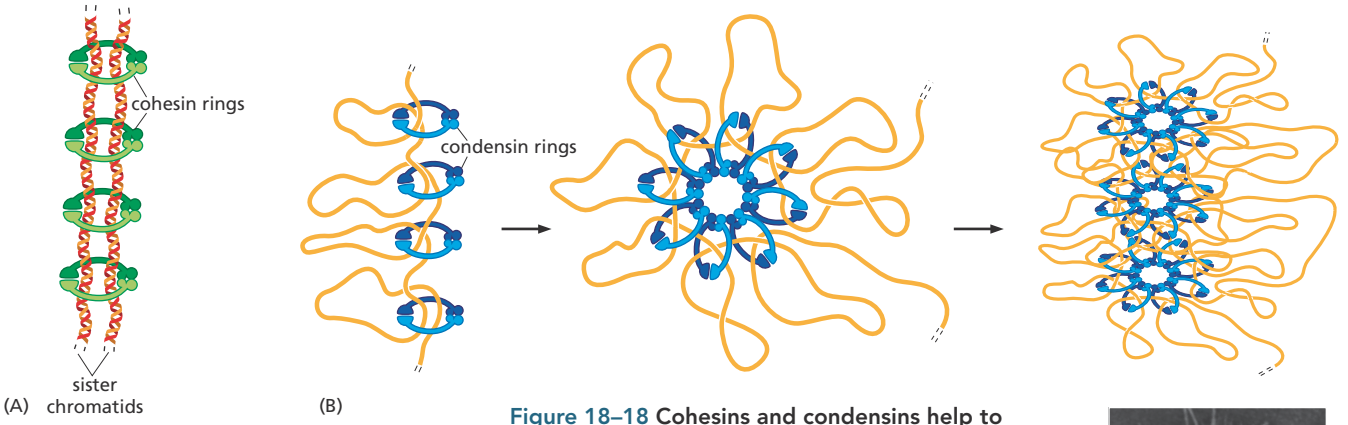


Figure 18–18 Cohesins and condensins help to configure duplicated chromosomes for segregation. (A) Cohesins tie together the two adjacent sister chromatids in each duplicated chromosome. They are thought to form large protein rings that surround the sister chromatids, preventing them from coming apart, until the rings are broken late in mitosis. (B) Condensins help coil each sister chromatid (in other words, each DNA double helix) into a smaller, more compact structure that can be more easily segregated during mitosis. (C) A scanning electron micrograph of a condensed, duplicated mitotic chromosome, consisting of two sister chromatids joined along their length. The constricted region (*arrow*) is the centromere, where each chromatid will attach to the mitotic spindle, which pulls the sister chromatids apart toward the end of mitosis. (C, courtesy of Terry D. Allen.)

The *contractile ring* consists mainly of actin filaments and myosin filaments arranged in a ring around the equator of the cell (see Chapter 17). It starts to assemble just beneath the plasma membrane toward the end of mitosis. As the ring contracts, it pulls the membrane inward, thereby dividing the cell in two (see Figure 18–19). We discuss later how plant cells, which have a cell wall to contend with, divide their cytoplasm by a very different mechanism.

M Phase Occurs in Stages

Although M phase proceeds as a continuous sequence of events, it is traditionally divided into a series of stages. The first five stages of M phase—prophase, prometaphase, metaphase, anaphase, and telophase—constitute **mitosis**, which was originally defined as the period in which the chromosomes are visible (because they have become condensed). *Cytokinesis*, which constitutes the final stage of M phase, begins before mitosis ends. The stages of M phase are summarized in **Panel 18–1** (pp. 622–623). Together, they form a dynamic sequence in which many independent cycles—involving the chromosomes, cytoskeleton, and centrosomes—are coordinated to produce two genetically identical daughter cells (**Movie 18.4** and **Movie 18.5**).

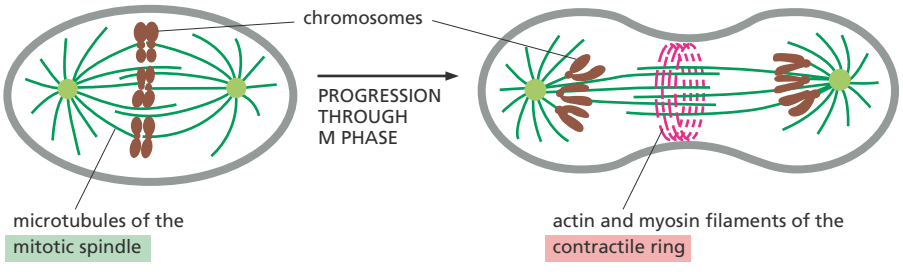


Figure 18–19 Two transient cytoskeletal structures mediate M phase in animal cells. The mitotic spindle assembles first to separate the duplicated chromosomes. Then, the contractile ring assembles to divide the cell in two. Whereas the mitotic spindle is based on microtubules, the contractile ring is based on actin and myosin filaments. Plant cells use a very different mechanism to divide the cytoplasm, as we discuss later.

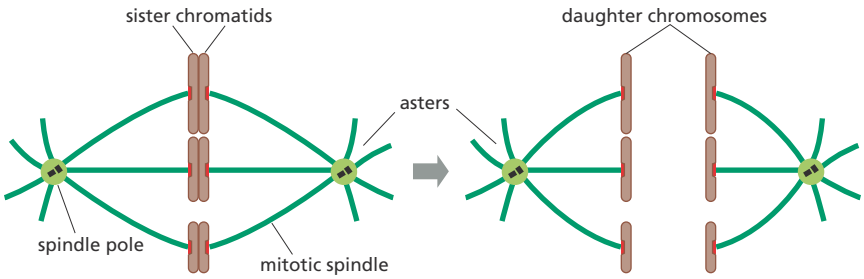


Figure 18–20 Sister chromatids separate at the beginning of anaphase. The mitotic spindle then pulls the resulting chromosomes to opposite poles of the cell.

MITOSIS

Before nuclear division, or mitosis, begins, each chromosome has been duplicated and consists of two identical sister chromatids, held together along their length by cohesin proteins (see Figure 18–18A). During mitosis, the cohesin proteins are cleaved, the sister chromatids split apart, and the resulting daughter chromosomes are pulled to opposite poles of the cell by the mitotic spindle (**Figure 18–20**). In this section, we examine how the mitotic spindle assembles and functions. We discuss how the dynamic instability of microtubules and the activity of microtubule-associated motor proteins contribute to both the assembly of the spindle and its ability to segregate the duplicated chromosomes. We then consider the mechanism that operates during mitosis to ensure the synchronous separation of these chromosomes. Finally we discuss how the daughter nuclei form.

Centrosomes Duplicate To Help Form the Two Poles of the Mitotic Spindle

Before M phase begins, two critical events must be completed: DNA must be fully replicated, and, in animal cells, the centrosome must be duplicated. The **centrosome** is the principal *microtubule-organizing center* in animal cells. It duplicates so that it can help form the two poles of the mitotic spindle and so that each daughter cell can receive its own centrosome.

Centrosome duplication begins at the same time as DNA replication. The process is triggered by the same Cdks—G_1/S-Cdk and S-Cdk—that initiate DNA replication. Initially, when the centrosome duplicates, both copies remain together as a single complex on one side of the nucleus. As mitosis begins, however, the two centrosomes separate, and each nucleates a radial array of microtubules called an **aster**. The two asters move to opposite sides of the nucleus to form the two poles of the mitotic spindle (**Figure 18–21**). The process of centrosome duplication and separation is known as the **centrosome cycle**.

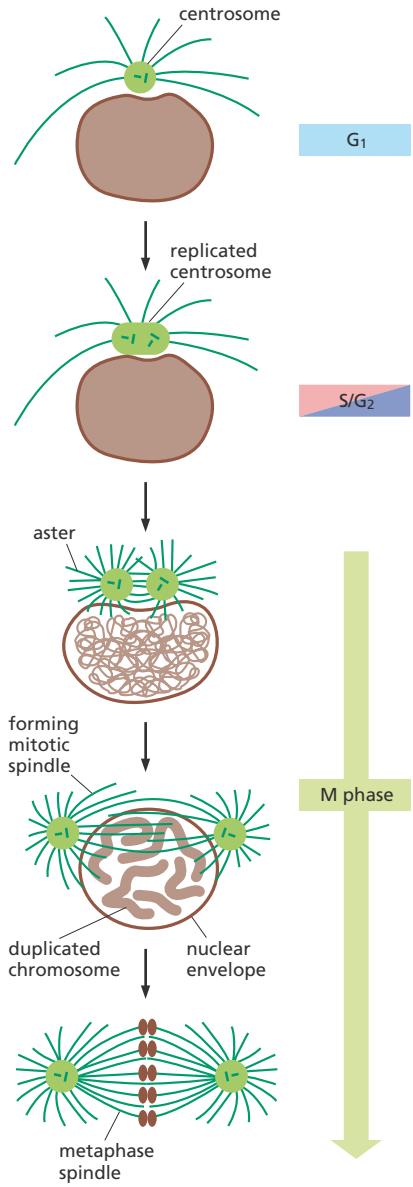
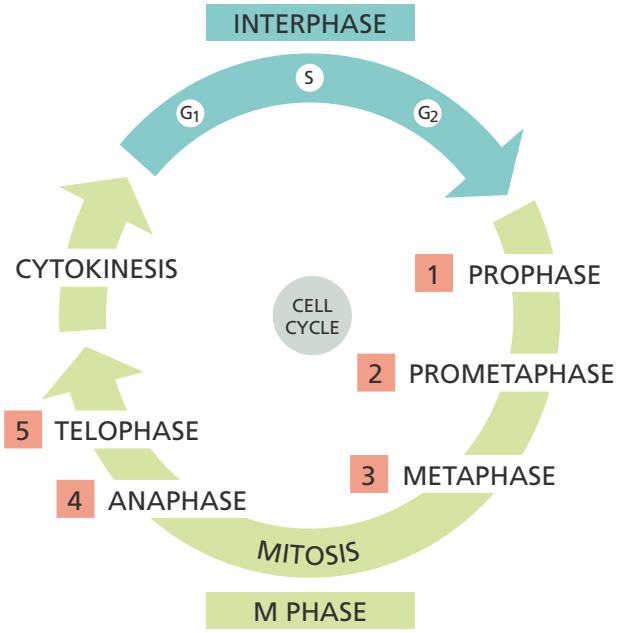


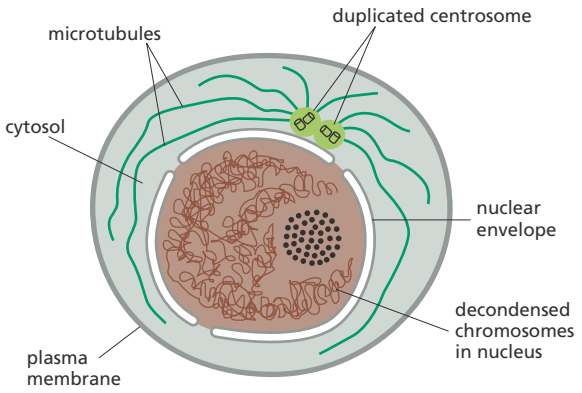
Figure 18–21 The centrosome in an interphase cell duplicates to form the two poles of a mitotic spindle. In most animal cells in interphase (G_1, S, and G_2), a centriole pair (shown here as a pair of *dark green bars*) is associated with the centrosome matrix (*light green*), which nucleates microtubule outgrowth. (The volume of the centrosome matrix is exaggerated in this diagram for clarity.) Centrosome duplication begins at the start of S phase and is complete by the end of G_2. Initially, the two centrosomes remain together, but, in early M phase, they separate, and each nucleates its own aster of microtubules. The centrosomes then move apart, and the microtubules that interact between the two asters elongate preferentially to form a bipolar mitotic spindle, with an aster at each pole. When the nuclear envelope breaks down, the spindle microtubules are able to interact with the duplicated chromosomes.

CELL DIVISION AND THE CELL CYCLE



The division of a cell into two daughters occurs in the M phase of the cell cycle. M phase consists of nuclear division, or mitosis, and cytoplasmic division, or cytokinesis. In this figure, M phase has been greatly expanded for clarity. Mitosis is itself divided into five stages, and these, together with cytokinesis, are described in this panel.

INTERPHASE



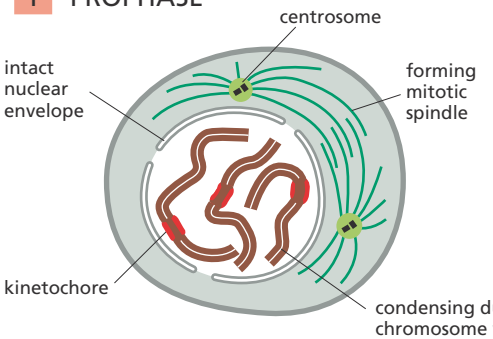
During interphase, the cell increases in size. The DNA of the chromosomes is replicated, and the centrosome is duplicated.

In the light micrographs of dividing animal cells shown in this panel, chromosomes are stained *orange* and microtubules are *green*.

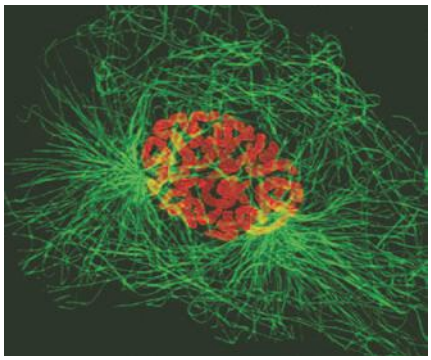
(Micrographs courtesy of Julie Canman and Ted Salmon; "Metaphase" from cover of *J. Cell. Sci.* 115(9), 2002. With permission from The Company of Biologists Ltd; "Telophase" from J.C. Canman et al., *Nature* 424:1074–1078, 2003. With permission from Macmillan Publishers Ltd.)

MITOSIS

1 PROPHASE



At prophase, the duplicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have begun to move apart. For simplicity, only three chromosomes are drawn.



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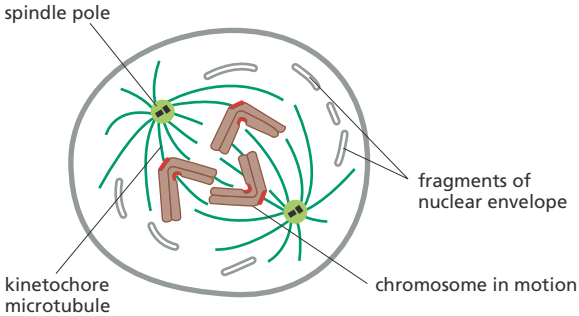
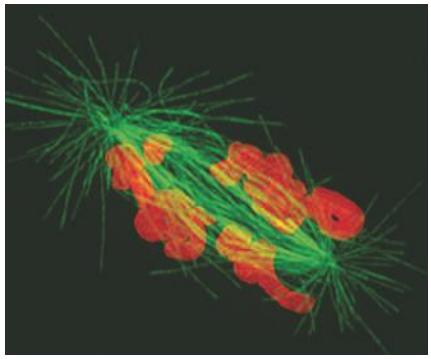
MITOSIS

2 PROMETAPHASE



Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.



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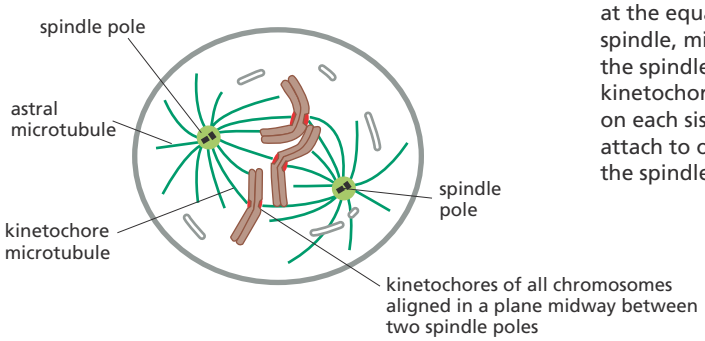
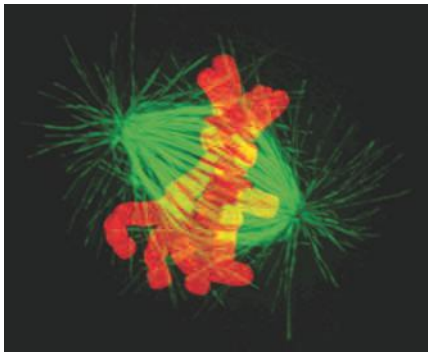
MITOSIS

3 METAPHASE



At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules on each sister chromatid attach to opposite poles of the spindle.



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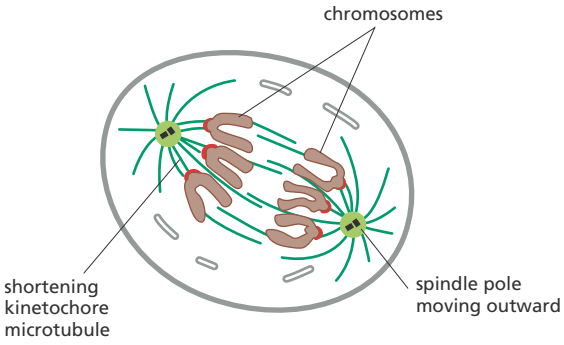
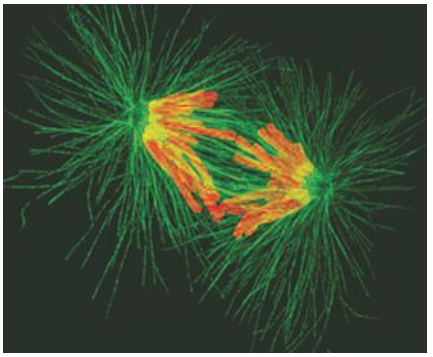
MITOSIS

4 ANAPHASE



At anaphase, the sister chromatids synchronously separate and are pulled slowly toward the spindle pole to which they are attached. The kinetochore microtubules get shorter, and the spindle poles also move apart, both contributing to chromosome segregation.



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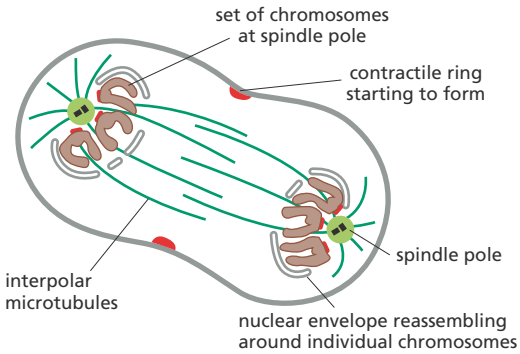
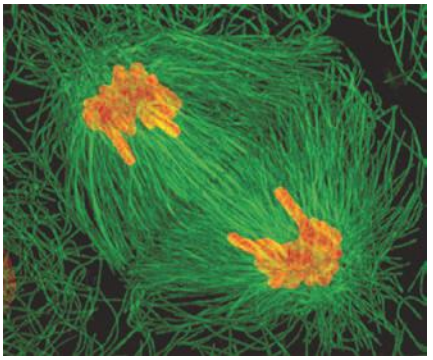
MITOSIS

5 TELOPHASE



During telophase, the two sets of chromosomes arrive at the poles of the spindle. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with the assembly of the contractile ring.



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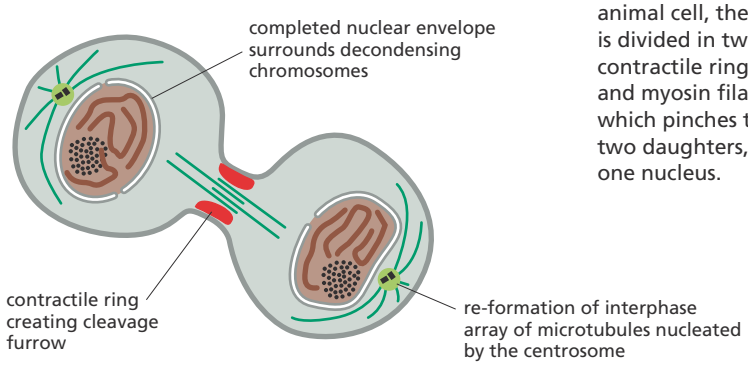
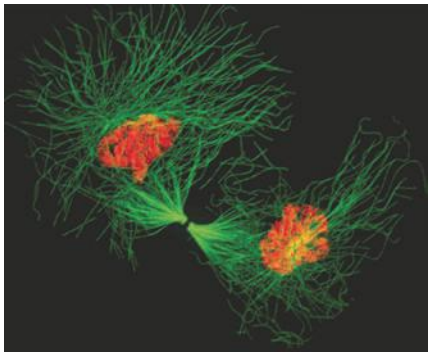
CYTOKINESIS

CYTOKINESIS



During cytokinesis of an animal cell, the cytoplasm is divided in two by a contractile ring of actin and myosin filaments, which pinches the cell into two daughters, each with one nucleus.



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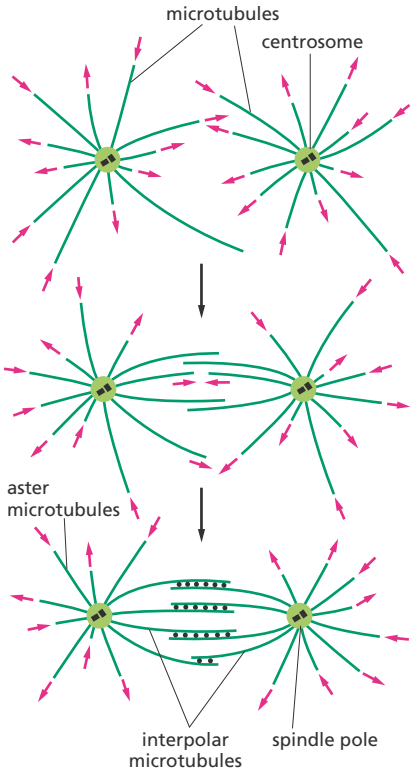


Figure 18–22 A bipolar mitotic spindle is formed by the selective stabilization of interacting microtubules. New microtubules grow out in random directions from the two centrosomes. The two ends of a microtubule (by convention, called the plus and the minus ends) have different properties, and it is the minus end that is anchored in the centrosome (discussed in Chapter 17). The free plus ends are dynamically unstable and switch suddenly from uniform growth (*outward-pointing red arrows*) to rapid shrinkage (*inward-pointing red arrows*). When two microtubules from opposite centrosomes interact in an overlap zone, motor proteins and other microtubule-associated proteins cross-link the microtubules together (*black dots*) in a way that stabilizes the plus ends by decreasing the probability of their depolymerization.

The Mitotic Spindle Starts to Assemble in Prophase

The mitotic spindle begins to form in **prophase**. This assembly of the highly dynamic spindle depends on the remarkable properties of microtubules. As discussed in Chapter 17, microtubules continuously polymerize and depolymerize by the addition and loss of their tubulin subunits, and individual filaments alternate between growing and shrinking—a process called *dynamic instability* (see Figure 17–13). At the start of mitosis, the stability of microtubules decreases—in part because M-Cdk phosphorylates microtubule-associated proteins that influence the stability of the microtubules. As a result, during prophase, rapidly growing and shrinking microtubules extend in all directions from the two centrosomes, exploring the interior of the cell.

Some of the microtubules growing from one centrosome interact with the microtubules from the other centrosome (see Figure 18–21). This interaction stabilizes the microtubules, preventing them from depolymerizing, and it joins the two sets of microtubules together to form the basic framework of the **mitotic spindle**, with its characteristic bipolar shape (**Movie 18.6**). The two centrosomes that give rise to these microtubules are now called **spindle poles**, and the interacting microtubules are called *interpolar microtubules* (**Figure 18–22**). The assembly of the spindle is driven, in part, by motor proteins associated with the interpolar microtubules that help to cross-link the two sets of microtubules.

In the next stage of mitosis, the duplicated chromosomes attach to the spindle microtubules in such a way that, when the sister chromatids separate, they will be drawn to opposite poles of the cell.

Chromosomes Attach to the Mitotic Spindle at Prometaphase

Prometaphase starts abruptly with the disassembly of the nuclear envelope, which breaks up into small membrane vesicles. This process is triggered by the phosphorylation and consequent disassembly of nuclear pore proteins and the intermediate filament proteins of the nuclear lamina, the network of fibrous proteins that underlies and stabilizes the nuclear envelope (see Figure 17–8). The spindle microtubules, which have been lying in wait outside the nucleus, now gain access to the duplicated chromosomes and capture them (see Panel 18–1, pp. 622–623).

The spindle microtubules grab hold of the chromosomes at **kinetochores**, protein complexes that assemble on the centromere of each condensed chromosome during late prophase (**Figure 18–23**). Each duplicated chromosome has two kinetochores—one on each sister chromatid—which face in opposite directions. Kinetochores recognize the special DNA sequence present at the centromere: if this sequence is altered, kinetochores fail to assemble and, consequently, the chromosomes fail to segregate properly during mitosis.

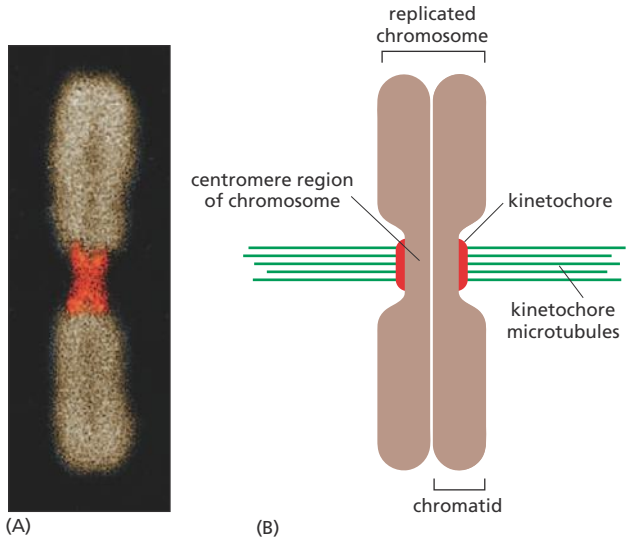


Figure 18–23 Kinetochores attach chromosomes to the mitotic spindle. (A) A fluorescence micrograph of a duplicated mitotic chromosome. The DNA is stained with a fluorescent dye, and the kinetochores are stained *red* with fluorescent antibodies that recognize kinetochore proteins. These antibodies come from patients with scleroderma (a disease that causes progressive overproduction of connective tissue in skin and other organs), who, for unknown reasons, produce antibodies against their own kinetochore proteins. (B) Schematic drawing of a mitotic chromosome showing its two sister chromatids attached to kinetochore microtubules, which bind to the kinetochore by their plus ends. Each kinetochore forms a plaque on the surface of the centromere. (A, courtesy of B.R. Brinkley.)

Once the nuclear envelope has broken down, a randomly probing microtubule encountering a kinetochore will bind to it, thereby capturing the chromosome. This kinetochore microtubule links the chromosome to a spindle pole (see Figure 18–23 and Panel 18–1, pp. 622–623). Because kinetochores on sister chromatids face in opposite directions, they tend to attach to microtubules from opposite poles of the spindle, so that each duplicated chromosome becomes linked to both spindle poles. The attachment to opposite poles, called **bi-orientation**, generates tension on the kinetochores, which are being pulled in opposite directions. This tension signals to the sister kinetochores that they are attached correctly and are ready to be separated. The cell-cycle control system monitors this tension to ensure correct chromosome attachment (see Figure 18–3), a safeguard we discuss in detail shortly.

The number of microtubules attached to each kinetochore varies among species: each human kinetochore binds 20–40 microtubules, for example, whereas a yeast kinetochore binds just one. The three classes of microtubules that form the mitotic spindle are differently colored in **Figure 18–24**.

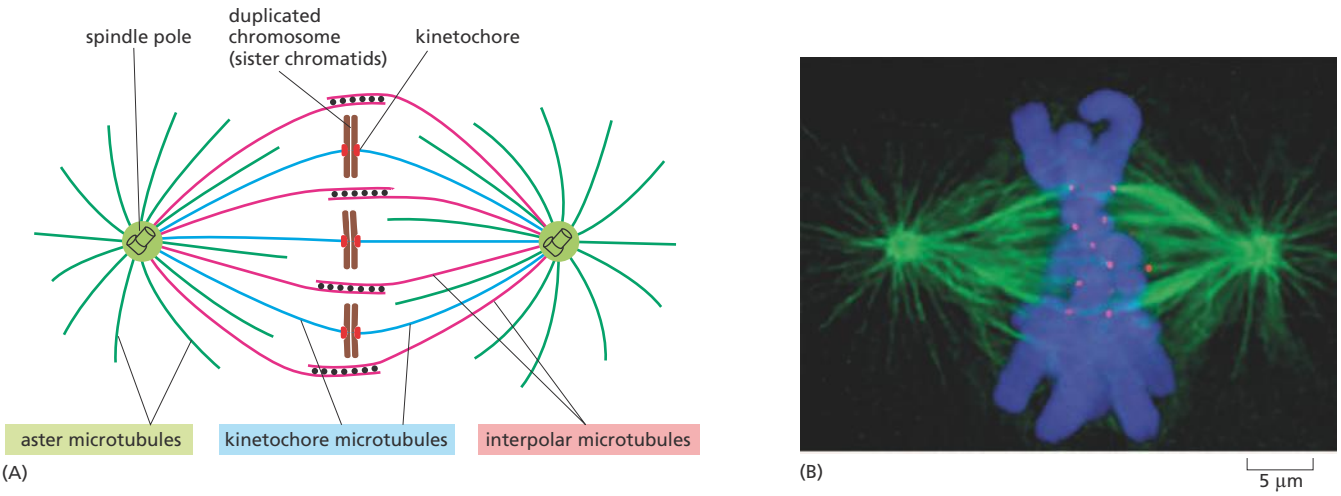


Figure 18–24 Three classes of microtubules make up the mitotic spindle. (A) Schematic drawing of a spindle with chromosomes attached, showing the three types of spindle microtubules: astral microtubules, kinetochore microtubules, and interpolar microtubules. In reality, the chromosomes are much larger than shown, and usually multiple microtubules are attached to each kinetochore. (B) Fluorescence micrograph of duplicated chromosomes at the metaphase plate of a real mitotic spindle. In this image, kinetochores are labeled in *red*, microtubules in *green*, and chromosomes in *blue*. (B, from A. Desai, *Curr. Biol.* 10:R508, 2000. With permission from Elsevier.)

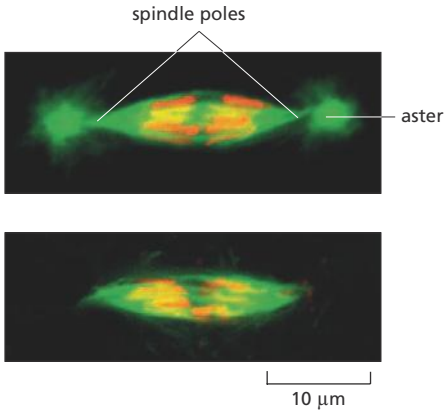


Figure 18–25 Motor proteins and chromosomes can direct the assembly of a functional bipolar spindle in the absence of centrosomes. In these fluorescence micrographs of embryos of the insect *Sciara*, the microtubules are stained *green* and the chromosomes *red*. The top micrograph shows a normal spindle formed with centrosomes in a normally fertilized embryo. The bottom micrograph shows a spindle formed without centrosomes in an embryo that initiated development without fertilization and thus lacks the centrosome normally provided by the sperm when it fertilizes the egg. Note that the spindle with centrosomes has an aster at each pole, whereas the spindle formed without centrosomes does not. As shown, both types of spindles are able to segregate the chromosomes. (From B. de Saint Phalle and W. Sullivan, *J. Cell Biol.* 141:1383–1391, 1998. With permission from The Rockefeller University Press.)

Chromosomes Assist in the Assembly of the Mitotic Spindle

Chromosomes are more than passive passengers in the process of spindle assembly: they can themselves stabilize and organize microtubules into functional mitotic spindles. In cells without centrosomes—including all plant cells and some animal cell types—the chromosomes nucleate microtubule assembly, and motor proteins then move and arrange the microtubules and chromosomes into a bipolar spindle. Even in animal cells that normally have centrosomes, a bipolar spindle can still be formed in this way if the centrosomes are removed (**Figure 18–25**). In cells with centrosomes, the chromosomes, motor proteins, and centrosomes work together to form the mitotic spindle.

Chromosomes Line Up at the Spindle Equator at Metaphase

During prometaphase, the duplicated chromosomes, now attached to the mitotic spindle, begin to move about, as if jerked first this way and then that. Eventually, they align at the equator of the spindle, halfway between the two spindle poles, thereby forming the *metaphase plate*. This event defines the beginning of **metaphase** (**Figure 18–26**). Although the forces that act to bring the chromosomes to the equator are not completely understood, both the continual growth and shrinkage of the microtubules and the action of microtubule motor proteins are required. A continuous balanced addition and loss of tubulin subunits is also required to maintain the metaphase spindle: when tubulin addition to the ends of microtubules is blocked by the drug colchicine, tubulin loss continues until the metaphase spindle disappears.

The chromosomes gathered at the equator of the metaphase spindle oscillate back and forth, continually adjusting their positions, indicating that the tug-of-war between the microtubules attached to opposite poles

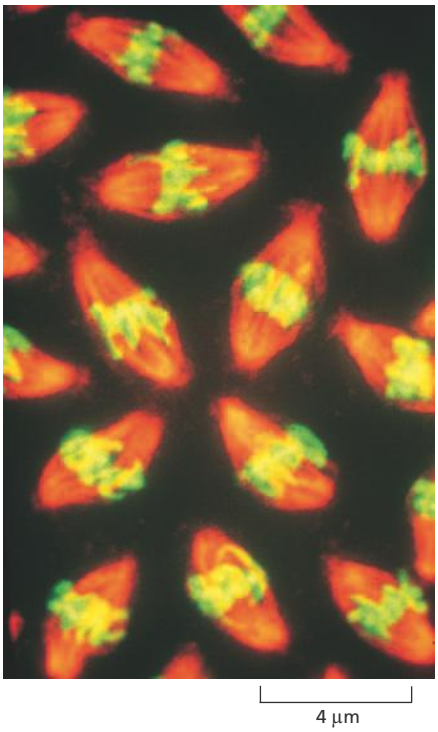


Figure 18–26 During metaphase, duplicated chromosomes gather halfway between the two spindle poles. This fluorescence micrograph shows multiple mitotic spindles at metaphase in a fruit fly (*Drosophila*) embryo. The microtubules are stained *red,* and the chromosomes are stained *green*. At this stage of *Drosophila* development, there are multiple nuclei in one large cytoplasmic compartment, and all of the nuclei divide synchronously, which is why all of the nuclei shown here are at the same metaphase stage of the cell cycle (**Movie 18.7**). Metaphase spindles are usually pictured in two dimensions, as they are here; when viewed in three dimensions, however, the chromosomes are seen to be gathered at a platelike region at the equator of the spindle—the so-called metaphase plate. (Courtesy of William Sullivan.)

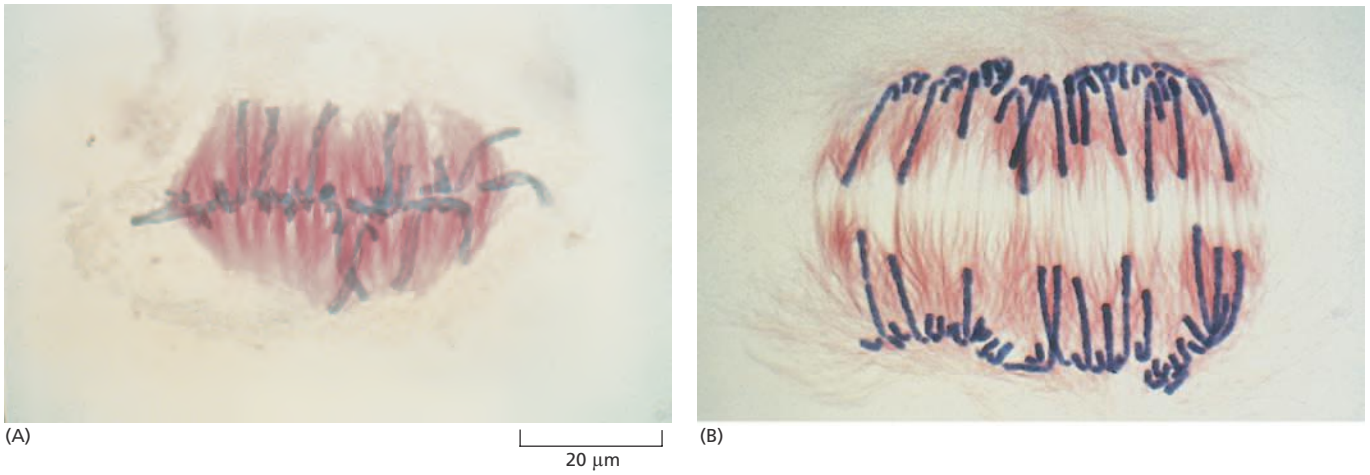


Figure 18–27 Sister chromatids separate at anaphase. In the transition from metaphase (A) to anaphase (B), sister chromatids (stained *blue*) suddenly separate, allowing the resulting chromosomes to move toward opposite poles, as seen in these plant cells stained with gold-labeled antibodies to label the microtubules (*red*). Plant cells generally do not have centrosomes and therefore have less sharply defined spindle poles than animal cells (see Figure 18–34); nonetheless, spindle poles are present here at the top and bottom of each micrograph, although they cannot be seen. (Courtesy of Andrew Bajer.)

of the spindle continues to operate after the chromosomes are all aligned. If one of the pair of kinetochore attachments is artificially severed with a laser beam during metaphase, the entire duplicated chromosome immediately moves toward the pole to which it remains attached. Similarly, if the attachment between sister chromatids is cut, the two chromosomes separate and move toward opposite poles. These experiments show that the duplicated chromosomes are not simply deposited at the metaphase plate. They are suspended there under tension. In anaphase, that tension will pull the sister chromatids apart.

Proteolysis Triggers Sister-Chromatid Separation at Anaphase

Anaphase begins abruptly with the breakage of the cohesin linkages that hold sister chromatids together (see Figure 18–18A). This release allows each chromatid—now considered a chromosome—to be pulled to the spindle pole to which it is attached (**Figure 18–27**). This movement segregates the two identical sets of chromosomes to opposite ends of the spindle (see Panel 18–1, pp. 622–623).

The cohesin linkage is destroyed by a protease called *separase*. Before anaphase begins, this protease is held in an inactive state by an inhibitory protein called *securin*. At the beginning of anaphase, securin is targeted for destruction by APC—the same protein complex, discussed earlier, that marks M cyclin for degradation. Once securin has been removed, separase is then free to sever the cohesin linkages (**Figure 18–28**).

Chromosomes Segregate During Anaphase

Once the sister chromatids separate, the resulting chromosomes are pulled to the spindle pole to which they are attached. They all move at the same speed, which is typically about 1 μm per minute. The movement is the consequence of two independent processes that depend on different parts of the mitotic spindle. The two processes are called *anaphase A* and *anaphase B*, and they occur more or less simultaneously. In anaphase A, the kinetochore microtubules shorten and the attached chromosomes

QUESTION 18–6

If fine glass needles are used to manipulate a chromosome inside a living cell during early M phase, it is possible to trick the kinetochores on the two sister chromatids into attaching to the same spindle pole. This arrangement is normally unstable, but the attachments can be stabilized if the needle is used to gently pull the chromosome so that the microtubules attached to both kinetochores (via the same spindle pole) are under tension. What does this suggest to you about the mechanism by which kinetochores normally become attached and stay attached to microtubules from opposite spindle poles? Is the finding consistent with the possibility that a kinetochore is programmed to attach to microtubules from a particular spindle pole? Explain your answers.

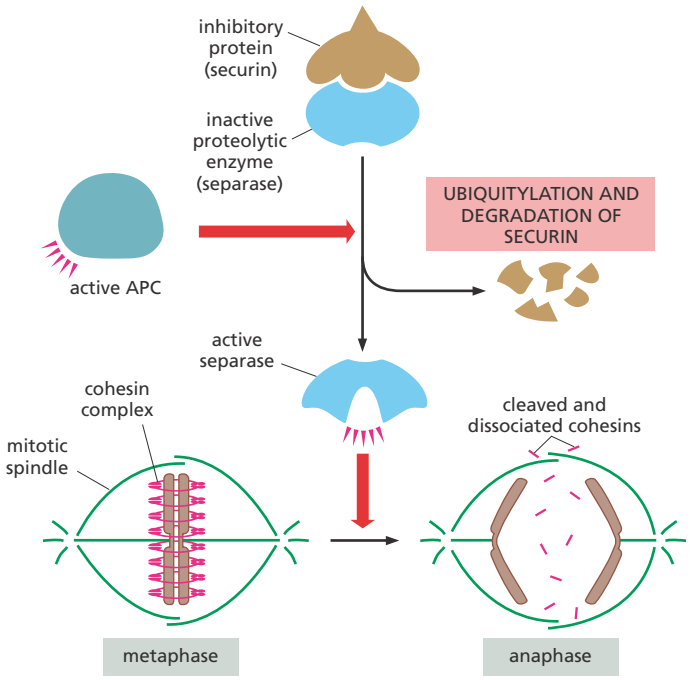


Figure 18–28 APC triggers the separation of sister chromatids by promoting the destruction of cohesins. APC indirectly triggers the cleavage of the cohesins that hold sister chromatids together. It catalyzes the ubiquitylation and destruction of an inhibitory protein called securin. Securin inhibits the activity of a proteolytic enzyme called separase; when freed from securin, separase cleaves the cohesin complexes, allowing the mitotic spindle to pull the sister chromatids apart.

move poleward. In anaphase B, the spindle poles themselves move apart, further segregating the two sets of chromosomes (**Figure 18–29**).

The driving force for the movements of anaphase A is thought to be provided mainly by the loss of tubulin subunits from both ends of the kinetochore microtubules. The driving forces in anaphase B are thought to be provided by two sets of motor proteins—members of the kinesin and dynein families—operating on different types of spindle microtubules (see Figure 17–21). Kinesin proteins act on the long, overlapping interpolar microtubules, sliding the microtubules from opposite poles past one another at the equator of the spindle and pushing the spindle poles apart. Dynein proteins, anchored to the cell cortex that underlies the plasma membrane, pull the poles apart (see Figure 18–29B).

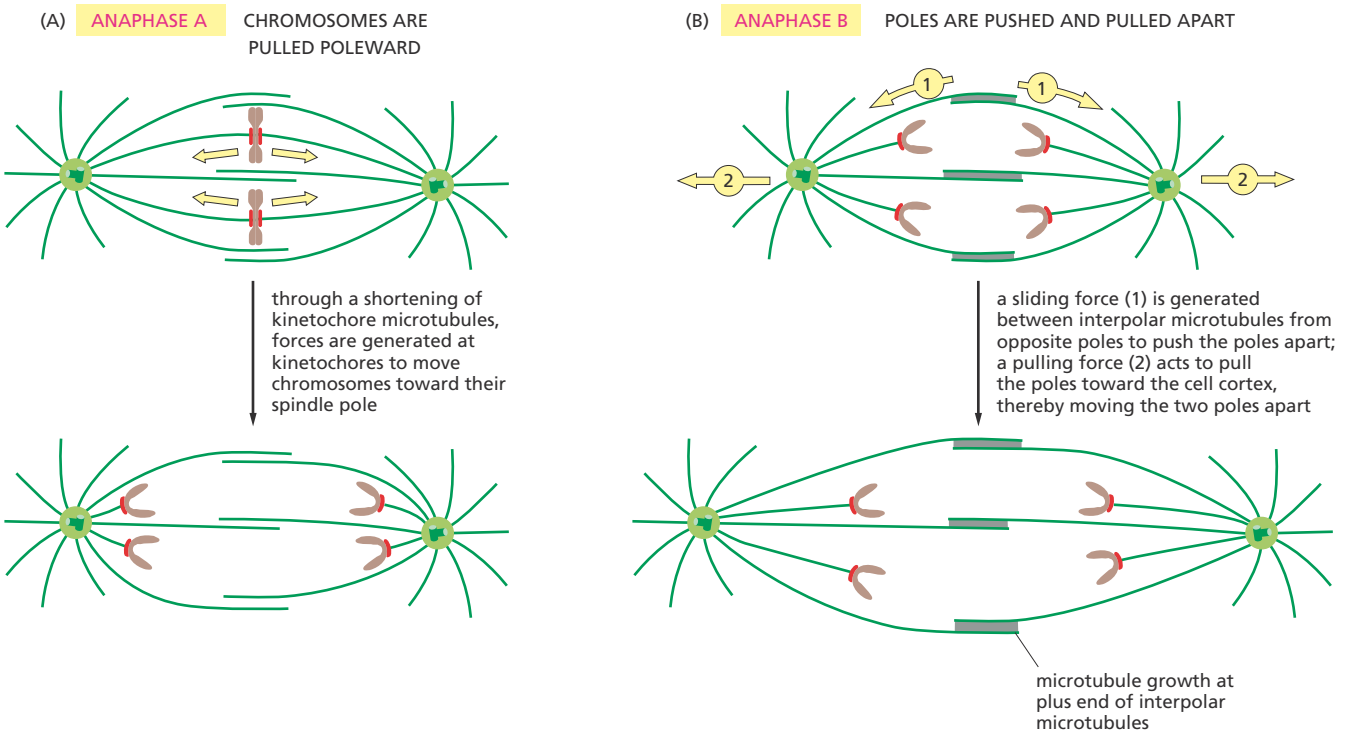


Figure 18–29 Two processes segregate daughter chromosomes at anaphase. In *anaphase A*, the daughter chromosomes are *pulled* toward opposite poles as the kinetochore microtubules depolymerize. The force driving this movement is generated mainly at the kinetochore. In *anaphase B*, the two spindle poles move apart as the result of two separate forces: (1) the elongation and sliding of the interpolar microtubules past one another *pushes* the two poles apart, and (2) forces exerted on the outward-pointing astral microtubules at each spindle pole *pull* the poles away from each other, toward the cell cortex. Both forces are thought to depend on the action of motor proteins associated with the microtubules.

An Unattached Chromosome Will Prevent Sister-Chromatid Separation

If a dividing cell were to begin to segregate its chromosomes before all the chromosomes were properly attached to the spindle, one daughter cell would receive an incomplete set of chromosomes, while the other would receive a surplus. Both situations could be lethal. Thus, a dividing cell must ensure that every last chromosome is attached properly to the spindle before it completes mitosis. To monitor chromosome attachment, the cell makes use of a negative signal: unattached chromosomes send a "stop" signal to the cell-cycle control system. Although only some of the details are known, the signal inhibits further progress through mitosis by blocking the activation of the APC (see Figure 18–28). Without active APC, the sister chromatids remain glued together. Thus, none of the duplicated chromosomes can be pulled apart until every chromosome has been positioned correctly on the mitotic spindle. This so-called *spindle assembly checkpoint* thereby controls the onset of anaphase, as well as the exit from mitosis, as mentioned earlier (see Figure 18–12).

The Nuclear Envelope Re-forms at Telophase

By the end of anaphase, the daughter chromosomes have separated into two equal groups, one at each pole of the spindle. During **telophase**, the final stage of mitosis, the mitotic spindle disassembles, and a nuclear envelope reassembles around each group of chromosomes to form the two daughter nuclei (**Movie 18.8**). Vesicles of nuclear membrane first cluster around individual chromosomes and then fuse to re-form the nuclear envelope (see Panel 18–1, pp. 622–623). During this process, the nuclear pore proteins and nuclear lamins that were phosphorylated during prometaphase are now dephosphorylated, which allows them to reassemble and the nuclear envelope and lamina to re-form (**Figure 18–30**). Once the nuclear envelope has re-formed, the pores pump in nuclear proteins, the nucleus expands, and the condensed chromosomes decondense into their interphase state. As a consequence of this decondensation, gene transcription is able to resume. A new nucleus has been created, and mitosis is complete. All that remains is for the cell to complete its division into two separate daughter cells.

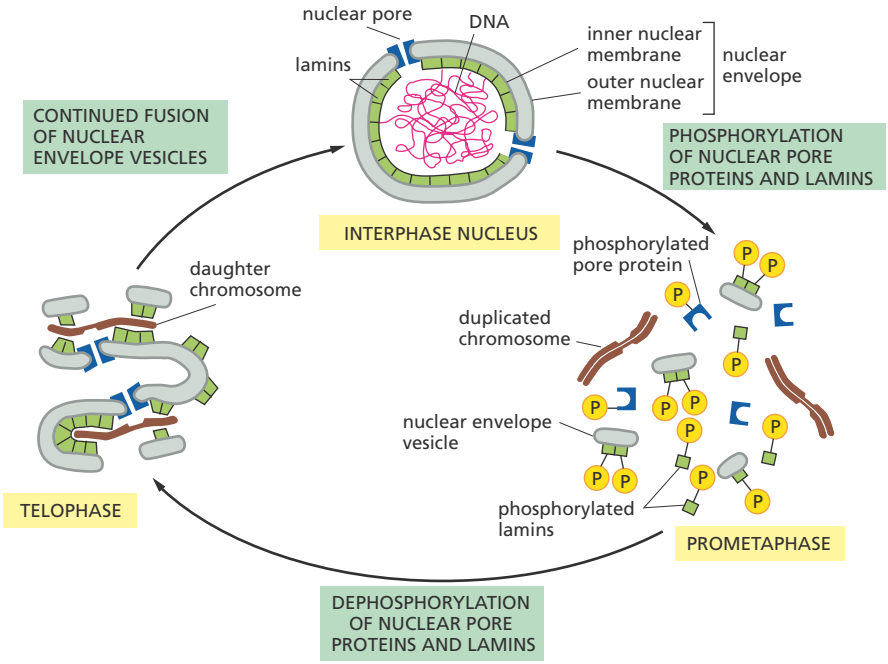


Figure 18–30 The nuclear envelope breaks down and re-forms during mitosis. The phosphorylation of nuclear pore proteins and lamins helps trigger the disassembly of the nuclear envelope at prometaphase. Dephosphorylation of these proteins at telophase helps reverse the process.

QUESTION 18–7

Consider the events that lead to the formation of the new nucleus at telophase. How do nuclear and cytosolic proteins become properly re-sorted so that the new nucleus contains nuclear proteins but not cytosolic proteins?

CYTOKINESIS

Cytokinesis, the process by which the cytoplasm is cleaved in two, completes M phase. It usually begins in anaphase but is not completed until the two daughter nuclei have formed in telophase. Whereas mitosis depends on a transient microtubule-based structure, the mitotic spindle, cytokinesis in animal cells depends on a transient structure based on actin and myosin filaments, the *contractile ring* (see Figure 18–19). Both the plane of cleavage and the timing of cytokinesis, however, are determined by the mitotic spindle.

The Mitotic Spindle Determines the Plane of Cytoplasmic Cleavage

The first visible sign of cytokinesis in animal cells is a puckering and furrowing of the plasma membrane that occurs during anaphase (**Figure 18–31**). The furrowing invariably occurs in a plane that runs perpendicular to the long axis of the mitotic spindle. This positioning ensures that the *cleavage furrow* cuts between the two groups of segregated chromosomes, so that each daughter cell receives an identical and complete set of chromosomes. If the mitotic spindle is deliberately displaced (using a fine glass needle) as soon as the furrow appears, the furrow disappears and a new one develops at a site corresponding to the new spindle location and orientation. Once the furrowing process is well under way, however, cleavage proceeds even if the mitotic spindle is artificially sucked out of the cell or depolymerized using the drug colchicine.

How does the mitotic spindle dictate the position of the cleavage furrow? The mechanism is still uncertain, but it appears that, during anaphase, the overlapping interpolar microtubules that form the *central spindle* recruit and activate proteins that signal to the cell cortex to initiate the assembly of the contractile ring at a position midway between the spindle poles. Because these signals originate in the anaphase spindle, this mechanism also contributes to the timing of cytokinesis in late mitosis.

When the mitotic spindle is located centrally in the cell—the usual situation in most dividing cells—the two daughter cells produced will be of equal size. During embryonic development, however, there are some instances in which the dividing cell moves its mitotic spindle to an asymmetrical position, and, consequently, the furrow creates two daughter cells that differ in size. In most of these *asymmetric divisions*, the daughters also differ in the molecules they inherit, and they usually develop into different cell types.

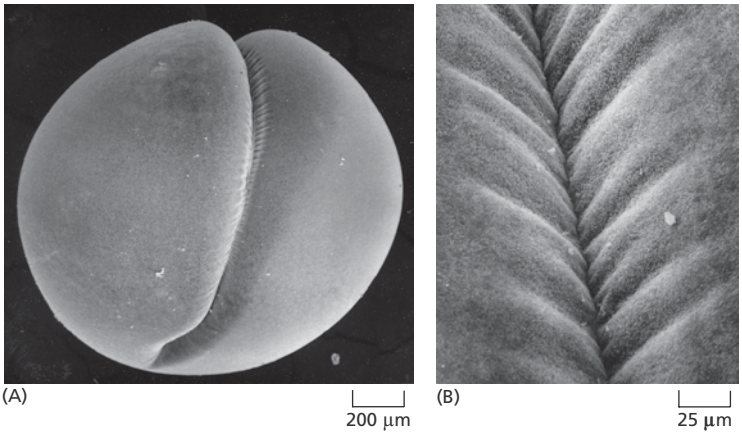
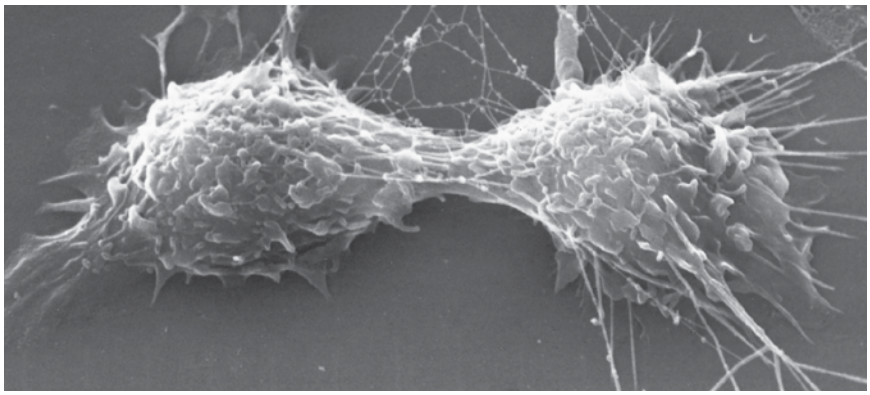
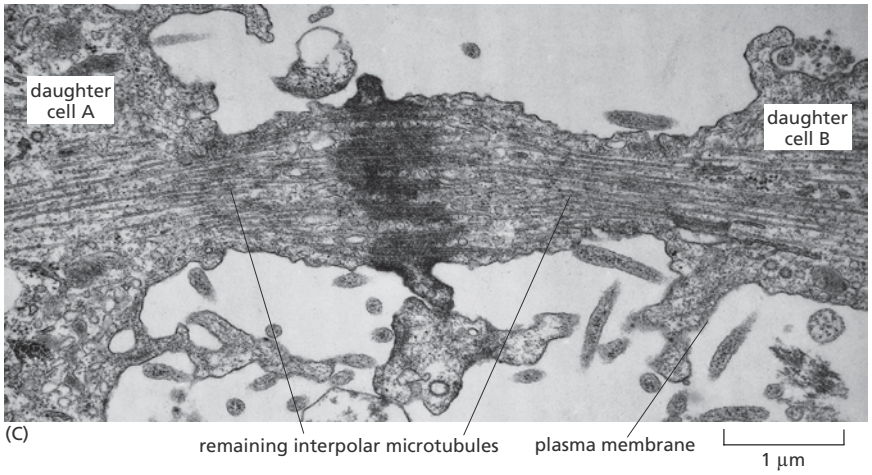


Figure 18–31 The cleavage furrow is formed by the action of the contractile ring underneath the plasma membrane. In these scanning electron micrographs of a dividing fertilized frog egg, the cleavage furrow is unusually well defined. (A) Low-magnification view of the egg surface. (B) A higher-magnification view of the cleavage furrow. (From H.W. Beams and R.G. Kessel, *Am. Sci.* 64:279–290, 1976. With permission of Sigma Xi.)





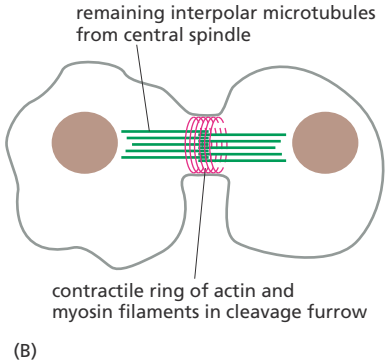


Figure 18–32 The contractile ring divides the cell in two. (A) Scanning electron micrograph of an animal cell in culture in the last stages of cytokinesis. (B) Schematic diagram of the midregion of a similar cell showing the contractile ring beneath the plasma membrane and the remains of the two sets of interpolar microtubules. (C) A conventional electron micrograph of a dividing animal cell. Cleavage is almost complete, but the daughter cells remain attached by a thin strand of cytoplasm containing the remains of the overlapping interpolar microtubules of the central mitotic spindle. (A, courtesy of Guenter Albrecht-Buehler; C, courtesy of J.M. Mullins.)

The Contractile Ring of Animal Cells Is Made of Actin and Myosin Filaments

The **contractile ring** is composed mainly of an overlapping array of actin filaments and myosin filaments (**Figure 18–32**). It assembles at anaphase and is attached to membrane-associated proteins on the cytoplasmic face of the plasma membrane. Once assembled, the contractile ring is capable of exerting a force strong enough to bend a fine glass needle inserted into the cell before cytokinesis. Much of this force is generated by the sliding of the actin filaments against the myosin filaments. Unlike the stable association of actin and myosin filaments in muscle fibers, however, the contractile ring is a transient structure: it assembles to carry out cytokinesis, gradually becomes smaller as cytokinesis progresses, and disassembles completely once the cell has been cleaved in two.

Cell division in many animal cells is accompanied by large changes in cell shape and a decrease in the adherence of the cell to its neighbors, to the extracellular matrix, or to both. These changes result, in part, from the reorganization of actin and myosin filaments in the cell cortex, only one aspect of which is the assembly of the contractile ring. Mammalian fibroblasts in culture, for example, spread out flat during interphase, as a result of the strong adhesive contacts they make with the surface they are growing on—called the *substratum*. As the cells enter M phase, however, they round up. The cells change shape in part because some of the plasma membrane proteins responsible for attaching the cells to the substratum—the *integrins* (discussed in Chapter 20)—become phosphorylated and thus weaken their grip. Once cytokinesis is complete, the daughter cells reestablish their strong contacts with the substratum and

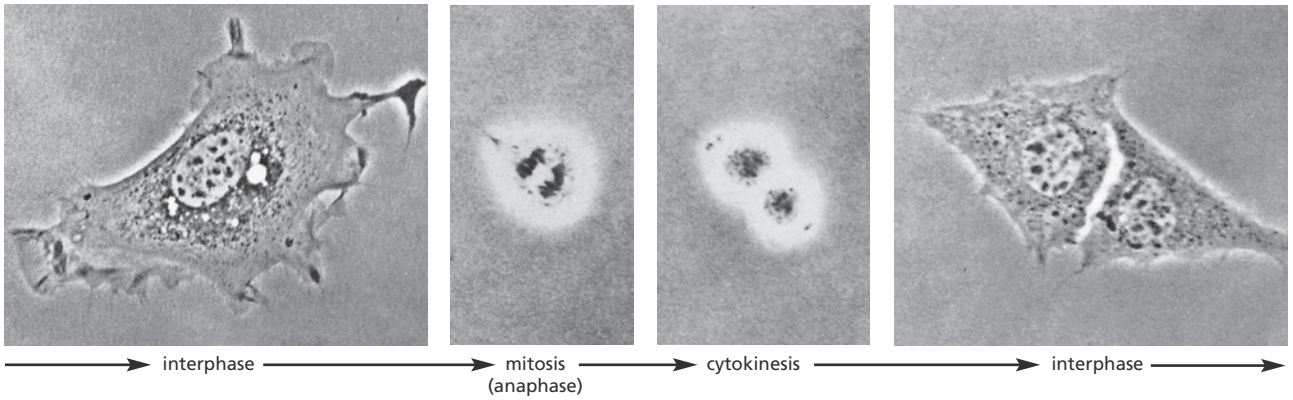


Figure 18–33 Animal cells change shape during M phase. In these micrographs of a mouse fibroblast dividing in culture, the same cell was photographed at successive times. Note how the cell rounds up as it enters mitosis; the two daughter cells then flatten out again after cytokinesis is complete. (Courtesy of Guenter Albrecht-Buehler.)

flatten out again (**Figure 18–33**). When cells divide in an animal tissue, this cycle of attachment and detachment presumably allows the cells to rearrange their contacts with neighboring cells and with the extracellular matrix, so that the new cells produced by cell division can be accommodated within the tissue.

Cytokinesis in Plant Cells Involves the Formation of a New Cell Wall

The mechanism of cytokinesis in higher plants is entirely different from that in animal cells, presumably because plant cells are surrounded by a tough cell wall (discussed in Chapter 20). The two daughter cells are separated not by the action of a contractile ring at the cell surface but instead by the construction of a new wall that forms inside the dividing cell. The positioning of this new wall precisely determines the position of the two daughter cells relative to neighboring cells. Thus, the planes of cell division, together with cell enlargement, determine the final form of the plant.

The new cell wall starts to assemble in the cytoplasm between the two sets of segregated chromosomes at the start of telophase. The assembly process is guided by a structure called the **phragmoplast**, which is formed by the remains of the interpolar microtubules at the equator of the old mitotic spindle. Small membrane-enclosed vesicles, largely derived from the Golgi apparatus and filled with polysaccharides and glycoproteins required for the cell-wall matrix, are transported along the microtubules to the phragmoplast. Here, they fuse to form a disclike, membrane-enclosed structure, which expands outward by further vesicle fusion until it reaches the plasma membrane and original cell wall, thereby dividing the cell in two (**Figure 18–34**). Later, cellulose microfibrils are laid down within the matrix to complete the construction of the new cell wall.

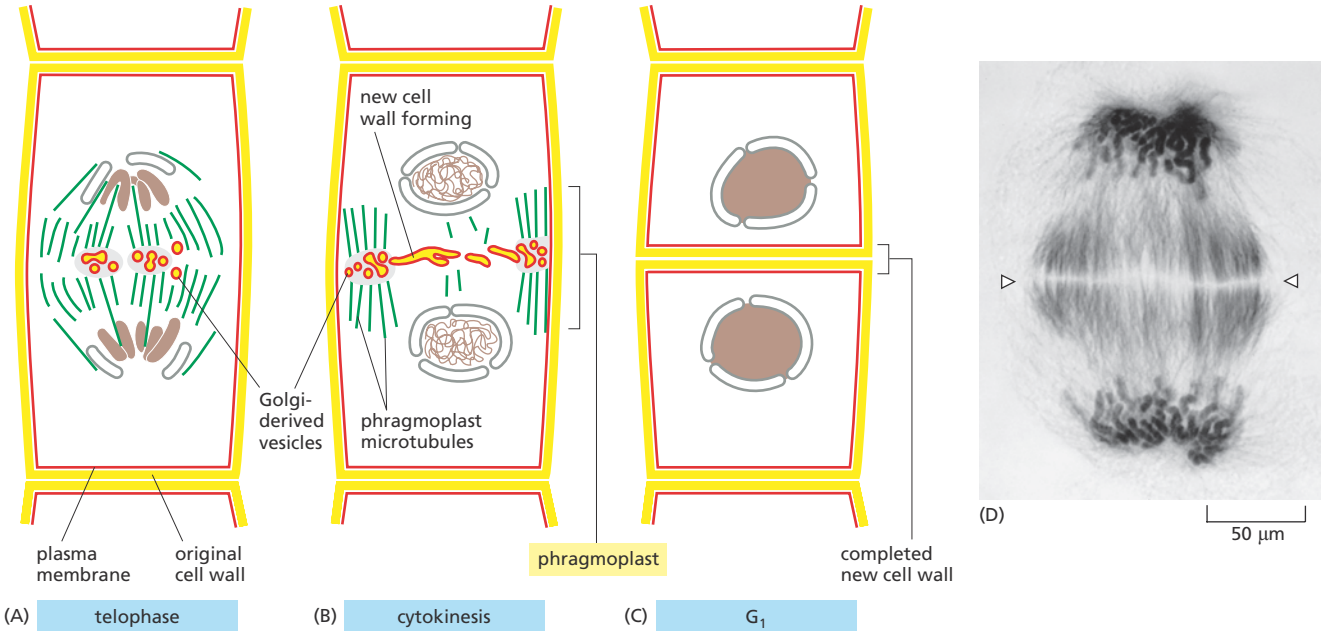
QUESTION 18–8

Draw a detailed view of the formation of the new cell wall that separates the two daughter cells when a plant cell divides (see Figure 18–34). In particular, show where the membrane proteins of the Golgi-derived vesicles end up, indicating what happens to the part of a protein in the Golgi vesicle membrane that is exposed to the interior of the Golgi vesicle. (Refer to Chapter 11 if you need a reminder of membrane structure.)

Membrane-Enclosed Organelles Must Be Distributed to Daughter Cells When a Cell Divides

Organelles such as mitochondria and chloroplasts cannot assemble spontaneously from their individual components; they arise only from the growth and division of the preexisting organelles. Likewise, endoplasmic reticulum (ER) and Golgi apparatus also derive from preexisting organelle fragments. How, then, are these various membrane-enclosed organelles segregated when the cell divides so that each daughter gets some?

Mitochondria and chloroplasts are usually present in large numbers and will be safely inherited if, on average, their numbers simply double once



each cell cycle. The ER in interphase cells is continuous with the nuclear membrane and is organized by the microtubule cytoskeleton (see Figure 17–20A). Upon entry into M phase, the reorganization of the microtubules releases the ER; in most cells, the released ER remains intact during mitosis and is cut in two during cytokinesis. The Golgi apparatus fragments during mitosis; the fragments associate with the spindle microtubules via motor proteins, thereby hitching a ride into the daughter cells as the spindle elongates in anaphase. Other components of the cell—including the other membrane-enclosed organelles, ribosomes, and all of the soluble proteins—are inherited randomly when the cell divides.

Having discussed how cells divide, we now turn to the general problem of how the size of an animal or an organ is determined, which leads us to consider how cell number and cell size are controlled.

CONTROL OF CELL NUMBERS AND CELL SIZE

A fertilized mouse egg and a fertilized human egg are similar in size—about 100 µm in diameter. Yet an adult mouse is much smaller than an adult human. What are the differences between the control of cell behavior in humans and mice that generate such big differences in size? The same fundamental question can be asked about each organ and tissue in an individual's body. What adjustment of cell behavior explains the length of an elephant's trunk or the size of its brain or its liver? These questions are largely unanswered, but it is at least possible to say what the ingredients of an answer must be. Three fundamental processes largely determine organ and body size: cell growth, cell division, and cell death. Each of these processes, in turn, depends on programs intrinsic to the individual cell, regulated by signals from other cells in the body.

In this section, we first consider how organisms eliminate unwanted cells by a form of programmed cell death called *apoptosis*. We then discuss how extracellular signals balance cell death, cell growth, and cell division—thereby helping control the size of an animal and its organs. We conclude the section with a brief discussion of the extracellular signals that help keep these three processes in check.

Figure 18–34 Cytokinesis in a plant cell is guided by a specialized microtubule-based structure called the phragmoplast. At the beginning of telophase, after the chromosomes have segregated, a new cell wall starts to assemble inside the cell at the equator of the old spindle (A). The interpolar microtubules of the mitotic spindle remaining at telophase form the *phragmoplast* and guide vesicles, derived from the Golgi apparatus, toward the equator of the spindle. The vesicles, which are filled with cell-wall material, fuse to form the growing new cell wall that grows outward to reach the plasma membrane and original cell wall (B). The pre-existing plasma membrane and the membrane surrounding the new cell wall (both shown in *red*) then fuse, completely separating the two daughter cells (C). A light micrograph of a plant cell in telophase is shown in (D) at a stage corresponding to (A). The cell has been stained to show both the microtubules and the two sets of chromosomes segregated at the two poles of the spindle. The location of the growing new cell wall is indicated by the arrowheads. (D, courtesy of Andrew Bajer.)

QUESTION 18–9

The Golgi apparatus is thought to be partitioned into the daughter cells at cell division by a random distribution of fragments that are created at mitosis. Explain why random partitioning of chromosomes would not work.

Apoptosis Helps Regulate Animal Cell Numbers

The cells of a multicellular organism are members of a highly organized community. The number of cells in this community is tightly regulated—not simply by controlling the rate of cell division, but also by controlling the rate of cell death. If cells are no longer needed, they can commit suicide by activating an intracellular death program—a process called **programmed cell death**. In animals, the most common form of programmed cell death is called **apoptosis** (from a Greek word meaning "falling off," as leaves fall from a tree).

The amount of apoptosis that occurs in both developing and adult animal tissues can be astonishing. In the developing vertebrate nervous system, for example, more than half of some types of nerve cells normally die soon after they are formed. In a healthy adult human, billions of cells die in the bone marrow and intestine every hour. It seems remarkably wasteful for so many cells to die, especially as the vast majority are perfectly healthy at the time they kill themselves. What purposes does this massive cell suicide serve?

In some cases, the answers are clear. Mouse paws—and our own hands and feet—are sculpted by apoptosis during embryonic development: they start out as spadelike structures, and the individual fingers and toes separate because the cells between them die (**Figure 18–35**). In other cases, cells die when the structure they form is no longer needed. When a tadpole changes into a frog at metamorphosis, the cells in the tail die, and the tail, which is not needed in the frog, disappears (**Figure 18–36**). In these cases, the unneeded cells die largely through apoptosis.

In adult tissues, cell death usually exactly balances cell division, unless the tissue is growing or shrinking. If part of the liver is removed in an adult rat, for example, liver cells proliferate to make up the loss. Conversely, if a rat is treated with the drug phenobarbital, which stimulates liver cell division, the liver enlarges. However, when the phenobarbital treatment is stopped, apoptosis in the liver greatly increases until the organ has returned to its original size, usually within a week or so. Thus, the liver is kept at a constant size through regulation of both the cell death rate and the cell birth rate.

Apoptosis Is Mediated by an Intracellular Proteolytic Cascade

Cells that die as a result of acute injury typically swell and burst, spilling their contents all over their neighbors, a process called *cell necrosis* (**Figure 18–37A**). This eruption triggers a potentially damaging inflammatory response. By contrast, a cell that undergoes apoptosis dies neatly, without damaging its neighbors. A cell in the throes of apoptosis may

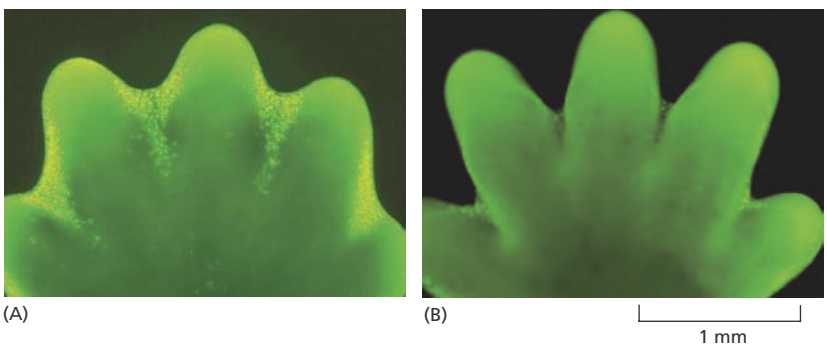


Figure 18–35 Apoptosis in the developing mouse paw sculpts the digits. (A) The paw in this mouse embryo has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green dots* between the developing digits. (B) This cell death eliminates the tissue between the developing digits, as seen in the paw shown one day later. Here, few, if any, apoptotic cells can be seen—demonstrating how quickly apoptotic cells can be cleared from a tissue. (From W. Wood et al., *Development* 127:5245–5252, 2000. With permission from The Company of Biologists Ltd.)

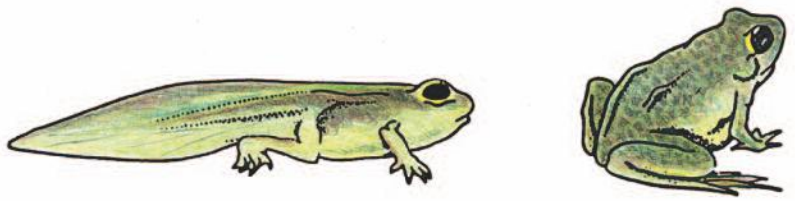


Figure 18–36 As a tadpole changes into a frog, the cells in its tail are induced to undergo apoptosis. All of the changes that occur during metamorphosis, including the induction of apoptosis in the tadpole tail, are stimulated by an increase in thyroid hormone in the blood.

develop irregular bulges—or *blebs*—on its surface; but it then shrinks and condenses (**Figure 18–37B**). The cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear DNA breaks up into fragments (**Movie 18.9**). Most importantly, the cell surface is altered in such a manner that it immediately attracts phagocytic cells, usually specialized phagocytic cells called macrophages (see Figure 15–32B). These cells engulf the apoptotic cell before it spills its contents (**Figure 18–37C**). This rapid removal of the dying cell avoids the damaging consequences of cell necrosis, and it also allows the organic components of the apoptotic cell to be recycled by the cell that ingests it.

The molecular machinery responsible for apoptosis, which seems to be similar in most animal cells, involves a family of proteases called **caspases**. These enzymes are made as inactive precursors, called *procaspases*, which are activated in response to signals that induce apoptosis (**Figure 18–38A**). Two types of caspases work together to take a cell apart. *Initiator caspases* cleave, and thereby activate, downstream *executioner caspases*. Some of these executioner caspases then activate additional executioners, kicking off an amplifying, proteolytic cascade;

QUESTION 18–10

Why do you think apoptosis occurs by a different mechanism from the cell death that occurs in cell necrosis? What might be the consequences if apoptosis were not achieved in so neat and orderly a fashion, whereby the cell destroys itself from within and avoids leakage of its contents into the extracellular space?

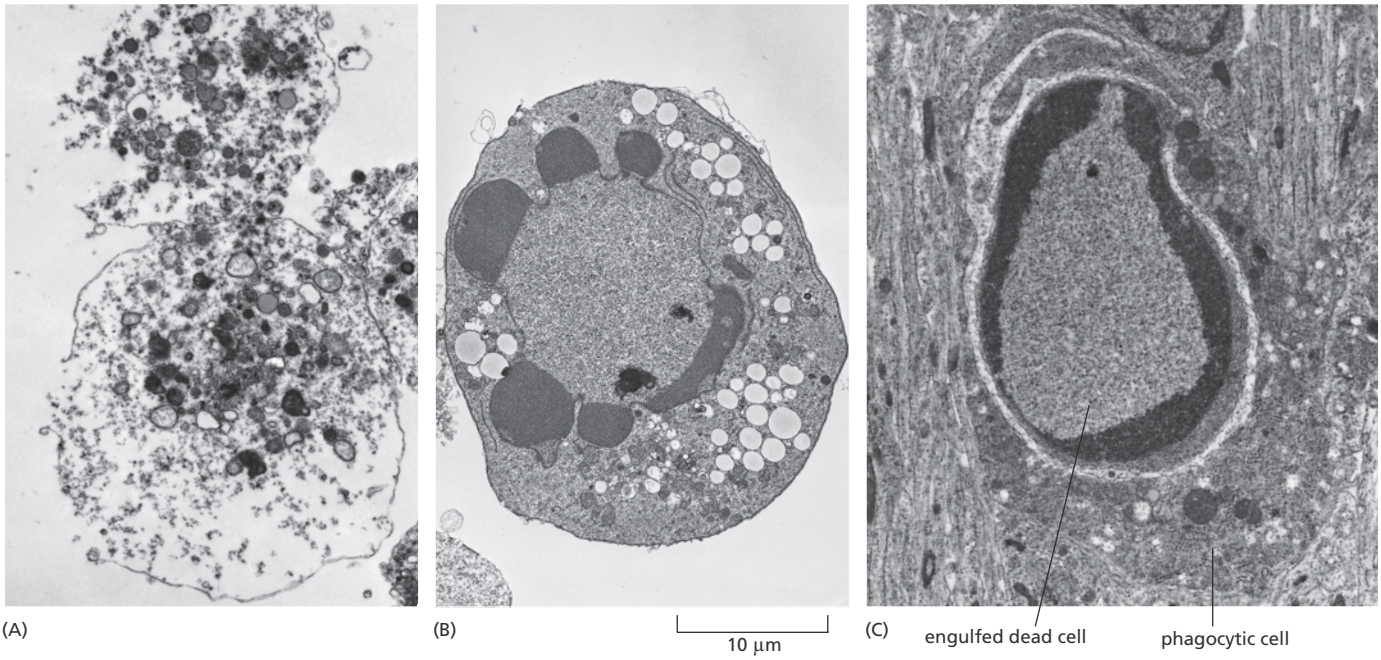


Figure 18–37 Cells undergoing apoptosis die quickly and cleanly. Electron micrographs showing cells that have died by necrosis (A) or by apoptosis (B and C). The cells in (A) and (B) died in a culture dish, whereas the cell in (C) died in a developing tissue and has been engulfed by a phagocytic cell. Note that the cell in (A) seems to have exploded, whereas those in (B) and (C) have condensed but seem relatively intact. The large vacuoles seen in the cytoplasm of the cell in (B) are a variable feature of apoptosis. (Courtesy of Julia Burne.)

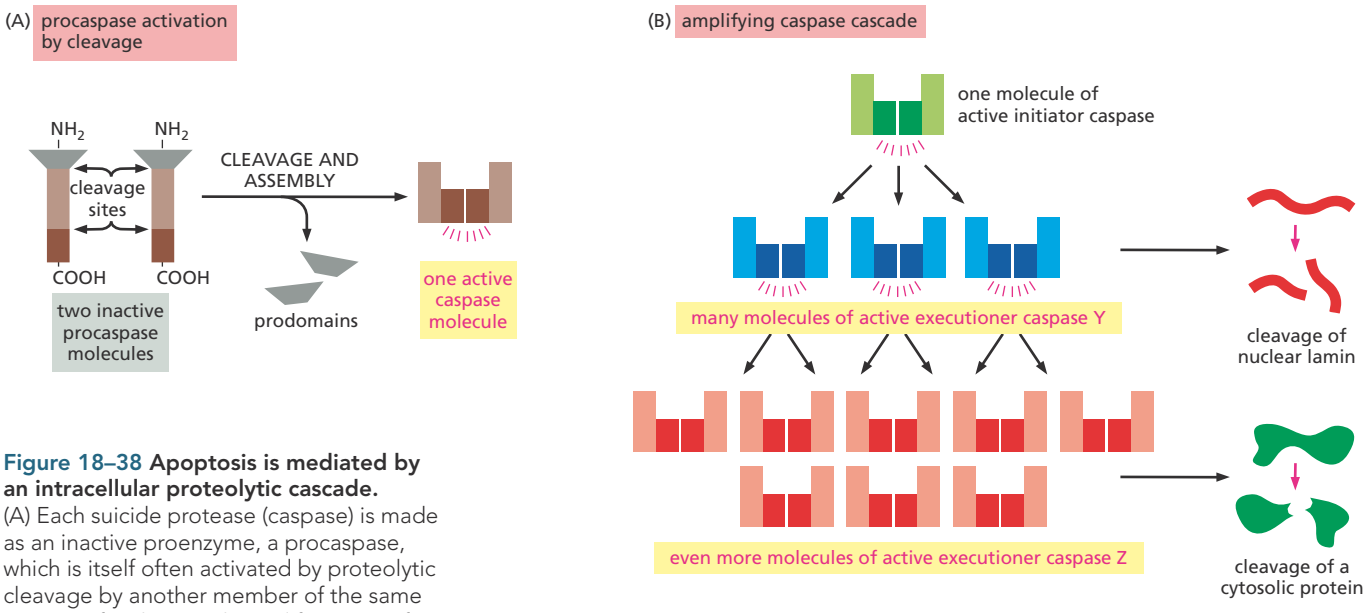


Figure 18–38 Apoptosis is mediated by an intracellular proteolytic cascade. (A) Each suicide protease (caspase) is made as an inactive proenzyme, a procaspase, which is itself often activated by proteolytic cleavage by another member of the same protease family. Two cleaved fragments from each of two procaspase molecules associate to form an active caspase, which is formed from two small and two large subunits; the two prodomains are usually discarded. (B) Each activated initiator caspase molecule can cleave many executioner procaspase molecules, thereby activating them, and these can activate even more procaspase molecules. In this way, an initial activation of a small number of initiator caspase molecules can lead, via an amplifying chain reaction (a cascade), to the explosive activation of a large number of executioner caspase molecules. Some of the activated executioner caspases break down key proteins in the cell, such as the nuclear lamins, leading to the controlled death of the cell. The proteolytic cascade begins when initiator procaspases are activated, as we discuss shortly.

others dismember other key proteins in the cell (**Figure 18–38B**). For example, one executioner caspase targets the lamin proteins that form the nuclear lamina underlying the nuclear envelope; this cleavage causes the irreversible breakdown of the nuclear lamina, which allows nucleases to enter the nucleus and break down the DNA. In this way, the cell dismantles itself quickly and cleanly, and its corpse is rapidly taken up and digested by another cell.

Activation of the apoptotic program, like entry into a new stage of the cell cycle, is usually triggered in an all-or-none fashion. The caspase cascade is not only destructive and self-amplifying but also irreversible; once a cell reaches a critical point along the path to destruction, it cannot turn back. Thus it is important that the decision to die be tightly controlled.

The Intrinsic Apoptotic Death Program Is Regulated by the Bcl2 Family of Intracellular Proteins

All nucleated animal cells contain the seeds of their own destruction: in these cells, inactive procaspases lie waiting for a signal to destroy the cell. It is therefore not surprising that caspase activity is tightly regulated inside the cell to ensure that the death program is held in check until it is needed—for example, to eliminate cells that are superfluous, mislocated, or badly damaged.

The main proteins that regulate the activation of caspases are members of the **Bcl2 family** of intracellular proteins. Some members of this protein family promote caspase activation and cell death, whereas others inhibit these processes. Two of the most important death-inducing family members are proteins called *Bax* and *Bak*. These proteins—which are activated in response to DNA damage or other insults—promote cell death by inducing the release of the electron-transport protein cytochrome *c* from mitochondria into the cytosol. Other members of the Bcl2 family (including Bcl2 itself) inhibit apoptosis by preventing Bax and Bak from releasing cytochrome *c*. The balance between the activities of pro-apoptotic and anti-apoptotic members of the Bcl2 family largely determines whether a mammalian cell lives or dies by apoptosis.

The cytochrome *c* molecules released from mitochondria activate initiator procaspases—and induce cell death—by promoting the assembly of a large, seven-armed, pinwheel-like protein complex called an *apoptosome*.

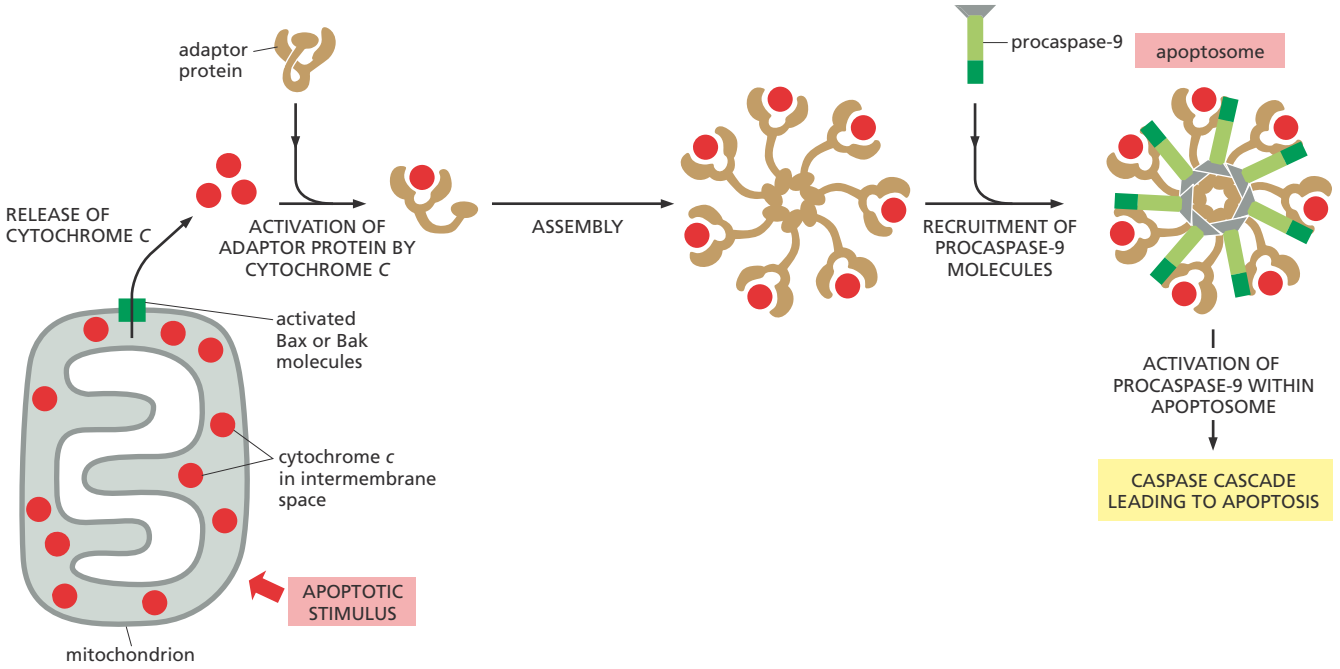


Figure 18–39 Bax and Bak are death-promoting members of the Bcl2 family of intracellular proteins that can trigger apoptosis by releasing cytochrome *c* from mitochondria. When Bak or Bax proteins are activated by an apoptotic stimulus, they aggregate in the outer mitochondrial membrane, leading to the release of cytochrome *c* by an unknown mechanism. The cytochrome *c* is released into the cytosol from the mitochondrial intermembrane space (along with other proteins in this space—not shown). Cytochrome *c* then binds to an adaptor protein, causing it to assemble into a seven-armed complex. This complex then recruits seven molecules of a specific initiator procaspase (procaspase-9) to form a structure called an apoptosome. The procaspase-9 proteins become activated within the apoptosome and then go on to activate executioner procaspases in the cytosol, leading to a caspase cascade and apoptosis.

The apoptosome then recruits and activates a particular initiator procaspase, which then triggers a caspase cascade that leads to apoptosis (**Figure 18–39**).

Extracellular Signals Can Also Induce Apoptosis

Sometimes the signal to commit suicide is not generated internally, but instead comes from a neighboring cell. Some of these extracellular signals activate the cell death program by affecting the activity of members of the Bcl2 family of proteins. Others stimulate apoptosis more directly by activating a set of cell-surface receptor proteins known as *death receptors*.

One particularly well-understood death receptor, called *Fas*, is present on the surface of a variety of mammalian cell types. Fas is activated by a membrane-bound protein, called *Fas ligand*, present on the surface of specialized immune cells called *killer lymphocytes*. These killer cells help regulate immune responses by inducing apoptosis in other immune cells that are unwanted or are no longer needed—and activating Fas is one way they do so. The binding of Fas ligand to its receptor triggers the assembly of a death-inducing signaling complex, which includes specific initiator procaspases that, when activated, launch a caspase cascade that leads to cell death (**Figure 18–40**).

Animal Cells Require Extracellular Signals to Survive, Grow, and Divide

In a multicellular organism, the fate of individual cells is controlled by signals from other cells. For either tissue growth or cell replacement, cells must grow before they divide. Nutrients are not enough for an animal cell to survive, grow, or divide. It must also receive chemical signals from other cells, usually its neighbors. Such controls ensure that a cell survives only when it is needed and divides only when another cell is required, either to allow tissue growth or to replace cell loss.

Most of the extracellular signal molecules that influence cell survival, cell growth, and cell division are either soluble proteins secreted by other cells or proteins that are bound to the surface of other cells or to the

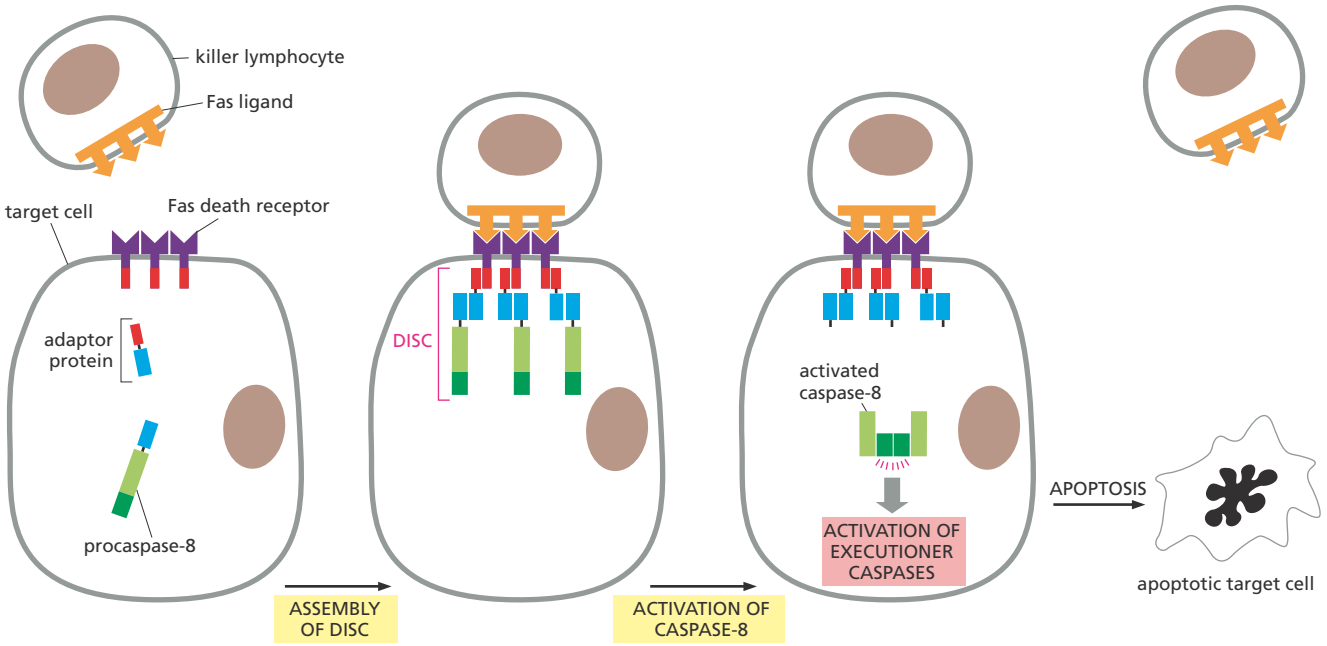


Figure 18–40 Activated death receptors initiate an intracellular signaling pathway that leads to apoptosis. Fas ligand on the surface of a killer lymphocyte activates Fas receptors on the surface of a target cell. This triggers the assembly of a collection of intracellular proteins to form a death-inducing signaling complex (DISC), which includes a specific initiator procaspase (procaspase-8 or 10). The procaspases cleave and activate one another, and the resulting active caspases then activate executioner procaspases in the cytosol, leading to a caspase proteolytic cascade and apoptosis.

extracellular matrix. Although most act positively to stimulate one or more of these cell processes, some act negatively to inhibit a particular process. The positively acting signal proteins can be classified, on the basis of their function, into three major categories:

1. **Survival factors** promote cell survival, largely by suppressing apoptosis.
2. **Mitogens** stimulate cell division, primarily by overcoming the intracellular braking mechanisms that tend to block progression through the cell cycle.
3. **Growth factors** stimulate cell growth (an increase in cell size and mass) by promoting the synthesis and inhibiting the degradation of proteins and other macromolecules.

These categories are not mutually exclusive, as many signal molecules have more than one of these functions. Unfortunately, the term "growth factor" is often used as a catch-all phrase to describe a protein with any of these functions. Indeed, the phrase "cell growth" is frequently used inappropriately to mean an increase in cell number, which is more correctly termed "cell proliferation."

In the following three sections, we examine each of these types of signal molecules in turn.

Survival Factors Suppress Apoptosis

Animal cells need signals from other cells just to survive. If deprived of such survival factors, cells activate a caspase-dependent intracellular suicide program and die by apoptosis. This requirement for signals from other cells helps ensure that cells survive only when and where they are needed. Many types of nerve cells, for example, are produced in excess in the developing nervous system and then compete for limited amounts of survival factors that are secreted by the target cells they contact. Those nerve cells that receive enough survival factor live, while the others die by apoptosis. In this way, the number of surviving nerve cells is automatically adjusted to match the number of cells with which they connect (**Figure 18–41**). A similar dependence on survival signals from

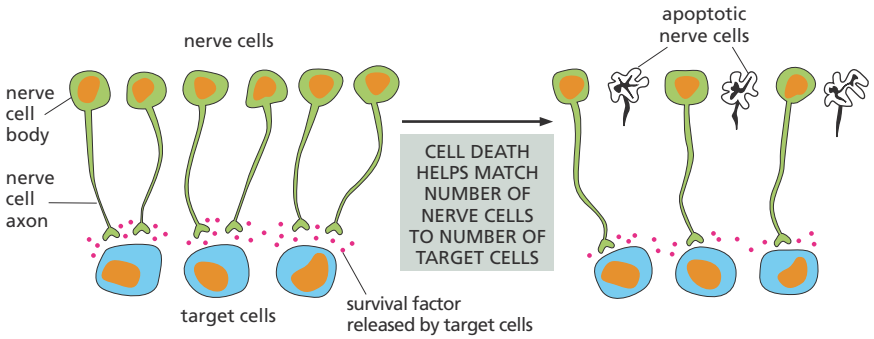


Figure 18–41 Cell death can help adjust the number of developing nerve cells to the number of target cells they contact. If more nerve cells are produced than can be supported by the limited amount of survival factor released by the target cells, some cells will receive insufficient amounts of survival factor to keep their suicide program suppressed and will undergo apoptosis. This strategy of overproduction followed by culling can help ensure that all target cells are contacted by nerve cells and that the "extra" nerve cells are automatically eliminated.

neighboring cells is thought to help control cell numbers in other tissues, both during development and in adulthood.

Survival factors usually act by activating cell-surface receptors. Once activated, the receptors turn on intracellular signaling pathways that keep the apoptotic death program suppressed, usually by regulating members of the Bcl2 family of proteins. Some survival factors, for example, increase the production of Bcl2, a protein that suppresses apoptosis (**Figure 18–42**).

Mitogens Stimulate Cell Division by Promoting Entry into S Phase

Most mitogens are secreted signal proteins that bind to cell-surface receptors. When activated by mitogen binding, these receptors initiate various intracellular signaling pathways (discussed in Chapter 16) that stimulate cell division. As we saw earlier, these signaling pathways act mainly by releasing the molecular brakes that block the transition from the G_1 phase of the cell cycle into S phase (see Figure 18–14).

Most mitogens have been identified and characterized by their effects on cells in culture. One of the first mitogens identified in this way was *platelet-derived growth factor*, or *PDGF*, the effects of which are typical of many others discovered since. When blood clots form (in a wound, for example), blood platelets incorporated in the clots are stimulated to release PDGF. PDGF then binds to receptor tyrosine kinases (discussed in Chapter 16) in surviving cells at the wound site, stimulating the cells to proliferate and help heal the wound. Similarly, if part of the liver is lost through surgery or acute injury, a mitogen called *hepatocyte growth factor* helps stimulate the surviving liver cells to proliferate.

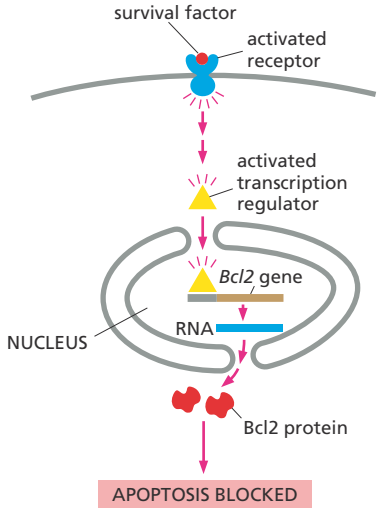


Figure 18–42 Survival factors often suppress apoptosis by regulating Bcl2 family members. In this case, the survival factor binds to cell-surface receptors that activate an intracellular signaling pathway, which in turn activates a transcription regulator in the cytosol. This protein moves to the nucleus, where it activates the gene encoding Bcl2, a protein that inhibits apoptosis.

Growth Factors Stimulate Cells to Grow

The growth of an organism or organ depends on cell growth as much as on cell division. If cells divided without growing, they would get progressively smaller, and there would be no increase in total cell mass. In single-celled organisms such as yeasts, both cell growth and cell division require only nutrients. In animals, by contrast, both cell growth and cell division depend on signals from other cells. Cell growth, unlike cell division, does not depend on the cell-cycle control system. Indeed, many

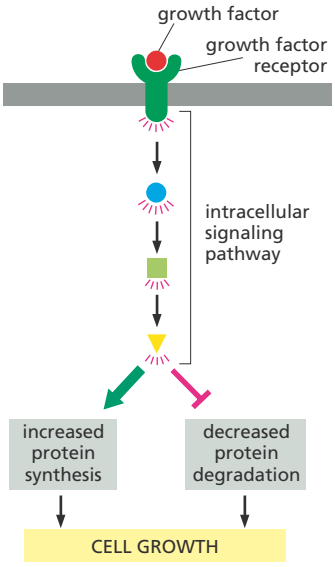


Figure 18–43 Extracellular growth factors increase the synthesis and decrease the degradation of macromolecules. This change leads to a net increase in macromolecules and thereby cell growth (see also Figure 16–39).

animal cells, including nerve cells and most muscle cells, do most of their growing after they have terminally differentiated and permanently stopped dividing.

Like most survival factors and mitogens, most extracellular growth factors bind to cell-surface receptors that activate intracellular signaling pathways. These pathways lead to the accumulation of proteins and other macromolecules. Growth factors both increase the rate of synthesis of these molecules and decrease their rate of degradation (**Figure 18–43**).

Some extracellular signal proteins, including PDGF, can act as both growth factors and mitogens, stimulating both cell growth and progression through the cell cycle. Such proteins help ensure that cells maintain their appropriate size as they proliferate.

Compared to cell division, there has been surprisingly little study of how cell size is controlled in animals. As a result, it remains a mystery how different cell types in the same animal grow to be so different in size (**Figure 18–44**).

Some Extracellular Signal Proteins Inhibit Cell Survival, Division, or Growth

The extracellular signal proteins that we have discussed so far—survival factors, mitogens, and growth factors—act positively to increase the size of organs and organisms. Some extracellular signal proteins, however, act to oppose these positive regulators and thereby inhibit tissue growth. *Myostatin*, for example, is a secreted signal protein that normally inhibits the growth and proliferation of the precursor cells (myoblasts) that fuse to form skeletal muscle cells during mammalian development. When the gene that encodes myostatin is deleted in mice, their muscles grow to be several times larger than normal, because both the number and the size of muscle cells is increased. Remarkably, two breeds of cattle that were bred for large muscles turned out to have mutations in the gene encoding myostatin (**Figure 18–45**).

Cancers are similarly the products of mutations that set cells free from the normal "social" controls operating on cell survival, growth, and proliferation. Because cancer cells are generally less dependent than normal cells on signals from other cells, they can out-survive, outgrow, and outdivide their normal neighbors, producing tumors that can kill their host (see Chapter 20).

In our discussions of cell division, we have thus far focused entirely on the ordinary divisions that produce two daughter cells, each with a full and identical complement of the parent cell's genetic material. There is, however, a different and highly specialized type of cell division called

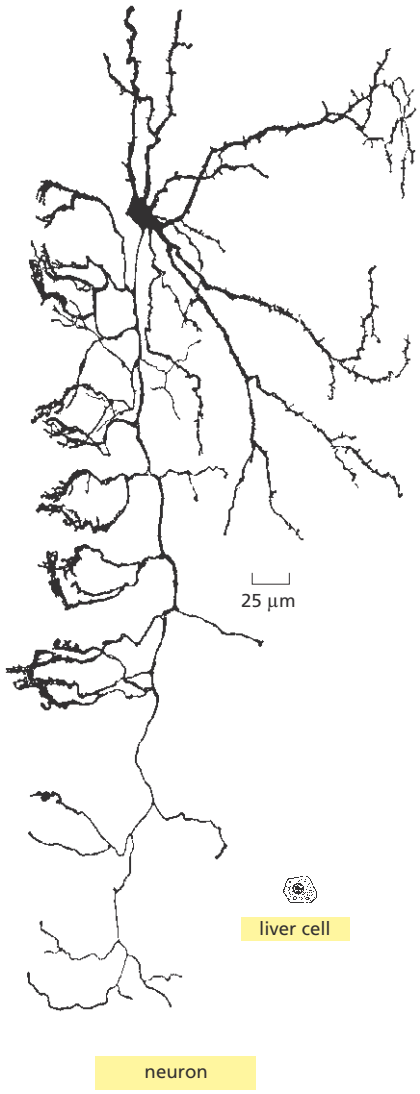


Figure 18–44 The cells in an animal can differ greatly in size. The neuron and liver cell shown here are drawn at the same scale and both contain the same amount of DNA. A neuron grows progressively larger after it has terminally differentiated and permanently stopped dividing. During this time, the ratio of cytoplasm to DNA increases enormously—by a factor of more than 10^5 for some neurons. (Neuron adapted from S. Ramón y Cajal, Histologie du Système Nerveux de l'Homme et de Vertébrés, 1909–1911. Paris: Maloine; reprinted, Madrid: C.S.I.C., 1972.)

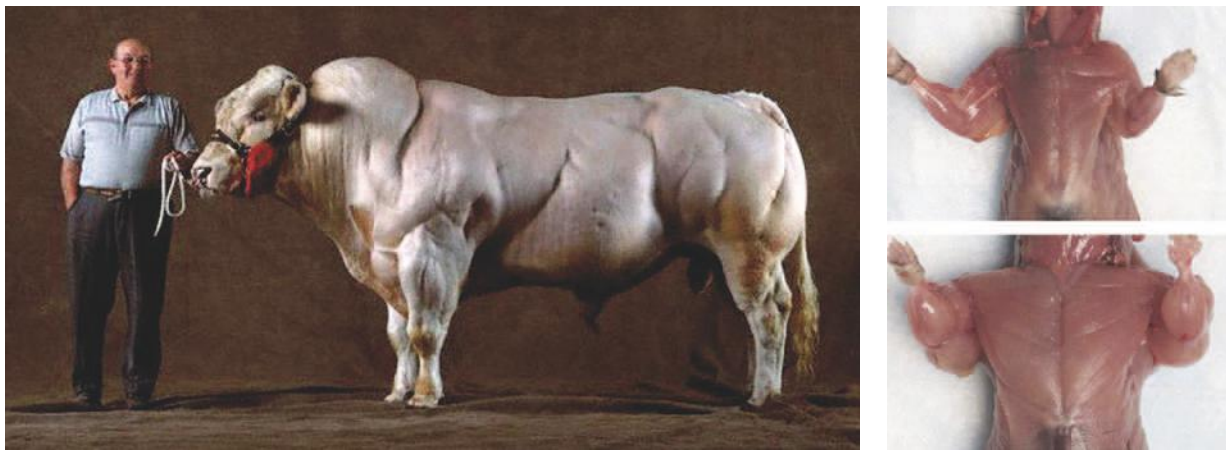


Figure 18–45 Mutation of the *myostatin* gene leads to a dramatic increase in muscle mass. (A) This Belgian Blue was produced by cattle breeders and was only later found to have a mutation in the *myostatin* gene. (B) Mice purposely made deficient in the same gene also have remarkably big muscles. A normal mouse is shown at the top for comparison with the muscular mutant shown at the bottom. (A, from H.L. Sweeney, *Sci. Am.* 291:62–69, 2004. With permission from Scientific American. B, from S.-J. Lee, *PLoS ONE* 2:e789, 2007.)

meiosis, which is required for sexual reproduction in eukaryotes. In the next chapter, we describe the special features of meiosis and how they underlie the genetic principles that define the laws of inheritance.

ESSENTIAL CONCEPTS

- The eukaryotic cell cycle consists of several distinct phases. In interphase, the cell grows and the nuclear DNA is replicated; in M phase, the nucleus divides (mitosis) followed by the cytoplasm (cytokinesis).
- In most cells, interphase consists of an S phase when DNA is duplicated, plus two gap phases—G_1 and G_2. These gap phases give proliferating cells more time to grow and prepare for S phase and M phase.
- The cell-cycle control system coordinates events of the cell cycle by sequentially and cyclically switching on and off the appropriate parts of the cell-cycle machinery.
- The cell-cycle control system depends on cyclin-dependent protein kinases (Cdks), which are cyclically activated by the binding of cyclin proteins and by phosphorylation and dephosphorylation; when activated, Cdks phosphorylate key proteins in the cell.
- Different cyclin–Cdk complexes trigger different steps of the cell cycle: M-Cdk drives the cell into mitosis; G_1-Cdk drives it through G_1; G_1/S-Cdk and S-Cdk drive it into S phase.
- The control system also uses protein complexes, such as APC, to trigger the destruction of specific cell-cycle regulators at particular stages of the cycle.
- The cell-cycle control system can halt the cycle at specific transition points to ensure that intracellular and extracellular conditions are favorable and that each step is completed before the next is started. Some of these control mechanisms rely on Cdk inhibitors that block the activity of one or more cyclin–Cdk complexes.
- S-Cdk initiates DNA replication during S phase and helps ensure that the genome is copied only once. The cell-cycle control system can delay cell-cycle progression during G_1 or S phase to prevent cells from replicating damaged DNA. It can also delay the start of M phase to ensure that DNA replication is complete.

- Centrosomes duplicate during S phase and separate during G_2. Some of the microtubules that grow out of the duplicated centrosomes interact to form the mitotic spindle.
- When the nuclear envelope breaks down, the spindle microtubules capture the duplicated chromosomes and pull them in opposite directions, positioning the chromosomes at the equator of the metaphase spindle.
- The sudden separation of sister chromatids at anaphase allows the chromosomes to be pulled to opposite poles; this movement is driven by the depolymerization of spindle microtubules and by microtubule-associated motor proteins.
- A nuclear envelope re-forms around the two sets of segregated chromosomes to form two new nuclei, thereby completing mitosis.
- In animal cells, cytokinesis is mediated by a contractile ring of actin filaments and myosin filaments, which assembles midway between the spindle poles; in plant cells, by contrast, a new cell wall forms inside the parent cell to divide the cytoplasm in two.
- In animals, extracellular signals regulate cell numbers by controlling cell survival, cell growth, and cell proliferation.
- Most animal cells require survival signals from other cells to avoid apoptosis—a form of cell suicide mediated by a proteolytic caspase cascade; this strategy helps ensure that cells survive only when and where they are needed.
- Animal cells proliferate only if stimulated by extracellular mitogens produced by other cells; mitogens release the normal intracellular brakes that block progression from G_1 or G_0 into S phase.
- For an organism or an organ to grow, cells must grow as well as divide; animal cell growth depends on extracellular growth factors that stimulate protein synthesis and inhibit protein degradation.
- Some extracellular signal molecules inhibit rather than promote cell survival, cell growth, or cell division.
- Cancer cells fail to obey these normal "social" controls on cell behavior and therefore outgrow, out-divide, and out-survive their normal neighbors.

KEY TERMS

anaphase
anaphase-promoting complex (APC)
apoptosis
aster
Bcl2 family
bi-orientation
caspase
Cdk (cyclin-dependent protein kinase)
Cdk inhibitor protein
cell cycle
cell-cycle control system
centrosome
centrosome cycle
chromosome condensation
cohesin
condensin
contractile ring
cyclin
cytokinesis
G_1-Cdk
G_1 cyclin
G_1 phase
G_2 phase
G_1/S-Cdk
G_1/S cyclin
growth factor
interphase
kinetochore
M-Cdk
M cyclin
M phase
metaphase
mitogen
mitosis
mitotic spindle
p53
phragmoplast
programmed cell death
prometaphase
prophase
S-Cdk
S cyclin
S phase
sister chromatid
spindle pole
survival factor
telophase

QUESTIONS

QUESTION 18–11

Roughly, how long would it take a single fertilized human egg to make a cluster of cells weighing 70 kg by repeated divisions, if each cell weighs 1 nanogram just after cell division and each cell cycle takes 24 hours? Why does it take very much longer than this to make a 70-kg adult human?

QUESTION 18–12

The shortest eukaryotic cell cycles of all—shorter even than those of many bacteria—occur in many early animal embryos. These so-called cleavage divisions take place without any significant increase in the weight of the embryo. How can this be? Which phase of the cell cycle would you expect to be most reduced?

QUESTION 18–13

One important biological effect of a large dose of ionizing radiation is to halt cell division.

A. How does this occur?

B. What happens if a cell has a mutation that prevents it from halting cell division after being irradiated?

C. What might be the effects of such a mutation if the cell is not irradiated?

D. An adult human who has reached maturity will die within a few days of receiving a radiation dose large enough to stop cell division. What does that tell you (other than that one should avoid large doses of radiation)?

QUESTION 18–14

If cells are grown in a culture medium containing radioactive thymidine, the thymidine will be covalently incorporated into the cell's DNA during S phase. The radioactive DNA can be detected in the nuclei of individual cells by autoradiography (i.e., by placing a photographic emulsion over the cells, radioactive cells will activate the emulsion and be labeled by black dots when looked at under a microscope). Consider a simple experiment in which cells are radioactively labeled by this method for only a short period (about 30 minutes). The radioactive thymidine medium is then replaced with one containing unlabeled thymidine, and the cells are grown for some additional time. At different time points after replacement of the medium, cells are examined in a microscope. The fraction of cells in mitosis (which can be easily recognized because the cells have rounded up and their chromosomes are condensed) that have radioactive DNA in their nuclei is then determined and plotted as a function of time after the labeling with radioactive thymidine (**Figure Q18–14**).

A. Would all cells (including cells at all phases of the cell cycle) be expected to contain radioactive DNA after the labeling procedure?

B. Initially there are no mitotic cells that contain radioactive DNA (see Figure Q18–14). Why is this?

C. Explain the rise and fall and then rise again of the curve.

D. Estimate the length of the G_2 phase from this graph.

QUESTION 18–15

One of the functions of M-Cdk is to cause a precipitous drop in M-cyclin concentration halfway through M phase. Describe the consequences of this sudden decrease and suggest possible mechanisms by which it might occur.

QUESTION 18–16

Figure 18–5 shows the rise of cyclin concentration and the rise of M-Cdk activity in cells as they progress through the cell cycle. It is remarkable that the cyclin concentration rises slowly and steadily, whereas M-Cdk activity increases suddenly. How do you think this difference arises?

QUESTION 18–17

What is the order in which the following events occur during cell division:

A. anaphase

B. metaphase

C. prometaphase

D. telophase

E. lunar phase

F. mitosis

G. prophase

Where does cytokinesis fit in?

QUESTION 18–18

The lifetime of a microtubule in a mammalian cell, between its formation by polymerization and its spontaneous disappearance by depolymerization, varies with the stage of the cell cycle. For an actively proliferating cell, the average lifetime is 5 minutes in interphase and 15 seconds in mitosis. If the average length of a microtubule in interphase is 20 μm, how long will it be during mitosis, assuming that the rates of microtubule elongation due to the addition of tubulin subunits in the two phases are the same?

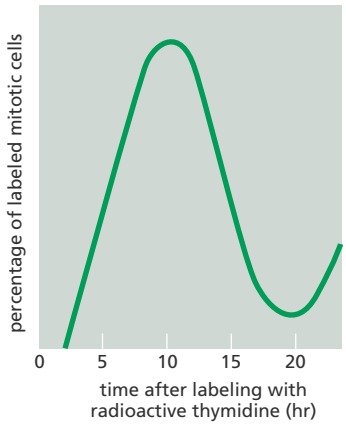


Figure Q18–14

QUESTION 18–19

The balance between plus-end-directed and minus-end-directed motor proteins that bind to interpolar microtubules in the overlap region of the mitotic spindle is thought to help determine the length of the spindle. How might each type of motor protein contribute to the determination of spindle length?

QUESTION 18–20

Sketch the principal stages of mitosis, using Panel 18–1 (pp. 622–623) as a guide. Color one sister chromatid and follow it through mitosis and cytokinesis. What event commits this chromatid to a particular daughter cell? Once initially committed, can its fate be reversed? What may influence this commitment?

QUESTION 18–21

The polar movement of chromosomes during anaphase A is associated with microtubule shortening. In particular, microtubules depolymerize at the ends at which they are attached to the kinetochores. Sketch a model that explains how a microtubule can shorten and generate force yet remain firmly attached to the chromosome.

QUESTION 18–22

Rarely, both sister chromatids of a replicated chromosome end up in one daughter cell. How might this happen? What could be the consequences of such a mitotic error?

QUESTION 18–23

Which of the following statements are correct? Explain your answers.

A. Centrosomes are replicated before M phase begins.

B. Two sister chromatids arise by replication of the DNA of the same chromosome and remain paired as they line up on the metaphase plate.

C. Interpolar microtubules attach end-to-end and are therefore continuous from one spindle pole to the other.

D. Microtubule polymerization and depolymerization and microtubule motor proteins are all required for DNA replication.

E. Microtubules nucleate at the centromeres and then connect to the kinetochores, which are structures at the centrosome regions of chromosomes.

QUESTION 18–24

An antibody that binds to myosin prevents the movement of myosin molecules along actin filaments (the interaction between actin and myosin is described in Chapter 17). How do you suppose the antibody exerts this effect? What might be the result of injecting this antibody into cells (A) on the movement of chromosomes at anaphase or (B) on cytokinesis? Explain your answers.

QUESTION 18–25

Look carefully at the electron micrographs in Figure 18–37. Describe the differences between the cell that died by necrosis and those that died by apoptosis. How do the pictures confirm the differences between the two processes? Explain your answer.

QUESTION 18–26

Which of the following statements are correct? Explain your answers.

A. Cells do not pass from G_1 into M phase of the cell cycle unless there are sufficient nutrients to complete an entire cell cycle.

B. Apoptosis is mediated by special intracellular proteases, one of which cleaves nuclear lamins.

C. Developing neurons compete for limited amounts of survival factors.

D. Some vertebrate cell-cycle control proteins function when expressed in yeast cells.

E. The enzymatic activity of a Cdk protein is determined both by the presence of a bound cyclin and by the phosphorylation state of the Cdk.

QUESTION 18–27

Compare the rules of cell behavior in an animal with the rules that govern human behavior in society. What would happen to an animal if its cells behaved as people normally behave in our society? Could the rules that govern cell behavior be applied to human societies?

QUESTION 18–28

In his highly classified research laboratory, Dr. Lawrence M. is charged with the task of developing a strain of dog-sized rats to be deployed behind enemy lines. In your opinion, which of the following strategies should Dr. M. pursue to increase the size of rats?

A. Block all apoptosis.

B. Block p53 function.

C. Overproduce growth factors, mitogens, or survival factors.

D. Obtain a taxi driver's license and switch careers.

Explain the likely consequences of each option.

QUESTION 18–29

PDGF is encoded by a gene that can cause cancer when expressed inappropriately. Why do cancers not arise at wounds in which PDGF is released from platelets?

QUESTION 18–30

What do you suppose happens in mutant cells that

A. cannot degrade M-cyclin?

B. always express high levels of p21?

C. cannot phosphorylate Rb?

QUESTION 18–31

Liver cells proliferate excessively both in patients with chronic alcoholism and in patients with liver cancer. What are the differences in the mechanisms by which cell proliferation is induced in these diseases?