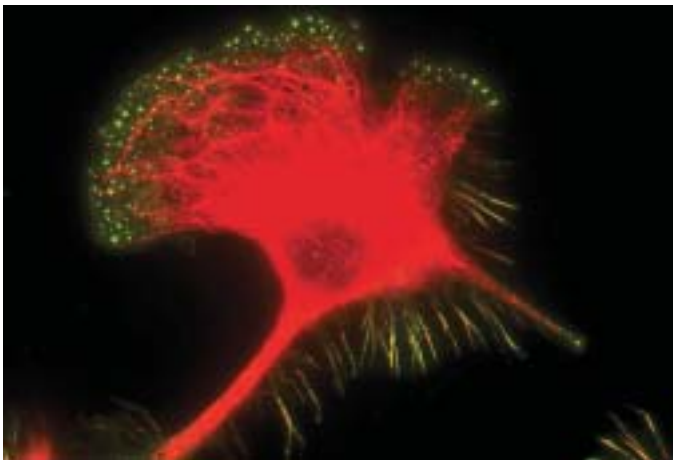
19

MICROFILAMENTS AND INTERMEDIATE FILAMENTS



The macrophage cytoskeleton. Prominent structures include a network of intermediate filaments (red) and the punctate distributions of cell adhesions (yellow) containing both actin and vimentin. [Courtesy of J. Evans.]

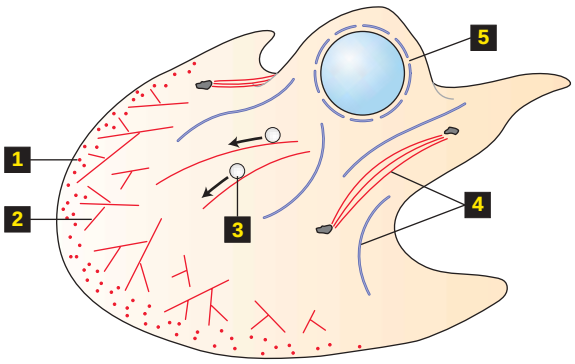
The ability of cells to migrate is one of the crowning achievements of evolution. Primitive cells probably lacked such self-generated movement (motility), depending on currents in the primordial milieu to move them about. In multicellular organisms, however, the migration of single cells and groups of cells from one part of an embryo to another is critical to the development of the organism. In adult animals, single cells search out foreign invaders as part of a host's defenses against infection; on the other hand, uncontrolled cell migration is an ominous sign of a cancerous cell. Some bacterial cells can move by the beating of flagella powered by a rotary motor in the cell membrane (see Figure 3-22b). Motile eukaryotic cells, however, use different mechanisms to generate movement.

Even stationary cells, which predominate in the body, may exhibit dramatic changes in their morphology—the contraction of muscle cells, the elongation of nerve axons, the formation of cell-surface protrusions, the constriction of a dividing cell in mitosis. Even more subtle than these movements are those that take place within cells—the active separation of chromosomes, the streaming of cytosol, the transport of membrane vesicles. These internal movements are essential elements in the growth and differentiation of cells, carefully controlled by the cell to take place at specified times and in particular locations.

The **cytoskeleton,** a cytoplasmic system of fibers, is critical to cell motility. In Chapter 5, we introduced the three types of cytoskeletal fibers—**microfilaments, intermediate filaments,** and **microtubules**—and considered their roles in supporting cell membranes and organizing the cell contents (see Figure 5-29). All these fibers are polymers built from small protein subunits held together by noncovalent bonds. Instead of being a disordered array, the cytoskeleton is organized into discrete structures—primarily bundles, geodesic-dome-like networks, and gel-like lattices. In this chapter, we extend our earlier consideration of actin microfilaments and intermediate filaments (Figure 19-1). Both of these cytoskeletal components are usually attached to plasma membrane proteins and form a skeleton that helps support the plasma membrane. However, actin filaments participate in several types of cell movements, whereas intermediate filaments are not directly engaged in cell movements.

All cell movements are a manifestation of mechanical work; they require a fuel (ATP) and proteins that convert the energy stored in ATP into motion. Cells have evolved two basic mechanisms for generating movement. One mechanism entails the assembly and disassembly of microfilaments and microtubules; it is responsible for many changes in cell shape. The other mechanism requires a special class of enzymes called **motor proteins,** first described in Chapter 3. These proteins use energy from ATP to walk or slide along a microfilament or a microtubule and ferry organelles and vesicles with them. A few movements require both the action of motor proteins and cytoskeleton rearrangements. In this chapter, we also cover **myosin,** the motor protein that interacts with actin, building on our earlier description of the

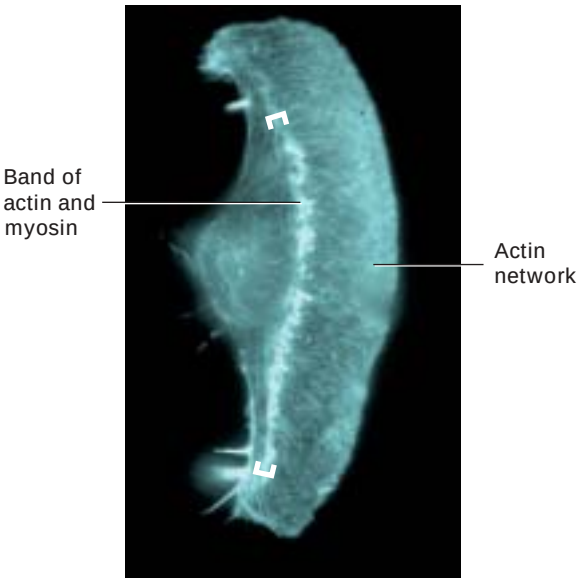
OUTLINE



	CYTOSKELETAL COMPONENT	CELL FUNCTION
1	Actin dynamics	Membrane extension
2	Filament networks: bundles	Cell structure
3	Myosin motors	Contractility and vesicle transport
4	Actin bundles and intermediate filaments	Cell adhesion
5	Lamin network	Nuclear structure

▲ FIGURE 19-1 **Overview of the actin and intermediate filament cytoskeletons and their functions.** The actin cytoskeletal machinery (red) is responsible for maintaining cell shape and generating force for movements. Polymerization and depolymerization of actin filaments (1) drives the membrane forward, whereas actin cross-linking proteins organize bundles and networks of filaments (2) that support overall cell shape. Movements within the cell and contractions at the cell membrane (3) are produced by myosin motor proteins. The actin (red) and intermediate filament (purple) cytoskeletons integrate a cell and its contents with other cells in tissues (4) through attachments to cell adhesions. Another type of intermediate filament, the nuclear lamins (5) are responsible for maintaining the structure of the nucleus.

MEDIA CONNECTIONS
Video: Actin Filaments in a Lamellipodium of a Fish Keratinocyte



◀ FIGURE 19-2 **Actin cytoskeleton in a moving cell.** Fish keratinocytes are among the fastest crawling cells. Two actin-containing structures work together to generate the force for movement. A network of actin filaments in the front of the cell pushes the membrane forward. Meanwhile, the cell body is pulled by a band of myosin and actin (bracketed). This arrangement of actin and myosin is typical in a moving cell. [From T. M. Svitkina et al., 1997, *J. Cell Biol.* **139**:397.]

structure of myosin II and its role in muscle contraction. Myosin II belongs to a large family of proteins found in both animals and plants. The functions of three myosins (I, II, and V) are well established, but the activities of the others are still largely unknown. A discussion of microtubules, the third type of cytoskeletal fiber, and their motor proteins is deferred until Chapter 20.

19.1 Actin Structures

As we saw in Chapter 5, the *actin cytoskeleton* is organized into various large structures that extend throughout the cell. Because it is so big, the actin cytoskeleton can easily change cell morphology just by assembling or disassembling itself. In preceding chapters, we have seen examples of large protein complexes in which the number and positions of the subunits are fixed. For example, all ribosomes have the same number of protein and RNA components, and their three-dimensional geometry is invariant. However, the actin cytoskeleton is different—the lengths of filaments vary greatly, the filaments are cross-linked into imperfect bundles and networks, and the ratio of cytoskeletal proteins is not rigidly maintained. This organizational flexibility of the actin cytoskeleton permits a cell to assume many shapes and to vary them easily. In moving cells, the cytoskeleton must assemble rapidly and does not always have a chance to form well-organized, highly ordered structures.

In this section, we consider the properties of monomeric and polymeric actin, as well as the various proteins that assemble actin filaments into large structures. With this basic understanding of the actin cytoskeleton established, we examine in Section 19.2 how a cell can tailor this framework to carry out various tasks requiring motion of the entire cell or subcellular parts.

Actin Is Ancient, Abundant, and Highly Conserved

Actin is the most abundant intracellular protein in most eukaryotic cells. In muscle cells, for example, actin comprises 10 percent by weight of the total cell protein; even in nonmuscle cells, actin makes up 1–5 percent of the cellular protein. The cytosolic concentration of actin in nonmuscle cells ranges from 0.1 to 0.5 mM; in special structures such as microvilli, however, the local actin concentration can be 5 mM.

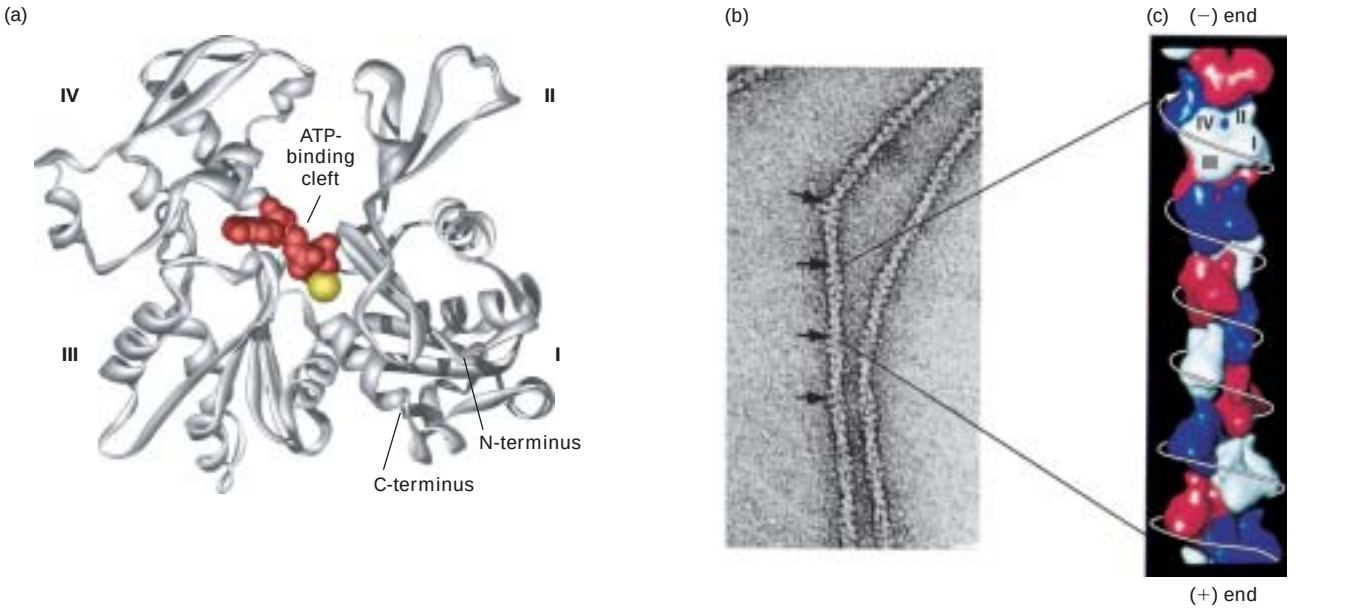
To grasp how much actin cells contain, consider a typical liver cell, which has 2×10^4 insulin receptor molecules but approximately 5×10^8, or half a billion, actin molecules. The high concentration of actin compared with other cell proteins is a common feature of all cytoskeletal proteins. Because they form structures that cover large parts of the cell interior, these proteins are among the most abundant proteins in a cell.

A moderate-sized protein with a molecular weight of 42,000, actin is encoded by a large, highly conserved gene family. Actin arose from a bacterial ancestor and then evolved further as eukaryotic cells became specialized. Some single-celled organisms such as rod-shaped bacteria, yeasts, and amebas have one or two actin genes, whereas many multicellular organisms contain multiple actin genes. For instance, humans have six actin genes, which encode **isoforms** of the protein, and some plants have more than 60 actin genes, although most are **pseudogenes.** In vertebrates, the four α-actin isoforms present in various muscle cells and the β-actin and γ-actin isoforms present in nonmuscle cells differ at only four or five positions. Although these differences among isoforms seem minor, the isoforms have different functions: α-actin is associated with contractile structures; γ-actin accounts for filaments in stress fibers; and β-actin is at the front, or *leading edge*, of moving cells where actin filaments polymerize (Figure 19-2). Sequencing of actins from different sources has revealed that they are among the most conserved proteins in a cell, comparable with histones, the structural proteins of chromatin (Chapter 10). The sequences of actins from amebas and from animals are identical at 80 percent of the positions.

G-Actin Monomers Assemble into Long, Helical F-Actin Polymers

Actin exists as a globular monomer called *G-actin* and as a filamentous polymer called *F-actin*, which is a linear chain of G-actin subunits. (The microfilaments visualized in a cell by electron microscopy are F-actin filaments plus any bound proteins.) Each actin molecule contains a Mg^{2+} ion complexed with either ATP or ADP. Thus there are four states of actin: ATP–G-actin, ADP–G-actin, ATP–F-actin, and ADP–F-actin. Two of these forms, ATP–G-actin and ADP–F-actin, predominate in a cell. The importance of the interconversion between the ATP and the ADP forms of actin in the assembly of the cytoskeleton is discussed later.

Although G-actin appears globular in the electron microscope, x-ray crystallographic analysis reveals that it is separated into two lobes by a deep cleft (Figure 19-3a). The lobes and the cleft compose the *ATPase fold*, the site where ATP



▲ FIGURE 19-3 **Structures of monomeric G-actin and F-actin filament.** (a) Model of a β-actin monomer from a nonmuscle cell shows it to be a platelike molecule (measuring 5.5 × 5.5 × 3.5 nm) divided by a central cleft into two approximately equally sized lobes and four subdomains, numbered I–IV. ATP (red) binds at the bottom of the cleft and contacts both lobes (the yellow ball represents Mg^{2+}). The N- and C-termini lie in subdomain I. (b) In the electron microscope, negatively stained actin filaments appear as long, flexible, and twisted strands of beaded subunits. Because of the twist, the filament appears alternately thinner (7 nm diameter) and thicker (9 nm diameter) (arrows). (c) In one model of the arrangement of subunits in an actin filament, the subunits lie in a tight helix along the filament, as indicated by the arrow. One repeating unit consists of 28 subunits (13 turns of the helix), covering a distance of 72 nm. Only 14 subunits are shown in the figure. The ATP-binding cleft is oriented in the same direction (*top*) in all actin subunits in the filament. The end of a filament with an exposed binding cleft is designated the (−)end; the opposite end is the (+) end. [Part (a) adapted from C. E. Schutt et al., 1993, *Nature* **365**:810; courtesy of M. Rozycki. Part (b) courtesy of R. Craig. Part (c) see M. F. Schmid et al., 1994, *J. Cell Biol.* **124:**341; courtesy of M. Schmid.]

and Mg^{2+} are bound. In actin, the floor of the cleft acts as a hinge that allows the lobes to flex relative to each other. When ATP or ADP is bound to G-actin, the nucleotide affects the conformation of the molecule. In fact, without a bound nucleotide, G-actin denatures very quickly.

The addition of ions—Mg^{2+}, K^+, or Na^+—to a solution of G-actin will induce the polymerization of G-actin into F-actin filaments. The process is also reversible: F-actin depolymerizes into G-actin when the ionic strength of the solution is lowered. The F-actin filaments that form in vitro are indistinguishable from microfilaments isolated from cells, indicating that other factors such as accessory proteins are not required for polymerization in vivo. The assembly of G-actin into F-actin is accompanied by the hydrolysis of ATP to ADP and P_i; however, as discussed later, ATP hydrolysis affects the kinetics of polymerization but is not necessary for polymerization to take place.

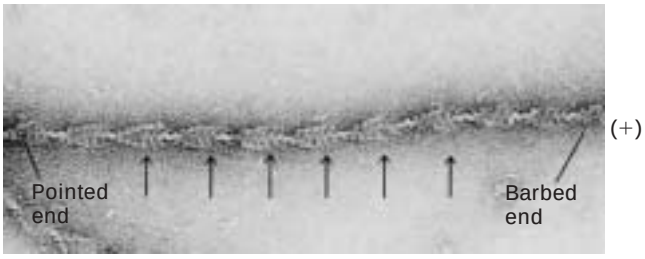
When negatively stained by uranyl acetate for electron microscopy, F-actin appears as twisted strings of beads whose diameter varies between 7 and 9 nm (Figure 19-3b). From the results of x-ray diffraction studies of actin filaments and the actin monomer structure shown in Figure 19-3a, scientists have produced a model of an actin filament in which the subunits are organized as a tightly wound helix (Figure 19-3c). In this arrangement, each subunit is surrounded by four other subunits, one above, one below, and two to one side. Each subunit corresponds to a bead seen in electron micrographs of actin filaments.

The ability of G-actin to polymerize into F-actin and of F-actin to depolymerize into G-actin is an important property of actin. In this chapter, we will see how the reversible assembly of actin lies at the core of many cell movements.

F-Actin Has Structural and Functional Polarity

All subunits in an actin filament point toward the same end of the filament. Consequently, a filament exhibits **polarity;** that is, one end differs from the other. By convention, the end at which the ATP-binding cleft of the terminal actin subunit is exposed to the surrounding solution is designated the (−) end. At the opposite end, the (+) end, the cleft contacts the neighboring actin subunit and is not exposed (see Figure 19-3c).

Without the atomic resolution afforded by x-ray crystallography, the cleft in an actin subunit and therefore the polarity of a filament are not detectable. However, the polarity of actin filaments can be demonstrated by electron microscopy in "decoration" experiments, which exploit the ability of myosin to bind specifically to actin filaments. In this type of experiment, an excess of myosin S1, the globular head domain of myosin, is mixed with actin filaments and binding is permitted to take place. Myosin attaches to the sides of a filament with a slight tilt. When all the actin subunits are bound by myosin, the filament appears coated ("decorated") with arrowheads that all point toward one end of the filament (Figure 19-4). Because myosin binds to actin filaments and not to microtubules or intermediate filaments, arrowhead decoration is one criterion by which actin filaments are identified among the other cytoskeletal fibers in electron micrographs of thin-sectioned cells.



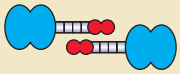
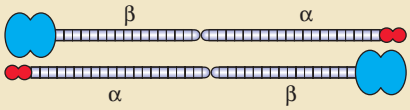
▲ EXPERIMENTAL FIGURE 19-4 **Decoration demonstrates the polarity of an actin filament.** Myosin S1 head domains bind to actin subunits in a particular orientation. When bound to all the subunits in a filament, S1 appears to spiral around the filament. This coating of myosin heads produces a series of arrowhead-like decorations, most easily seen at the wide views of the filament. The polarity in decoration defines a pointed (−) end and a barbed (+) end; the former corresponds to the top of the model in Figure 19-3c. [Courtesy of R. Craig.]

CH-Domain and Other Proteins Organize Microfilaments into Bundles and Networks

Actin filaments can form a tangled network of filaments in vitro. However, *actin cross-linking proteins* are required to assemble filaments into the stable networks and bundles that provide a supportive framework for the plasma membrane (Figure 19-5). Some actin cross-linking proteins (e.g., fimbrin and fascin) are monomeric proteins that contain two actin-binding domains in a single polypeptide chain. But many cross-linking proteins, particularly those that form networks of filaments, consist of two or more polypeptide chains, each of which contains a single actin-binding domain. Multiple actin-binding sites in these proteins are generated by their assembly into dimers or other oligomers. Actin bundles and networks are often stabilized by several different actin cross-linking proteins.

Many actin cross-linking proteins belong to the *calponin homology–domain superfamily* (Table 19-1). Each of these CH-domain proteins has a pair of actin-binding domains whose sequence is homologous to that of calponin, a muscle protein. The actin-binding domains are separated by repeats of helical coiled-coil or β-sheet immunoglobulin motifs. The organization of the actin-binding sites in these proteins determines whether they organize filaments into bundles or networks. When the binding sites are arranged in tandem, as in fimbrin and α-actinin, the bound actin filaments are packed tightly and align into bundles (see Figure 19-5a) in cell adhesions and extensions. However, if binding sites are spaced apart at the ends of flexible arms, as in filamin, spectrin, and dystrophin, then cross-links can form

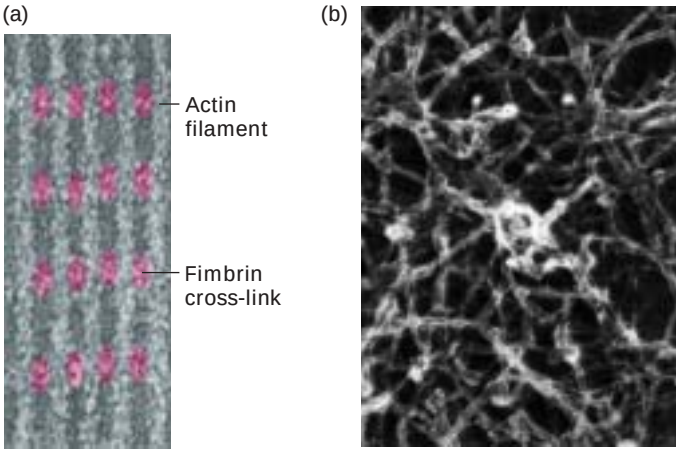
TABLE 19-1 Selected Actin-Binding Proteins

Protein	MW	Domain Organization*	Location
CH-DOMAIN SUPERFAMILY			
Fimbrin	68,000		Microvilli, stereocilia, adhesion plaques, yeast actin cables
α-Actinin	102,000		Filopodia, lamellipodia, stress fibers, adhesion plaques
Spectrin	α: 280,000 β: 246,000–275,000		Cortical networks
Dystrophin	427,000		Muscle cortical networks
Filamin	280,000		Filopodia, pseudopodia, stress fibers
OTHERS			
Fascin	55,000		Filopodia, lamellipodia, stress fibers, microvilli, acrosomal process
Villin	92,000		Microvilli in intestinal and kidney brush border

*Blue = actin-binding domains; red = calmodulin-like Ca^{2+}-binding domains; purple = α-helical repeats; green = β-sheet repeats; orange = other domains.

between orthogonally arranged and loosely packed filaments (see Figure 19-5b). The large networks thus formed fill the cytoplasm and give it a gel-like character. Because these proteins also bind membrane proteins, the networks are generally found in the cortical region adjacent to the plasma membrane. In proteins that form networks of filaments, repeats of different protein motifs determine the length of the arms and thus the spacing and orientation between filaments.

Although CH-domain proteins form the majority of actin cross-linking proteins, other proteins that bind to different sites on actin play equally important roles in organizing actin filaments. One such protein, fascin, is found in many actin bundles including stress fibers, cell-surface microvilli, and the sensory bristles that cover the body of the fruit fly *Drosophila*. The important structural role of fascin is illustrated by the effects of mutations in the *singed* gene, which encodes fascin in *Drosophila*. In *singed* mutants, sensory bristles are bent and deformed, evidence that fascin is responsible for maintaining the rigidity of actin bundles in the core of each bristle.



▲ FIGURE 19-5 **Actin cross-linking proteins bridging pairs of actin filaments.** (a) When cross-linked by fimbrin (red), a short protein, actin filaments pack side by side to form a bundle. (b) Long cross-linking proteins such as filamin are flexible and can thus cross-link actin filaments into a network. [Part (a) courtesy of D. Hanein. Part (b) courtesy of J. Hartwig.]

KEY CONCEPTS OF SECTION 19.1

Actin Structures

- A major component of the cytoskeleton, actin is the most abundant intracellular protein in eukaryotic cells and is highly conserved.
- The various actin isoforms exhibit minor sequence differences but generally perform different functions.
- F-actin is a helical filamentous polymer of globular G-actin subunits all oriented in the same direction (see Figure 19-3).
- Actin filaments are polarized with one end, the (−) end, containing an exposed ATP-binding site.
- Actin filaments are organized into bundles and networks by a variety of bivalent cross-linking proteins (see Figure 19-5).
- Many actin cross-linking proteins belong to the CH-domain superfamily (see Table 19-1). They include the bundle-forming proteins fimbrin and α-actinin and the larger network-forming proteins spectrin, dystrophin, and filamin.

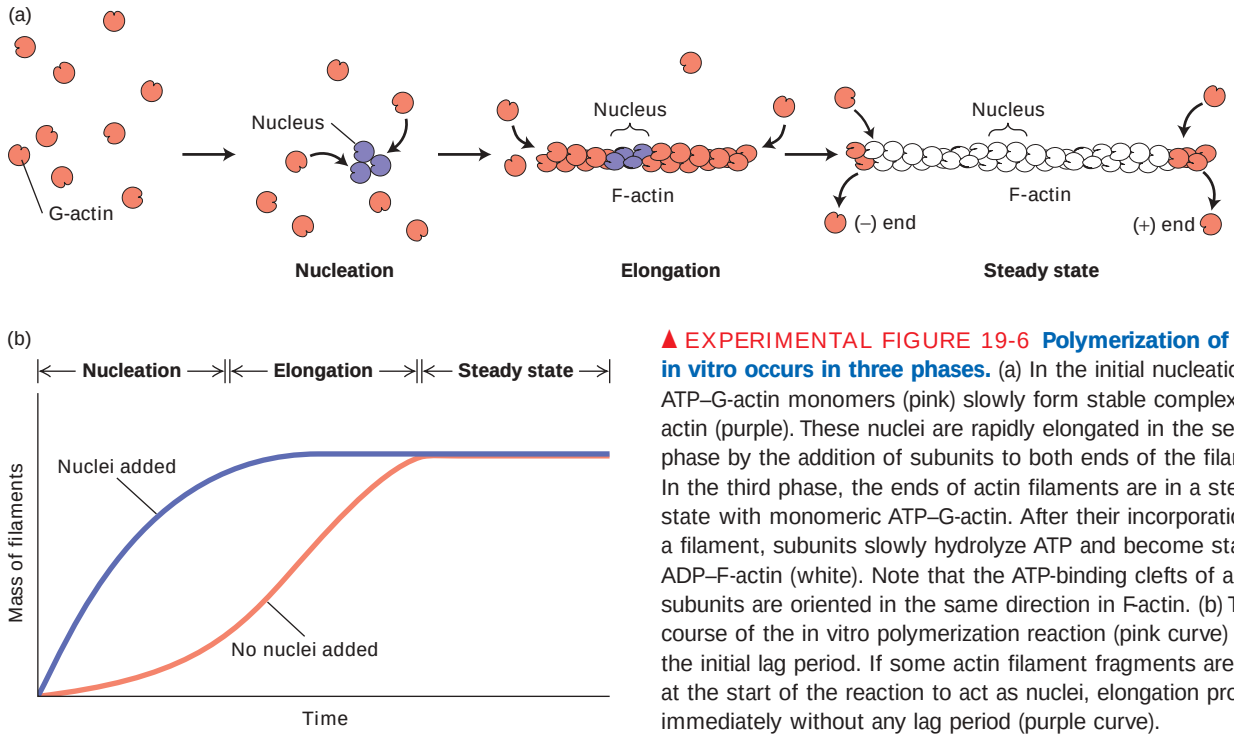
19.2 The Dynamics of Actin Assembly

As mentioned previously, the actin cytoskeleton is not a static, unchanging structure consisting of bundles and networks of filaments. Rather, the microfilaments in a cell are constantly shrinking or growing in length, and bundles and meshworks of microfilaments are continually forming and dissolving. These changes in the organization of actin filaments generate forces that cause equally large changes in the shape of a cell. In this section, we consider the mechanism of actin polymerization and the regulation of this process, which is largely responsible for the dynamic nature of the cytoskeleton.

Actin Polymerization in Vitro Proceeds in Three Steps

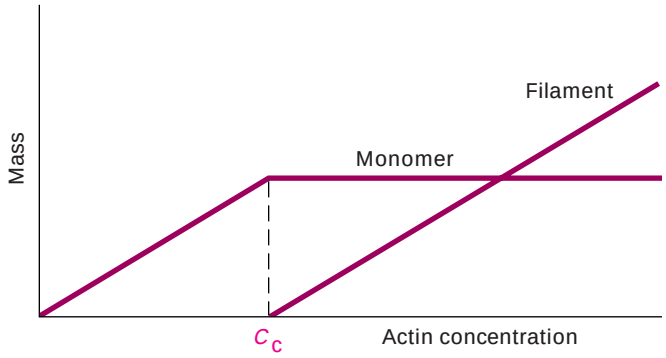
The in vitro polymerization of G-actin to form F-actin filaments can be monitored by viscometry, sedimentation, fluorescence spectroscopy, and fluorescence microscopy. When actin filaments become long enough to become entangled, the viscosity of the solution increases, which is measured as a decrease in its flow rate in a viscometer. The basis of the sedimentation assay is the ability of ultracentrifugation (100,000*g* for 30 minutes) to pellet F-actin but not G-actin. The third assay makes use of G-actin covalently labeled with a fluorescent dye; the fluorescence spectrum of the modified G-actin monomer changes when it is polymerized into F-actin. Finally, growth of the labeled filaments can be imaged with a fluorescence microscope. These assays are useful in kinetic studies of actin polymerization and during purification of actin-binding proteins, which cross-link or depolymerize actin filaments.

The in vitro polymerization of G-actin proceeds in three sequential phases (Figure 19-6a). The first *nucleation phase* is marked by a lag period in which G-actin aggregates into short, unstable oligomers. When the oligomer reaches a cer-



▲ EXPERIMENTAL FIGURE 19-6 **Polymerization of G-actin in vitro occurs in three phases.** (a) In the initial nucleation phase, ATP–G-actin monomers (pink) slowly form stable complexes of actin (purple). These nuclei are rapidly elongated in the second phase by the addition of subunits to both ends of the filament. In the third phase, the ends of actin filaments are in a steady state with monomeric ATP–G-actin. After their incorporation into a filament, subunits slowly hydrolyze ATP and become stable ADP–F-actin (white). Note that the ATP-binding clefts of all the subunits are oriented in the same direction in F-actin. (b) Time course of the in vitro polymerization reaction (pink curve) reveals the initial lag period. If some actin filament fragments are added at the start of the reaction to act as nuclei, elongation proceeds immediately without any lag period (purple curve).

MEDIA CONNECTIONS
Focus Animation: Actin Polymerization



▲ EXPERIMENTAL FIGURE 19-7 **Concentration of G-actin determines filament formation.** The critical concentration (C_c) is the concentration of G-actin monomers in equilibrium with actin filaments. At monomer concentrations below the C_c, no polymerization takes place. At monomer concentrations above the C_c, filaments assemble until the monomer concentration reaches C_c.

tain length (three or four subunits), it can act as a stable seed, or nucleus, which in the second *elongation phase* rapidly increases in length by the addition of actin monomers to both of its ends. As F-actin filaments grow, the concentration of G-actin monomers decreases until equilibrium is reached between filaments and monomers. In this third *steady-state phase*, G-actin monomers exchange with subunits at the filament ends, but there is no net change in the total mass of filaments. The kinetic curves presented in Figure 19-6b show that the lag period can be eliminated by the addition of a small number of F-actin nuclei to the solution of G-actin.

When the steady-state phase has been reached, the concentration of the pool of unassembled subunits is called the *critical concentration*, C_c. This parameter is the dissociation constant, the ratio of the "on" and "off" rate constants, and it measures the concentration of G-actin where the addition of subunits is balanced by the dissociation of subunits; that is, the on rate equals the off rate. Under typical in vitro conditions, the C_c of G-actin is 0.1 μM. Above this value, a solution of G-actin will polymerize; below this value, a solution of F-actin will depolymerize (Figure 19-7).

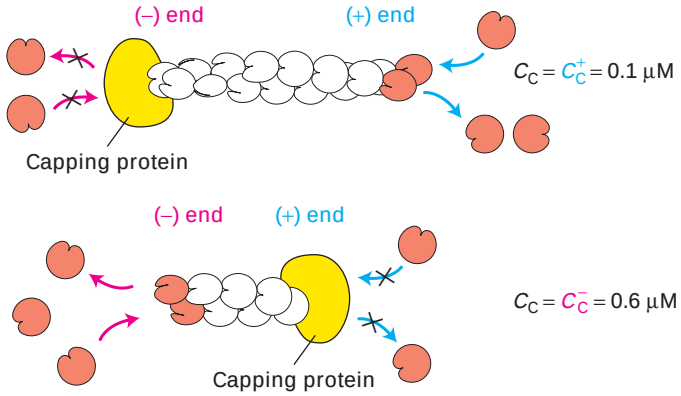
After ATP–G-actin monomers are incorporated into a filament, the bound ATP is slowly hydrolyzed to ADP. As a result of this hydrolysis, most of the filament consists of ADP–F-actin, but some ATP–F-actin is found at one end (see next subsection). However, ATP hydrolysis is not essential for polymerization to take place, as evidenced by the ability of G-actin containing ADP or a nonhydrolyzable ATP analog to polymerize into filaments.

Actin Filaments Grow Faster at (+) End Than at (−) End

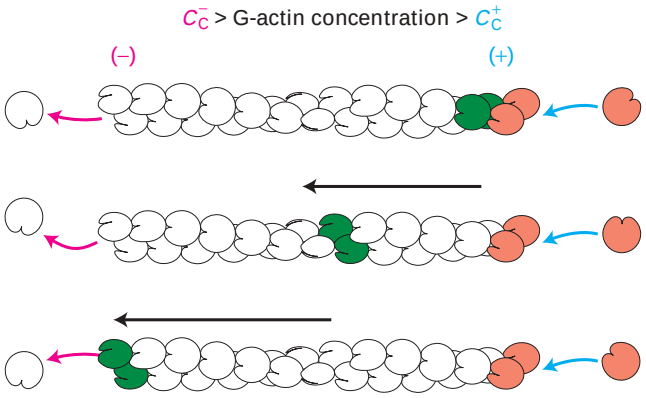
We saw earlier that myosin decoration experiments reveal an inherent structural polarity of F-actin (see Figure 19-4). This polarity is also manifested by the different rates at which ATP-G-actin adds to the two ends. One end of the filament, the (+) end, elongates 5–10 times as fast as does the opposite, or (−), end. The unequal growth rates can be demonstrated by a simple experiment in which myosin-decorated actin filaments nucleate the polymerization of G-actin. Electron microscopy of the elongated filaments reveals bare sections at both ends, corresponding to the added undecorated G-actin. The newly polymerized (undecorated) actin is 5–10 times as long at the (+) end as at the (−) end of the filaments (Figure 19-8a).

(a)

(b)



▲ EXPERIMENTAL FIGURE 19-8 **Myosin decoration and capping proteins demonstrate unequal growth rates at the two ends of an actin filament.** (a) When short myosin-decorated filaments are the nuclei for actin polymerization, the resulting elongated filaments have a much longer undecorated (+) end than (−) end. This result indicates that G-actin monomers are added much faster at the (+) end than at the (−) end. (b) Blocking the (+) or (−) ends of a filament with actin-capping proteins permits growth only at the opposite end. In polymerization assays with capped filaments, the critical concentration (C_c) is determined by the unblocked growing end. Such assays show that the C_c at the (+) end is much lower than the C_c at the (−) end. [Part (a) courtesy of T. Pollard.]



▲ FIGURE 19-9 **Treadmilling of actin filaments.** At G-actin concentrations intermediate between the C_c values for the (−) and (+) ends, actin subunits can flow through the filaments by attaching preferentially to the (+) end and dissociating preferentially from the (−) end of the filament. This treadmilling phenomenon occurs in some moving cells. The oldest subunits in a treadmilling filament lie at the (−) end.

The difference in elongation rates at the opposite ends of an actin filament is caused by a difference in C_c values at the two ends. This difference can be measured by blocking one or the other end with proteins that "cap" the ends of actin filaments. If the (+) end of an actin filament is capped, it can elongate only from its (−) end; conversely, elongation takes place only at the (+) end when the (−) end of a filament is blocked (Figure 19-8b). Polymerization assays of such capped filaments have shown that the C_c is about six times lower for polymerization at the (+) end than for addition at the (−) end.

As a result of the difference in the C_c values for the (+) and (−) ends of a filament, we can make the following predictions: at ATP–G-actin concentrations below C_c^+, there is no filament growth; at G-actin concentrations between C_c^+ and C_c^-, growth is only at the (+) end; and, at G-actin concentrations above C_c^-, there is growth at both ends, although it is faster at the (+) end than at the (−) end. When the steady-state phase is reached at G-actin concentrations intermediate between the C_c values for the (+) and the (−) ends, subunits continue to be added at the (+) end and lost from the (−) end (Figure 19-9). In this situation, the length of the filament remains constant, with the newly added subunits traveling through the filament, as if on a treadmill, until they reach the (−) end, where they dissociate. Turnover of actin filaments at the leading edge of some migrating cells probably occurs by a *treadmilling* type of mechanism, with subunits added to filaments near the leading edge of the cell and lost from the other end toward the rear.

Toxins Perturb the Pool of Actin Monomers

Several toxins shift the usual equilibrium between actin monomers and filaments. Two unrelated toxins, cytochalasin D and latrunculin promote the dissociation of filaments, though by different mechanisms. Cytochalasin D, a fungal alkaloid, depolymerizes actin filaments by binding to the (+) end of F-actin, where it blocks further addition of subunits. Latrunculin, a toxin secreted by sponges, binds G-actin and inhibits it from adding to a filament end. Exposure to either toxin thus increases the monomer pool. When cytochalasin is added to live cells, the actin cytoskeleton disappears and cell movements such as locomotion and cytokinesis are inhibited. These observations were among the first that implicated actin filaments in cell motility.

In contrast, the monomer–polymer equilibrium is shifted in the direction of filaments by jasplakolinode, another sponge toxin, and by phalloidin, which is isolated from *Amanita phalloides* (the "angel of death" mushroom). Phalloidin poisons a cell by binding at the interface between subunits in F-actin, thereby locking adjacent subunits together and preventing actin filaments from depolymerizing. Even when actin is diluted below its critical concentration, phalloidin-stabilized filaments will not depolymerize. Fluorescence-labeled phalloidin, which binds only to F-actin, is commonly used to stain actin filaments for light microscopy.

Actin Polymerization Is Regulated by Proteins That Bind G-Actin

In the artificial world of a test tube, experimenters can start the polymerization process by adding salts to G-actin or can depolymerize F-actin by simply diluting the filaments. Cells, however, must maintain a nearly constant cytosolic ionic concentration and thus employ a different mechanism for controlling actin polymerization. The cellular regulatory mechanism includes several actin-binding proteins that either promote or inhibit actin polymerization. Here, we consider two such proteins that have been isolated and characterized.

Inhibition of Actin Assembly by Thymosin β_4 Calculations based on the C_c of G-actin (0.1 μM), a typical cytosolic total actin concentration (0.5 mM), and the ionic conditions of the cell indicate that nearly all cellular actin should exist as filaments; there should be very little G-actin. Actual measurements, however, show that as much as 40 percent of actin in an animal cell is unpolymerized. What keeps the cellular concentration of G-actin above its C_c? The most likely explanation is that cytosolic proteins sequester actin, holding it in a form that is unable to polymerize.

Because of its abundance in the cytosol and ability to bind ATP–G-actin (but not F-actin), *thymosin β_4* is considered to be the main actin-sequestering protein in cells. A small protein (5000 MW), thymosin binds ATP–G-actin in a 1:1 complex. The binding of thymosin β_4 blocks the ATP-binding site in G-actin, thereby preventing its polymerization. In platelets, the concentration of thymosin β_4 is 0.55 mM, approximately twice the concentration of unpolymerized actin (0.22 mM). At these concentrations, approximately 70 percent of the monomeric actin in a platelet should be sequestered by thymosin β_4.

Thymosin β_4 ($T\beta_4$) functions like a buffer for monomeric actin, as represented in the following reaction:

$$\text{F-actin} \rightleftharpoons \text{G-actin} \rightleftharpoons T\beta_4 \,\Delta\, \text{G-actin}/T\beta_4$$

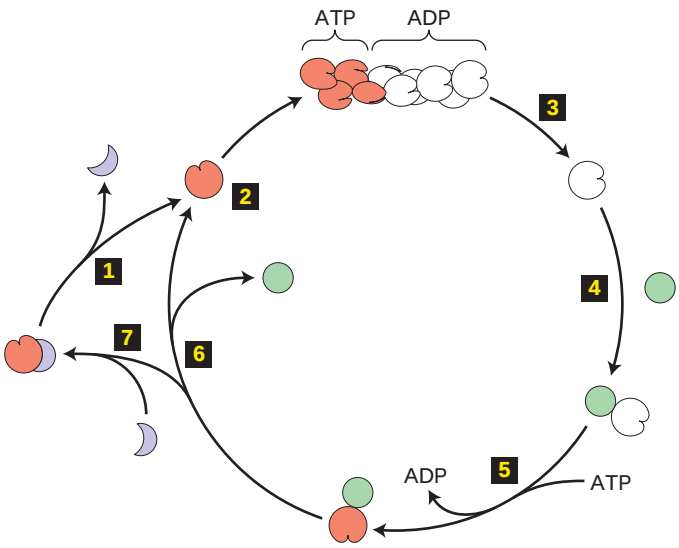
In a simple equilibrium, an increase in the cytosolic concentration of thymosin β_4 would increase the concentration of sequestered actin subunits and correspondingly decrease F-actin, because actin filaments are in equilibrium with actin monomers. This effect of thymosin β_4 on the cellular F-actin level has been experimentally demonstrated in live cells.

Promotion of Actin Assembly by Profilin Another cytosolic protein, *profilin* (15,000 MW), also binds ATP-actin monomers in a stable 1:1 complex. At most, profilin can buffer 20 percent of the unpolymerized actin in cells, a level too low for it to act as an effective sequestering protein. Rather than sequestering actin monomers, the main function of profilin probably is to promote the assembly of actin filaments in cells. It appears to do so by several mechanisms.

First, profilin promotes the assembly of actin filaments by acting as a nucleotide-exchange factor. Profilin is the only actin-binding protein that allows the exchange of ATP for ADP. When G-actin is complexed with other proteins, ATP or ADP is trapped in the ATP-binding cleft of actin. However, because profilin binds to G-actin at a site opposite the ATP-binding cleft, it can recharge ADP-actin monomers released from a filament, thereby replenishing the pool of ATP-actin (Figure 19-10).

Second, as a complex with G-actin, profilin is postulated to assist in the addition of monomers to the (+) end of an actin filament. This hypothesis is consistent with the three-dimensional structure of the profilin–actin complex in which profilin is bound to the part of an actin monomer opposite the ATP-binding end, thereby leaving it free to associate with the (+) end of a filament (see Figure 19-3). After the complex binds transiently to the filament, the profilin dissociates from actin.

Finally, profilin also interacts with membrane components taking part in cell–cell signaling, suggesting that it may be particularly important in controlling actin assembly at the plasma membrane. For example, profilin binds to the membrane phospholipid phosphoinositol 4,5-bisphosphate (PIP_2); this interaction prevents the binding of profilin to G-actin. (As discussed in Chapter 13, PIP_2 is hydrolyzed in response to certain extracellular signals.) In addition, profilin binds to proline-rich sequences that are commonly found in membrane-associated signaling proteins such as Vasp and Mena. This interaction, which does not inhibit the binding of profilin to G-actin, localizes profilin–actin complexes to the membrane.



▲ FIGURE 19-10 **Model of the complementary roles of profilin and thymosin β_4 in regulating polymerization of G-actin.** Actin subunits (pink) complexed with thymosin β4 (purple) dissociate (1)and add to the end of a filament (2). In the filament, ATP is hydrolyzed to ADP, the ADP-associated subunit eventually dissociates from the opposite end of the filament (3), the ADP-G-actin forms a complex with profilin (green) (4), and ATP exchanges with ADP to form ATP-G-actin (5). Profilin delivers actin monomers to the (+) end of actin filaments (6) or thymosin β4 sequesters the ATP-G-actin into a polymerization ready pool of subunits (7).

Filament-Binding Severing Proteins Create New Actin Ends

A second group of proteins, which bind to actin filaments, control the length of actin filaments by breaking them into shorter fragments and generating new filament ends for polymerization (Table 19-2). A valuable clue that led to the discovery of these severing proteins came from studies of amebas. Viscosity measurements and light-microscope observations demonstrated that during ameboid movement the cytosol flows forward in the center of the cell and then turns into a gel when it reaches the front end of the cell. As discussed later, this "sol to gel" transformation depends on the assembly of new actin filaments in the front part of a moving ameba and the disassembly of old actin filaments in the rear part. Because the actin concentration in a cell favors the formation of filaments, the breakdown of existing actin filaments and filament networks requires the assistance of severing proteins such as *gelsolin* and *cofilin*.

Severing proteins are thought to break an actin filament by stabilizing a change in the conformation of the subunit to which it binds; the resulting strain on the intersubunit bonds leads to its breakage. In support of this hypothesis are electron micrographs showing that an actin filament with bound cofilin is severely twisted. After a severing protein breaks a filament at one site, it remains bound at the (+) end of one of the resulting fragments, where it prevents the addition or exchange of actin subunits, an activity called *capping*. The (−) ends of fragments remain uncapped and are rapidly shortened. Thus severing promotes turnover of actin

TABLE 19-2 Some Cytosolic Proteins That Control Actin Polymerization

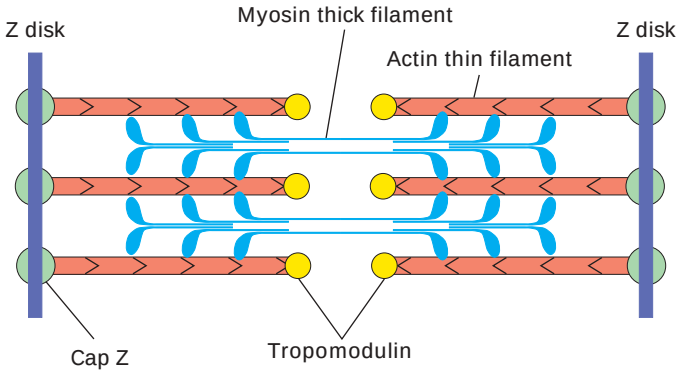
Protein	MW	Activity
Cofilin	15,000	Dissociation from (−) end
Severin	40,000	Severing, capping [(+) end]
Gelsolin	87,000	Severing, capping [(+) end]
CapZ capping protein	36,000 (α) 32,000 (β)	Capping [(+) end]
Tropomodulin	40,000	Capping [(−) end]
Arp2/3 complex	200,000	Capping [(−) end], side binding and nucleation

filaments by creating new (−) ends and causes disintegration of an actin network, although many filaments remain cross-linked. The turnover of actin filaments promoted by severing proteins is necessary not only for cell locomotion but also for cytokinesis.

The capping and severing proteins are regulated by several signaling pathways. For example, both cofilin and gelsolin bind PIP_2 in a way that inhibits their binding to actin filaments and thus their severing activity. Hydrolysis of PIP_2 by phospholipase C releases these proteins and induces rapid severing of filaments. The reversible phosphorylation and dephosphorylation of cofilin also regulates its activity, and the severing activity of gelsolin is activated by an increase in cytosolic Ca^{2+} to about 10^{-6} M. The counteracting influence of different signaling molecules, Ca^{2+}, and PIP_2 permits the reciprocal regulation of these proteins. In Section 19.4, we consider how extracellular signals coordinate the activities of different actin-binding proteins, including severing proteins, in cell migration.

Actin-Capping Proteins Stabilize F-Actin

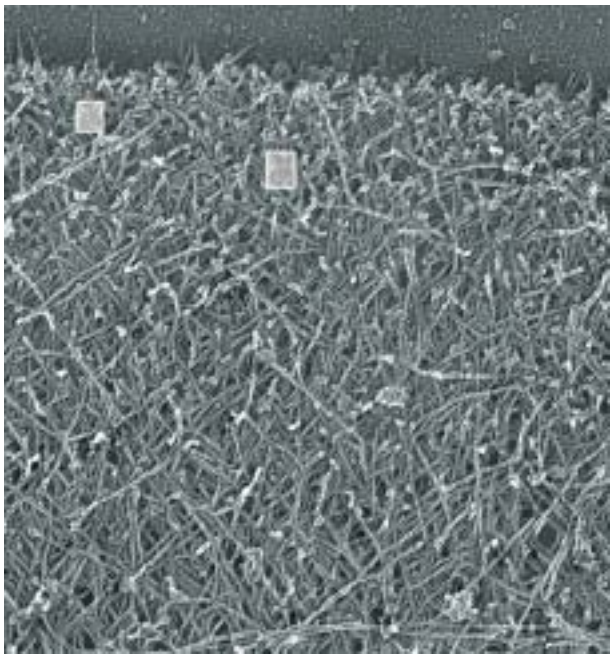
Another group of proteins can cap the ends of actin filaments but, unlike severing proteins, cannot break filaments to create new ends. One such protein, *CapZ*, binds the (+) ends of actin filaments independently of Ca^{2+} and prevents the addition or loss of actin subunits from the (+) end. Capping by this protein is inhibited by PIP_2, suggesting that its activity is regulated by the same signaling pathways that control cofilin and profilin. *Tropomodulin*, which is unrelated to CapZ in sequence, caps the (−) ends of actin filaments. Its capping activity is enhanced in the presence of tropomyosin, which suggests that the two proteins function as a complex to stabilize a filament. An actin filament that is capped at both ends is effectively stabilized, undergoing neither addition nor loss of subunits. Such capped actin filaments are needed in places where the organization of the cytoskeleton is unchanging, as in a muscle sarcomere (Figure 19-11) or at the erythrocyte membrane.

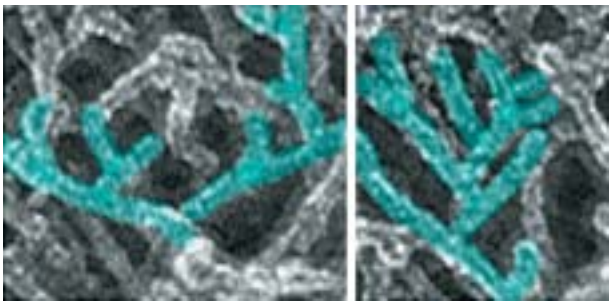


▲ FIGURE 19-11 **Diagram of sarcomere in skeletal muscle showing location of actin-capping proteins.** CapZ (green) caps the (+) ends of actin thin filaments, which are located at the Z disk separating adjacent sarcomeres. Tropomodulin (yellow) caps the (−) ends of thin filaments, located toward the center of a sarcomere. The presence of these two proteins at opposite ends of a thin filament prevents actin subunits from dissociating during muscle contraction.

Arp2/3 Assembles Branched Filaments

A family of *actin-related proteins* (Arps), exhibiting 50 percent sequence similarity with actin, has been identified in many eukaryotic organisms. One group of Arps, a complex of seven proteins called Arp2/3, stimulates actin assembly in vivo. (Another Arp group that is associated with microtubules and a microtubule motor protein is discussed in the next chapter.) Isolated from cell extracts on the basis of its ability to bind profilin, the Arp2/3 complex binds at 70° to the side of an actin filament to nucleate a daughter filament. The combination of mother and daughter filaments creates a branched network in which Arp2/3 is located at the branch points (Figure 19-12). As a result, the newly created ends of filaments elongate and create the force to push the membrane forward.





◀ FIGURE 19-12 **Branched actin filaments with Arp2/3 at the branch points.** An extensive network of actin filaments fills the cytoplasm at the leading edge of a keratinocyte. Within selected areas of the network, highly branched filaments (green) are seen. At each branch point lies the Arp2/3 complex. [From T. M. Svitkina and G. G. Borisy, 1999, *J. Cell Biol.* **145**:1009; courtesy of T. M. Svitkina and G. G. Borisy.]

MEDIA CONNECTIONS
Video: Direct Observation of Actin Filament Branching Mediated by Arp2/3 Complex

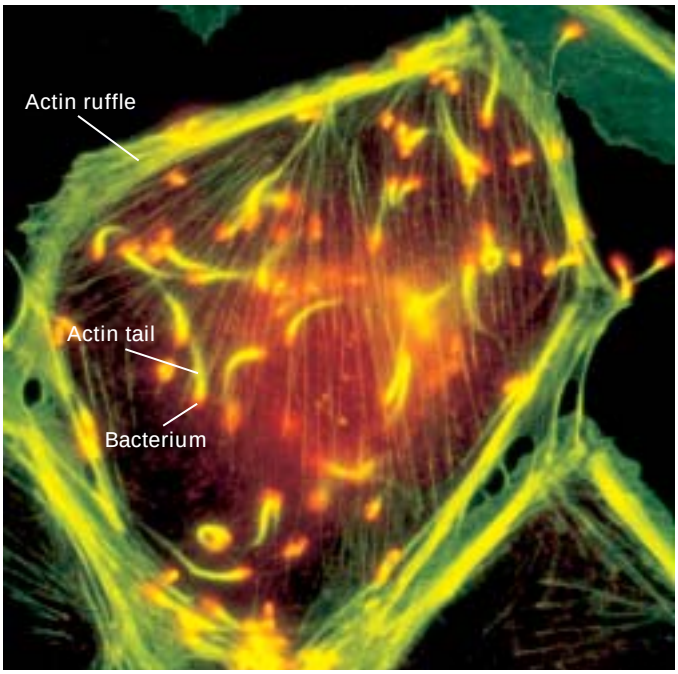
Branching is stimulated by the WASp family of proteins under the control of the Rho GTPases. Actin cross-linking proteins such as filamin stabilize the branched network, whereas actin-severing proteins such as cofilin disassemble the branched structures.

Intracellular Movements and Changes in Cell Shape Are Driven by Actin Polymerization

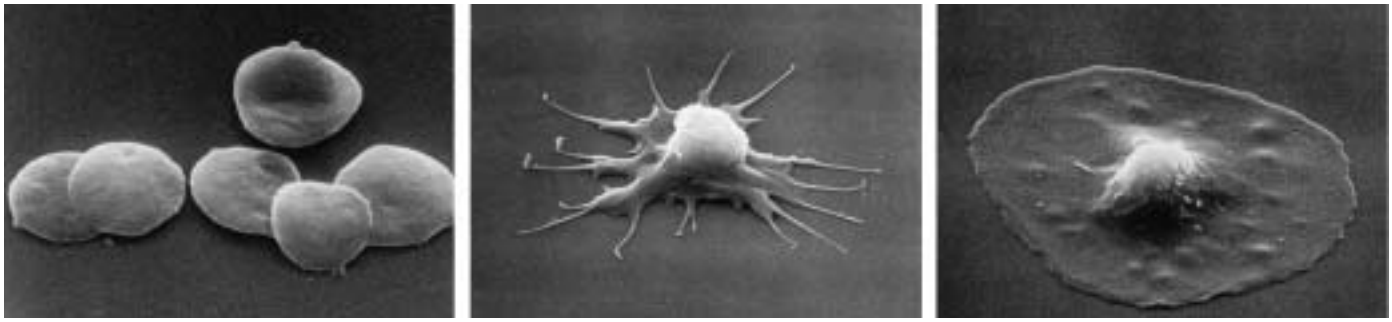
By manipulating actin polymerization and depolymerization, the cell can create forces that produce several types of movement. As noted previously and described in detail later, actin polymerization at the leading edge of a moving cell is critical to cell migration. Here, we consider other examples of cell movement that most likely result from actin polymerization—one concerning infection and the other blood clotting.

Most infections are spread by bacteria or viruses that are liberated when an infected cell lyses. However, some bacteria and viruses escape from a cell on the end of a polymerizing actin filament. Examples include *Listeria monocytogenes*, a bacterium that can be transmitted from a pregnant woman to the fetus, and vaccinia, a virus related to the smallpox virus. When such organisms infect mammalian cells, they move through the cytosol at rates approaching 11 μm/min. Fluorescence microscopy revealed that a meshwork of short actin filaments follows a moving bacterium or virus like the plume of a rocket exhaust (Figure 19-13). These observations suggested that actin generates the force necessary for movement.

The first hints about how actin mediates bacterial movement were provided by a microinjection experiment in which fluorescence-labeled G-actin was injected into *Listeria*-infected cells. In the microscope, the labeled monomers could be seen incorporating into the tail-like meshwork at the end



▲ EXPERIMENTAL FIGURE 19-13 **Fluorescence microscopy implicates actin in movement of *Listeria* in infected fibroblasts.** Bacteria (red) are stained with an antibody specific for a bacterial membrane protein that binds cellular profilin and is essential for infectivity and motility. Behind each bacterium is a "tail" of actin (green) stained with fluorescent phalloidin. Numerous bacterial cells move independently within the cytosol of an infected mammalian cell. Infection is transmitted to other cells when a spike of cell membrane, generated by a bacterium, protrudes into a neighboring cell and is engulfed by a phagocytotic event. [Courtesy of J. Theriot and T. Mitchison.]

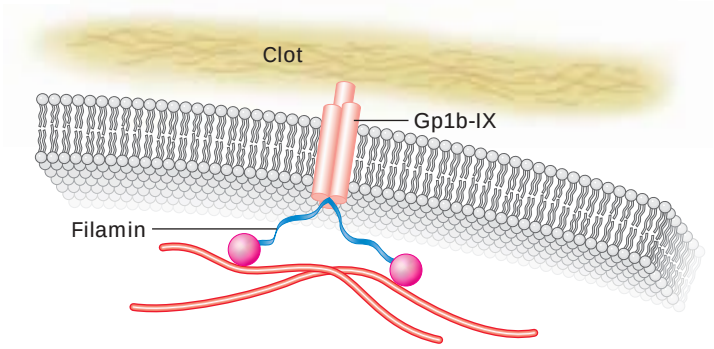


▲ EXPERIMENTAL FIGURE 19-14 **Platelets change shape during blood clotting.** Resting cells have a discoid shape (*left*). When exposed to clotting agents, the cells settle on the substratum, extend numerous filopodia (*center*), and then spread out (*right*). The changes in morphology result from complex rearrangements of the actin cytoskeleton, which is cross-linked to the plasma membrane. [Courtesy of J. White.]

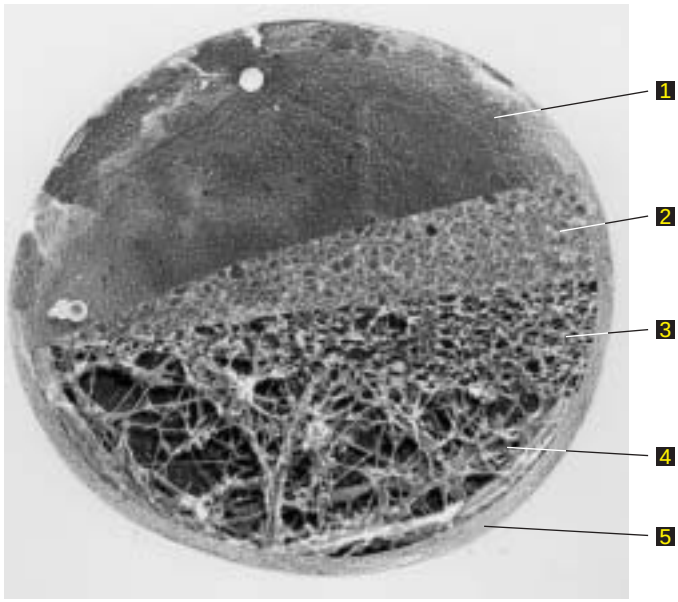
nearest the bacterium, with a simultaneous loss of actin throughout the tail. This result showed that actin polymerizes into filaments at the base of the bacterium and suggested that, as the tail-like meshwork assembles, it pushes the bacterium ahead. Findings from studies with mutant bacteria indicate that the interaction of cellular Arp 2/3 with a bacterial membrane protein promotes actin polymerization at the end of the tail nearest the bacterium. Recent studies have detected rocket tails trailing common cytoplasmic vesicles such as endosomes. Such observations suggest that actin polymerization may underlie the movement of endosomes in the cytoplasm.

During blood clotting, complicated rearrangements of the cytoskeleton in activated platelets dramatically change the cell shape and promote clot formation (Figure 19-14). The cytoskeleton of an unactivated platelet consists of a rim of microtubules (the *marginal band*), a membrane skeleton, and a cytosolic actin network. The membrane skeleton in

(a)



(b)



▲ FIGURE 19-15 **Cross-linkage of actin filament networks to the platelet plasma membrane.** In platelets, a three-dimensional network of actin filaments is attached to the integral membrane glycoprotein complex Gp1b-IX by filamin. Gp1b-IX also binds to proteins in a blood clot outside the platelet. Platelets also possess a two-dimensional cortical network of actin and spectrin similar to that underlying the erythrocyte membrane (see Figure 5-31). (b) This composite picture of the actin cytoskeleton in a resting platelet shows the different arrangements of microfilaments. Beneath the plasma membrane (1) lies a two-dimensional network of filaments (2) cross-linked by spectrin. Filamin organizes the filaments into a three-dimensional gel (3), forming the cortex of the cell. A lattice of filament bundles (4) forms adhesions to the underlying substratum. The disk shape of the cell is maintained by a ring of microtubules (5) at the cell periphery. [Part (b) courtesy of John Hartwig.]

platelets is somewhat similar to the cortical cytoskeleton in erythrocytes (see Figure 5-31). A critical difference between erythrocytes and platelet cytoskeletons is the presence in the platelet of the second network of actin filaments, which are organized by filamin cross-links into a three-dimensional gel (Figure 19-15). The gel fills the cytosol of a platelet and is anchored by filamin to a glycoprotein complex (Gp1b-IX) in the platelet membrane. Gp1b-IX not only binds filamin but also is the membrane receptor for two blood-clotting proteins. Through Gp1b-IX and an integrin receptor, forces generated during rearrangements of the actin cytoskeleton in platelets can be transmitted to a developing clot. Several examples of similar connections between the cytoskeleton and components of the extracellular matrix are described in Chapter 6.

KEY CONCEPTS OF SECTION 19.2

The Dynamics of Actin Assembly

- Within cells, the actin cytoskeleton is dynamic, with filaments able to grow and shrink rapidly.
- Polymerization of G-actin in vitro is marked by a lag period during which nucleation occurs. Eventually, a polymerization reaction reaches a steady state in which the rates of addition and loss of subunits are equal (see Figure 19-10).
- The concentration of actin monomers in equilibrium with actin filaments is the critical concentration (C_c). At a G-actin concentration above C_c, there is net growth of filaments; at concentrations below C_c, there is net depolymerization of filaments.
- Actin filaments grow considerably faster at their (+) end than at their (−) end, and the C_c for monomer addition to the (+) end is lower than that for addition at the (−) end.
- The assembly, length, and stability of actin filaments are controlled by specialized actin-binding proteins that can sever filaments or cap the ends or both. These proteins are in turn regulated by various mechanisms.
- The complementary actions of thymosin β_4 and profilin are critical to regulating the actin cytoskeleton near the cell membrane (see Figure 19-10).
- The regulated polymerization of actin can generate forces that move certain bacteria and viruses or cause changes in cell shape.

19.3 Myosin-Powered Cell Movements

We now examine the function of different myosin motor proteins in nonmuscle cells and muscle. As discussed in Chapter 3, interactions between myosin II and actin filaments are responsible for muscle contraction. At first, scientists thought that most cell movements were caused by a contractile mechanism similar to the sliding of actin and myosin filaments in muscle cells. This idea was based on several properties of at least some nonmuscle cells: the ability of cytosolic extracts to undergo contractile-like movements, the presence of actin and myosin II, and the existence of structures similar to muscle sarcomeres. However, the results of later biochemical studies led to the extraction of "unusual" forms of myosin that differed from myosin II in structure, location, and enzymatic properties.

As biologists investigated various types of cell movements, it became clear that myosin II mediates only a few types, such as cytokinesis and muscle contraction. Other types of cell movements, including vesicle transport, membrane extension, and the movement of chromosomes, require either other myosin isoforms, other motor proteins such as kinesin or dynein, or actin polymerization. In this section, we first consider the properties of various myosins and some of their functions in nonmuscle cells. *Contraction,* the special form of movement resulting from the interaction of actin and myosin II, is most highly evolved in skeletal muscle cells. However, somewhat similar contractile events entailing less organized systems are found in nonmuscle cells. After reviewing the highly ordered structure of actin and myosin filaments in the sarcomere of skeletal muscle, we describe the primary mechanisms for regulating contraction.

Myosins Are a Large Superfamily of Mechanochemical Motor Proteins

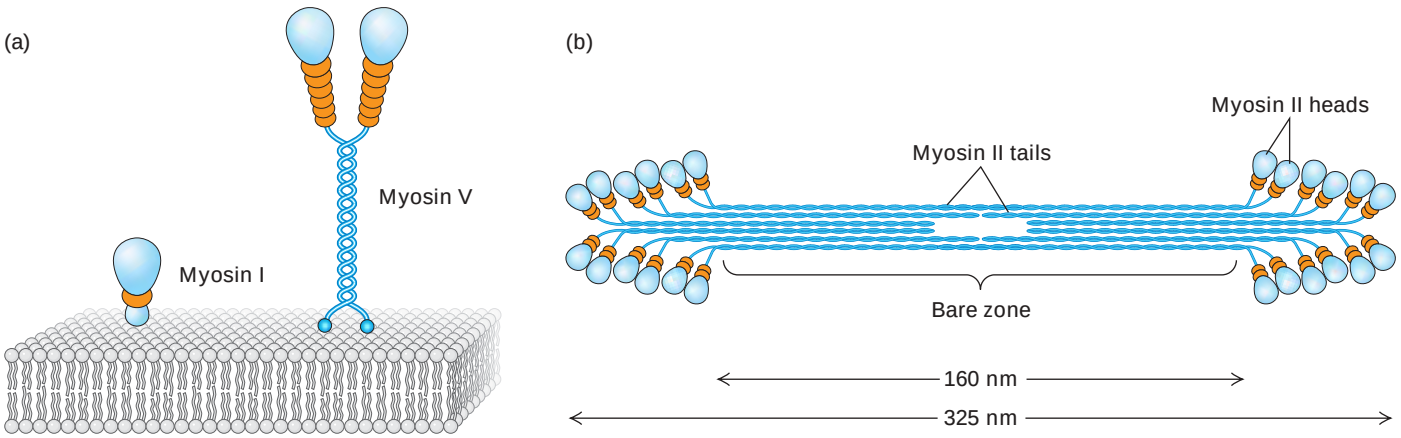
Eight members of the myosin gene family have been identified by genomic analysis (Chapter 9). Three family members—*myosin I, myosin II,* and *myosin V*—are present in nearly all eukaryotic cells and are the best understood. Although the specific activities of these myosins differ, they all function as motor proteins. As already noted, myosin II powers muscle contraction, as well as cytokinesis. Myosins I and V take part in cytoskeleton–membrane interactions, such as the transport of membrane vesicles.

Researchers are currently uncovering the activities of the remaining myosins. Genetic analysis has revealed that myosins VI, VII, and XV have functions associated with hearing and hair cell stereocilia structure. Plants do not have the same myosins as animal cells. Three myosins (VII, XI, and XIII) are exclusively expressed in plants. Myosin XI, which may be the fastest myosin of all, is implicated in the cytoplasmic streaming seen in green algae and higher plants (Table 19-3).

All myosins consist of one or two heavy chains and several light chains, which generally have a regulatory function. A characteristic head, neck, and tail domain organization is found in all myosin heavy chains. Myosin II and myosin V are dimers in which α-helical sequences in the tail of each heavy chain associate to form a rodlike coiled-coil structure. In contrast some myosins, including myosin I, are monomers because their heavy chains lack this α-helical sequence. All myosin *head domains* have ATPase activity and

TABLE 19-3 Myosins

Type	Heavy Chain (MW)	Structure	Step Size (nm)	Activity
I	110,000–150,000		10–14	Membrane binding, endocytic vesicles
II	220,000		5–10	Filament sliding
V	170,000–220,000		36	Vesicle transport
VI	140,000		30	Endocytosis
XI	170,000–260,000		35	Cytoplasmic streaming



▲ FIGURE 19-16 **Functions of myosin tail domains.** (a) Myosin I and myosin V are localized to cellular membranes by undetermined sites in their tail domains. As a result, these myosins are associated with intracellular membrane vesicles or the cytoplasmic face of the plasma membrane. (b) In contrast, the coiled-coil tail domains of myosin II molecules pack side by side, forming a thick filament from which the heads project. In a skeletal muscle, the thick filament is bipolar. Heads are at the ends of the thick filament and are separated by a bare zone, which consists of the side-by-side tails.

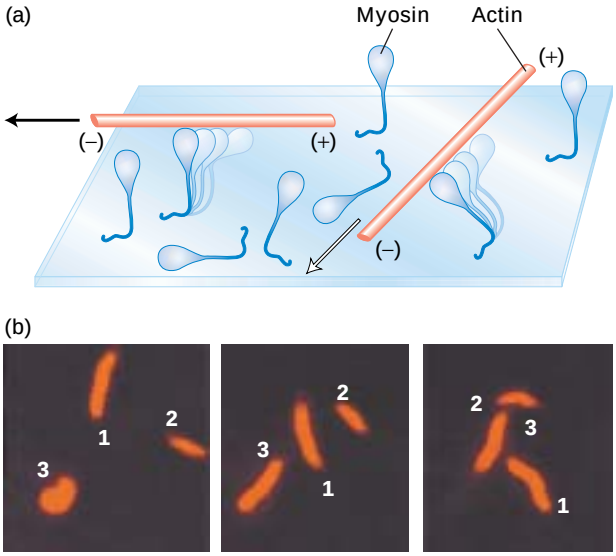
in conjunction with the *neck domain* couple ATP hydrolysis to movement of a myosin molecule along an actin filament via a common mechanism involving cyclical binding and hydrolysis of ATP and attachment/detachment of myosin and actin (see Figures 3-24 and 3-25).

The role of a particular myosin in vivo is related to its *tail domain*. For example, the tail domains of myosins I, V, VI, and XI bind the plasma membrane or the membranes of intracellular organelles; as a result, these molecules have membrane-related activities (Figure 19-16a). In contrast, the coiled-coil tail domains of myosin II dimers associate to form bipolar thick filaments in which the heads are located at both ends of the filament and are separated by a central bare zone devoid of heads (Figure 19-16b). The close packing of myosin molecules into thick filaments, which are a critical part of the contractile apparatus in skeletal muscle, allows many myosin head domains to interact simultaneously with actin filaments.

The number and type of light chains bound in the neck region vary among the different myosins (see Table 19-3). The light chains of myosin I and myosin V are **calmodulin,** a Ca^{2+}-binding regulatory subunit in many intracellular enzymes (see Figure 3-28). Myosin II contains two different light chains called essential and regulatory light chains (see Figure 3-24); both are Ca^{2+}-binding proteins but differ from calmodulin in their Ca^{2+}-binding properties. All myosins are regulated in some way by Ca^{2+}; however, because of the differences in their light chains, the different myosins exhibit different responses to Ca^{2+} signals in the cell.

Myosin Heads Walk Along Actin Filaments in Discrete Steps

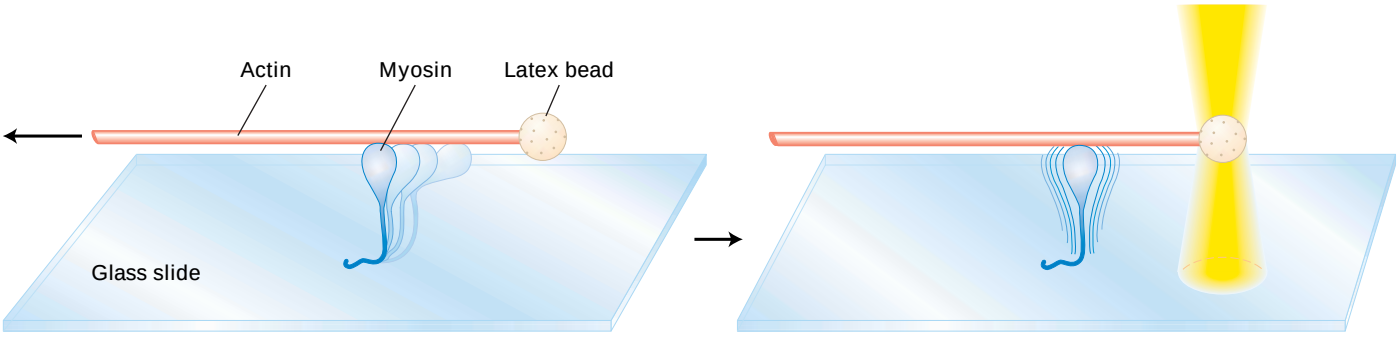
Unraveling the mechanism of myosin-powered movement was greatly aided by development of in vitro motility assays. In one such assay, the *sliding-filament assay,* the movement of fluorescence-labeled actin filaments along a bed of myosin molecules is observed in a fluorescence microscope. Because the myosin molecules are tethered to a coverslip, they cannot move; thus any force generated by interaction of myosin heads with actin filaments forces the filaments to move relative to the myosin (Figure 19-17a). If ATP is present, added actin filaments can be seen to glide along the surface of the coverslip; if ATP is absent, no filament movement is observed. This movement is caused by a myosin head (bound to the coverslip) "walking" toward the (+) end of a filament; thus filaments move with the (−) end in the lead. [The one exception is myosin VI, which moves in the opposite direction, toward the (−) end; so the (+) end of a moving filament is in the lead.] The rate at which myosin moves an actin filament can be determined from video camera recordings of sliding-filament assays (Figure 19-17b). The velocity of filament movement can vary widely, depending on the myosin tested and the assay conditions (e.g., ionic strength, ATP and Ca^{2+} concentrations, temperature).



▲ EXPERIMENTAL FIGURE 19-17 **Sliding-filament assay is used to detect myosin-powered movement.**
(a) After myosin molecules are adsorbed onto the surface of a glass coverslip, excess myosin is removed; the coverslip then is placed myosin-side down on a glass slide to form a chamber through which solutions can flow. A solution of actin filaments, made visible by staining with rhodamine-labeled phalloidin, is allowed to flow into the chamber. (The coverslip in the diagram is shown inverted from its orientation on the flow chamber to make it easier to see the positions of the molecules.) In the presence of ATP, the myosin heads walk toward the (+) end of filaments by the mechanism illustrated in Figure 3-25. Because myosin tails are immobilized, walking of the heads causes sliding of the filaments. Movement of individual filaments can be observed in a fluorescence light microscope. (b) These photographs show the positions of three actin filaments (numbered 1, 2, 3) at 30-second intervals recorded by video microscopy. The rate of filament movement can be determined from such recordings. [Part (b) courtesy of M. Footer and S. Kron.]

MEDIA CONNECTIONS
Technique Animation: In Vitro Motility Assay

The most critical feature of myosin is its ability to generate a force that powers movements. Researchers have used a device called an *optical trap* to measure the forces generated by single myosin molecules (Figure 19-18). The results of optical-trap studies show that myosin II moves in discrete steps, approximately 5–10 nm long, and generates 3–5 piconewtons (pN) of force, approximately the same force as that exerted by gravity on a single bacterium. This force is sufficient to cause myosin thick filaments to slide past actin thin filaments during muscle contraction or to transport a membrane-bounded vesicle through the cytoplasm. With a step size of 5 nm, myosin would bind to every actin subunit on one strand of the filament. Some evidence suggests that ATP hydrolysis and myosin walking are closely coupled, with myosin taking a discrete step for every ATP molecule hydrolyzed.



▲ EXPERIMENTAL FIGURE 19-18 **Optical trap determines force generated by a single myosin molecule.** In an optical trap, the beam of an infrared laser focused by a light microscope on a latex bead (or any other object that does not absorb infrared light) captures and holds the bead in the center of the beam. The strength of the force holding the bead is adjusted by increasing or decreasing the intensity of the laser beam. In this experiment, a bead is attached to the end of an actin filament. With the optical trap turned off, the actin filament and its attached bead move in response to the force generated by myosin adsorbed on the coverslip, as in Figure 19-17. When the optical trap is turned on, it captures the filament, through the bead, and holds the filament to the surface of a myosin-coated coverslip. The force exerted by a single myosin molecule on an actin filament is measured from the force needed to hold the bead in the optical trap. A computer-controlled electronic feedback system keeps the bead centered in the trap, and myosin-generated movement of the bead is counteracted by the opposing force of the trap. The distance traveled by the actin filament is measured from the displacement of the bead in the trap.

The neck domains in different myosins vary in length and number of associated light chains. Generally, the longer the neck domain of a myosin, the greater its *step size* (i.e., the distance traveled along an actin filament in one step). Because the neck region is the lever arm of myosin, a longer neck would lead to a longer distance traveled by the arm. For instance, myosin II, with a short neck, has from 5- to 10-nm steps, whereas myosin V, with a long neck, has much longer 36-nm steps. The correlation between step size and neck length has been further supported by experiments in which the neck domain is lengthened by recombinant methods. However, the correlation between neck length and step size is not absolute, as evidenced by myosin VI, which moves in 30-nm steps, although it has a neck domain shorter than that of myosin II.

Myosin-Bound Vesicles Are Carried Along Actin Filaments

Among the many movements exhibited by cells, vesicle translocation has been one of the most fascinating to cell biologists. In early studies of the cytoplasm, researchers found that certain particles, now known to be membrane-bounded vesicles, moved in straight lines within the cytosol, sometimes stopping and then resuming movement, at times after changing direction. This type of behavior could not be caused by diffusion, because the movement was clearly not random. Therefore, researchers reasoned, there must be tracks, most likely actin filaments or microtubules, along which the particles travel, as well as some type of motor to power the movement.

In the sliding-filament assay, walking of the myosin head along an actin filament causes the filament to move because the myosin tail is immobilized. In cells, however, the situation is often reversed: when part of an extensive network, actin filaments are largely immobile, whereas myosin is free to move. In this case, if the tail of a myosin molecule binds to the membrane of a vesicle and the head walks along a filament, the vesicle will be carried along as "cargo." Here, we present evidence that some myosins, including myosins I, V, and VI, do just that. We also consider the related process of cytoplasmic streaming, which is most likely powered by myosin XI.

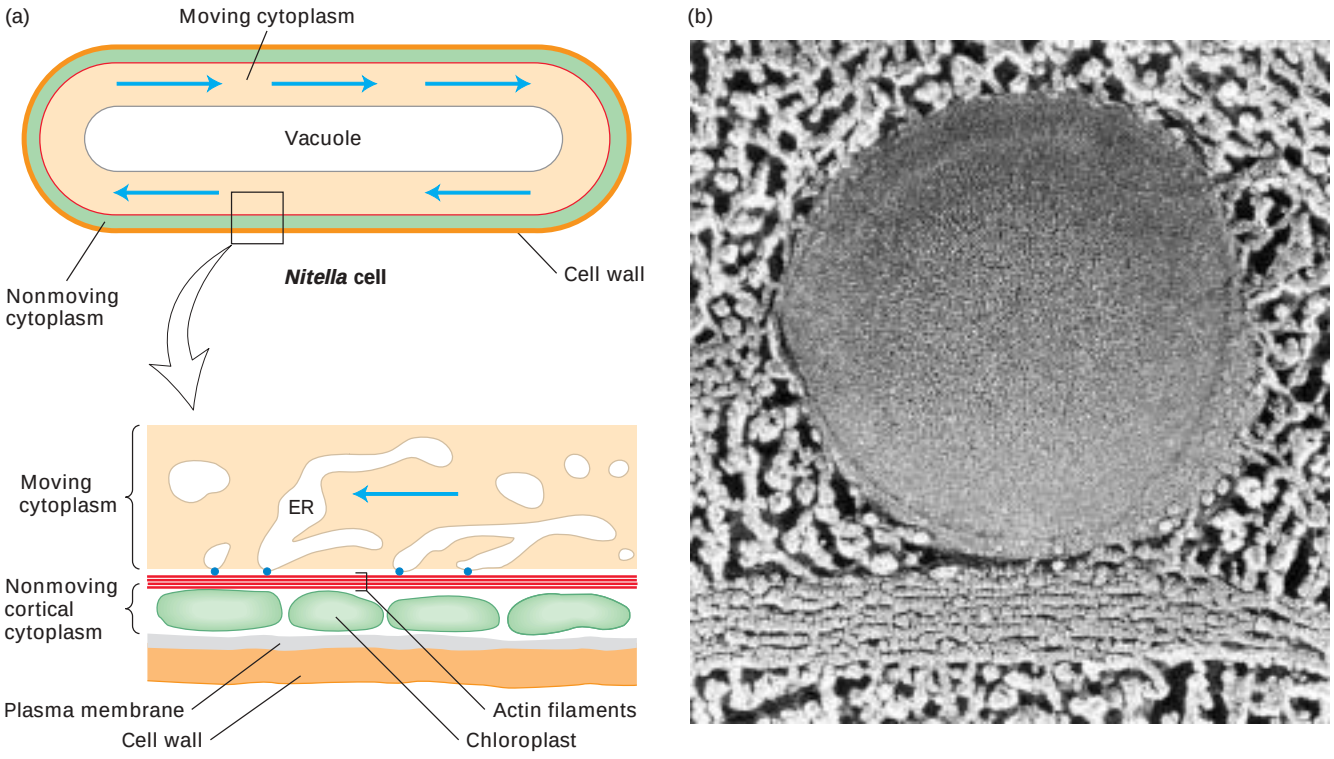
Vesicle Trafficking (Myosins I, V, and VI) Findings from studies with amebas provided the initial clues that myosin I participates in vesicle transport. Indeed the first myosin I molecule to be identified and characterized was from these organisms; subsequently, the cDNA sequences of three myosin I genes were identified in *Acanthameba*, a common soil ameba. Using antibodies specific for each myosin I isoform, researchers found that the isoforms are localized to different membrane structures in the cell. For example, myosin IA is associated with small cytoplasmic vesicles. Myosin IC, in contrast, is found at the plasma membrane and at the contractile vacuole, a vesicle that regulates the osmolarity of the cytosol by fusing with the plasma membrane. The introduction of antibodies against myosin IC into a living ameba prevents transport of the vacuole to the membrane; as a result, the vacuole expands uncontrollably, eventually bursting the cell. In addition, myosin I in animal cells serves as a membrane–microfilament linkage in microvilli, another example of a membrane-associated function.

Several types of evidence suggest that myosin V also participates in the intracellular transport of membrane-bounded vesicles. For example, mutations in the myosin V gene in yeast disrupt protein secretion and lead to an accumulation of vesicles in the cytoplasm. Vertebrate brain tissue is rich in myosin V, which is concentrated on Golgi stacks. This association with membranes is consistent with the effects of myosin V mutations in mice. Such mutations are associated with defects in synaptic transmission and eventually cause death from seizures. Myosin VI also is implicated in membrane trafficking of vesicles.

Unlike myosin in a thick filament where multiple heads interact with the same actin filament, cytoplasmic myosins work alone in carrying their membrane cargos. How do these myosins move without dissociating from the filament? The answer lies in the *duty ratio*, the fraction of time spent attached to the filament during the ATPase cycle. Myosins with a high duty ratio, such as myosins V and VI, are bound to actin filaments for most of the ATP cycle. Consequently, these myosins process or move along a filament for considerable distances with little danger of falling off.

Cytoplasmic Streaming (Myosin XI) In large, cylindrical green algae such as *Nitella* and *Chara*, cytosol flows rapidly, at a rate approaching 4.5 mm/min, in an endless loop around the inner circumference of the cell (Figure 19-19). This cytoplasmic streaming is a principal mechanism for distributing cellular metabolites, especially in large cells such as plant cells and amebas. This type of movement probably represents an exaggerated version of the smaller-scale movements exhibited during the transport of membrane vesicles.

Close inspection of objects caught in the flowing cytosol, such as the endoplasmic reticulum (ER) and other membrane-bounded vesicles, show that the velocity of streaming increases from the cell center (zero velocity) to the cell periphery. This gradient in the rate of flow is most easily explained if the motor generating the flow lies at the membrane. In electron micrographs, bundles of actin filaments can be seen aligned along the length of the cell, lying across chloroplasts embedded at the membrane. Attached to the actin bundles are vesicles of the ER network. The bulk cytosol is propelled by myosin attached to parts of the ER lying along the stationary actin filaments. The flow



▲ FIGURE 19-19 **Cytoplasmic streaming in cylindrical giant algae.** (a) The center of a *Nitella* cell is filled with a single large water-filled vacuole, which is surrounded by a layer of moving cytoplasm (indicated by blue arrows). A nonmoving layer of cortical cytoplasm filled with chloroplasts lies just under the plasma membrane (enlarged lower figure). On the inner side of this layer are bundles of stationary actin filaments (red), all oriented with the same polarity. A motor protein (blue dots), most likely myosin XI, carries parts of the endoplasmic reticulum (ER) along the actin filaments. The movement of the ER network propels the entire viscous cytoplasm, including organelles that are enmeshed in the ER network. (b) An electron micrograph of the cortical cytoplasm shows a large vesicle connected to an underlying bundle of actin filaments. This vesicle, which is part of the ER network, contacts the stationary actin filaments and moves along them by a myosin motor protein. [Part (b) from B. Kachar.]

rate of the cytosol in *Nitella* is at least 15 times as fast as the movement produced by any other myosin. This evidence and other evidence suggest that cytoplasmic streaming is powered by myosin XI, one of the fastest moving myosins.

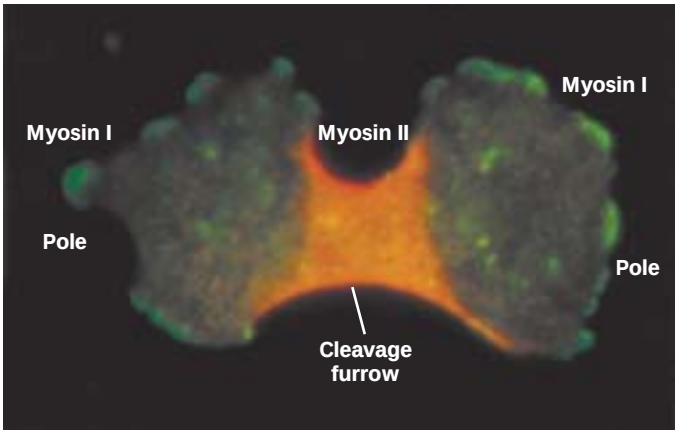
Actin and Myosin II Form Contractile Bundles in Nonmuscle Cells

Nonmuscle cells contain prominent **contractile bundles** composed of actin and myosin II filaments. The contractile bundles of nonmuscle cells, which may be transitory or permanent differ in several ways from the noncontractile bundles of actin described earlier in this chapter (see Figure 19-5). Interspersed among the actin filaments of a contractile bundle is myosin II, which is responsible for their contractility. When isolated from cells, these bundles contract on the addition of ATP. Contractile bundles are always located adjacent to the plasma membrane as a sheet or belt, whereas noncontractile actin bundles form the core of membrane projections (e.g., microvilli and filopodia).

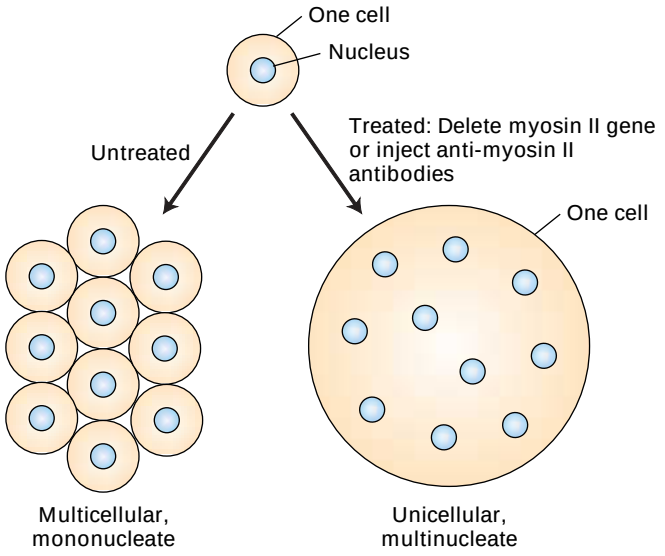
In epithelial cells, contractile bundles are most commonly found as a *circumferential belt*, which encircles the inner surface of the cell at the level of the adherens junction (see Figure 6-5). *Stress fibers*, which are seen along the ventral surfaces of cells cultured on artificial (glass or plastic) surfaces or in extracellular matrices, are a second type of contractile bundle. The ends of stress fibers terminate at **integrin**-containing focal adhesions, special structures that attach a cell to the underlying substratum (see Figure 6-26). Circumferential belts and stress fibers contain several proteins found in smooth muscle, and both exhibit some organizational features resembling muscle sarcomeres. Thus, both these structures appear to function in cell adhesion and cell movement.

A third type of contractile bundle, referred to as a *contractile ring*, is a transient structure that assembles at the equator of a dividing cell, encircling the cell midway between the poles of the spindle. As division of the cytoplasm (**cytokinesis**) proceeds, the diameter of the contractile ring decreases; so the cell is pinched into two parts by a deepening cleavage furrow. Dividing cells stained with antibodies against myosin I and myosin II show that myosin II is localized to the contractile ring, whereas myosin I is at the cell poles (Figure 19-20). This localization indicates that myosin II but not myosin I takes part in cytokinesis.

The results of experiments in which active myosin II is eliminated from the cell demonstrate that cytokinesis is indeed dependent on myosin II (Figure 19-21). In one type of experiment, anti-myosin II antibodies are microinjected into one cell of a sea urchin embryo at the two-cell stage. In other experiments, expression of myosin II is inhibited by deletion of the myosin gene or by antisense inhibition of myosin mRNA expression. In all cases, a cell lacking myosin II replicates to form a multinucleated syncytium because cytokinesis, but not chromosome separation, is inhibited. Without myosin II, cells fail to assemble a contractile ring, although other events in the cell cycle proceed normally.



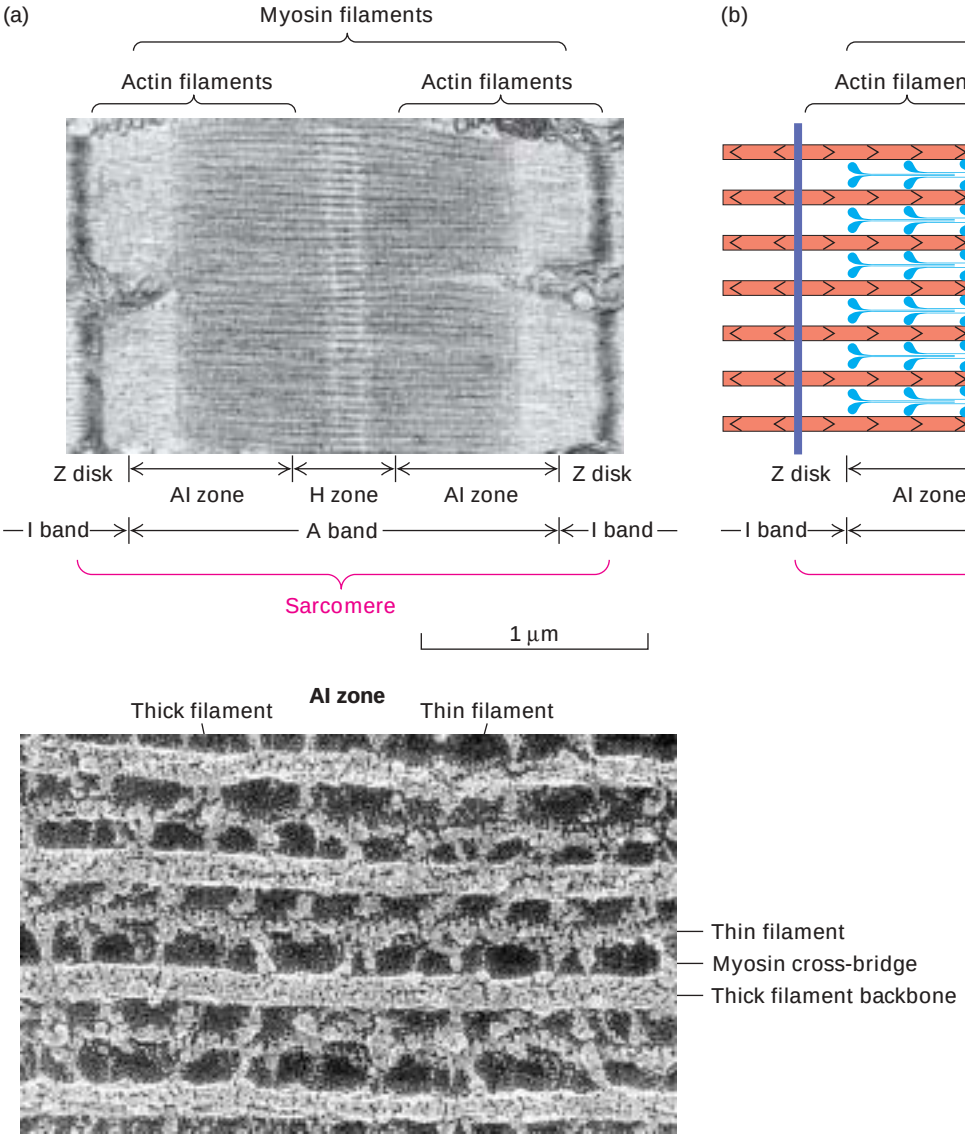
▲ EXPERIMENTAL FIGURE 19-20 **Fluorescent antibodies reveal the localization of myosin I and myosin II during cytokinesis.** Fluorescence micrograph of a *Dictyostelium* ameba during cytokinesis reveals that myosin II (red) is concentrated in the cleavage furrow, whereas myosin I (green) is localized at the poles of the cell. The cell was stained with antibodies specific for myosin I and myosin II, with each antibody preparation linked to a different fluorescent dye. [Courtesy of Y. Fukui.]



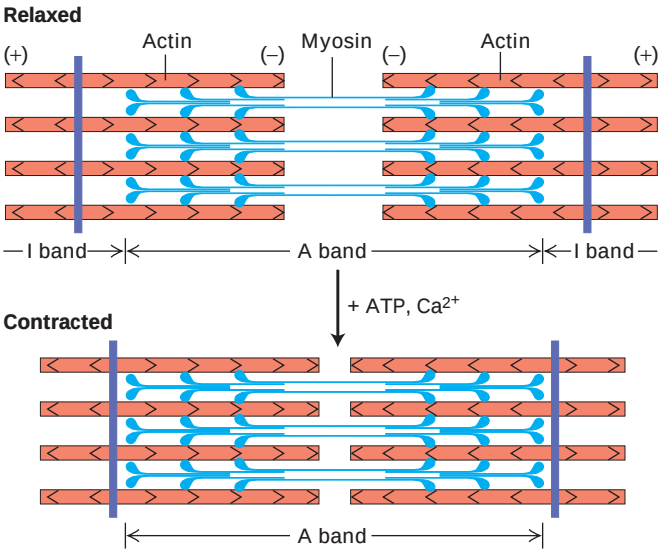
▲ EXPERIMENTAL FIGURE 19-21 **Inhibition of myosin II demonstrates that it is required for cytokinesis.** The activity of myosin II can be inhibited either by deleting its gene or by microinjecting anti-myosin II antibodies into a cell. A cell that lacks myosin II is able to replicate its DNA and nucleus but fails to divide; as a result, the cell forms a large, multinucleate syncytium over a period of time. In comparison, an untreated cell during the same period continues to divide, forming a multicellular ball of cells in which each cell contains a single nucleus.

Organized Thick and Thin Filaments in Skeletal Muscle Slide Past One Another During Contraction

Muscle cells have evolved to carry out one highly specialized function—contraction. Muscle contractions must occur quickly and repetitively, and they must occur through long distances and with enough force to move large loads. A typical skeletal muscle cell, called a myofiber, is cylindrical, large (1–40 mm in length and 10–50 μm in width), and multinucleated (containing as many as 100 nuclei). The cytoplasm is packed with a regular repeating array of filament bundles organized into a specialized structure called a **sarcomere.** A chain of sarcomeres, each about 2 μm long in resting muscle, constitutes a *myofibril.* The sarcomere is both the structural and the functional unit of skeletal muscle. During contraction, the sarcomeres are shortened to about 70 percent of their uncontracted, resting length. Electron microscopy and biochemical analysis have shown that each sarcomere contains two types of filaments: *thick filaments,* composed of myosin II, and *thin filaments,* containing actin (Figure 19-22).



▲ FIGURE 19-22 **Structure of the sarcomere.** (a) Electron micrograph of mouse striated muscle in longitudinal section, showing one sarcomere. On either side of the Z disks are the lightly stained I bands, composed entirely of actin filaments. These thin filaments extend from both sides of the Z disk to interdigitate with the dark-stained myosin thick filaments in the A band. The region containing both thick and thin filaments (the AI zone) is darker than the area containing only myosin thick filaments (the H zone). (b) Diagram of a sarcomere. The (+) ends of actin filaments are attached to the Z disks. (c) Electron micrograph showing actin-myosin cross-bridges in the AI zone of a striated flight muscle of an insect. This image shows a nearly crystalline array of thick myosin and thin actin filaments. The muscle was in the rigor state at preparation. Note that the myosin heads protruding from the thick filaments connect with the actin filaments at regular intervals. [Part (a) courtesy of S. P. Dadoune. Part (c) courtesy of M. Reedy.]



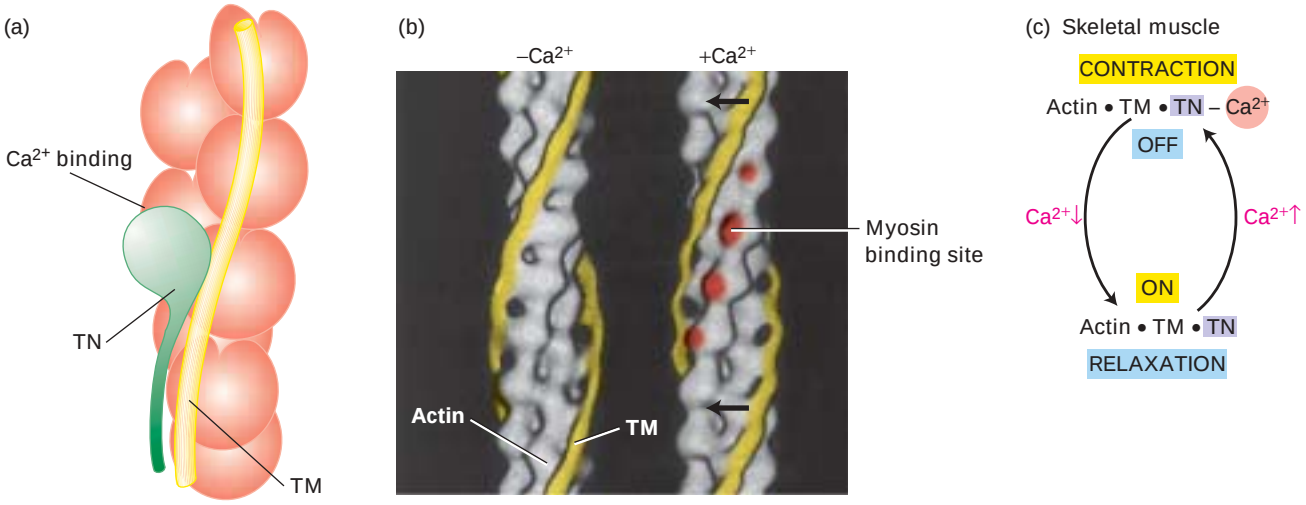
▲ FIGURE 19-23 **The sliding-filament model of contraction in striated muscle.** The arrangement of thick myosin and thin actin filaments in the relaxed state is shown in the upper diagram. In the presence of ATP and Ca^{2+}, the myosin heads extending from the thick filaments walk toward the (+) ends of the thin filaments. Because the thin filaments are anchored at the Z disks (purple), movement of myosin pulls the actin filaments toward the center of the sarcomere, shortening its length in the contracted state as shown in the lower diagram.

To understand how a muscle contracts, consider the interactions between one myosin head (among the hundreds in a thick filament) and a thin (actin) filament as diagrammed in Figure 3-25. During these cyclical interactions, also called the *cross-bridge cycle*, the hydrolysis of ATP is coupled to the movement of a myosin head toward the Z disk, which corresponds to the (+) end of the thin filament. Because the thick filament is bipolar, the action of the myosin heads at opposite ends of the thick filament draws the thin filaments toward the center of the thick filament and therefore toward the center of the sarcomere (Figure 19-23). This movement shortens the sarcomere until the ends of the thick filaments abut the Z disk or the (−) ends of the thin filaments overlap at the center of the A band. Contraction of an intact muscle results from the activity of hundreds of myosin heads on a single thick filament, amplified by the hundreds of thick and thin filaments in a sarcomere and thousands of sarcomeres in a muscle fiber.

Contraction of Skeletal Muscle Is Regulated by Ca^{2+} and Actin-Binding Proteins

Like many cellular processes, skeletal muscle contraction is initiated by an increase in the cytosolic Ca^{2+} concentration. As described in Chapter 7, the Ca^{2+} concentration of the cytosol is normally kept low, below 0.1 μM. In nonmuscle cells, Ca^{2+} ATPases in the plasma membrane maintain this low concentration. In contrast, in skeletal muscle cells, a

MEDIA CONNECTIONS
Video: Three-Dimensional Animation of an Actin-Myosin Crossbridge Regulated by Tropomyosin-Troponin



▲ FIGURE 19-24 **Actin-dependent regulation of skeletal muscle contraction.** (a) Model of the tropomyosin-troponin (TM-TN) regulatory complex on a thin filament. TN, a clublike complex of TN-C, TN-I, and TN-T subunits, is bound to the long α-helical TM molecule. (b) Three-dimensional electron-microscopic reconstructions of the TM helix (yellow) on a thin filament from scallop muscle. TM in its "off" state (*left*) shifts to its new position (arrow) in the "on" state (*right*) when the Ca^{2+} concentration increases. This movement exposes myosin-binding sites (red) on actin. TN is not shown in this representation. (c) Regulation of skeletal muscle contraction by Ca^{2+} binding to TN. Note that the TM-TN complex remains bound to the thin filament whether muscle is relaxed or contracted. [Part (b) adapted from W. Lehman, R. Craig, and P. Vibert, 1993, *Nature* **123**:313; courtesy of P. Vibert.]

low cytosolic Ca^{2+} level is maintained primarily by a unique Ca^{2+} ATPase that continually pumps Ca^{2+} ions from the cytosol into the **sarcoplasmic reticulum (SR),** a specialized endoplasmic reticulum in the muscle-cell cytosol (see Figure 7-7). This activity establishes a reservoir of Ca^{2+} in the SR.

The arrival of a nerve impulse at a neuromuscular junction leads to the opening of voltage-gated Ca^{2+} channels in the SR membrane (see Figure 7-45). The ensuing release of Ca^{2+} from the SR raises the cytosolic Ca^{2+} concentration surrounding myofibrils sufficiently to trigger contraction. In skeletal muscle, the cytosolic Ca^{2+} concentration influences the interaction of four accessory proteins with actin thin filaments. The position of these proteins on the thin filaments in turn controls myosin–actin interactions.

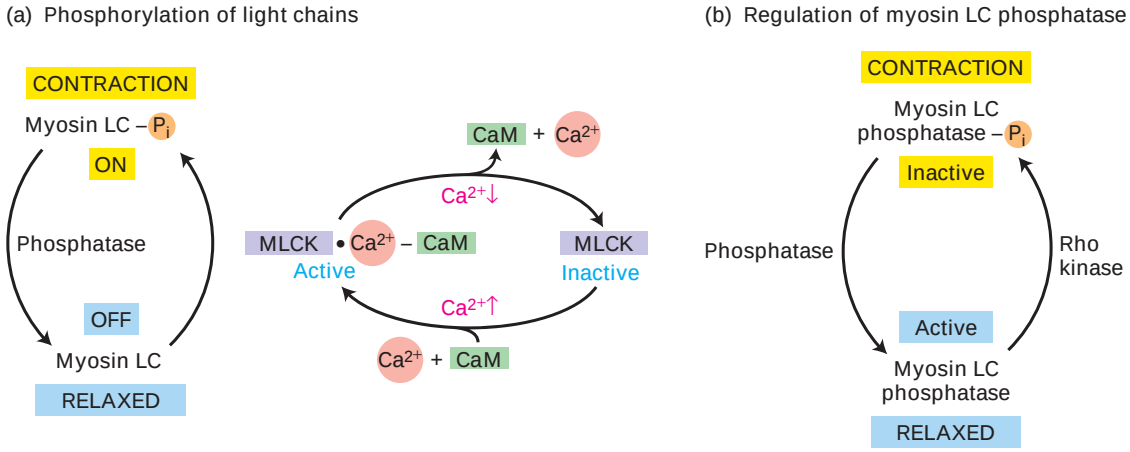
Tropomyosin (TM) is a ropelike molecule, about 40 nm in length; TM molecules are strung together head to tail, forming a continuous chain along each actin thin filament (Figure 19-24a). Associated with tropomyosin is *troponin* (TN), a complex of the three subunits, TN-T, TN-I, and TN-C. Troponin-C is the calcium-binding subunit of troponin. Similar in sequence to calmodulin and the myosin light chains, TN-C controls the position of TM on the surface of an actin filament through the TN-I and TN-T subunits.

Scientists currently think that, under the control of Ca^{2+} and TN, TM can occupy two positions on a thin filament—an "off" state and an "on" state. In the absence of Ca^{2+} (the off state), myosin can bind to a thin filament, but the TM-TN complex prevents myosin from sliding along the thin filament. Binding of Ca^{2+} ions to TN-C triggers a slight movement of TM that exposes the myosin-binding sites on actin (Figure 19-24b). At Ca^{2+} concentrations $> 10^{-6}$ M, the inhibition exerted by the TM-TN complex is relieved, and contraction occurs. The Ca^{2+}-dependent cycling between on and off states in skeletal muscle is summarized in Figure 19-24c.

Myosin-Dependent Mechanisms Regulate Contraction in Smooth Muscle and Nonmuscle Cells

A smooth muscle cell contains large, loosely aligned contractile bundles that resemble the contractile bundles in epithelial cells and contractile ring during cytokinesis. Although specialized for generating force to restrict blood vessels, propel food down the gut, and restrict airway passages, the contractile apparatus of smooth muscle and its regulation constitute a valuable model for undertanding how myosin activity is regulated in a nonmuscle cell. As we have just seen, skeletal muscle contraction is regulated by cycling of actin between on and off states. In contrast, smooth muscle contraction is regulated by cycling of myosin II between on and off states. Contraction of smooth muscle and nonmuscle cells is regulated by intracellular Ca^{2+} levels in response to many extracellular signaling molecules.

Calcium-Dependent Activation of Myosin II Contraction of vertebrate smooth muscle is regulated primarily by a complex pathway in which the *myosin regulatory light chain (LC)* undergoes phosphorylation and dephosphorylation. When the regulatory light chain is unphosphorylated, myosin II is inactive. The smooth muscle contracts when the regulatory LC is phosphorylated by the enzyme *myosin LC kinase* (Figure 19-25a). Because this enzyme is activated by Ca^{2+}, the cytosolic Ca^{2+} level indirectly regulates the extent of LC phosphorylation and hence contraction. The Ca^{2+}-dependent regulation of myosin LC kinase activity is mediated through



▲ FIGURE 19-25 **Myosin-dependent mechanisms for regulating smooth muscle contraction.** (a) In vertebrate smooth muscle, phosphorylation of the myosin regulatory light chains by Ca^{2+}-dependent myosin LC kinase activates contraction. At Ca^{2+} concentrations $<10^{-6}$M, the myosin LC kinase is inactive, and a myosin LC phosphatase, which is not dependent on Ca^{2+} for activity, dephosphorylates the myosin LC, causing muscle relaxation. (b) Activation of Rho kinase leads to phosphorylation and inactivation of the myosin LC phosphatase. Various extracellular signaling molecules induce activation of Rho kinase in both smooth muscle and nonmuscle cells.

calmodulin. Calcium first binds to calmodulin, and the Ca^{2+}/calmodulin complex then binds to myosin LC kinase and activates it. Because this mode of regulation relies on the diffusion of Ca^{2+} and the action of protein kinases, contraction is much slower in smooth muscle than in skeletal muscle.

The role of activated myosin LC kinase can be demonstrated by microinjecting a kinase inhibitor into smooth muscle cells. Even though the inhibitor does not block the rise in the cytosolic Ca^{2+} level that follows the arrival of a nerve impulse, injected cells cannot contract. The effect of the inhibitor can be overcome by microinjecting a proteolytic fragment of myosin LC kinase that is active even in the absence of Ca^{2+}-calmodulin (this treatment also does not affect Ca^{2+} levels).

Signal-Induced Activation of Myosin II by Rho Kinase Unlike skeletal muscle, which is stimulated to contract solely by nerve impulses, smooth muscle cells and nonmuscle cells are regulated by many types of external signals in addition to nervous stimuli. For example, norepinephrine, angiotensin, endothelin, histamine, and other signaling molecules can modulate or induce the contraction of smooth muscle or elicit changes in the shape and adhesion of nonmuscle cells by triggering various signal-transduction pathways. Some of these pathways lead to an increase in the cytosolic Ca^{2+} level; as previously described, this increase can stimulate myosin activity by activating myosin LC kinase (see Figure 19-25a).

Other signaling pathways activate *Rho kinase,* which can stimulate myosin activity in two ways. First, Rho kinase can phosphorylate myosin LC phosphatase (see Figure 19-25b), thereby inhibiting its activity. With the phosphatase inactivated, the level of myosin LC phosphorylation and thus myosin activity increase. In addition, Rho kinase directly activates myosin by phosphorylating the regulatory light chain. Note that Ca^{2+} plays no role in the regulation of myosin activity by Rho kinase.

KEY CONCEPTS OF SECTION 19.3

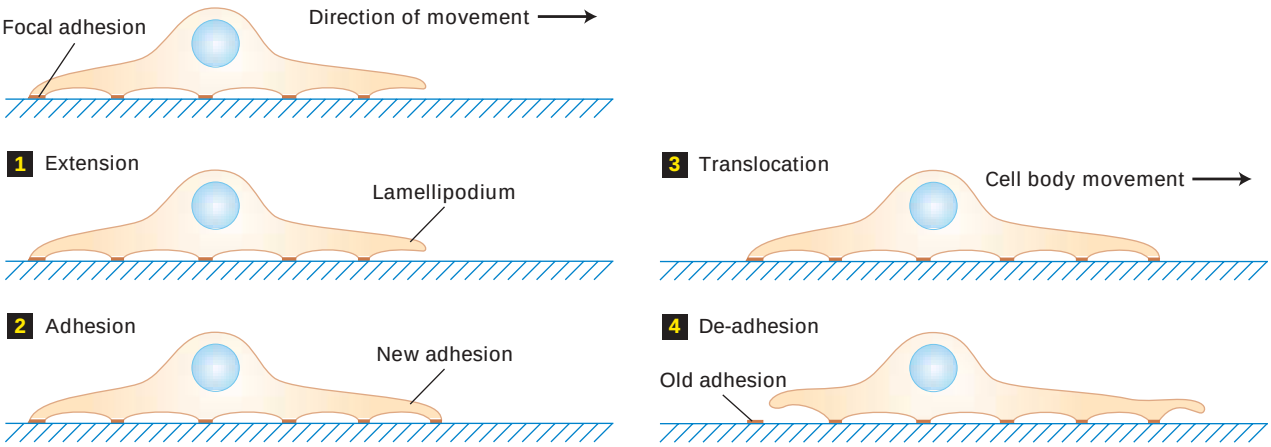
Myosin-Powered Cell Movements

- All myosin isoforms can interact with actin filaments through their head domains, but their cellular roles differ, depending on their tail domains (see Table 19-3).
- Movement of actin filaments by myosin can be directly monitored in the sliding-filament assay (see Figure 19-17).
- Myosins I, V, and VI power intracellular translocation of some membrane-limited vesicles along actin filaments. A similar process is responsible for cytoplasmic streaming, which is probably mediated by myosin XI, one of the fastest moving myosins (see Figure 19-19).
- In nonmuscle cells, actin filaments and myosin II form contractile bundles that have a primitive sarcomere-like organization. Common examples are the circumferential belt present in epithelial cells and the stress fibers in cells cultured on plastic or glass surfaces; in the latter case, they may be an artifact. Both structures function in cell adhesion.
- The contractile ring, a transient bundle of actin and myosin II, forms in a dividing cell and pinches the cell into two halves in cytokinesis.
- In skeletal muscle cells, actin thin filaments and myosin thick filaments are organized into highly ordered structures, called sarcomeres (see Figure 19-22). The (+) end of the thin filaments is attached to the Z disk, the demarcation between adjacent sarcomeres.
- During skeletal muscle contraction, myosin heads at each end of a thick filament walk along thin filaments toward the Z disks bounding a sarcomere. The force generated by myosin movement pulls the thin filaments toward the center of the sarcomere, shortening its length (see Figure 19-23).
- The rapid rise in cytosolic Ca^{2+} induced by nerve stimulation of a skeletal muscle changes the interaction between actin filaments and tropomyosin, exposing the myosin-binding sites and thus permitting contraction to occur (see Figure 19-24).
- Contraction of smooth muscle and nonmuscle cells is triggered by phosphorylation of the myosin regulatory light chains either by myosin LC kinase, in response to a rise in cytosolic Ca^{2+}, or by Rho kinase, in response to external signals (see Figure 19-25).

19.4 Cell Locomotion

We have now examined the different mechanisms used by cells to create movement—from the assembly of actin filaments and the formation of actin-filament bundles and networks to the contraction of bundles of actin and myosin and the sliding of single myosin molecules along an actin filament. These mechanisms are thought to constitute the major processes whereby cells generate the forces needed to migrate. *Cell locomotion* results from the coordination of motions generated by different parts of a cell. These motions are complex, but their major features can be revealed by fluorescent antibody-labeling techniques combined with fluorescence microscopy.

A property exhibited by all moving cells is polarity; that is, certain structures always form at the front of the cell, whereas others are found at the rear. Cell migration is initiated by the formation of a large, broad membrane protrusion at the leading edge of a cell. Video microscopy reveals that a major feature of this movement is the polymerization of actin at the membrane. In addition, actin filaments at the leading edge are rapidly cross-linked into bundles and networks in a protruding region, called a *lamellipodium* in vertebrate cells. In some cases, slender, fingerlike membrane projections, called *filopodia,* also are extended from the leading edge. These structures then form stable contacts with the underlying surface and prevent the membrane from retracting. In this section, we take a closer look at how cells employ the various force-generating processes to move across a



▲ FIGURE 19-26 **Steps in keratinocyte movement.** In a fast-moving cell such as a fish epidermal cell, movement begins with the extension of one or more lamellipodia from the leading edge of the cell (1); some lamellipodia adhere to the substratum by focal adhesions (2). Then the bulk of the cytoplasm in the cell body flows forward (3). The trailing edge of the cell remains attached to the substratum until the tail eventually detaches and retracts into the cell body (4). See text for more discussion.

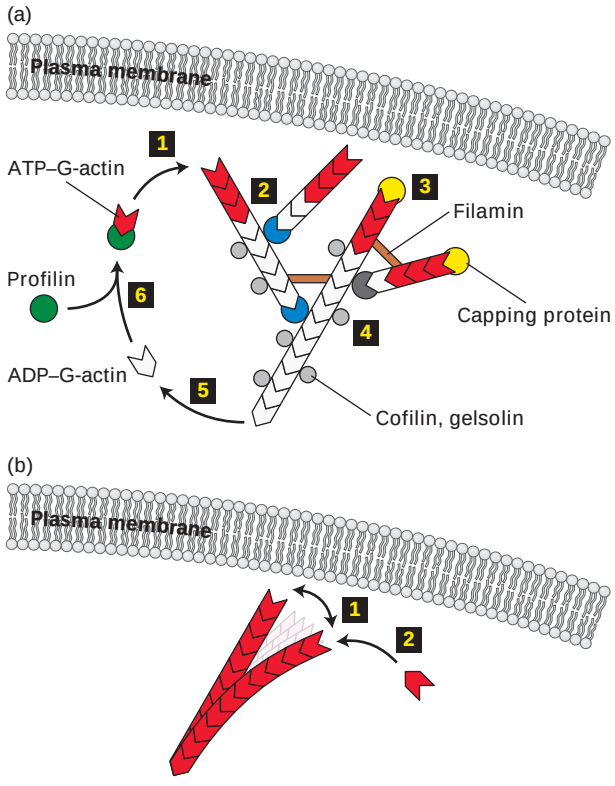
MEDIA CONNECTIONS
Overview Animation: Cell Motility
Video: Mechanics of Fish Keratinocyte Migration

surface. We also consider the role of signaling pathways in coordinating and integrating the actions of the cytoskeleton, a major focus of current research.

Cell Movement Coordinates Force Generation with Cell Adhesion

A moving keratinocyte (skin cell) and a moving fibroblast (connective tissue cell) display the same sequence of changes in cell morphology—initial extension of a membrane protrusion, attachment to the substratum, forward flow of cytosol, and retraction of the rear of the cell (Figure 19-26).

Membrane Extension The network of actin filaments at the leading edge (see Figure 19-12) is a type of a cellular engine that pushes the membrane forward by an actin polymerization–based mechanism (Figure 19-27a). The key



▶ FIGURE 19-27 **Forces produced by assembly of the actin network.** (a) As shown in this diagram, actin filaments are assembled into a branched network in which the ends of filaments approach the plasma membrane at an acute angle. ATP–G-actin (red) adds to the filament end and pushes the membrane forward (1). The Arp2/3 complex (blue) binds to sides of filaments (2) and forms a branch at a 70° angle from the filament. With time, filaments ends are capped by capping protein (yellow) (3); the ATP–G-actin subunits convert into ADP–G-actin subunits (white) (4) and dissociate from the filament through the action of the severing proteins cofilin and gelsolin (gray) (5). The released ADP–G-actin subunits form complexes with profilin (green) (6) to regenerate ATP–G-actin subunits. (b) The network of actin filaments supports the elongation of filaments and the generation of pushing forces. An actin filament is stiff but can bend from thermal fluctuations. In the elastic Brownian ratchet model, bending of filaments at the leading edge (1), where the (+) ends contact the membrane, creates space at the membrane for subunits to bind to the ends of filaments (2). The elastic recoil force of the filaments then pushes the membrane forward. [Part (a) adapted from T. M. Svitkina and G. G. Borisy, 1999, *J. Cell Biol.* **145**:1009.]

step in generating the force is (step 1) the addition of actin subunits at the ends of filaments close to the membrane. New filament ends are created (step 2) by branches formed by the Arp 2/3 complex. The branched network of filaments are stabilized by cross-linking proteins such as filamin. As the filaments grow, the ATP-actin subunits are converted into ADP-actin subunts. Consequently, (step 3) capping protein caps the (+) ends of filaments, and (step 4) cofilin and gelsolin fragment actin filaments and (step 5) cause actin subunits to dissociate. Profilin converts the ADP-actin monomers into a polymerization-competent ATP-actin monomer ready to participate in the next cycle.

A mechanism to explain what propels the membrane forward, called the *elastic Brownian ratchet model*, is based on the elastic mechanical property of an actin filament (Figure 19-27b). Electron micrographs show that the ends of actin filaments abut against the membrane, leaving no space for an actin subunit to bind. However, thermal energy causes a filament to bend, creating room for subunit addition. Because actin filaments have the same stiffness as that of a plastic rod, the energy stored in bending straightens the filament. The concerted action of numerous filaments undergoing similar movements and their cross-linkage into a mechanically strong network generate sufficient force (several piconewtons) to push the membrane forward.

Cell–Substrate Adhesions When the membrane has been extended and the cytoskeleton has been assembled, the membrane becomes firmly attached to the substratum. Time-lapse microscopy shows that actin bundles in the leading edge become anchored to the attachment site, which quickly develops into a focal adhesion. The attachment serves two purposes: it prevents the leading lamella from retracting and it attaches the cell to the substratum, allowing the cell to push forward.

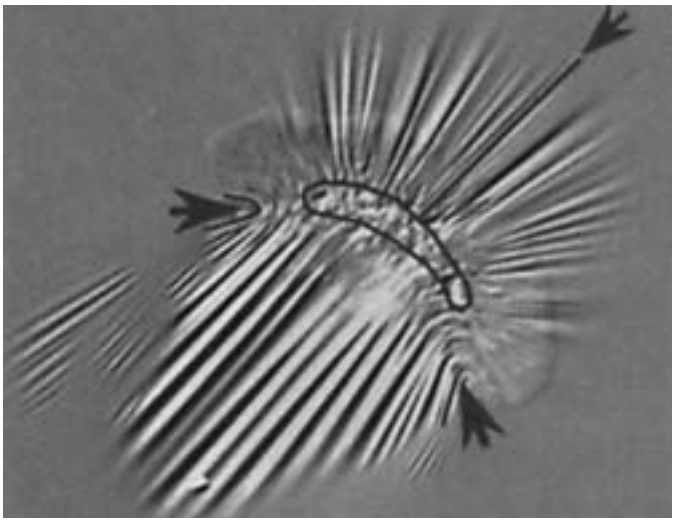
Cell Body Translocation After the forward attachments have been made, the bulk contents of the cell body are translocated forward (see Figure 19-26). How this translocation is accomplished is unknown; one speculation is that the nucleus and the other organelles are embedded in the cytoskeleton and that myosin-dependent cortical contraction moves the cytoplasm forward. The involvement of myosin-dependent cortical contraction in cell migration is supported by the localization of myosin II. Associated with the movement is a transverse band of myosin II and actin filaments at the boundary between the lamellipodia and the cell body (Figure 19-28).

Breaking Cell Attachments Finally, in the last step of movement (de-adhesion), the focal adhesions at the rear of the cell are broken and the freed tail is brought forward. In the light microscope, the tail is seen to "snap" loose from its connections—perhaps by the contraction of stress fibers in the tail or by elastic tension—but it leaves a little bit of its membrane behind, still firmly attached to the substratum.

The ability of a cell to move corresponds to a balance between the mechanical forces generated by the cytoskeleton and the resisting forces generated by cell adhesions. Cells cannot move if they are either too strongly attached or not

(a)

(b)



▲ EXPERIMENTAL FIGURE 19-28 **Contractile forces are generated by a moving cell.** (a) A fluorescence micrograph of a keratinocyte shows that the network of actin filaments (blue) is located at the front of the cell, whereas myosin II (red) is at the rear of the cell. However, both are located in a band (white) that traverses the cell just anterior to the nucleus. Contraction of this band is postulated to pull the cell body forward. (b) A moving cell exerts traction forces on the substratum. A keratinocyte plated on a thin silicon membrane exerts lateral forces from contraction of the cell body and causes the membrane to buckle. [Part (a) from T. M. Svitkina and G. G. Borisy, 1999, *J. Cell Biol.* **145**:1009; courtesy of T. M. Svitkina. Part (b) from K. Burton et al., 1999, *Mol. Biol. Cell* **10**:3745; courtesy of D. L. Taylor.]

attached to a surface. This relation can be demonstrated by measuring the rate of movement in cells that express varying levels of integrins, the cell-adhesion molecules that mediate most cell–matrix interactions (Chapter 6). Such measurements show that the fastest migration occurs at an intermediate level of adhesion, with the rate of movement falling off at high and low levels of adhesion. Cell locomotion thus results from traction forces exerted by the cell on the underlying substratum. The traction forces can be detected by the effects of cells on extremely thin sheets of silicon (Figure 19-28b). As a cell moves forward, contractile forces exerted at the front and the back of the cell cause the membrane to buckle. On a stiffer membrane that resists deformation, the buckling forces will be transformed into the forward movement of the cell.

Ameboid Movement Entails Reversible Gel–Sol Transitions of Actin Networks

Amebas are large, highly motile protozoans whose forward movement exhibits the same basic steps as those characterizing the movement of keratinocytes. Ameboid movement is initiated when the plasma membrane balloons forward to form a *pseudopodium*, or "false foot," which is similar to a lamellipodium in a vertebrate cell. As the pseudopodium attaches to the substratum, it fills with cytosol that is flowing forward through the cell. In the last step in movement, the rear of the ameba is pulled forward, breaking its attachments to the substratum.

Movement of an ameba is accompanied by changes in the viscosity of its cytosol, which cycles between sol and gel states. The central region of cytoplasm, the *endoplasm*, is a fluid, which flows rapidly toward the front of the cell, filling the pseudopodium. Here, the endoplasm is converted into the *ectoplasm*, a gel that forms the cortex, just beneath the plasma membrane. As the cell crawls forward, the ectoplasmic gel at the tail end of the cell is converted back into endoplasmic sol, only to be converted once again into ectoplasm when it again reaches the front of the cell. This cycling between sol and gel states continues only when the cell migrates.

The transformation between sol and gel states results from the disassembly and reassembly of actin microfilament networks in the cytosol. Several actin-binding proteins probably control this process and hence the viscosity of the cytosol. Profilin at the front of the cell promotes actin polymerization, and α-actinin and filamin form gel-like actin networks in the more viscous ectoplasm, as discussed earlier. Conversely, proteins such as cofilin sever actin filaments to form the more fluid endoplasm.

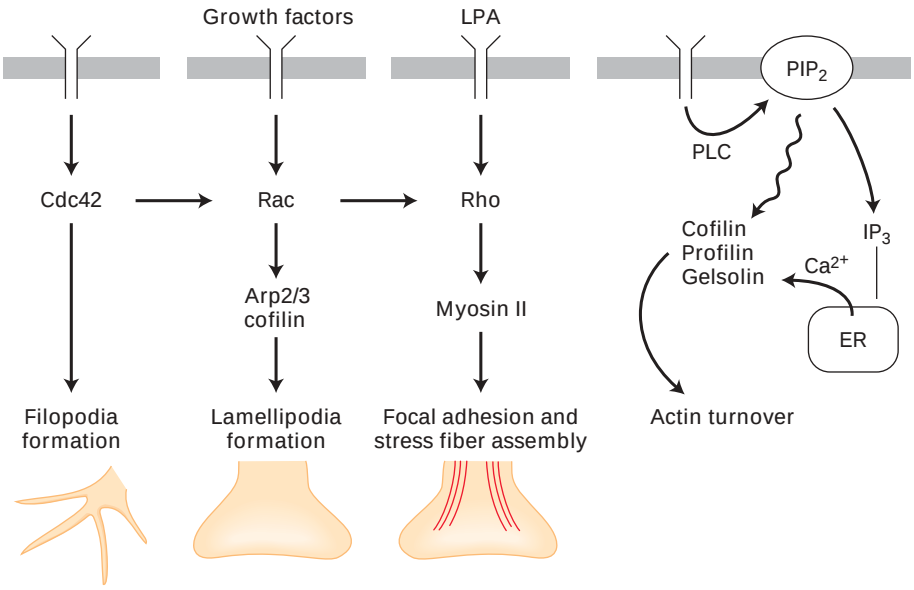
External Signals and Various Signaling Pathways Coordinate Events That Lead to Cell Migration

A striking feature of a moving cell is its polarity: a cell has a front and a back. When a cell makes a turn, a new leading lamellipodium or pseudopodium forms in the new direction. If these extensions form in all directions, as in myosin I ameba mutants, then the cell is unable to pick a new direction of movement. To sustain movement in a particular direction, a cell requires signals to coordinate events at the front of the cell with events at the back and, indeed, signals to tell the cell where its front is. In this section, we present several examples of how external signals activate cell migration and control the direction of movement.

Activation of Filopodia, Membrane Ruffles, and Stress Fibers by Growth Factors Certain growth factors in a fresh wound stimulate a quiescent cultured fibroblast to grow and divide by forming filopodia and lamellipodia at its leading edge and later to assemble stress fibers and focal adhesions. Similar signal-induced events are thought to take place in the wound-healing response of fibroblasts in vivo, the development of cells in embryos, and the metastasis of cancer cells. These events require the polymerization of actin filaments, the activation of myosin molecules, and the assembly of actin bundles and networks. The cytoskeletal rearrangements that are a part of the wound-healing response of fibroblasts include intracellular signaling pathways directed by Rac, Rho, and Cdc42, all Ras-like molecules belonging to the **GTPase superfamily** of switch proteins. These pathways are activated by the binding of growth factors to receptor tyrosine kinases, a class of cell-surface receptors described in Chapter 13.

The roles of Ras-related proteins were revealed by simple microinjection experiments. When Rac was microinjected into a fibroblast, the membrane immediately started to form upward projections called *ruffles;* focal adhesions and stress fibers formed 5–10 minutes later. Injection of an inactive form of Rac inhibited all reorganization of the actin cytoskeleton when growth factors were added to the cell. When Rho, rather than Rac, was injected, it mimicked the mitogenic effects of lysophosphatidic acid (LPA), a chemokine in serum and a potent stimulator of platelet aggregation. Both Rho and LPA induced the assembly of stress fibers and focal adhesions within 2 minutes but did not induce membrane ruffling.

These findings lead to a model in which extracellular factors trigger Ras-linked signal-transduction pathways that activate actin polymerization at the leading-edge membrane as an early event and the formation of focal adhesions as a later event (Figure 19-29). If this model is correct, then the inhibition of stress-fiber assembly should not affect membrane ruffling. To test the model, Rac and ADP-ribosylase, an enzyme that inactivates Rho by covalently attaching ADP to it, were co-injected into a fibroblast. As predicted, membrane ruffles were formed, but the assembly of stress fibers was blocked. These observations suggest that Rho-dependent events such as stress-fiber formation are "downstream" of control by Rac. The results of later experiments in which Cdc42 was microinjected into fibroblasts showed that this protein controlled an earlier step, the formation of filopodia. Thus the sequence of events in wound healing begins with the participation of filopodia and lamellipodia during the



▲ FIGURE 19-29 **Role of signal-transduction pathways in cell locomotion and the organization of the cytoskeleton.** Extracellular signals are transmitted across the plasma membrane by receptors specific for different factors. One set of growth factors induces actin polymerization at the leading edge through a Rac- and Cdc42-dependent pathway (*left*); another set of factors acts downstream through a Rho-dependent pathway to induce the assembly of focal adhesions and cortical contraction (*center*). Adhesion of a cell to the extracellular matrix triggers a parallel signaling pathway that induces the activation of profilin, cofilin, and gelsolin (*right*). Triggering of this pathway activates phospholipase C (PLC), which hydrolyzes PIP_2 in the membrane. The subsequent increase in cytosolic Ca^{2+} stimulates actin turnover.

migration of cells into the wound and the formation of focal adhesions and stress fibers to close the wound.

An important aspect of locomotion is how movement is coordinated in response to different stimuli. For example, the assembly of the branched actin network at the membrane is enhanced by the action of several signaling pathways and their adapter proteins. The branching activity of the Arp2/3 complex is activated by an adapter protein, WASp, under the control of the Cdc42 GTPase. In addition, as discussed previously, the hydrolysis of PIP_2 by phospholipase C releases profilin, cofilin, and gelsolin from the membrane. In another pathway, inositol 1,4,5-trisphosphate (IP_3), a by-product of PIP_2 hydrolysis, stimulates the release of Ca^{2+} ions from the endoplasmic reticulum into the cytosol; this increase in Ca^{2+} ions activates myosin II and the severing activity of gelsolin. These parallel pathways thus stimulate both actin severing and filament growth, thereby increasing actin turnover (Figure 19-29, *right*).

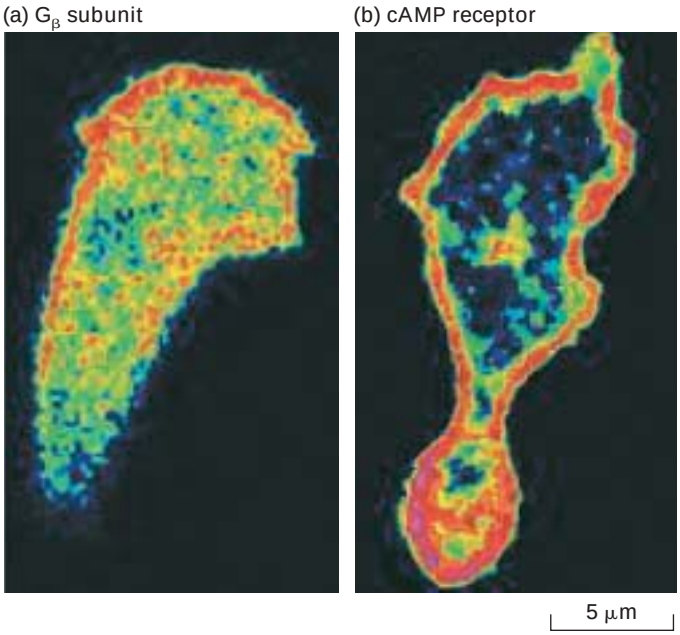
Steering of Migrating Cells by Chemotactic Molecules

Under certain conditions, extracellular chemical cues guide the locomotion of a cell in a particular direction. In some cases, the movement is guided by insoluble molecules in the underlying substratum. In other cases, the cell senses soluble molecules and follows them, along a concentration gradient, to their source. The latter response is called **chemotaxis.** One of the best-studied examples of chemotaxis is the migration of *Dictyostelium* amebas along an increasing concentration of cAMP. Following cAMP to its source, the amebas aggregate into a slug and then differentiate into a fruiting body. Many other cells also display chemotactic movements. For example, leukocytes are guided by a tripeptide secreted by many bacterial cells. In the development of skeletal muscle, a secreted protein signal called scatter factor guides the migration of myoblasts to the proper locations in limb buds (Chapter 22).

Despite the variety of different chemotactic molecules—sugars, peptides, cell metabolites, cell-wall or membrane lipids—they all work through a common and familiar mechanism: binding to cell-surface receptors, activation of intracellular signaling pathways, and re-modeling of the cytoskeleton through the activation or inhibition of various actin-binding proteins. The central question is, How do cell-surface receptors detect as small as a 2 percent difference in the concentration of chemotactic molecules across the length of the cell? To direct cell migration, an external chemoattractant gradient must somehow induce internal gradients that lead to polarization of the actin cytoskeleton.

Coincident Gradients of Chemoattractants, Activated G Proteins, and Ca^{2+}

Micrographs of cAMP receptors tagged with green fluorescent protein (GFP) show that the receptors are distributed uniformally along the length of an ameba cell (Figure 19-30). Therefore an internal gradient must be established by another component of the signalling pathway. Because cAMP receptors signal through trimeric G proteins, a subunit of the trimeric G protein and other downstream signaling proteins were tagged with GFP. Fluorescence micrographs show that the concentration of trimeric G proteins is higher in the direction of the chemoattractant. Trimeric G proteins coupled to cAMP receptors can activate pathways



◀ EXPERIMENTAL FIGURE 19-30
Chemoattractant is used to demonstrate signal-induced gradient of G_β subunit. False color images of amebas expressing (a) a GFP-tagged G_β subunit and (b) a GFP-tagged cAMP receptor, which served as a control. When an external source of chemoattractant was placed near the cells (at the top of the photographs), the cells turned toward the source. (a) The G_β subunit became concentrated at the leading edge of the cell, closest to the chemoattractant, and depleted from the tail. (b) In contrast, the cAMP receptor retained a uniform distribution in the cell membrane. [W. F. Loomis and R. H. Insall, 1999, *Nature* **401**:440–441; courtesy of Peter Devreotes Laboratory.]

leading to the activation of Arp 2/3 through the mediator protein WASp or through other pathways that increase cytosolic Ca^{2+} (see Figure 13-29).

Findings from studies with fluorescent dyes that act as internal Ca^{2+} sensors indicate that a cytosolic gradient of Ca^{2+} also is established in migrating cells, with the lowest concentration at the front of the cell and the highest concentration at the rear. Moreover, if a pipette containing a chemoattractant is placed to the side of a migrating leukocyte, the overall concentration of cytosolic Ca^{2+} first increases and then the Ca^{2+} gradient reorients, with the lowest concentration on the side of the cell closest to the pipette, causing the cell to turn toward the chemotactic source. After the chemoattractant is removed, the cell continues to move in the direction of its newly established Ca^{2+} gradient (see Figure 5-47).

We have seen that many actin-binding proteins, including myosins I and II, gelsolin, α-actinin, and fimbrin, are regulated by Ca^{2+}. Hence the cytosolic Ca^{2+} gradient may regulate the sol-to-gel transitions that take place in cell movement. The low Ca^{2+} concentration at the front of the cell would favor the formation of actin networks by activating myosin I, inactivating actin-severing proteins, and reversing the inhibition of Ca^{2+}-regulated actin cross-linking proteins. The high Ca^{2+} concentration at the rear of the cell would cause actin networks to disassemble and a sol to form by activating gelsolin or would cause cortical actin networks to contract by activating myosin II. Thus an internal gradient of Ca^{2+} would contribute to the turnover of actin filaments in migrating cells.

KEY CONCEPTS OF SECTION 19.4

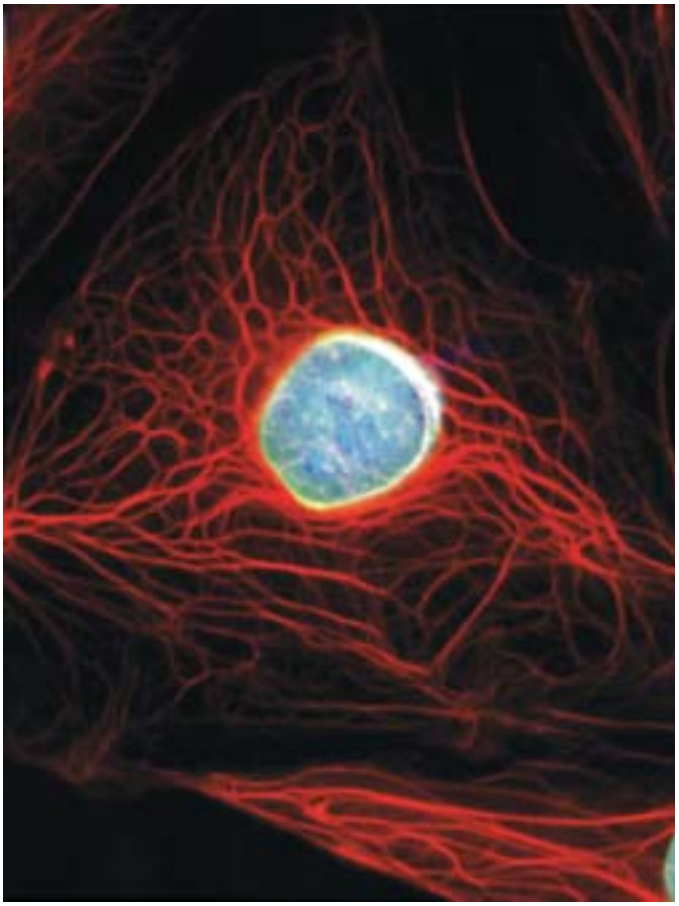
Cell Locomotion

- Migrating cells undergo a series of characteristic events: extension of a lamellipodium or pseudopodium, adhesion of the extended leading edge to the substratum, forward flow (streaming of the cytosol), and retraction of the cell body (see Figure 19-26).
- Cell locomotion is probably through a common mechanism including actin polymerization and branching–generated movement at the leading edge, assembly of adhesion structures, and cortical contraction mediated by myosin II (see Figure 19-28).
- External signals (e.g., growth factors and chemoattractants) induce the assembly and organization of the cytoskeleton and the establishment of an internal gradient of trimeric G proteins and calcium (see Figure 19-29). The resulting polarization of the cell leads to locomotion.

19.5 Intermediate Filaments

In the remainder of this chapter, we consider the properties of intermediate filaments (IFs) and the cytoskeletal structures that they form in cells. Intermediate filaments are found in nearly all animals but not in in plants and fungi. The association of intermediate filaments with the nuclear and plasma membranes suggests that their principal function is structural (Figure 19-31). In epithelium, for instance, intermediate filaments provide mechanical support for the plasma membrane where it comes into contact with other cells or with the extracellular matrix. In epidermal cells (outer layer of skin) and the axons of neurons (Figure 19-32), intermediate filaments are at least 10 times as abundant as microfilaments or microtubules, the other components of the cytoskeleton.

Much of the following discussion about intermediate filaments will seem familiar because their cellular organization is similar to that of the actin microfilaments discussed in preceding sections. These two types of cytoskeletal fibers are



▲ EXPERIMENTAL FIGURE 19-31 **Staining with fluorochrome-tagged antibodies reveals cellular distribution of keratin and lamin intermediate filaments.** In this fluorescent micrograph of a PtK2 cell doubly stained with anti-keratin and anti-lamin antibodies, a meshwork of lamin intermediate filaments (blue) can be seen underlying the nuclear membrane. The cytoplasmic keratin cytoskeleton (red) extends from the nuclear membrane to the plasma membrane. [Courtesy of R. D. Goldman.]

also similar in that they are usually associated with cell membranes. Unlike microfilaments and microtubules, however, intermediate filaments do not contribute to cell motility. There are no known examples of IF-dependent cell movements or of motor proteins that move along intermediate filaments.

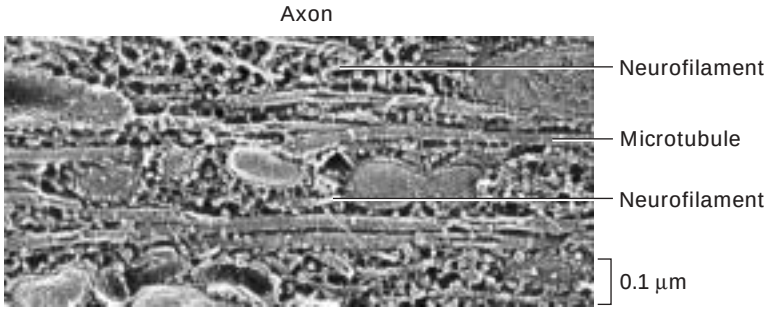
Intermediate Filaments Differ in Stability, Size, and Structure from Other Cytoskeletal Fibers

Several physical and biochemical properties distinguish intermediate filaments from microfilaments and microtubules. To begin with, intermediate filaments are extremely stable. Even after extraction with solutions containing detergents and high concentrations of salts, most intermediate filaments in a cell remain intact, whereas microfilaments and microtubules depolymerize into their soluble subunits. In fact, most IF purification methods employ these treatments to free intermediate filaments from other proteins. Intermediate filaments also differ in size from the other two cytoskeletal fibers. Indeed, their name derives from their 10-nm diameter—smaller than microtubules (24 nm) but larger than microfilaments (7 nm) (see Figure 5-29). Moreover, in contrast with the globular actin and tubulin subunits, which polymerize into microfilaments and hollow microtubules, respectively, IF subunits are α-helical rods that assemble into ropelike filaments. Finally, IF subunits do not bind nucleotides, and their assembly into intermediate filaments does not involve the hydrolysis of ATP or GTP, as does the polymerization of G-actin and tubulin. However, many of the details concerning the assembly of intermediate filaments in cells remain speculative.

IF Proteins Are Classified According to Their Distributions in Specific Tissues

In higher vertebrates, the subunits composing intermediate filaments constitute a superfamily of highly α helical proteins that are found in the cytoplasm of different tissues and at the nuclear membrane. The superfamily is divided into four groups on the basis of similarities in sequence and their patterns of expression in cells (Table 19-4). Unlike the actin and tubulin isoforms, the various classes of IF proteins are widely divergent in sequence and vary greatly in molecular weight. We introduce the four groups here and consider their functions in various cells in more detail later.

The most ubiquitous group of IFs are the **lamins**. In contrast with the cytosolic location of the other four classes of IF proteins, lamins are found exclusively in the nucleus. Of the three nuclear lamins, two are alternatively spliced products encoded by a common gene, whereas the third is encoded by a separate gene. A single lamin gene is found in the



▶ EXPERIMENTAL FIGURE 19-32 **Deep-etching reveals microtubules and intermediate filaments in a neuronal axon.** Neurofilaments and microtubules in a quick-frozen frog axon are visualized by the deep-etching technique. Several 24-nm-diameter microtubules run longitudinally; thinner, 10-nm-diameter intermediate filaments also run longitudinally. Occasional connections link the two types of cytoskeletal fibers. [From N. Hirokawa, 1982, *J. Cell Biol.* **94**:129; courtesy of N. Hirokawa.]

TABLE 19-4 Primary Intermediate Filaments in Mammals

IF Protein	MW (10^{-3})*	Filament Form	Tissue Distribution
Nuclear Lamins			
Lamin A	70	Homopolymer	Nucleus
Lamin B	67	Homopolymer	Nucleus
Lamin C	67	Homopolymer	Nucleus
Keratins†			
Acidic keratins	40–57	Heteropolymers	Epithelia
Basic keratins	53–67	Heteropolymers	Epithelia
Type III Intermediate Filaments			
Vimentin	57	Homo- and heteropolymers	Mesenchyme (fibroblasts)
Desmin	53	Homo- and heteropolymers	Muscle
Glial fibrillary acidic protein	50	Homo- and heteropolymers	Glial cells, astrocytes
Peripherin	57	Homo- and heteropolymers	Peripheral and central neurons
Neurofilaments			
NF-L	62	Homopolymers	Mature neurons
NF-M	102	Heteropolymers	Mature neurons
NF-H	110	Heteropolymers	Mature neurons
Internexin	66	—	Developing CNS

*Intermediate filaments show species-dependent variations in molecular weight (MW).
†More than 15 isoforms of both acidic and basic keratins are known.

Drosophila genome; none are in the yeast genome. Because the lamin, but not the cytosolic, groups of IFs are expressed in *Drosophila,* lamins are probably the evolutionary precursor of the IF superfamily.

Epithelial cells express acidic and basic **keratins.** They associate in a 1:1 ratio to form heterodimers, which assemble into heteropolymeric keratin filaments; neither type alone can assemble into a keratin filament. The keratins are the most diverse classes of IF proteins, with a large number of keratin isoforms being expressed. These isoforms can be divided into two groups: about 10 keratins are specific for "hard" epithelial tissues, which give rise to nails, hair, and wool; and about 20, called *cytokeratins,* are more generally found in the epithelia that line internal body cavities. Each type of epithelium always expresses a characteristic combination of acidic and basic keratins.

Four proteins are classified as **type III** IF proteins. Unlike the keratins, the type III proteins can form both homo- and heteropolymeric IF filaments. The most widely distributed of all IF proteins is *vimentin,* which is typically expressed in leukocytes, blood vessel endothelial cells, some epithelial cells, and mesenchymal cells such as fibroblasts (see the illustration at the beginning of this chapter). Vimentin filaments help support cellular membranes. Vimentin networks may also help keep the nucleus and other organelles in a defined place within the cell. Vimentin is frequently associated with microtubules and, as noted earlier, the network of vimentin filaments parallels the microtubule network (see Figure 1-15). The other type III IF proteins have a much more limited distribution. *Desmin* filaments in muscle cells are responsible for stabilizing sarcomeres in contracting muscle. *Glial fibrillary acidic protein* forms filaments in the glial cells that surround neurons and in astrocytes. *Peripherin* is found in neurons of the peripheral nervous system, but little is known about it.

The core of neuronal axons is filled with **neurofilaments (NFs),** each a heteropolymer composed of three polypeptides—NF-L, NF-M, and NF-H—which differ greatly in molecular weight (see Figure 19-32 and Table 19-4). Neurofilaments are responsible for the radial growth of an axon and thus determine axonal diameter, which is directly related to the speed at which it conducts impulses. The influence of the number of

neurofilaments on impulse conduction is highlighted by a mutation in quails named *quiver,* which blocks the assembly of neurofilaments. As a result, the velocity of nerve conduction is severely reduced. Also present in axons are microtubules, which direct axonal elongation.

MEDICINE

Because of their characteristic distributions, IF proteins are useful in the diagnosis and treatment of certain tumors. In a tumor, cells lose their normal appearance, and thus their origin cannot be identified by their morphology. However, tumor cells retain many of the differentiated properties of the cells from which they are derived, including the expression of particular IF proteins. With the use of fluorescence-tagged antibodies specific for those IF proteins, diagnosticians can often determine whether a tumor originated in epithelial, mesenchymal, or neuronal tissue.

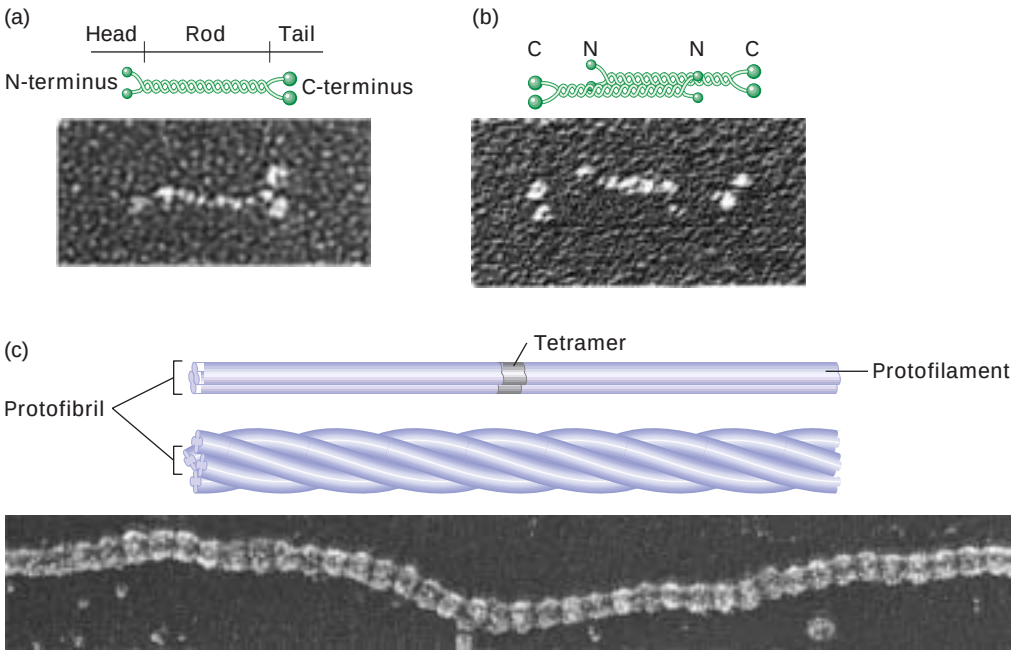
For example, the most common malignant tumors of the breast and gastrointestinal tract contain keratins and lack vimentin; thus they are derived from epithelial cells (which contain keratins but not vimentin) rather than from the underlying stromal mesenchymal cells (which contain vimentin but not keratins). Because epithelial cancers and mesenchymal cancers are sensitive to different treatments, identifying the IF proteins in a tumor cell helps a physician select the most effective treatment for destroying the tumor. ▮

All IF Proteins Have a Conserved Core Domain and Are Organized Similarly into Filaments

Besides having in common an ability to form filaments 10 nm in diameter, all IF subunit proteins have a common domain structure: a central α-helical core flanked by globular N- and C-terminal domains. The core helical domain, which is conserved among all IF proteins, consists of four long α helices separated by three nonhelical, "spacer" regions. The α-helical segments pair to form a coiled-coil dimer.

In electron micrographs, an IF-protein dimer appears as a rodlike molecule with globular domains at the ends; two dimers associate laterally into a tetramer (Figure 19-33a, b). The results of labeling experiments with antibodies to the N- or C-terminal domain indicate that the polypeptide chains are parallel in a dimer, whereas the dimers in a tetramer have an antiparallel orientation. The next steps in assembly are not well understood but seem to include the end-to-end association of tetramers to form long protofilaments, which aggregate laterally into a loose bundle of protofibrils. Compaction of a protofibril yields a mature 10-nm-diameter filament with the N- and C-terminal globular domains of the tetramers forming beaded clusters along the surface (Figure 19-33c). Interestingly, because the tetramer is symmetric, an intermediate filament may not have a polarity, as does an actin filament or a microtubule. This idea is supported by findings from experiments showing that vimentin subunits can incorporate along the length, as well as the ends, of a filament.

Although the α-helical core is common to all IF proteins, the N- and C-terminal domains of different types of IF proteins vary greatly in molecular weight and sequence. Partly because of this lack of sequence conservation, scientists initially speculated that the N- and C-terminal domains do not have roles in IF assembly. The results of several subsequent experiments, however, proved this hypothesis to be partly incorrect. For instance, if the N-terminal domain of an IF



▶ EXPERIMENTAL FIGURE 19-33 **Electron microscopy visualizes intermediate structures in the assembly of intermediate filaments.** Shown here are electron micrographs and drawings of IF protein dimers and tetramers and of mature intermediate filaments from *Ascaris,* an intestinal parasitic worm. (a) IF proteins form parallel dimers with a highly conserved coiled-coil core domain and globular tails and heads, which are variable in length and sequence. (b) A tetramer is formed by antiparallel, staggered side-by-side aggregation of two identical dimers. (c) Tetramers aggregate end-to-end and laterally into a protofibril. In a mature filament, consisting of four protofibrils, the globular domains form beaded clusters on the surface. [Adapted from N. Geisler et al., 1998, *J. Mol. Biol.* **282**:601; courtesy of Ueli Aebi.]

protein is shortened, either by proteolysis or by deletion mutagenesis, the truncated protein cannot assemble into filaments. (Keratins are an exception; they form filaments even if both terminal domains are absent.) The prevailing view now is that the N-terminal domain plays an important role in the assembly of most intermediate filaments. Even though the C-terminal domain is dispensable for IF assembly, it seems to affect the organization of IF cytoskeletons in a cell. Thus these domains may control lateral interactions within an intermediate filament, as well as interactions between intermediate filaments and other cellular components.

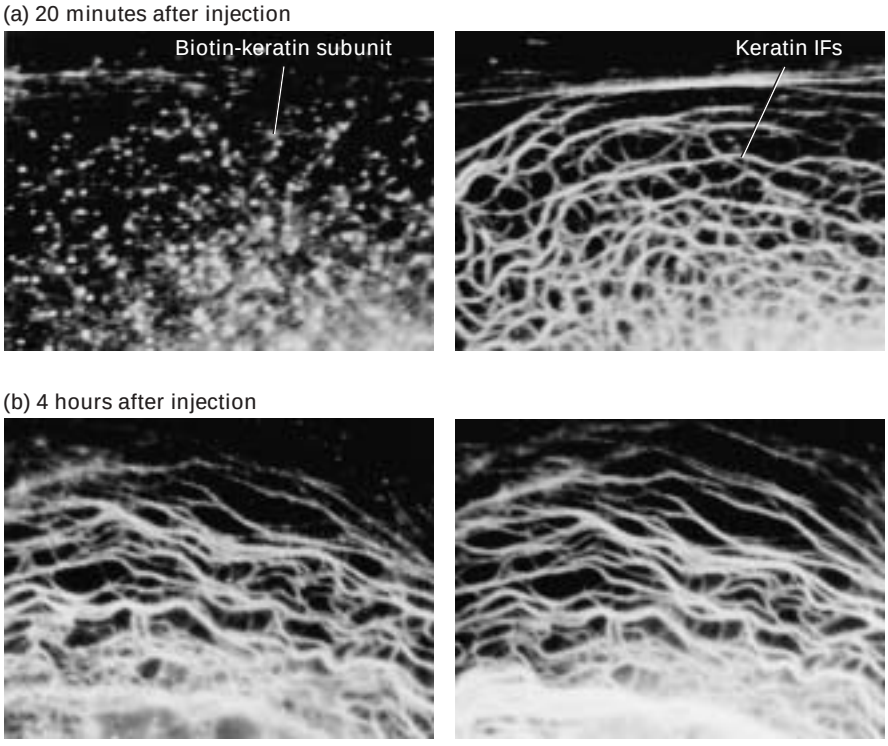
Identity of IF Subunits Whether IF monomers, dimers, or tetramers constitute the immediate subunit for assembly of filaments, analogous to G-actin monomers in the assembly of microfilaments, is still unresolved. The main supporting evidence for the involvement of the IF tetramer comes from cell fractionation experiments showing that, although most vimentin in cultured fibroblasts is polymerized into filaments, 1–5 percent of the protein exists as a soluble pool of tetramers. The presence of a tetramer pool suggests that vimentin monomers are rapidly converted into dimers, which rapidly form tetramers.

Homo- and Heteropolymeric Filaments Some IF proteins form homopolymeric filaments; others form only heteropolymeric filaments with other proteins in their class; and some can form both homo- and heteropolymeric filaments. Some IF proteins, but not the keratins, can form heteropolymers with IF proteins in another class. NF-L self-associates to form a homopolymer, but NF-H and NF-M commonly coassemble with the NF-L backbone, and so most neurofilaments contain all three proteins. Spacer sequences in the coiled-coil regions of IF dimers or sequences in the diverse N- or C-terminal domains or both are most likely responsible for determining whether particular IF proteins assemble into heteropolymers or homopolymers. In fact, mutations in these regions generate mutated IF polypeptides that can form heterooligomers with normal IF proteins. These hybrid molecules often "poison" IF polymerization by blocking assembly at an intermediate stage. The ability of mutated IF proteins to block IF assembly has proved extremely useful in studies of the function of intermediate filaments in a cell. At the end of the chapter, we look at how such mutations in keratins have revealed the role of keratin filaments in the epidermis.

Intermediate Filaments Are Dynamic

Although intermediate filaments are clearly more stable than microtubules and microfilaments, IF proteins have been shown to exchange with the existing IF cytoskeleton. In one experiment, a biotin-labeled type I keratin was injected into fibroblasts; within 2 hours after injection, the labeled protein had been incorporated into the already existing keratin cytoskeleton (Figure 19-34). The results of this experiment and others demonstrate that IF subunits in a soluble pool are able to add themselves to preexisting filaments and that subunits are able to dissociate from intact filaments.

The relative stability of intermediate filaments presents special problems in mitotic cells, which must reorganize all



◀ EXPERIMENTAL FIGURE 19-34
Chemical labeling and fluorescent staining reveal the incorporation of type I keratin into existing IF cytoskeleton. Monomeric type I keratin was purified, chemically labeled with biotin, and microinjected into living fibroblast cells. The cells were then fixed at different times after injection and stained with a fluorescent antibody to biotin and with antibodies to keratin. (a) At 20 minutes after injection, the injected biotin-labeled keratin is concentrated in small foci scattered through the cytoplasm (*left*) and has not been integrated into the endogenous keratin cytoskeleton (*right*). (b) By 4 hours, the biotin-labeled subunits (*left*) and the keratin filaments (*right*) display identical patterns, indicating that the microinjected protein has become incorporated into the existing cytoskeleton. [From R. K. Miller, K. Vistrom, and R. D. Goldman, 1991, *J. Cell Biol.* **113**:843; courtesy of R. D. Goldman.]

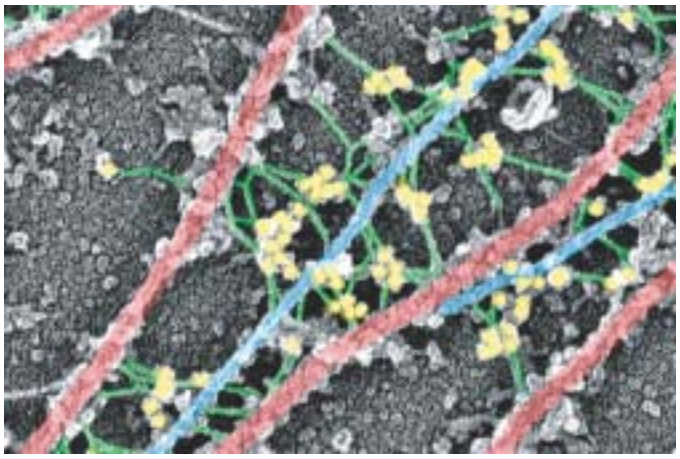
three cytoskeletal networks in the course of the cell cycle. In particular, breakdown of the nuclear envelope early in mitosis depends on the disassembly of the lamin filaments that form a meshwork supporting the membrane. As discussed in Chapter 21, the phosphorylation of nuclear lamins by Cdc2, a **cyclin-dependent kinase** that becomes active early in mitosis (prophase), induces the disassembly of intact filaments and prevents their reassembly. Later in mitosis (telophase), removal of these phosphates by specific phosphatases promotes lamin reassembly, which is critical to reformation of a nuclear envelope around the daughter chromosomes. The opposing actions of kinases and phosphatases thus provide a rapid mechanism for controlling the assembly state of lamin intermediate filaments. Other intermediate filaments undergo similar disassembly and reassembly in the cell cycle.

Various Proteins Cross-Link Intermediate Filaments to One Another and to Other Cell Structures

Intermediate filament–associated proteins (IFAPs) cross-link intermediate filaments with one another, forming a bundle or a network, and with other cell structures, including the plasma membrane. Only a few IFAPs have been identified to date, but many more will undoubtedly be discovered as researchers focus attention on the proteins that control IF organization and assembly. Unlike actin-binding proteins or microtubule-associated proteins, none of the known IFAPs sever or cap intermediate filaments, sequester IF proteins in a soluble pool, or act as a motor protein. Rather, IFAPs appear to play a role in organizing the IF cytoskeleton, integrating the IF cytoskeleton with both the microfilament and the microtubule cytoskeletons, and attaching the IF cytoskeleton to the nuclear membrane and plasma membrane, especially at cell junctions.

A physical linkage between intermediate filaments and microtubules can be detected with certain drugs. Treatment of cells with high concentrations of colchicine causes the complete dissolution of microtubules after a period of several hours. Although vimentin filaments in colchicine-treated cells remain intact, they clump into disorganized bundles near the nucleus. This finding demonstrates that the organization of vimentin filaments is dependent on intact microtubules and suggests the presence of proteins linking the two types of filaments. In other studies, IFs have been shown to be cross-linked to actin filaments.

One family of IFAPs, the **plakins,** is responsible for linking IFs with both microtubules and microfilaments. One plakin family member is *plectin,* a 500,000-MW protein that has been shown to cross-link intermediate filaments with microtubules and actin filaments in vitro. Plectin also interacts with other cytoskeletal proteins, including spectrin, microtubule-associated proteins, and lamin B. Immunoelectron microscopy reveals gold-labeled antibodies to plectin decorating short, thin connections between microtubules and vimentin,



▲ EXPERIMENTAL FIGURE 19-35 **Gold-labeled antibody allows visualization of plectin cross-links between intermediate filaments and microtubules.** In this immunoelectron micrograph of a fibroblast cell, microtubules are highlighted in red; intermediate filaments, in blue; and the short connecting fibers between them, in green. Staining with gold-labeled antibodies to plectin (yellow) reveals that these fibers contain plectin. [From T. M. Svitkina, A. B. Verkhovsky, and G. G. Borisy, 1996, *J. Cell Biol.* **135**:991; courtesy of T. M. Svitkina.]

indicating the presence of plectin in these cross-links (Figure 19-35). The N-terminus of plectin and other plakins contains a calponin-homology (CH) domain similar to that in fimbrin and other actin cross-linking proteins. This finding suggests that some plakins form cross-links between actin microfilaments and intermediate filaments.

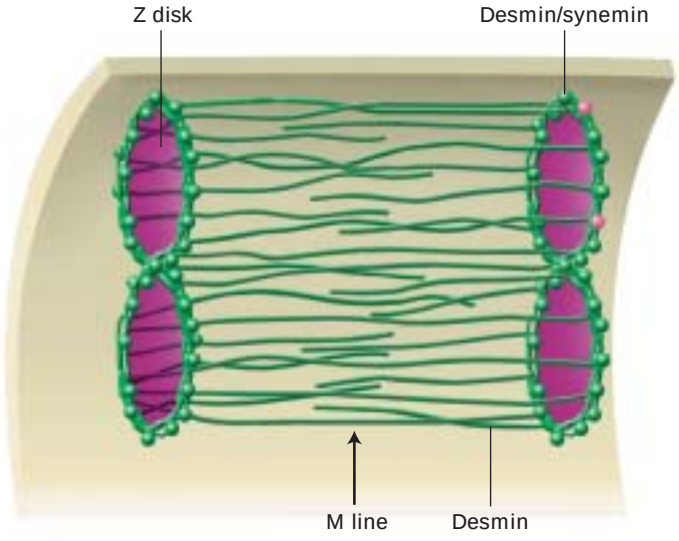
Cross-links between microtubules and neurofilaments are seen in micrographs of nerve-cell axons (see Figure 19-32). Although the identity of these connections in axons is unknown, they may be IFAPs whose function is to cross-link neurofilaments and microtubules into a stable cytoskeleton. Alternatively, these connections to microtubules may be the long arms of NF-H, which is known to bind microtubules.

IF Networks Form Various Supportive Structures and Are Connected to Cellular Membranes

A network of intermediate filaments is often found as a laminating layer adjacent to a cellular membrane, where it provides mechanical support. The best example is the **nuclear lamina** along the inner surface of the nuclear membrane (see Figure 21-16). This supporting network is composed of lamin A and lamin C filaments cross-linked into an orthogonal lattice, which is attached by lamin B to the inner nuclear membrane through interactions with a lamin B receptor, an IFAP, in the membrane. Like the membrane skeleton of the plasma membrane, the lamin nuclear skeleton not only supports the inner nuclear membrane but also provides sites where nuclear pores and interphase chromosomes attach. Thus, the nuclear lamins organize the nuclear contents from the outside in.

In addition to forming the nuclear lamina, intermediate filaments are typically organized in the cytosol as an extended system that stretches from the nuclear envelope to the plasma membrane (see Figure 19-31). Some intermediate filaments run parallel to the cell surface, whereas others traverse the cytosol; together they form an internal framework that helps support the shape and resilience of the cell. The results of in vitro binding experiments suggest that, at the plasma membrane, vimentin filaments bind two proteins: ankyrin, the actin-binding protein associated with the Na^+/K^+ ATPase in nonerythroid cells, and plectin, which also binds to α6β4 integrin in certain cell junctions (Chapter 6). Through these two IFAPs, the vimentin cytoskeleton is attached to the plasma membrane, providing a flexible structural support.

In muscle, a lattice composed of a band of desmin filaments surrounds the sarcomere (Figure 19-36). The desmin filaments encircle the Z disk and are cross-linked to the plasma membrane by several IFAPs, including paranemin and ankyrin. Longitudinal desmin filaments cross to neighboring Z disks within the myofibril, and connections between desmin filaments around Z disks in adjacent myofibrils serve to cross-link myofibrils into bundles within a muscle cell. The lattice is also attached to the sarcomere through interactions with myosin thick filaments. Because the desmin filaments lie outside the sarcomere, they do not actively participate in generating contractile forces. Rather, desmin plays an essential structural role in maintaining muscle integrity. In transgenic mice lacking desmin, for example, this supporting architecture is disrupted and muscles are misaligned.



▲ FIGURE 19-36 **Diagram of desmin filaments in muscle.** These type III intermediate filaments encircle the Z disk and make additional connections to neighboring Z disks. The alignment of desmin filaments with the muscle sarcomere is held in place at the Z disk by a collar of desmin/synemin heteropolymers.

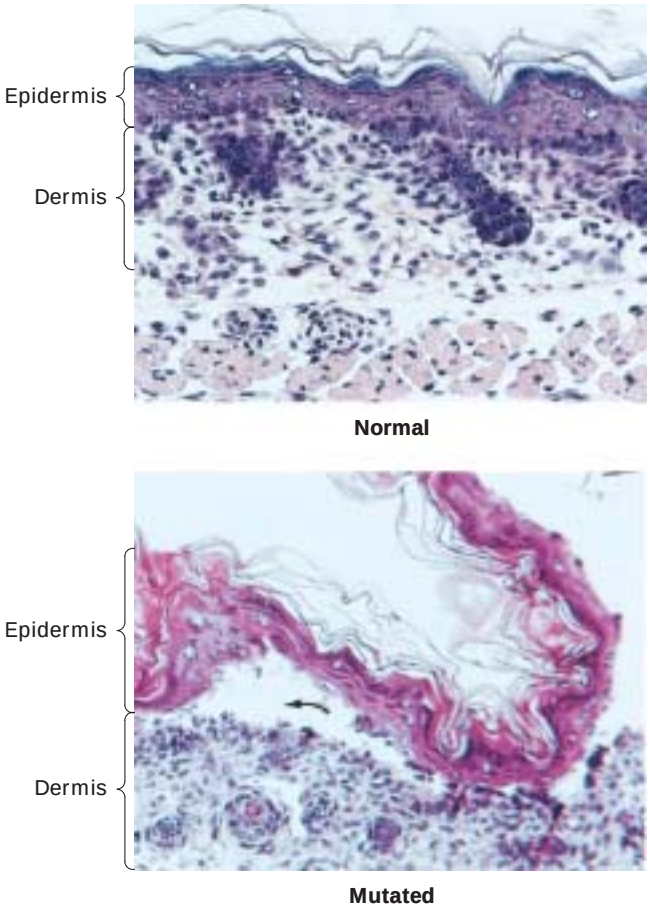
In Chapter 6, we describe the linkage between keratin filaments in epithelial cells and two types of anchoring junctions: *desmosomes,* which mediate cell–cell adhesion, and *hemidesmosomes,* which are responsible for attaching cells to the underlying extracellular matrix. In the electron microscope, both junctions appear as darkly staining proteinaceous plaques that are bound to the cytosolic face of the plasma membrane and attached to bundles of keratin filaments (see Figure 6-8). The keratin filaments in one cell are thus indirectly connected to those in a neighboring cell by desmosomes or to the extracellular matrix by hemidesmosomes. As a result of these connections, shearing forces are distributed from one region of a cell layer to the entire sheet of epithelial cells, providing strength and rigidity to the entire epithelium. Without the supporting network of intermediate filaments, an epithelium remains intact, but the cells are easily damaged by abrasive forces. Like actin microfilaments, which are attached to a third type of cell junction in epithelial cells, intermediate filaments form a flexible but resilient framework that gives structural support to an epithelium.

Disruption of Keratin Networks Causes Blistering

The epidermis is a tough outer layer of tissue, which acts as a water-tight barrier to prevent desiccation and serves as a protection against abrasion. In epidermal cells, bundles of keratin filaments are cross-linked by *filaggrin,* an IFAP, and are anchored at their ends to desmosomes. As epidermal cells differentiate, the cells condense and die, but the keratin filaments remain intact, forming the structural core of the dead, keratinized layer of skin. The structural integrity of keratin is essential in order for this layer to withstand abrasion.

MEDICINE

In humans and mice, the K4 and K14 keratin isoforms form heterodimers that assemble into protofilaments. A mutant K14 with deletions in either the N- or the C-terminal domain can form heterodimers in vitro but does not assemble into protofilaments. The expression of such mutant keratin proteins in cells causes IF networks to break down into aggregates. Transgenic mice that express a mutant K14 protein in the basal stem cells of the epidermis display gross skin abnormalities, primarily blistering of the epidermis, that resemble the human skin disease *epidermolysis bullosa simplex* (EBS). Histological examination of the blistered area reveals a high incidence of dead basal cells. Death of these cells appears to be caused by mechanical trauma from rubbing of the skin during movement of the limbs. Without their normal bundles of keratin filaments, the mutant basal cells become fragile and easily damaged, causing the overlying epidermal layers to delaminate and blister (Figure 19-37). Like the role of desmin filaments in supporting muscle tissue, the general role of keratin filaments appears to be to maintain the structural integrity of epithelial tissues by mechanically reinforcing the connections between cells. ▮



▲ EXPERIMENTAL FIGURE 19-37 **Transgenic mice carrying a mutant keratin gene exhibit blistering similar to that in the human disease epidermolysis bullosa simplex.** Histological sections through the skin of a normal mouse and a transgenic mouse carrying a mutant K14 keratin gene are shown. In the normal mouse, the skin consists of a hard outer epidermal layer covering and in contact with the soft inner dermal layer. In the skin from the transgenic mouse, the two layers are separated (arrow) due to weakening of the cells at the base of the epidermis. [From P. Coulombe et al., 1991, *Cell* **66**:1301; courtesy of E. Fuchs.]

KEY CONCEPTS OF SECTION 19.5

Intermediate Filaments

- Intermediate filaments are present only in cells that display a multicellular organization. An essential role of intermediate filaments is to distribute tensile forces across cells in a tissue.
- Unlike microtubules and microfilaments, intermediate filaments are assembled from a large number of different IF proteins. These proteins are divided into four major types based on their sequences and tissue distribution. The lamins are expressed in all cells, whereas the other types are expressed in specific tissues (see Table 19-4).
- The assembly of intermediate filaments probably proceeds through several intermediate structures, which associate by lateral and end-to-end interactions (see Figure 19-33).
- Although intermediate filaments are much more stable than microfilaments and microtubules, they readily exchange subunits from a soluble pool.
- The phosphorylation of intermediate filaments early in mitosis leads to their disassembly; they reassemble late in mitosis after dephosphorylation of the subunits.
- The organization of intermediate filaments into networks and bundles, mediated by various IFAPs, provides structural stability to cells. IFAPs also cross-link intermediate filaments to the plasma and nuclear membranes, microtubules, and microfilaments.
- Major degenerative diseases of skin, muscle, and neurons are caused by disruption of the IF cytoskeleton or its connections to other cell structures.

PERSPECTIVES FOR THE FUTURE

With the list of proteins encoded by the genome growing ever larger, a major challenge is to understand how they contribute to intracellular motility and cell movements. Historically, knowledge of motility has been built on a largely biochemical and structural foundation. The major biochemical components of the actin cytoskeleton have been identified, and their roles in defining the structure of the cytoskeleton and regulation are being discovered. However, motility is a dynamic process in which function is also built on the foundation of mechanics.

Dynamics implies a change in structure with time and includes movements of proteins and protein structures. From Newton's laws of physics, movements are linked with forces, but we are only now becoming capable of studying forces at the molecular and cellular levels. Much has changed from the pioneering studies of muscle physiologists who measured muscle fiber contractile forces with tension gauges and of experimental cell physiologists who estimated the contractile force of the contractile ring from the bending of glass needles. These pioneering studies lead directly to modern studies that incorporate elegant physical techniques such as optical traps capable of measuring piconewton forces or that deduce stiffness from images of microtubules and microfilaments buckling against an unmovable object. At the heart of these discoveries lies the capability of the light microscope to image single molecules and to watch the binding of individual ligands and enzyme action on individual nucleotides.

But to understand fully how a cell moves, we must be able to measure forces in the cell. In vivo methods that measure force directly are not yet invented. What is needed? A mechanical sensor, perhaps engineered from a fluorescent protein, that can change fluorescence when a force is applied. Such a technique would be the analog of experiments in

which fluorescently labeled actin was microinjected into a live cell and scientists watched for the first time the actin cytoskeleton treadmill at the leading edge of the cell.

A second challenge is to understand how a cell moves in its natural environment—between layers of cells in a three-dimensional extracellular matrix. The story of cell motility is one shaped by studies of cells moving on a flat surface. The flat world imposes a geometry in which interactions with the substratum is through adhesions on the ventral surface of the cell while the free, noninteracting surface defines the dorsal surface. Consider, instead, movement through a gel of matrix. When surrounded by matrix, cells lose their stereotypic flat shape and take on tubular spindle shape. With adhesions on all sides, cells corkscrew through the gel of matrix molecules. The adhesions are smaller than those formed with a flat surface, which itself makes studying adhesions more difficult. Furthermore, the techniques for estimating the forces that a cell must exert to crawl on a flat surface cannot be applied to a three-dimensional situation. Squeezing through a gel adds several dimensions of complexity to our understanding how cell motility and adhesion are coupled.

KEY TERMS

actin-related proteins (Arps) *788*
calponin homology–domain superfamily *782*
cell locomotion *800*
chemotaxis *804*
contractile bundles *796*
critical concentration *785*
cytoskeleton *779*
F-actin *781*
G-actin *781*
intermediate filaments *779*
keratins *807*
lamellipodium *800*
lamins *806*
microfilaments *779*
motor protein *779*
myosin head domain *791*
myosin tail domain *793*
myosin LC kinase *799*
plakins *810*
profilin *787*
sarcomere *797*
sliding-filament assay *793*
thick filaments *797*
thin filaments *797*
thymosin β_4 *786*
treadmilling *786*

REVIEW THE CONCEPTS

1. Actin filaments have a defined polarity. What is filament polarity? How is it generated at the subunit level? How is filament polarity detectable?

2. In cells, actin filaments form bundles and/or networks. How do cells form such structures, and what specifically determines whether actin filaments will form a bundle or a network?

3. Much of our understanding of actin assembly in the cell is derived from experiments using purified actin in vitro. What techniques may be used to study actin assembly in vitro? Explain how each of these techniques works.

4. The predominant forms of actin inside a cell are ATP-G-actin and ADP-F-actin. Explain how the interconversion of the nucleotide state is coupled to the assembly and disassembly of actin subunits. What would be the consequence for actin filament assembly/disassembly if a mutation prevented actin's ability to bind ATP? What would be the consequence if a mutation prevented actin's ability to hydrolyze ATP?

5. Actin filaments at the leading edge of a crawling cell are believed to undergo treadmilling. What is treadmilling, and what accounts for this assembly behavior?

6. Although purified actin can reversibly assemble in vitro, various actin-binding proteins regulate the assembly of actin filaments in the cell. Predict the effect on a cell's actin cytoskeleton if function-blocking antibodies against each of the following were independently microinjected into cells: profilin, thymosin β4, gelsolin, tropomodulin, and the Arp2/3 complex.

7. There are at least 17 different types of myosin. What properties do all types share, and what makes them different?

8. The ability of myosin to walk along an actin filament may be observed with the aid of an appropriately equipped microscope. Describe how such assays are typically performed. Why is ATP required in these assays? How may such assays be used to determine the direction of myosin movement or the force produced by myosin?

9. Contractile bundles occur in nonmuscle cells, although the structures are less organized than the sarcomeres of muscle cells. What is the purpose of nonmuscle contractile bundles?

10. Contraction of both skeletal and smooth muscle is triggered by an increase in cytosolic Ca^{2+}. Compare the mechanisms by which each type of muscle converts a rise in Ca^{2+} into contraction.

11. Several types of cells utilize the actin cytoskeleton to power locomotion across surfaces. What types of cells have been utilized as models for the study of locomotion? What sequential morphological changes do each of these model cells exhibit as they move across a surface? How are actin filaments involved in each of these morphological changes?

12. To move in a specific direction, migrating cells must utilize extracellular cues to establish which portion of the cell will act as the front and which will act as the back. Describe how G proteins and Ca^{2+} gradients appear to be involved in the signaling pathways used by migrating cells to determine direction of movement.

13. Unlike actin filaments, intermediate filaments do not exhibit polarity. Explain how the structure of intermediate filament subunits and the relationship between assembled

subunits in an intermediate filament produce a filament lacking polarity.

14. Compared to actin filaments, intermediate filaments are relatively stable. However, cells can induce intermediate filament disassembly when needed. How does this disassembly occur, and why is it necessary?

15. Animal cells contain proteins that could be considered to serve as intermediate filament-associated proteins. Several such intermediate filament-associated proteins have now been identified. What functions do these proteins carry out in cells? To what other cellular structures do intermediate filament-associated proteins bind?

ANALYZE THE DATA

Understanding of actin filaments has been greatly facilitated by the ability of scientists to purify actin and actin-binding proteins and the ability to assemble actin filaments in vitro. Following are various experimental approaches designed to characterize actin assembly and the effects of actin-binding proteins on actin assembly.

a. The graph in part (a) of the figure depicts the actin polymerization rate at the plus (+) and minus (−) ends of rabbit actin as a function of actin concentration. Assume that you could add actin filaments of a predefined length to rabbit actin maintained at the concentrations labeled A, B, and C in the figure. Diagram the appearance of the filaments after a 10-minute incubation at each of the indicated actin concentrations, if the original filaments are depicted as follows:

Original filament: +_____−

Make sure to mark the location of the original (+) and (−) ends of the filament on your diagrams.

b. A novel actin-binding protein (X) is overexpressed in certain highly malignant cancers. You wish to determine if protein X caps actin filaments at the (+) or (−) end. You incubate an excess of protein X with various concentrations of G-actin under conditions that induce polymerization. Control samples are incubated in the absence of protein X. The results are shown in part (b) of the figure. How can you conclude from these data that protein X binds to the (+) end of actin filaments? Design an experiment, using myosin S1 fragments and electron microscopy, to corroborate the conclusion that protein X binds to the (+) end. What results would you expect if this conclusion is correct?

c. An in vitro system was developed to study actin assembly and disassembly in nonmuscle cells. In this study, tissue culture cells were incubated for several hours with [^{35}S]methionine so that all the actin monomers in each filament were labeled. Actin filaments were then collected by differential centrifugation and put into a buffer containing one of three different cytosolic extracts (A, B, or C). The amounts of soluble actin in each sample were monitored over time (see part (c) of the figure). What do these data indicate about the effects of A, B, and C on the assembly and disassembly of actin filaments?