CHAPTER 16

The Cytoskeleton

IN THIS CHAPTER

For cells to function properly, they must organize themselves in space and interact mechanically with each other and with their environment. They have to be correctly shaped, physically robust, and properly structured internally. Many have to change their shape and move from place to place. All cells have to be able to rearrange their internal components as they grow, divide, and adapt to changing circumstances. These spatial and mechanical functions depend on a remarkable system of filaments called the **cytoskeleton** (**Figure 16–1**).

The cytoskeleton's varied functions depend on the behavior of three families of protein filaments—*actin filaments, microtubules,* and *intermediate filaments.* Each type of filament has distinct mechanical properties, dynamics, and biological roles, but all share certain fundamental features. Just as we require our ligaments, bones, and muscles to work together, so all three cytoskeletal filament systems must normally function collectively to give a cell its strength, its shape, and its ability to move.

In this chapter, we describe the function and conservation of the three main filament systems. We explain the basic principles underlying filament assembly and disassembly, and how other proteins interact with the filaments to alter their dynamics, enabling the cell to establish and maintain internal order, to shape and remodel its surface, and to move organelles in a directed manner from one place to another. Finally, we discuss how the integration and regulation of the cytoskeleton allows a cell to move to new locations.

FUNCTION AND ORIGIN OF THE CYTOSKELETON

The three major cytoskeletal filaments are responsible for different aspects of the cell's spatial organization and mechanical properties. Actin filaments determine the shape of the cell's surface and are necessary for whole-cell locomotion; they also drive the pinching of one cell into two. Microtubules determine the positions of membrane-enclosed organelles, direct intracellular transport, and form the mitotic spindle that segregates chromosomes during cell division. Intermediate filaments provide mechanical strength. All of these cytoskeletal filaments interact with hundreds of accessory proteins that regulate and link the filaments to other cell components, as well as to each other. The accessory proteins are essential for the controlled assembly of the cytoskeletal filaments in particular locations, and they include the *motor proteins*, remarkable molecular machines that convert the energy of ATP hydrolysis into mechanical force that can either move organelles along the filaments or move the filaments themselves.

In this section, we discuss the general features of the proteins that make up the filaments of the cytoskeleton. We focus on their ability to form intrinsically

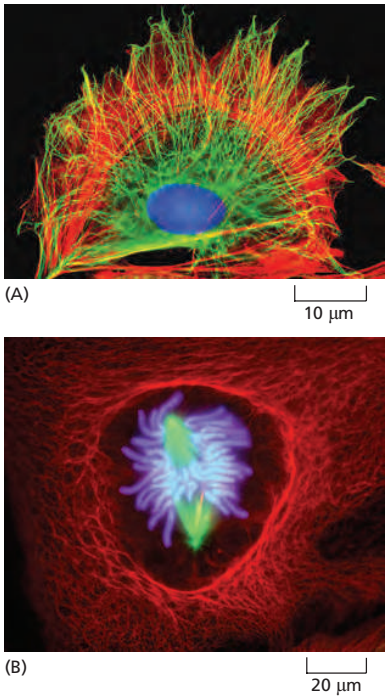


Figure 16–1 The cytoskeleton. (A) A cell in culture has been fixed and labeled to show its cytoplasmic arrays of microtubules *(green)* and actin filaments *(red)*. (B) This dividing cell has been labeled to show its spindle microtubules *(green)* and surrounding cage of intermediate filaments *(red)*. The DNA in both cells is labeled in *blue*. (A, courtesy of Albert Tousson; B, courtesy of Conly Rieder.)

polarized and self-organized structures that are highly dynamic, allowing the cell to rapidly modify cytoskeletal structure and function under different conditions.

Cytoskeletal Filaments Adapt to Form Dynamic or Stable Structures

Cytoskeletal systems are dynamic and adaptable, organized more like ant trails than interstate highways. A single trail of ants may persist for many hours, extending from the ant nest to a delectable picnic site, but the individual ants within the trail are anything but static. If the ant scouts find a new and better source of food, or if the picnickers clean up and leave, the dynamic structure adapts with astonishing rapidity. In a similar way, large-scale cytoskeletal structures can change or persist, according to need, lasting for lengths of time ranging from less than a minute up to the cell's lifetime. But the individual macromolecular components that make up these structures are in a constant state of flux. Thus, like the alteration of an ant trail, a structural rearrangement in a cell requires little extra energy when conditions change.

Regulation of the dynamic behavior and assembly of cytoskeletal filaments allows eukaryotic cells to build an enormous range of structures from the three basic filament systems. The micrographs in **Panel 16–1** illustrate some of these structures. Actin filaments underlie the plasma membrane of animal cells, providing strength and shape to its thin lipid bilayer. They also form many types of cell-surface projections. Some of these are dynamic structures, such as the *lamellipodia* and *filopodia* that cells use to explore territory and move around. More stable arrays allow cells to brace themselves against an underlying substratum and enable muscle to contract. The regular bundles of *stereocilia* on the surface of hair cells in the inner ear contain stable bundles of actin filaments that tilt as rigid rods in response to sound, and similarly organized *microvilli* on the surface of intestinal epithelial cells vastly increase the apical cell-surface area to enhance nutrient absorption. In plants, actin filaments drive the rapid streaming of cytoplasm inside cells.

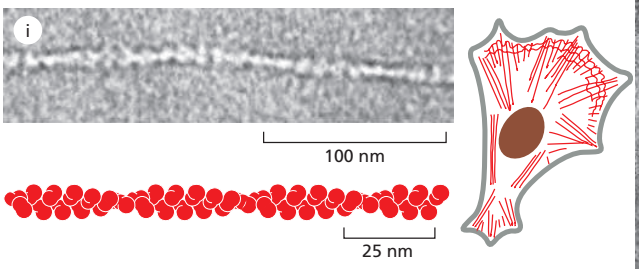
Microtubules, which are frequently found in a cytoplasmic array that extends to the cell periphery, can quickly rearrange themselves to form a bipolar *mitotic spindle* during cell division. They can also form *cilia*, which function as motile whips or sensory devices on the surface of the cell, or tightly aligned bundles that serve as tracks for the transport of materials down long neuronal axons. In plant cells, organized arrays of microtubules help to direct the pattern of cell wall synthesis, and in many protozoans they form the framework upon which the entire cell is built.

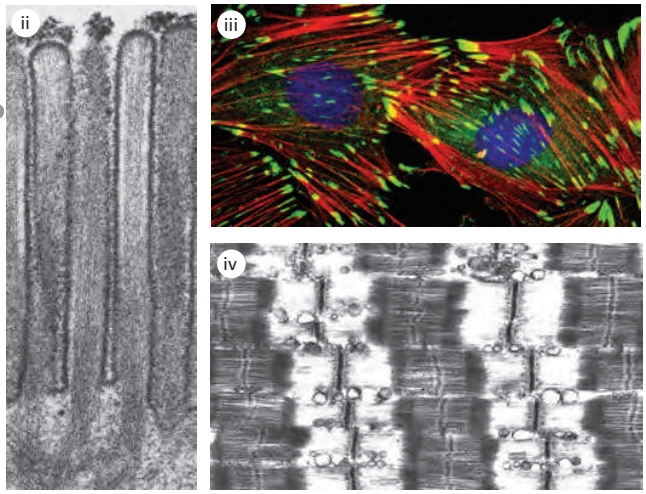
Intermediate filaments line the inner face of the nuclear envelope, forming a protective cage for the cell's DNA; in the cytosol, they are twisted into strong cables that can hold epithelial cell sheets together or help nerve cells to extend long and robust axons, and they allow us to form tough appendages such as hair and fingernails.

An important and dramatic example of rapid reorganization of the cytoskeleton occurs during cell division, as shown in **Figure 16–2** for a fibroblast growing in a tissue-culture dish. After the chromosomes have replicated, the interphase microtubule array that spreads throughout the cytoplasm is reconfigured into the bipolar *mitotic spindle*, which segregates the two copies of each chromosome into daughter nuclei. At the same time, the specialized actin structures that enable the fibroblast to crawl across the surface of the dish rearrange so that the cell stops moving and assumes a more spherical shape. Actin and its associated motor protein myosin then form a belt around the middle of the cell, the *contractile ring*, which constricts like a tiny muscle to pinch the cell in two. When division is complete, the cytoskeletons of the two daughter fibroblasts reassemble into their interphase structures to convert the two rounded-up daughter cells into smaller versions of the flattened, crawling mother cell.

Many cells require rapid cytoskeletal rearrangements for their normal functioning during interphase as well. For example, the *neutrophil*, a type of white

ACTIN FILAMENTS

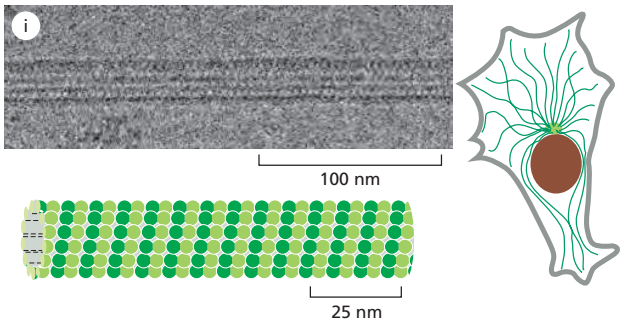


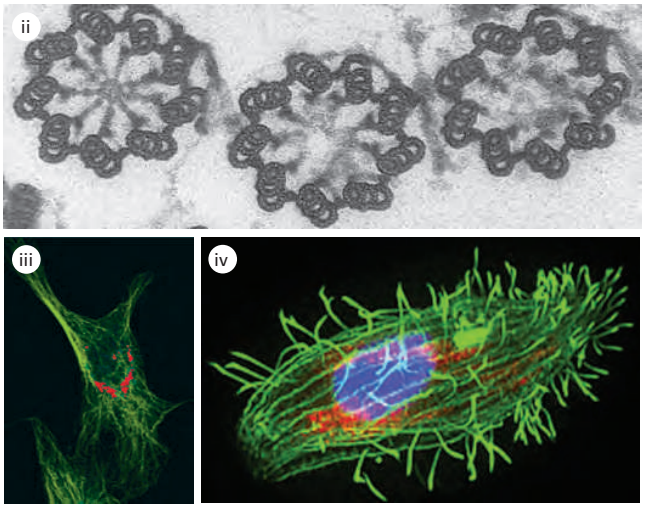


Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures with a diameter of 8 nm that organize into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane. (i) Single actin filament; (ii) microvilli; (iii) stress fibers (*red*) terminating in focal adhesions (*green*); (iv) striated muscle.

Micrographs courtesy of R. Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); K. Burridge (iii).

MICROTUBULES

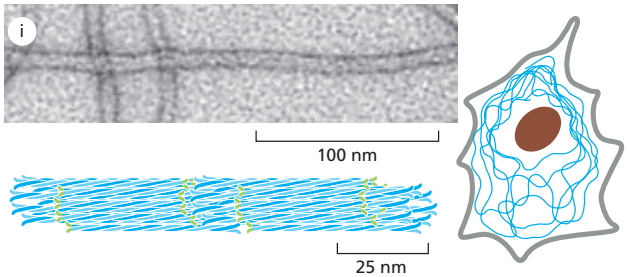


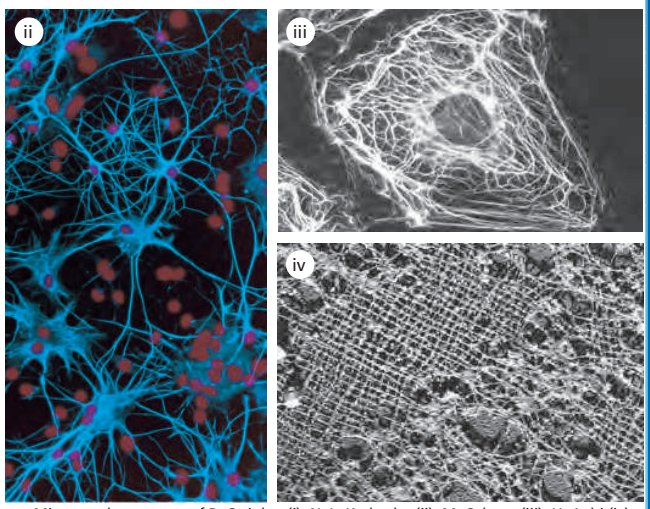


Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and frequently have one end attached to a microtubule-organizing center (MTOC) called a *centrosome*. (i) Single microtubule; (ii) cross section at the base of three cilia showing triplet microtubules; (iii) interphase microtubule array (*green*) and organelles (*red*); (iv) ciliated protozoan.

Micrographs courtesy of R. Wade (i); D.T. Woodrow and R.W. Linck (ii); D. Shima (iii); D. Burnette (iv).

INTERMEDIATE FILAMENTS





Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell–cell junction to another, thereby strengthening the entire epithelium. (i) Individual intermediate filaments; (ii) Intermediate filaments (*blue*) in neurons and (iii) epithelial cell; (iv) nuclear lamina.

Micrographs courtesy of R. Quinlan (i); N. L. Kedersha (ii); M. Osborn (iii); U. Aebi (iv).

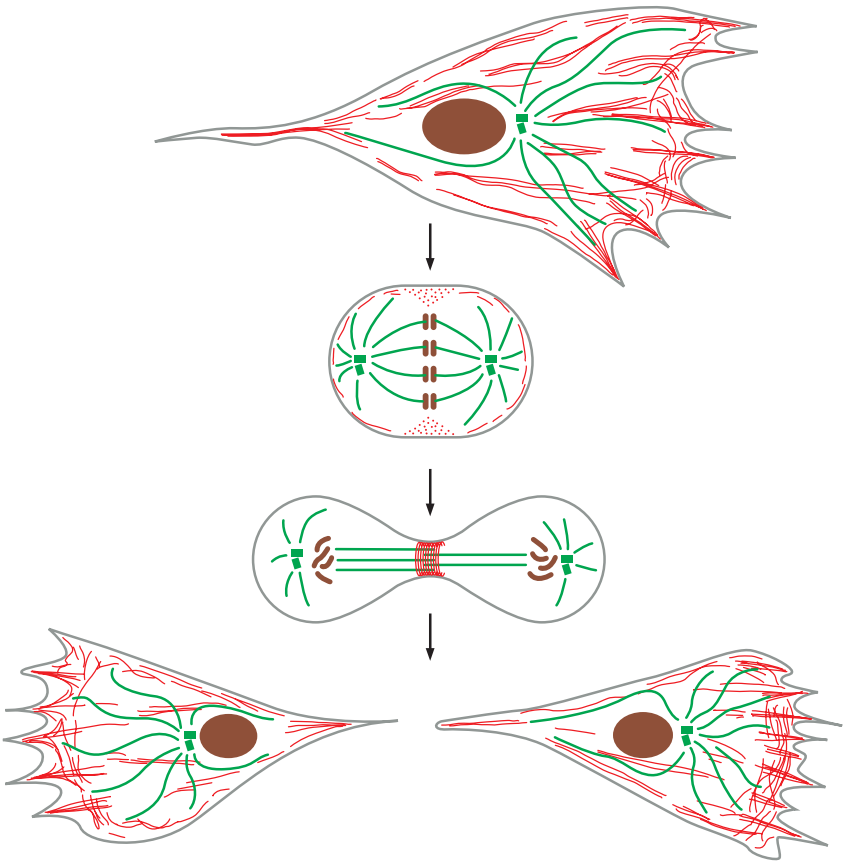


Figure 16–2 Diagram of changes in cytoskeletal organization associated with cell division. The crawling fibroblast drawn here has a polarized, dynamic actin cytoskeleton (shown in *red*) that assembles lamellipodia and filopodia to push its leading edge toward the right. The polarization of the actin cytoskeleton is assisted by the microtubule cytoskeleton *(green)*, consisting of long microtubules that emanate from a single microtubule-organizing center located in front of the nucleus. When the cell divides, the polarized microtubule array rearranges to form a bipolar mitotic spindle, which is responsible for aligning and then segregating the duplicated chromosomes *(brown)*. The actin filaments form a contractile ring at the center of the cell that pinches the cell in two after the chromosome segregation. After cell division is complete, the two daughter cells reorganize both the microtubule and actin cytoskeletons into smaller versions of those that were present in the mother cell, enabling them to crawl their separate ways.

blood cell, chases and engulfs bacterial and fungal cells that accidentally gain access to the normally sterile parts of the body, as through a cut in the skin. Like most crawling cells, neutrophils advance by extending a protrusive structure filled with newly polymerized actin filaments. When the elusive bacterial prey moves in a different direction, the neutrophil is poised to reorganize its polarized protrusive structures within seconds (**Figure 16–3**).

The Cytoskeleton Determines Cellular Organization and Polarity

In cells that have achieved a stable, differentiated morphology—such as mature neurons or epithelial cells—the dynamic elements of the cytoskeleton must also provide stable, large-scale structures for cellular organization. On specialized epithelial cells that line organs such as the intestine and the lung, cytoskeletal-based cell-surface protrusions including microvilli and cilia are able to maintain a constant location, length, and diameter over the entire lifetime of the cell. For the actin bundles at the cores of microvilli on intestinal epithelial cells, this is only a few days. But the actin bundles at the cores of stereocilia on the hair cells of the inner ear must maintain their stable organization for the entire lifetime of the animal, since these cells do not turn over. Nonetheless, the individual actin filaments

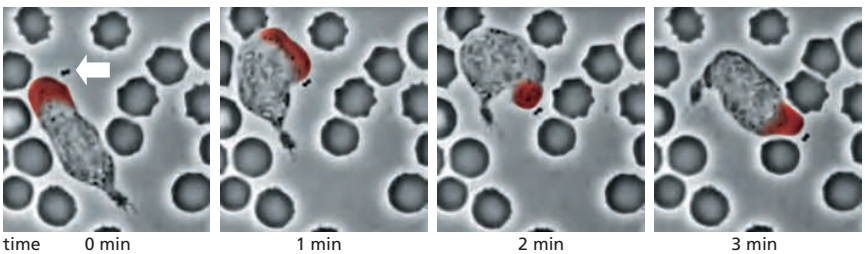


Figure 16–3 A neutrophil in pursuit of bacteria. In this preparation of human blood, a clump of bacteria *(white arrow)* is about to be captured by a neutrophil. As the bacteria move, the neutrophil quickly reassembles the dense actin network at its leading edge (highlighted in *red*) to push toward the location of the bacteria (**Movie 16.1**). Rapid disassembly and reassembly of the actin cytoskeleton in this cell enables it to change its orientation and direction of movement within a few minutes. (From a video recorded by David Rogers.)

remain strikingly dynamic and are continuously remodeled and replaced every 48 hours, even within stable cell-surface structures that persist for decades.

Besides forming stable, specialized cell-surface protrusions, the cytoskeleton is also responsible for large-scale cellular polarity, enabling cells to tell the difference between top and bottom, or front and back. The large-scale polarity information conveyed by cytoskeletal organization is often maintained over the lifetime of the cell. Polarized epithelial cells use organized arrays of microtubules, actin filaments, and intermediate filaments to maintain the critical differences between the *apical surface* and the *basolateral surface*. They also must maintain strong adhesive contacts with one another to enable this single layer of cells to serve as an effective physical barrier (**Figure 16–4**).

Filaments Assemble from Protein Subunits That Impart Specific Physical and Dynamic Properties

Cytoskeletal filaments can reach from one end of the cell to the other, spanning tens or even hundreds of micrometers. Yet the individual protein molecules that form the filaments are only a few nanometers in size. The cell builds the filaments by assembling large numbers of the small subunits, like building a skyscraper out of bricks. Because these subunits are small, they can diffuse rapidly in the cytosol, whereas the assembled filaments cannot. In this way, cells can undergo rapid structural reorganizations, disassembling filaments at one site and reassembling them at another site far away.

Actin filaments and microtubules are built from subunits that are compact and globular—*actin subunits* for actin filaments and *tubulin subunits* for microtubules—whereas intermediate filaments are made up of smaller subunits that are themselves elongated and fibrous. All three major types of cytoskeletal filaments form as helical assemblies of subunits (see Figure 3–22) that self-associate, using a combination of end-to-end and side-to-side protein contacts. Differences in the structures of the subunits and the strengths of the attractive forces between

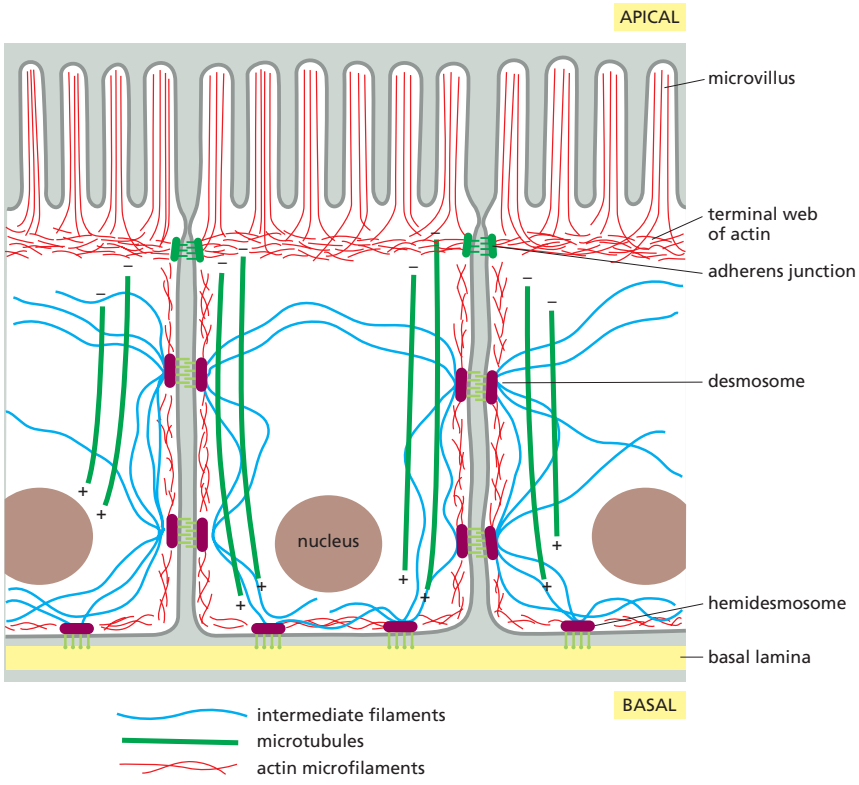


Figure 16–4 Organization of the cytoskeleton in polarized epithelial cells. All the components of the cytoskeleton cooperate to produce the characteristic shapes of specialized cells, including the epithelial cells that line the small intestine, diagrammed here. At the apical (upper) surface, facing the intestinal lumen, bundled actin filaments *(red)* form microvilli that increase the cell surface area available for absorbing nutrients from food. Below the microvilli, a circumferential band of actin filaments is connected to cell–cell adherens junctions that anchor the cells to each other. Intermediate filaments *(blue)* are anchored to other kinds of adhesive structures, including desmosomes and hemidesmosomes, that connect the epithelial cells into a sturdy sheet and attach them to the underlying extracellular matrix; these structures are discussed in Chapter 19. Microtubules *(green)* run vertically from the top of the cell to the bottom and provide a global coordinate system that enables the cell to direct newly synthesized components to their proper locations.

them produce important differences in the stability and mechanical properties of each type of filament. Whereas covalent linkages between their subunits hold together the backbones of many biological polymers—including DNA, RNA, and proteins—it is weak noncovalent interactions that hold together the three types of cytoskeletal polymers. Consequently, their assembly and disassembly can occur rapidly, without covalent bonds being formed or broken.

The subunits of actin filaments and microtubules are asymmetrical and bind to one another head-to-tail so that they all point in one direction. This subunit polarity gives the filaments structural polarity along their length, and makes the two ends of each polymer behave differently. In addition, actin and tubulin subunits are both enzymes that catalyze the hydrolysis of a nucleoside triphosphate—ATP and GTP, respectively. As we discuss later, the energy derived from nucleotide hydrolysis enables the filaments to remodel rapidly. By controlling when and where actin and microtubules assemble, the cell harnesses the polar and dynamic properties of these filaments to generate force in a specific direction, to move the leading edge of a migrating cell forward, for example, or to pull chromosomes apart during cell division. In contrast, the subunits of intermediate filaments are symmetrical, and thus do not form polarized filaments with two different ends. Intermediate filament subunits also do not catalyze the hydrolysis of nucleotides. Nevertheless, intermediate filaments can be disassembled rapidly when required. In mitosis , for example, kinases phosphorylate the subunits, leading to their dissociation.

Cytoskeletal filaments in living cells are not built by simply stringing subunits together in single file. A thousand tubulin subunits lined up end-to-end, for example, would span the diameter of a small eukaryotic cell, but a filament formed in this way would lack the strength to avoid breakage by ambient thermal energy, unless each subunit in the filament was bound extremely tightly to its two neighbors. Such tight binding would limit the rate at which the filaments could disassemble, making the cytoskeleton a static and less useful structure. To provide both strength and adaptability, microtubules are built of 13 **protofilaments**—linear strings of subunits joined end-to-end—that associate with one another laterally to form a hollow cylinder. The addition or loss of a subunit at the end of one protofilament makes or breaks a small number of bonds. In contrast, loss of a subunit from the middle of the filament requires breaking many more bonds, while breaking it in two requires breaking bonds in multiple protofilaments all at the same time (**Figure 16–5**). The greater energy required to break multiple noncovalent bonds simultaneously allows microtubules to resist thermal breakage, while allowing rapid subunit addition and loss at the filament ends. Helical actin filaments are much thinner and therefore require much less energy to break. However, multiple actin filaments are often bundled together inside cells, providing mechanical strength, while allowing dynamic behavior of filament ends.

As with other specific protein–protein interactions, many hydrophobic interactions and noncovalent bonds hold the subunits in a cytoskeletal filament together (see Figure 3–4). The locations and types of subunit–subunit contacts differ for the different filaments. Intermediate filaments, for example, assemble by forming strong lateral contacts between α-helical coiled-coils, which extend over most of the length of each elongated fibrous subunit. Because the individual subunits are staggered in the filament, intermediate filaments form strong, ropelike structures that tolerate stretching and bending to a greater extent than do either actin filaments or microtubules (**Figure 16–6**).

Accessory Proteins and Motors Regulate Cytoskeletal Filaments

The cell regulates the length and stability of its cytoskeletal filaments, as well as their number and geometry. It does so largely by regulating their attachments to one another and to other components of the cell, so that the filaments can form a wide variety of higher-order structures. Direct covalent modification of the filament subunits regulates some filament properties, but most of the regulation is performed by hundreds of accessory proteins that determine the spatial

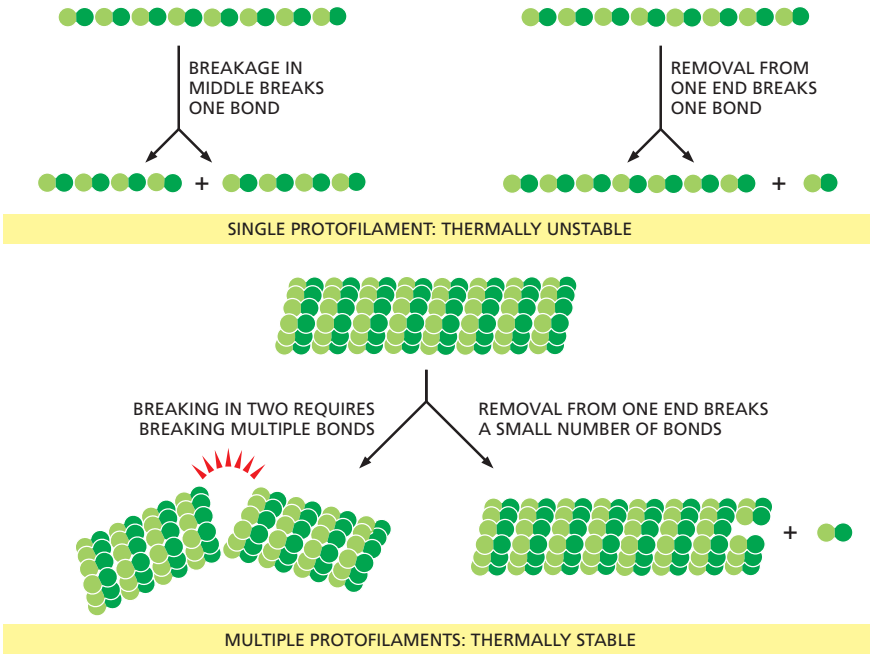


Figure 16–5 The thermal stability of cytoskeletal filaments with dynamic ends. A protofilament consisting of a single strand of subunits is thermally unstable, since breakage of a single bond between subunits is sufficient to break the filament. In contrast, formation of a cytoskeletal filament from more than one protofilament allows the ends to be dynamic, while enabling the filaments themselves to be resistant to thermal breakage. In a microtubule, for example, removing a single subunit dimer from the end of the filament requires breaking noncovalent bonds with a maximum of three other subunits, whereas fracturing the filament in the middle requires breaking noncovalent bonds in all thirteen protofilaments.

distribution and the dynamic behavior of the filaments, converting information received through signaling pathways into cytoskeletal action. These accessory proteins bind to the filaments or their subunits to determine the sites of assembly of new filaments, to regulate the partitioning of polymer proteins between filament and subunit forms, to change the kinetics of filament assembly and disassembly, to harness energy to generate force, and to link filaments to one another or to other cell structures such as organelles and the plasma membrane. In these processes, the accessory proteins bring cytoskeletal structure under the control of extracellular and intracellular signals, including those that trigger the dramatic transformations of the cytoskeleton that occur during each cell cycle. Acting together, the accessory proteins enable a eukaryotic cell to maintain a highly organized but flexible internal structure and, in many cases, to move.

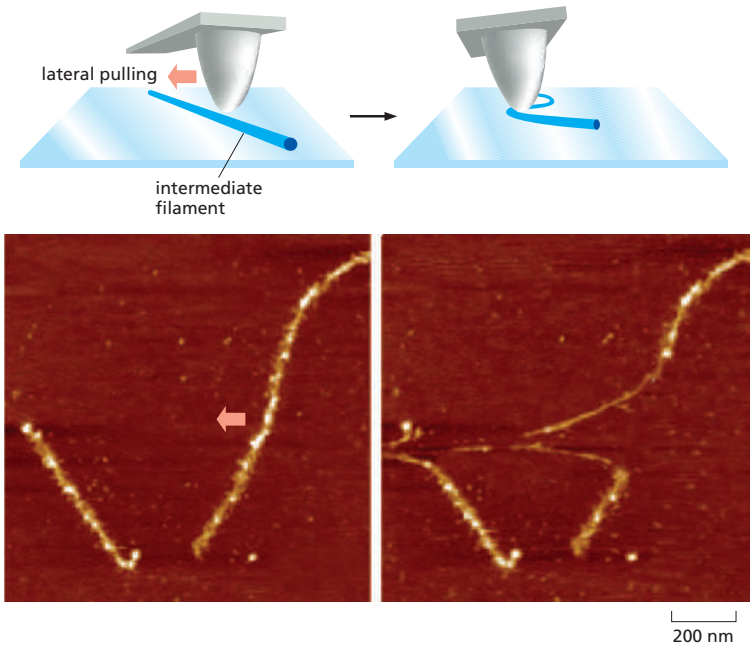


Figure 16–6 Flexibility and stretch in an intermediate filament. Intermediate filaments are formed from elongated fibrous subunits with strong lateral contacts, resulting in resistance to stretching forces. When a tiny mechanical probe is dragged across an intermediate filament, the filament is stretched over three times its length before it breaks, as illustrated by the fluorescently labeled filaments in the photomicrographs. This technique is termed atomic force microscopy (see Figure 9–33). (Adapted from L. Kreplak et al., *J. Mol. Biol.* 354:569–577, 2005. With permission from Elsevier.)

Among the most fascinating proteins that associate with the cytoskeleton are the **motor proteins**. These proteins bind to a polarized cytoskeletal filament and use the energy derived from repeated cycles of ATP hydrolysis to move along it. Dozens of different motor proteins coexist in every eukaryotic cell. They differ in the type of filament they bind to (either actin or microtubules), the direction in which they move along the filament, and the "cargo" they carry. Many motor proteins carry membrane-enclosed organelles—such as mitochondria, Golgi stacks, or secretory vesicles—to their appropriate locations in the cell. Other motor proteins cause cytoskeletal filaments to exert tension or to slide against each other, generating the force that drives such phenomena as muscle contraction, ciliary beating, and cell division.

Cytoskeletal motor proteins that move unidirectionally along an oriented polymer track are reminiscent of some other proteins and protein complexes discussed elsewhere in this book, such as DNA and RNA polymerases, helicases, and ribosomes. All of these proteins have the ability to use chemical energy to propel themselves along a linear track, with the direction of sliding dependent on the structural polarity of the track. All of them generate motion by coupling nucleoside triphosphate hydrolysis to a large-scale conformational change (see Figure 3–75).

Bacterial Cell Organization and Division Depend on Homologs of Eukaryotic Cytoskeletal Proteins

While eukaryotic cells are typically large and morphologically complex, bacterial cells are usually only a few micrometers long and assume simple shapes such as spheres or rods. Bacteria also lack elaborate networks of intracellular membrane-enclosed organelles. Historically, biologists assumed that a cytoskeleton was not necessary in such simple cells. We now know, however, that bacteria contain homologs of all three of the eukaryotic cytoskeletal filaments. Furthermore, bacterial actins and tubulins are more diverse than their eukaryotic versions, both in the types of assemblies they form and in the functions they carry out.

Nearly all bacteria and many archaea contain a homolog of tubulin called FtsZ, which can polymerize into filaments and assemble into a ring (called the Z-ring) at the site where the **septum** forms during cell division (**Figure 16–7**). Although the Z-ring persists for many minutes, the individual filaments within it are highly dynamic, with an average filament half-life of about thirty seconds. As the bacterium divides, the Z-ring becomes smaller until it has completely disassembled. FtsZ filaments in the Z-ring are thought to generate a bending force that drives the membrane invagination necessary to complete cell division. The Z-ring may also serve as a site for localization of enzymes required for building the septum between the two daughter cells.

Many bacteria also contain homologs of actin. Two of these, MreB and Mbl, are found primarily in rod-shaped or spiral-shaped cells where they assemble to form dynamic patches that move circumferentially along the length of the cell (**Figure 16–8**A). These proteins contribute to cell shape by serving as a scaffold to direct the synthesis of the peptidoglycan cell wall, in much the same way that microtubules help organize the synthesis of the cellulose cell wall in higher

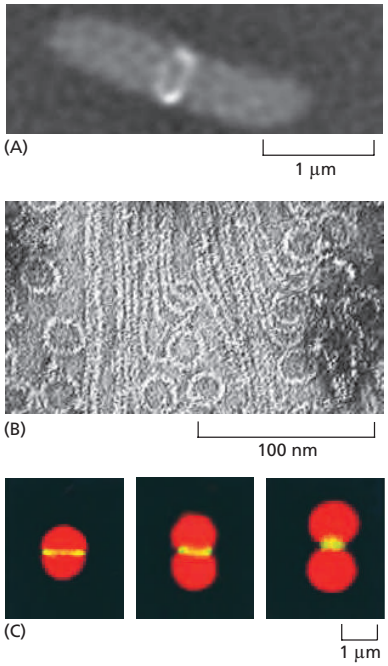


Figure 16–7 The bacterial FtsZ protein, a tubulin homolog in prokaryotes. (A) A band of FtsZ protein forms a ring in a dividing bacterial cell. This ring has been labeled by fusing the FtsZ protein to green fluorescent protein (GFP), which allows it to be observed in living *E. coli* cells with a fluorescence microscope. (B) FtsZ filaments and circles, formed *in vitro*, as visualized using electron microscopy. (C) Dividing chloroplasts *(red)* from a red alga also cleave using a protein ring made from FtsZ *(yellow)*. (A, from X. Ma, D.W. Ehrhardt and W. Margolin, *Proc. Natl Acad. Sci. USA* 93:12998–13003, 1996; B, from H.P. Erickson et al., *Proc. Natl Acad. Sci. USA* 93:519–523, 1996. Both with permission from National Academy of Sciences; C, from S. Miyagishima et al., *Plant Cell* 13:2257–2268, 2001, with permission from American Society of Plant Biologists.)

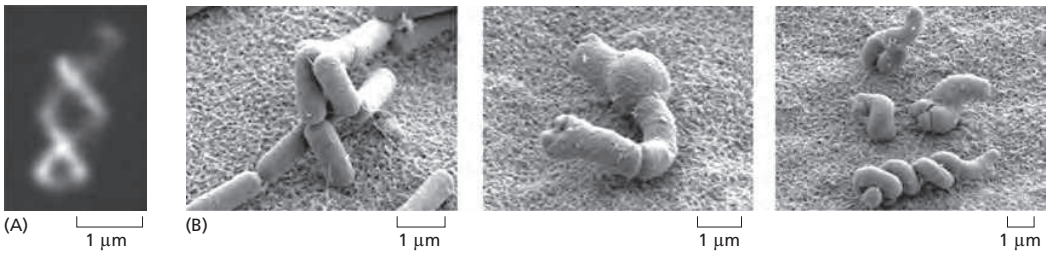


Figure 16–8 Actin homologs in bacteria determine cell shape. (A) The MreB protein forms abundant patches made up of many short, interwoven linear or helical filaments that are seen to move circumferentially along the length of the bacterium and are associated with sites of cell wall synthesis. (B) The common soil bacterium *Bacillus subtilis* normally forms cells with a regular rodlike shape when viewed by scanning electron microscopy *(left).* In contrast, *B. subtilis* cells lacking the actin homolog MreB or Mbl grow in distorted or twisted shapes and eventually die *(center* and *right).* (A, from P. Vats and L. Rothfield, *Proc. Natl Acad. Sci. USA* 104:17795–17800, 2007. With permission from National Academy of Sciences; B, from A. Chastanet and R. Carballido-Lopez, *Front. Biosci.* 4S:1582–1606, 2012. With permission Frontiers in Bioscience.)

plant cells (see Figure 19–65). As with FtsZ, MreB and Mbl filaments are highly dynamic, with half-lives of a few minutes, and nucleotide hydrolysis accompanies the polymerization process. Mutations disrupting MreB or Mbl expression cause extreme abnormalities in cell shape and defects in chromosome segregation (Figure 16–8B).

Relatives of MreB and Mbl have more specialized roles. A particularly intriguing bacterial actin homolog is ParM, which is encoded by a gene on certain bacterial plasmids that also carry genes responsible for antibiotic resistance and cause the spread of multidrug resistance in epidemics. Bacterial plasmids typically encode all the gene products that are necessary for their own segregation, presumably as a strategy to ensure their inheritance and propagation in bacterial hosts following plasmid replication. ParM assembles into filaments that associate at each end with a copy of the plasmid, and growth of the ParM filament pushes the replicated plasmid copies apart (**Figure 16–9**). This spindle-like structure apparently arises from the selective stabilization of filaments that bind to specialized proteins recruited to the origins of replication on the plasmids. A distant relative of both tubulin and FtsZ, called TubZ, has a similar function in other bacterial species.

Thus, self-association of nucleotide-binding proteins into dynamic filaments is used in all cells, and the actin and tubulin families are very ancient, predating the split between the eukaryotic and bacterial kingdoms.

At least one bacterial species, *Caulobacter crescentus,* appears to harbor a protein with significant structural similarity to the third major class of cytoskeletal filaments found in animal cells, the intermediate filaments. A protein called crescentin forms a filamentous structure that influences the unusual crescent shape of this species; when the gene encoding crescentin is deleted, the *Caulobacter* cells grow as straight rods (**Figure 16–10**).

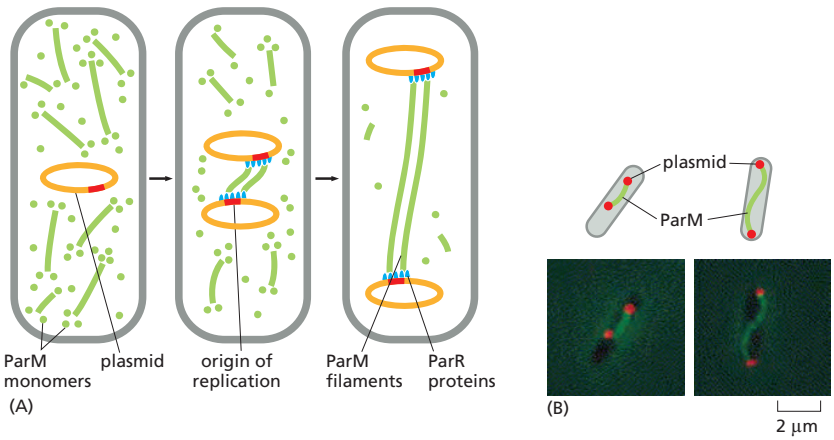


Figure 16–9 Role of the actin homolog ParM in plasmid segregation in bacteria. (A) Some bacterial drug-resistance plasmids *(orange)* encode an actin homolog, ParM, that will spontaneously nucleate to form small, dynamic filaments *(green)* throughout the bacterial cytoplasm. A second plasmid-encoded protein called ParR *(blue)* binds to specific DNA sequences in the plasmid and also stabilizes the dynamic ends of the ParM filaments. When the plasmid duplicates, both ends of the ParM filaments become stabilized, and the growing ParM filaments push the duplicated plasmids to opposite ends of the cell. (B) In these bacterial cells harboring a drug-resistance plasmid, the plasmids are labeled in *red* and the ParM protein in *green*. Left, a short ParM filament bundle connects the two daughter plasmids shortly after their duplication. Right, the fully assembled ParM filament has pushed the duplicated plasmids to the cell poles. (A, adapted from E.C. Garner, C.S. Campbell and R.D. Mullins, *Science* 306:1021–1025, 2004; B, from J. Møller-Jensen et al., *Mol. Cell* 12:1477–1487, 2003. With permission from Elsevier.)

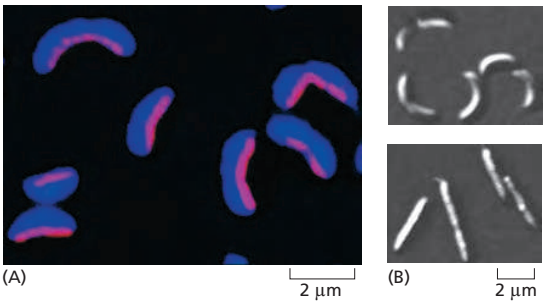


Figure 16–10 *Caulobacter* and crescentin. The sickle-shaped bacterium *Caulobacter crescentus* expresses a protein, crescentin, with a series of coiled-coil domains similar in size and organization to the domains of eukaryotic intermediate filaments. (A) The crescentin protein forms a fiber (labeled in *red*) that runs down the inner side of the curving bacterial cell wall. (B) When the gene is disrupted, the bacteria grow as straight rods *(bottom)*. (From N. Ausmees, J.R. Kuhn and C. Jacobs-Wagner, *Cell* 115:705–713, 2003. With permission from Elsevier.)

Summary

The cytoplasm of eukaryotic cells is spatially organized by a network of protein filaments known as the cytoskeleton. This network contains three principal types of filaments: actin filaments, microtubules, and intermediate filaments. All three types of filaments form as helical assemblies of subunits that self-associate using a combination of end-to-end and side-to-side protein contacts. Differences in the structure of the subunits and the manner of their self-assembly give the filaments different mechanical properties. Subunit assembly and disassembly constantly remodel all three types of cytoskeletal filaments. Actin and tubulin (the subunits of actin filaments and microtubules, respectively) bind and hydrolyze nucleoside triphosphates (ATP and GTP, respectively), and assemble head-to-tail to generate polarized filaments capable of generating force. In living cells, accessory proteins modulate the dynamics and organization of cytoskeletal filaments, resulting in complex events such as cell division or migration, and generating elaborate cellular architecture to form polarized tissues such as epithelia. Bacterial cells also contain homologs of actin, tubulin, and intermediate filaments that form dynamic structures that help control cell shape and division.

ACTIN AND ACTIN-BINDING PROTEINS

The actin cytoskeleton performs a wide range of functions in diverse cell types. Each actin subunit, sometimes called globular or G-actin, is a 375-amino-acid polypeptide carrying a tightly associated molecule of ATP or ADP (**Figure 16–11**A). Actin is extraordinarily well conserved among eukaryotes. The amino acid sequences of actins from different eukaryotic species are usually about 90% identical. Small variations in actin amino acid sequence can cause significant functional differences: In vertebrates, for example, there are three isoforms of actin, termed α, β, and γ, that differ slightly in their amino acid sequences and have distinct functions. α-Actin is expressed only in muscle cells, while β- and γ-actins are found together in almost all non-muscle cells.

Actin Subunits Assemble Head-to-Tail to Create Flexible, Polar Filaments

Actin subunits assemble head-to-tail to form a tight, right-handed helix, forming a structure about 8 nm wide called filamentous or F-actin (Figure 16–11B and C). Because the asymmetrical actin subunits of a filament all point in the same direction, filaments are polar and have structurally different ends: a slower-growing *minus end* and a faster-growing *plus end*. The minus end is also referred to as the "pointed end" and the plus end as the "barbed end," because of the "arrowhead" appearance of the complex formed between actin filaments and the motor protein myosin (**Figure 16–12**). Within the filament, the subunits are positioned with their nucleotide-binding cleft directed toward the minus end.

Individual actin filaments are quite flexible. The stiffness of a filament can be characterized by its *persistence length*, the minimum filament length at which random thermal fluctuations are likely to cause it to bend. The persistence length of an actin filament is only a few tens of micrometers. In a living cell, however,

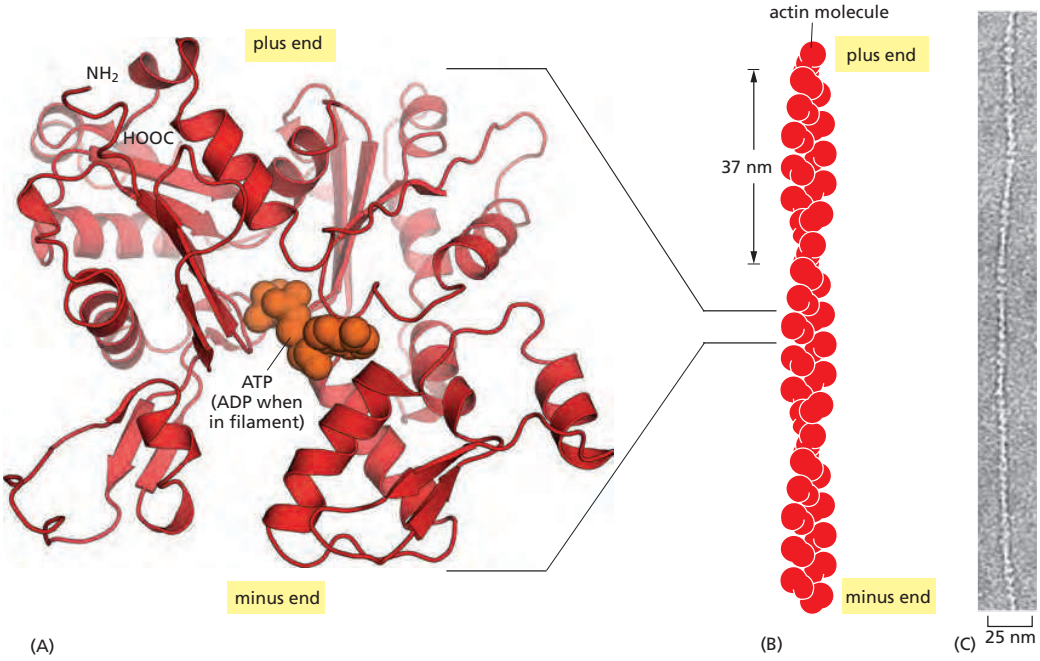


Figure 16–11 The structures of an actin monomer and actin filament. (A) The actin monomer has a nucleotide (either ATP or ADP) bound in a deep cleft in the center of the molecule. (B) Arrangement of monomers in a filament consisting of two protofilaments, held together by lateral contacts, which wind around each other as two parallel strands of a helix, with a twist repeating every 37 nm. All the subunits within the filament have the same orientation. (C) Electron micrograph of negatively stained actin filament. (C, courtesy of Roger Craig.)

accessory proteins cross-link and bundle the filaments together, making large-scale actin structures that are much more rigid than an individual actin filament.

Nucleation Is the Rate-Limiting Step in the Formation of Actin Filaments

The regulation of actin filament formation is an important mechanism by which cells control their shape and movement. Small oligomers of actin subunits can assemble spontaneously, but they are unstable and disassemble readily because each monomer is bound to only one or two other monomers. For a new actin filament to form, subunits must assemble into an initial aggregate, or nucleus, that is stabilized by multiple subunit–subunit contacts and can then elongate rapidly by addition of more subunits. This process is called filament *nucleation*.

Many features of actin nucleation and polymerization have been studied with purified actin in a test tube (**Figure 16–13**). The instability of smaller actin aggregates creates a kinetic barrier to nucleation. When polymerization is initiated, this barrier results in a lag phase during which no filaments are observed. During this lag phase, however, a few of the small, unstable aggregates succeed in making the transition to a more stable form that resembles an actin filament. This leads to a

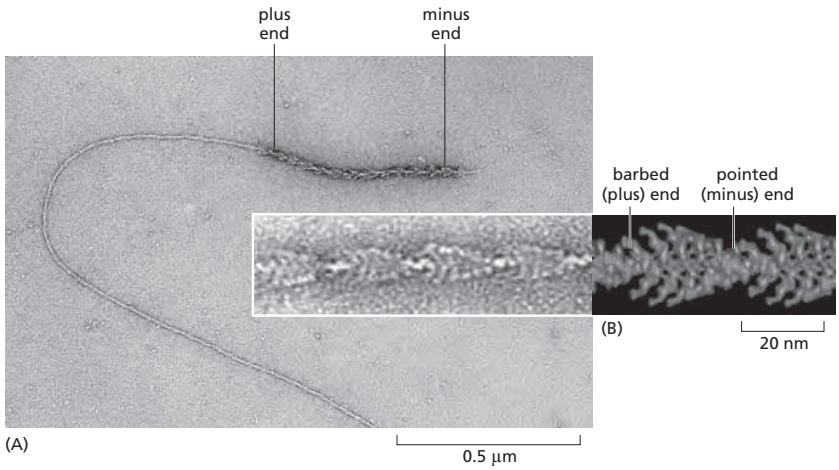


Figure 16–12 Structural polarity of the actin filament. (A) This electron micrograph shows an actin filament polymerized from a short actin filament seed that was decorated with myosin motor domains, resulting in an arrowhead pattern. The filament has grown much faster at the barbed (plus) end than at the pointed (minus) end. (B) Enlarged image and model showing the arrowhead pattern. (A, courtesy of Tom Pollard; B, adapted from M. Whittaker, B.O. Carragher and K.A. Milligan, *Ultramicro.* 54:245–260, 1995.)

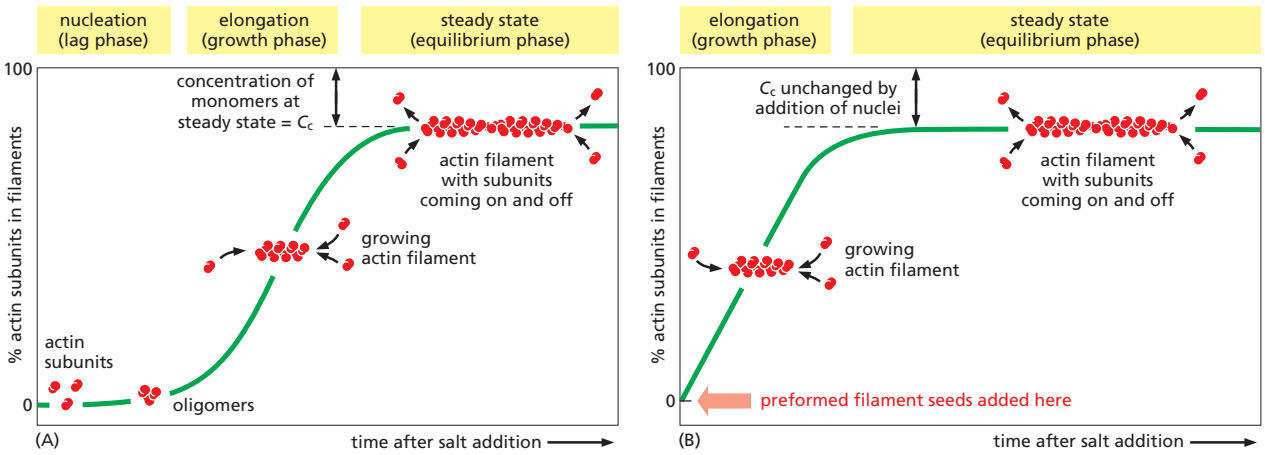


Figure 16–13 The time course of actin polymerization in a test tube. (A) Polymerization of pure actin subunits into filaments occurs after a lag phase. (B) Polymerization occurs more rapidly in the presence of preformed fragments of actin filaments, which act as nuclei for filament growth. As indicated, the % free subunits after polymerization reflects the critical concentration (C_c), at which there is no net change in polymer. Actin polymerization is often studied by observing the change in the light emission from a fluorescent probe, called pyrene, that has been covalently attached to the actin. Pyrene-actin fluoresces more brightly when it is incorporated into actin filaments.

phase of rapid filament elongation during which subunits are added quickly to the ends of the nucleated filaments (Figure 16–13A). Finally, as the concentration of actin monomers declines, the system approaches a steady state at which the rate of addition of new subunits to the filament ends exactly balances the rate of subunit dissociation. The concentration of free subunits left in solution at this point is called the *critical concentration*, C_c. As explained in **Panel 16–2**, the value of the critical concentration is equal to the rate constant for subunit loss divided by the rate constant for subunit addition—that is, $C_c = k_{off}/k_{on}$, which is equal to the dissociation constant, K_d, and the inverse of the equilibrium constant, K (see Figure 3–44). In a test tube, the C_c for actin polymerization—that is, the free actin monomer concentration at which the fraction of actin in the polymer stops increasing—is about 0.2 μM. Inside the cell, the concentration of unpolymerized actin is much higher than this, and the cell has evolved mechanisms to prevent most of its monomeric actin from assembling into filaments, as we discuss later.

The lag phase in filament growth is eliminated if preexisting seeds (such as fragments of actin filaments that have been chemically cross-linked) are added to the solution at the beginning of the polymerization reaction (Figure 16–13B). The cell takes great advantage of this nucleation requirement: it uses special proteins to catalyze filament nucleation at specific sites, thereby determining the location at which new actin filaments are assembled.

Actin Filaments Have Two Distinct Ends That Grow at Different Rates

Due to the uniform orientation of asymmetric actin subunits in the filament, the structures at its two ends are different. This orientation makes the two ends of each polymer different in ways that have a profound effect on filament growth rates. The kinetic rate constants for actin subunit association and dissociation—k_{on} and k_{off}, respectively—are much greater at the plus end than the minus end. This can be seen when an excess of purified actin monomers is allowed to assemble onto polarity-marked filaments—the plus end of the filament elongates up to ten times faster (see Figure 16–12). If filaments are rapidly diluted so that the free subunit concentration drops below the critical concentration, the plus end also depolymerizes faster.

It is important to note, however, that the two ends of an actin filament have the same net affinity for actin subunits, if all of the subunits are in the same nucleotide

state. Addition of a subunit to either end of a filament of n subunits results in a filament of $n + 1$ subunits. Thus, the free-energy difference, and therefore the equilibrium constant (and the critical concentration), must be the same for addition of subunits at either end of the polymer. In this case, the ratio of the rate constants, k_{off}/k_{on}, must be identical at the two ends, even though the absolute values of these rate constants are very different at each end (see Panel 16–2).

The cell takes advantage of actin filament dynamics and polarity to do mechanical work. Filament elongation proceeds spontaneously when the free-energy change (ΔG) for addition of the soluble subunit is less than zero. This is the case when the concentration of subunits in solution exceeds the critical concentration. A cell can couple an energetically unfavorable process to this spontaneous process; thus, the cell can use free energy released during spontaneous filament polymerization to move an attached load. For example, by orienting the fast-growing plus ends of actin filaments toward its leading edge, a motile cell can push its plasma membrane forward, as we discuss later.

ATP Hydrolysis Within Actin Filaments Leads to Treadmilling at Steady State

Thus far in our discussion of actin filament dynamics, we have ignored the critical fact that actin can catalyze the hydrolysis of the nucleoside triphosphate ATP. For free actin subunits, this hydrolysis proceeds very slowly; however, it is accelerated when the subunits are incorporated into filaments. Shortly after ATP hydrolysis occurs, the free phosphate group is released from each subunit, but the ADP remains trapped in the filament structure. Thus, two different types of filament structures can exist, one with the "T form" of the nucleotide bound (ATP), and one with the "D form" bound (ADP).

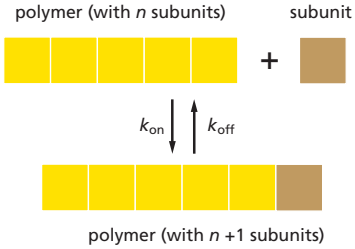
When the nucleotide is hydrolyzed, much of the free energy released by cleavage of the phosphate–phosphate bond is stored in the polymer. This makes the free-energy change for dissociation of a subunit from the D-form polymer more negative than the free-energy change for dissociation of a subunit from the T-form polymer. Consequently, the ratio of k_{off}/k_{on} for the D-form polymer, which is numerically equal to its critical concentration [C_c(D)], is larger than the corresponding ratio for the T-form polymer. Thus, C_c(D) is greater than C_c(T). At certain concentrations of free subunits, D-form polymers will therefore shrink while T-form polymers grow.

In living cells, most soluble actin subunits are in the T form, as the free concentration of ATP is about tenfold higher than that of ADP. However, the longer the time that subunits have been in the actin filament, the more likely they are to have hydrolyzed their ATP. Whether the subunit at each end of a filament is in the T or the D form depends on the rate of this hydrolysis compared with the rate of subunit addition. If the concentration of actin monomers is greater than the critical concentration for both the T-form and D-form polymer, then subunits will add to the polymer at both ends before the nucleotides in the previously added subunits are hydrolyzed; as a result, the tips of the actin filament will remain in the T form. On the other hand, if the subunit concentration is less than the critical concentrations for both the T-form and D-form polymer, then hydrolysis may occur before the next subunit is added and both ends of the filament will be in the D form and will shrink. At intermediate concentrations of actin subunits, it is possible for the rate of subunit addition to be faster than nucleotide hydrolysis at the plus end, but slower than nucleotide hydrolysis at the minus end. In this case, the plus end of the filament remains in the T conformation, while the minus end adopts the D conformation. The filament then undergoes a net addition of subunits at the plus end, while simultaneously losing subunits from the minus end. This leads to the remarkable property of filament **treadmilling** (**Figure 16–14**; see Panel 16–2).

At a particular intermediate subunit concentration, the filament growth at the plus end exactly balances the filament shrinkage at the minus end. Under these conditions, the subunits cycle rapidly between the free and filamentous states,

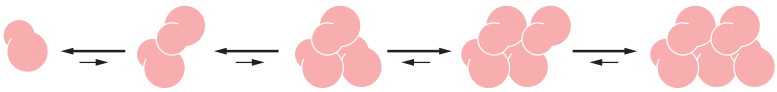
ON RATES AND OFF RATES

A linear polymer of protein molecules, such as an actin filament or a microtubule, assembles (polymerizes) and disassembles (depolymerizes) by the addition and removal of subunits at the ends of the polymer. The rate of addition of these subunits (called monomers) is given by the rate constant k_{on}, which has units of M^{-1} sec^{-1}. The rate of loss is given by k_{off} (units of sec^{-1}).



NUCLEATION

A helical polymer is stabilized by multiple contacts between adjacent subunits. In the case of actin, two actin molecules bind relatively weakly to each other, but addition of a third actin monomer to form a trimer makes the entire group more stable.



Further monomer addition can take place onto this trimer, which therefore acts as a nucleus for polymerization. For tubulin, the nucleus is larger and has a more complicated structure (possibly a ring of 13 or more tubulin molecules)—but the principle is the same.

The assembly of a nucleus is relatively slow, which explains the lag phase seen during polymerization. The lag phase can be reduced or abolished entirely by adding premade nuclei, such as fragments of already polymerized microtubules or actin filaments.

THE CRITICAL CONCENTRATION

The number of monomers that add to the polymer (actin filament or microtubule) per second will be proportional to the concentration of the free subunit ($k_{on}C$), but the subunits will leave the polymer end at a constant rate (k_{off}) that does not depend on C. As the polymer grows, subunits are used up, and C is observed to drop until it reaches a constant value, called the critical concentration (C_c). At this concentration, the rate of subunit addition equals the rate of subunit loss.

At this equilibrium,

$$k_{on}\,C = k_{off}$$

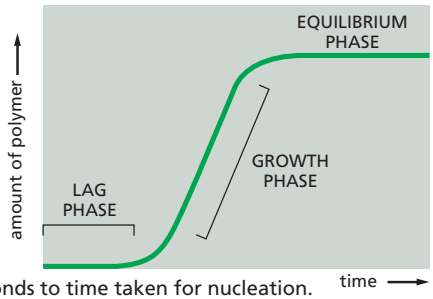
so that

$$C_c = \frac{k_{off}}{k_{on}} = K_d$$

(where K_d is the dissociation constant; see Figure 3–44).

TIME COURSE OF POLYMERIZATION

The assembly of a protein into a long helical polymer such as a cytoskeletal filament or a bacterial flagellum typically shows the following time course:



The lag phase corresponds to time taken for nucleation.

The growth phase occurs as monomers add to the exposed ends of the growing filament, causing filament elongation.

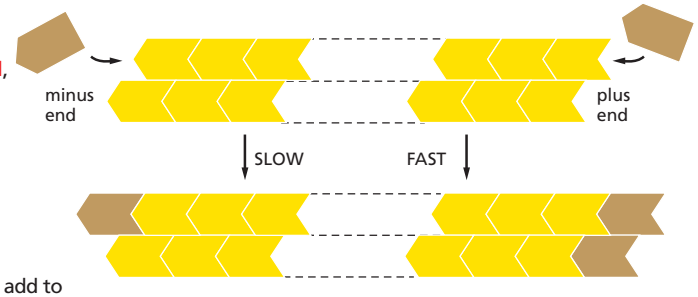
The equilibrium phase, or steady state, is reached when the growth of the polymer due to monomer addition precisely balances the shrinkage of the polymer due to disassembly back to monomers.

PLUS AND MINUS ENDS

The two ends of an actin filament or microtubule polymerize at different rates. The fast-growing end is called the plus end, whereas the slow-growing end is called the minus end. The difference in the rates of growth at the two ends is made possible by changes in the conformation of each subunit as it enters the polymer.

This conformational change affects the rates at which subunits add to the two ends.

Even though k_{on} and k_{off} will have different values for the plus and minus ends of the polymer, their ratio k_{off}/k_{on}—and hence C_c—must be the same at both ends for a simple polymerization reaction (no ATP or GTP hydrolysis). This is because exactly the same subunit interactions are broken when a subunit is lost at either end, and the final state of the subunit after dissociation is identical. Therefore, the ΔG for subunit loss, which determines the equilibrium constant for its association with the end, is identical at both ends: if the plus end grows four times faster than the minus end, it must also shrink four times faster. Thus, for $C > C_c$, both ends grow; for $C < C_c$, both ends shrink.



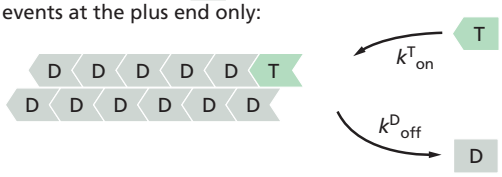
The nucleoside triphosphate hydrolysis that accompanies actin and tubulin polymerization removes this constraint.

NUCLEOTIDE HYDROLYSIS

Each actin molecule carries a tightly bound ATP molecule that is hydrolyzed to a tightly bound ADP molecule soon after its assembly into the polymer. Similarly, each tubulin molecule carries a tightly bound GTP that is converted to a tightly bound GDP molecule soon after the molecule assembles into the polymer.

Hydrolysis of the bound nucleotide reduces the binding affinity of the subunit for neighboring subunits and makes it more likely to dissociate from each end of the filament (see Figure 16–44 for a possible mechanism). It is usually the T form that adds to the filament and the D form that leaves.

Considering events at the plus end only:



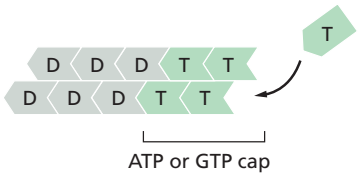
As before, the polymer will grow until $C = C_c$. For illustrative purposes, we can ignore k^D_{on} and k^T_{off} since they are usually very small, so that polymer growth ceases when

$$k^T_{on}\, C = k^D_{off} \quad \text{or} \quad C_c = \frac{k^D_{off}}{k^T_{on}}$$

This is a steady state and not a true equilibrium, because the ATP or GTP that is hydrolyzed must be replenished by a nucleotide exchange reaction of the free subunit (D → T).

ATP CAPS AND GTP CAPS

The rate of addition of subunits to a growing actin filament or microtubule can be faster than the rate at which their bound nucleotide is hydrolyzed. Under such conditions, the end has a "cap" of subunits containing the nucleoside triphosphate—an ATP cap on an actin filament or a GTP cap on a microtubule.



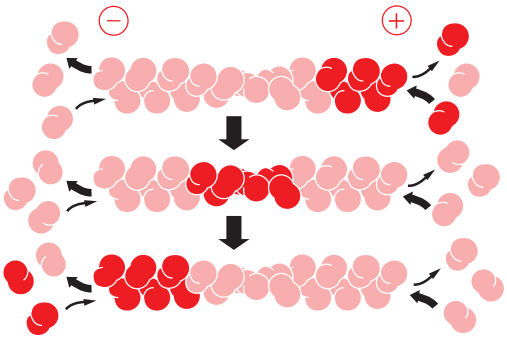
DYNAMIC INSTABILITY and TREADMILLING are two behaviors observed in cytoskeletal polymers. Both are associated with nucleoside triphosphate hydrolysis. Dynamic instability is believed to predominate in microtubules, whereas treadmilling may predominate in actin filaments.

TREADMILLING

One consequence of the nucleotide hydrolysis that accompanies polymer formation is to change the critical concentration at the two ends of the polymer. Since k^D_{off} and k^T_{on} refer to different reactions, their ratio k^D_{off}/k^T_{on} need not be the same at both ends of the polymer, so that:

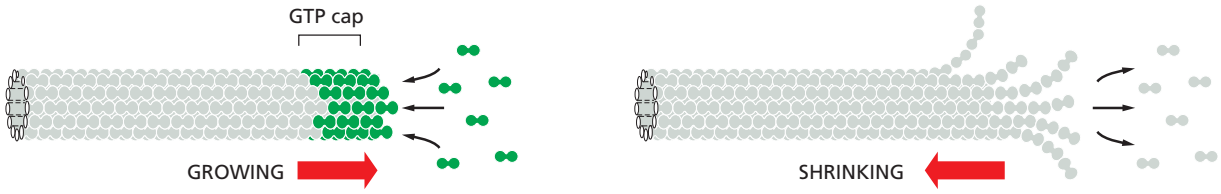
$$C_c \text{ (minus end)} > C_c \text{ (plus end)}$$

Thus, if both ends of a polymer are exposed, polymerization proceeds until the concentration of free monomer reaches a value that is above C_c for the plus end but below C_c for the minus end. At this steady state, subunits undergo a net assembly at the plus end and a net disassembly at the minus end at an identical rate. The polymer maintains a constant length, even though there is a net flux of subunits through the polymer, known as treadmilling.



DYNAMIC INSTABILITY

Microtubules depolymerize about 100 times faster from an end containing GDP-tubulin than from one containing GTP-tubulin. A GTP cap favors growth, but if it is lost, then depolymerization ensues.



Individual microtubules can therefore alternate between a period of slow growth and a period of rapid disassembly, a phenomenon called dynamic instability.

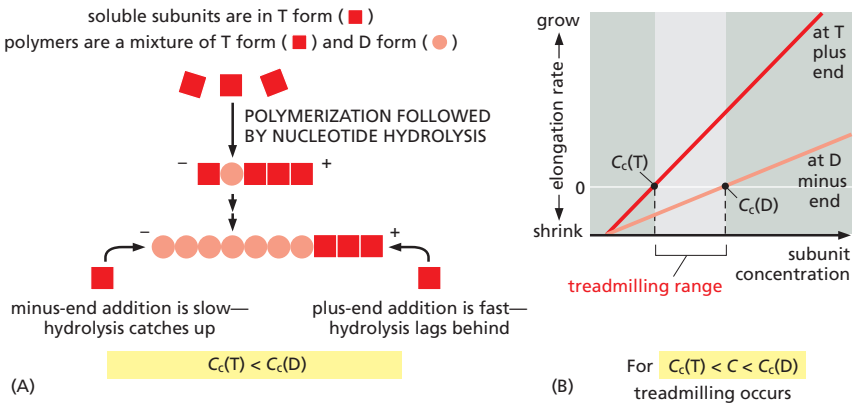


Figure 16–14 Treadmilling of an actin filament, made possible by the ATP hydrolysis that follows subunit addition. (A) Explanation for the different critical concentrations (C_c) at the plus and minus ends. Subunits with bound ATP (T-form subunits) polymerize at both ends of a growing filament, and then undergo nucleotide hydrolysis within the filament. As the filament grows, elongation is faster than hydrolysis at the plus end in this example, and the terminal subunits at this end are therefore always in the T form. However, hydrolysis is faster than elongation at the minus end, and so terminal subunits at this end are in the D form. (B) Treadmilling occurs at intermediate concentrations of free subunits. The critical concentration for polymerization on a filament end in the T form is lower than for a filament end in the D form. If the actual subunit concentration is somewhere between these two values, the plus end grows while the minus end shrinks, resulting in treadmilling.

while the total length of the filament remains unchanged. This "steady-state treadmilling" requires a constant consumption of energy in the form of ATP hydrolysis.

The Functions of Actin Filaments Are Inhibited by Both Polymer-stabilizing and Polymer-destabilizing Chemicals

Chemical compounds that stabilize or destabilize actin filaments are important tools in studies of the filaments' dynamic behavior and function in cells. The *cytochalasins* are fungal products that prevent actin polymerization by binding to the plus end of actin filaments. *Latrunculin* prevents actin polymerization by binding to actin subunits. The *phalloidins* are toxins isolated from the *Amanita* mushroom that bind tightly all along the side of actin filaments and stabilize them against depolymerization. All of these compounds cause dramatic changes in the actin cytoskeleton and are toxic to cells, indicating that the function of actin filaments depends on a dynamic equilibrium between filaments and actin monomers (**Table 16–1**).

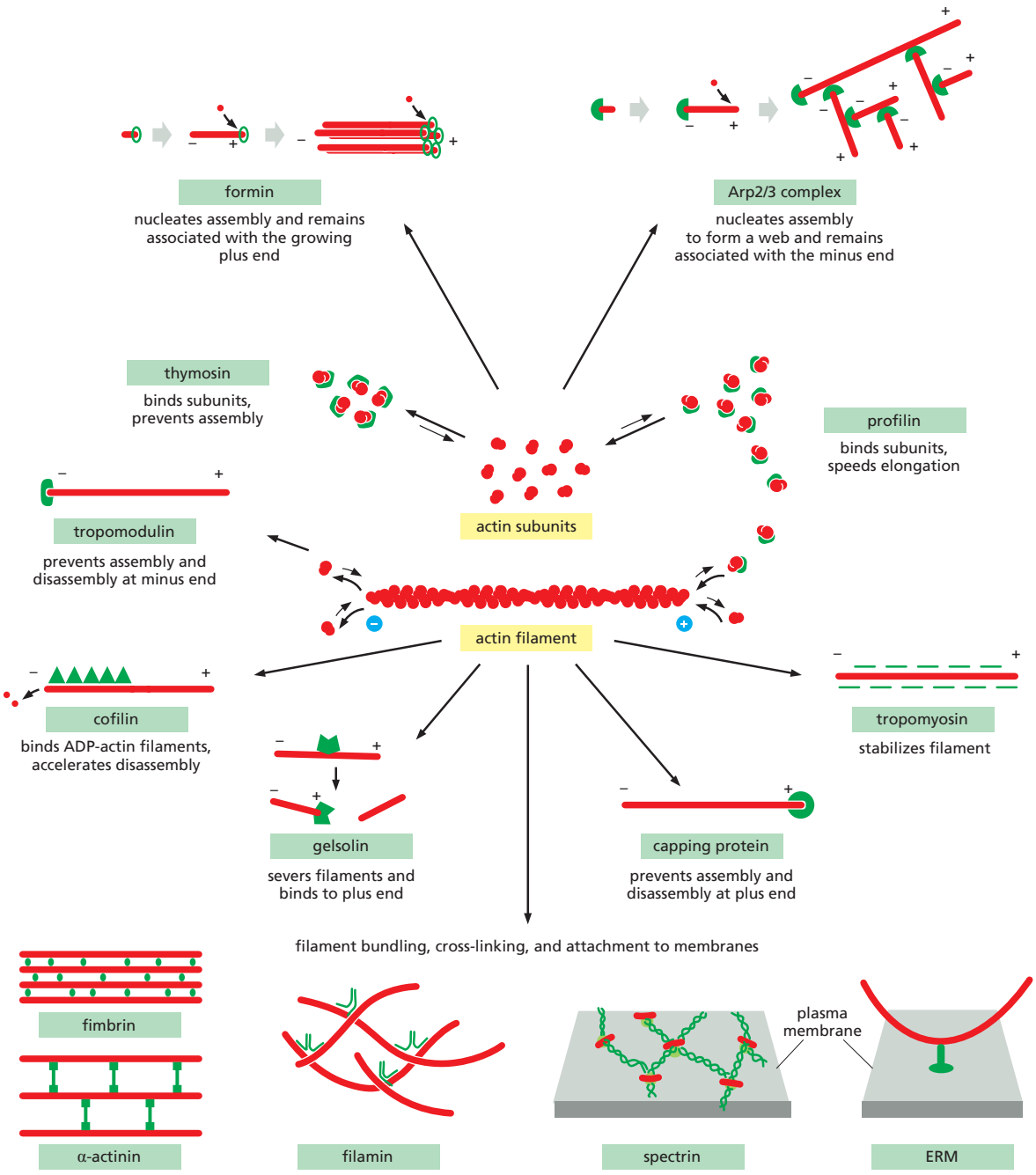
Actin-Binding Proteins Influence Filament Dynamics and Organization

In a test tube, polymerization of actin is controlled simply by its concentration, as described above, and by pH and the concentrations of salts and ATP. Within a cell, however, actin behavior is also regulated by numerous accessory proteins that bind actin monomers or filaments (summarized in **Panel 16–3**). At steady state

TABLE 16–1 Chemical Inhibitors of Actin and Microtubules

Chemical	Effect on filaments	Mechanism	Original source
Actin			
Latrunculin	Depolymerizes	Binds actin subunits	Sponges
Cytochalasin B	Depolymerizes	Caps filament plus ends	Fungi
Phalloidin	Stabilizes	Binds along filaments	*Amanita* mushroom
Microtubules			
Taxol® (paclitaxel)	Stabilizes	Binds along filaments	Yew tree
Nocodazole	Depolymerizes	Binds tubulin subunits	Synthetic
Colchicine	Depolymerizes	Caps filament ends	Autumn crocus

ACTIN FILAMENTS



Some of the major accessory proteins of the actin cytoskeleton. Except for the myosin motor proteins, an example of each major type is shown. Each of these is discussed in the text. However, most cells contain more than a hundred different actin-binding proteins, and it is likely that there are important types of actin-associated proteins that are not yet recognized.

in vitro, when the monomer concentration is 0.2 μM, filament half-life, a measure of how long an individual actin monomer spends in a filament as it treadmills, is approximately 30 minutes. In a non-muscle vertebrate cell, actin half-life in filaments is only 30 seconds, demonstrating that cellular factors modify the dynamic behavior of actin filaments. Actin-binding proteins dramatically alter actin filament dynamics and organization through spatial and temporal control of monomer availability, filament nucleation, elongation, and depolymerization. In the following sections, we describe the ways in which these accessory proteins modify actin function in the cell.

Monomer Availability Controls Actin Filament Assembly

In most non-muscle vertebrate cells, approximately 50% of the actin is in filaments and 50% is soluble—and yet the soluble monomer concentration is 50–200 μM, well above the critical concentration. Why does so little of the actin polymerize into filaments? The reason is that the cell contains proteins that bind to the actin monomers and make polymerization much less favorable (an action similar to that of the drug latrunculin). A small protein called *thymosin* is the most abundant of these proteins. Actin monomers bound to thymosin are in a locked state, where they cannot associate with either the plus or minus ends of actin filaments and can neither hydrolyze nor exchange their bound nucleotide.

How do cells recruit actin monomers from this buffered storage pool and use them for polymerization? The answer depends on another monomer-binding protein called *profilin*. Profilin binds to the face of the actin monomer opposite the ATP-binding cleft, blocking the side of the monomer that would normally associate with the filament minus end, while leaving exposed the site on the monomer that binds to the plus end (**Figure 16–15**). When the profilin–actin complex binds a free plus end, a conformational change in actin reduces its affinity for profilin and the profilin falls off, leaving the actin filament one subunit longer. Profilin competes with thymosin for binding to individual actin monomers. Thus, by regulating the local activity of profilin, cells can control the movement of actin subunits from the sequestered thymosin-bound pool onto filament plus ends.

Several mechanisms regulate profilin activity, including profilin phosphorylation and profilin binding to inositol phospholipids. These mechanisms can define the sites where profilin acts. For example, profilin is required for filament assembly at the plasma membrane, where it is recruited by an interaction with acidic membrane phospholipids. At this location, extracellular signals can activate profilin to produce local actin polymerization and the extension of actin-rich motile structures such as filopodia and lamellipodia.

Actin-Nucleating Factors Accelerate Polymerization and Generate Branched or Straight Filaments

In addition to the availability of active actin subunits, a second prerequisite for cellular actin polymerization is filament nucleation. Proteins that contain actin monomer binding motifs linked in tandem mediate the simplest mechanism of filament nucleation. These actin-nucleating proteins bring several actin subunits together to form a seed. In most cases, actin nucleation is catalyzed by one of two different types of factors: the Arp 2/3 complex or the formins. The first of these is a complex of proteins that includes two *actin-related proteins*, or *ARPs*, each of which is about 45% identical to actin. The **Arp 2/3 complex** nucleates actin filament growth from the minus end, allowing rapid elongation at the plus end (**Figure 16–16**A and B). The complex can attach to the side of another actin filament while remaining bound to the minus end of the filament that it has nucleated, thereby building individual filaments into a treelike web (Figure 16–16C and D).

Formins are dimeric proteins that nucleate the growth of straight, unbranched filaments that can be cross-linked by other proteins to form parallel bundles. Each formin subunit has a binding site for monomeric actin, and the formin dimer appears to nucleate actin filament polymerization by capturing two monomers.

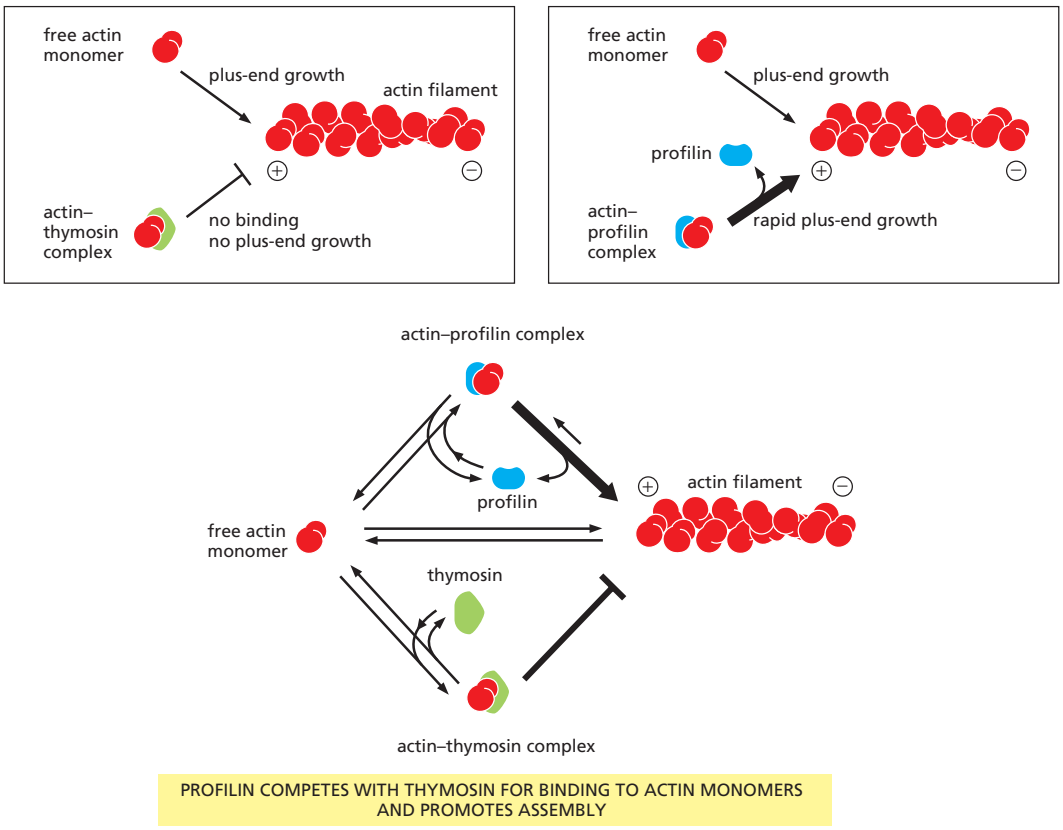


Figure 16–15 Effects of thymosin and profilin on actin polymerization. An actin monomer bound to thymosin is sterically prevented from binding to and elongating the plus end of an actin filament (*left*). An actin monomer bound to profilin, on the other hand, is capable of elongating a filament (*right*). Thymosin and profilin cannot both bind to a single actin monomer at the same time. In a cell in which most of the actin monomer is bound to thymosin, the activation of a small amount of profilin can produce rapid filament assembly. As indicated (*bottom*), profilin binds to actin monomers that are transiently released from the thymosin-bound monomer pool, shuttles them onto the plus ends of actin filaments, and is then released and recycled for further rounds of filament elongation.

As the newly nucleated filament grows, the formin dimer remains associated with the rapidly growing plus end while still allowing the addition of new subunits at that end (**Figure 16–17**). This mechanism of filament assembly is clearly different from that used by the Arp 2/3 complex, which remains stably bound to the filament minus end, preventing subunit addition or loss at that end. Formin-dependent actin filament growth is strongly enhanced by the association of actin monomers with profilin (**Figure 16–18**).

Like profilin activation, actin filament nucleation by Arp 2/3 complexes and formins occurs primarily at the plasma membrane, and the highest density of actin filaments in most cells is at the cell periphery. The layer just beneath the plasma membrane is called the **cell cortex**, and the actin filaments in this region determine the shape and movement of the cell surface, allowing the cell to change its shape and stiffness rapidly in response to changes in its external environment.

Actin-Filament-Binding Proteins Alter Filament Dynamics

Actin filament behavior is regulated by two major classes of binding proteins: those that bind along the side of a filament and those that bind to the ends (see Panel 16–3). Side-binding proteins include *tropomyosin*, an elongated protein that binds simultaneously to six or seven adjacent actin subunits along each of the two grooves of the helical actin filament. In addition to stabilizing and stiffening

the filament, the binding of tropomyosin can prevent the actin filament from interacting with other proteins; this aspect of tropomyosin function is important in the control of muscle contraction, as we discuss later.

An actin filament that stops growing and is not specifically stabilized in the cell will depolymerize rapidly, particularly at its plus end, once the actin molecules

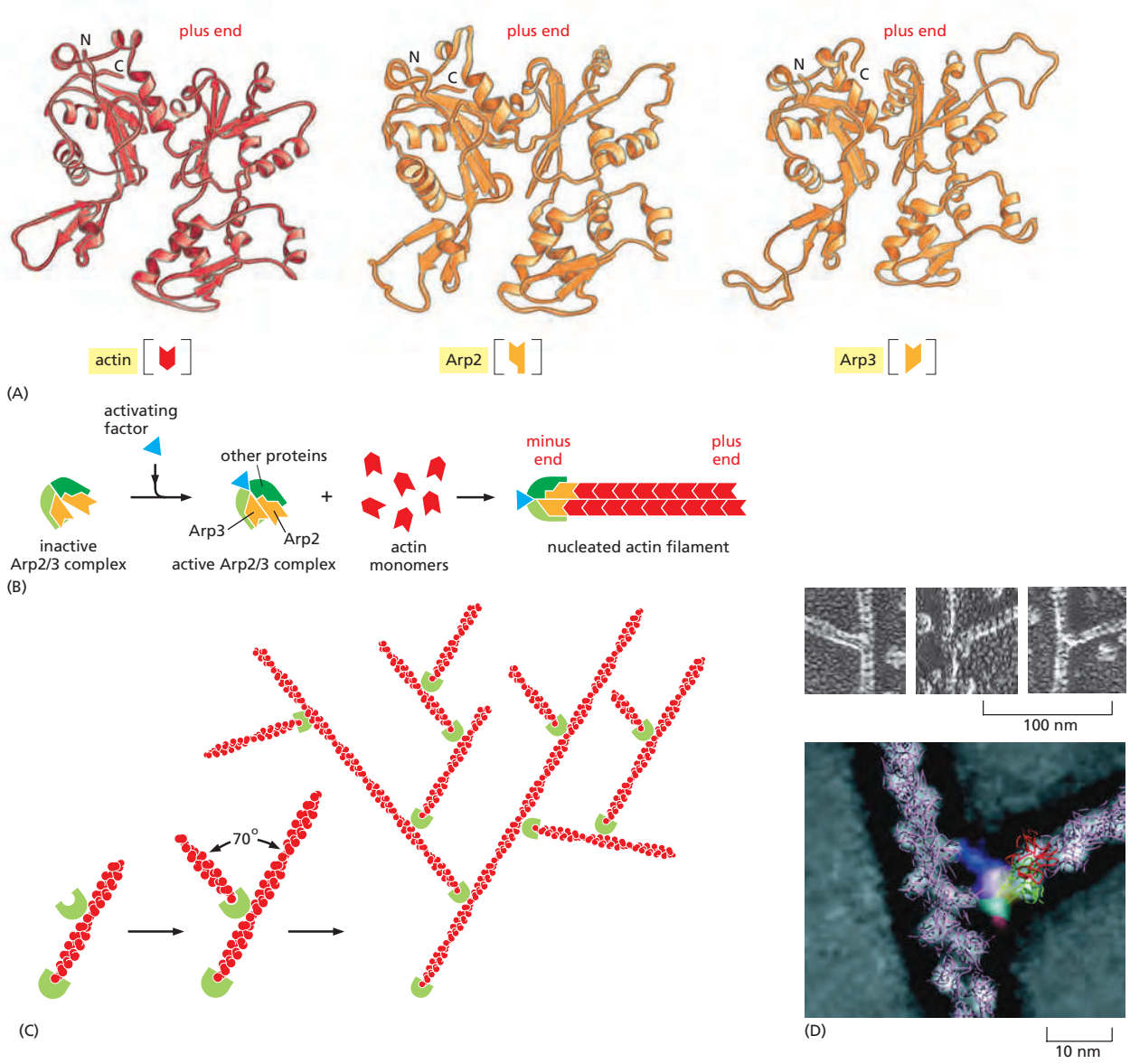


Figure 16–16 Nucleation and actin web formation by the Arp 2/3 complex. (A) The structures of Arp2 and Arp3 compared to the structure of actin. Although the face of the molecule equivalent to the plus end *(top)* in both Arp2 and Arp3 is very similar to the plus end of actin itself, differences on the sides and minus end prevent these actin-related proteins from forming filaments on their own or coassembling into filaments with actin. (B) A model for actin filament nucleation by the Arp 2/3 complex. In the absence of an activating factor, Arp2 and Arp3 are held by their accessory proteins in an orientation that prevents them from nucleating a new actin filament. When an activating factor (indicated by the *blue triangle*) binds the complex, Arp2 and Arp3 are brought together into a new configuration that resembles the plus end of an actin filament. Actin subunits can then assemble onto this structure, bypassing the rate-limiting step of filament nucleation. (C) The Arp 2/3 complex nucleates filaments most efficiently when it is bound to the side of a preexisting actin filament. The result is a filament branch that grows at a 70° angle relative to the original filament. Repeated rounds of branching nucleation result in a treelike web of actin filaments. (D) Top, electron micrographs of branched actin filaments formed by mixing purified actin subunits with purified Arp 2/3 complexes. Bottom, reconstructed image of a branch where the crystal structures of actin *(pink)* and the Arp 2/3 complex have been fitted to the electron density. The mother filament runs from top to bottom, and the daughter filament branches off to the right where the Arp 2/3 complex binds to three actin subunits in the mother filament. (D, top, from R.D. Mullins et al., *Proc. Natl Acad. Sci. USA* 95:6181–6186, 1998, with permission from National Academy of Sciences; botttom, from N. Volkmann et al., *Science* 293:2456–2459, 2001, with permission from AAAS.)

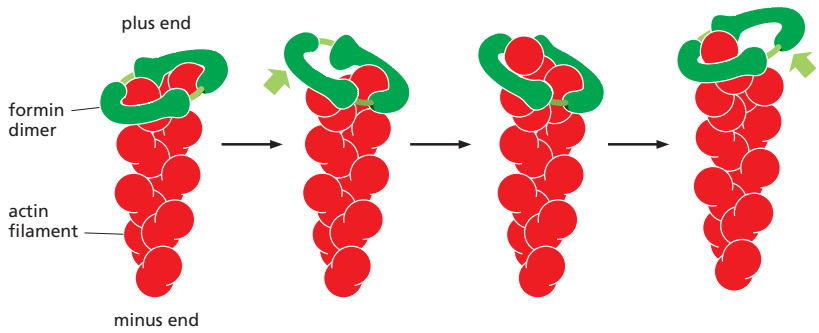


Figure 16–17 Actin elongation mediated by formins. Formin proteins *(green)* form a dimeric complex that can nucleate the formation of a new actin filament *(red)* and remain associated with the rapidly growing plus end as it elongates. The formin protein maintains its binding to one of the two actin subunits exposed at the plus end as it allows each new subunit to assemble. Only part of the large dimeric formin molecule is shown here. Other regions regulate its activity and link it to particular structures in the cell. Many formins are indirectly connected to the cell plasma membrane and aid the insertional polymerization of the actin filament directly beneath the membrane surface.

have hydrolyzed their ATP. The binding of plus-end *capping protein* (also called *CapZ* for its location in the muscle Z band) stabilizes an actin filament at its plus end by rendering it inactive, greatly reducing the rates of filament growth and depolymerization (**Figure 16–19**). At the minus end, an actin filament may be capped by the Arp 2/3 complex that was responsible for its nucleation, although many minus ends in a typical cell are released from the Arp 2/3 complex and are uncapped.

Tropomodulin, best known for its function in the capping of exceptionally long-lived actin filaments in muscle, binds tightly to the minus ends of actin filaments that have been coated and thereby stabilized by tropomyosin. It can also transiently cap pure actin filaments and significantly reduce their elongation and depolymerization rates. A large family of tropomodulin proteins regulates actin filament length and stability in many cell types.

For maximum effect, proteins that bind the side of actin filaments coat the filament completely, and must therefore be present in high amounts. In contrast, end-binding proteins can affect filament dynamics even when they are present at very low levels. Since subunit addition and loss occur primarily at filament ends, one molecule of an end-binding protein per actin filament (roughly one molecule per 200–500 actin subunits) can be enough to transform the architecture of an actin filament network.

Severing Proteins Regulate Actin Filament Depolymerization

Another important mechanism of actin filament regulation depends on proteins that break an actin filament into many smaller filaments, thereby generating a large number of new filament ends. The fate of these new ends depends on the presence of other accessory proteins. Under some conditions, newly formed ends nucleate filament elongation, thereby accelerating the assembly of new filament structures. Under other conditions, severing promotes the depolymerization of old filaments, speeding up the depolymerization rate by tenfold or more. In addition, severing changes the physical and mechanical properties of the cytoplasm: stiff, large bundles and gels become more fluid.

One class of actin-severing proteins is the *gelsolin superfamily*. These proteins are activated by high levels of cytosolic Ca^{2+}. Gelsolin interacts with the side of the actin filament and contains subdomains that bind to two different sites: one that is exposed on the surface of the filament and one that is hidden between adjacent

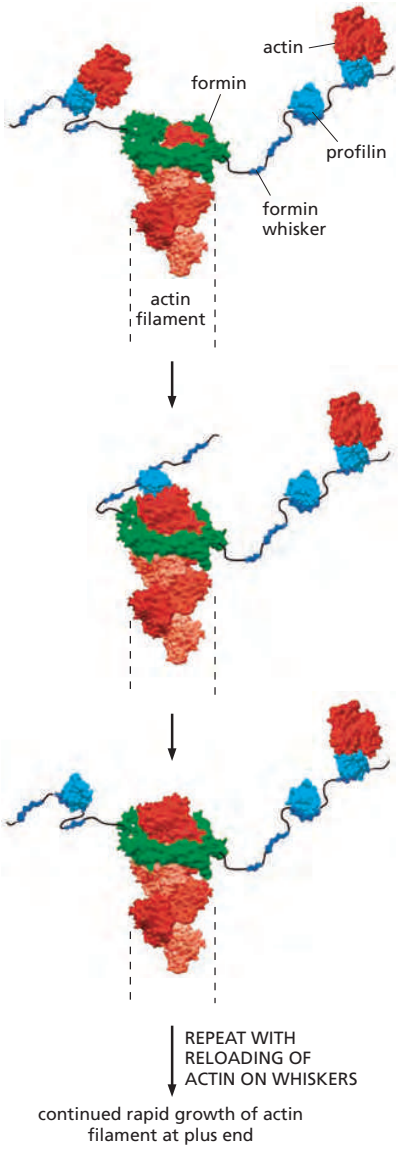


Figure 16–18 Profilin and formins. Some members of the formin protein family have unstructured domains or "whiskers" that contain several binding sites for profilin or the profilin–actin complex. These flexible domains serve as a staging area for addition of actin to the growing plus end of the actin filament when formin is bound. Under some conditions, this can enhance the rate of actin filament elongation so that filament growth is faster than that expected for a diffusion-controlled reaction, and faster in the presence of formin and profilin than the rate for pure actin alone (see also Figure 3–78).

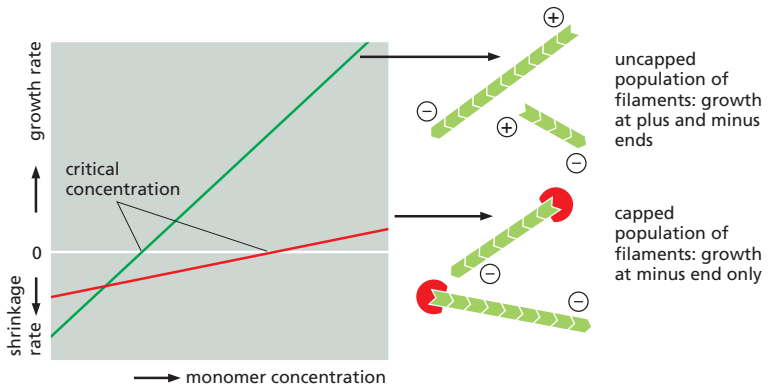


Figure 16–19 Filament capping and its effects on filament dynamics. A population of uncapped filaments adds and loses subunits at both the plus and minus ends, resulting in rapid growth or shrinkage, depending on the concentration of available free monomers (*green* line). In the presence of a protein that caps the plus end (*red* line), only the minus end is able to add or lose subunits; consequently, filament growth will be slower at all monomer concentrations above the critical concentration, and filament shrinkage will be slower at all monomer concentrations below the critical concentration. In addition, the critical concentration for the population shifts to that of the filament minus end.

subunits. According to one model, gelsolin binds the side of an actin filament until a thermal fluctuation creates a small gap between neighboring subunits, at which point gelsolin inserts itself into the gap to break the filament. After the severing event, gelsolin remains attached to the actin filament and caps the new plus end.

Another important actin-filament destabilizing protein, found in all eukaryotic cells, is *cofilin*. Also called *actin depolymerizing factor*, cofilin binds along the length of the actin filament, forcing the filament to twist a little more tightly (**Figure 16–20**). This mechanical stress weakens the contacts between actin subunits in the filament, making the filament brittle and more easily severed by thermal motions, generating filament ends that undergo rapid disassembly. As a result, most of the actin filaments inside cells are shorter lived than are filaments formed from pure actin in a test tube.

Cofilin binds preferentially to ADP-containing actin filaments rather than to ATP-containing filaments. Since ATP hydrolysis is usually slower than filament assembly, the newest actin filaments in the cell still contain mostly ATP and are resistant to depolymerization by cofilin. Cofilin therefore tends to dismantle the older filaments in the cell. As we will discuss later, the cofilin-mediated disassembly of old but not new actin filaments is critical for the polarized, directed growth of the actin network that is responsible for unidirectional cell crawling and the intracellular motility of pathogens. Actin filaments can be protected from cofilin by tropomyosin binding. Thus, the dynamics of actin in different subcellular locations depends on the balance of stabilizing and destabilizing accessory proteins.

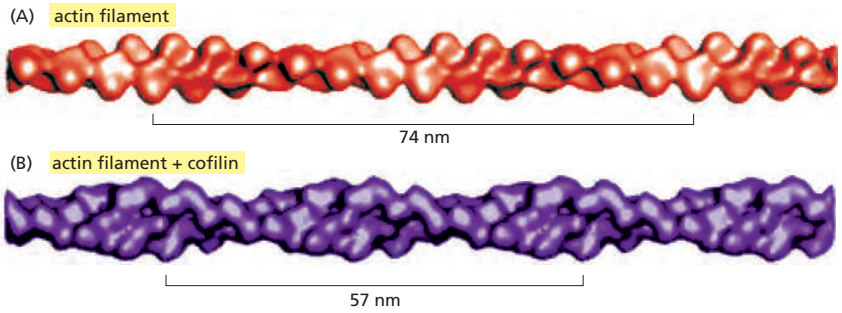


Figure 16–20 Twisting of an actin filament induced by cofilin. (A) Three-dimensional reconstruction from cryoelectron micrographs of filaments made of pure actin. The bracket shows the span of two twists of the actin helix. (B) Reconstruction of an actin filament coated with cofilin, which binds in a 1:1 stoichiometry to actin subunits all along the filament. Cofilin is a small protein (14 kD) compared to actin (43 kD), and so the filament appears only slightly thicker. The energy of cofilin binding serves to deform the actin filament, twisting it more tightly and reducing the distance spanned by each twist of the helix. (From A. McGough et al., *J. Cell Biol.* 138:771–781, 1997. With permission from the authors.)

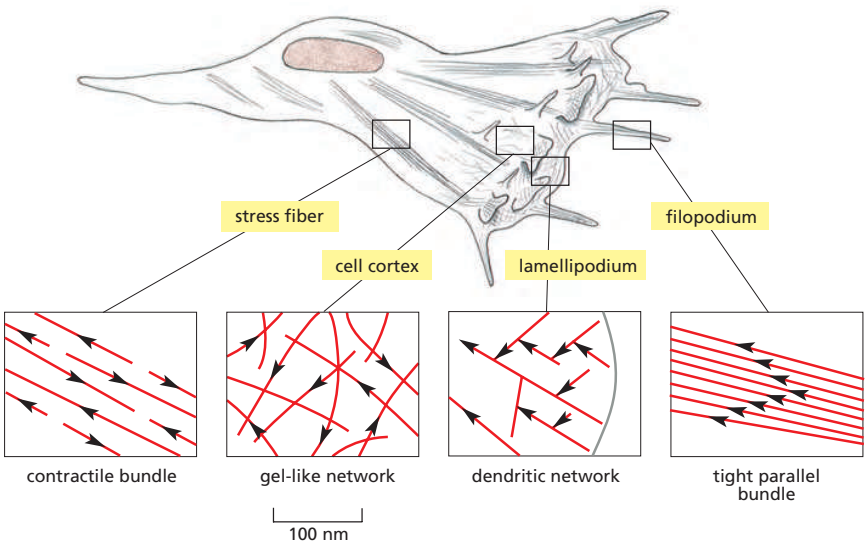


Figure 16–21 Actin arrays in a cell. A fibroblast crawling in a tissue-culture dish is shown with four areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in *red*, with arrowheads pointing toward the minus end. Stress fibers are contractile and exert tension. The actin cortex underlies the plasma membrane and consists of gel-like networks or dendritic actin networks that enable membrane protrusion at lamellopodia. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environment.

Higher-Order Actin Filament Arrays Influence Cellular Mechanical Properties and Signaling

Actin filaments in animal cells are organized into several types of arrays: dendritic networks, bundles, and weblike (gel-like) networks (**Figure 16–21**). Different structures are initiated by the action of distinct nucleating proteins: the actin filaments of dendritic networks are nucleated by the Arp 2/3 complex, while bundles are made of the long, straight filaments produced by formins. The proteins nucleating the filaments in the gel-like networks are not yet well defined.

The structural organization of different actin networks depends on specialized accessory proteins. As explained earlier, Arp 2/3 organizes filaments into dendritic networks by attaching filament minus ends to the side of other filaments. Other actin filament structures are assembled and maintained by two classes of proteins: *bundling proteins,* which cross-link actin filaments into a parallel array, and *gel-forming proteins*, which hold two actin filaments together at a large angle to each other, thereby creating a looser meshwork. Both bundling and gel-forming proteins generally have two similar actin-filament-binding sites, which can either be part of a single polypeptide chain or contributed by each of two polypeptide chains held together in a dimer (**Figure 16–22**). The spacing and arrangement of these two filament-binding domains determine the type of actin structure that a given cross-linking protein forms.

Each type of bundling protein also determines which other molecules can interact with the cross-linked actin filaments. Myosin II is the motor protein that enables stress fibers and other contractile arrays to contract. The very close packing of actin filaments caused by the small monomeric bundling protein *fimbrin* apparently excludes myosin, and thus the parallel actin filaments held together by fimbrin are not contractile. On the other hand, α-actinin cross-links oppositely

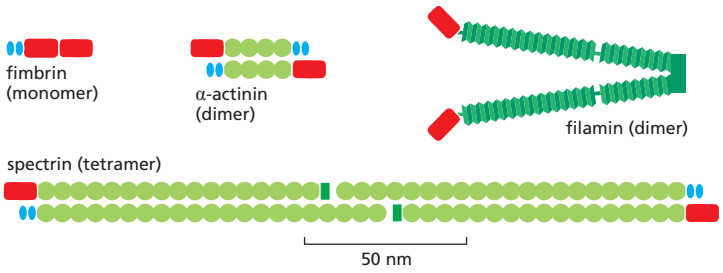


Figure 16–22 The modular structures of four actin-cross-linking proteins. Each of the proteins shown has two actin-binding sites *(red)* that are related in sequence. Fimbrin has two directly adjacent actin-binding sites, so that it holds its two actin filaments very close together (14 nm apart), aligned with the same polarity (see Figure 16–23A). The two actin-binding sites in α-actinin are separated by a spacer around 30 nm long, so that it forms more loosely packed actin bundles (see Figure 16–23A). Filamin has two actin-binding sites with a V-shaped linkage between them, so that it cross-links actin filaments into a network with the filaments oriented almost at right angles to one another (see Figure 16–24). Spectrin is a tetramer of two α and two β subunits, and the tetramer has two actin-binding sites spaced about 200 nm apart (see Figure 10–38).

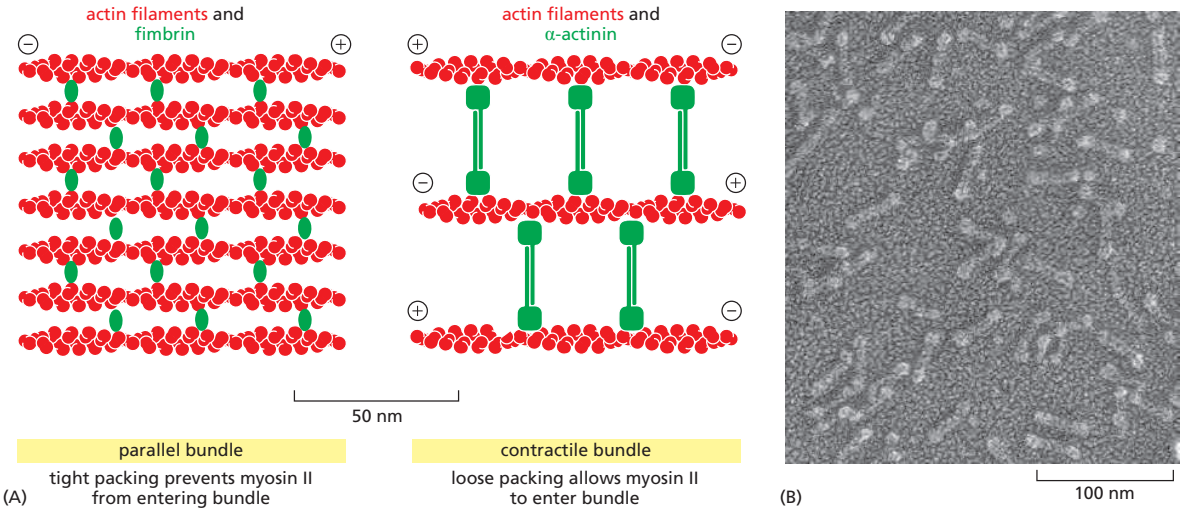


Figure 16–23 The formation of two types of actin filament bundles. (A) Fimbrin cross-links actin filaments into tight bundles, which exclude the motor protein myosin II from participating in the assembly. In contrast, α-actinin, which is a homodimer, cross-links actin filaments into loose bundles, which allow myosin (not shown) to incorporate into the bundle. Fimbrin and α-actinin tend to exclude one another because of the very different spacing of the actin filament bundles that they form. (B) Electron micrograph of purified α-actinin molecules. (B, courtesy of John Heuser.)

polarized actin filaments into loose bundles, allowing the binding of myosin and formation of contractile actin bundles (**Figure 16–23**). Because of the very different spacing and orientation of the actin filaments, bundling by fimbrin automatically discourages bundling by α-actinin, and vice versa, so that the two types of bundling protein are mutually exclusive.

The bundling proteins that we have discussed so far have straight, stiff connections between their two actin-filament-binding domains. Other actin cross-linking proteins have either a flexible or a stiff, bent connection between their two binding domains, allowing them to form actin filament webs or gels, rather than actin bundles. *Filamin* (see Figure 16–22) promotes the formation of a loose and highly viscous gel by clamping together two actin filaments roughly at right angles (**Figure 16–24**A). Cells require the actin gels formed by filamin to extend the thin, sheetlike membrane projections called *lamellipodia* that help them to crawl across solid surfaces. In humans, mutations in the filamin A gene cause defects in nerve-cell migration during early embryonic development. Cells in the periventricular region of the brain fail to migrate to the cortex and instead form nodules, causing a syndrome called periventricular heterotopia (Figure 16–24B). Interestingly, in addition to binding actin, filamins have been reported to interact with a large number of cellular proteins of great functional diversity, including membrane receptors for signaling molecules, and filamin mutations can also lead to defects in development of bone, the cardiovascular system, and other organs. Thus, filamins may also function as signaling scaffolds by connecting and coordinating a wide variety of cellular processes with the actin cytoskeleton.

A very different, well-studied web-forming protein is *spectrin*, which was first identified in red blood cells. Spectrin is a long, flexible protein made out of four elongated polypeptide chains (two α subunits and two β subunits), arranged so that the two actin-filament-binding sites are about 200 nm apart (compared with 14 nm for fimbrin and about 30 nm for α-actinin; see Figure 16–23). In the red blood cell, spectrin is concentrated just beneath the plasma membrane, where it forms a two-dimensional weblike network held together by short actin filaments whose precise lengths are tightly regulated by capping proteins at each end; spectrin links this web to the plasma membrane because it has separate binding sites for peripheral membrane proteins, which are themselves positioned near the lipid bilayer by integral membrane proteins (see Figure 10–38). The resulting network creates a strong, yet flexible cell cortex that provides mechanical support for the overlying plasma membrane, allowing the red blood cell to spring back to its original shape after squeezing through a capillary. Close relatives of spectrin are found in the cortex of most other vertebrate cell types, where they also help to shape and stiffen the surface membrane. A particularly striking example of spectrin's role

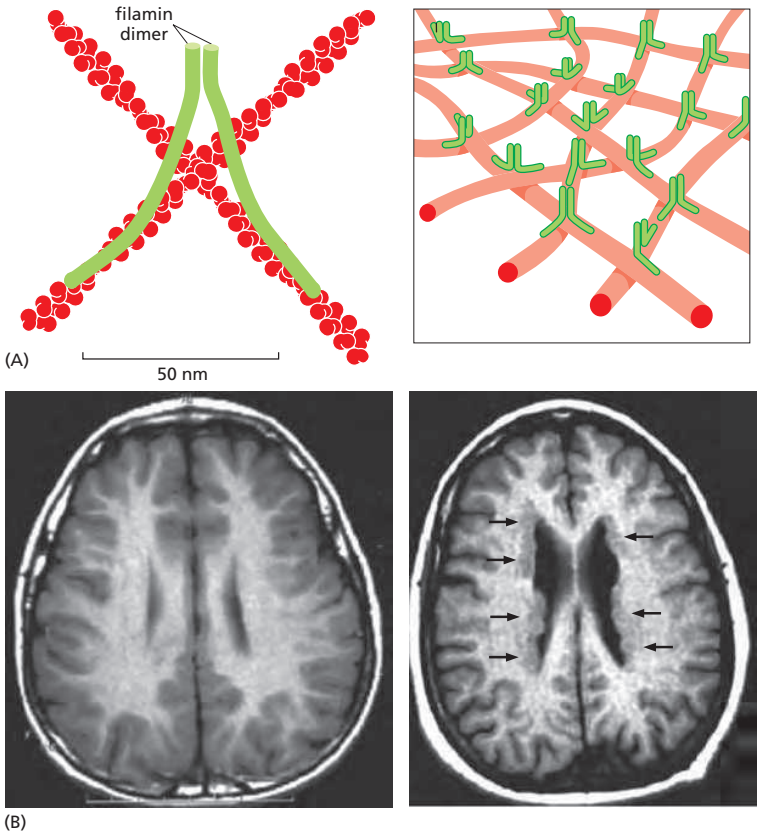


Figure 16–24 Filamin cross-links actin filaments into a three-dimensional network and is required for normal neuronal migration. (A) Each filamin homodimer is about 160 nm long when fully extended and forms a flexible, high-angle link between two adjacent actin filaments. A set of actin filaments cross-linked by filamin forms a mechanically strong web or gel. (B) Magnetic resonance imaging of a normal human brain *(left)* and of a patient with periventricular heterotopia *(right)* caused by mutation in the filamin A gene. In contrast to the smooth ventricular surface in the normal brain, a rough zone of cortical neurons (arrowheads) is seen along the lateral walls of the ventricles, representing neurons that have failed to migrate to the cortex during brain development. Remarkably, although many neurons are not in the right place, the intelligence of affected individuals is frequently normal or only mildly compromised, and the major clinical syndrome is epilepsy that often starts in the second decade of life. (B, adapted from Y. Feng and C.A. Walsh, *Nat. Cell Biol.* 6:1034–1038, 2004. With permission from Macmillan Publishers Ltd.)

in promoting mechanical stability is the long, thin axon of neurons in the nematode worm *Caenorhabditis elegans*, where spectrin is required to keep them from breaking during the twisting motions the worms make during crawling.

The connections of the cortical actin cytoskeleton to the plasma membrane are only partially understood. Members of the *ERM* family (named for its first three members, ezrin, radixin, and moesin), help organize membrane domains through their ability to interact with transmembrane proteins and the underlying cytoskeleton. In so doing, they not only provide structural links to strengthen the cell cortex, but also regulate the activities of signal transduction pathways. Moesin also increases cortical stiffness to promote cell rounding during mitosis. Measurements by atomic force microscopy indicate that the cell cortex remains soft during mitosis when moesin is depleted. ERM proteins are thought to bind to and organize the cortical actin cytoskeleton in a variety of contexts, thereby affecting the shape and stiffness of the membrane as well as the localization and activity of signaling molecules.

Bacteria Can Hijack the Host Actin Cytoskeleton

The importance of accessory proteins in actin-based motility and force production is illustrated beautifully by studies of certain bacteria and viruses that use components of the host-cell actin cytoskeleton to move through the cytoplasm. The cytoplasm of mammalian cells is extremely viscous, containing organelles and cytoskeletal elements that inhibit diffusion of large particles like bacteria or viruses. To move around in a cell and invade neighboring cells, several pathogens, including *Listeria monocytogenes* (which causes a rare but serious form of food poisoning), overcome this problem by recruiting and activating the Arp 2/3 complex at their surface. The Arp 2/3 complex nucleates the assembly of actin filaments that generate a substantial force and push the bacterium through the

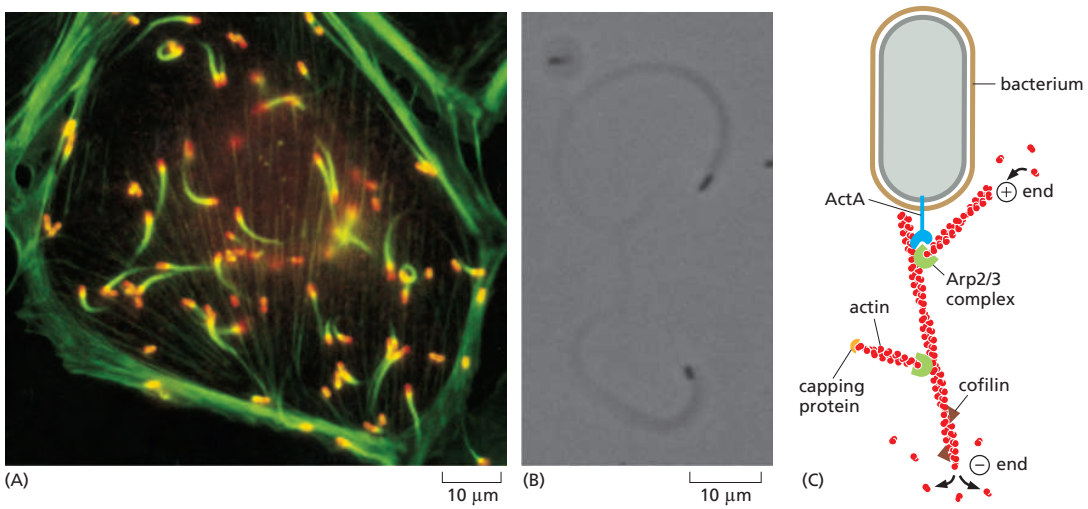


Figure 16–25 The actin-based movement of *Listeria monocytogenes*. (A) Fluorescence micrograph of an infected cell that has been stained to reveal bacteria in *red* and actin filaments in *green*. Note the cometlike tail of actin filaments behind each moving bacterium. Regions of overlap between red and green fluorescence appear *yellow*. (B) *Listeria* motility can be reconstituted in a test tube with ATP and just four purified proteins: actin, Arp 2/3 complex, capping protein, and cofilin. This micrograph shows the dense actin tails behind bacteria *(black)*. (C) The ActA protein on the bacterial surface activates the Arp 2/3 complex to nucleate new filament assembly along the sides of existing filaments. Filaments grow at their plus end until capped by capping protein. Actin is recycled through the action of cofilin, which enhances depolymerization at the minus ends of the filaments. By this mechanism, polymerization is focused at the rear surface of the bacterium, propelling it forward (see Movie 23.7). (A, courtesy of Julie Theriot and Tim Mitchison; B, from T.P. Loisel et al., *Nature* 401:613–616, 1999. With permission from Macmillan Publishers Ltd.)

cytoplasm at rates of up to 1 μm/sec, leaving behind a long actin "comet tail" (**Figure 16–25**; see also Figures 23–28 and 23–29). This motility can be reconstituted in a test tube by adding the bacteria to a mixture of pure actin, Arp 2/3 complex, cofilin, and capping protein, illustrating how actin polymerization dynamics generate movement through spatial regulation of filament assembly and disassembly. As we shall see, actin-based movement of this sort also underlies membrane protrusion at the leading edge of motile cells.

Summary

Actin is a highly conserved cytoskeletal protein that is present in high concentrations in nearly all eukaryotic cells. Nucleation presents a kinetic barrier to actin polymerization, but once formed, actin filaments undergo dynamic behavior due to hydrolysis of the bound nucleotide ATP. Actin filaments are polarized and can undergo treadmilling when a filament assembles at the plus end while simultaneously depolymerizing at the minus end. In cells, actin filament dynamics are regulated at every step, and the varied forms and functions of actin depend on a versatile repertoire of accessory proteins. Approximately half of the actin is kept in a monomeric form through association with sequestering proteins such as thymosin. Nucleation factors such as the Arp 2/3 complex and formins promote formation of branched and parallel filaments, respectively. Interplay between proteins that bind or cap actin filaments and those that promote filament severing or depolymerization can slow or accelerate the kinetics of filament assembly and disassembly. Another class of accessory proteins assembles the filaments into larger ordered structures by cross-linking them to one another in geometrically defined ways. Connections between these actin arrays and the plasma membrane of cells give an animal cell mechanical strength and permit the elaboration of cortical cellular structures such as lamellipodia, filopodia, and microvilli. By inducing actin filament polymerization at their surface, intracellular pathogens can hijack the host-cell cytoskeleton and move around inside the cell.

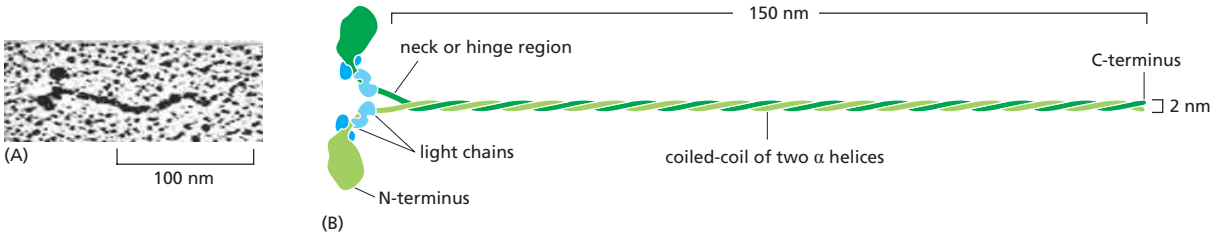


Figure 16–26 Myosin II. (A) The two globular heads and long tail of a myosin II molecule shadowed with platinum can be seen in this electron micrograph. (B) A myosin II molecule is composed of two heavy chains (each about 2000 amino acids long; *green*) and four light chains *(blue).* The light chains are of two distinct types, and one copy of each type is present on each myosin head. Dimerization occurs when the two α helices of the heavy chains wrap around each other to form a coiled-coil, driven by the association of regularly spaced hydrophobic amino acids (see Figure 3–9). The coiled-coil arrangement makes an extended rod in solution, and this part of the molecule forms the tail. (A, courtesy of David Shotton.)

MYOSIN AND ACTIN

A crucial feature of the actin cytoskeleton is that it can form contractile structures that cross-link and slide actin filaments relative to one another through the action of **myosin** motor proteins. In addition to driving muscle contraction, actin–myosin assemblies perform important functions in non-muscle cells.

Actin-Based Motor Proteins Are Members of the Myosin Superfamily

The first motor protein to be identified was skeletal muscle myosin, which generates the force for muscle contraction. This protein, now called *myosin II*, is an elongated protein formed from two heavy chains and two copies of each of two light chains. Each heavy chain has a globular head domain at its N-terminus that contains the force-generating machinery, followed by a very long amino acid sequence that forms an extended coiled-coil that mediates heavy-chain dimerization (**Figure 16–26**). The two light chains bind close to the N-terminal head domain, while the long coiled-coil tail bundles itself with the tails of other myosin molecules. These tail–tail interactions form large, bipolar "thick filaments" that have several hundred myosin heads, oriented in opposite directions at the two ends of the thick filament (**Figure 16–27**).

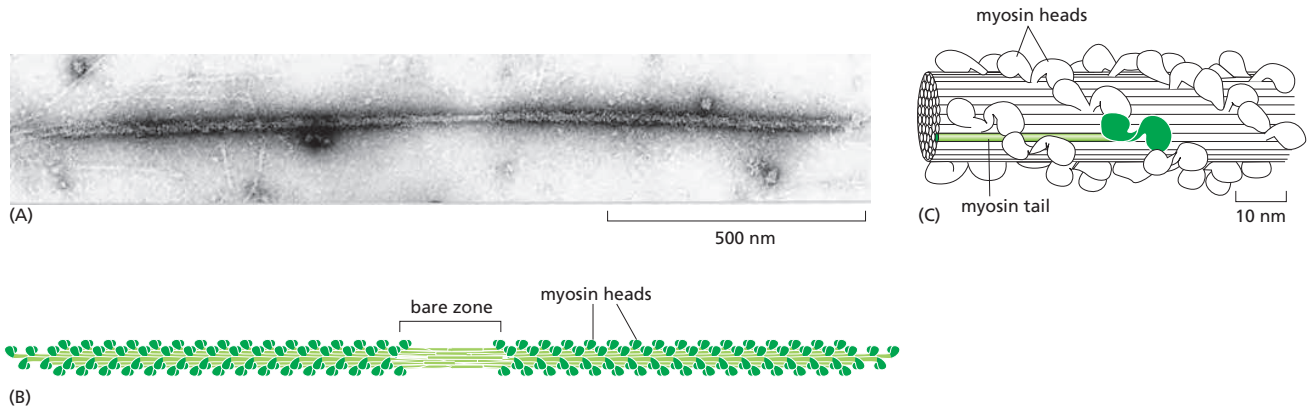


Figure 16–27 The myosin II bipolar thick filament in muscle. (A) Electron micrograph of a myosin II thick filament isolated from frog muscle. Note the central bare zone, which is free of head domains. (B) Schematic diagram, not drawn to scale. The myosin II molecules aggregate by means of their tail regions, with their heads projecting to the outside of the filament. The bare zone in the center of the filament consists entirely of myosin II tails. (C) A small section of a myosin II filament as reconstructed from electron micrographs. An individual myosin molecule is highlighted in *green*. The cytoplasmic myosin II filaments in non-muscle cells are much smaller, although similarly organized (see Figure 16–39). (A, courtesy of Murray Stewart; C, based on R.A. Crowther, R. Padrón and R. Craig, *J. Mol. Biol*. 184:429–439, 1985. With permission from Academic Press.)

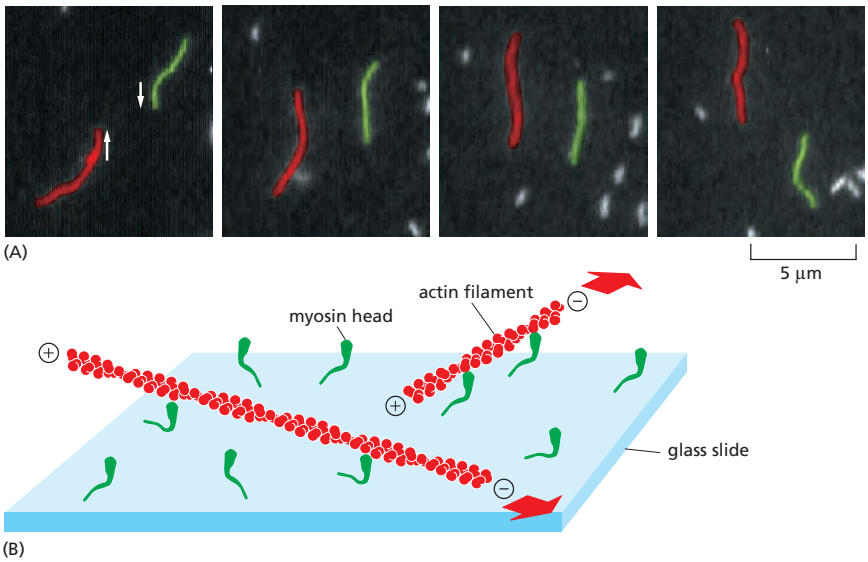


Figure 16–28 Direct evidence for the motor activity of the myosin head. In this experiment, purified myosin heads were attached to a glass slide, and then actin filaments labeled with fluorescent phalloidin were added and allowed to bind to the myosin heads. (A) When ATP was added, the actin filaments began to glide along the surface, owing to the many individual steps taken by each of the dozens of myosin heads bound to each filament. The video frames shown in this sequence were recorded about 0.6 second apart; the two actin filaments shown (one *red* and one *green*) were moving in opposite directions at a rate of about 4 µm/sec. (B) Diagram of the experiment. The large *red* arrows indicate the direction of actin filament movement (Movie 16.2). (A, courtesy of James Spudich.)

Each myosin head binds and hydrolyzes ATP, using the energy of ATP hydrolysis to walk toward the plus end of an actin filament (Figure 16–28). The opposing orientation of the heads in the thick filament makes the filament efficient at sliding pairs of oppositely oriented actin filaments toward each other, shortening the muscle. In skeletal muscle, in which carefully arranged actin filaments are aligned in "thin filament" arrays surrounding the myosin thick filaments, the ATP-driven sliding of actin filaments results in a powerful contraction. Cardiac and smooth muscle contain myosin II molecules that are similarly arranged, although different genes encode them.

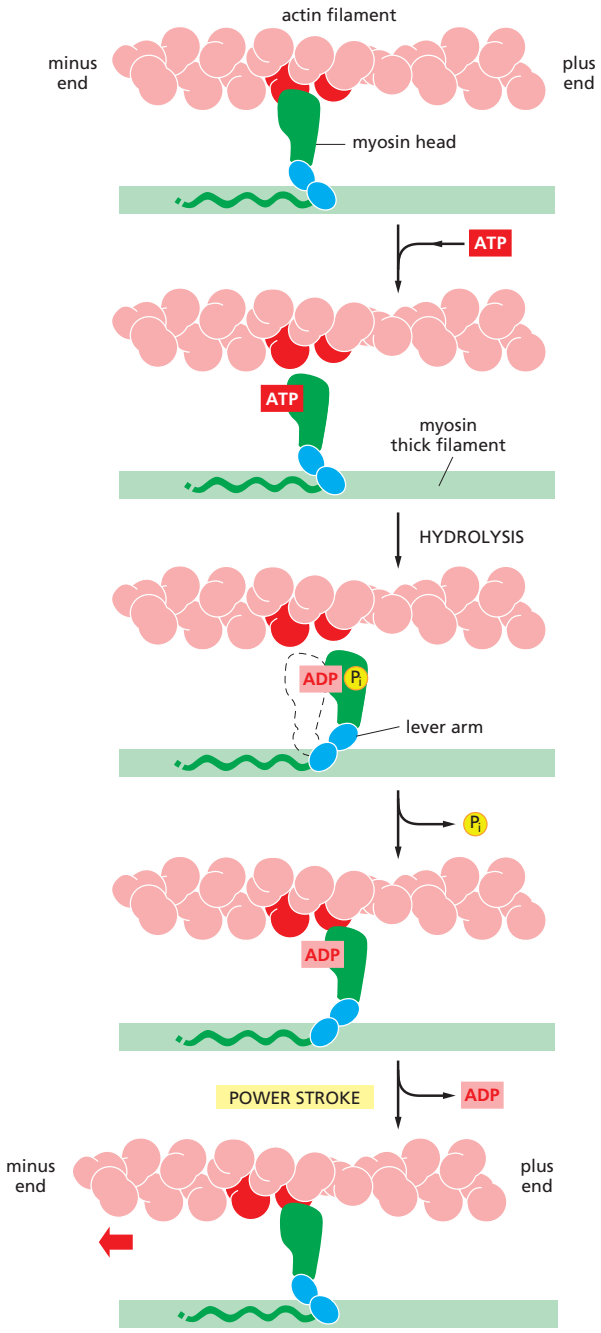
Myosin Generates Force by Coupling ATP Hydrolysis to Conformational Changes

Motor proteins use structural changes in their ATP-binding sites to produce cyclic interactions with a cytoskeletal filament. Each cycle of ATP binding, hydrolysis, and release propels them forward in a single direction to a new binding site along the filament. For myosin II, each step of the movement along actin is generated by the swinging of an 8.5-nm-long α helix, or *lever arm*, which is structurally stabilized by the binding of light chains. At the base of this lever arm next to the head, there is a pistonlike helix that connects movements at the ATP-binding cleft in the head to small rotations of the so-called converter domain. A small change at this point can swing the helix like a long lever, causing the far end of the helix to move by about 5.0 nm.

These changes in the conformation of the myosin are coupled to changes in its binding affinity for actin, allowing the myosin head to release its grip on the actin filament at one point and snatch hold of it again at another. The full mechanochemical cycle of nucleotide binding, nucleotide hydrolysis, and phosphate release (which causes the "power stroke") produces a single step of movement (Figure 16–29). At low ATP concentrations, the interval between the force-producing step and the binding of the next ATP is long enough that single steps can be observed (Figure 16–30).

Sliding of Myosin II Along Actin Filaments Causes Muscles to Contract

Muscle contraction is the most familiar and best-understood form of movement in animals. In vertebrates, running, walking, swimming, and flying all depend on the rapid contraction of skeletal muscle on its scaffolding of bone, while involuntary



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

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

COCKED The cleft closes like a clam shell around the ATP molecule, triggering a movement in the lever arm that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate (P_i) remain tightly bound to the protein.

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

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

Figure 16–29 The cycle of structural changes used by myosin II to walk along an actin filament. In the myosin II cycle, the head remains bound to the actin filament for only about 5% of the entire cycle time, allowing many myosins to work together to move a single actin filament (**Movie 16.3**). (Based on I. Rayment et al., *Science* 261:50–58, 1993.)

movements such as heart pumping and gut peristalsis depend on the contraction of cardiac muscle and smooth muscle, respectively. All these forms of muscle contraction depend on the ATP-driven sliding of highly organized arrays of actin filaments against arrays of myosin II filaments.

Skeletal muscle was a relatively late evolutionary development, and muscle cells are highly specialized for rapid and efficient contraction. The long, thin muscle fibers of skeletal muscle are actually huge single cells that form during

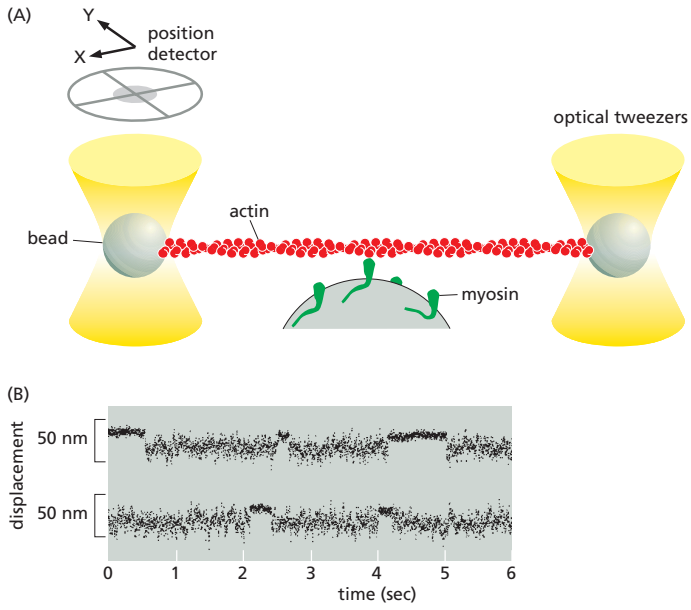
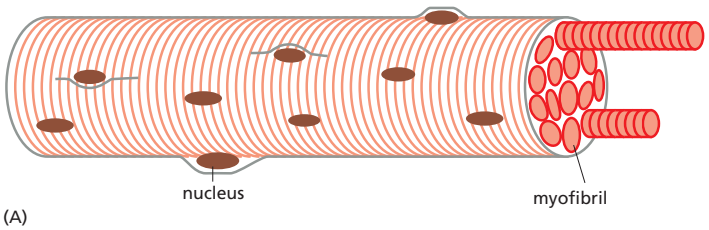


Figure 16–30 The force of a single myosin molecule moving along an actin filament measured using an optical trap. (A) Schematic of the experiment, showing an actin filament with beads attached at both ends and held in place by focused beams of light called optical tweezers (Movie 16.4). The tweezers trap and move the bead, and can also be used to measure the force exerted on the bead through the filament. In this experiment, the filament was positioned over another bead to which myosin II motors were attached, and the optical tweezers were used to determine the effects of myosin binding on movement of the actin filament. (B) These traces show filament movement in two separate experiments. Initially, when the actin filament is unattached to myosin, thermal motion of the filament produces noisy fluctuations in filament position. When a single myosin binds to the actin filament, thermal motion decreases abruptly and a roughly 10-nm displacement results from movement of the filament by the motor. The motor then releases the filament. Because the ATP concentration is very low in this experiment, the myosin remains attached to the actin filament for much longer than it would in a muscle cell. (Adapted from C. Rüegg et al., *Physiology* 17:213–218, 2002. With permission from the American Physiological Society.)

development by the fusion of many separate cells. The large muscle cell retains the many nuclei of the contributing cells. These nuclei lie just beneath the plasma membrane (Figure 16–31). The bulk of the cytoplasm inside is made up of myofibrils, which is the name given to the basic contractile elements of the muscle cell. A **myofibril** is a cylindrical structure 1–2 μm in diameter that is often as long as the muscle cell itself. It consists of a long, repeated chain of tiny contractile units—called *sarcomeres*, each about 2.2 μm long—which give the vertebrate myofibril its striated appearance (Figure 16–32).

Each sarcomere is formed from a miniature, precisely ordered array of parallel and partly overlapping thin and thick filaments. The *thin filaments* are composed of actin and associated proteins, and they are attached at their plus ends to a *Z disc* at each end of the sarcomere. The capped minus ends of the actin filaments extend in toward the middle of the sarcomere, where they overlap with *thick filaments*, the bipolar assemblies formed from specific muscle isoforms of myosin II (see Figure 16–27). When this region of overlap is examined in cross section by electron microscopy, the myosin filaments are arranged in a regular hexagonal lattice, with the actin filaments evenly spaced between them (Figure 16–33). Cardiac muscle and smooth muscle also contain sarcomeres, although the organization is not as regular as that in skeletal muscle.

Figure 16–31 Skeletal muscle cells (also called muscle fibers). (A) These huge multinucleated cells form by the fusion of many muscle cell precursors, called myoblasts. Here, a single muscle cell is depicted. In an adult human, a muscle cell is typically 50 μm in diameter and can be up to several centimeters long. (B) Fluorescence micrograph of rat muscle, showing the peripherally located nuclei *(blue)* in these giant cells. Myofibrils are stained *red*. (B, courtesy of Nancy L. Kedersha.)



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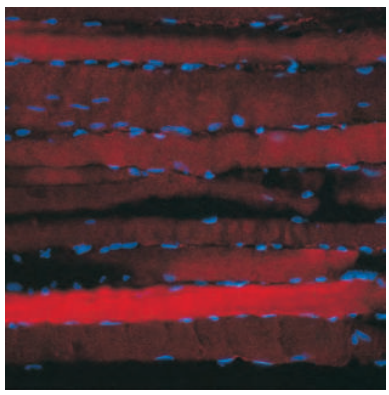

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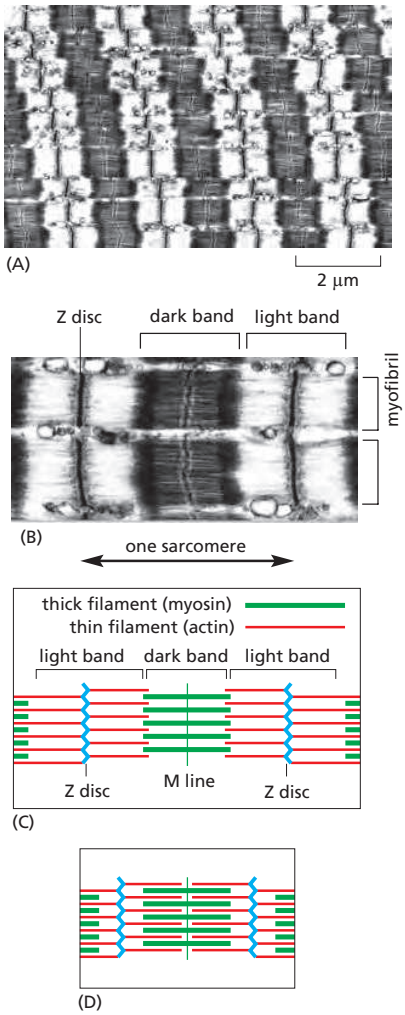


Figure 16–32 Skeletal muscle myofibrils. (A) Low-magnification electron micrograph of a longitudinal section through a skeletal muscle cell of a rabbit, showing the regular pattern of cross-striations. The cell contains many myofibrils aligned in parallel (see Figure 16–31). (B) Detail of the skeletal muscle shown in (A), showing portions of two adjacent myofibrils and the definition of a sarcomere (*black* arrow). (C) Schematic diagram of a single sarcomere, showing the origin of the dark and light bands seen in the electron micrographs. The Z discs, at each end of the sarcomere, are attachment sites for the plus ends of actin filaments (thin filaments); the M line, or midline, is the location of proteins that link adjacent myosin II filaments (thick filaments) to one another. (D) When the sarcomere contracts, the actin and myosin filaments slide past one another without shortening. (A and B, courtesy of Roger Craig.)

Sarcomere shortening is caused by the myosin filaments sliding past the actin thin filaments, with no change in the length of either type of filament (see Figure 16–32C and D). Bipolar thick filaments walk toward the plus ends of two sets of thin filaments of opposite orientations, driven by dozens of independent myosin heads that are positioned to interact with each thin filament. Because there is no coordination among the movements of the myosin heads, it is critical that they remain tightly bound to the actin filament for only a small fraction of each ATPase cycle so that they do not hold one another back. Each myosin thick filament has about 300 heads (294 in frog muscle), and each head cycles about five times per second in the course of a rapid contraction—sliding the myosin and actin filaments past one another at rates of up to 15 μm/sec and enabling the sarcomere to shorten by 10% of its length in less than one-fiftieth of a second. The rapid synchronized shortening of the thousands of sarcomeres lying end-to-end in each myofibril enables skeletal muscle to contract rapidly enough for running and flying, or for playing the piano.

Accessory proteins produce the remarkable uniformity in filament organization, length, and spacing in the sarcomere (**Figure 16–34**). The actin filament plus ends are anchored in the Z disc, which is built from CapZ and α-actinin; the Z disc caps the filaments (preventing depolymerization), while holding them together in a regularly spaced bundle. The precise length of each thin filament is influenced by a protein of enormous size, called *nebulin*, which consists almost entirely of a repeating 35-amino-acid actin-binding motif. Nebulin stretches from the Z disc toward the minus end of each thin filament, which is capped and stabilized by tropomodulin. Although there is some slow exchange of actin subunits at both ends of the muscle thin filament, such that the components of the thin filament turn over with a half-life of several days, the actin filaments in sarcomeres are remarkably stable compared with those found in most other cell types, whose dynamic actin filaments turn over with half-lives of a few minutes or less.

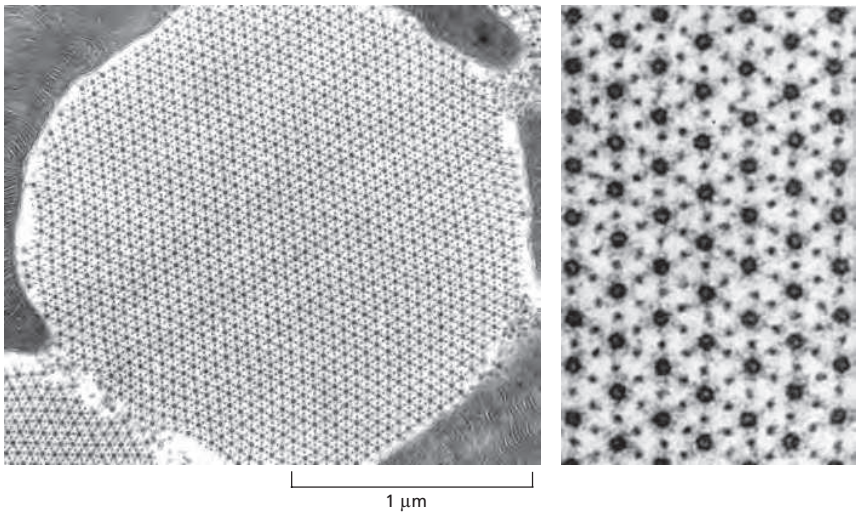


Figure 16–33 Electron micrographs of an insect flight muscle viewed in cross section. The myosin and actin filaments are packed together with almost crystalline regularity. Unlike their vertebrate counterparts, these myosin filaments have a hollow center, as seen in the enlargement on the right. The geometry of the hexagonal lattice is slightly different in vertebrate muscle. (From J. Auber, *J. de Microsc.* 8:197–232, 1969. With permission from Societé Française de Microscopie Électronique.)

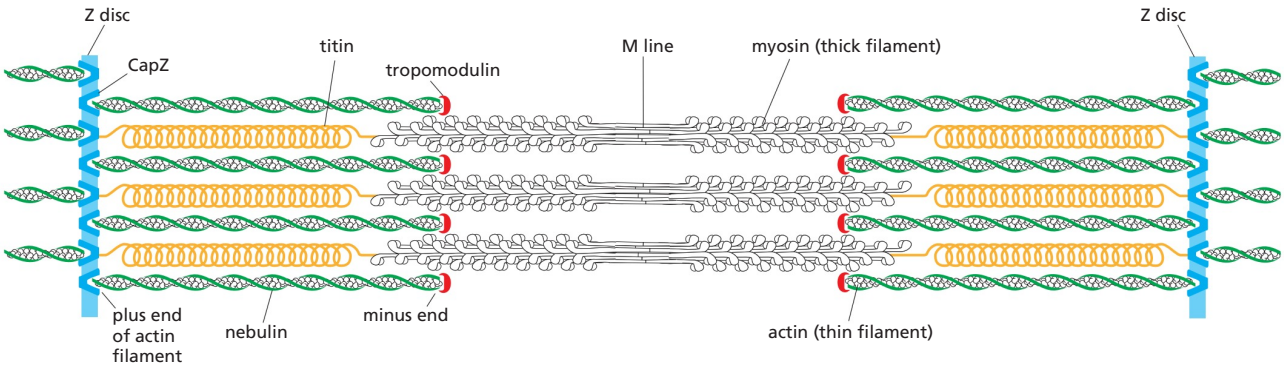


Figure 16–34 Organization of accessory proteins in a sarcomere. Each giant titin molecule extends from the Z disc to the M line—a distance of over 1 μm. Part of each titin molecule is closely associated with a myosin thick filament (which switches polarity at the M line); the rest of the titin molecule is elastic and changes length as the sarcomere contracts and relaxes. Each nebulin molecule is exactly the length of a thin filament. The actin filaments are also coated with tropomyosin and troponin (not shown; see Figure 16–36) and are capped at both ends. Tropomodulin caps the minus end of the actin filaments, and CapZ anchors the plus end at the Z disc, which also contains α-actinin (not shown).

Opposing pairs of an even longer template protein, called *titin*, position the thick filaments midway between the Z discs. Titin acts as a molecular spring, with a long series of immunoglobulin-like domains that can unfold one by one as stress is applied to the protein. A springlike unfolding and refolding of these domains keeps the thick filaments poised in the middle of the sarcomere and allows the muscle fiber to recover after being overstretched. In *C. elegans*, whose sarcomeres are longer than those in vertebrates, titin is longer as well, suggesting that it serves also as a molecular ruler, determining in this case the overall length of each sarcomere.

A Sudden Rise in Cytosolic Ca^{2+} Concentration Initiates Muscle Contraction

The force-generating molecular interaction between myosin thick filaments and actin thin filaments takes place only when a signal passes to the skeletal muscle from the nerve that stimulates it. Immediately upon arrival of the signal, the muscle cell needs to be able to contract very rapidly, with all the sarcomeres shortening simultaneously. Two major features of the muscle cell make extremely rapid contraction possible. First, as previously discussed, the individual myosin motor heads in each thick filament spend only a small fraction of the ATP cycle time bound to the filament and actively generating force, so many myosin heads can act in rapid succession on the same thin filament without interfering with one another. Second, a specialized membrane system relays the incoming signal rapidly throughout the entire cell. The signal from the nerve triggers an action potential in the muscle cell plasma membrane (discussed in Chapter 11), and this electrical excitation spreads swiftly into a series of membranous folds—the transverse tubules, or *T tubules*—that extend inward from the plasma membrane around each myofibril. The signal is then relayed across a small gap to the *sarcoplasmic reticulum*, an adjacent weblike sheath of modified endoplasmic reticulum that surrounds each myofibril like a net stocking (**Figure 16–35**A and B).

When the incoming action potential activates a Ca^{2+} channel in the T-tubule membrane, Ca^{2+} influx triggers the opening of Ca^{2+}-release channels in the sarcoplasmic reticulum (Figure 16–35C). Ca^{2+} flooding into the cytosol then initiates the contraction of each myofibril. Because the signal from the muscle cell plasma membrane is passed within milliseconds (via the T tubules and sarcoplasmic reticulum) to every sarcomere in the cell, all of the myofibrils in the cell contract at once. The increase in Ca^{2+} concentration is transient because the Ca^{2+} is rapidly pumped back into the sarcoplasmic reticulum by an abundant, ATP-dependent Ca^{2+}-pump (also called a Ca^{2+}-ATPase) in its membrane (see Figure 11–13). Typically, the cytoplasmic Ca^{2+} concentration is restored to resting levels within 30 msec, allowing the myofibrils to relax. Thus, muscle contraction depends on two processes that consume enormous amounts of ATP: filament sliding, driven by the ATPase of the myosin motor domain, and Ca^{2+} pumping, driven by the Ca^{2+}-pump.

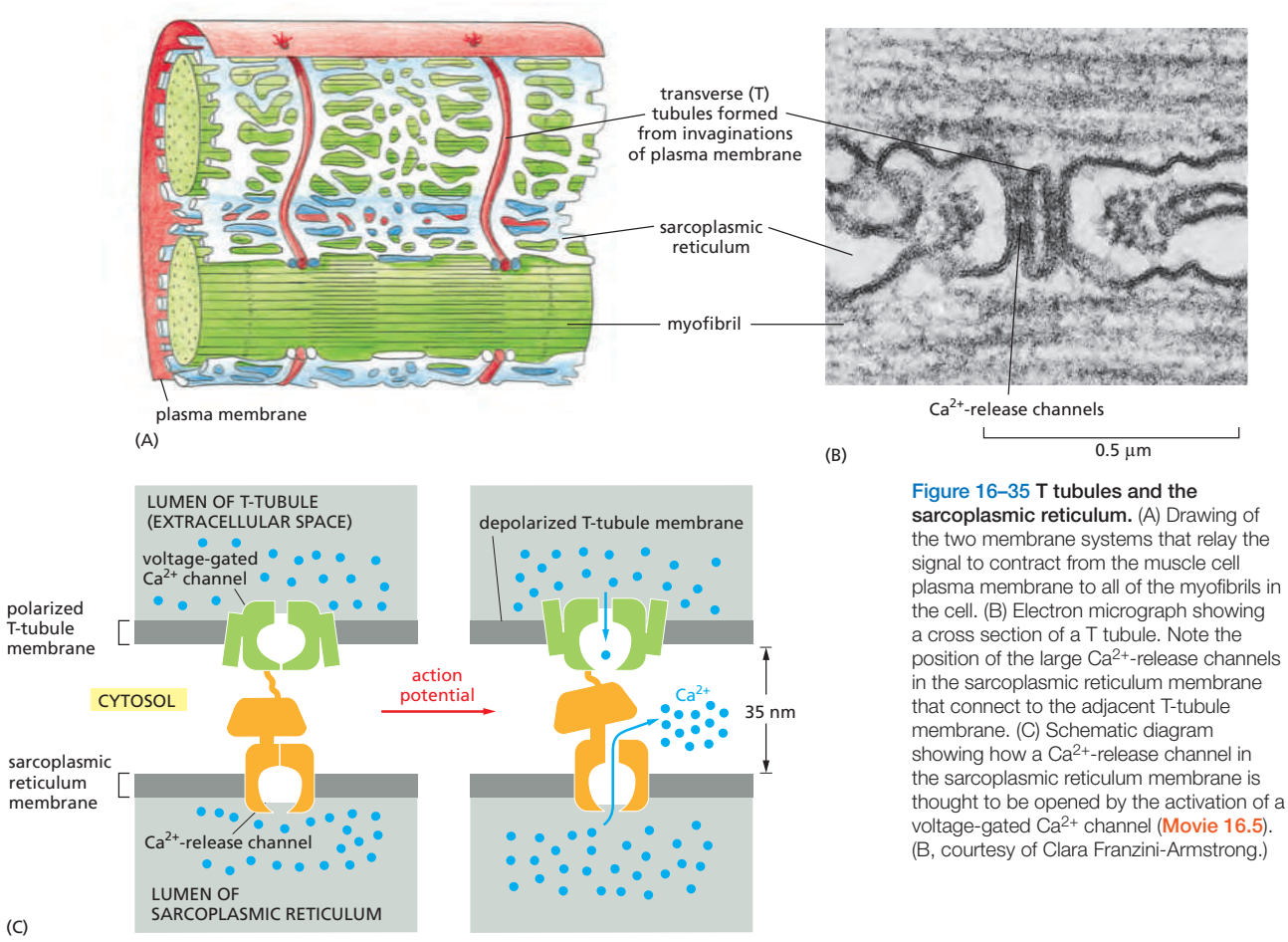


Figure 16–35 T tubules and the sarcoplasmic reticulum. (A) Drawing of the two membrane systems that relay the signal to contract from the muscle cell plasma membrane to all of the myofibrils in the cell. (B) Electron micrograph showing a cross section of a T tubule. Note the position of the large Ca^{2+}-release channels in the sarcoplasmic reticulum membrane that connect to the adjacent T-tubule membrane. (C) Schematic diagram showing how a Ca^{2+}-release channel in the sarcoplasmic reticulum membrane is thought to be opened by the activation of a voltage-gated Ca^{2+} channel (Movie 16.5). (B, courtesy of Clara Franzini-Armstrong.)

The Ca^{2+}-dependence of vertebrate skeletal muscle contraction, and hence its dependence on commands transmitted via nerves, is due entirely to a set of specialized accessory proteins that are closely associated with the actin thin filaments. One of these accessory proteins is a muscle form of *tropomyosin*, the elongated protein that binds along the groove of the actin filament helix. The other is *troponin*, a complex of three polypeptides, troponins T, I, and C (named for their tropomyosin-binding, inhibitory, and Ca^{2+}-binding activities, respectively). Troponin I binds to actin as well as to troponin T. In a resting muscle, the troponin I–T complex pulls the tropomyosin out of its normal binding groove into a position along the actin filament that interferes with the binding of myosin heads, thereby preventing any force-generating interaction. When the level of Ca^{2+} is raised, troponin C—which binds up to four molecules of Ca^{2+}—causes troponin I to release its hold on actin. This allows the tropomyosin molecules to slip back into their normal position so that the myosin heads can walk along the actin filaments (Figure 16–36). Troponin C is closely related to the ubiquitous Ca^{2+}-binding protein calmodulin (see Figure 15–33); it can be thought of as a specialized form of calmodulin that has acquired binding sites for troponin I and troponin T, thereby ensuring that the myofibril responds extremely rapidly to an increase in Ca^{2+} concentration.

In smooth muscle cells, so called because they lack the regular striations of skeletal muscle, contraction is also triggered by an influx of calcium ions, but the regulatory mechanism is different. Smooth muscle forms the contractile portion of the stomach, intestine, and uterus, as well as the walls of arteries and many other structures requiring slow and sustained contractions. Smooth muscle is

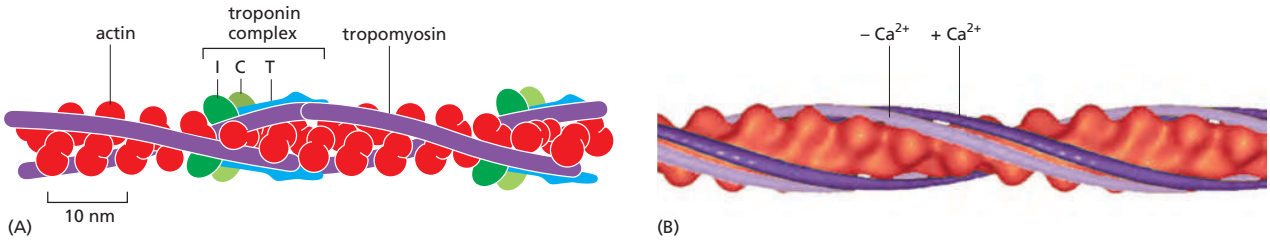


Figure 16–36 The control of skeletal muscle contraction by troponin. (A) A skeletal-muscle-cell thin filament, showing the positions of tropomyosin and troponin along the actin filament. Each tropomyosin molecule has seven evenly spaced regions with similar amino acid sequences, each of which is thought to bind to an actin subunit in the filament. (B) Reconstructed cryoelectron microscopy image of an actin filament showing the relative position of a superimposed tropomyosin strand in the presence *(dark purple)* or absence *(light purple)* of calcium. (A, adapted from G.N. Phillips, J.P. Fillers and C. Cohen, *J. Mol. Biol.* 192:111–131, 1986. With permission from Academic Press; B, adapted from C. Xu et al., *Biophys. J.* 77: 985–992, 1999. With permission from Elsevier.)

composed of sheets of highly elongated spindle-shaped cells, each with a single nucleus. Smooth muscle cells do not express the troponins. Instead, elevated intracellular Ca^{2+} levels regulate contraction by a mechanism that depends on calmodulin (Figure 16–37). Ca^{2+}-bound calmodulin activates myosin light-chain kinase (MLCK), thereby inducing the phosphorylation of smooth muscle myosin on one of its two light chains. When the light chain is phosphorylated, the myosin head can interact with actin filaments and cause contraction; when it is dephosphorylated, the myosin head tends to dissociate from actin and becomes inactive.

The phosphorylation events that regulate contraction in smooth muscle cells occur relatively slowly, so that maximum contraction often requires nearly a second (compared with the few milliseconds required for contraction of a skeletal muscle cell). But rapid activation of contraction is not important in smooth

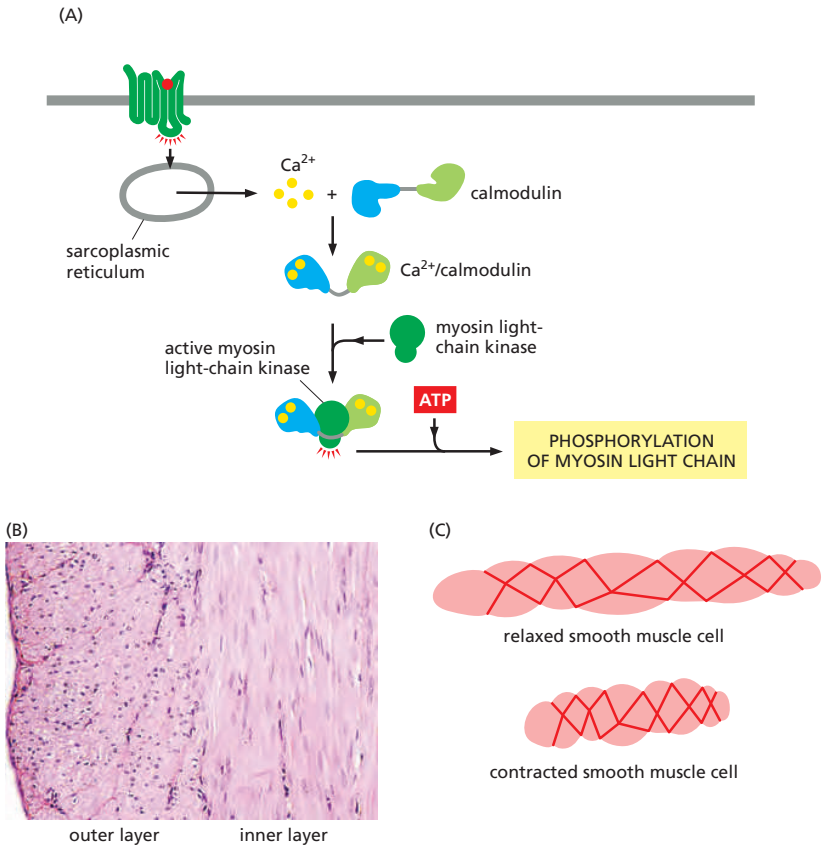


Figure 16–37 Smooth muscle contraction. (A) Upon muscle stimulation by activation of cell-surface receptors, Ca^{2+} released into the cytoplasm from the sarcoplasmic reticulum (SR) binds to calmodulin (see Figure 15–29). Ca^{2+}-bound calmodulin then binds myosin light-chain kinase (MLCK), which phosphorylates myosin light chain, stimulating myosin activity. Non-muscle myosin is regulated by the same mechanism (see Figure 16–39). (B) Smooth muscle cells in a cross section of cat intestinal wall. The outer layer of smooth muscle is oriented with the long axis of its cells extending parallel along the length of the intestine, and upon contraction will shorten the intestine. The inner layer is oriented circularly around the intestine and when contracted will cause the intestine to become narrower. Contraction of both layers squeezes material through the intestine, much like squeezing toothpaste out of a tube. (C) A model for the contractile apparatus in a smooth muscle cell, with bundles of contractile filaments containing actin and myosin *(red)* oriented obliquely to the long axis of the cell. Their contraction greatly shortens the cell. Only a few of the many bundles are shown. (B, courtesy of Gwen V. Childs.)

muscle: its myosin II hydrolyzes ATP about 10 times more slowly than skeletal muscle myosin, producing a slow cycle of myosin conformational changes that results in slow contraction.

Heart Muscle Is a Precisely Engineered Machine

The heart is the most heavily worked muscle in the body, contracting about 3 billion (3×10^9) times during the course of a human lifetime (**Movie 16.6**). Heart cells express several specific isoforms of cardiac muscle myosin and cardiac muscle actin. Even subtle changes in these cardiac-specific contractile proteins—changes that would not cause any noticeable consequences in other tissues—can cause serious heart disease (**Figure 16–38**).

The normal cardiac contractile apparatus is such a highly tuned machine that a tiny abnormality anywhere in the works can be enough to gradually wear it down over years of repetitive motion. *Familial hypertrophic cardiomyopathy* is a common cause of sudden death in young athletes. It is a genetically dominant inherited condition that affects about two out of every thousand people, and it is associated with heart enlargement, abnormally small coronary vessels, and disturbances in heart rhythm (cardiac arrhythmias). The cause of this condition is either any one of over 40 subtle point mutations in the genes encoding cardiac β myosin heavy chain (almost all causing changes in or near the motor domain) or one of about a dozen mutations in other genes encoding contractile proteins—including myosin light chains, cardiac troponin, and tropomyosin. Minor missense mutations in the cardiac actin gene cause another type of heart condition, called *dilated cardiomyopathy*, which can also result in early heart failure.

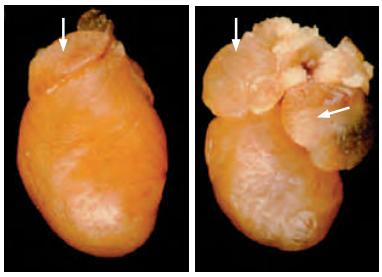
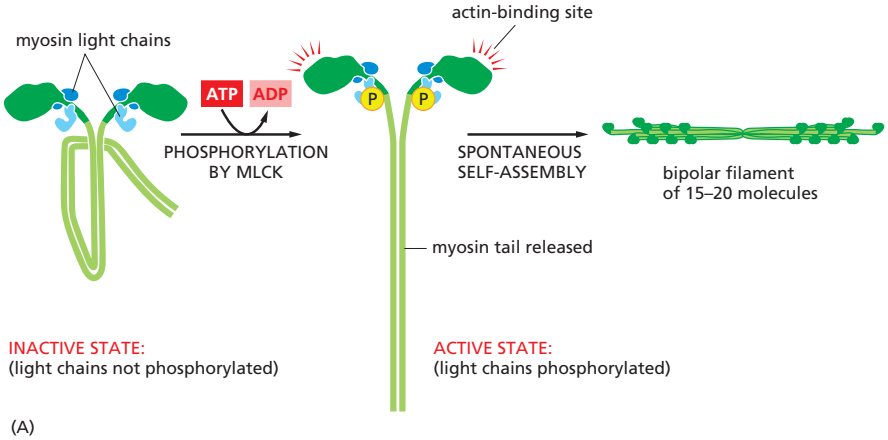


Figure 16–38 Effect on the heart of a subtle mutation in cardiac myosin. *Left*, normal heart from a 6-day-old mouse pup. *Right*, heart from a pup with a point mutation in both copies of its cardiac myosin gene, changing Arg403 to Gln. The arrows indicate the atria. In the heart from the pup with the cardiac myosin mutation, both atria are greatly enlarged (hypertrophic), and the mice die within a few weeks of birth. (From D. Fatkin et al., *J. Clin. Invest.* 103:147–153, 1999. With permission from The American Society for Clinical Investigation.)

Actin and Myosin Perform a Variety of Functions in Non-Muscle Cells

Most non-muscle cells contain small amounts of contractile actin–myosin II bundles that form transiently under specific conditions and are much less well organized than muscle fibers. Non-muscle contractile bundles are regulated by myosin phosphorylation rather than the troponin mechanism (**Figure 16–39**). These contractile bundles function to provide mechanical support to cells, for example, by assembling into cortical **stress fibers** that connect the cell to the extracellular



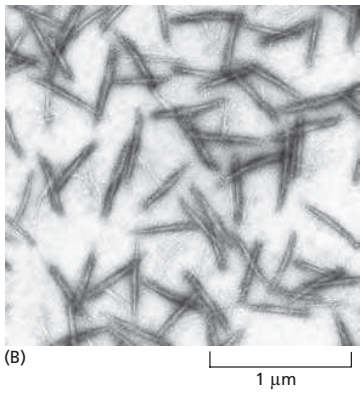


Figure 16–39 Light-chain phosphorylation and the regulation of the assembly of myosin II into thick filaments. (A) The controlled phosphorylation by the enzyme myosin light-chain kinase (MLCK) of one of the two light chains (the so-called regulatory light chain, shown in *light blue*) on non-muscle myosin II in a test tube has at least two effects: it causes a change in the conformation of the myosin head, exposing its actin-binding site, and it releases the myosin tail from a "sticky patch" on the myosin head, thereby allowing the myosin molecules to assemble into short, bipolar, thick filaments. Smooth muscle is regulated by the same mechanism (see Figure 16–37). (B) Electron micrograph of negatively stained short filaments of myosin II that have been induced to assemble in a test tube by phosphorylation of their light chains. These myosin II filaments are much smaller than those found in skeletal muscle cells (see Figure 16–27). (B, courtesy of John Kendrick-Jones.)

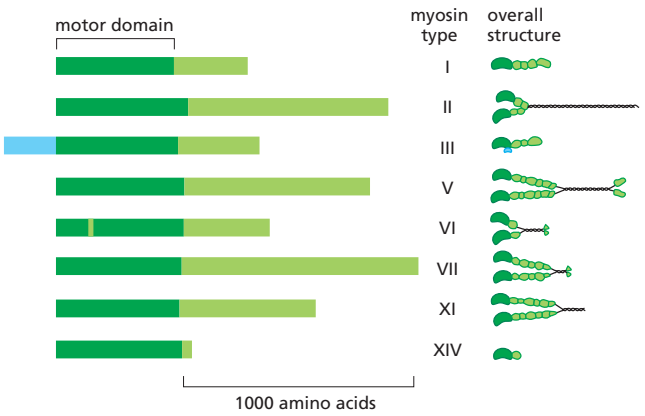


Figure 16–40 Myosin superfamily members. Comparison of the domain structure of the heavy chains of some myosin types. All myosins share similar motor domains (shown in *dark green*), but their C-terminal tails *(light green)* and N-terminal extensions *(light blue)* are very diverse. On the right are depictions of the molecular structure for these family members. Many myosins form dimers, with two motor domains per molecule, but a few (such as I, III, and XIV) seem to function as monomers, with just one motor domain. Myosin VI, despite its overall structural similarity to other family members, is unique in moving toward the minus end (instead of the plus end) of an actin filament. The small insertion within its motor head domain, not found in other myosins, is probably responsible for this change in direction.

matrix through *focal adhesions* or by forming a *circumferential belt* in an epithelial cell, connecting it to adjacent cells through *adherens junctions* (discussed in Chapter 19). As described in Chapter 17, actin and myosin II in the *contractile ring* generate the force for cytokinesis, the final stage in cell division. Finally, as discussed later, contractile bundles also contribute to the adhesion and forward motion of migrating cells.

Non-muscle cells also express a large family of other myosin proteins, which have diverse structures and functions in the cell. Following the discovery of conventional muscle myosin, a second member of the family was found in the freshwater amoeba *Acanthamoeba castellanii*. This protein had a different tail structure and seemed to function as a monomer, and so it was named *myosin I* (for one-headed). Conventional muscle myosin was renamed *myosin II* (for two-headed). Subsequently, many other myosin types were discovered. The heavy chains generally start with a recognizable myosin motor domain at the N-terminus and then diverge widely with a variety of C-terminal tail domains (**Figure 16–40**). The myosin family includes a number of one-headed and two-headed varieties that are about equally related to myosin I and myosin II, and the nomenclature now reflects their approximate order of discovery (myosin III through at least myosin XVIII). Sequence comparisons among diverse eukaryotes indicate that there are at least 37 distinct myosin families in the superfamily. All of the myosins except one move toward the plus end of an actin filament, although they do so at different speeds. The exception is myosin VI, which moves toward the minus end. The myosin tails (and the tails of motor proteins generally) have apparently diversified during evolution to permit the proteins to bind other subunits and to interact with different cargoes.

Some myosins are found only in plants, and some are found only in vertebrates. Most, however, are found in all eukaryotes, suggesting that myosins arose early in eukaryotic evolution. The human genome includes about 40 myosin genes. Nine of the human myosins are expressed primarily or exclusively in the hair cells of the inner ear, and mutations in five of them are known to cause hereditary deafness. These extremely specialized myosins are important for the construction and function of the complex and beautiful bundles of actin found in stereocilia that project from the apical surface of these cells (see Figure 9–51); these cellular protrusions tilt in response to sound and convert sound waves into electrical signals.

The functions of most of the myosins remain to be determined, but several are well characterized. The myosin I proteins often contain either a second actin-binding site or a membrane-binding site in their tails, and they are generally involved in intracellular organization—including the protrusion of actin-rich structures at the cell surface, such as microvilli (see Panel 16–1 and Figure 16–4), and endocytosis. Myosin V is a two-headed myosin with a large step size (**Figure 16–41**A) and is involved in organelle transport along actin filaments. In contrast to myosin II motors, which work in ensembles and are attached only transiently to actin filaments so as not to interfere with one another, myosin V moves continuously,

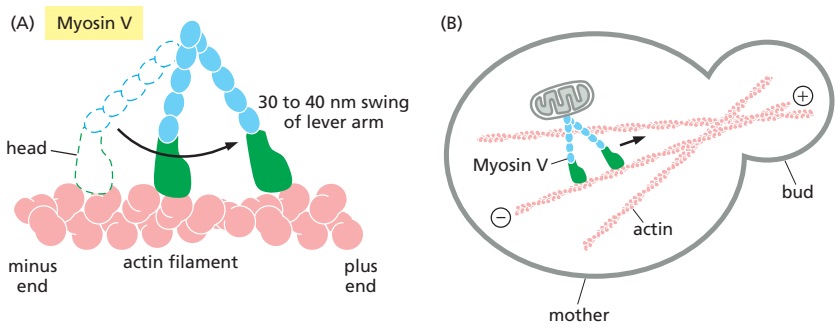


Figure 16–41 Myosin V carries cargo along actin filaments. (A) The lever arm of myosin V is long, allowing it to take a bigger step along an actin filament than myosin II (see Figure 16–29). (B) Myosin V transports cargo and organelles along actin cables, in this example moving a mitochondrion into the growing bud of a yeast cell.

or *processively*, along actin filaments without letting go. Myosin V functions are well studied in the yeast *Saccharomyces cerevisiae*, which undergoes a stereotypical pattern of growth and division called budding. Actin cables in the mother cell point toward the bud, where actin is found in patches that concentrate where cell wall growth is taking place. Myosin V motors carry a wide range of cargoes—including mRNA, endoplasmic reticulum, and secretory vesicles—along the actin cables and into the bud. In addition, myosin V mediates the correct partitioning of organelles such as peroxisomes and mitochondria between mother and daughter cells (see Figure 16–41B).

Summary

Using their neck domain as a lever arm, myosins convert ATP hydrolysis into mechanical work to move along actin filaments in a stepwise fashion. Skeletal muscle is made up of myofibrils containing thousands of sarcomeres assembled from highly ordered arrays of actin and myosin II filaments, together with many accessory proteins. Muscle contraction is stimulated by calcium, which causes the actin-filament-associated protein tropomyosin to move, uncovering myosin binding sites and allowing the filaments to slide past one another. Smooth muscle and non-muscle cells have less well-ordered contractile bundles of actin and myosin, which are regulated by myosin light-chain phosphorylation. Myosin V transports cargo by walking along actin filaments.

MICROTUBULES

Microtubules are structurally more complex than actin filaments, but they are also highly dynamic and play comparably diverse and important roles in the cell. Microtubules are polymers of the protein **tubulin**. The tubulin subunit is itself a heterodimer formed from two closely related globular proteins called *α-tubulin* and *β-tubulin,* each comprising 445–450 amino acids, which are tightly bound together by noncovalent bonds (**Figure 16–42**A). These two tubulin proteins are found only in this heterodimer, and each α or β monomer has a binding site for one molecule of GTP. The GTP that is bound to α-tubulin is physically trapped at the dimer interface and is never hydrolyzed or exchanged; it can therefore be considered to be an integral part of the tubulin heterodimer structure. The nucleotide on the β-tubulin, in contrast, may be in either the GTP or the GDP form and is exchangeable within the soluble (unpolymerized) tubulin dimer.

Tubulin is found in all eukaryotic cells, and it exists in multiple isoforms. Yeast and human tubulins are 75% identical in amino acid sequence. In mammals, there are at least six forms of α-tubulin and a similar number of β-tubulins, each encoded by a different gene. The different forms of tubulin are very similar, and they generally copolymerize into mixed microtubules in the test tube. However, they can have distinct locations in cells and tissues and perform subtly different functions. As a striking example, mutations in a particular human β-tubulin gene give rise to a paralytic eye-movement disorder due to loss of ocular nerve function. Numerous human neurological diseases have been linked to specific mutations in different tubulin genes.

Microtubules Are Hollow Tubes Made of Protofilaments

A microtubule is a hollow cylindrical structure built from 13 parallel protofilaments, each composed of αβ-tubulin heterodimers stacked head to tail and then folded into a tube (Figure 16–42B–D). Microtubule assembly generates two new types of protein–protein contacts. Along the longitudinal axis of the microtubule, the "top" of one β-tubulin molecule forms an interface with the "bottom" of the α-tubulin molecule in the adjacent heterodimer. This interface is very similar to the interface holding the α and β monomers together in the dimer subunit, and the binding energy is high. Perpendicular to these interactions, neighboring protofilaments form lateral contacts. In this dimension, the main lateral contacts are between monomers of the same type (α–α and β–β). As longitudinal and lateral contacts are repeated during assembly, a slight stagger in lateral contacts gives rise to the helical microtubule lattice. Because multiple contacts within the lattice hold most of the subunits in a microtubule in place, the addition and loss of subunits occurs almost exclusively at the microtubule ends (see Figure 16–5). These multiple contacts among subunits make microtubules stiff and difficult to bend. The persistence length of a microtubule is several millimeters, making microtubules the stiffest and straightest structural elements found in most animal cells.

The subunits in each protofilament in a microtubule all point in the same direction, and the protofilaments themselves are aligned in parallel (see Figure

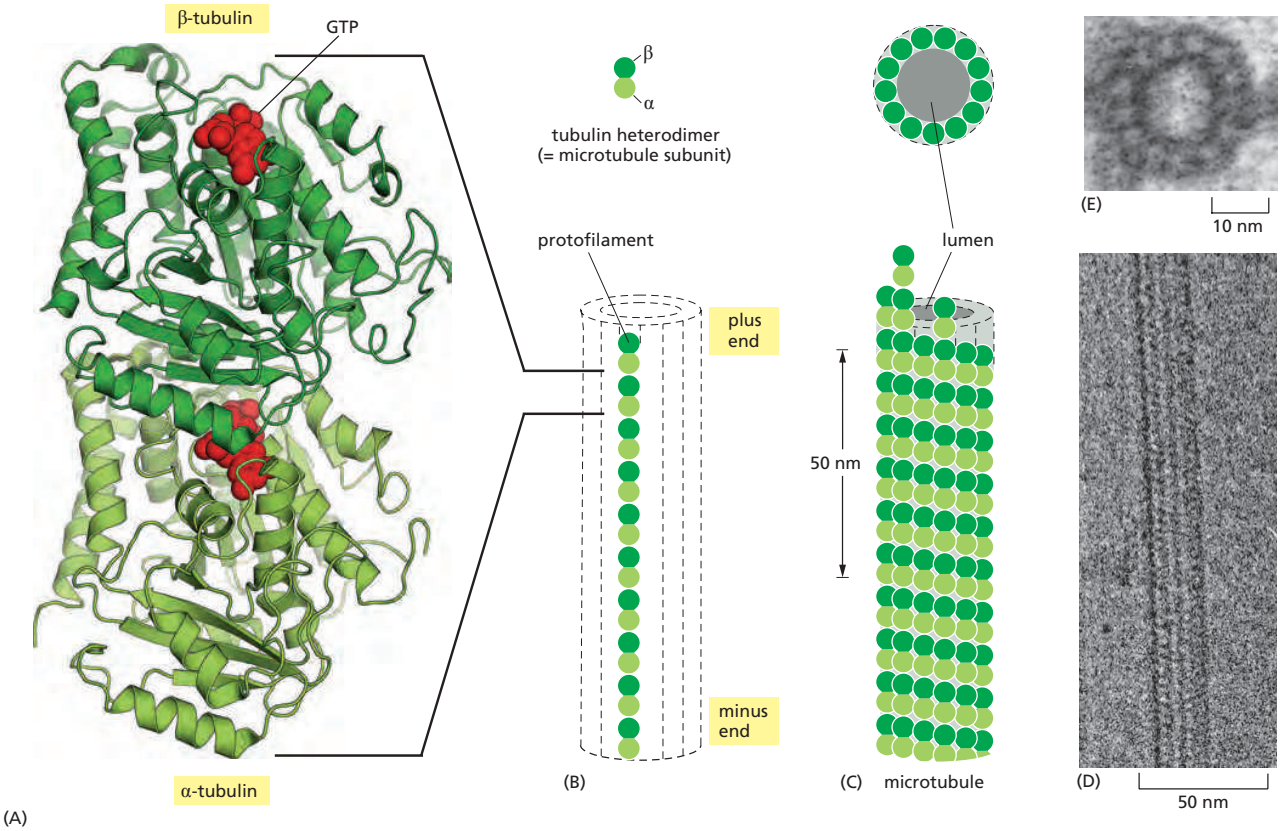


Figure 16–42 The structure of a microtubule and its subunit. (A) The subunit of each protofilament is a tubulin heterodimer, formed from a tightly linked pair of α- and β-tubulin monomers. The GTP molecule in the α-tubulin monomer is so tightly bound that it can be considered an integral part of the protein. The GTP molecule in the β-tubulin monomer, however, is less tightly bound and has an important role in filament dynamics. Both nucleotides are shown in *red*. (B) One tubulin subunit (αβ-heterodimer) and one protofilament are shown schematically. Each protofilament consists of many adjacent subunits with the same orientation. (C) The microtubule is a stiff hollow tube formed from 13 protofilaments aligned in parallel. (D) A short segment of a microtubule viewed in an electron microscope. (E) Electron micrograph of a cross section of a microtubule showing a ring of 13 distinct protofilaments. (D, courtesy of Richard Wade; E, courtesy of Richard Linck.)

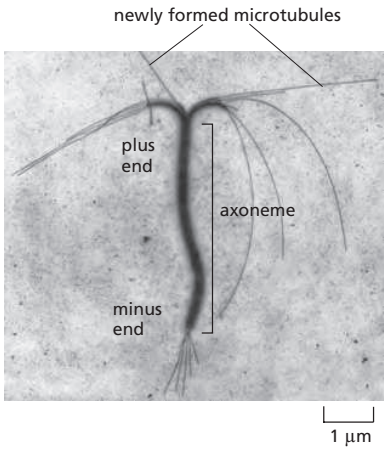


Figure 16–43 The preferential growth of microtubules at the plus end. Microtubules grow faster at one end than at the other. A stable bundle of microtubules obtained from the core of a cilium (called an axoneme) was incubated for a short time with tubulin subunits under polymerizing conditions. Microtubules grew fastest from the plus end of the microtubule bundle, the end at the *top* in this micrograph. (Courtesy of Gary Borisy.)

16–42). Therefore, the microtubule lattice itself has a distinct structural polarity, with α-tubulins exposed at the minus end and β-tubulins exposed at the plus end. As for actin filaments, the regular, parallel orientation of their subunits gives microtubules structural and dynamic polarity (**Figure 16–43**), with plus ends growing and shrinking more rapidly.

Microtubules Undergo Dynamic Instability

Microtubule dynamics, like those of actin filaments, are profoundly influenced by the binding and hydrolysis of nucleotide—GTP in this case. GTP hydrolysis occurs only within the β-tubulin subunit of the tubulin dimer. It proceeds very slowly in free tubulin subunits but is accelerated when they are incorporated into microtubules. Following GTP hydrolysis, the free phosphate group is released and the GDP remains bound to β-tubulin within the microtubule lattice. Thus, as in the case of actin filaments, two different types of microtubule structures can exist, one with the "T form" of the nucleotide bound (GTP) and one with the "D form" bound (GDP). The energy of nucleotide hydrolysis is stored as elastic strain in the polymer lattice, making the free-energy change for dissociation of a subunit from the D-form polymer more negative than the free-energy change for dissociation of a subunit from the T-form polymer. In consequence, the ratio of k_{off}/k_{on} for GDP-tubulin (its critical concentration $[C_c(D)]$) is much higher than that of GTP-tubulin. Thus, under physiological conditions, GTP-tubulin tends to polymerize and GDP-tubulin to depolymerize.

Whether the tubulin subunits at the very end of a microtubule are in the T or the D form depends on the relative rates of GTP hydrolysis and tubulin addition. If the rate of subunit addition is high—and thus the filament is growing rapidly—then it is likely that a new subunit will be added to the polymer before the nucleotide in the previously added subunit has been hydrolyzed. In this case, the tip of the polymer remains in the T form, forming a *GTP cap*. However, if the rate of subunit addition is low, hydrolysis may occur before the next subunit is added, and the tip of the filament will then be in the D form. If GTP-tubulin subunits assemble at the end of the microtubule at a rate similar to the rate of GTP hydrolysis, then hydrolysis will sometimes "catch up" with the rate of subunit addition and transform the end to a D form. This transformation is sudden and random, with a certain probability per unit time that depends on the concentration of free GTP-tubulin subunits.

Suppose that the concentration of free tubulin is intermediate between the critical concentration for a T-form end and the critical concentration for a D-form end (that is, above the concentration necessary for T-form assembly, but below that for the D form). Now, any end that happens to be in the T form will grow, whereas any end that happens to be in the D form will shrink. On a single microtubule, an end might grow for a certain length of time in a T form, but then suddenly change to the D form and begin to shrink rapidly, even while the free subunit concentration is held constant. At some later time, it might then regain a T-form end and begin to grow again. This rapid interconversion between a growing and shrinking state, at a uniform free subunit concentration, is called **dynamic instability** (**Figure 16–44**A and **Figure 16–45**; see Panel 16–2). The change from growth to shrinkage is called a *catastrophe*, while the change to growth is called a *rescue*.

In a population of microtubules, at any instant some of the ends are in the T form and some are in the D form, with the ratio depending on the hydrolysis rate and the free subunit concentration. *In vitro*, the structural difference between a T-form end and a D-form end is dramatic. Tubulin subunits with GTP bound to

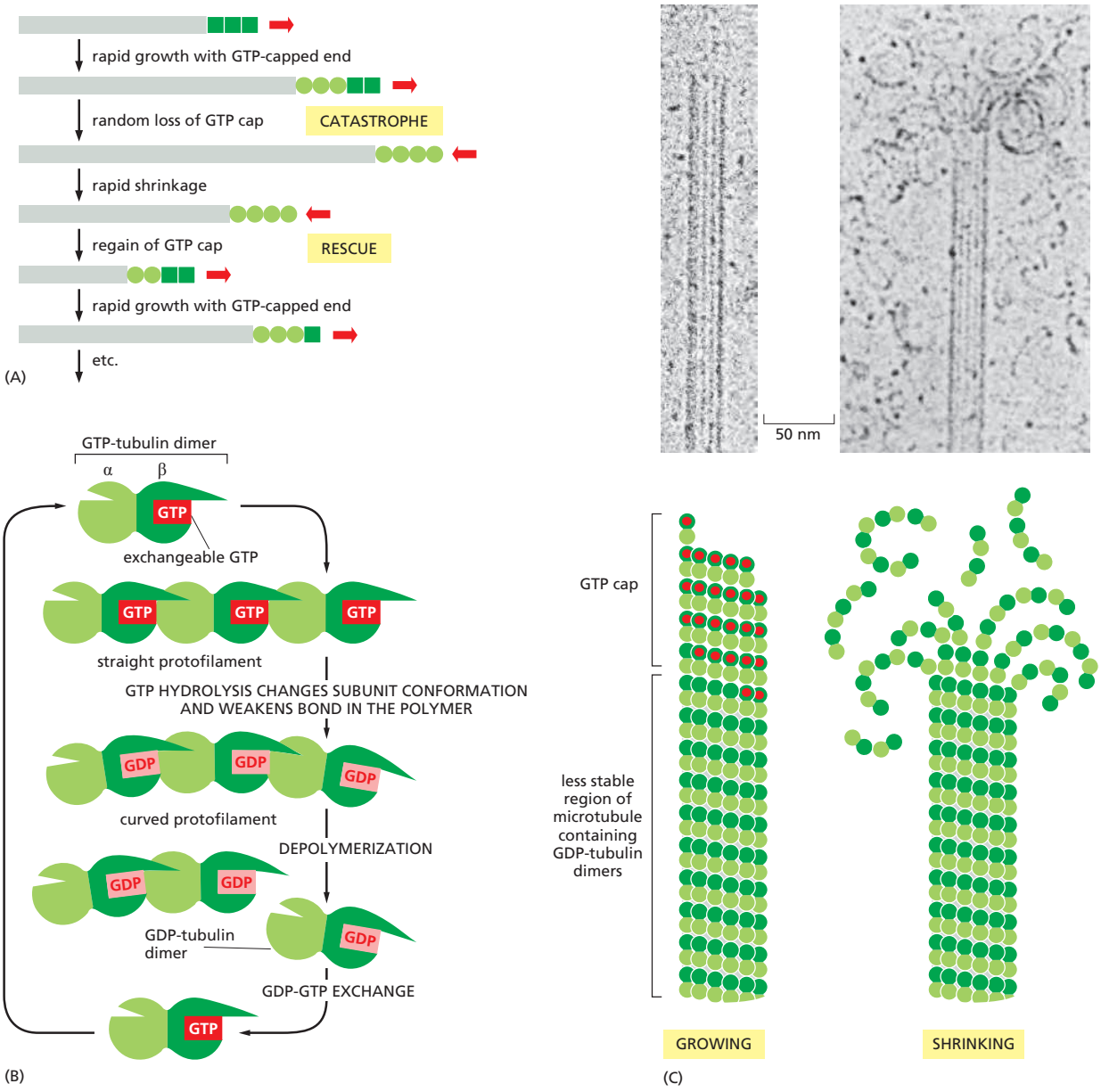


Figure 16–44 Dynamic instability due to the structural differences between a growing and a shrinking microtubule end. (A) If the free tubulin concentration in solution is between the critical concentrations of the GTP- and GDP-bound forms, a single microtubule end may undergo transitions between a growing state and a shrinking state. A growing microtubule has GTP-containing subunits at its end, forming a GTP cap. If nucleotide hydrolysis proceeds more rapidly than subunit addition, the cap is lost and the microtubule begins to shrink, an event called a "catastrophe." But GTP-containing subunits may still add to the shrinking end, and if enough add to form a new cap, then microtubule growth resumes, an event called "rescue." (B) Model for the structural consequences of GTP hydrolysis in the microtubule lattice. The addition of GTP-containing tubulin subunits to the end of a protofilament causes the end to grow in a linear conformation that can readily pack into the cylindrical wall of the microtubule. Hydrolysis of GTP after assembly changes the conformation of the subunits and tends to force the protofilament into a curved shape that is less able to pack into the microtubule wall. (C) In an intact microtubule, protofilaments made from GDP-containing subunits are forced into a linear conformation by the many lateral bonds within the microtubule wall, given a stable cap of GTP-containing subunits. Loss of the GTP cap, however, allows the GDP-containing protofilaments to relax into their more curved conformation. This leads to a progressive disruption of the microtubule. Above the drawings of a growing and a shrinking microtubule, electron micrographs show actual microtubules in each of these two states. Note particularly the curling, disintegrating GDP-containing protofilaments at the end of the shrinking microtubule. (C, from E.M. Mandelkow, E. Mandelkow and R.A. Milligan, *J. Cell Biol.* 114:977–991, 1991. With permission from The Rockefeller University Press.)

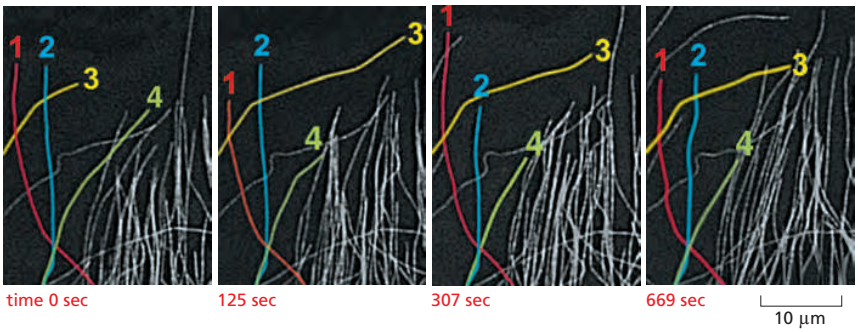


Figure 16–45 Direct observation of the dynamic instability of microtubules in a living cell. Microtubules in a newt lung epithelial cell were observed after the cell was injected with a small amount of rhodamine-labeled tubulin. Notice the dynamic instability of microtubules at the edge of the cell. Four individual microtubules are highlighted for clarity; each of these shows alternating shrinkage and growth (Movie 16.7). (Courtesy of Wendy C. Salmon and Clare Waterman-Storer.)

the β-monomer produce straight protofilaments that make strong and regular lateral contacts with one another. But the hydrolysis of GTP to GDP is associated with a subtle conformational change in the protein, which makes the protofilaments curved (Figure 16–44B). On a rapidly growing microtubule, the GTP cap is thought to constrain the curvature of the protofilaments, and the ends appear straight. But when the terminal subunits have hydrolyzed their nucleotides, this constraint is removed, and the curved protofilaments spring apart. This cooperative release of the energy of hydrolysis stored in the microtubule lattice causes the curled protofilaments to peel off rapidly, and curved oligomers of GDP-containing tubulin are seen near the ends of depolymerizing microtubules (Figure 16–44C).

Microtubule Functions Are Inhibited by Both Polymer-stabilizing and Polymer-destabilizing Drugs

Chemical compounds that impair polymerization or depolymerization of microtubules are powerful tools for investigating the roles of these polymers in cells. Whereas *colchicine* and *nocodazole* interact with tubulin subunits and lead to microtubule depolymerization, *Taxol* binds to and stabilizes microtubules, causing a net increase in tubulin polymerization (see Table 16–1). Drugs like these have a rapid and profound effect on the organization of the microtubules in living cells. Both microtubule-depolymerizing drugs (such as nocodazole) and microtubule-polymerizing drugs (such as Taxol) preferentially kill dividing cells, since microtubule dynamics are crucial for correct function of the mitotic spindle (discussed in Chapter 17). Some of these drugs efficiently kill certain types of tumor cells in a human patient, although not without toxicity to rapidly dividing normal cells, including those in the bone marrow, intestine, and hair follicles. Taxol in particular has been widely used to treat cancers of the breast and lung, and it is frequently successful in treatment of tumors that are resistant to other chemotherapeutic agents.

A Protein Complex Containing γ-Tubulin Nucleates Microtubules

Because formation of a microtubule requires the interaction of many tubulin heterodimers, the concentration of tubulin subunits required for spontaneous nucleation of microtubules is very high. Microtubule nucleation therefore requires help from other factors. While α- and β-tubulins are the regular building blocks of microtubules, another type of tubulin, called *γ-tubulin*, is present in much smaller amounts than α- and β-tubulin and is involved in the nucleation of microtubule growth in organisms ranging from yeasts to humans. Microtubules are generally nucleated from a specific intracellular location known as a **microtubule-organizing center** (**MTOC**) where γ-tubulin is most enriched. Nucleation in many cases depends on the **γ-tubulin ring complex** (**γ-TuRC**). Within this complex, two accessory proteins bind directly to the γ-tubulin, along with several other proteins that help create a spiral ring of γ-tubulin molecules, which serves as a template that creates a microtubule with 13 protofilaments (**Figure 16–46**).

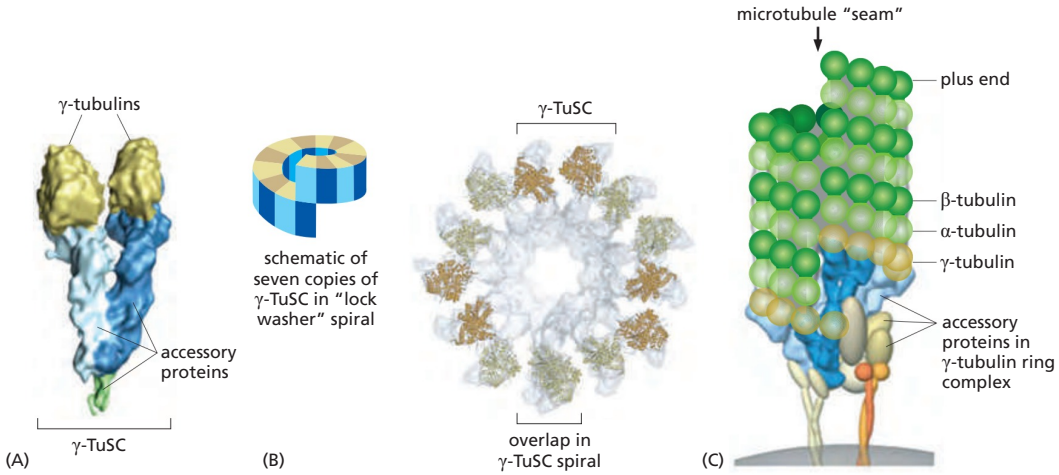


Figure 16–46 Microtubule nucleation by the γ-tubulin ring complex. (A) Two copies of γ-tubulin associate with a pair of accessory proteins to form the γ-tubulin small complex (γ-TuSC). This image was generated by high-resolution electron microscopy of individual purified complexes. (B) Seven copies of the γ-TuSC associate to form a spiral structure in which the last γ-tubulin lies beneath the first, resulting in 13 exposed γ-tubulin subunits in a circular orientation that matches the orientation of the 13 protofilaments in a microtubule. (C) In many cell types, the γ-TuSC spiral associates with additional accessory proteins to form the γ-tubulin ring complex (γ-TuRC), which is likely to nucleate the minus end of a microtubule as shown here. Note the longitudinal discontinuity between two protofilaments, which results from the spiral orientation of the γ-tubulin subunits. Microtubules often have one such "seam" breaking the otherwise uniform helical packing of the protofilaments. (A and B, from J.M. Kollman et al., *Nature* 466:879–883, 2010. With permission from Macmillan Publishers Ltd.)

Microtubules Emanate from the Centrosome in Animal Cells

Many animal cells have a single, well-defined MTOC called the **centrosome**, which is located near the nucleus and from which microtubules are nucleated at their minus ends, so the plus ends point outward and continuously grow and shrink, probing the entire three-dimensional volume of the cell. A centrosome typically recruits more than fifty copies of γ-TuRC. In addition, γ-TuRC molecules are found in the cytoplasm, and centrosomes are not absolutely required for microtubule nucleation, since destroying them with a laser pulse does not prevent microtubule nucleation elsewhere in the cell. A variety of proteins have been identified that anchor γ-TuRC to the centrosome, but mechanisms that activate microtubule nucleation at MTOCs and at other sites in the cell are poorly understood.

Embedded in the centrosome are the **centrioles**, a pair of cylindrical structures arranged at right angles to each other in an L-shaped configuration (**Figure 16–47**). A centriole consists of a cylindrical array of short, modified microtubules arranged into a barrel shape with striking ninefold symmetry (**Figure 16–48**). Together with a large number of accessory proteins, the centrioles organize the *pericentriolar material*, where microtubule nucleation takes place. As described in Chapter 17, the centrosome duplicates and splits into two parts before mitosis, each containing a duplicated centriole pair. The two centrosomes move to opposite sides of the nucleus when mitosis begins, and they form the two poles of the mitotic spindle (see Panel 17–1).

Microtubule organization varies widely among different species and cell types. In budding yeast, microtubules are nucleated at an MTOC that is embedded in the nuclear envelope as a small, multilayered structure called the *spindle pole body*, also found in other fungi and diatoms. Higher-plant cells appear to nucleate microtubules at sites distributed all around the nuclear envelope and at the cell cortex. Neither fungi nor most plant cells contain centrioles. Despite these differences, all these cells seem to use γ-tubulin to nucleate their microtubules.

In cultured animal cells, the aster-like configuration of microtubules is robust, with dynamic plus ends pointing outward toward the cell periphery and stable minus ends collected near the nucleus. The system of microtubules radiating from

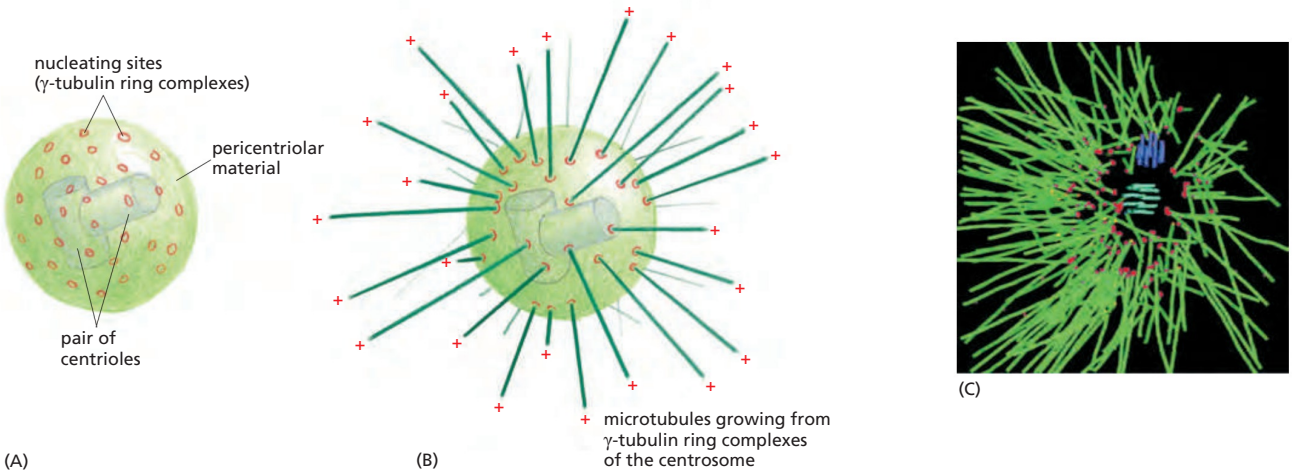


Figure 16–47 The centrosome. (A) The centrosome is the major MTOC of animal cells. Located in the cytoplasm next to the nucleus, it consists of an amorphous matrix of fibrous proteins to which the γ-tubulin ring complexes that nucleate microtubule growth are attached. This matrix is organized by a pair of centrioles, as described in the text. (B) A centrosome with attached microtubules. The minus end of each microtubule is embedded in the centrosome, having grown from a γ-tubulin ring complex, whereas the plus end of each microtubule is free in the cytoplasm. (C) In a reconstructed image of the MTOC from a *C. elegans* cell, a dense thicket of microtubules can be seen emanating from the centrosome. (C, from E.T. O'Toole et al., *J. Cell Biol.* 163:451–456, 2003. With permission from the authors.)

the centrosome acts as a device to survey the outlying regions of the cell and to position the centrosome at its center. Even in an isolated cell fragment lacking the centrosome, dynamic microtubules arrange themselves into a star-shaped array with the microtubule minus ends clustered at the center by minus-end-binding proteins (**Figure 16–49**). This ability of the microtubule cytoskeleton to find the center of the cell establishes a general coordinate system, which is then used to position many organelles within the cell. Highly differentiated cells with complex morphologies such as neurons, muscles, and epithelial cells must use additional measuring mechanisms to establish their more elaborate internal coordinate systems. Thus, for example, when an epithelial cell forms cell–cell junctions and becomes highly polarized, the microtubule minus ends move to a region near the

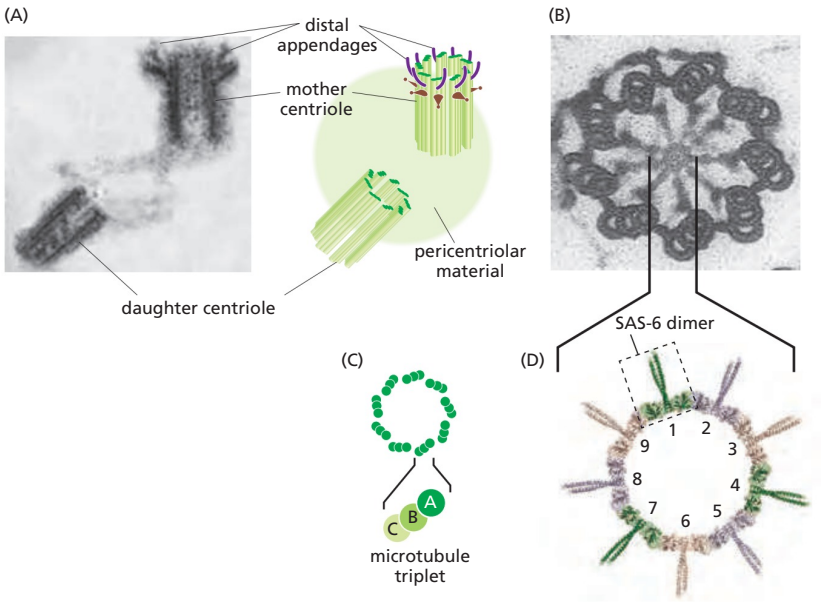


Figure 16–48 A pair of centrioles in the centrosome. (A) An electron micrograph of a thin section of an isolated centrosome showing the mother centriole with its distal appendages and the adjacent daughter centriole, which formed through a duplication event during S phase (see Figure 17–26). In the centrosome, the centriole pair is surrounded by a dense matrix of pericentriolar material from which microtubules nucleate. Centrioles also function as basal bodies to nucleate the formation of ciliary axonemes (see Figure 16–68). (B) Electron micrograph of a cross section through a centriole in the cortex of a protozoan. Each centriole is composed of nine sets of triplet microtubules arranged to form a cylinder. (C) Each triplet contains one complete microtubule (the A microtubule) fused to two incomplete microtubules (the B and C microtubules). (D) The centriolar protein SAS-6 forms a coiled-coil dimer. Nine SAS-6 dimers can self-associate to form a ring. Located at the hub of the centriole cartwheel structure, the SAS-6 ring is thought to generate the ninefold symmetry of the centriole. (A, from from M. Paintrand, et al. *J. Struct. Biol.* 108:107, 1992. With permission from Elsevier; B, courtesy of Richard Linck; D, courtesy of Michel Steinmetz.)

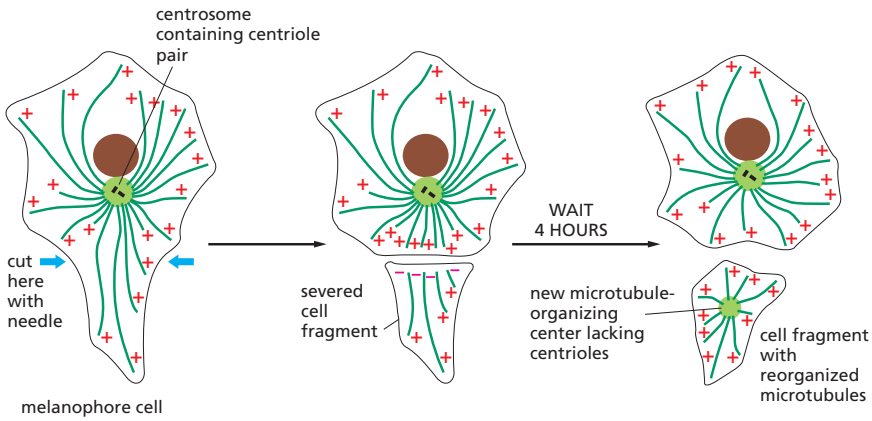


Figure 16–49 A microtubule array can find the center of a cell. After the arm of a fish pigment cell is cut off with a needle, the microtubules in the detached cell fragment reorganize so that their minus ends end up near the center of the fragment, buried in a new microtubule-organizing center.

apical plasma membrane. From this asymmetrical location, a microtubule array extends along the long axis of the cell, with plus ends directed toward the basal surface (see Figure 16–4).

Microtubule-Binding Proteins Modulate Filament Dynamics and Organization

Microtubule polymerization dynamics are very different in cells than in solutions of pure tubulin. Microtubules in cells exhibit a much higher polymerization rate (typically 10–15 μm/min, relative to about 1.5 μm/min with purified tubulin at similar concentrations), a greater catastrophe frequency, and extended pauses in microtubule growth, a dynamic behavior rarely observed in pure tubulin solutions. These and other differences arise because microtubule dynamics inside the cell are governed by a variety of proteins that bind tubulin dimers or microtubules, as summarized in **Panel 16–4**.

Proteins that bind to microtubules are collectively called **microtubule-associated proteins**, or **MAPs**. Some MAPs can stabilize microtubules against disassembly. A subset of MAPs can also mediate the interaction of microtubules with other cell components. This subset is prominent in neurons, where stabilized microtubule bundles form the core of the axons and dendrites that extend from the cell body (**Figure 16–50**). These MAPs have at least one domain that binds to the microtubule surface and another that projects outward. The length of the projecting domain can determine how closely MAP-coated microtubules pack together, as demonstrated in cells engineered to overproduce different MAPs. Cells overexpressing MAP2, which has a long projecting domain, form bundles of stable microtubules that are kept widely spaced, while cells overexpressing tau, a MAP with a much shorter projecting domain, form bundles of more closely packed microtubules (**Figure 16–51**). MAPs are the targets of several protein kinases, and phosphorylation of a MAP can control both its activity and localization inside cells.

Microtubule Plus-End-Binding Proteins Modulate Microtubule Dynamics and Attachments

Cells contain numerous proteins that bind the ends of microtubules and thereby influence microtubule stability and dynamics. These proteins can influence the

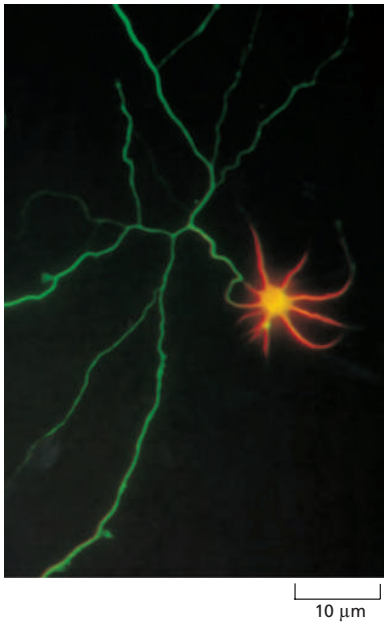
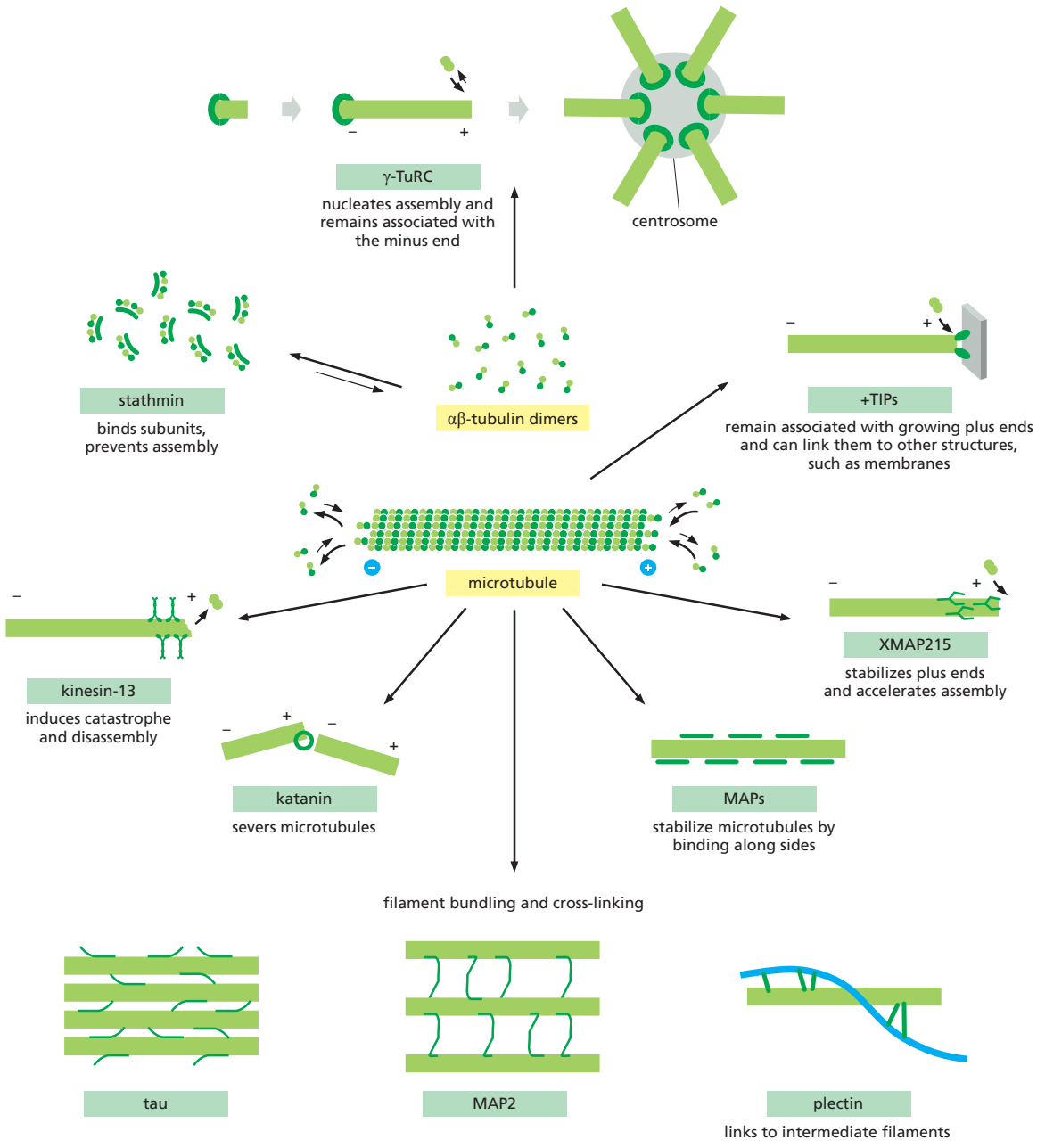


Figure 16–50 Localization of MAPs in the axon and dendrites of a neuron. This immunofluorescence micrograph shows the distribution of the proteins tau *(green)* and MAP2 *(orange)* in a hippocampal neuron in culture. Whereas tau staining is confined to the axon (long and branched in this neuron), MAP2 staining is confined to the cell body and its dendrites. The antibody used here to detect tau binds only to unphosphorylated tau; phosphorylated tau is also present in dendrites. (Courtesy of James W. Mandell and Gary A. Banker.)

MICROTUBULES



Some of the major accessory proteins of the microtubule cytoskeleton. Except for two classes of motor proteins, an example of each major type is shown. Each of these is discussed in the text. However, most cells contain more than a hundred different microtubule-binding proteins, and — as for the actin-associated proteins — it is likely that there are important types of microtubule-associated proteins that are not yet recognized.

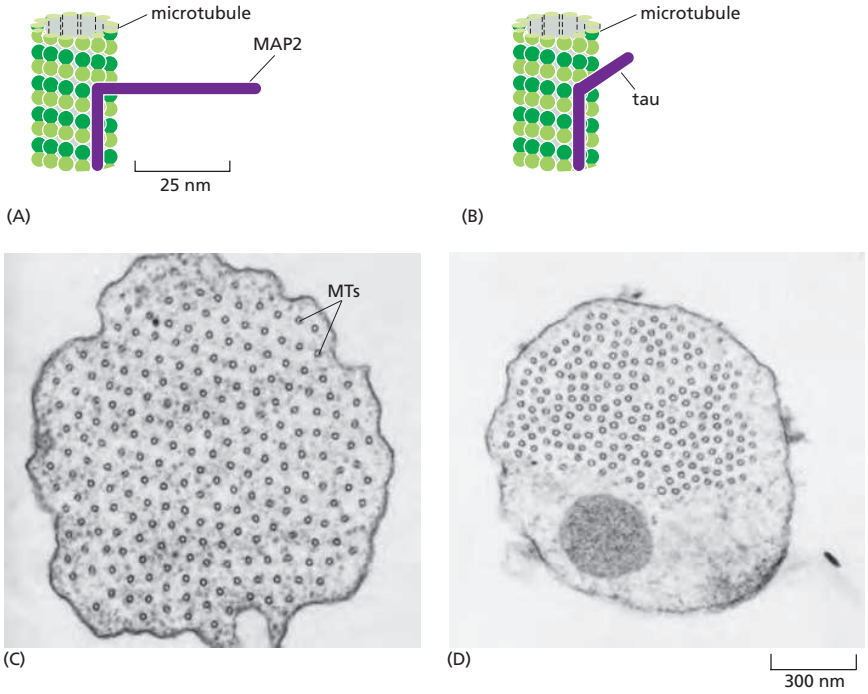


Figure 16–51 Organization of microtubule bundles by MAPs. (A) MAP2 binds along the microtubule lattice at one of its ends and extends a long projecting arm with a second microtubule-binding domain at the other end. (B) Tau possesses a shorter microtubule cross-linking domain. (C) Electron micrograph showing a cross section through a microtubule bundle in a cell overexpressing MAP2. The regular spacing of the microtubules (MTs) in this bundle results from the constant length of the projecting arms of the MAP2. (D) Similar cross section through a microtubule bundle in a cell overexpressing tau. Here the microtubules are spaced more closely together than they are in (C) because of tau's relatively short projecting arm. (C and D, courtesy of J. Chen et al., *Nature* 360:674–677, 1992. With permission from Macmillan Publishers Ltd.)

rate at which a microtubule switches from a growing to a shrinking state (the frequency of catastrophes) or from a shrinking to a growing state (the frequency of rescues). For example, members of a family of kinesin-related proteins known as *catastrophe factors* (or kinesin-13) bind to microtubule ends and appear to pry protofilaments apart, lowering the normal activation-energy barrier that prevents a microtubule from springing apart into the curved protofilaments that are characteristic of the shrinking state (**Figure 16–52**). Another protein, called Nezha or Patronin, protects microtubule minus ends from the effects of catastrophe factors.

While very few microtubule minus-end-binding proteins have been characterized, a large subset of MAPs has been identified that are enriched at microtubule plus ends. A particularly ubiquitous example is *XMAP215*, which has close homologs in organisms that range from yeast to humans. XMAP215 binds free tubulin subunits and delivers them to the plus end, thereby promoting microtubule polymerization and simultaneously counteracting catastrophe factor activity (see Figure 16–52). The phosphorylation of XMAP215 during mitosis inhibits

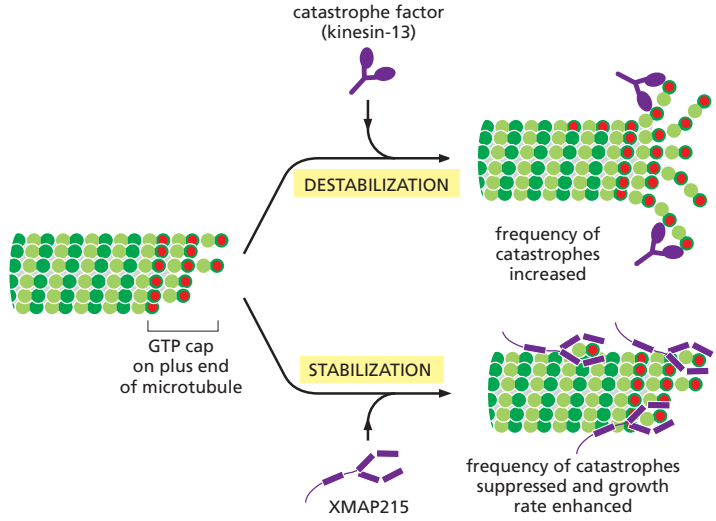


Figure 16–52 The effects of proteins that bind to microtubule ends. The transition between microtubule growth and shrinkage is controlled in cells by a variety of proteins. Catastrophe factors such as kinesin-13, a member of the kinesin motor protein superfamily, bind to microtubule ends and pry them apart, thereby promoting depolymerization. On the other hand, a MAP such as XMAP215 stabilizes the end of a growing microtubule (XMAP stands for *Xenopus* microtubule-associated protein, and the number refers to its molecular mass in kilodaltons). XMAP215 binds tubulin dimers and delivers them to the microtubule plus end, thereby increasing the microtubule growth rate and suppressing catastrophes.

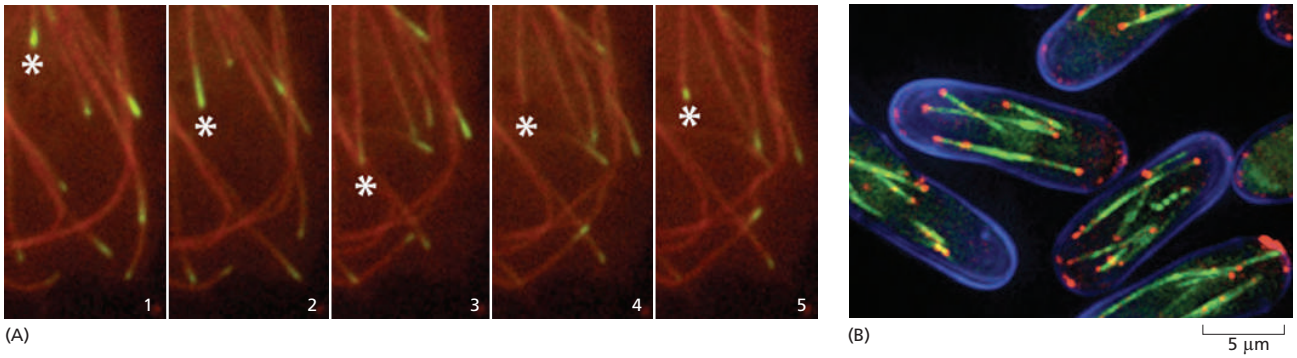


Figure 16–53 +TIP proteins found at the growing plus ends of microtubules. (A) Frames from a fluorescence time-lapse movie of the edge of a cell expressing fluorescently labeled tubulin that incorporates into microtubules *(red)* as well as the +TIP protein EB1 tagged with a different color *(green)*. The same microtubule is marked (asterisk) in successive movie frames. When the microtubule is growing (frames 1, 2), EB1 is associated with the tip. When the microtubule undergoes a catastrophe and begins shrinking, EB1 is lost (frames 3, 4). The labeled EB1 is regained when growth of the microtubule is rescued (frame 5). See Movie 16.8. (B) In the fission yeast *Schizosaccharomyces pombe*, the plus ends of the microtubules *(green)* are associated with the homolog of EB1 *(red)* at the two poles of the rod-shaped cells. (A, courtesy of Anna Akhmanova and Ilya Grigoriev; B, courtesy of Takeshi Toda.)

its activity and shifts the balance of its competition with catastrophe factors. This shift results in a tenfold increase in the dynamic instability of microtubules during mitosis, a transition that is critical for the efficient construction of the mitotic spindle (discussed in Chapter 17).

In many cells, the minus ends of microtubules are stabilized by association with a capping protein or the centrosome, or else they serve as microtubule depolymerization sites. The plus ends, in contrast, efficiently explore and probe the entire cell space. Microtubule-associated proteins called *plus-end tracking proteins (+TIPs)* accumulate at these active ends and appear to rocket around the cell as passengers at the ends of rapidly growing microtubules, dissociating from the ends when the microtubules begin to shrink (**Figure 16–53**).

The kinesin-related catastrophe factors and XMAP215 mentioned above behave as +TIPs and act to modulate the growth and shrinkage of the microtubule end to which they are attached. Other +TIPs control microtubule positioning by helping to capture and stabilize the growing microtubule end at specific cellular targets, such as the cell cortex or the kinetochore of a mitotic chromosome. EB1 and its relatives, small dimeric proteins that are highly conserved in animals, plants, and fungi, are key players in this process. EB1 proteins do not actively move toward plus ends, but rather recognize a structural feature of the growing plus end (see Figure 16–53). Several of the +TIPs depend on EB1 proteins for their plus-end accumulation and also interact with each other and with the microtubule lattice. By attaching to the plus end, these factors allow the cell to harness the energy of microtubule polymerization to generate pushing forces that can be used for positioning the spindle, chromosomes, or organelles.

Tubulin-Sequestering and Microtubule-Severing Proteins Destabilize Microtubules

As it does with actin monomers, the cell sequesters unpolymerized tubulin subunits to maintain a pool of active subunits at a level near the critical concentration. One molecule of the small protein *stathmin* (also called Op18) binds to two tubulin heterodimers and prevents their addition to the ends of microtubules (**Figure 16–54**). Stathmin thus decreases the effective concentration of tubulin subunits that are available for polymerization (an action analogous to that of the drug colchicine), and enhances the likelihood that a growing microtubule will switch to the shrinking state. Phosphorylation of stathmin inhibits its binding to tubulin, and signals that cause stathmin phosphorylation can increase the rate of microtubule elongation and suppress dynamic instability. Stathmin has been implicated in the regulation of both cell proliferation and cell death. Interestingly, mice lacking stathmin develop normally but are less fearful than wild-type mice, reflecting a role for stathmin in neurons of the amygdala, where it is normally expressed at high levels.

Severing is another mechanism employed by the cell to destabilize microtubules. To sever a microtubule, thirteen longitudinal bonds must be broken, one for each protofilament. The protein *katanin*, named after the Japanese word for

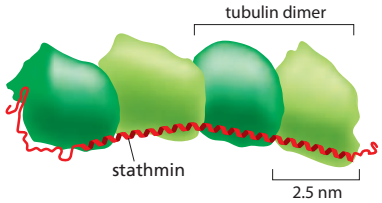


Figure 16–54 Sequestration of tubulin by stathmin. Structural studies with electron microscopy and crystallography suggest that the elongated stathmin protein binds along the side of two tubulin heterodimers. (Adapted from M.O. Steinmetz et al., *EMBO J.* 19:572–580, 2000. With permission from John Wiley and Sons.)

"sword," accomplishes this demanding task (**Figure 16–55**). Katanin is made up of two subunits: a smaller subunit that hydrolyzes ATP and performs the actual severing, and a larger one that directs katanin to the centrosome. Katanin releases microtubules from their attachment to a microtubule-organizing center and is thought to contribute to the rapid microtubule depolymerization observed at the poles of spindles during mitosis. It may also be involved in microtubule release and depolymerization in proliferating cells in interphase and in postmitotic cells such as neurons.

Two Types of Motor Proteins Move Along Microtubules

Like actin filaments, microtubules also use motor proteins to transport cargo and perform a variety of other functions within the cell. There are two major classes of microtubule-based motors, **kinesins** and **dyneins**. **Kinesin-1**, also called "conventional kinesin," was first purified from squid neurons, where it carries membrane-enclosed organelles away from the cell body toward the axon terminal by walking toward the plus end of microtubules. Kinesin-1 is similar to myosin II in having two heavy chains per active motor; these form two globular head motor domains that are held together by an elongated coiled-coil tail that is responsible for heavy-chain dimerization. One kinesin-1 light chain associates with each heavy chain through its tail domain and mediates cargo binding. Like myosin, kinesin is a member of a large protein superfamily, for which the motor domain is the common element (**Figure 16–56**). The yeast *Saccharomyces cerevisiae* has six distinct kinesins. The nematode *C. elegans* has 20 kinesins, and humans have 45.

There are at least fourteen distinct families in the kinesin superfamily. Most of them have the motor domain at the N-terminus of the heavy chain and walk toward the plus end of the microtubule. One family has the motor domain at the C-terminus and walks in the opposite direction, toward the minus end of the microtubule, while kinesin-13 has a central motor domain and does not walk at all, but uses the energy of ATP hydrolysis to depolymerize microtubule ends, as described above (see Figure 16–52). Some kinesin heavy chains are homodimers, and others are heterodimers. Most kinesins have a binding site in the tail for another microtubule; alternatively, they may link the motor to a membrane-enclosed organelle via a light chain or an adaptor protein. Many of the kinesin superfamily members have specific roles in mitotic spindle formation and in chromosome segregation during cell division.

In kinesin-1, instead of the rocking of a lever arm, small movements at the nucleotide-binding site regulate the docking and undocking of the motor head domain to a long linker region. This acts to throw the second head forward along

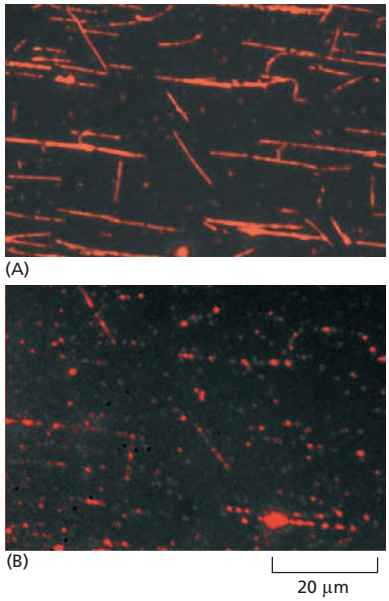


Figure 16–55 Microtubule severing by katanin. Taxol stabilized, rhodamine-labeled microtubules were adsorbed on the surface of a glass slide, and purified katanin was added along with ATP. (A) There are a few breaks in the microtubules 30 seconds after the addition of katanin. (B) The same field 3 minutes after the addition of katanin. The filaments have been severed in many places, leaving a series of small fragments at the previous locations of the long microtubules. (From J.J. Hartman et al., *Cell* 93:277–287, 1998. With permission from Elsevier.)

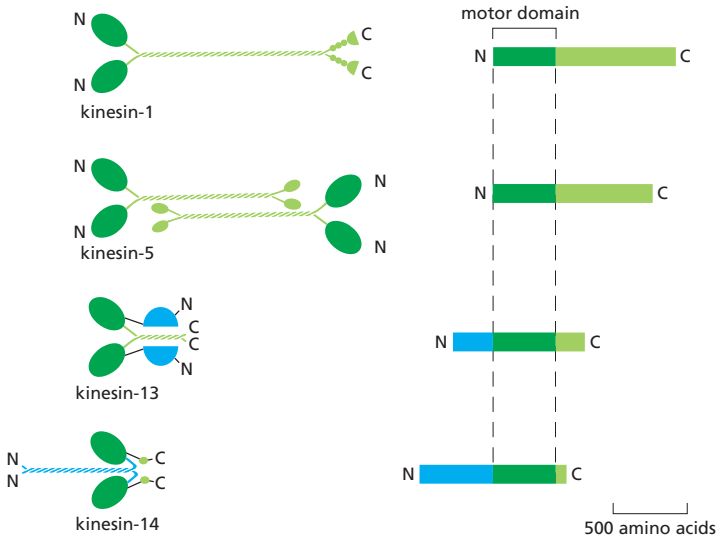


Figure 16–56 Kinesin and kinesin-related proteins. Structures of four kinesin superfamily members. As in the myosin superfamily, only the motor domains are conserved. Kinesin-1 has the motor domain at the N-terminus of the heavy chain. The middle domain forms a long coiled-coil, mediating dimerization. The C-terminal domain forms a tail that attaches to cargo, such as a membrane-enclosed organelle. Kinesin-5 forms tetramers where two dimers associate by their tails. The bipolar kinesin-5 tetramer is able to slide two microtubules past each other, analogous to the activity of the bipolar thick filaments formed by myosin II. Kinesin-13 has its motor domain located in the middle of the heavy chain. It is a member of a family of kinesins that have lost typical motor activity and instead bind to microtubule ends to promote depolymerization (see Figure 16–52). Kinesin-14 is a C-terminal kinesin that includes the *Drosophila* protein Ncd and the yeast protein Kar3. These kinesins generally travel in the opposite direction from the majority of kinesins, toward the minus end instead of the plus end of a microtubule.

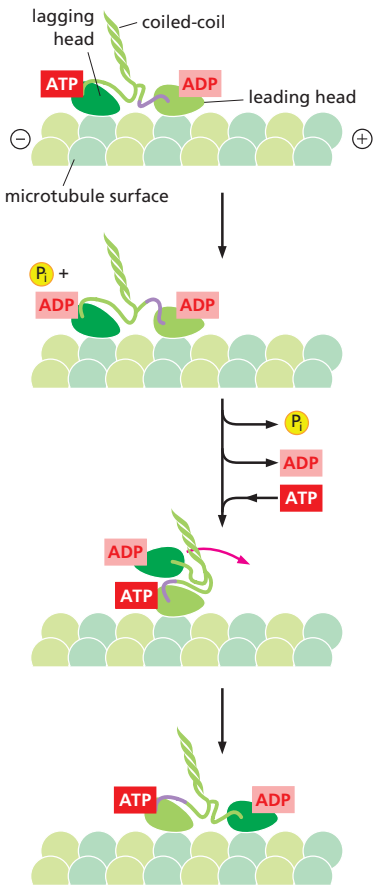


Figure 16–57 The mechanochemical cycle of kinesin. Kinesin-1 is a dimer of two nucleotide-binding motor domains (heads) that are connected through a long coiled-coil tail (see Figure 16–56). The two kinesin motor domains work in a coordinated manner; during a kinesin "step," the rear head detaches from its tubulin binding site, passes the partner motor domain, and then rebinds to the next available tubulin binding site. Using this "hand-over-hand" motion, the kinesin dimer can move for long distances on the microtubule without completely letting go of its track.

At the start of each step, one of the two kinesin motor domain heads, the rear or lagging head *(dark green)*, is tightly bound to the microtubule and to ATP, while the front or leading head is loosely bound to the microtubule with ADP in its binding site. The forward displacement of the rear motor domain is driven by the dissociation of ADP and binding of ATP in the leading head (between panels 2 and 3 in this drawing). The binding of ATP to this motor domain causes a small peptide called the "neck linker" to shift from a rearward-pointing to a forward-pointing conformation (the neck linker is drawn here as a *purple* connecting line between the leading motor domain and the intertwined coiled-coil). This shift pulls the rear head forward, once it has detached from the microtubule with ADP bound [detachment requires ATP hydrolysis and phosphate (P_i) release]. The kinesin molecule is now poised for the next step, which proceeds by an exact repeat of the process shown (Movie 16.9).

the protofilament to a binding site 8 nm closer to the microtubule plus end, which is the distance between tubulin dimers of a protofilament. The nucleotide-hydrolysis cycles in the two heads are closely coordinated, so that this cycle of linker docking and undocking allows the two-headed motor to move in a hand-over-hand (or head-over-head) stepwise manner (Figure 16–57).

The **dyneins** are a family of minus-end directed microtubule motors unrelated to the kinesins. They are composed of one, two, or three heavy chains (that include the motor domain) and a large and variable number of associated intermediate, light-intermediate, and light chains. The dynein family has two major branches (Figure 16–58). The first branch contains the *cytoplasmic dyneins*, which are homodimers of two heavy chains. Cytoplasmic dynein 1 is encoded by a single gene in almost all eukaryotic cells, but is missing from flowering plants and some algae. It is used for organelle and mRNA trafficking, for positioning the centrosome and nucleus during cell migration, and for construction of the microtubule spindle in mitosis and meiosis. Cytoplasmic dynein 2 is found only in eukaryotic organisms that have cilia and is used to transport material from the tip to the base of the cilia, a process called intraflagellar transport. *Axonemal dyneins* (also called *ciliary dyneins*) comprise the second branch and include monomers, heterodimers, and heterotrimers, with one, two, or three motor-containing heavy chains, respectively. They are highly specialized for the rapid and efficient sliding movements of microtubules that drive the beating of cilia and flagella (discussed later).

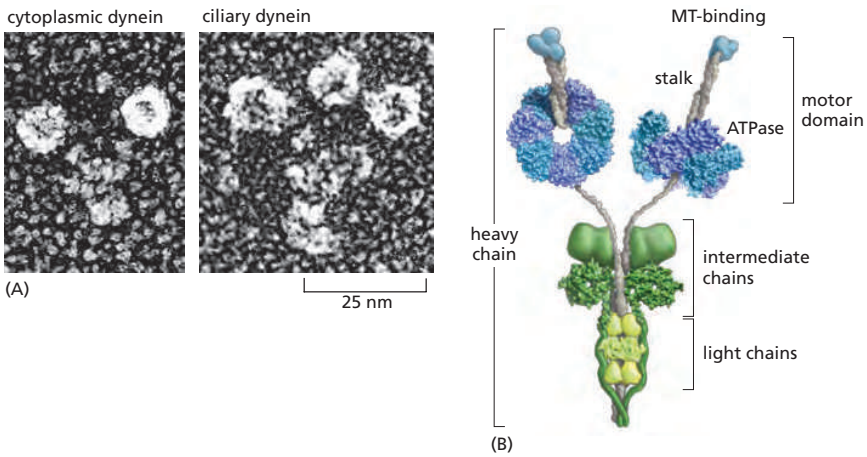


Figure 16–58 Dyneins. (A) Freeze-etch electron micrographs of a molecule of cytoplasmic dynein and a molecule of ciliary (axonemal) dynein. Like myosin II and kinesin-1, cytoplasmic dynein is a two-headed molecule. The ciliary dynein shown is from a protozoan and has three heads; ciliary dynein from animals has two heads. Note that the dynein head is very large compared with the head of either myosin or kinesin. (B) Schematic depiction of cytoplasmic dynein showing the two heavy chains *(blue and gray)* that contain domains for microtubule (MT) binding and ATP hydrolysis, connected by a long stalk. Bound to the heavy chain are multiple intermediate chains *(dark green)* and light chains *(light green)* that help to mediate many of dynein's functions. (A, courtesy of John Heuser; B, adapted from R. Vale, *Cell* 112:467–480, 2003. With permission from Cell Press.)

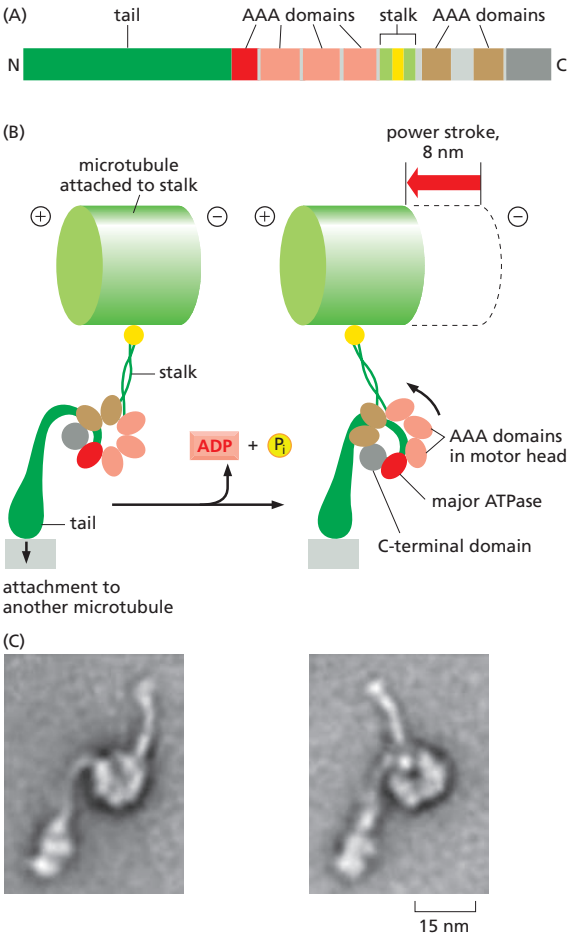


Figure 16–59 The power stroke of dynein. (A) The organization of the domains in each dynein heavy chain. This is a huge polypeptide, containing nearly 4000 amino acids. The number of heavy chains in a dynein is equal to its number of motor heads. (B) Illustration of dynein c, a monomeric axonemal dynein found in the unicellular green alga *Chlamydomonas reinhardtii.* The large dynein motor head is a planar ring containing a C-terminal domain *(gray)* and six AAA domains, four of which retain ATP-binding sequences, but only one of which *(dark red)* has the major ATPase activity. Extending from the head are a long, coiled-coil stalk with the microtubule-binding site at the tip, and a tail that attaches to an adjacent microtubule in the axoneme. In the ATP-bound state, the stalk is detached from the microtubule, but ATP hydrolysis causes stalk–microtubule attachment *(left)*. Subsequent release of ADP and phosphate (P_i) then leads to a large conformational "power stroke" involving rotation of the head and stalk relative to the tail *(right)*. Each cycle generates a step of about 8 nm, thereby contributing to flagellar beating (see Figure 16–65). In the case of cytoplasmic dynein, the tail is attached to a cargo such as a vesicle, and a single power stroke transports the cargo about 8-nm along the microtubule toward its minus end (see Figure 16–60). (C) Electron micrographs of purified monomeric dyneins in two different conformations representing different steps in the mechanochemical cycle. (C, from S.A. Burgess et al., *Nature* 421:715–718, 2003. With permission from Macmillan Publishers Ltd.)

Dyneins are the largest of the known molecular motors, and they are also among the fastest: axonemal dyneins attached to a glass slide can move microtubules at the rate of 14 μm/sec. The dynein motor is structurally unrelated to myosins and kinesins, but still follows the general rule of coupling nucleotide hydrolysis to microtubule binding and unbinding as well as to a force-generating conformational change (**Figure 16–59**).

Microtubules and Motors Move Organelles and Vesicles

A major function of cytoskeletal motors in interphase cells is the transport and positioning of membrane-enclosed organelles (**Movie 16.10**). Kinesin was originally identified as the protein responsible for fast *anterograde axonal transport*, the rapid movement of mitochondria, secretory vesicle precursors, and various synapse components down the microtubule highways of the axon to the distant nerve terminals. Cytoplasmic dynein was identified as the motor responsible for transport in the opposite direction, *retrograde axonal transport*. Although organelles in most cells need not cover such long distances, their polarized transport is equally necessary. A typical microtubule array in an interphase cell is oriented with the minus ends near the center of the cell at the centrosome and the plus ends extending to the cell periphery. Thus, centripetal movements of organelles or vesicles toward the cell center require the action of minus-end directed cytoplasmic dynein motors, whereas centrifugal movements toward the periphery require plus-end directed kinesin motors. Interestingly, in animal cells, nearly all minus-end directed transport is driven by the single cytoplasmic dynein 1 motor, whereas 15 different kinesins are used for plus-end directed transport.

A clear example of the effect of microtubules and microtubule motors on the behavior of intracellular membranes is their role in organizing the endoplasmic reticulum (ER) and the Golgi apparatus. The network of ER membrane tubules aligns with microtubules and extends almost to the edge of the cell (**Movie 16.11**), whereas the Golgi apparatus is located near the centrosome. When cells are treated with a drug that depolymerizes microtubules, such as colchicine or nocodazole, the ER collapses to the center of the cell, while the Golgi apparatus fragments and disperses throughout the cytoplasm. *In vitro*, kinesins can tether ER-derived membranes to preformed microtubule tracks and walk toward the microtubule plus ends, dragging the ER membranes out into tubular protrusions and forming a membranous web that looks very much like the ER in cells. Conversely, dyneins are required for positioning the Golgi apparatus near the cell center of animal cells; they do this by moving Golgi vesicles along microtubule tracks toward the microtubules' minus ends at the centrosome.

The different tails and their associated light chains on specific motor proteins allow the motors to attach to their appropriate organelle cargo. Membrane-associated motor receptors that are sorted to specific membrane-enclosed compartments interact directly or indirectly with the tails of the appropriate kinesin family members. Many viruses take advantage of microtubule motor-based transport during infection and use kinesin to move from their site of replication and assembly to the plasma membrane, from which they are poised to infect neighboring cells. An outer-membrane protein of *Vaccinia* virus, for example, contains an amino acid motif that mediates binding to kinesin-1 light chain and transport along microtubules to the plasma membrane. Interestingly, this motif is present in over 450 human proteins, one-third of which are associated with human diseases. Thus, kinesin transports a diverse set of cargoes involved in a wide range of important cellular functions.

For dynein, a large macromolecular assembly often mediates attachment to membranes. Cytoplasmic dynein, itself a huge protein complex, requires association with a second large protein complex called *dynactin* to translocate organelles effectively. The dynactin complex includes a short, actin-like filament that forms from the actin-related protein Arp1 (distinct from Arp2 and Arp3, the components of the Arp 2/3 complex involved in the nucleation of conventional actin filaments) (**Figure 16–60**). A number of other proteins also contribute to dynein cargo binding and motor regulation, and their function is especially important in neurons, where defects in microtubule-based transport have been linked to neurological diseases. A striking example is smooth brain, or lissencephaly, a human disorder in which cells fail to migrate to the cerebral cortex of the developing brain. One type of lissencephaly is caused by defects in Lis1, a dynein-binding protein required for nuclear migration in several species. In the normal brain, migration of the nucleus directs the developing neural cell body toward its correct position in the cortex. In the absence of Lis1, however, the nuclei of migrating neurons fail to attach to dynein, resulting in nuclear-migration defects. Dynein is required continuously for neuronal function, as mutations in a dynactin subunit or in the tail region of cytoplasmic dynein lead to neuronal degeneration in humans and mice. These effects are associated with decreased retrograde axonal transport and provide strong evidence for the importance of robust axonal transport in neuronal viability.

The cell can regulate the activity of motor proteins and thereby cause either a change in the positioning of its membrane-enclosed organelles or whole-cell movements. Fish melanocytes provide one of the most dramatic examples. These giant cells, which are responsible for rapid changes in skin coloration in several species of fish, contain large pigment granules that can alter their location in response to neuronal or hormonal stimulation (**Figure 16–61**). The pigment granules aggregate or disperse by moving along an extensive network of microtubules that are anchored at the centrosome by their minus ends. The tracking of individual pigment granules reveals that the inward movement is rapid and smooth, while the outward movement is jerky, with frequent backward steps. Both dynein and kinesin microtubule motors are associated with the pigment granules. The

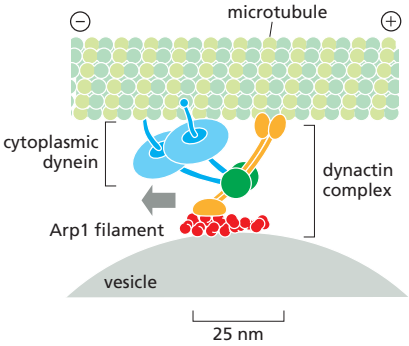


Figure 16–60 Dynactin mediates the attachment of dynein to a membrane-enclosed organelle. Dynein requires the presence of a large number of accessory proteins to associate with membrane-enclosed organelles. Dynactin is a large complex that includes components that bind weakly to microtubules, components that bind to dynein itself, and components that form a small, actin-like filament made of the actin-related protein Arp1.

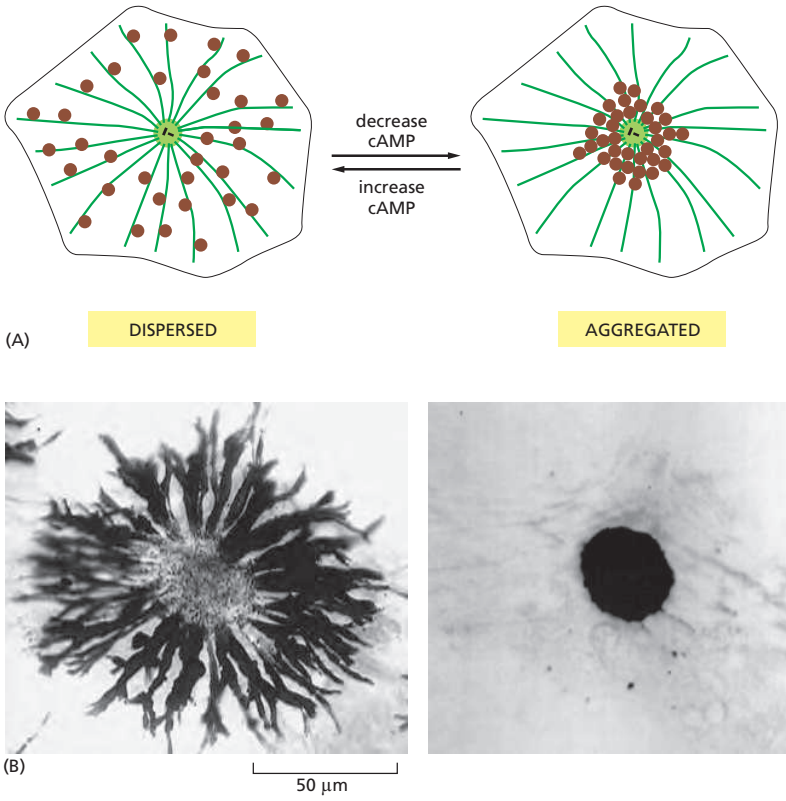


Figure 16–61 Regulated melanosome movements in fish pigment cells. These giant cells, which are responsible for changes in skin coloration in several species of fish, contain large pigment granules, or melanosomes *(brown).* The melanosomes can change their location in the cell in response to a hormonal or neuronal stimulus. (A) Schematic view of a pigment cell, showing the dispersal and aggregation of melanosomes in response to an increase or decrease in intracellular cyclic AMP (cAMP), respectively. Both redistributions of melanosomes occur along microtubules. (B) Bright-field images of a single cell in a scale of an African cichlid fish, showing its melanosomes either dispersed throughout the cytoplasm *(left)* or aggregated in the center of the cell *(right).* (B, courtesy of Leah Haimo.)

jerky outward movements may result from a tug-of-war between the two opposing microtubule motor proteins, with the stronger kinesin winning out overall. When intracellular cyclic AMP levels decrease, kinesin is inactivated, leaving dynein free to drag the pigment granules rapidly toward the cell center, changing the fish's color. In a similar way, the movement of other membrane organelles coated with particular motor proteins is controlled by a complex balance of competing signals that regulate both motor protein attachment and activity.

Construction of Complex Microtubule Assemblies Requires Microtubule Dynamics and Motor Proteins

The construction of the mitotic spindle and the neuronal cytoskeleton are important and fascinating examples of the power of organization by teams of motor proteins interacting with dynamic cytoskeletal filaments. As described in Chapter 17, mitotic spindle assembly depends on reorganization of the interphase array of microtubules to form a bipolar array of microtubules, with their minus ends focused at the poles and their plus ends overlapping in the center or connecting to chromosomes. Spindle assembly depends on the coordinated actions of several motor proteins and other factors that modulate polymerization dynamics (see Figures 17–23 and 17–25).

Neurons also contain complex cytoskeletal structures. As they differentiate, neurons send out specialized processes that will either receive electrical signals (*dendrites*) or transmit electrical signals (*axons*) (see Figure 16–50). The beautiful and elaborate branching morphology of axons and dendrites enables neurons to form tremendously complex signaling networks, interacting with many other cells simultaneously and making possible the complicated behavior of the higher animals. Both axons and dendrites (collectively called *neurites*) are filled with bundles of microtubules that are critical to both their structure and their function.

In axons, all the microtubules are oriented in the same direction, with their minus end pointing back toward the cell body and their plus end pointing toward the axon terminals (**Figure 16–62**). The microtubules do not reach from the cell

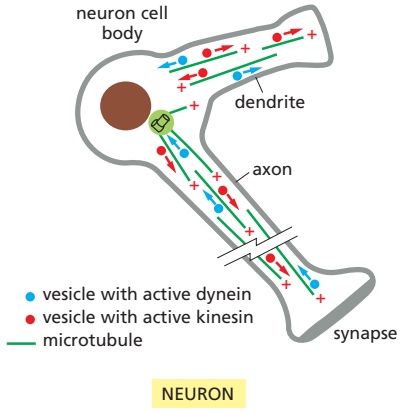


Figure 16–62 Microtubule organization in a neuron. In a neuron, microtubule organization is complex. In the axon, all microtubules share the same polarity, with the plus ends pointing outward toward the axon terminus. No single microtubule stretches the entire length of the axon; instead, short overlapping segments of parallel microtubules make the tracks for fast axonal transport. In dendrites, the microtubules are of mixed polarity, with some plus ends pointing outward and some pointing inward. Vesicles can associate with both kinesin and dynein and move in either direction along the microtubules in axons and dendrites, depending on which motor is active.

body all the way to the axon terminals; each is typically only a few micrometers in length, but large numbers are staggered in an overlapping array. These aligned microtubule tracks act as a highway to transport specific proteins, protein-containing vesicles, and mRNAs to the axon terminals, where synapses are constructed and maintained. The longest axon in the human body reaches from the base of the spinal cord to the foot and is up to a meter in length. By comparison, dendrites are generally much shorter than axons. The microtubules in dendrites lie parallel to one another but their polarities are mixed, with some pointing their plus ends toward the dendrite tip, while others point back toward the cell body, reminiscent of the antiparallel microtubule array of the mitotic spindle.

Motile Cilia and Flagella Are Built from Microtubules and Dyneins

Just as myofibrils are highly specialized and efficient motility machines built from actin and myosin filaments, cilia and flagella are highly specialized and efficient motility structures built from microtubules and dynein. Both cilia and flagella are hairlike cell appendages that have a bundle of microtubules at their core. **Flagella** are found on sperm and many protozoa. By their undulating motion, they enable the cells to which they are attached to swim through liquid media. **Cilia** are organized in a similar fashion, but they beat with a whiplike motion that resembles the breaststroke in swimming. Ciliary beating can either propel single cells through a fluid (as in the swimming of the protozoan *Paramecium*) or can move fluid over the surface of a group of cells in a tissue. In the human body, huge numbers of cilia ($10^9/cm^2$ or more) line our respiratory tract, sweeping layers of mucus, trapped particles of dust, and bacteria up to the mouth where they are swallowed and ultimately eliminated. Likewise, cilia along the oviduct help to sweep eggs toward the uterus.

The movement of a cilium or a flagellum is produced by the bending of its core, which is called the **axoneme**. The axoneme is composed of microtubules and their associated proteins, arranged in a distinctive and regular pattern. Nine special doublet microtubules (comprising one complete and one partial microtubule fused together so that they share a common tubule wall) are arranged in a ring around a pair of single microtubules (**Figure 16–63**). Almost all forms of motile eukaryotic flagella and cilia (from protozoans to humans) have this characteristic arrangement. The microtubules extend continuously for the length of the axoneme, which can be 10–200 μm. At regular positions along the length of the microtubules, accessory proteins cross-link the microtubules together.

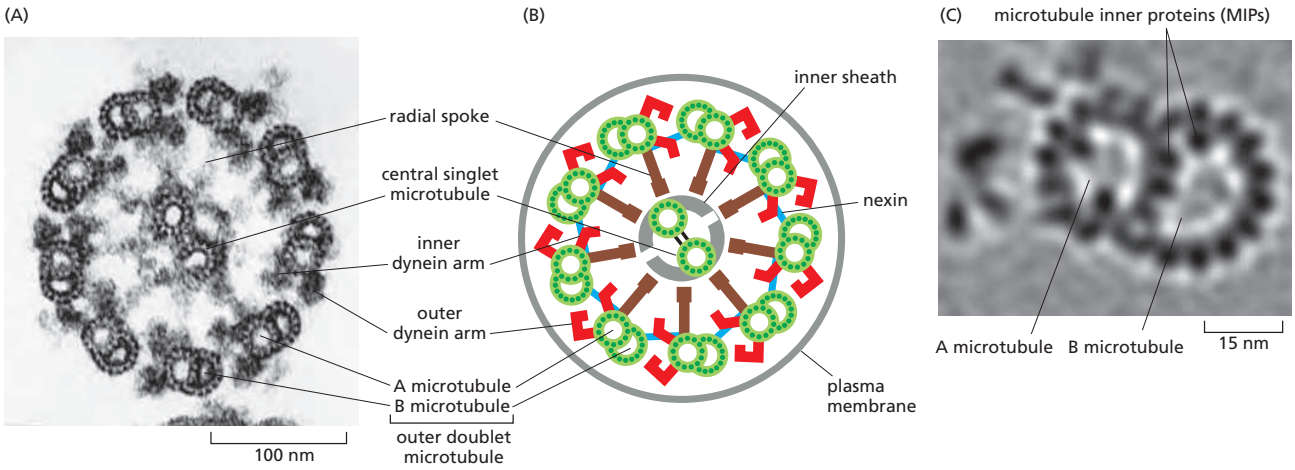


Figure 16–63 The arrangement of microtubules in a flagellum or cilium. (A) Electron micrograph of the flagellum of a green-alga cell (*Chlamydomonas*) shown in cross section, illustrating the distinctive "9 + 2" arrangement of microtubules. (B) Diagram of the parts of a flagellum or cilium. The various projections from the microtubules link the microtubules together and occur at regular intervals along the length of the axoneme. (C) High-resolution electron tomography image of an outer doublet microtubule showing structural details and features inside the microtubules called microtubule inner proteins (MIPs). (A, courtesy of Lewis Tilney; C, courtesy of Daniela Nicastro.)

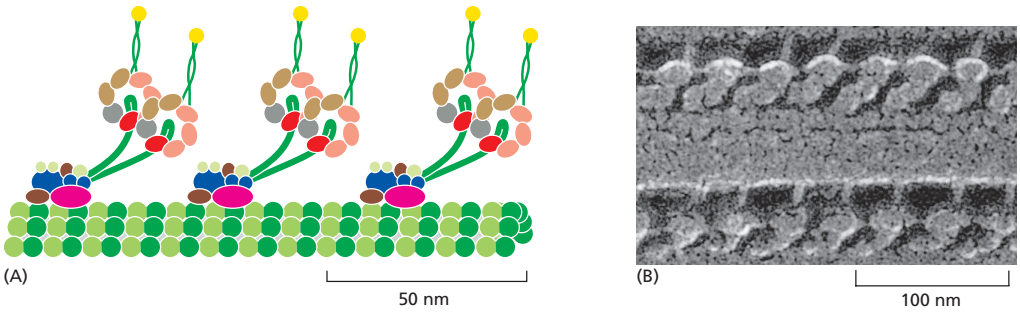


Figure 16–64 Ciliary dynein. Ciliary (axonemal) dynein is a large protein assembly (nearly 2 million daltons) composed of 9–12 polypeptide chains, the largest of which is the heavy chain of more than 500,000 daltons. (A) The heavy chains form the major portion of the globular head and stem domains, and many of the smaller chains are clustered around the base of the stem. There are two heads in the outer dynein in metazoans (shown here), but three heads in protozoa, each formed from their own heavy chain. The tail of the molecule binds tightly to an A microtubule, while the large globular heads have an ATP-dependent binding site for a B microtubule (see Figure 16–63). When the heads hydrolyze their bound ATP, they move toward the minus end of the B microtubule, thereby producing a sliding force between the adjacent microtubule doublets in a cilium or flagellum (see Figure 16–59). (B) Freeze-etch electron micrograph of a cilium showing the dynein arms projecting at regular intervals from the doublet microtubules. (B, courtesy of John Heuser.)

Molecules of *axonemal dynein* form bridges between the neighboring doublet microtubules around the circumference of the axoneme (**Figure 16–64**). When the motor domain of this dynein is activated, the dynein molecules attached to one microtubule doublet (see Figure 16–59) attempt to walk along the adjacent microtubule doublet, tending to force the adjacent doublets to slide relative to one another, much as actin thin filaments slide during muscle contraction. However, the presence of other links between the microtubule doublets prevents this sliding, and the dynein force is instead converted into a bending motion (**Figure 16–65**).

In humans, hereditary defects in axonemal dynein cause a condition called primary ciliary dyskinesia or Kartagener's syndrome. This syndrome is characterized by inversion of the normal asymmetry of internal organs (sinus inversus) due to disruption of fluid flow in the developing embryo, male sterility due to immotile sperm, and a high susceptibility to lung infections due to paralyzed cilia being unable to clear the respiratory tract of debris and bacteria.

Bacteria also swim using cell-surface structures called flagella, but these do not contain microtubules or dynein and do not wave or beat. Instead, *bacterial flagella* are long, rigid helical filaments, made up of repeating subunits of the protein flagellin. The flagella rotate like propellers, driven by a special rotary motor embedded in the bacterial cell wall. The use of the same name to denote these two very different types of swimming apparatus is an unfortunate historical accident.

Primary Cilia Perform Important Signaling Functions in Animal Cells

Many cells possess a shorter, nonmotile counterpart of cilia and flagella called the *primary cilium*. Primary cilia can be viewed as specialized cellular compartments or organelles that perform a wide range of cellular functions, but share

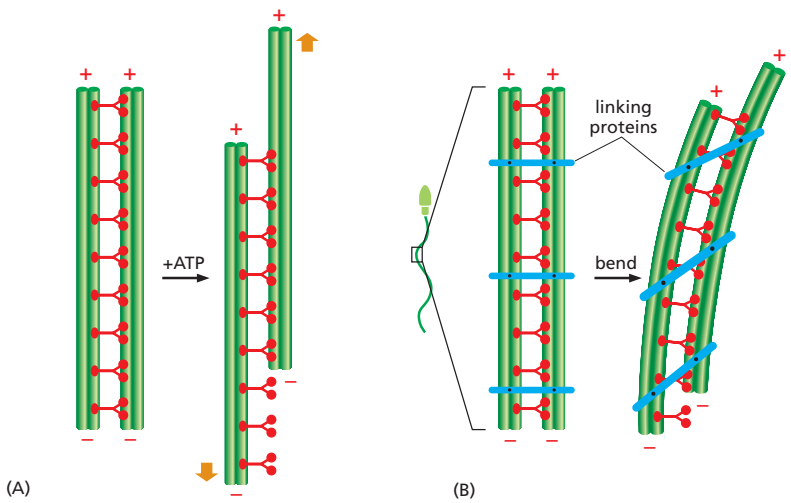


Figure 16–65 The bending of an axoneme. (A) When axonemes are exposed to the proteolytic enzyme trypsin, the linkages holding neighboring doublet microtubules together are broken. In this case, the addition of ATP allows the motor action of the dynein heads to slide one pair of doublet microtubules against the other pair. (B) In an intact axoneme (such as in a sperm), flexible protein links prevent the sliding of the doublet. The motor action therefore causes a bending motion, creating waves or beating motions.

many structural features with motile cilia. Both motile and nonmotile cilia are generated during interphase at plasma-membrane-associated structures called *basal bodies* that firmly root them at the cell surface. At the core of each basal body is a centriole, the same structure found embedded at the center of animal centrosomes, with nine groups of fused triplet microtubules arranged in a cartwheel (see Figure 16–48). Centrioles are multifunctional, contributing to assembly of the mitotic spindle in dividing cells but migrating to the plasma membrane of interphase cells to template the nucleation of the axoneme (**Figure 16–66**). Because no protein translation occurs in cilia, construction of the axoneme requires intraflagellar transport (IFT), a transport system discovered in the green algae *Chlamydomonas*. Analogous to the axon, motors move cargoes in both anterograde and retrograde directions, in this case driven by kinesin-2 and cytoplasmic dynein 2, respectively.

Primary cilia are found on the surface of almost all cell types, where they sense and respond to the exterior environment, functions best understood in the context of smell and sight. In the nasal epithelium, cilia protruding from dendrites of olfactory neurons are the site of both odorant reception and signal amplification. Similarly, the rod and cone cells of the vertebrate retina possess a primary cilium equipped with an expanded tip called the outer segment, which is specialized for converting light into a neural signal (see Figure 15–38). Maintenance of the outer segment requires continuous IFT-mediated transport of large quantities of lipids and proteins into the cilium, at rates of up to 2000 molecules per minute. The links between cilia function and the senses of sight and smell are underscored by Bardet-Biedl syndrome, a set of disorders associated with defects in IFT, the cilium, or the basal body. Patients with Bardet-Biedl syndrome cannot smell and suffer from retinal degeneration. Other characteristics of this multifaceted disorder include hearing loss, polycystic kidney disease, diabetes, obesity, and polydactyly, suggesting that primary cilia have functions in many aspects of human physiology.

Summary

Microtubules are stiff polymers of tubulin molecules. They assemble by addition of GTP-containing tubulin subunits to the free end of a microtubule, with one end (the plus end) growing faster than the other. Hydrolysis of the bound GTP takes place

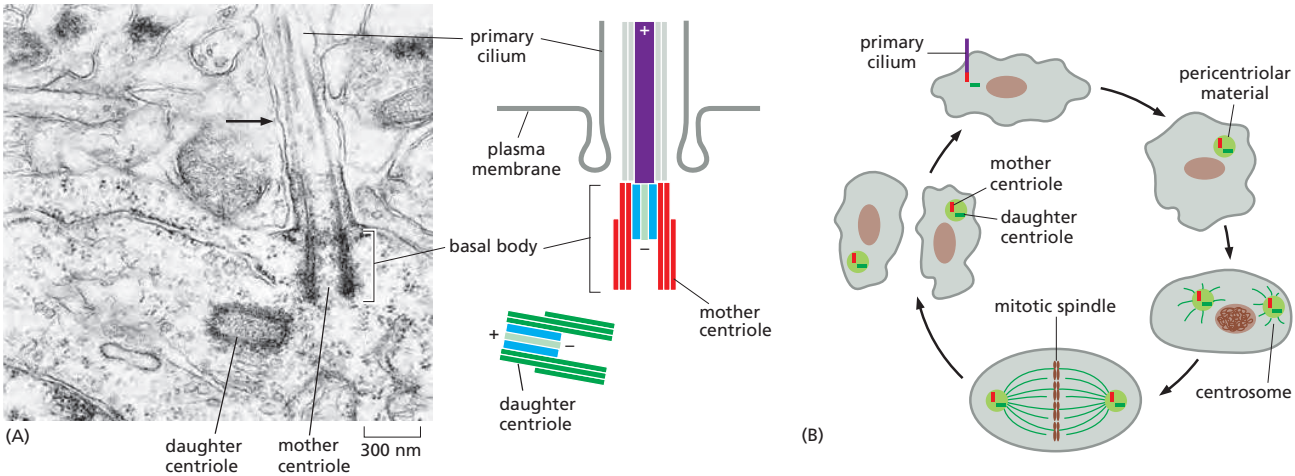


Figure 16–66 Primary cilia. (A) Electron micrograph and diagram of the basal body of a mouse neuron primary cilium. The axoneme of the primary cilium (*black arrow*) is nucleated by the mother centriole at the basal body, which localizes at the plasma membrane near the cell surface. (B) Centrioles function alternately as basal bodies and as the core of centrosomes. Before a cell enters the cell division cycle, the primary cilium is shed or resorbed. The centrioles recruit pericentriolar material and duplicate during S phase, generating two centrosomes, each of which contains a pair of centrioles. The centrosomes nucleate microtubules and localize to the poles of the mitotic spindle. Upon exit from mitosis, a primary cilium again grows from the mother centriole. (A, courtesy of Josef Spacek.)

after assembly and weakens the bonds that hold the microtubule together. Microtubules are dynamically unstable and liable to catastrophic disassembly, but they can be stabilized in cells by association with other structures. Microtubule-organizing centers such as centrosomes protect the minus ends of microtubules and continually nucleate the formation of new microtubules. Microtubule-associated proteins (MAPs) stabilize microtubules, and those that localize to the plus end (+TIPs) can alter the dynamic properties of the microtubule or mediate their interaction with other structures. Counteracting the stabilizing activity of MAPs are catastrophe factors, such as kinesin-13 proteins, that act to peel apart microtubule ends. Other kinesin family members as well as dynein use the energy of ATP hydrolysis to move unidirectionally along a microtubule. The motor dynein moves toward the minus end of microtubules, and its sliding of axonemal microtubules underlies the beating of cilia and flagella. Primary cilia are nonmotile sensory organs found on many cell types.

INTERMEDIATE FILAMENTS AND SEPTINS

All eukaryotic cells contain actin and tubulin. But the third major type of cytoskeletal protein, the *intermediate filament*, forms a cytoplasmic filament only in some metazoans—including vertebrates, nematodes, and mollusks. Intermediate filaments are particularly prominent in the cytoplasm of cells that are subject to mechanical stress and are generally not found in animals that have rigid exoskeletons, such as arthropods and echinoderms. It seems that intermediate filaments impart mechanical strength to tissues for the squishier animals.

Cytoplasmic intermediate filaments are closely related to their ancestors, the much more prevalent *nuclear lamins*, which are found in many eukaryotes but missing from unicellular organisms. The nuclear lamins form a meshwork lining the inner membrane of the nuclear envelope, where they provide anchorage sites for chromosomes and nuclear pores. Several times during metazoan evolution, lamin genes have apparently duplicated, and the duplicates have evolved to produce ropelike, cytoplasmic intermediate filaments. In contrast to the highly conserved actins and tubulin isoforms that are encoded by a handful of genes, different families of intermediate filaments are much more diverse and are encoded by 70 different human genes with distinct, cell type-specific functions (**Table 16–2**).

TABLE 16–2 Major Types of Intermediate Filament Proteins in Vertebrate Cells

Types of intermediate filament	Component polypeptides	Location
Nuclear	Lamins A, B, and C	Nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	Vimentin	Many cells of mesenchymal origin
	Desmin	Muscle
	Glial fibrillary acidic protein	Glial cells (astrocytes and some Schwann cells)
	Peripherin	Some neurons
Epithelial	Type I keratins (acidic)	Epithelial cells and their derivatives (e.g., hair and nails)
	Type II keratins (neutral/basic)	
Axonal	Neurofilament proteins (NF-L, NF-M, and NF-H)	Neurons

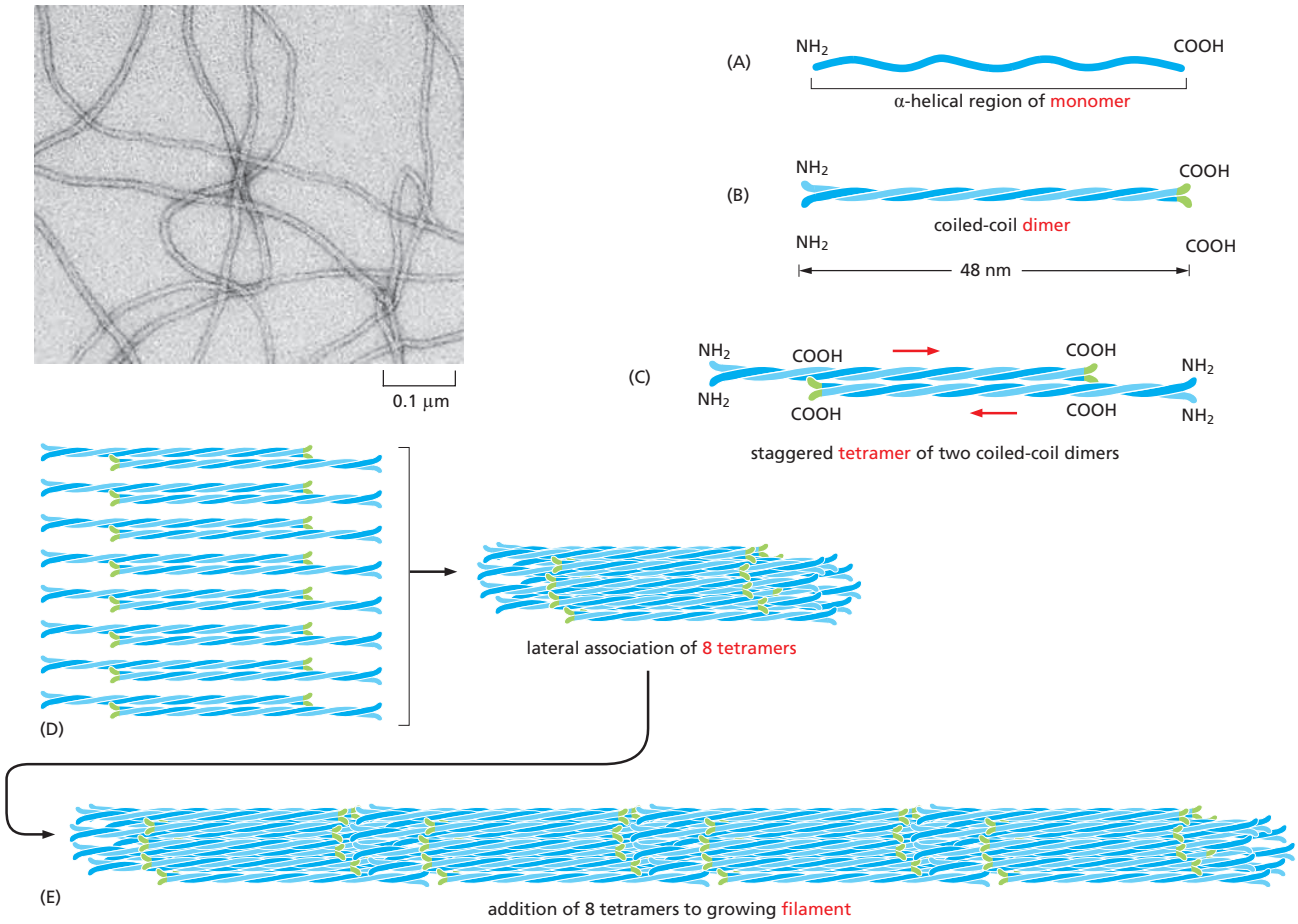


Figure 16–67 A model of intermediate filament construction. The monomer shown in (A) pairs with another monomer to form a dimer (B), in which the conserved central rod domains are aligned in parallel and wound together into a coiled-coil. (C) Two dimers then line up side by side to form an antiparallel tetramer of four polypeptide chains. Dimers and tetramers are the soluble subunits of intermediate filaments. (D) Within each tetramer, the two dimers are offset with respect to one another, thereby allowing it to associate with another tetramer. (E) In the final 10-nm ropelike filament, tetramers are packed together in a helical array, which has 16 dimers (32 coiled-coils) in cross section. Half of these dimers are pointing in each direction. An electron micrograph of intermediate filaments is shown on the upper left (**Movie 16.12**). (Electron micrograph courtesy of Roy Quinlan.)

Intermediate Filament Structure Depends on the Lateral Bundling and Twisting of Coiled-Coils

Although their amino- and carboxy-terminal domains differ, all intermediate filament family members are elongated proteins with a conserved central α-helical domain containing 40 or so heptad repeat motifs that form an extended coiled-coil structure with another monomer (see Figure 3–9). A pair of parallel dimers then associates in an antiparallel fashion to form a staggered tetramer (**Figure 16–67**). Unlike actin or tubulin subunits, intermediate filament subunits do not contain a binding site for a nucleotide. Furthermore, since the tetrameric subunit is made up of two dimers pointing in opposite directions, its two ends are the same. The assembled intermediate filament therefore lacks the overall structural polarity that is critical for actin filaments and microtubules. The tetramers pack together laterally to form the filament, which includes eight parallel protofilaments made up of tetramers. Each individual intermediate filament therefore has a cross section of 32 individual α-helical coils. This large number of polypeptides all lined up together, with the strong lateral hydrophobic interactions typical of coiled-coil proteins, gives intermediate filaments a ropelike character. They can be easily bent, with a persistence length of less than one micrometer (compared

to several millimeters for microtubules and about ten micrometers for actin), but they are extremely difficult to break and can be stretched to over three times their length (see Figure 16–6).

Less is understood about the mechanism of assembly and disassembly of intermediate filaments than of actin filaments and microtubules. In pure protein solutions, intermediate filaments are extremely stable due to tight association of subunits, but some types of intermediate filaments, including *vimentin*, form highly dynamic structures in cells such as fibroblasts. Protein phosphorylation probably regulates their disassembly, in much the same way that phosphorylation regulates the disassembly of nuclear lamins in mitosis (see Figure 12–18). As evidence for rapid turnover, labeled subunits microinjected into tissue-culture cells incorporate into intermediate filaments within a few minutes. Remodeling of the intermediate filament network accompanies events requiring dynamic cellular reorganization, such as division, migration, and differentiation.

Intermediate Filaments Impart Mechanical Stability to Animal Cells

Keratins are the most diverse intermediate filament family: there are about 20 found in different types of human epithelial cells and about 10 more that are specific to hair and nails; analysis of the human genome sequence has revealed that there are 54 distinct keratins. Every keratin filament is made up of an equal mixture of type I (acidic) and type II (neutral/basic) keratin proteins; these form a heterodimer filament subunit (see Figure 16–67). Cross-linked keratin networks held together by disulfide bonds can survive even the death of their cells, forming tough coverings for animals, as in the outer layer of skin and in hair, nails, claws, and scales. The diversity in keratins is clinically useful in the diagnosis of epithelial cancers (carcinomas), as the particular set of keratins expressed gives an indication of the epithelial tissue in which the cancer originated and thus can help to guide the choice of treatment.

A single epithelial cell may produce multiple types of keratins, and these copolymerize into a single network (**Figure 16–68**). Keratin filaments impart mechanical strength to epithelial tissues in part by anchoring the intermediate filaments at sites of cell–cell contact, called *desmosomes*, or cell–matrix contact, called *hemidesmosomes* (see Figure 16–4). We discuss these important adhesive structures in Chapter 19. Accessory proteins, such as *filaggrin*, bundle keratin filaments in differentiating cells of the epidermis to give the outermost layers of the

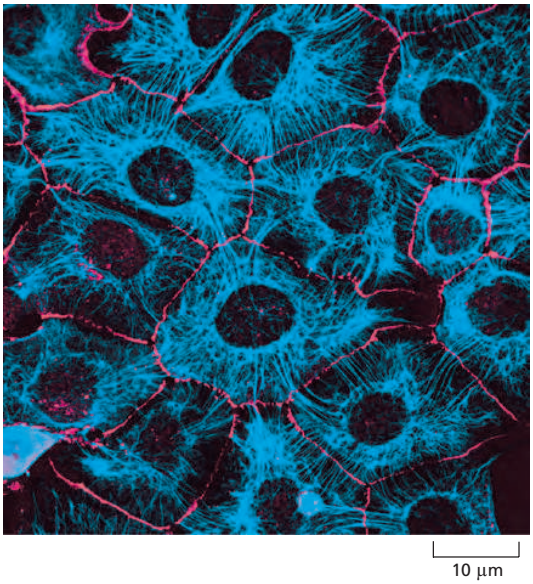


Figure 16–68 Keratin filaments in epithelial cells. Immunofluorescence micrograph of the network of keratin filaments *(blue)* in a sheet of epithelial cells in culture. The filaments in each cell are indirectly connected to those of its neighbors by desmosomes (discussed in Chapter 19). A second protein *(red)* has been stained to reveal the location of the cell boundaries. (Courtesy of Kathleen Green and Evangeline Amargo.)

skin their special toughness. Individuals with mutations in the gene encoding filaggrin are strongly predisposed to dry skin diseases such as eczema.

Mutations in keratin genes cause several human genetic diseases. For example, when defective keratins are expressed in the basal cell layer of the epidermis, they produce a disorder called *epidermolysis bullosa simplex*, in which the skin blisters in response to even very slight mechanical stress, which ruptures the basal cells (**Figure 16–69**). Other types of blistering diseases, including disorders of the mouth, esophageal lining, and the cornea of the eye, are caused by mutations in the different keratins whose expression is specific to those tissues. All of these maladies are typified by cell rupture as a consequence of mechanical trauma and a disorganization or clumping of the keratin filament cytoskeleton. Many of the specific mutations that cause these diseases alter the ends of the central rod domain, demonstrating the importance of this particular part of the protein for correct filament assembly.

Members of another family of intermediate filaments, called **neurofilaments**, are found in high concentrations along the axons of vertebrate neurons (**Figure 16–70**). Three types of neurofilament proteins (NF-L, NF-M, and NF-H) coassemble *in vivo*, forming heteropolymers. The NF-H and NF-M proteins have lengthy C-terminal tail domains that bind to neighboring filaments, generating aligned arrays with a uniform interfilament spacing. During axonal growth, new neurofilament subunits are incorporated all along the axon in a dynamic process that involves the addition of subunits along the filament length as well as the ends. After an axon has grown and connected with its target cell, the diameter of the axon may increase as much as fivefold. The level of neurofilament gene expression seems to directly control axonal diameter, which in turn influences how fast electrical signals travel down the axon. In addition, neurofilaments provide strength and stability to the long cell processes of neurons.

The neurodegenerative disease amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease) is associated with an accumulation and abnormal assembly of neurofilaments in motor neuron cell bodies and in the axon, aberrations that may interfere with normal axonal transport. The degeneration of the axons leads to muscle weakness and atrophy, which is usually fatal. The overexpression of human NF-L or NF-H in mice results in mice that have an ALS-like disease. However, a causative link between neurofilament pathology and ALS has not been firmly established.

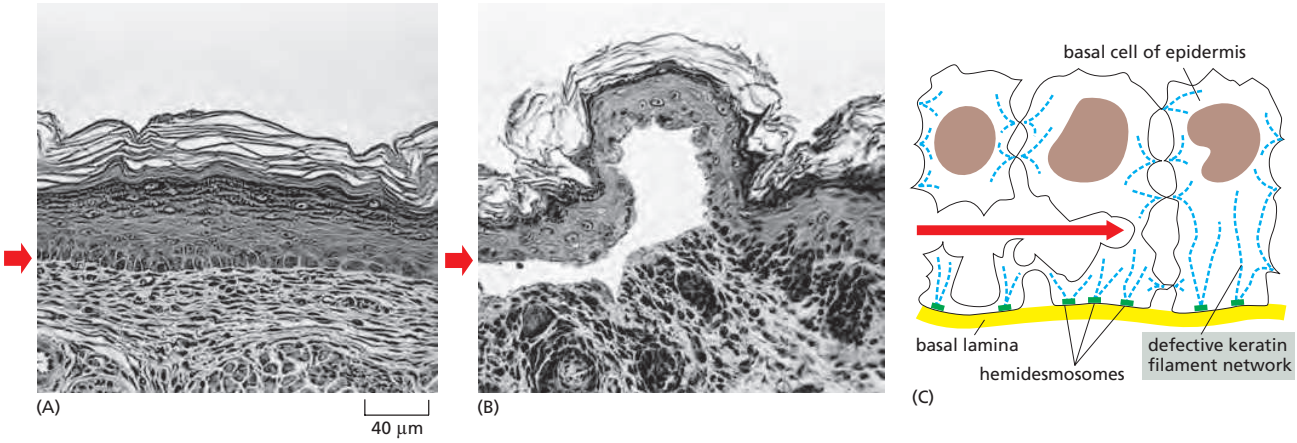


Figure 16–69 Blistering of the skin caused by a mutant keratin gene. A mutant gene encoding a truncated keratin protein (lacking both the N- and C-terminal domains) was expressed in a transgenic mouse. The defective protein assembles with the normal keratins and thereby disrupts the keratin filament network in the basal cells of the skin. Light micrographs of cross sections of (A) normal and (B) mutant skin show that the blistering results from the rupturing of cells in the basal layer of the mutant epidermis (short *red* arrows). (C) A sketch of three cells in the basal layer of the mutant epidermis, as observed by electron microscopy. As indicated by the *red* arrow, the cells rupture between the nucleus and the hemidesmosomes (discussed in Chapter 19), which connect the keratin filaments to the underlying basal lamina. (From P.A. Coulombe et al., *J. Cell Biol*. 115:1661–1674, 1991. With permission from The Rockefeller University Press.)

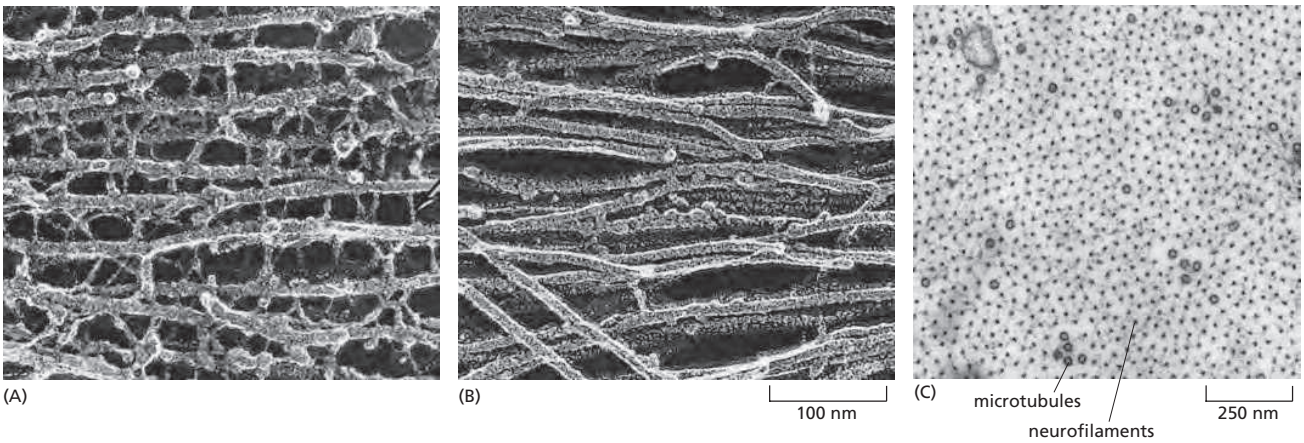


Figure 16–70 Two types of intermediate filaments in cells of the nervous system. (A) Freeze-etch electron microscopic image of neurofilaments in a nerve cell axon, showing the extensive cross-linking through protein cross-bridges—an arrangement believed to give this long cell process great tensile strength. The cross-bridges are formed by the long, nonhelical extensions at the C-terminus of the largest neurofilament protein (NF-H). (B) Freeze-etch image of glial filaments in glial cells, showing that these intermediate filaments are smooth and have few cross-bridges. (C) Conventional transmission electron micrograph of a cross section of an axon showing the regular side-to-side spacing of the neurofilaments, which greatly outnumber the microtubules. (A and B, courtesy of Nobutaka Hirokawa; C, courtesy of John Hopkins.)

The vimentin-like filaments are a third family of intermediate filaments. *Desmin*, a member of this family, is expressed in skeletal, cardiac, and smooth muscle, where it forms a scaffold around the Z disc of the sarcomere (see Figure 16–34). Mice lacking desmin show normal initial muscle development, but adults have various muscle-cell abnormalities, including misaligned muscle fibers. In humans, mutations in desmin are associated with various forms of muscular dystrophy and cardiac myopathy, illustrating the important role of desmin in stabilizing muscle fibers.

Besides their well-established role in maintaining the mechanical stability of the nucleus, it is becoming increasingly evident that one class of lamins, the A-type, together with many proteins of the nuclear envelope, are scaffolds for proteins that control myriad cellular processes including transcription, chromatin organization, and signal transduction. The majority of *laminopathies* are associated with mutant versions of lamin A and include tissue-specific diseases. Skeletal and cardiac abnormalities might be explained by a weakened nuclear envelope leading to cell damage and death, but laminopathies are also thought to arise from pathogenic and tissue-specific alterations in gene expression.

Linker Proteins Connect Cytoskeletal Filaments and Bridge the Nuclear Envelope

The intermediate filament network is linked to the rest of the cytoskeleton by members of a family of proteins called *plakins*. Plakins are large and modular, containing multiple domains that connect cytoskeletal filaments to each other and to junctional complexes. *Plectin* is a particularly interesting example. In addition to bundling intermediate filaments, it links the intermediate filaments to microtubules, actin filament bundles, and filaments of the motor protein myosin II; it also helps attach intermediate filament bundles to adhesive structures at the plasma membrane (**Figure 16–71**).

Plectin and other plakins can interact with protein complexes that connect the cytoskeleton to the nuclear interior. These complexes consist of SUN proteins

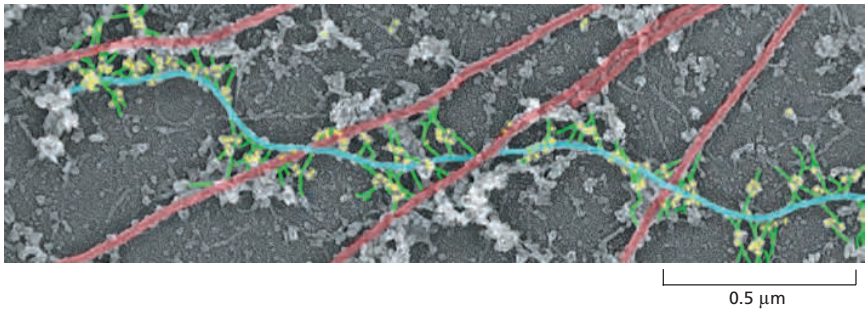


Figure 16–71 Plectin cross-linking of diverse cytoskeletal elements. Plectin *(green)* is seen here making cross-links from intermediate filaments *(blue)* to microtubules *(red)*. In this electron micrograph, the dots *(yellow)* are gold particles linked to anti-plectin antibodies. The entire actin filament network was removed to reveal these proteins. (From T.M. Svitkina et al., *J. Cell Biol.* 135:991–1007, 1996. With permission from The Rockefeller University Press.)

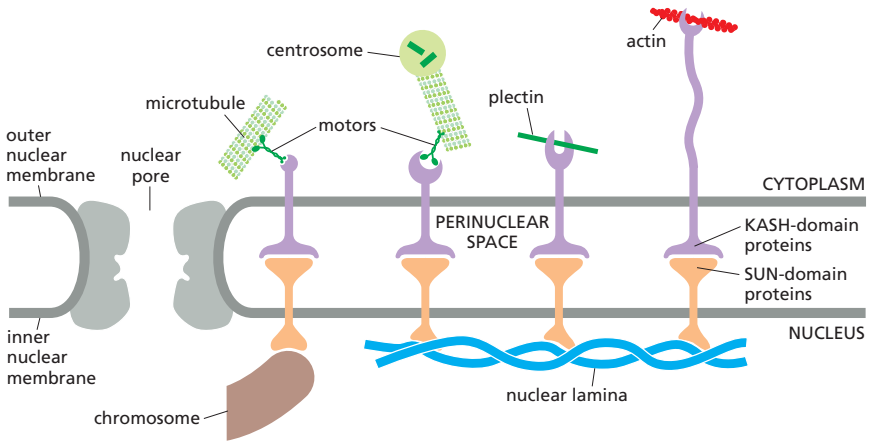


Figure 16–72 SUN–KASH protein complexes connect the nucleus and cytoplasm through the nuclear envelope. The cytoplasmic cytoskeleton is linked across the nuclear envelope to the nuclear lamina or chromosomes through SUN and KASH proteins *(orange* and *purple*, respectively). The SUN and KASH domains of these proteins bind within the lumen of the nuclear envelope. From the inner nuclear envelope, SUN proteins connect to the nuclear lamina or chromosomes. KASH proteins in the outer nuclear envelope connect to the cytoplasmic cytoskeleton by binding microtubule motor proteins, actin filaments, or plectin.

of the inner nuclear membrane and KASH proteins (also called nesprins) of the outer nuclear membrane (**Figure 16–72**). SUN and KASH proteins bind to each other within the lumen of the nuclear envelope, forming a bridge that connects the nuclear and cytoplasmic cytoskeletons. Inside the nucleus, the SUN proteins bind to the nuclear lamina or chromosomes, whereas in the cytoplasm, KASH proteins can bind directly to actin filaments and indirectly to microtubules and intermediate filaments through association with motor proteins and plakins, respectively. This linkage serves to mechanically couple the nucleus to the cytoskeleton and is involved in many cellular functions, including chromosome movements inside the nucleus during meiosis, nuclear and centrosome positioning, nuclear migration, and global cytoskeletal organization.

Mutations in the gene for plectin cause a devastating human disease that combines epidermolysis bullosa (caused by disruption of skin keratin filaments), muscular dystrophy (caused by disruption of desmin filaments), and neurodegeneration (caused by disruption of neurofilaments). Mice lacking a functional plectin gene die within a few days of birth, with blistered skin and abnormal skeletal and heart muscles. Thus, although plectin may not be necessary for the initial formation and assembly of intermediate filaments, its cross-linking action is required to provide cells with the strength they need to withstand the mechanical stresses inherent to vertebrate life.

Septins Form Filaments That Regulate Cell Polarity

GTP-binding proteins called *septins* serve as an additional filament system in all eukaryotes except terrestrial plants. Septins assemble into nonpolar filaments that form rings and cagelike structures, which act as scaffolds to compartmentalize membranes into distinct domains, or recruit and organize the actin and microtubule cytoskeletons. First identified in budding yeast, septin filaments localize to the neck between a dividing yeast mother cell and its growing bud (**Figure 16–73**A). At this location, septins block the movement of proteins from one side of the bud neck to the other, thereby concentrating cell growth preferentially within the bud. Septins also recruit the actin–myosin machinery that forms the contractile ring required for cytokinesis. In animal cells, septins function in cell division, migration, and vesicle trafficking. In primary cilia, for example, a ring of septin filaments assembles at the base of the cilium and serves as a diffusion barrier at the plasma membrane, restricting the movement of membrane proteins and establishing a specific composition in the ciliary membrane (Figure 16–73B and C). Reduction of septin levels impairs primary cilium formation and signaling.

There are 7 septin genes in yeast and 13 in human, and septin proteins fall into four groups on the basis of sequence relationships. In a test tube, purified septins assemble into symmetrical hetero-hexamers or hetero–octamers that

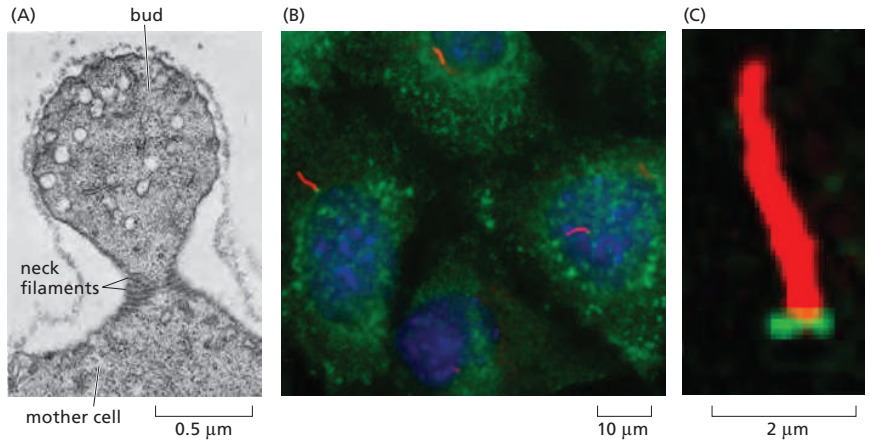


Figure 16–73 Cell compartmentalization by septins. (A) Septins form filaments in the neck region between a mother yeast cell and bud. (B) In this photomicrograph of human cultured cells, the DNA is stained *blue* and septins are labeled in *green*. The microtubules of primary cilia are labeled with an antibody that recognizes a modified (acetylated) form of tubulin *(red)* that is enriched in the axoneme. (C) A magnified image reveals a collar of septin at the base of the cilium. (A, from B. Byers and L. Goetsch, *J. Cell Biol.* 69:717–721, 1976. With permission from Rockefeller University Press. B and C, from Q. Hu et al., *Science* 329:436–439, 2010. With permission from AAAS.)

form nonpolar paired filaments (**Figure 16–74**). GTP binding is required for the folding of septin polypeptides, but the role of GTP hydrolysis in septin function is not understood. Septin structures assemble and disassemble inside cells, but they are not as dynamic as actin filaments and microtubules.

Summary

Whereas tubulin and actin have been highly conserved in evolution, intermediate filament proteins are very diverse. There are many tissue-specific forms of intermediate filaments in the cytoplasm of animal cells, including keratin filaments in epithelial cells, neurofilaments in nerve cells, and desmin filaments in muscle cells. The primary function of these filaments is to provide mechanical strength. Septins comprise an additional system of filaments that organize compartments inside cells.

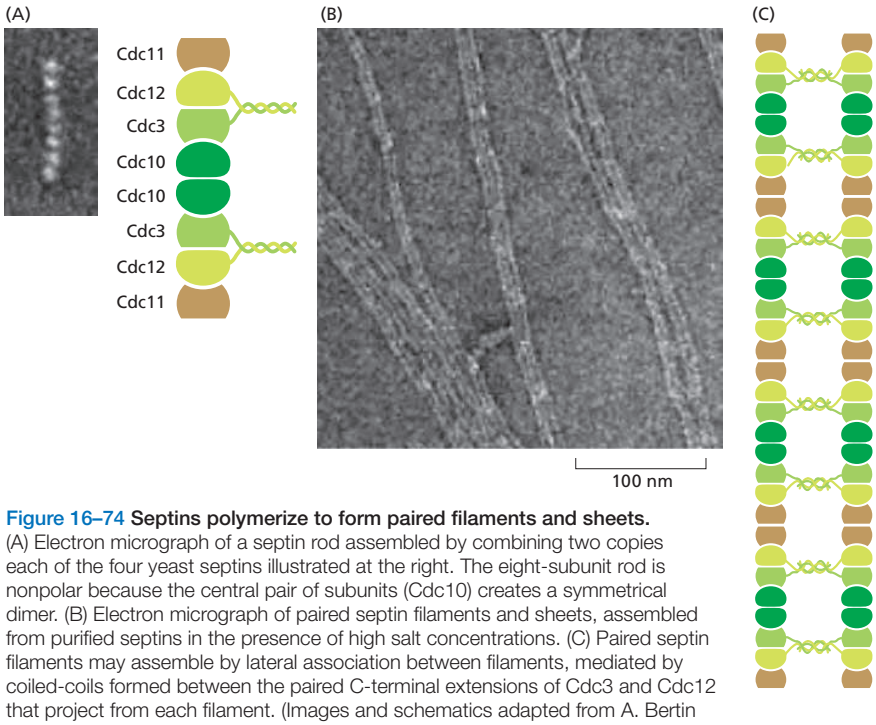


Figure 16–74 Septins polymerize to form paired filaments and sheets. (A) Electron micrograph of a septin rod assembled by combining two copies each of the four yeast septins illustrated at the right. The eight-subunit rod is nonpolar because the central pair of subunits (Cdc10) creates a symmetrical dimer. (B) Electron micrograph of paired septin filaments and sheets, assembled from purified septins in the presence of high salt concentrations. (C) Paired septin filaments may assemble by lateral association between filaments, mediated by coiled-coils formed between the paired C-terminal extensions of Cdc3 and Cdc12 that project from each filament. (Images and schematics adapted from A. Bertin et al., *Proc. Natl Acad. Sci. USA* 105:8274–8279, 2008. With permission from the National Academy of Sciences.)

CELL POLARIZATION AND MIGRATION

A central challenge in cell biology is to understand how multiple individual molecular components collaborate to produce complex cell behaviors. The process of cell migration, which we describe in this final section, relies on the coordinated deployment of the components and processes that we have explored in this chapter: the dynamic assembly and disassembly of cytoskeletal polymers, the regulation and modification of their structure by polymer-associated proteins, and the actions of motor proteins moving along the polymers or exerting tension against them. How does the cell coordinate all these activities to define its polarity and enable it to crawl?

Many Cells Can Crawl Across a Solid Substratum

Many cells move by crawling over surfaces rather than by using cilia or flagella to swim. Predatory amoebae crawl continuously in search of food, and they can easily be observed to attack and devour smaller ciliates and flagellates in a drop of pond water (see Movie 1.4). In animals, almost all cell locomotion occurs by crawling, with the notable exception of swimming sperm. During embryogenesis, the structure of an animal is created by the migrations of individual cells to specific target locations and by the coordinated movements of whole epithelial sheets (discussed in Chapter 21). In vertebrates, *neural crest cells* are remarkable for their long-distance migrations from their site of origin in the neural tube to a variety of sites throughout the embryo (see Movie 21.5). Long-distance crawling is fundamental to the construction of the entire nervous system: it is in this way that the actin-rich growth cones at the advancing tips of developing axons travel to their eventual synaptic targets, guided by combinations of soluble signals and signals bound to cell surfaces and extracellular matrix along the way.

The adult animal also seethes with crawling cells. Macrophages and neutrophils crawl to sites of infection and engulf foreign invaders as a critical part of the innate immune response. Osteoclasts tunnel into bone, forming channels that are filled in by the osteoblasts that follow after them, in a continuous process of bone remodeling and renewal. Similarly, fibroblasts migrate through connective tissues, remodeling them where necessary and helping to rebuild damaged structures at sites of injury. In an ordered procession, the cells in the epithelial lining of the intestine travel up the sides of the intestinal villi, replacing absorptive cells lost at the tip of the villus. Unfortunately, cell crawling also has a role in many cancers, when cells in a primary tumor invade neighboring tissues and crawl into blood vessels or lymph vessels and then emerge at other sites in the body to form metastases.

Cell migration is a complex process that depends on the actin-rich cortex beneath the plasma membrane. Three distinct activities are involved: *protrusion*, in which the plasma membrane is pushed out at the front of the cell; *attachment*, in which the actin cytoskeleton connects across the plasma membrane to the substratum; and *traction*, in which the bulk of the trailing cytoplasm is drawn forward (**Figure 16–75**). In some crawling cells, such as keratocytes from the fish epidermis, these activities occur simultaneously, and the cells seem to glide forward smoothly without changing shape. In other cells, such as fibroblasts, these activities are more independent, and the locomotion is jerky and irregular.

Actin Polymerization Drives Plasma Membrane Protrusion

The first step in locomotion, protrusion of a leading edge, frequently relies on forces generated by actin polymerization pushing the plasma membrane outward. Different cell types generate different types of protrusive structures, including filopodia (also known as microspikes) and lamellipodia. These are filled with dense cores of filamentous actin, which excludes membrane-enclosed organelles. The structures differ primarily in the way in which the actin is organized by actin-cross-linking proteins (see Figure 16–22).

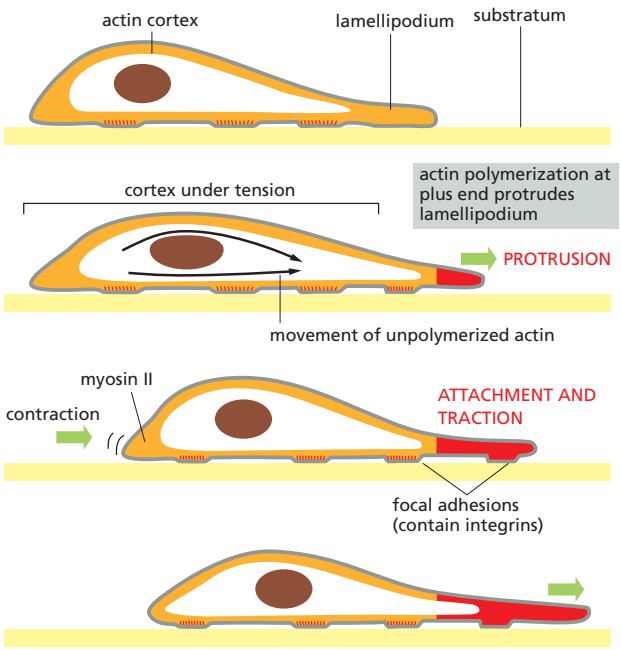


Figure 16–75 A model of how forces generated in the actin-rich cortex move a cell forward. The actin-polymerization-dependent protrusion and firm attachment of a lamellipodium at the leading edge of the cell move the edge forward (*green* arrows at front) and stretch the actin cortex. Contraction at the rear of the cell propels the body of the cell forward (*green* arrow at back) to relax some of the tension (traction). New focal contacts are made at the front, and old ones are disassembled at the back as the cell crawls forward. The same cycle can be repeated, moving the cell forward in a stepwise fashion. Alternatively, all steps can be tightly coordinated, moving the cell forward smoothly. The newly polymerized cortical actin is shown in *red*.

Filopodia, formed by migrating growth cones of neurons and some types of fibroblasts, are essentially one-dimensional. They contain a core of long, bundled actin filaments, which are reminiscent of those in microvilli but longer and thinner, as well as more dynamic. **Lamellipodia**, formed by epithelial cells and fibroblasts, as well as by some neurons, are two-dimensional, sheetlike structures. They contain a cross-linked mesh of actin filaments, most of which lie in a plane parallel to the solid substratum. **Invadopodia** and related structures known as podosomes represent a third type of actin-rich protrusion. These extend in three dimensions and are important for cells to cross tissue barriers, as when a metastatic cancer cell invades the surrounding tissue. Invadopodia contain many of the same actin-regulatory components as filopodia and lamellipodia, and they also degrade the extracellular matrix, which requires the delivery of vesicles containing matrix-degrading proteases.

A distinct form of membrane protrusion called **blebbing** is often observed *in vivo* or when cells are cultured on a pliable extracellular matrix substratum. Blebs form when the plasma membrane detaches locally from the underlying actin cortex, thereby allowing cytoplasmic flow to push the membrane outward (Figure 16–76). Bleb formation also depends on hydrostatic pressure within the cell, which is generated by the contraction of actin and myosin assemblies. Once blebs have extended, actin filaments reassemble on the bleb membrane to form a new

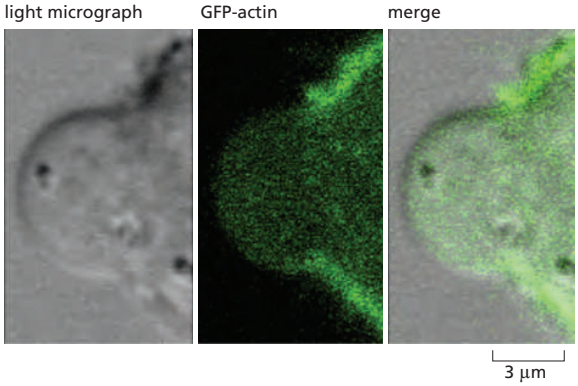


Figure 16–76 Membrane bleb induced by disruption of the actin cortex. On the left is a light micrograph showing a spherical membrane protrusion or bleb induced by laser ablation of a small region of the actin cortex. The cortex is labeled *green* in the *middle* image by expression of GFP-actin. (Courtesy of Ewa Paluch.)

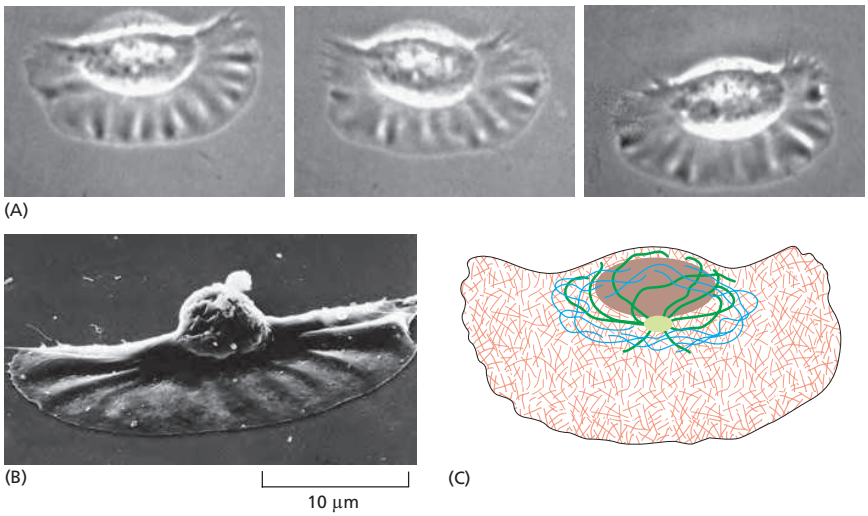


Figure 16–77 Migratory keratocytes from a fish epidermis. (A) Light micrographs of a keratocyte in culture, taken about 15 seconds apart. This cell is moving at about 15 μm/min (**Movie 16.13** and see Movie 1.1). (B) Keratocyte seen by scanning electron microscopy, showing its broad, flat lamellipodium and small cell body, including the nucleus, carried up above the substratum at the rear. (C) Distribution of cytoskeletal filaments in this cell. Actin filaments *(red)* fill the large lamellipodium and are responsible for the cell's rapid movement. Microtubules *(green)* and intermediate filaments *(blue)* are restricted to the regions close to the nucleus. (A and B, courtesy of Juliet Lee.)

actin cortex. Recruitment of myosin II and contraction of actin and myosin can then power retraction of membrane blebs. Alternatively, extension of new blebs from old ones can drive cell migration.

Lamellipodia Contain All of the Machinery Required for Cell Motility

Lamellipodia have been particularly well studied in the epithelial cells of the epidermis of fish and frogs; these epithelial cells are known as *keratocytes* because of their abundant keratin filaments. These cells normally cover the animal by forming an epithelial sheet, and they are specialized to close wounds very rapidly, moving at rates of up to 30 μm/min. When cultured as individual cells, keratocytes assume a distinctive shape with a very large lamellipodium and a small, trailing cell body that is not attached to the substratum (**Figure 16–77**). Fragments of this lamellipodium can be sliced off with a micropipette. Although the fragments generally lack microtubules and membrane-enclosed organelles, they continue to crawl normally, looking like tiny keratocytes.

The dynamic behavior of actin filaments in keratocyte lamellipodia can be studied by labeling a small patch of actin and examining its fate. This reveals that, while the lamellipodia crawl forward, the actin filaments remain stationary with respect to the substratum. The actin filaments in the meshwork are mostly oriented with their plus ends facing forward. The minus ends are frequently attached to the sides of other actin filaments by Arp 2/3 complexes (see Figure 16–16), helping to form the two-dimensional web (**Figure 16–78**). The web as a whole is undergoing treadmilling, assembling at the front and disassembling at the back, reminiscent of the treadmilling that occurs in individual actin filaments discussed previously (see Figure 16–14).

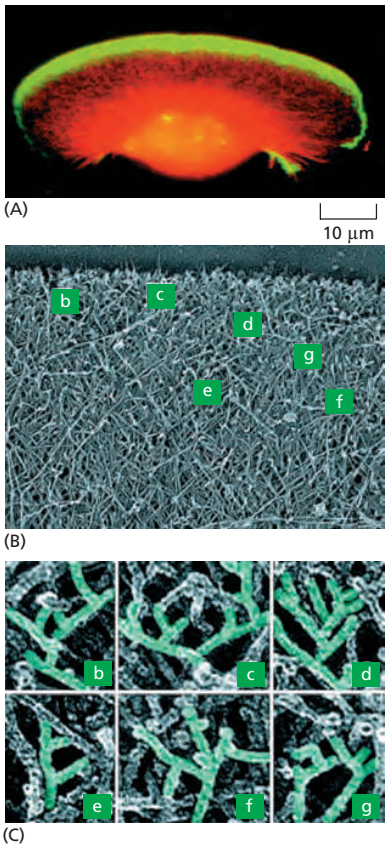


Figure 16–78 Actin filament nucleation and web formation by the Arp 2/3 complex in lamellipodia. (A) A keratocyte with actin filaments labeled in *red* by fluorescent phalloidin and the Arp 2/3 complex labeled in *green* with an antibody against one of its subunits. The Arp 2/3 complex is highly concentrated near the front of the lamellipodium, where actin nucleation is most active. (B) Electron micrograph of a platinum-shadowed replica of the leading edge of a keratocyte, showing the dense actin filament meshwork. The labels denote areas enlarged in (C). (C) Close-up views of the marked regions of the actin web at the leading edge shown in (B). Numerous branched filaments can be seen, with the characteristic 70° angle formed when the Arp 2/3 complex nucleates a new actin filament off the side of a preexisting filament (see Figure 16–16). (From T. Svitkina and G. Borisy, *J. Cell Biol.* 145:1009–1026, 1999. With permission from the authors.)

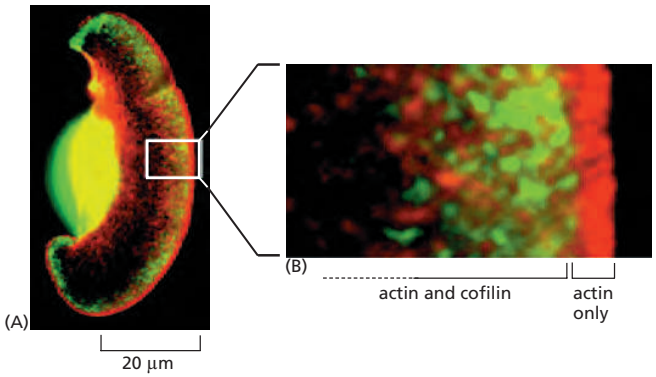


Figure 16–79 Cofilin in lamellipodia. (A) A keratocyte with actin filaments labeled in *red* by fluorescent phalloidin, and cofilin labeled in *green* with a fluorescent antibody. Although the dense actin meshwork reaches all the way through the lamellipodium, cofilin is not found at the very leading edge. (B) Close-up view of the region marked with the *white* rectangle in (A). The actin filaments closest to the leading edge, which are also the ones that have formed most recently and that are most likely to contain ATP-actin (rather than ADP-actin), are generally not associated with cofilin. (From T. Svitkina and G. Borisy, *J. Cell Biol.* 145:1009–1026, 1999. With permission from the authors.)

Maintenance of unidirectional motion by lamellipodia is thought to require the cooperation and mechanical integration of several factors. Filament nucleation is localized at the leading edge, with new actin filament growth occurring primarily in that location to push the plasma membrane forward. Most filament depolymerization occurs at sites located well behind the leading edge. Because *cofilin* (see Figure 16–20) binds cooperatively and preferentially to actin filaments containing ADP-actin (the D form), the new T-form filaments generated at the leading edge should be resistant to depolymerization by cofilin (**Figure 16–79**). As the filaments age and ATP hydrolysis proceeds, cofilin can efficiently disassemble the older filaments. Thus, the delayed ATP hydrolysis by filamentous actin is thought to provide the basis for a mechanism that maintains an efficient, unidirectional treadmilling process in the lamellipodium (**Figure 16–80**); it also explains the intracellular movement of bacterial pathogens such as *Listeria* (see Figure 16–25).

Myosin Contraction and Cell Adhesion Allow Cells to Pull Themselves Forward

Forces generated by actin filament polymerization at the front of a migrating cell are transmitted to the underlying substratum to drive cell motion. For the leading

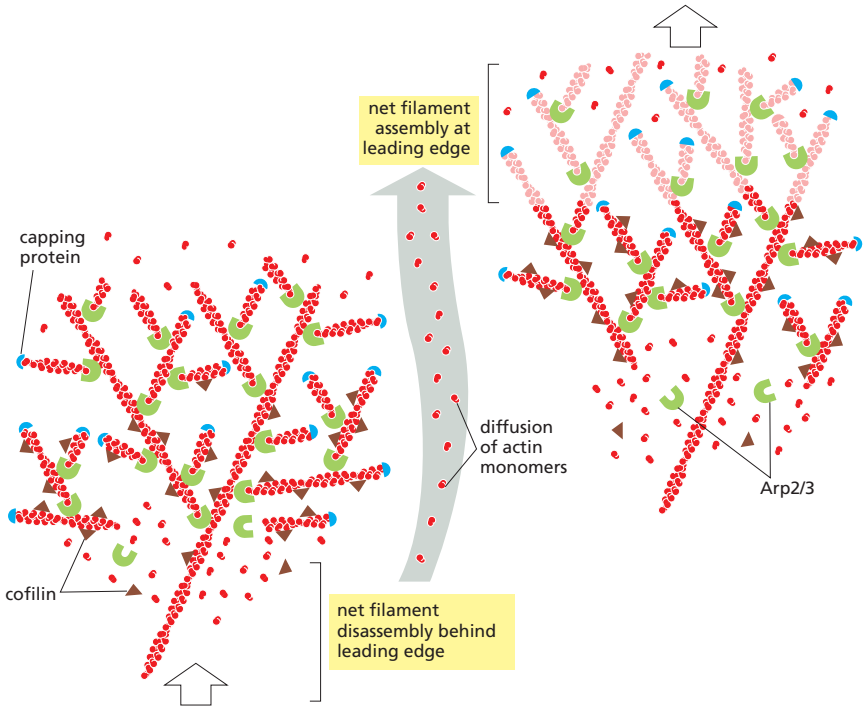


Figure 16–80 A model for protrusion of the actin meshwork at the leading edge. Two time points during advance of the lamellipodium are illustrated, with newly assembled structures at the later time point shown in a lighter color. Nucleation is mediated by the Arp 2/3 complex at the front. Newly nucleated actin filaments are attached to the sides of preexisting filaments, primarily at a 70° angle. Filaments elongate, pushing the plasma membrane forward because of some sort of anchorage of the array behind. At a steady rate, actin filament plus ends become capped. After newly polymerized actin subunits hydrolyze their bound ATP in the filament lattice, the filaments become susceptible to depolymerization by cofilin. This cycle causes a spatial separation between net filament assembly at the front and net filament disassembly at the rear, so that the actin filament network as a whole can move forward, even though the individual filaments within it remain stationary with respect to the substratum. Not all of the actin disassembles, however, and actin at the rear of the lamellipodium contributes to subsequent steps of migration together with myosin.

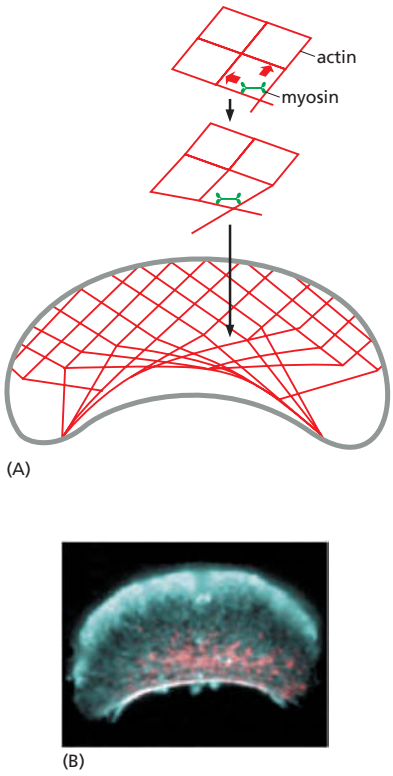


Figure 16–81 Contribution of myosin II to polarized cell motility. (A) Myosin II bipolar filaments bind to actin filaments in the lamellipodial meshwork and cause network contraction. The myosin-driven reorientation of the actin filaments forms an actin bundle that recruits more myosin II and helps generate the contractile forces required for retraction of the trailing edge of the moving cell. (B) A fragment of the large lamellipodium of a keratocyte can be separated from the main cell body either by surgery with a micropipette or by treating the cell with certain drugs. Many of these fragments continue to move rapidly, with the same overall cytoskeletal organization as the intact keratocytes. Actin *(blue)* forms a protrusive meshwork at the front of the fragment. Myosin II *(pink)* is gathered into a band at the rear. (From A. Verkhovsky et al., *Curr. Biol.* 9:11–20, 1999. With permission from Elsevier.)

edge of a migrating cell to advance, protrusion of the membrane must be followed by adhesion to the substratum at the front. Conversely, in order for the cell body to follow, contraction must be coupled with de-adhesion at the rear of the cell. The processes contributing to migration are therefore tightly regulated in space and time, with actin polymerization, dynamic adhesions, and myosin contraction being employed to coordinate movement. Myosin II operates in at least two ways to assist cell migration. The first is by helping to connect the actin cytoskeleton to the substratum through integrin-mediated adhesions. Forces generated by both actin polymerization and myosin activity create tension at attachment sites, promoting their maturation into *focal adhesions*, which are dynamic assemblies of structural and signaling proteins that link the migrating cell to the extracellular matrix (see Figure 19–59). A second mechanism involves bipolar myosin II filaments, which associate with the actin filaments at the rear of the lamellipodium and pull them into a new orientation—from nearly perpendicular to the leading edge to almost parallel to the leading edge. This sarcomere-like contraction prevents protrusion, and it pinches in the sides of the locomoting lamellipodium, helping to gather in the sides of the cell as it moves forward (**Figure 16–81**).

Actin-mediated protrusions can only push the leading edge of the cell forward if there are strong interactions between the actin network and the focal adhesions that link the cell to the substrate. When these interactions are disengaged, polymerization pressure at the leading edge and myosin-dependent contraction cause the actin network to slip back, resulting in a phenomenon known as retrograde flow (**Figure 16–82**).

The traction forces generated by locomoting cells exert a significant pull on the substratum. By growing cells on a surface coated with tiny flexible posts, the force exerted on the substratum can be calculated by measuring the deflection of each post from its vertical position (**Figure 16–83**). In a living animal, most crawling cells move across a semiflexible substratum made of extracellular matrix, which can be deformed and rearranged by these cell forces. Conversely, mechanical tension or stretching applied externally to a cell will cause it to assemble stress fibers and focal adhesions, and become more contractile. Although poorly understood, this two-way mechanical interaction between cells and their physical environment is thought to help vertebrate tissues organize themselves.

Cell Polarization Is Controlled by Members of the Rho Protein Family

Cell migration requires long-distance communication and coordination between one end of a cell and the other. During directed migration, it is important that the front end of the cell remain structurally and functionally distinct from the back end. In addition to driving local mechanical processes such as protrusion at the front and retraction at the rear, the cytoskeleton is responsible for coordinating cell shape, organization, and mechanical properties from one end of the cell to the other, a distance that is typically tens of micrometers for animal cells.

In many cases, including but not limited to cell migration, large-scale cytoskeletal coordination takes the form of the establishment of cell polarity, where a cell builds different structures with distinct molecular components at the front

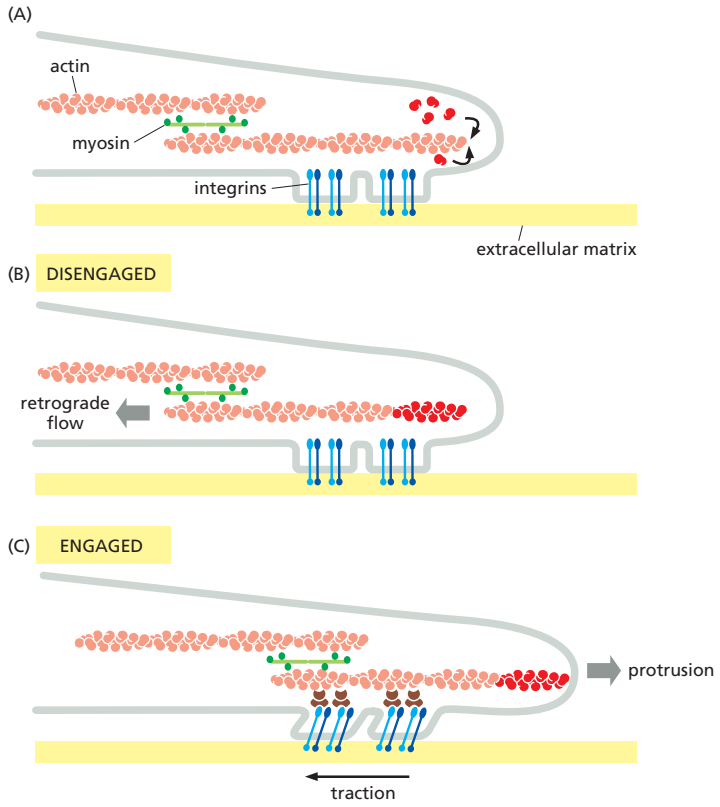


Figure 16–82 Control of cell–substratum adhesion at the leading edge of a migrating cell. (A) Actin monomers assemble on the barbed end of actin filaments at the leading edge. Transmembrane integrin proteins *(blue)* help form focal adhesions that link the cell membrane to the substrate. (B) If there is no interaction between the actin filaments and focal adhesions, the actin filament is driven rearward by newly assembled actin. Myosin motors *(green)* also contribute to filament movement. (C) Interactions between actin-binding adaptor proteins *(brown)* and integrins link the actin cytoskeleton to the substratum. Myosin-mediated contractile forces are then transmitted through the focal adhesion to generate traction on the extracellular matrix, and new actin polymerization drives the leading edge forward in a protrusion.

versus the back, or at the top versus the bottom. Cell locomotion requires an initial polarization of the cell to set it off in a particular direction. Carefully controlled cell-polarization processes are also required for oriented cell divisions in tissues and for formation of a coherent, organized multicellular structure. Genetic studies in yeast, flies, and worms have provided most of our current understanding of the molecular basis of cell polarity. The mechanisms that generate cell polarity in vertebrates are only beginning to be explored. In all known cases, however, the cytoskeleton has a central role, and many of the molecular components have been evolutionarily conserved.

The establishment of many kinds of cell polarity depends on the local regulation of the actin cytoskeleton by external signals. Many of these signals seem to converge inside the cell on a group of closely related monomeric GTPases that are members of the **Rho protein family**—*Cdc42*, *Rac*, and *Rho*. Like other monomeric GTPases, the Rho proteins act as molecular switches that cycle between an active GTP-bound state and an inactive GDP-bound state (see Figure 3–66). Activation of Cdc42 on the inner surface of the plasma membrane triggers actin polymerization and bundling to form filopodia. Activation of Rac promotes actin polymerization at the cell periphery, leading to the formation of sheetlike lamellipodial extensions. Activation of Rho promotes both the bundling of actin filaments with myosin II filaments into stress fibers and the clustering of integrins

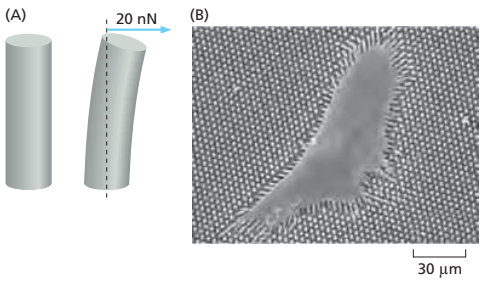


Figure 16–83 Traction forces exerted by a motile cell. (A) Tiny flexible pillars attached to the substratum bend in response to traction forces. (B) Scanning electron micrograph of a cell on a substratum coated with pillars that are 6.1 μm in height. Pillar deflections are used to calculate force vectors corresponding to inward pulling forces on the underlying substratum. (Adapted from J. Fu et al., *Nat. Methods* 7:733–736, 2010. With permission from Macmillan Publishers.)

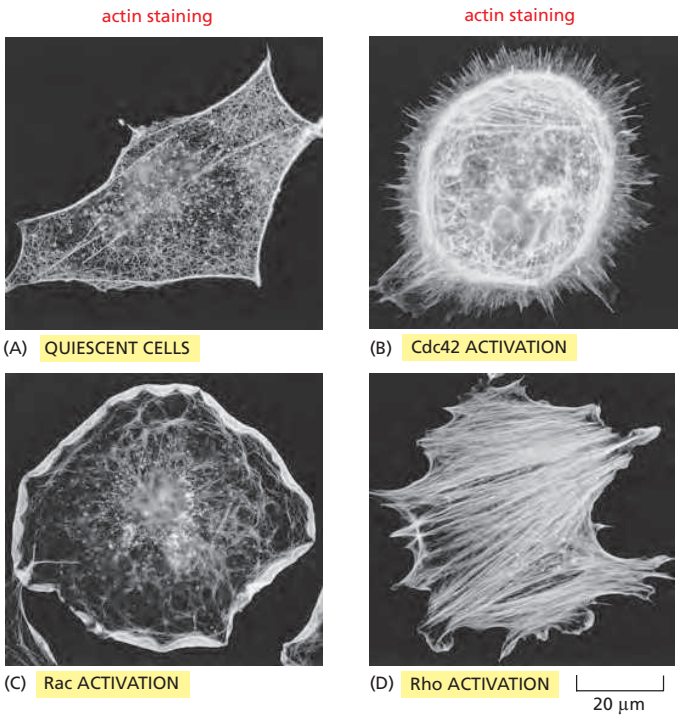


Figure 16–84 The dramatic effects of Cdc42, Rac, and Rho on actin organization in fibroblasts. In each case, the actin filaments have been labeled with fluorescent phalloidin. (A) Serum-starved fibroblasts have actin filaments primarily in the cortex, and relatively few stress fibers. (B) Microinjection of a constitutively activated form of Cdc42 causes the protrusion of many long filopodia at the cell periphery. (C) Microinjection of a constitutively activated form of Rac, a closely related monomeric GTPase, causes the formation of an enormous lamellipodium that extends from the entire circumference of the cell. (D) Microinjection of a constitutively activated form of Rho causes the rapid assembly of many prominent stress fibers. (From A. Hall, *Science* 279:509–514, 1998. With permission from AAAS.)

and associated proteins to form focal adhesions (**Figure 16–84**). These dramatic and complex structural changes occur because each of these three molecular switches has numerous downstream target proteins that affect actin organization and dynamics.

Some key targets of activated Cdc42 are members of the **WASp protein** family. Human patients deficient in WASp suffer from Wiskott-Aldrich Syndrome, a severe form of immunodeficiency in which immune system cells have abnormal actin-based motility and platelets do not form normally. Although WASp itself is expressed only in blood cells and immune system cells, other more ubiquitous versions enable activated Cdc42 to enhance actin polymerization in many cell types. WASp proteins can exist in an inactive folded conformation and an activated open conformation. Association with Cdc42-GTP stabilizes the open form of WASp, enabling it to bind to the Arp 2/3 complex and strongly enhance its actin-nucleating activity (see Figure 16–16). In this way, activation of Cdc42 increases actin nucleation.

Rac-GTP also activates WASp family members. Additionally, it activates the cross-linking activity of the gel-forming protein filamin and inhibits the contractile activity of the motor protein myosin II. It thereby stabilizes lamellipodia and inhibits the formation of contractile stress fibers (**Figure 16–85**A).

Rho-GTP has a very different set of targets. Instead of activating the Arp 2/3 complex to build actin networks, Rho-GTP turns on formin proteins to construct parallel actin bundles. At the same time, Rho-GTP activates a protein kinase that indirectly inhibits the activity of cofilin, leading to actin filament stabilization. The same protein kinase inhibits a phosphatase acting on myosin light chains (see Figure 16–39). The consequent increase in the net amount of myosin light chain phosphorylation increases the amount of contractile myosin motor protein activity in the cell, enhancing the formation of tension-dependent structures such as stress fibers (Figure 16–85B).

In some cell types, Rac-GTP activates Rho, usually at a rate that is slow compared to Rac's activation of the Arp 2/3 complex. This enables cells to use the Rac pathway to build a new actin structure while subsequently activating the Rho pathway to generate a contractility that builds up tension in this structure. This occurs, for example, during the formation and maturation of cell–cell contacts.

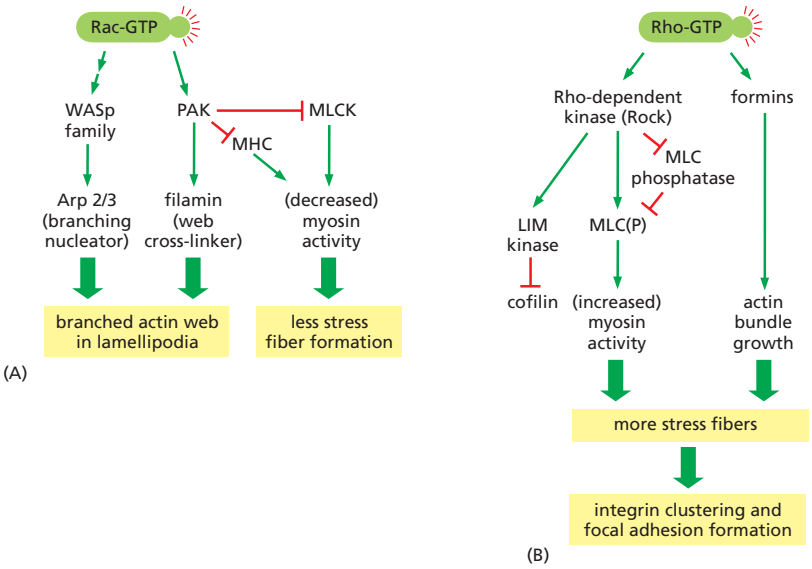


Figure 16–85 The contrasting effects of Rac and Rho activation on actin organization. (A) Activation of the small GTPase Rac leads to alterations in actin accessory proteins that tend to favor the formation of actin networks, as in lamellipodia. Several different pathways contribute independently. Rac-GTP activates members of the WASp protein family, which in turn activate actin nucleation and branched web formation by the Arp 2/3 complex. In a parallel pathway, Rac-GTP activates a protein kinase, PAK, which has several targets including the web-forming cross-linker filamin, which is activated by phosphorylation, and the myosin light chain kinase (MLCK), which is inhibited by phosphorylation. Inhibition of MLCK results in decreased phosphorylation of the myosin regulatory light chain and leads to myosin II filament disassembly and a decrease in contractile activity. In some cells, PAK also directly inhibits myosin II activity by phosphorylation of the myosin heavy chain (MHC). (B) Activation of the related GTPase Rho leads to nucleation of actin filaments by formins and increases contraction by myosin II, promoting the formation of contractile actin bundles such as stress fibers. Activation of myosin II by Rho requires a Rho-dependent protein kinase called Rock. This kinase inhibits the phosphatase that removes the activating phosphate groups from myosin II light chains (MLC); it may also directly phosphorylate the myosin light chains in some cell types. Rock also activates other protein kinases, such as LIM kinase, which in turn contributes to the formation of stable contractile actin filament bundles by inhibiting the actin depolymerizing factor cofilin. A similar signaling pathway is important for forming the contractile ring necessary for cytokinesis (see Figure 17–44).

As we will explore in more detail below, the communication between the Rac and Rho pathways also facilitates maintenance of the large-scale differences between the cell front and the cell rear during migration.

Extracellular Signals Can Activate the Three Rho Protein Family Members

The activation of the monomeric GTPases Rho, Rac, and Cdc42 occurs through an exchange of GTP for a tightly bound GDP molecule, catalyzed by guanine nucleotide exchange factors (GEFs). Of the many GEFs that have been identified in the human genome, some are specific for an individual Rho family GTPase, whereas others seem to act on multiple family members. Different GEFs are restricted to specific tissues and even specific subcellular locations, and they are sensitive to distinct kinds of regulatory inputs. GEFs can be activated by extracellular cues through cell-surface receptors, or in response to intracellular signals. GEFs may also act as scaffolds that direct GTPases to downstream effectors. Interestingly, several of the Rho family GEFs associate with the growing ends of microtubules by binding to one of the +TIPs. This provides a connection between the dynamics of the microtubule cytoskeleton and the large-scale organization of the actin cytoskeleton; such a connection is important for the overall integration of cell shape and movement.

External Signals Can Dictate the Direction of Cell Migration

Chemotaxis is the movement of a cell toward or away from a source of some diffusible chemical. These external signals act through Rho family proteins to set up large-scale cell polarity by influencing the organization of the cell motility apparatus. One well-studied example is the chemotactic movement of a class of white blood cells, called *neutrophils*, toward a source of bacterial infection. Receptor proteins on the surface of neutrophils enable them to detect very low concentrations of *N*-formylated peptides that are derived from bacterial proteins (only prokaryotes begin protein synthesis with *N*-formylmethionine). Using these receptors, neutrophils are guided to bacterial targets by their ability to detect a difference of only 1% in the concentration of these diffusible peptides on one side of the cell versus the other (**Figure 16–86**A).

In this case, and in the chemotaxis of *Dictyostelium* amoebae toward a source of cyclic AMP, binding of the chemoattractant to its G-protein-coupled receptor activates phosphoinositide 3-kinases (PI3Ks) (see Figure 15–52), which generate a signaling molecule [$PI(3,4,5)P_3$] that in turn activates the Rac GTPase. Rac

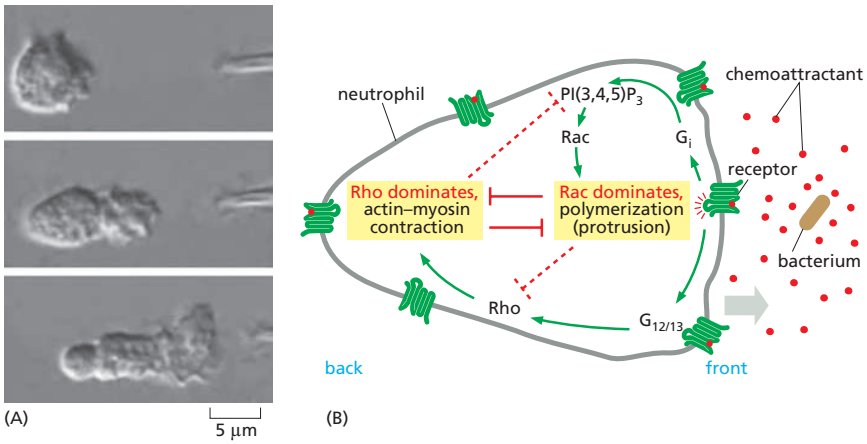


Figure 16–86 Neutrophil polarization and chemotaxis. (A) The pipette tip at the right is leaking a small amount of the bacterial peptide formyl-Met-Leu-Phe, which is recognized by the human neutrophil as the product of a foreign invader. The neutrophil quickly extends a new lamellipodium toward the source of the chemoattractant peptide *(top).* It then extends this lamellipodium and polarizes its cytoskeleton so that contractile myosin II is located primarily at the rear, opposite the position of the lamellipodium *(middle).* Finally, the cell crawls toward the source of the peptide *(bottom).* If a real bacterium were the source of the peptide, rather than an investigator's pipette, the neutrophil would engulf the bacterium and destroy it (see also Figure 16–3 and Movie 16.14). (B) Binding of bacterial molecules to G-protein-coupled receptors on the neutrophil stimulates directed motility. These receptors are found all over the surface of the cell, but are more likely to be bound to the bacterial ligand at the front. Two distinct signaling pathways contribute to the cell's polarization. At the front of the cell, stimulation of the Rac pathway leads, via the trimeric G protein G_i, to growth of protrusive actin networks. Second messengers within this pathway are short-lived, so protrusion is limited to the region of the cell closest to the stimulant. The same receptor also stimulates a second signaling pathway, via the trimeric G proteins G_{12} and G_{13}, that triggers the activation of Rho. The two pathways are mutually antagonistic. Since Rac-based protrusion is active at the front of the cell, Rho is activated only at the rear of the cell, stimulating contraction of the cell rear and assisting directed movement. (A, from O.D. Weiner et al., *Nat. Cell Biol.* 1:75–81, 1999. With permission from Macmillan Publishers Ltd.)

then activates the Arp 2/3 complex leading to lamellipodial protrusion. Through an unknown mechanism, accumulation of the polarized actin web at the leading edge causes further local enhancement of PI3K activity in a positive feedback loop, strengthening the induction of protrusion. The $PI(3,4,5)P_3$ that activates Rac cannot diffuse far from its site of synthesis, since it is rapidly converted back into $PI(4,5)P_2$ by a constitutively active lipid phosphatase. At the same time, binding of the chemoattractant ligand to its receptor activates another signaling pathway that turns on Rho and enhances myosin-based contractility. The two processes directly inhibit each other, such that Rac activation dominates in the front of the cell and Rho activation dominates in the rear (Figure 16–86B). This enables the cell to maintain its functional polarity with protrusion at the leading edge and contraction at the back.

Nondiffusible chemical cues attached to the extracellular matrix or to the surface of cells can also influence the direction of cell migration. When these signals activate receptors, they can cause increased cell adhesion and directed actin polymerization. Most long-distance cell migrations in animals, including neural-crest-cell migration and the travels of neuronal growth cones, depend on a combination of diffusible and nondiffusible signals to steer the locomoting cells or growth cones to their proper destinations.

Communication Among Cytoskeletal Elements Coordinates Whole-Cell Polarization and Locomotion

The interconnected cytoskeleton is crucial for cell migration. Although movement is driven primarily by actin polymerization and myosin contractility, septins and intermediate filaments also participate. For example, vimentin intermediate filament networks associate with integrins at focal adhesions, and vimentin-deficient fibroblasts display impaired mechanical stability, migration, and contractile capacity. Furthermore, disruption of linker proteins that connect different cytoskeletal elements, including several plakins and KASH proteins, leads to defects in cell polarization and migration. Thus, interactions among cytoplasmic filament systems, as well as mechanical linkage to the nucleus, are required for complex, whole-cell behaviors such as migration.

Cells also use microtubules to help organize persistent movement in a specific direction. In many locomoting cells, the position of the centrosome is influenced by the location of protrusive actin polymerization. Activation of receptors on the protruding front edge of a cell might locally activate dynein motor proteins that move the centrosome by pulling on its microtubules. Several effector proteins downstream of Rac and Rho modulate microtubule dynamics directly: for example, a protein kinase activated by Rac can phosphorylate (and thereby inhibit) the tubulin-binding protein stathmin (see Panel 16–4), thereby stabilizing microtubules.

In turn, microtubules influence actin rearrangements and cell adhesion. The centrosome nucleates a large number of dynamic microtubules, and its repositioning means that the plus ends of many of these microtubules extend into the protrusive region of the cell. Direct interactions with microtubules help guide focal adhesion dynamics in migrating cells. Microtubules might also influence actin filament formation by delivering Rac-GEFs that bind to the +TIPs traveling on growing microtubule ends. Microtubules also transport cargoes to and from the focal adhesions, thereby affecting their signaling and disassembly. Thus, microtubules reinforce the polarity information that the actin cytoskeleton receives from the outside world, allowing a sensitive response to weak signals and enabling motility to persist in the same direction for a prolonged period.

Summary

Whole-cell movements and the large-scale shaping and structuring of cells require the coordinated activities of all three basic filament systems along with a large variety of cytoskeletal accessory proteins, including motor proteins. Cell crawling—a widespread behavior important in embryonic development and also in wound healing, tissue maintenance, and immune system function in the adult animal—is a prime example of such complex, coordinated cytoskeletal action. For a cell to crawl, it must generate and maintain an overall structural polarity, which is influenced by external cues. In addition, the cell must coordinate protrusion at the leading edge (by assembly of new actin filaments), adhesion of the newly protruded part of the cell to the substratum, and forces generated by molecular motors to bring the cell body forward.

WHAT WE DON'T KNOW

- How is the cell cortex regulated locally and globally to coordinate its activities at different places on the cell surface? What determines, for example, where filopodia form?
- How are actin-regulatory proteins controlled spatially in the cytoplasm to generate multiple distinct types of actin arrays in the same cell?
- Are there biologically important processes occurring inside a microtubule?
- How can we account for the fact that there are many different kinesins and myosins in the cytoplasm but only one dynein?
- Mutations in the nuclear lamin proteins cause a large number of diseases called laminopathies. What do we not understand about the nuclear lamina that could account for this fact?

PROBLEMS

Which statements are true? Explain why or why not.

16–1 The role of ATP hydrolysis in actin polymerization is similar to the role of GTP hydrolysis in tubulin polymerization: both serve to weaken the bonds in the polymer and thereby promote depolymerization.

16–2 Motor neurons trigger action potentials in muscle cell membranes that open voltage-sensitive Ca^{2+} channels in T tubules, allowing extracellular Ca^{2+} to enter the cytosol, bind to troponin C, and initiate rapid muscle contraction.

16–3 In most animal cells, minus-end directed microtubule motors deliver their cargo to the periphery of the cell, whereas plus-end directed microtubule motors deliver their cargo to the interior of the cell.

Discuss the following problems.

16–4 The concentration of actin in cells is 50–100 times greater than the critical concentration observed for pure actin in a test tube. How is this possible? What prevents the actin subunits in cells from polymerizing into filaments? Why is it advantageous to the cell to maintain such a large pool of actin subunits?

16–5 Detailed measurements of sarcomere length and tension during isometric contraction in striated muscle provided crucial early support for the sliding-filament model of muscle contraction. Based on your understanding of the sliding-filament model and the structure of a sarcomere, propose a molecular explanation for the relationship of tension to sarcomere length in the portions of **Figure Q16–1** marked I, II, III, and IV. (In this muscle, the length of the myosin filament is 1.6 μm, and the lengths of the actin thin filaments that project from the Z discs are 1.0 μm.)

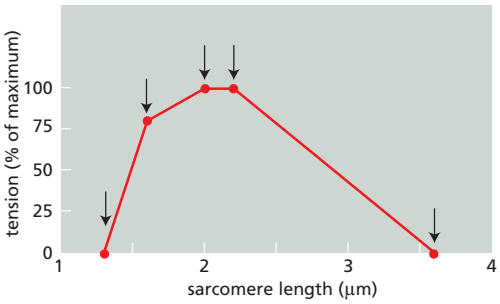


Figure Q16–1 Tension as a function of sarcomere length during isometric contraction (Problem 16–5).

16–6 At 1.4 mg/mL pure tubulin, microtubules grow at a rate of about 2 μm/min. At this growth rate, how many αβ-tubulin dimers (8 nm in length) are added to the ends of a microtubule each second?

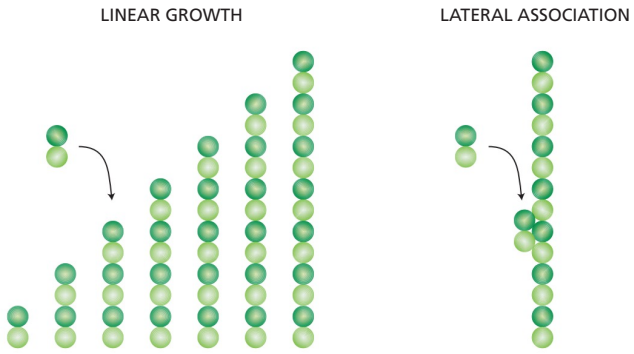


Figure Q16–2 Model for microtubule nucleation by pure αβ-tubulin dimers (Problem 16–7).

16–7 A solution of pure αβ-tubulin dimers is thought to nucleate microtubules by forming a linear protofilament about seven dimers in length. At that point, the probabilities that the next αβ-dimer will bind laterally or to the end of the protofilament are about equal. The critical event for microtubule formation is thought to be the first lateral association (**Figure Q16–2**). How does lateral association promote the subsequent rapid formation of a microtubule?

16–8 How does a centrosome "know" when it has found the center of the cell?

16–9 The movements of single motor-protein molecules can be analyzed directly. Using polarized laser light, it is possible to create interference patterns that exert a centrally directed force, ranging from zero at the center to a few piconewtons at the periphery (about 200 nm from the center). Individual molecules that enter the interference pattern are rapidly pushed to the center, allowing them to be captured and moved at the experimenter's discretion.

Using such "optical tweezers," single kinesin molecules can be positioned on a microtubule that is fixed to a coverslip. Although a single kinesin molecule cannot be seen optically, it can be tagged with a silica bead and tracked indirectly by following the bead (**Figure Q16–3**A). In the absence of ATP, the kinesin molecule remains at the center of the interference pattern, but with ATP it moves toward the plus end of the microtubule. As kinesin moves along the microtubule, it encounters the force of the interference pattern, which simulates the load kinesin carries during its actual function in the cell. Moreover, the pressure against the silica bead counters the effects of Brownian (thermal) motion, so that the position of the bead more accurately reflects the position of the kinesin molecule on the microtubule.

A trace of the movements of a kinesin molecule along a microtubule is shown in Figure Q16–3B.

A. As shown in Figure Q16–3B, all movement of kinesin is in one direction (toward the plus end of the microtubule). What supplies the free energy needed to ensure a unidirectional movement along the microtubule?

B. What is the average rate of movement of kinesin along the microtubule?

C. What is the length of each step that a kinesin takes as it moves along a microtubule?

D. From other studies it is known that kinesin has two globular domains that can each bind to β-tubulin, and that kinesin moves along a single protofilament in a microtubule. In each protofilament, the β-tubulin subunit repeats at 8-nm intervals. Given the step length and the interval between β-tubulin subunits, how do you suppose a kinesin molecule moves along a microtubule?

E. Is there anything in the data in Figure Q16–3B that tells you how many ATP molecules are hydrolyzed per step?

16–10 A mitochondrion 1 μm long can travel the 1 meter length of the axon from the spinal cord to the big toe in a day. The Olympic men's freestyle swimming record for 200 meters is 1.75 minutes. In terms of body lengths per day, who is moving faster: the mitochondrion or the Olympic record holder? (Assume that the swimmer is 2 meters tall.)

16–11 Cofilin preferentially binds to older actin filaments and promotes their disassembly. How does cofilin distinguish old filaments from new ones?

16–12 Why is it that intermediate filaments have identical ends and lack polarity, whereas actin filaments and microtubules have two distinct ends with a defined polarity?

16–13 How is the unidirectional motion of a lamellipodium maintained?

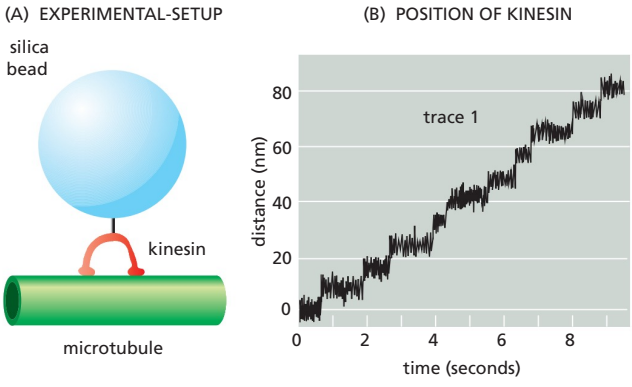


Figure Q16–3 Movement of kinesin along a microtubule (Problem 16–9). (A) Experimental set-up, with kinesin linked to a silica bead, moving along a microtubule. (B) Position of kinesin (as visualized by the position of the silica bead) relative to the center of the interference pattern, as a function of time of movement along the microtubule. The jagged nature of the trace results from Brownian motion of the bead.

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