16 The Cytoskeleton

For cells to function properly, they must organize themselves in space and interact mechanically 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. Eucaryotic cells have developed all these spatial and mechanical functions to a very high degree, and they depend on a remarkable system of filaments called the **cytoskeleton** (Figure 16–1).

The cytoskeleton pulls the chromosomes apart at mitosis and then splits the dividing cell into two. It drives and guides the intracellular traffic of organelles, ferrying materials from one part of the cell to another. It supports the fragile plasma membrane and provides the mechanical linkages that let the cell bear stresses and strains without being ripped apart as the environment shifts and changes. It enables cells such as sperm to swim and others, such as fibroblasts and white blood cells, to crawl across surfaces. It provides the machinery in the muscle cell for contraction and in the nerve cell to extend an axon and dendrites. It guides the growth of the plant cell wall and controls the amazing diversity of eucaryotic cell shapes.

The cytoskeleton's varied functions depend on the behavior of three families of protein molecules, which assemble to form three main types of filaments. Each type of filament has distinct mechanical properties, dynamics, and biological roles, but all three share certain fundamental principles. These principles provide the basis for a general understanding of how the cytoskeleton works and how the different elements cooperate. 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 begin by describing the three main types of filaments, the basic principles underlying their assembly and disassembly, and their individual peculiarities. We then describe how other proteins interact with the three main filament systems, enabling the cell to establish and maintain internal order, to shape and remodel its surface, to move organelles in a directed manner from one place to another, and—when appropriate—to move itself to new locations.

In This Chapter

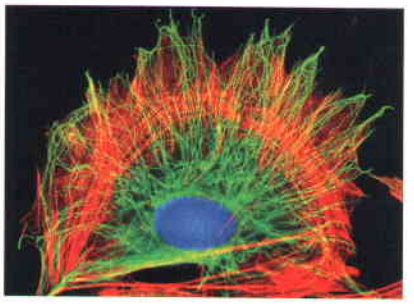


Figure 16–1 The cytoskeleton. A cell in culture has been fixed and labeled to show two of its major cytoskeletal systems, the microtubules (*green*) and the actin filaments (*red*). The DNA in the nucleus is labeled in *blue*. (Courtesy of Albert Tousson.)

THE SELF-ASSEMBLY AND DYNAMIC STRUCTURE OF CYTOSKELETAL FILAMENTS

Most animal cells have three types of cytoskeletal filaments that are responsible for the cells' spatial organization and mechanical properties. *Intermediate filaments* provide mechanical strength. *Microtubules* determine the positions of membrane-enclosed organelles and direct intracellular transport. *Actin filaments* determine the shape of the cell's surface and are necessary for whole-cell locomotion. But these cytoskeletal filaments would be ineffective without the hundreds of accessory proteins that link the filaments to other cell components, as well as to each other. This large set of *accessory proteins* is essential for the

controlled assembly of the cytoskeletal filaments in particular locations, and it includes 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 properties of the proteins that make up the filaments of the cytoskeleton. We focus on their ability to form intrinsically polarized and self-organized structures. We shall see that, because of the remarkable mechanisms that cause cytoskeletal filaments to be dynamic, the cell is able to respond rapidly to any eventuality that it may face.

Cytoskeletal Filaments Are Dynamic and Adaptable

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 rearranges itself with astonishing rapidity to deal with the new situation. 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 the cytoskeletal filaments allows eucaryotic cells to build an enormous range of structures from the three basic filament systems. The micrographs in **Panel 16–1** reveal some of these structures. Microtubules, which are frequently found in a star-like cytoplasmic array emanating from the center of an interphase cell, can quickly rearrange themselves to form a bipolar *mitotic spindle* during cell division. They can also form motile whips called *cilia* and *flagella* 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.

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 pull themselves around. The actin-based *contractile ring* assembles transiently to divide cells in two; 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.

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 spans throughout the cytoplasm is reconfigured into the bipolar *mitotic spindle*, which serves the critical function of accurately segregating the two copies of each replicated chromosome into two separate daughter nuclei. At the same time, the specialized actin structures that enable the fibroblast to crawl across the surface of the dish disassemble so that the cell stops moving, rounds up, 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

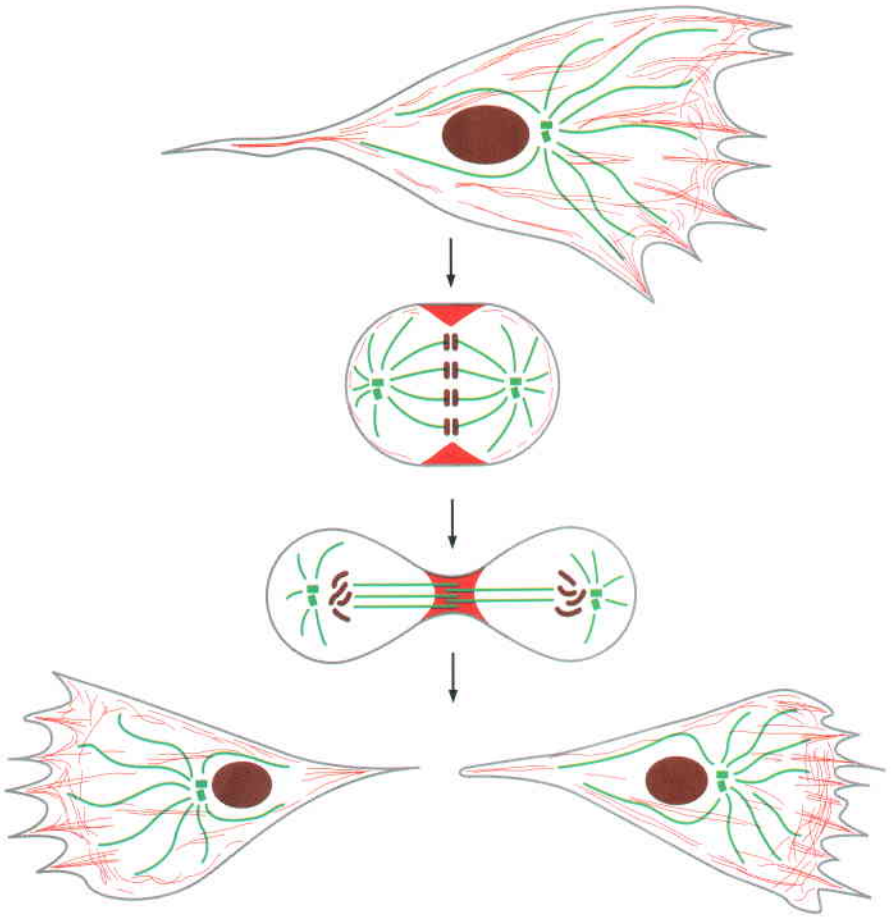


Figure 16–2 Rapid changes in cytoskeletal organization associated with cell division. The crawling fibroblast drawn here has a polarized, dynamic actin cytoskeleton (shown in *red*) that assembles to push its leading edge toward the right. The polarization of the actin cytoskeleton is assisted by the microtubule cytoskeleton (shown in *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 separating 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 chromosomes have separated. 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.

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. In a fibroblast, this sequence of events takes about an hour; in some cases, such as the early nuclear divisions in a *Drosophila* embryo, the actin and microtubule cytoskeletons can completely rearrange themselves within less than five minutes (**Figure 16–3**).

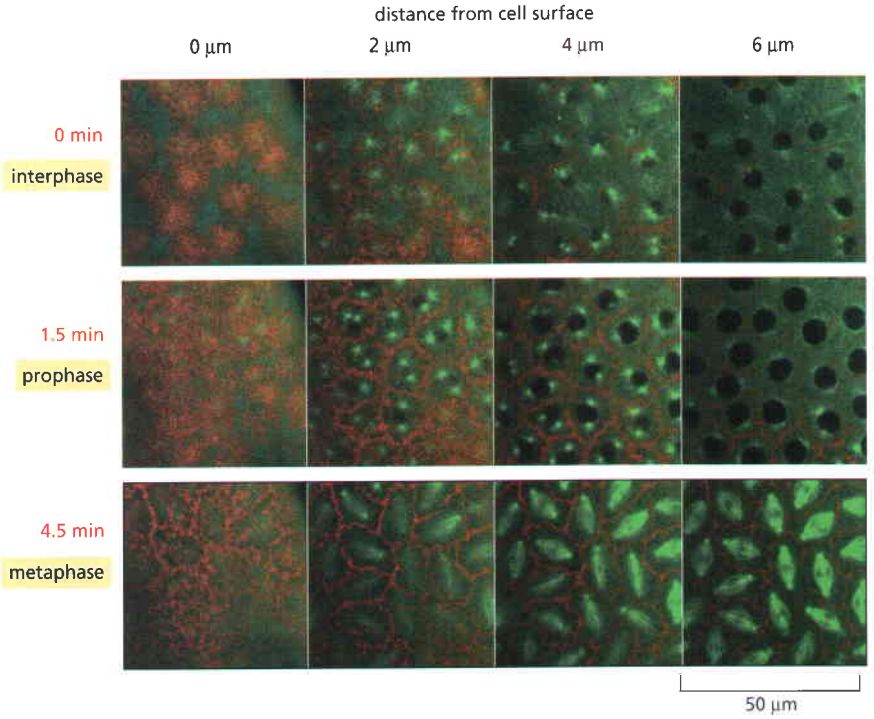
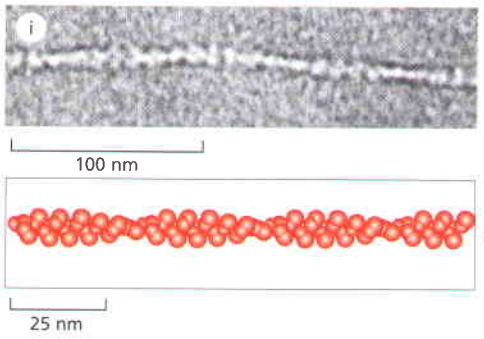
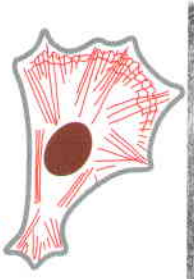
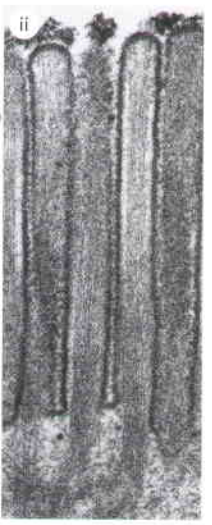


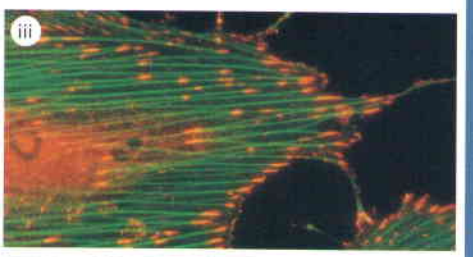
Figure 16–3 Rapid changes in cytoskeletal organization observed during the development of a *Drosophila* early embryo. <TTCT> In this giant multinuclear cell, the early nuclear divisions occur every 10 minutes or so in a common cytoplasm. The rapid rearrangements of the actin filaments *(red)* and microtubules *(green)*, seen here in a living embryo, are required to separate the chromosomes at mitosis, while keeping each nucleus from colliding with its neighbors. (Courtesy of William Sullivan.)

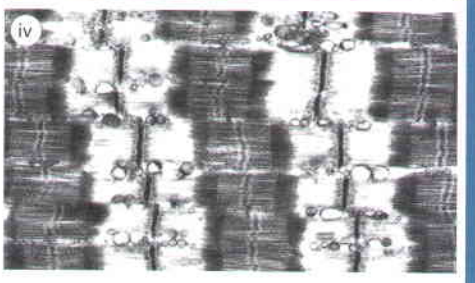
ACTIN FILAMENTS







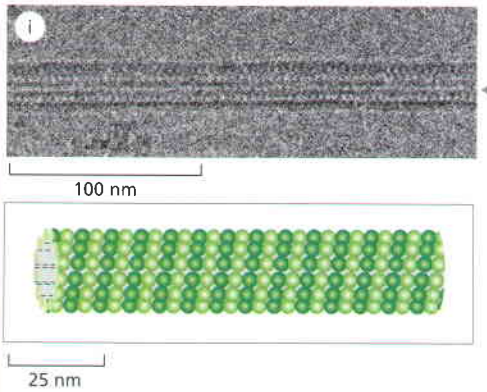


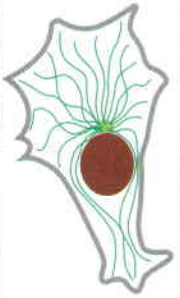


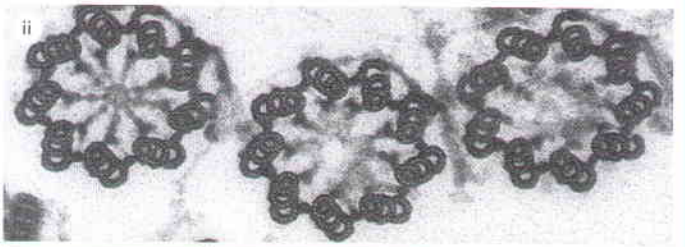
Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

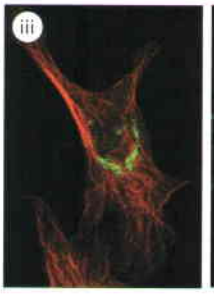
Micrographs courtesy of Roger Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); Keith Burridge (iii).

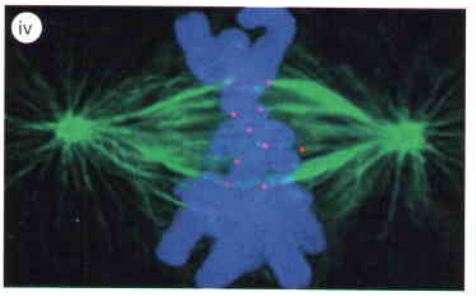
MICROTUBULES







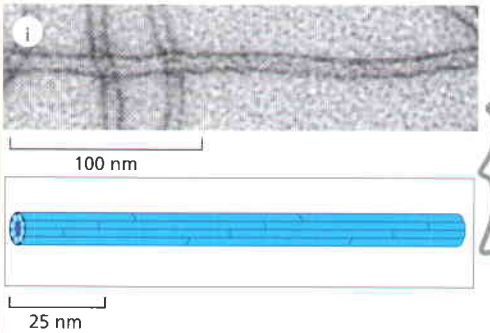


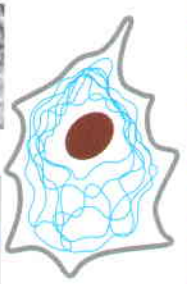


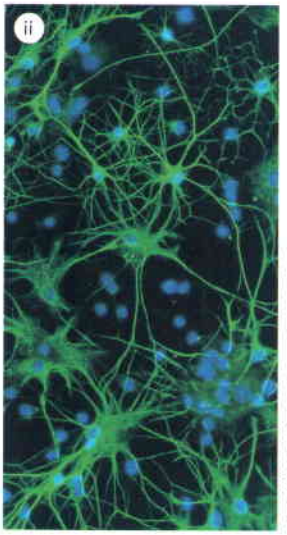
Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*.

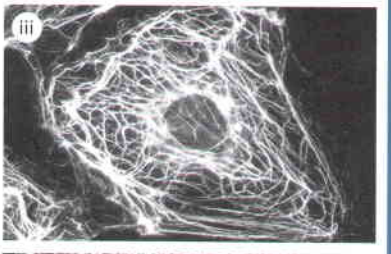
Micrographs courtesy of Richard Wade (i); D.T. Woodrow and R.W. Linck (ii); David Shima (iii); A. Desai (iv).

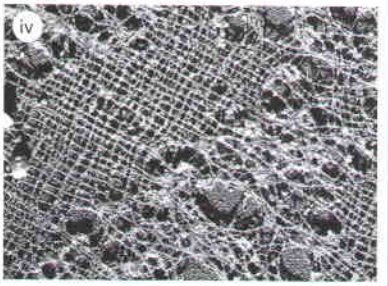
INTERMEDIATE FILAMENTS











Intermediate filaments are ropelike fibers with a diameter of around 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.

Micrographs courtesy of Roy Quinlan (i); Nancy L. Kedersha (ii); Mary Osborn (iii); Ueli Aebi (iv).

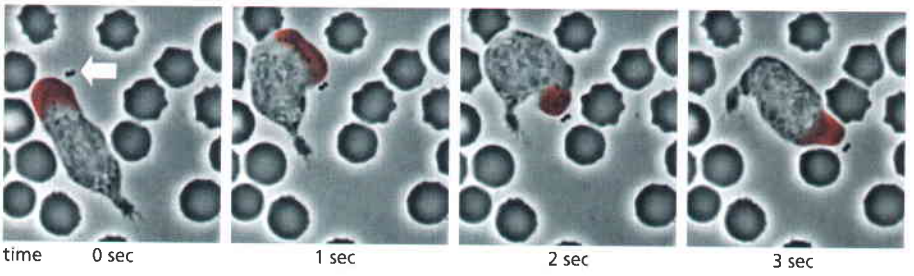


Figure 16–4 A neutrophil in pursuit of bacteria. <TGTA> In this preparation of human blood, a clump of bacteria (*white arrow*) is about to be captured by a neutrophil. As the bacteria move, the neutrophil quickly reassembles the dense actin network at the leading edge (*red*) to push toward the location of the bacteria. Rapid disassembly and reassembly of the actin cytoskeleton in this cell enables it to change its orientation and direction of movement within a few seconds. (From a video recorded by David Rogers.)

Many cells require rapid cytoskeletal rearrangements for their normal functioning during interphase as well. For example, the *neutrophil*, a type of white blood cell, chases and engulfs bacterial and fungal cells that accidentally gain access to the normally sterile parts of the body, as through cuts in the skin. Like most crawling cells, neutrophils advance by extending a protrusive structure at the leading edge 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 16–4**). Both of these kinds of rapid cytoskeletal rearrangements will be discussed in more detail in the final section of this chapter.

The Cytoskeleton Can Also Form Stable Structures

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 tissues 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 remain strikingly dynamic and are continuously remodeled and replaced on average every 48 hours, even within these 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 encoded by the organization of the cytoskeleton must also often be maintained over the lifetime of the cell. Polarized epithelial cells such as those found in the lining of the intestine, for example, use organized arrays of microtubules, actin filaments, and intermediate filaments to maintain the critical functional differences between the *apical surface* that absorbs nutrients from the lumen of the intestine where food passes by to the *basolateral surface* where the cells transfer nutrients through the plasma membrane to the bloodstream. 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 16–5**).

Even small, morphologically simple cells such as the budding yeast *Saccharomyces cerevisiae* need stable large-scale polarity. The most notable feature of the structure of these cells is the marked asymmetry, evident in the way they divide by budding to create a small daughter cell and a large mother cell. This asymmetry derives from the polar orientation of the cell's actin cytoskeleton. There are two types of actin filament assemblies in these cells: actin cables (long bundles of actin filaments) and actin patches (small assemblies of filaments associated with the cell cortex, marking sites of actin-driven endocytosis). Proliferating budding yeast cells must be highly polarized to allow the cell to grow a bud from a single site on the cell surface, as opposed to simply growing uniformly larger. In this process, the actin patches become highly concentrated at the growing tip of the bud, with the actin cables aligned and pointing toward

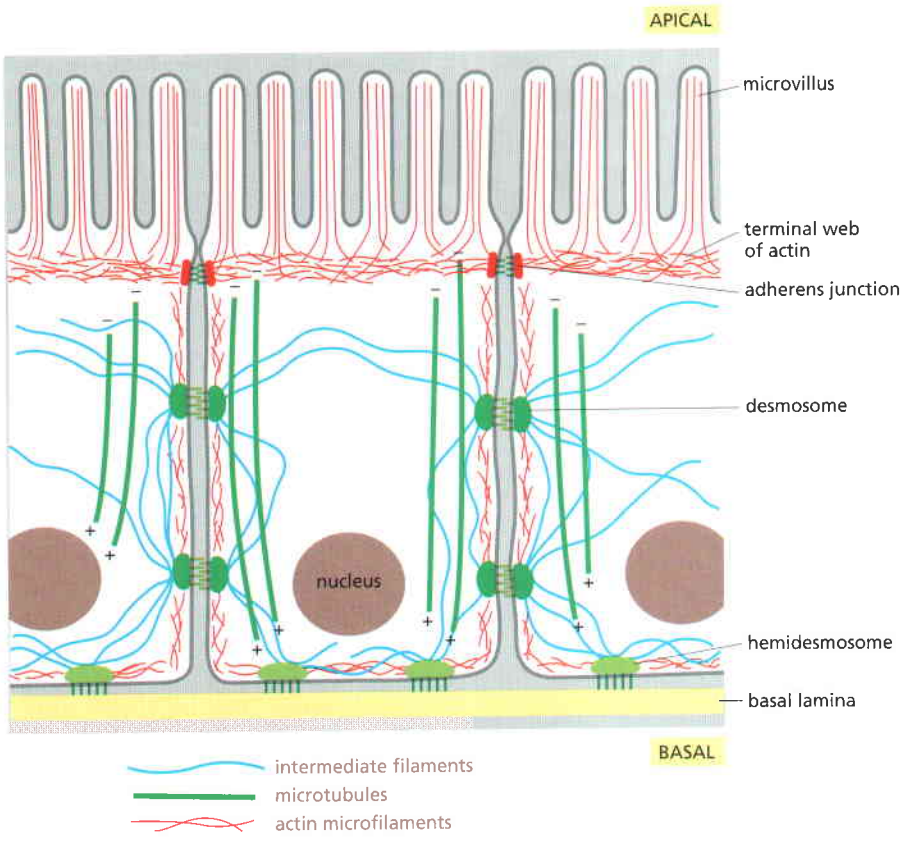


Figure 16–5 Organization of the cytoskeleton in polarized epithelial cells. All the components of the cytoskeleton cooperate to produce the characteristic shapes of specialized cells including the epithelial cells that line the small intestine. At the apical (upper) surface, facing the intestinal lumen, bundled actin filaments (*red*) form microvilli that increase the cell surface area available for absorbing nutrients from food. Just below the microvilli, a circumferential band of actin filaments contributes to forming cell-cell junctions that prevent the contents of the intestinal lumen from leaking into the body. 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 on the basal side of the cell; these important adhesive structures will be 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. This actin organization directs the secretion of new cell wall and other materials to the site of budding (**Figure 16–6**). The polarized organization of the actin structures in turn influences the orientation of the mitotic spindle, so that a complete set of replicated chromosomes can be delivered into the daughter cell at the end of the cell division process.

Each Type of Cytoskeletal Filament Is Constructed from Smaller Protein Subunits

Cytoskeletal structures frequently reach all the way from one end of the cell to the other, spanning tens or even hundreds of micrometers. Yet the individual protein molecules of the cytoskeleton are generally only a few nanometers in size. The cell builds the large structures by the repetitive assembly of large numbers of the small subunits, like building a skyscraper out of bricks. Because these subunits are small, they can diffuse rapidly within the cytoplasm, whereas the assembled filaments cannot. In this way, cells can undergo rapid

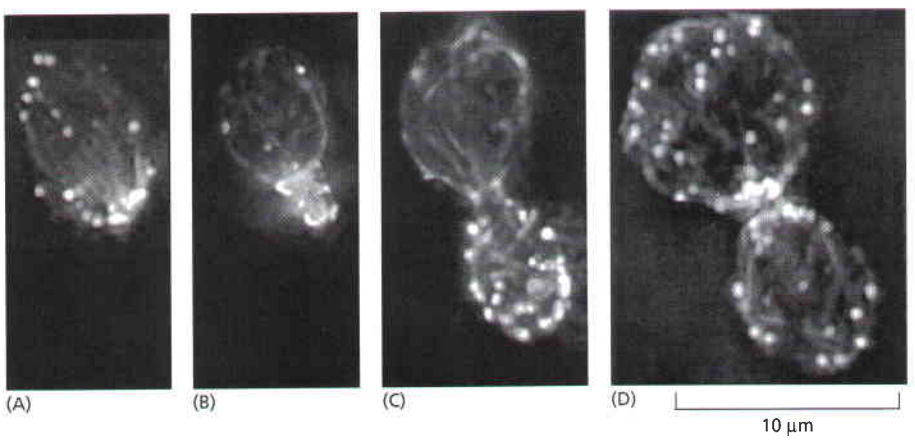


Figure 16–6 Polarity of actin patches and cables throughout the yeast cell cycle. Filamentous actin structures in the yeast cell, labeled here with fluorescent phalloidin, include actin patches (round bright spots) and actin cables (extended lines). (A) In a mother cell, before the formation of the bud, most of the patches become clustered at one end. The cables are lined up and point toward the cluster of patches, which is the site where the bud will emerge. (B) As the small bud grows, most patches remain within it. Cables in the mother cell continue to point toward this site of new cell wall growth. (C) Patches are almost uniformly distributed over the surface of a full-sized bud. Cables in the mother cell remain polarized. (D) Immediately after cell division, mother and daughter cells form new patches, which are concentrated near the division site, although both cells have randomly oriented cables. (From T.S. Karpova et al., *J. Cell Biol.* 142:1501–1517, 1998. With permission from The Rockefeller University Press.)

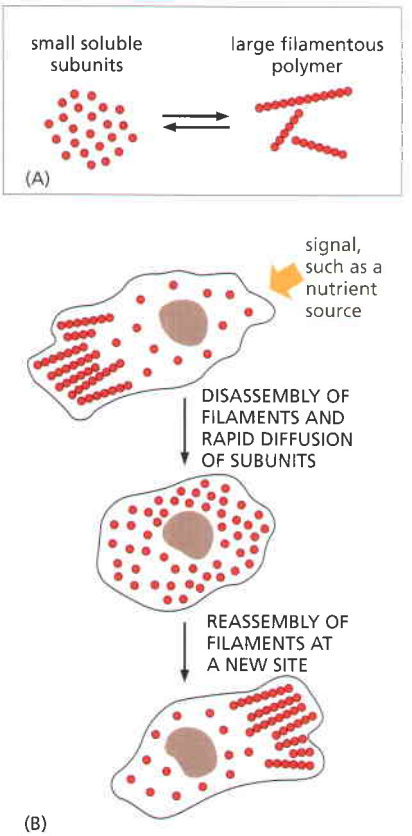


Figure 16–7 The cytoskeleton during changes in cell shape. The formation of protein filaments from much smaller protein subunits allows regulated filament assembly and disassembly to reshape the cytoskeleton. (A) Filament formation from a small protein. (B) Rapid reorganization of the cytoskeleton in a cell in response to an external signal.

structural reorganizations, disassembling filaments at one site and reassembling them at another site far away (**Figure 16–7**).

Intermediate filaments are made up of smaller subunits that are themselves elongated and fibrous, whereas actin filaments and microtubules are made of subunits that are compact and globular—*actin subunits* for actin filaments, *tubulin subunits* for microtubules. All three types of cytoskeletal filaments form as helical assemblies of subunits (see Figure 3–26) 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 them produce critical differences in the stability and mechanical properties of each type of filament.

Covalent linkages between their subunits hold together many biological polymers—including DNA, RNA, and proteins. In contrast, weak noncovalent interactions hold together the three types of cytoskeletal "polymers". Consequently, their assembly and disassembly can occur rapidly, without covalent bonds being formed or broken.

Within the cell, hundreds of different cytoskeleton-associated accessory proteins regulate the spatial 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 eucaryotic cell to maintain a highly organized but flexible internal structure and, in many cases, to move.

Filaments Formed from Multiple Protofilaments Have Advantageous Properties

In general, we can view the linking of protein subunits together to form a filament as a simple association reaction. A free subunit binds to the end of a filament that contains n subunits to generate a filament of length $n + 1$. The addition of each subunit to the end of the polymer creates a new end to which yet another subunit can bind. However, the robust cytoskeletal filaments in living cells are not built by simply stringing subunits together in a single straight file. A thousand tubulin monomers, for example, lined up end to end, would span the diameter of a small eucaryotic 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.

Cytoskeletal polymers combine strength with adaptability because they are built out of multiple **protofilaments**—long linear strings of subunits joined end to end—that associate with one another laterally. Typically, the protofilaments twist around one another in a helical lattice. The addition or loss of a subunit at the end of one protofilament makes or breaks one set of longitudinal bonds and either one or two sets of lateral bonds. In contrast, breakage of the composite filament in the middle requires breaking sets of longitudinal bonds in several

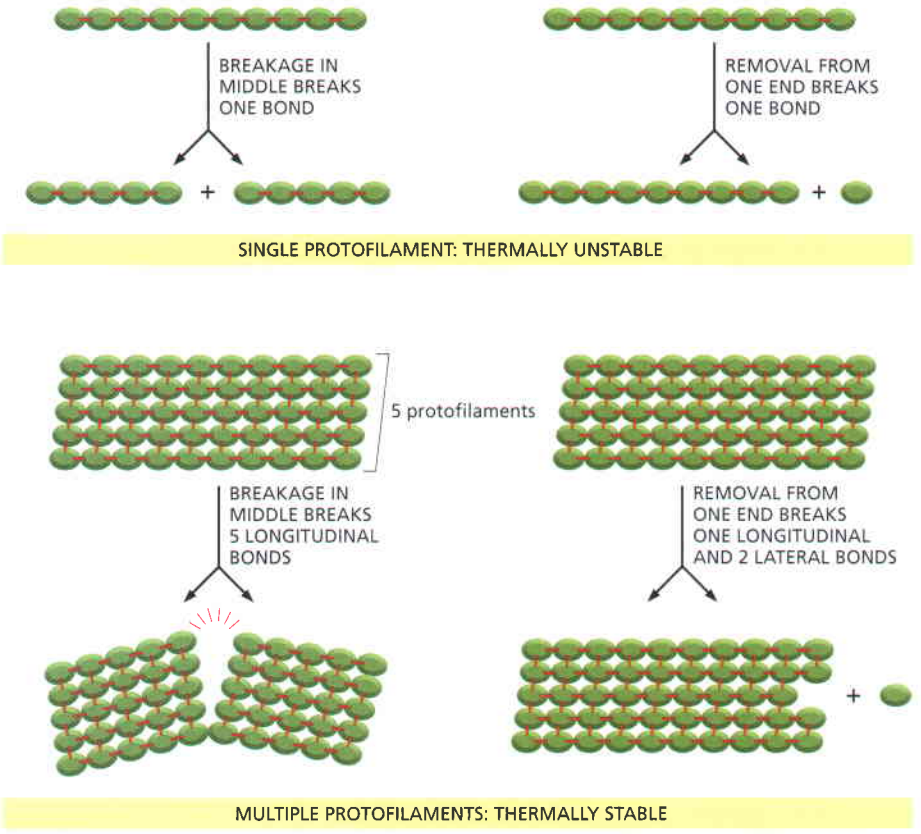


Figure 16–8 The thermal stability of cytoskeletal filaments with dynamic ends. Formation of a cytoskeletal filament from more than one protofilament allows the ends to be dynamic, while the filaments themselves are resistant to thermal breakage. In this hypothetical example, the stable filament is formed from five protofilaments. The bonds holding the subunits together in the filaments are shown in *red*.

protofilaments all at the same time (**Figure 16–8**). The large energy difference between these two processes allows most cytoskeletal filaments to resist thermal breakage, while leaving the filament ends as dynamic structures at which addition and loss of subunits can occur rapidly.

As with other specific protein–protein interactions, many hydrophobic interactions and weak 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 cytoskeletal 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 tolerate stretching and bending, forming strong rope-like structures (**Figure 16–9**). Microtubules, by contrast, are built from globular subunits held together primarily by longitudinal bonds, and the lateral bonds holding the 13 protofilaments together are comparatively weak. For this reason, microtubules break much more easily when they are bent than do intermediate filaments.

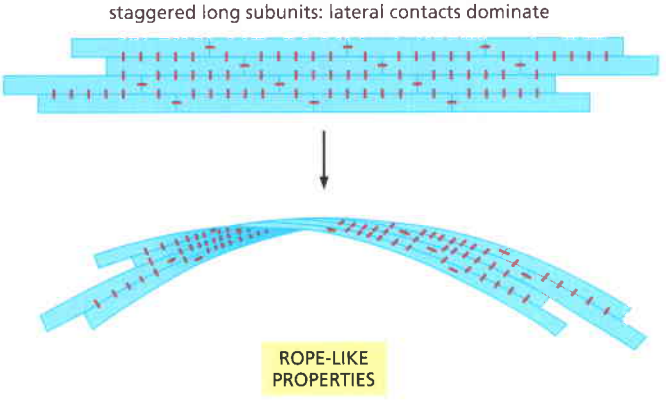


Figure 16–9 A strong filament formed from elongated fibrous subunits with strong lateral contacts. Intermediate filaments are formed in this way and are consequently especially resistant to stretching forces, although they are easily bent.

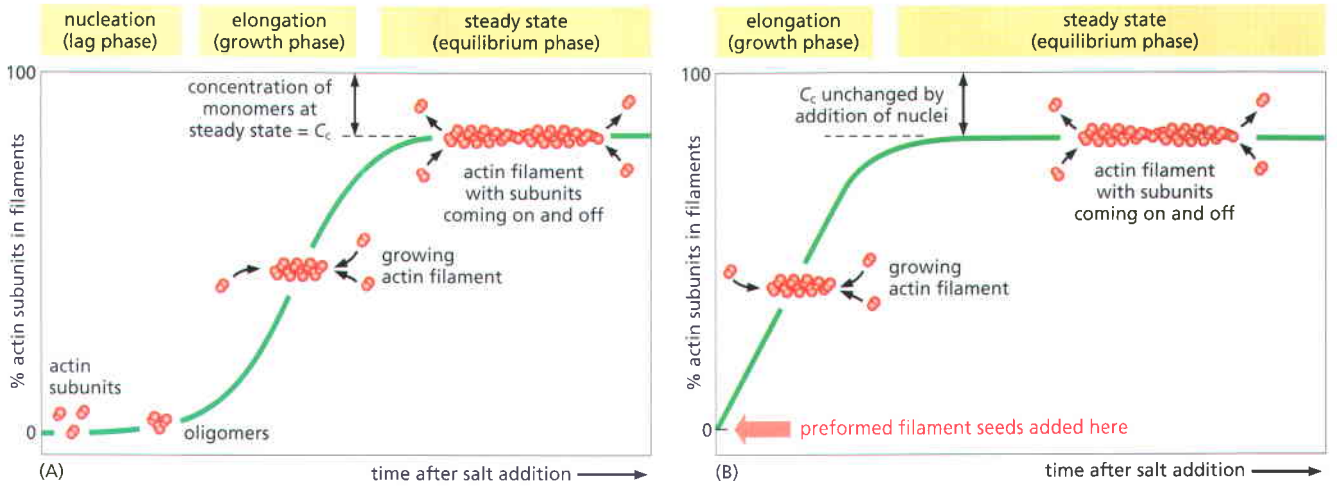


Figure 16–10 The time course of actin polymerization in a test tube. (A) Polymerization is begun by raising the salt concentration in a solution of pure actin subunits. (B) Polymerization is begun in the same way, but with preformed fragments of actin filaments present to act as nuclei for filament growth. As indicated, the % free subunits reflects the critical concentration (C_C), the point at which there is no net change in polymer.

Nucleation Is the Rate-Limiting Step in the Formation of a Cytoskeletal Polymer

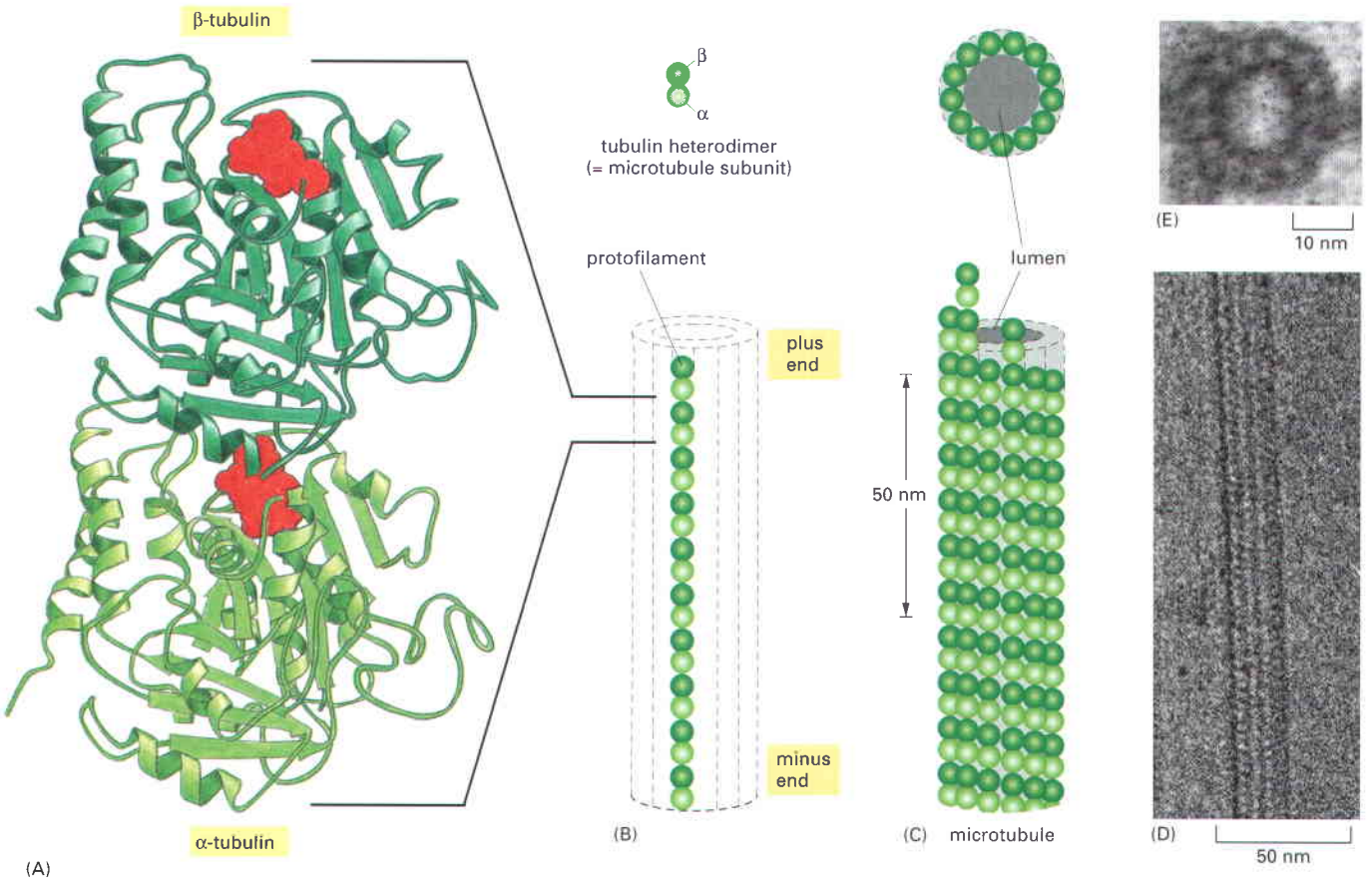
There is an important additional consequence of the multiple-protofilament organization of cytoskeletal polymers. Short oligomers composed of a few subunits can assemble spontaneously, but they are unstable and disassemble readily because each monomer is bonded only to a few other monomers. For a new large filament to form, subunits must assemble into an initial aggregate, or nucleus, that is stabilized by many subunit–subunit contacts and can then elongate rapidly by addition of more subunits. The initial process of nucleus assembly is called filament *nucleation*, and it can take quite a long time, depending on how many subunits must come together to form the nucleus.

The instability of smaller aggregates creates a kinetic barrier to nucleation, which is easily observed in a solution of pure actin or tubulin—the subunits of actin filaments and microtubules, respectively. When polymerization is initiated in a test tube containing a solution of pure individual subunits (by raising the temperature or raising the salt concentration), there is an initial 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 the more stable filament form, so that the lag phase is followed by a phase of rapid filament elongation, during which subunits add quickly onto the ends of the nucleated filaments (**Figure 16–10**A). Finally, 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 from the ends. The concentration of free subunits left in solution at this point is called the *critical concentration*, C_c. As explained in **Panel 16–2** (pp. 978–979), 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, $C_c = k_{off} / k_{on}$.

The lag phase in filament growth is eliminated if preexisting seeds (such as filament fragments that have been chemically cross-linked) are added to the solution at the beginning of the polymerization reaction (Figure 16–10B). 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 cytoskeletal filaments are assembled. Indeed, the regulation of filament nucleation is a primary way for cells to control their shape and their movement.

The Tubulin and Actin Subunits Assemble Head-to-Tail to Create Polar Filaments

Microtubules are formed from protein subunits of **tubulin**. The tubulin subunit is itself a heterodimer formed from two closely related globular proteins called *α-tubulin* and *β-tubulin*, tightly bound together by noncovalent bonds (**Figure**

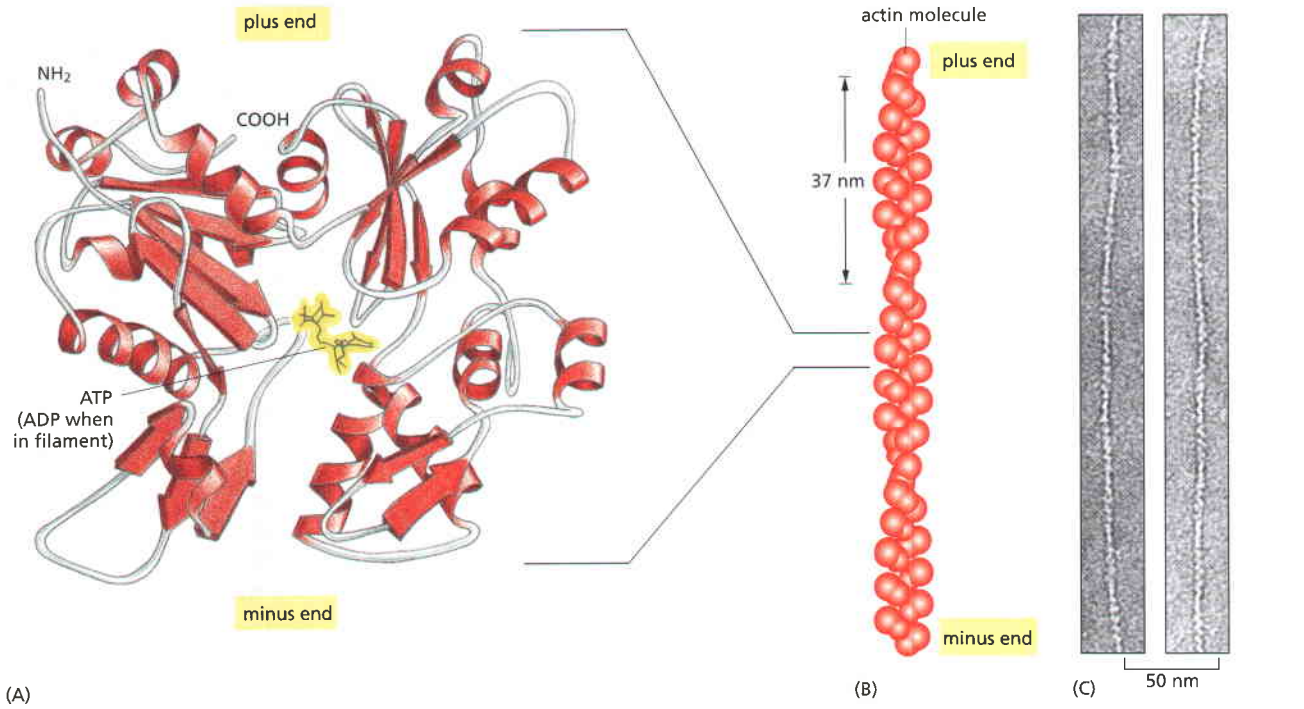


16–11). These two tubulin proteins are found only in this heterodimer. Each α or β monomer has a binding site for one molecule of GTP. The GTP that is bound to the α-tubulin monomer is physically trapped at the dimer interface and is never hydrolyzed or exchanged; it can therefore be considered to be 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 it is exchangeable. As we shall see, the hydrolysis of GTP at this site to produce GDP has an important effect on microtubule dynamics.

A microtubule is a hollow cylindrical structure built from 13 parallel protofilaments, each composed of alternating α-tubulin and β-tubulin molecules. When the tubulin heterodimers assemble to form the hollow cylindrical microtubule, they generate 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 strong. 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). Together, the longitudinal and lateral contacts are repeated in the regular helical lattice of the microtubule. 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 16–8). These multiple contacts among subunits make microtubules stiff and difficult to bend. The stiffness of a filament can be characterized by its *persistence length,* a property of the filament describing how long it must be before random thermal fluctuations are likely to cause it 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 (in Figure 16–11, for example, the α-tubulin is down and the β-tubulin up in each hetero-

Figure 16–11 The structure of a microtubule and its subunit. (A) The subunit of each protofilament is a tubulin heterodimer, formed from a very 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 (α–β 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.)



dimer). Therefore, the microtubule itself has a distinct structural polarity, with α-tubulins exposed at one end and β-tubulins exposed at the other end.

The actin subunit is a single globular polypeptide chain and is thus a monomer rather than a dimer. Like tubulin, each actin subunit has a binding site for a nucleotide, but for actin the nucleotide is ATP (or ADP) rather than GTP (or GDP) (**Figure 16–12**). As for tubulin, the actin subunits assemble head-to-tail to generate filaments with a distinct structural polarity. The actin filament can be considered to consist of two parallel protofilaments that twist around each other in a right-handed helix. Actin filaments are relatively flexible and easily bent compared with the hollow cylindrical microtubules, with a persistence length of only a few tens of micrometers. But in a living cell, accessory proteins (see below) crosslink and bundle them together, making these large-scale actin structures much stronger than an individual actin filament.

Figure 16–12 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. Although the filament is often described as a single helix of monomers, it can also be thought of as 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 micrographs of negatively stained actin filaments. (C, courtesy of Roger Craig.)

Microtubules and Actin Filaments Have Two Distinct Ends That Grow at Different Rates

The regular, parallel orientation of their subunits gives actin filaments and microtubules structural polarity. This orientation makes the two ends of each polymer different in ways that have a profound effect on filament growth rates. Addition of a subunit to either end of a filament of n subunits results in a filament of $n + 1$ subunits. In the absence of ATP or GTP hydrolysis, 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 forward and backward rate constants, k_{on}/k_{off}, must be identical at the two ends, even though the absolute values of these rate constants may be very different at each end.

In a structurally polar filament, the kinetic rate constants for association and dissociation—k_{on} and k_{off}, respectively—are often much greater at one end than at the other. Thus, if an excess of purified subunits is allowed to assemble onto marked fragments of preformed filaments, one end of each fragment elongates much faster than the other (**Figure 16–13**). If filaments are rapidly diluted so that the free subunit concentration drops below the critical concentration, the fast-growing end also depolymerizes fastest. The more dynamic of the two ends of a filament, where both growth and shrinkage are fast, is called the **plus end**, and the other end is called the **minus end**.

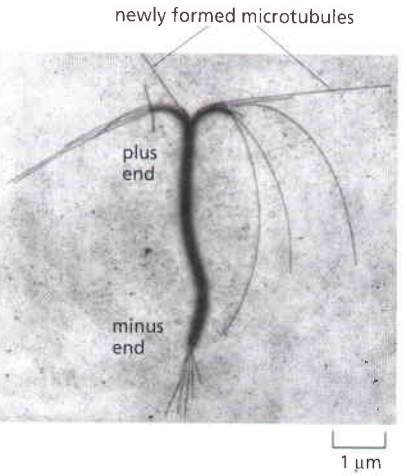


Figure 16–13 The preferential growth of microtubules at the plus end. Both microtubules and actin filaments grow faster at one end than at the other. In this case, a stable bundle of microtubules obtained from the core of a cilium (discussed later) was incubated for a short time with tubulin subunits under polymerizing conditions. Microtubules grow fastest from the plus end of the microtubule bundle, the end at the *top* in this micrograph. (Courtesy of Gary Borisy.)

On microtubules, α subunits are exposed at the minus end, and β subunits are exposed at the plus end. On actin filaments, the ATP-binding cleft on the monomer points toward the minus end. (For historical reasons, the plus ends of actin filaments are usually referred to as "barbed" ends, and minus ends as "pointed" ends, because of the arrowhead appearance of myosin heads when bound along the filament.)

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. Likewise, filament depolymerization proceeds spontaneously when this free energy change is greater than zero. A cell can couple an energetically unfavorable process to these spontaneous processes; thus, the cell can use free energy released during spontaneous filament polymerization or depolymerization to do mechanical work—in particular, to push or pull an attached load. For example, elongating microtubules can help push out membranes, and shrinking microtubules can help pull mitotic chromosomes away from their sisters during anaphase. Similarly, elongating actin filaments help protrude the leading edge of motile cells, as we discuss later.

Filament Treadmilling and Dynamic Instability Are Consequences of Nucleotide Hydrolysis by Tubulin and Actin

Thus far, our discussion of filament dynamics has ignored a critical fact that applies to both actin filaments and microtubules. In addition to their ability to form noncovalent polymers, the actin and tubulin subunits are both enzymes that can catalyze the hydrolysis of a nucleoside triphosphate, ATP or GTP, respectively. For the free subunits, this hydrolysis proceeds very slowly; however, it is accelerated when the subunits are incorporated into filaments. Shortly after incorporation of an actin or tubulin subunit into a filament, nucleotide hydrolysis occurs; the free phosphate group is released from each subunit, but the nucleoside diphosphate remains trapped in the filament structure. (On tubulin, the nucleotide-binding site lies at the interface between two neighboring subunits—see Figure 16–11, whereas in actin, the nucleotide is deep in a cleft near the center of the subunit—see Figure 16–12.) Thus, two different types of filament structures can exist, one with the "T form" of the nucleotide bound (ATP for actin, GTP for tubulin), and one with the "D form" bound (ADP for actin, GDP for tubulin).

When the nucleotide is hydrolyzed, much of the free energy released by cleavage of the high-energy phosphate–phosphate bond is stored in the polymer lattice. 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). For certain concentrations of free subunits, D-form polymers will therefore shrink while T-form polymers grow.

In living cells, most of the free subunits are in the T form, as the free concentration of both ATP and GTP is about ten-fold higher than that of ADP and GDP. The longer the time that subunits have been in the polymer lattice, the more likely they are to have hydrolyzed their bound nucleotide. Whether the subunit at the very 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 rate of subunit addition is high, that is if the filament is growing rapidly, then it is likely that

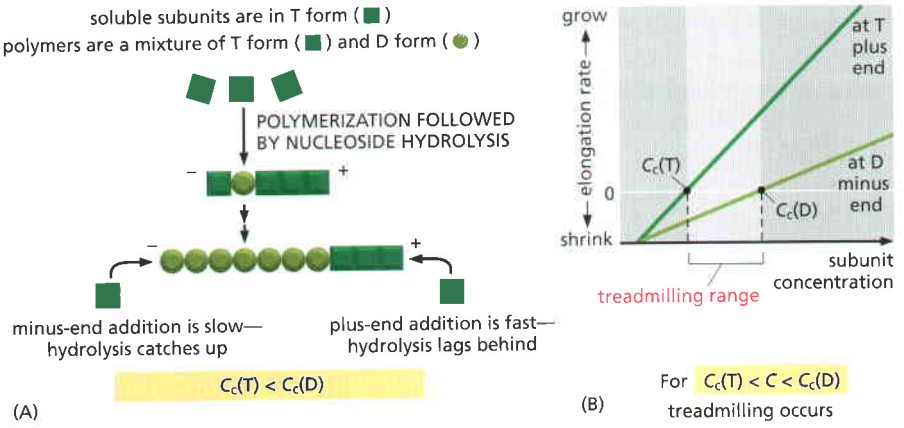


Figure 16–14 The treadmilling of an actin filament or microtubule, made possible by the nucleoside triphosphate hydrolysis that follows subunit addition. (A) Explanation for the different critical concentrations (C_c) at the plus and minus ends. Subunits with bound nucleoside triphosphate (T-form subunits) polymerize at both ends of a growing filament, and then undergo nucleotide hydrolysis in the filament lattice. 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.

a new subunit will add on to the polymer before the nucleotide in the previously added subunit has been hydrolyzed, so that the tip of the polymer remains in the T form, forming an *ATP cap* or *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.

The rate of subunit addition at the end of a filament is the product of the free subunit concentration and the rate constant k_{on}. The k_{on} is much faster for the plus end of a filament than for the minus end because of a structural difference between the two ends (see Panel 16–2). At an intermediate concentration of free subunits, it is therefore 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. As just explained, the D form has a higher critical concentration than the T form. (In other words, the D form leans more readily toward disassembly, while the T form leans more readily toward assembly). If the concentration of free subunits in solution is in an intermediate range—higher than the critical concentration of the T form (that is, the plus end), but lower than the critical concentration of the D form (that is, the minus end)—the filament adds subunits at the plus end, and simultaneously loses subunits from the minus end. This leads to the remarkable property of filament **treadmilling** (**Figure 16–14** and Panel 16–2).

During treadmilling, subunits are recruited at the plus end of the polymer in the T form and shed from the minus end in the D form. The ATP or GTP hydrolysis that occurs along the way gives rise to the difference in the free energy of the association/dissociation reactions at the plus and minus ends of the actin filament or microtubule and thereby makes treadmilling possible. At a particular intermediate subunit concentration, the filament growth at the plus end exactly balances the filament shrinkage at the minus end. Now, the subunits cycle rapidly between the free and filamentous states, while the total length of the filament remains unchanged. This "steady-state treadmilling" requires a constant consumption of energy in the form of nucleoside triphosphate hydrolysis. While the extent of treadmilling inside the cell is uncertain, the treadmilling of single filaments has been observed *in vitro* for actin, and a phenomenon that looks like treadmilling can be observed in live cells for individual microtubules (**Figure 16–15**).

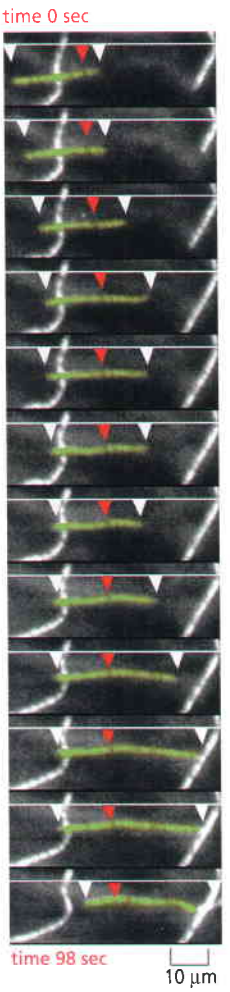
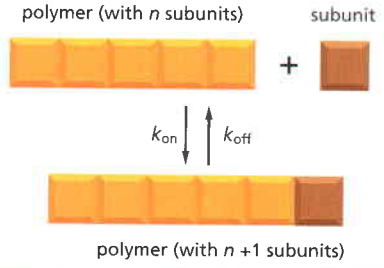


Figure 16–15 Treadmilling behavior of a microtubule, as observed in a living cell. A cell was injected with tubulin that had been covalently linked to the fluorescent dye rhodamine, so that approximately 1 tubulin subunit in 20 was fluorescent. The fluorescence of individual microtubules was then observed with a sensitive electronic camera. The microtubule shown appears to be sliding from *left* to *right*, but, in fact, the microtubule lattice remains stationary (as shown by a landmark within the microtubule lattice indicated by the *red* arrowhead), while the plus end (on the *right*) grows and the minus end (on the *left*) shrinks. The plus end also displays dynamic instability. (From C.M. Waterman-Storer and E.D. Salmon, *J. Cell Biol.* 139:417–434, 1997. With permission from The Rockefeller University Press.)

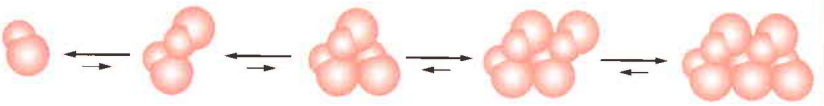
ON RATES AND OFF RATES

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,

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

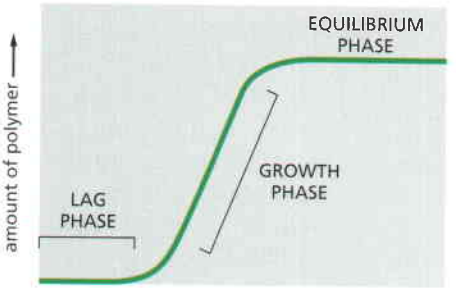
so that

$$C_c = \frac{k_{off}}{k_{on}} = \frac{1}{K}$$

(where K is the equilibrium constant for subunit addition; see Figure 3–43).

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.

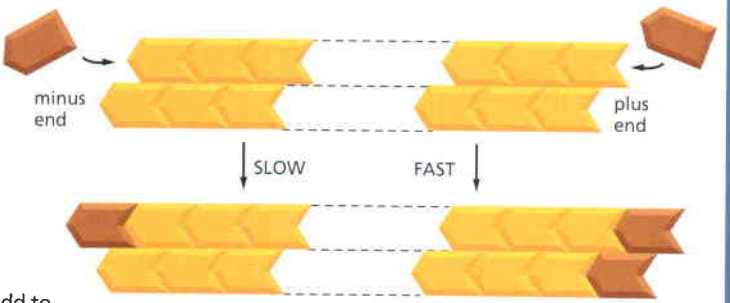
The equilibrium phase, or steady state, is reached when the growth of the polymer due to monomer addition precisely balances the shrinkage of the polymer due to disassembly back to monomers.

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.

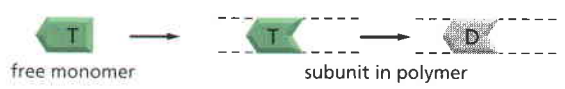
Even though k_{on} and k_{off} will have different values for the plus and minus ends of the polymer, their ratio k_{off}/k_{on}—and hence C_c—must be the same at both ends for a simple polymerization reaction (no ATP or GTP hydrolysis). This is because exactly the same subunit interactions are broken 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.

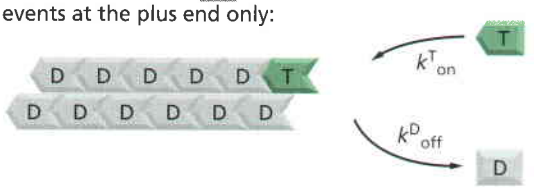
NUCLEOTIDE HYDROLYSIS

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 16–16 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:



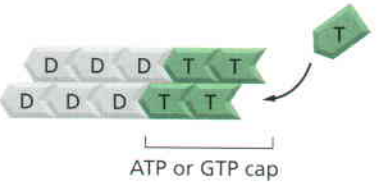
As before, the polymer will grow until $C = C_c$. For illustrative purposes, we can ignore k^D_{on} and k^T_{off} since they are usually very small, so that polymer growth ceases when

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

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.



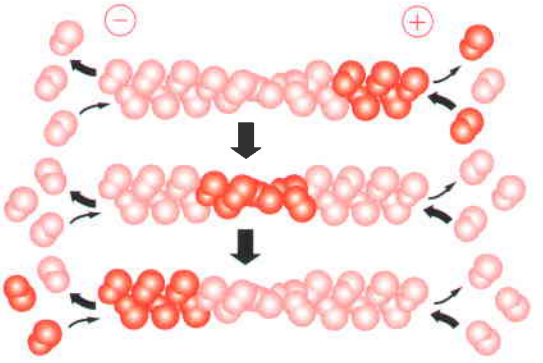
DYNAMIC INSTABILITY and TREADMILLING are two behaviors observed in cytoskeletal polymers. Both are associated with nucleoside triphosphate hydrolysis. Dynamic instability is believed to predominate in microtubules, whereas treadmilling may predominate in actin filaments.

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:

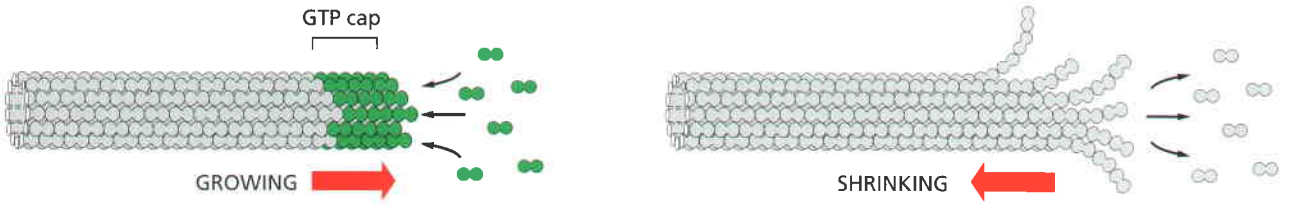
$$C_c \text{ (minus end)} > C_c \text{ (plus end)}$$

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.

The kinetic differences between the behavior of the T form and the D form have another important consequence for the behaviors of filaments. If the rate of subunit addition at one end is similar in magnitude to the rate of hydrolysis, there is a finite probability that this end will start out in a T form, but that hydrolysis will eventually "catch up" with the addition and transform the end to a D form. This transformation is sudden and random, with a certain probability per unit time.

Suppose that the concentration of free subunits is intermediate between the critical concentration for a T-form end and the critical concentration for a D-form end (that is, in the same range of concentrations where treadmilling is observed). 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 filament, 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 16–16**A). The change from growth to rapid shrinkage is called a *catastrophe,* while the change to growth is called a *rescue.* <CCCA>

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. The structural difference between a T-form end and a D-form end is dramatic. Tubulin subunits with GTP bound to 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 16–16B). 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 rings and curved oligomers of GDP-containing tubulin are seen near the ends of depolymerizing microtubules (Figure 16–16C).

Actin filaments also undergo length fluctuations but on a much smaller scale, so that at steady state the length fluctuates only a micrometer or so over several minutes, as compared to tens of micrometers for microtubules undergoing dynamic instability. In most eucaryotic cells, dynamic instability is thought to predominate in microtubules, whereas treadmilling may predominate in actin filaments.

Treadmilling and Dynamic Instability Aid Rapid Cytoskeletal Rearrangement <AAAT>

Both dynamic instability and treadmilling allow a cell to maintain the same overall filament content, while individual subunits constantly recycle between the filaments and the cytosol. How dynamic are the microtubules and actin filaments inside a living cell? Typically, a microtubule, with major structural differences between its growing and shrinking ends, switches between growth and shrinkage every few minutes. The ends of individual microtubules can therefore be seen in real time to exhibit dynamic instability (**Figure 16–17**). Because of their smaller size and denser packing, it is much more difficult to resolve the ends of individual actin filaments within living cells. With appropriate techniques based on fluorescence microscopy, however, one can show that actin filament turnover is typically rapid, with individual filaments persisting for only a few tens of seconds or minutes.

At first glance, the dynamic behavior of filaments seems like a waste of energy. To maintain a constant concentration of actin filaments and microtubules, most of which are undergoing a process of either treadmilling or dynamic instability, the cell must hydrolyze large amounts of nucleoside

triphosphate. As we explained with our ant-trail analogy at the beginning of the chapter, the advantage to the cell seems to be the spatial and temporal flexibility that is inherent in a structural system with constant turnover. Individual subunits are small and can diffuse very rapidly; an actin or tubulin subunit can diffuse across the diameter of a typical eucaryotic cell in several seconds. As noted

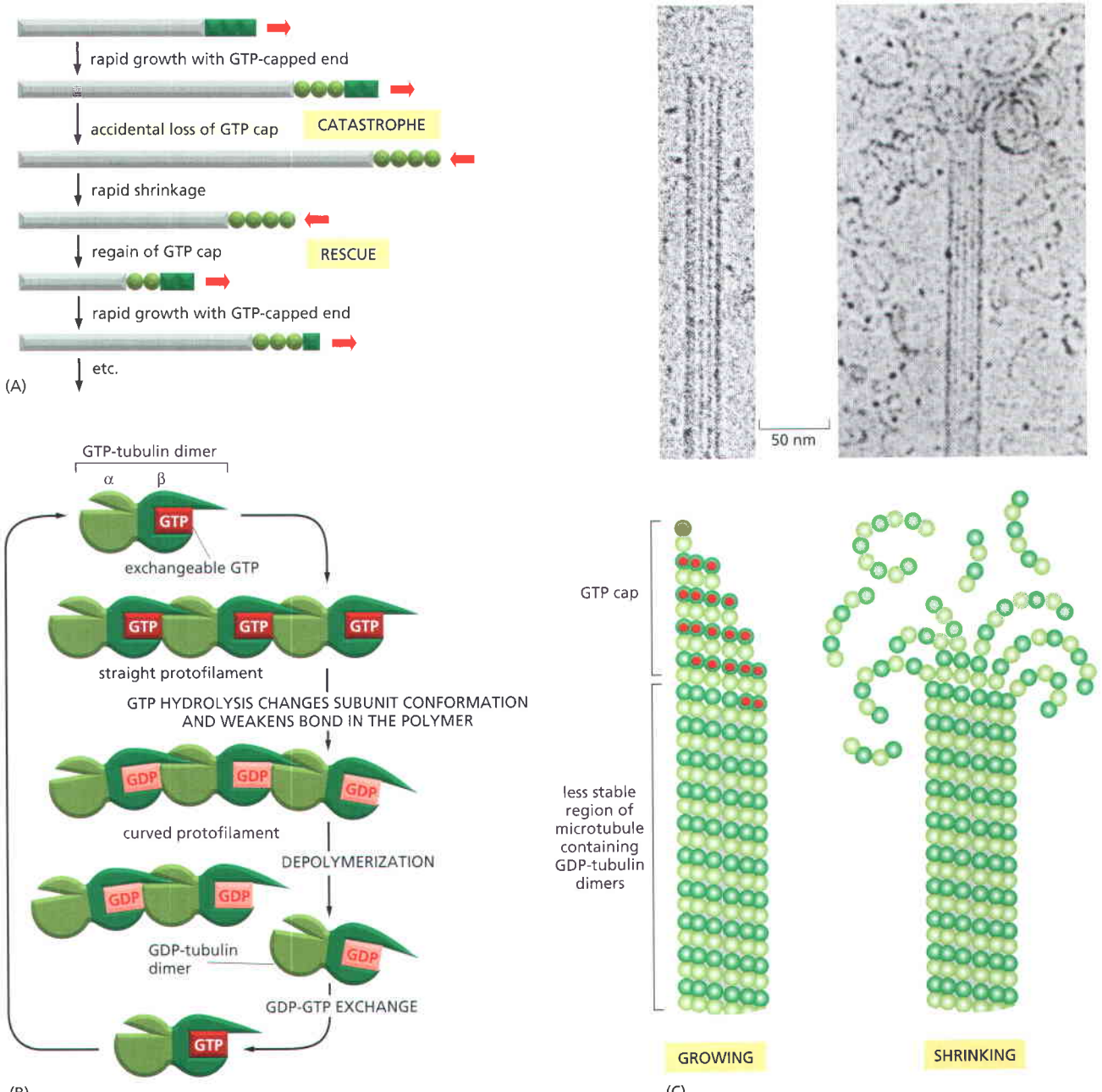


Figure 16–16 Dynamic instability due to the structural differences between a growing and a shrinking microtubule end. (A) If the free tubulin concentration in solution is between the critical values indicated in Figure 16–14B, a single microtubule end may undergo transitions between a growing state and 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, as observed in preparations in vitreous ice. Note particularly the curling, disintegrating GDP-containing protofilaments at the end of the shrinking microtubule. (C, courtesy of E.M. Mandelkow, E. Mandelkow and R.A. Milligan, *J. Cell Biol.* 114:977–991, 1991. With permission from The Rockefeller University Press.)

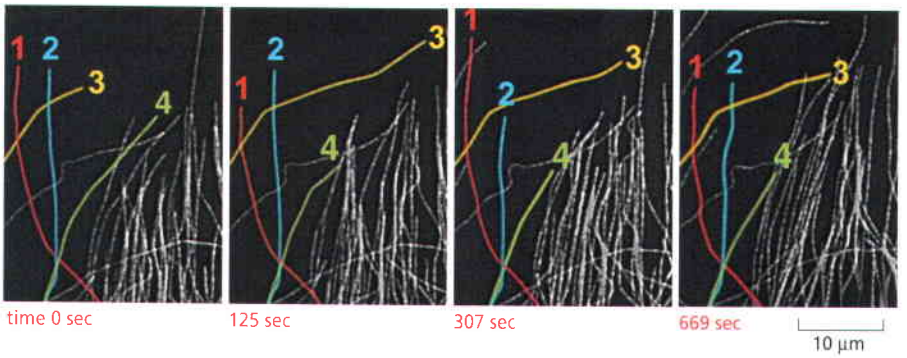


Figure 16–17 Direct observation of the dynamic instability of microtubules in a living cell. <AAAT> Microtubules in a newt lung epithelial cell were observed after the cell was injected with a small amount of rhodamine labeled tubulin, as in Figure 16–15. Notice the dynamic instability of microtubules at the edge of the cell. Four individual microtubules are highlighted for clarity; each of these shows alternating shrinkage and growth. (Courtesy of Wendy C. Salmon and Clare Waterman-Storer.)

previously, the rate-limiting step in the formation of a new filament is nucleation, so these rapidly diffusing subunits tend to assemble either on the ends of preexisting filaments or at particular sites where special proteins catalyze the nucleation step. The new filaments in either case are highly dynamic, and unless specifically stabilized, they have only a fleeting existence. By controlling where filaments are nucleated and selectively stabilized, a cell can control the location of its filament systems, and hence its structure. It seems that the cell is continually testing a wide variety of internal structures and only preserving those that are useful. When external conditions change, or when internal signals arise (as during the transitions in the cell cycle), the cell is poised to change its structure rapidly (see Figures 16–2 to 16–4).

Actin and tubulin have independently evolved their nucleoside triphosphate hydrolysis to enable their filaments to depolymerize readily after they have polymerized. These two proteins are completely unrelated in amino acid sequence: actin is distantly related in structure to the glycolytic enzyme hexokinase, whereas tubulin is distantly related to a large family of GTPases that includes the heterotrimeric G proteins and monomeric GTPases such as Ras (discussed in Chapter 3). In both protein families, the coupling between nucleotide hydrolysis and a protein conformational change that alters protein function appears to be evolutionarily very ancient; however, the purposes of that structural coupling have diverged over time to include signal transmission, catalysis, and regulation of the polymerization/depolymerization cycle.

In certain specialized structures, parts of the cytoskeleton become less dynamic. In a terminally differentiated cell such as a neuron, for example, it is desirable to maintain a consistent structure over time, and many of the actin filaments and microtubules are stabilized by association with other proteins. However, when new connections are made in the brain, as when the information you are reading now is transferred into long-term memory, even a cell as stable as a neuron can grow new elongated processes to make new synapses. To do this, a neuron requires the dynamic, exploratory activities of its cytoskeletal filaments.

Tubulin and Actin Have Been Highly Conserved During Eucaryotic Evolution

Tubulin is found in all eucaryotic 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 forms of β-tubulin, each encoded by a different gene. The different forms of tubulin are very similar, and they generally will copolymerize into mixed microtubules in the test tube. However, they can have distinct locations in a cell and perform subtly different functions. As a striking example, a specific form of β-tubulin forms the microtubules in six specialized touch-sensitive neurons in the nematode *Caenorhabditis elegans*. Mutations that eliminate this protein result in the loss of touch-sensitivity, with no apparent defect in other functions.

Like tubulin, actin is found in all eucaryotic cells. Most organisms have multiple genes encoding actin; humans have six. Actin is extraordinarily well conserved among eucaryotes. The amino acid sequences of actins from different species are usually about 90% identical. But, again like tubulin, small variations in actin amino acid sequence can cause significant functional differences. In vertebrates, there are three subtly different isoforms of actin, termed α, β, and γ, that differ slightly in their amino acid sequences. The α-actin is expressed only in muscle cells, while β and γ are found together in almost all nonmuscle cells. Yeast actin and *Drosophila* muscle actin are 89% identical, yet the expression of yeast actin in *Drosophila* results in a fly that looks normal but is unable to fly.

Why are the amino acid sequences of actin and tubulin so strictly conserved in eucaryotic evolution, whereas the sequences of most other cytoskeletal proteins, including intermediate filament proteins and the large families of accessory proteins that bind to actin or tubulin, are not? The likely explanation is that the need for large numbers of other proteins to interact with the entire surface of an actin filament or microtubule limits the variability of their structures. Genetic and biochemical studies in the yeast *Saccharomyces cerevisiae* have demonstrated that actin interacts directly with dozens of other proteins, and indirectly with even more (**Figure 16–18**). Thus, any mutation in actin that could result in a desirable change in its interaction with one protein might cause undesirable changes in its interactions with other proteins that bind at or near the same site. Over time, evolving organisms have found it more profitable to leave actin and tubulin alone, and to alter their binding partners instead.

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

All eucaryotic cells contain actin and tubulin. But the third major type of cytoskeletal protein, the *intermediate filament*, forms a cytplasmic filament in only some metazoans—including vertebrates, nematodes, and mollusks. Even in these organisms, intermediate filaments are not required in the cytoplasm of every cell type. The specialized glial cells (called oligodendrocytes) that make

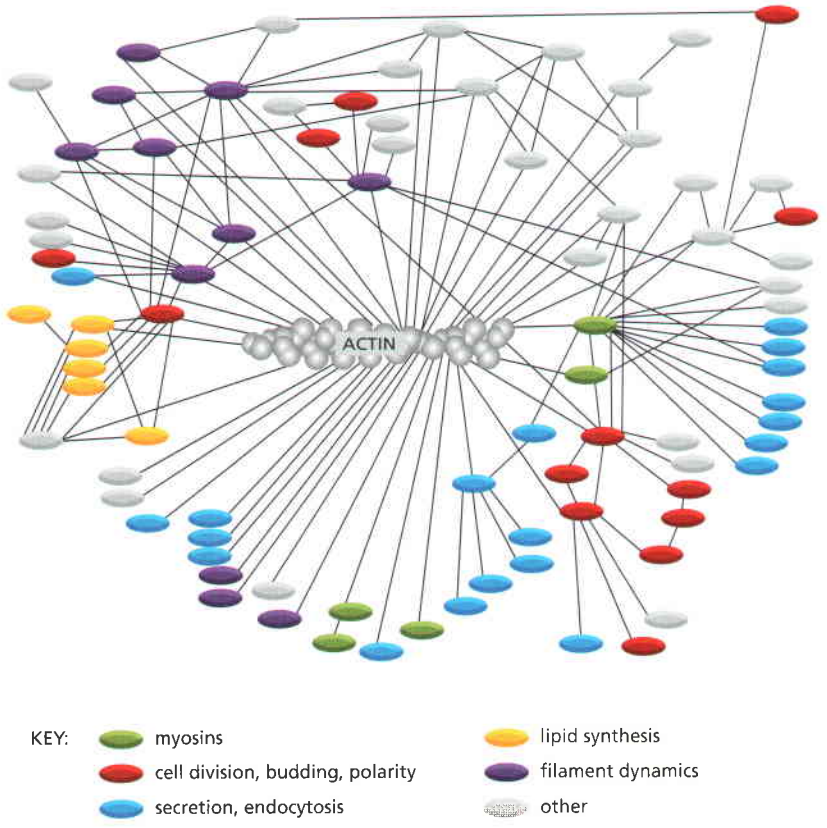


Figure 16–18 Actin at the crossroads. Actin binds to a very large variety of accessory proteins in all eucaryotic cells. This diagram shows most of the interactions that have been demonstrated, using either genetic or biochemical techniques, in the yeast *Saccharomyces cerevisiae*. Accessory proteins that operate in the same intracellular process are shown in the same color, as indicated in the key. (Adapted from D. Botstein et al., in The Molecular and Cellular Biology of the Yeast *Saccharomyces* [J.R. Broach, J.R. Pringle, E.W. Jones, eds.], Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1991.)

myelin in the vertebrate central nervous system, for example, do not contain such intermediate filaments. 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 play an important role in imparting mechanical strength to tissues for the squishier animals.

Cytoplasmic intermediate filaments are closely related to their ancestors, the much more prevalent *nuclear lamins*. The nuclear lamins are intermediate filament proteins that form a meshwork lining the inner membrane of the eucaryotic nuclear envelope, where they provide anchorage sites for chromosomes and nuclear pores (their dynamic behavior during cell division is discussed in Chapter 12). Several times during metazoan evolution, lamin genes have apparently duplicated, and the duplicates have evolved to produce rope-like, cytoplasmic intermediate filaments.

The individual polypeptides of **intermediate filaments** are elongated molecules with an extended central α-helical domain that forms a parallel coiled coil with another monomer. A pair of parallel dimers then associates in an antiparallel fashion to form a staggered tetramer. This tetramer represents the soluble subunit that is analogous to the αβ-tubulin dimer, or to the actin monomer (**Figure 16–19**). Unlike the actin or tubulin, the intermediate filament subunits do not contain a binding site for a nucleoside triphosphate.

Figure 16–19 A model of intermediate filament construction. <GCCA> The monomer shown in (A) pairs with an identical 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. The tetramer is the soluble subunit 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 rope-like 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. (Electron micrograph courtesy of Roy Quinlan.)

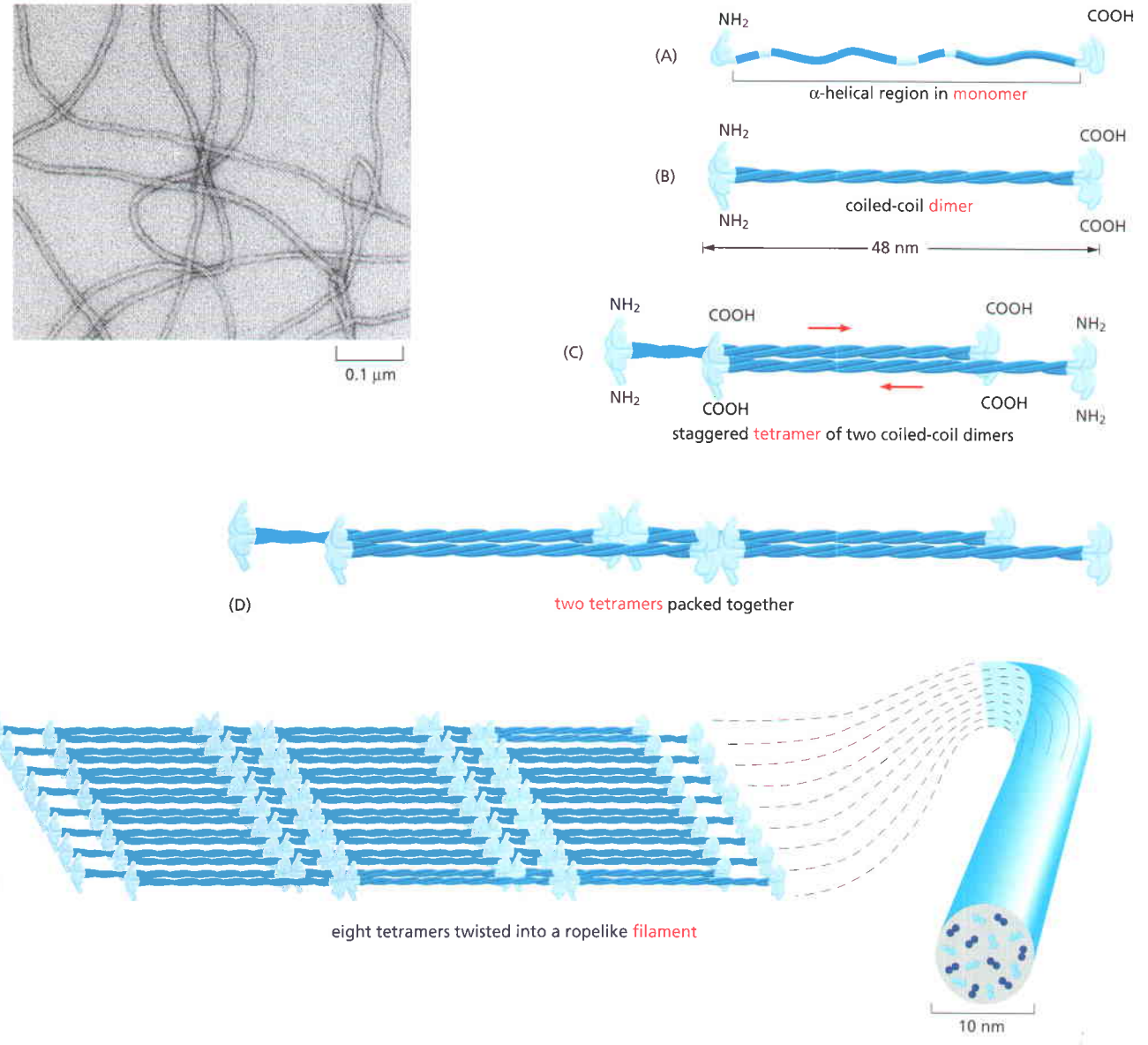


Table 16–1 Major Types of Intermediate Filament Proteins in Vertebrate Cells

TYPES OF IF	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 (basic)	
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

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 rope-like 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.

Less is understood about the mechanism of assembly and disassembly of intermediate filaments than of actin filaments and microtubules, but some types of intermediate filaments including *vimentin* form highly dynamic structures in cells such as fibroblasts. Under normal conditions, protein phosphorylation probably regulates their disassembly, in much the same way that phosphorylation regulates the disassembly of nuclear lamins in mitosis (see Figure 12–20). As evidence for rapid turnover, labeled subunits microinjected into tissue culture cells rapidly add themselves onto the existing intermediate filaments within a few minutes, while an injection of peptides derived from a conserved helical region of the subunit induces the rapid disassembly of the intermediate filament network. Interestingly, the latter injection can also induce the disassembly of the microtubule and actin filament networks in some cases, demonstrating that there is a fundamental mechanical integration of the three cytoskeletal systems in these cells.

Intermediate Filaments Impart Mechanical Stability to Animal Cells

Intermediate filaments come in a wide variety of types, with substantially more sequence variation in the subunit isoforms than occurs in the isoforms of actin or tubulin. A central α-helical domain has 40 or so heptad repeat motifs that form an extended coiled-coil structure (see Figure 3–9). This domain is similar in the different isoforms, but the N- and C-terminal globular domains can vary greatly.

Different families of intermediate filaments are expressed in different cell types (**Table 16–1**). **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 may be about 50 distinct keratins. Every keratin filament is made up of an equal mixture of type I (acidic) and type II (neutral/basic) keratin chains; these form heterodimers, two of which then join

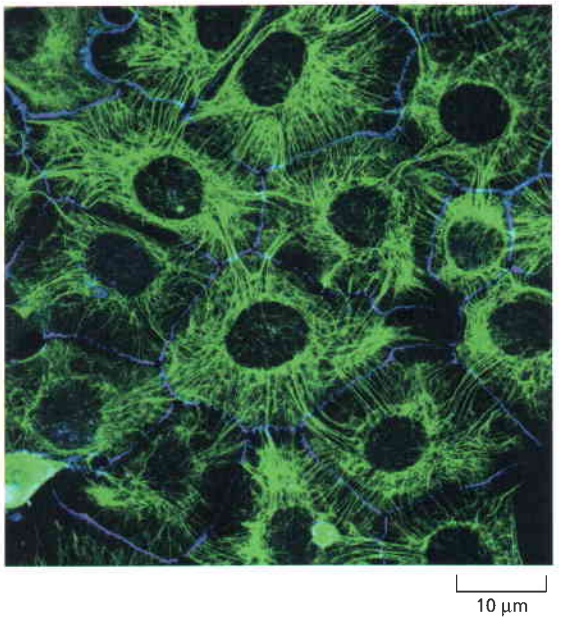


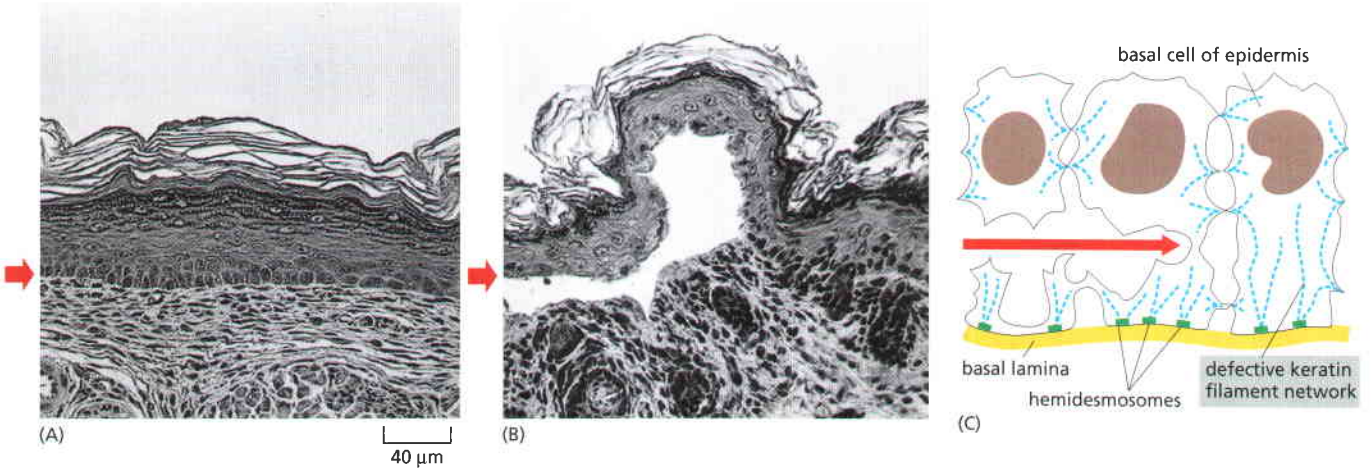
Figure 16–20 Keratin filaments in epithelial cells. Immunofluorescence micrograph of the network of keratin filaments *(green)* in a sheet of epithelial cells in culture. The filaments in each cell are indirectly connected to those of its neighbors by desmosomes (discussed in Chapter 19). A second protein *(blue)* has been stained to reveal the location of the cell boundaries. (Courtesy of Kathleen Green and Evangeline Amargo.)

to form the fundamental tetrameric subunit (see Figure 16–19). Cross-linked keratin networks held together by disulfide bonds may 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 16–20**). 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 16–5). We discuss these important adhesive structures in more detail in Chapter 19.

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 16–21**). 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

Figure 16–21 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 normal (A) and mutant (B) 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.)



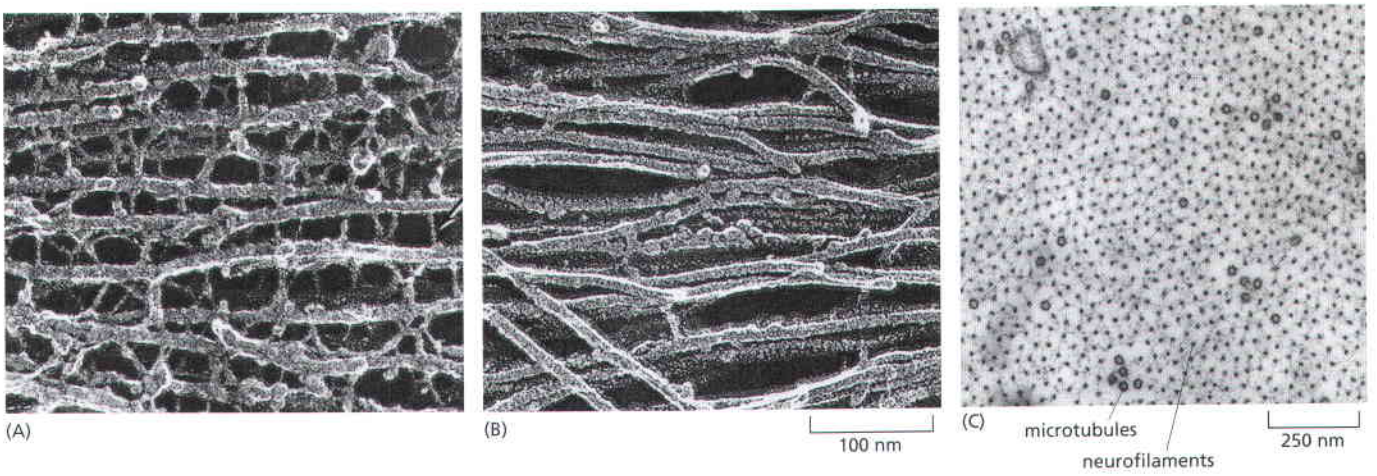


Figure 16–22 Two types of intermediate filaments in cells of the nervous system. (A) Freeze-etch electron microscopic image of neurofilaments in a nerve cell axon, showing the extensive cross-linking through protein cross-bridges—an arrangement believed to give this long cell process great tensile strength. 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 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 central rod domain, demonstrating the importance of this particular part of the protein for correct filament assembly.

A second family of intermediate filaments, called **neurofilaments**, is found in high concentrations along the axons of vertebrate neurons (**Figure 16–22**). Three types of neurofilament proteins (NF-L, NF-M, NF-H) coassemble *in vivo*, forming heteropolymers that contain NF-L plus one of the others. 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 addition of subunits at the filament 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.

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, which may interfere with normal axonal transport. The degeneration of the axons leads to muscle weakness and atrophy, which is usually fatal. The over-expression of human NF-L or NF-H in mice results in mice that have an ALS-like disease.

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. Mice lacking desmin show normal initial muscle development, but adults have various muscle cell abnormalities, including misaligned muscle fibers.

Drugs Can Alter Filament Polymerization

Because the survival of eucaryotic cells depends on a balanced assembly and disassembly of the highly conserved cytoskeletal filaments formed from actin and tubulin, the two types of filaments are frequent targets for natural toxins. These toxins are produced in self-defense by plants, fungi, or sponges that do not wish to be eaten but cannot run away from predators, and they generally disrupt the filament polymerization reaction. The toxin binds tightly to either the filament form or the free subunit form of a polymer, driving the assembly reaction in the direction that favors the form to which the toxin binds. For example, the drug *latrunculin*, extracted from the sea sponge *Latrunculia magnifica*, binds to actin monomers and prevents their assembly into filaments; it thereby causes a net depolymerization of actin filaments. In contrast, *phalloidin*, from the fungus *Amanita phalloides* (death cap), binds to and stabilizes actin filaments, causing a net increase in actin polymerization. (This attractive but inedible mushroom also expresses a second deadly toxin, the RNA polymerase II inhibitor α-amanitin.) Either change in actin filaments is very toxic for cells. Similarly, *colchicine*, from the autumn crocus (or meadow saffron), binds to and

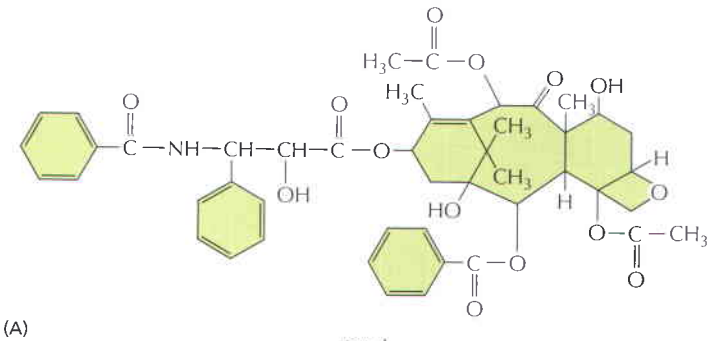
Table 16–2 Drugs That Affect Actin Filaments and Microtubules

ACTIN-SPECIFIC DRUGS	
Phalloidin	binds and stabilizes filaments
Cytochalasin	caps filament plus ends
Swinholide	severs filaments
Latrunculin	binds subunits and prevents their polymerization
MICROTUBULE-SPECIFIC DRUGS	
Taxol	binds and stabilizes microtubules
Colchicine, colcemid	binds subunits and prevents their polymerization
Vinblastine, vincristine	binds subunits and prevents their polymerization
Nocodazole	binds subunits and prevents their polymerization

stabilizes free tubulin, causing microtubule depolymerization. In contrast, *taxol,* extracted from the bark of a rare species of yew tree, binds to and stabilizes microtubules, causing a net increase in tubulin polymerization. These and some other natural products that are commonly used by cell biologists to manipulate the cytoskeleton are listed in **Table 16–2**.

Drugs like these have a rapid and profound effect on the organization of the cytoskeleton in living cells (**Figure 16–23**). They provided early evidence that the cytoskeleton is a dynamic structure, maintained by a rapid and continual exchange of subunits between the soluble and filamentous forms, and they revealed that this subunit flux is necessary for normal cytoskeletal function.

The drugs listed in Table 16–2 have been useful to cell biologists trying to probe the roles of actin and microtubules in various cell processes. Some of them are also used to treat cancer. Both microtubule-depolymerizing drugs (such as vinblastine) and microtubule-polymerizing drugs (such as taxol) preferentially kill dividing cells, since both microtubule assembly and disassembly are crucial for correct function of the mitotic spindle (discussed later in this chapter). 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.



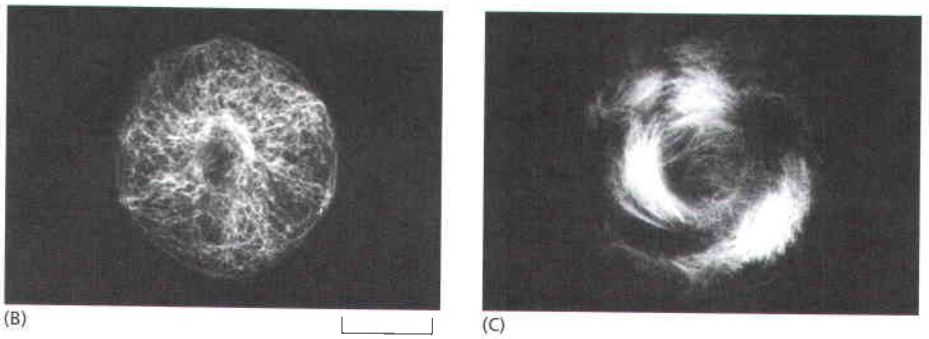


Figure 16–23 Effect of the drug taxol on microtubule organization. (A) Molecular structure of taxol. Recently, organic chemists have succeeded in synthesizing this complex molecule, which is widely used for cancer treatment. (B) Immunofluorescence micrograph showing the microtubule organization in a liver epithelial cell before the addition of taxol. (C) Microtubule organization in the same type of cell after taxol treatment. Note the thick circumferential bundles of microtubules around the periphery of the cell. (D) A Pacific yew tree, the natural source of taxol. (B, C from N.A. Gloushankova et al., *Proc. Natl Acad. Sci. U.S.A.* 91:8597–8601, 1994. With permission from National Academy of Sciences; D, courtesy of A.K. Mitchell 2001. © Her Majesty the Queen in Right of Canada, Canadian Forest Service.)

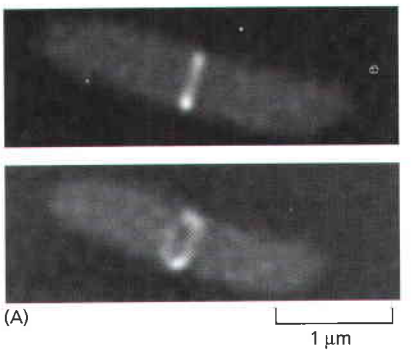
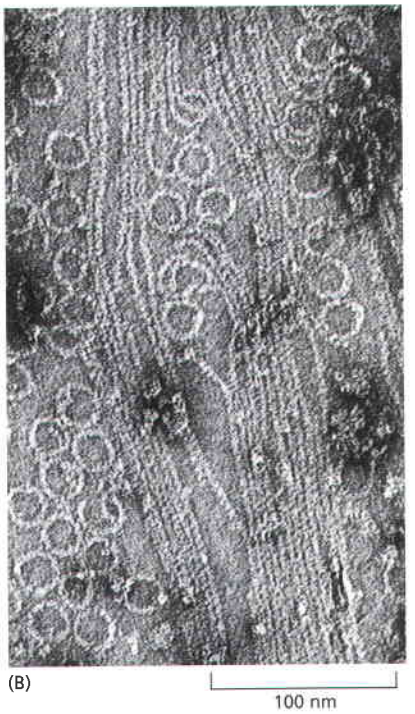


Figure 16–24 The bacterial FtsZ protein, a tubulin homolog in procaryotes. (A) A band of FtsZ protein forms a ring in a dividing bacterial cell. This ring has been labeled by fusing the FtsZ protein to the *g*reen *f*luorescent *p*rotein (GFP), which allows it to be observed in living *E. coli* cells with a fluorescence microscope. *Top*, side view shows the ring as a bar in the middle of the dividing cell. *Bottom*, rotated view showing the ring structure. (B) FtsZ filaments and rings, formed *in vitro*, as visualized using electron microscopy. Compare this image with that of the microtubule shown on the *right* in Figure 16–16C. (A, from X. Ma, D.W. Ehrhardt and W. Margolin, *Proc. Natl Acad. Sci. U.S.A.* 93:12998–13003, 1996; B, from H.A. Erickson et al., *Proc. Natl Acad. Sci. U.S.A.* 93:519–523, 1996. All with permission from National Academy of Sciences.)

Bacterial Cell Organization and Cell Division Depend on Homologs of the Eucaryotic Cytoskeleton



While eucaryotic cells are typically large and morphologically complex, bacterial cells are usually only a few micrometers long and assume simple, modest shapes such as spheres or rods. Bacteria also lack the elaborate networks of intracellular membrane-enclosed organelles such as the endoplasmic reticulum and Golgi apparatus. For many years, biologists assumed that the lack of a bacterial cytoskeleton was one reason for these striking differences between cell organization in the eucaryotic and bacterial kingdoms. This assumption was challenged with the discovery in the early 1990s that nearly all bacteria and many archaea contain a homolog of tubulin, FtsZ, that can polymerize into filaments and assemble into a ring (called the Z-ring) at the site where the septum forms during cell division (**Figure 16–24**).

The three-dimensional folded protein structure of FtsZ is remarkably similar to the structure of α or β tubulin and, like tubulin, hydrolysis of GTP is triggered by polymerization and causes a conformational change in the filament structure. Although the Z-ring itself persists for many minutes, the individual filaments within it are highly dynamic, with an average half-life of about thirty seconds. As the bacterium divides, the Z-ring becomes smaller until it has completely disassembled, and it is thought that the shrinkage of the Z-ring may contribute to the membrane invagination necessary for the completion of cell division. The Z-ring may also serve as a site for localization of specialized cell wall synthesis enzymes required for building the septum between the two daughter cells. The disassembled FtsZ subunits later reassemble at the new sites of septum formation in the daughter cells (**Figure 16–25**).

More recently, it has been found that many bacteria also contain homologs of actin. Two of these, MreB and Mbl, are found primarily in rod-shaped or spiral-shaped cells, and mutations disrupting their expression cause extreme abnormalities in cell shape and defects in chromosome segregation (**Figure 16–26**). MreB and Mbl filaments assemble *in vivo* to form large-scale spirals that

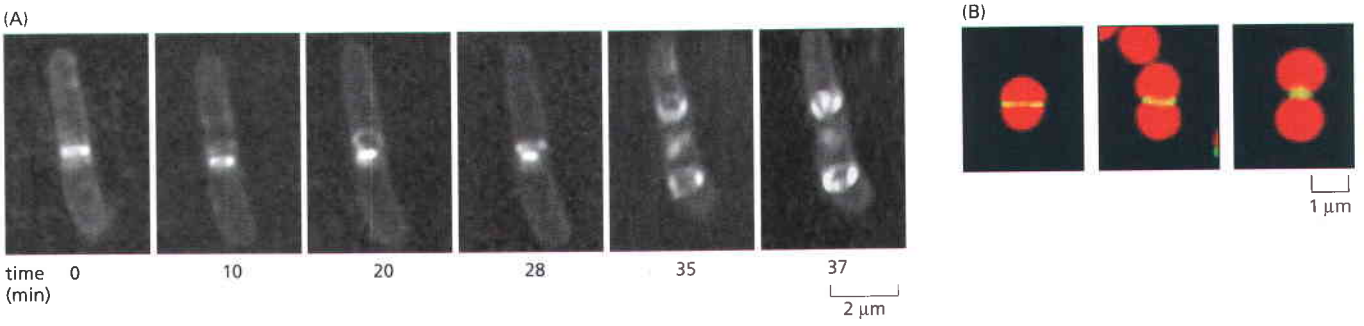


Figure 16–25 Rapid rearrangements of FtsZ through the bacterial cell cycle. (A) After chromosome segregation is complete, the ring formed by FtsZ at the middle of the cell becomes smaller as the cell pinches in two, much like the contractile ring formed by actin and myosin filaments in eucaryotic cells. The FtsZ filaments that have disassembled as the cells have separated then reassemble to form two new rings at the middle of the two daughter cells. (B) Dividing chloroplasts (*red*) from a red alga also make use of a protein ring made from FtsZ *(yellow)* for cleavage. (A, from Q. Sun and W. Margolin, *J. Bacteriol.* 180:2050–2056, 1998. With permission from American Society for Microbiology; B, from S. Miyagishima et al., *Plant Cell* 13:2257–2268, 2001. With permission from American Society of Plant Biologists.)

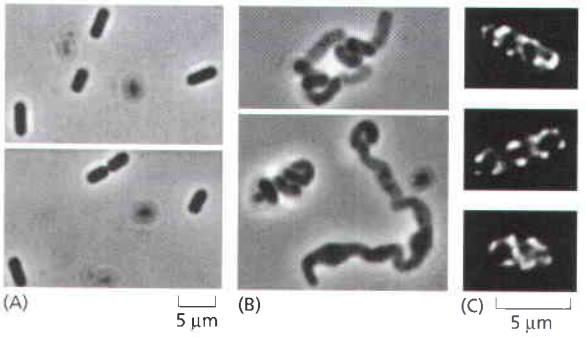


Figure 16–26 Actin homologs in bacteria determine cell shape. (A) The common soil bacterium *Bacillus subtilis* normally forms cells with a regular rod-like shape. (B) *B. subtilis* cells lacking the actin homolog Mbl grow into irregular twisted tubes and eventually die. (C) The Mbl protein forms long helices made of up many short filaments that run the length of the bacterial cell and help to direct the sites of cell wall synthesis. (From L.J. Jones, R. Carbadillo-Lopez and J. Errington, *Cell* 104: 913-922, 2001. With permission from Elsevier.)

span the length of the cell and apparently contribute to cell shape determination 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 plant cells (see Figure 19–82). As with FtsZ, the filaments within the MreB and Mbl spirals are highly dynamic, with half-lives of a few minutes; as for actin, ATP hydrolysis accompanies the polymerization process.

Diverse relatives of MreB and Mbl have more specialized roles. A particularly intriguing bacterial actin homolog is ParM, which is encoded on certain bacterial plasmids that also carry genes responsible for antibiotic resistance and frequently cause the spread of multi-drug 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 faithful inheritance and propagation in their bacterial hosts. *In vivo*, ParM assembles into a filamentous structure that associates at each end with a copy of the plasmid that encodes it, and growth of the ParM filament appears to push the replicated plasmid copies apart, rather like a mitotic spindle operating in reverse (**Figure 16–27**). Although ParM is a structural homolog of actin, its dynamic behavior differs significantly. ParM filaments undergo dramatic dynamic instability *in vitro*, more closely resembling microtubules than actin filaments in the way that they grow and shrink. The spindle-like structure is apparently built by the selective stabilization of spontaneously nucleated filaments that bind to specialized proteins recruited to the origins of replication on the plasmids.

The various bacterial actin homologs share similar molecular structures but their amino acid sequence similarity to each other is quite low (~10–15% identical residues). They assemble into filaments with distinct helical packing patterns, which may also have very different dynamic behaviors. Rather than using the same well-conserved actin for many different purposes, as eucaryotic cells do, bacteria have apparently opted to proliferate and specialize their actin homologs for distinct purposes.

It is now clear that the general principle of organizing cell structure by the self-association of nucleotide-binding proteins into dynamic helical filaments is used in all cells, and that the two major families of actin and tubulin are very

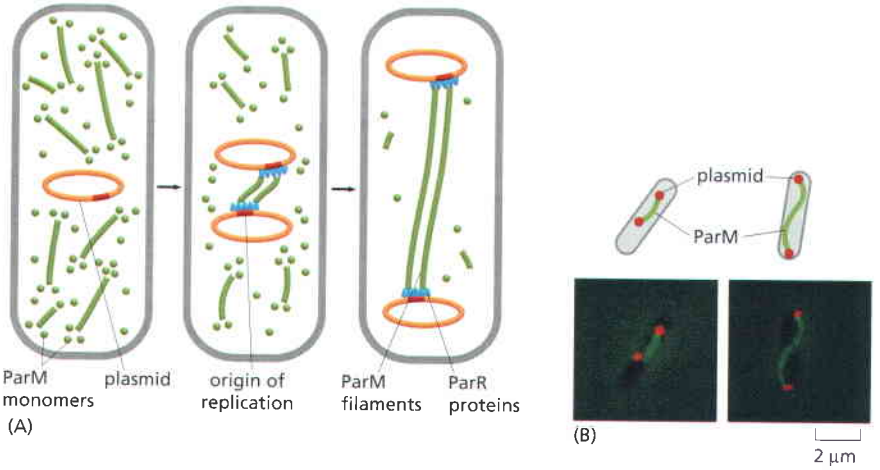


Figure 16–27 Role of the actin homolog ParM in plasmid segregation. (A) Some bacterial drug-resistance plasmids (*yellow*) encode an actin homolog, ParM, that will spontaneously nucleate to form small, dynamic filaments (*green*) throughout the bacterial cytoplasm. A second plasmid-encoded protein (*blue*) binds to specific DNA sequences in the plasmid, and also stabilizes the dynamic ends of the ParM filaments. When the plasmid has duplicated, so that the ParM filaments can be stabilized at both ends, the filaments grow and 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 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. With permission from AAAS; B, from J. Moller-Jensen et al., *Mol. Cell* 12:1477–1487, 2003. With permission from Elsevier.)

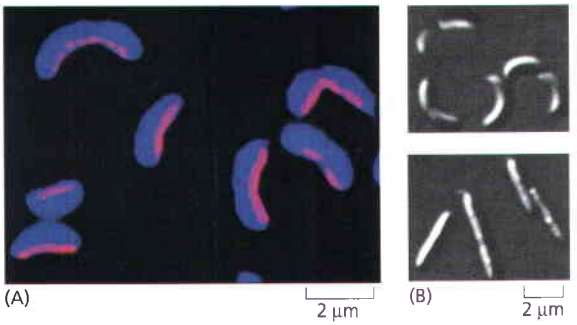


Figure 16–28 Caulobacter and crescentin. The sickle-shaped bacterium *Caulobacter crescentus* expresses a protein, crescentin, with a series of coiled-coil domains similar in size and organization to the domains of eucaryotic intermediate filaments. In cells, the crescentin protein forms a fiber that runs down the inner side of the curving bacterial cell wall. When the gene is disrupted, the bacteria are viable but grow in a straight rod-shaped form. (From N. Ausmees, J.R. Kuhn and C. Jacobs-Wagner, *Cell* 115:705–713, 2003. With permission from Elsevier.)

ancient, probably predating the split between the eucaryotic and bacterial kingdoms. However, the uses to which bacteria put their cytoskeletons appear somewhat different from their eucaryotic homologs. For example, in bacteria it is the tubulin (FtsZ) that is involved in *cytokinesis* (the pinching apart of a dividing cell into two daughters), while actin drives this process in eucaryotic cells. Conversely, eucaryotic microtubules are responsible for chromosome segregation, while bacterial actins (ParM and possibly MreB) help to segregate replicated DNA in bacteria.

At least one bacterial species with an unusual crescent shape, *Caulobacter crescentus*, even 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 seems to influence the cell shape; when the gene encoding crescentin is deleted, the *Caulobacter* cells grow as straight rods (**Figure 16–28**).

Since we now know that bacteria do in fact have sophisticated dynamic cytoskeletons, why then do they remain so small and morphologically simple? As yet there have been no *motor proteins* identified that walk along the bacterial filaments; perhaps the evolution of motor proteins was a critical step allowing morphological elaboration in the eucaryotes.

Summary

The cytoplasm of eucaryotic cells is spatially organized by a network of protein filaments known as the cytoskeleton. This network contains three principal types of filaments: microtubules, actin filaments, 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. Intermediate filaments are rope-like and easy to bend but hard to break. Microtubules are strong, rigid hollow tubes. Actin filaments are the thinnest of the three and are easy to break.

In living cells, the assembly and disassembly of their subunits constantly remodels all three types of cytoskeletal filaments. Microtubules and actin filaments add and lose subunits only at their ends, with one end (the plus end) growing faster than the other. Tubulin and actin (the subunits of microtubules and actin filaments, respectively) bind and hydrolyze nucleoside triphosphates (tubulin binds GTP and actin binds ATP). Nucleotide hydrolysis underlies the characteristic dynamic behavior of these two filaments. Actin filaments in cells seem to predominantly undergo treadmilling, where a filament assembles at one end while simultaneously disassembling at the other end. Microtubules in cells predominantly display dynamic instability, where a microtubule end undergoes alternating bouts of growth and shrinkage.

Whereas tubulin and actin have been strongly conserved in evolution, the family of intermediate filaments is very diverse. There are many tissue-specific forms found in the cytoplasm of animal cells, including keratin filaments in epithelial cells, neurofilaments in nerve cells, and desmin filaments in muscle cells. In all these cells, the primary job of intermediate filaments is to provide mechanical strength.

Bacterial cells also contain homologs of tubulin, actin and intermediate filaments that form dynamic filamentous structures involved in determining cell shape and in cell division.

HOW CELLS REGULATE THEIR CYTOSKELETAL FILAMENTS

Microtubules, actin filaments, and intermediate filaments are much more dynamic in cells than they are in the test tube. 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 a large array of accessory proteins that bind to either the filaments or their free subunits. Some of the most important accessory proteins associated with microtubules and actin filaments are outlined in **Panel 16–3** (pp. 994–995). This section describes how these accessory proteins modify the dynamics and structure of cytoskeletal filaments. We begin with a discussion of the way that microtubules and actin filaments are nucleated in cells, because this plays a major part in determining the overall organization of the cell's interior.

A Protein Complex Containing γ-Tubulin Nucleates Microtubules

While α- and β-tubulins are the regular building blocks of microtubules, another type of tubulin, called *γ-tubulin*, has a more specialized role. Present in much smaller amounts than α- and β-tubulin, this protein 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**). Antibodies against γ-tubulin stain the MTOC in virtually all species and cell types thus far examined.

Microtubules are nucleated at their minus end, with the plus end growing outward from each MTOC to create various types of microtubule arrays. A **γ-tubulin ring complex** (**γ-TuRC**) that is capable of nucleating microtubule growth in a test tube has been isolated from both insect and vertebrate cells. Two proteins, conserved from yeasts to humans, bind directly to the γ-tubulin, along with several other proteins that help create a ring of γ-tubulin molecules. This ring can be seen at the minus ends of the microtubules nucleated by γ-TuRC, and it is therefore thought to serve as a template that creates a microtubule with 13 protofilaments (**Figure 16–29**).

Microtubules Emanate from the Centrosome in Animal Cells

Most animal cells have a single, well-defined MTOC called the **centrosome**, located near the nucleus. From this focal point, the cytoplasmic microtubules emanate in a star-like, "astral" conformation. Microtubules are nucleated at the centrosome at their minus ends, so the plus ends point outward and grow toward the cell periphery. Microtubules nucleated at the centrosome continuously grow and shrink by dynamic instability, probing the entire three-dimensional volume of the cell. A centrosome is composed of a fibrous *centrosome*

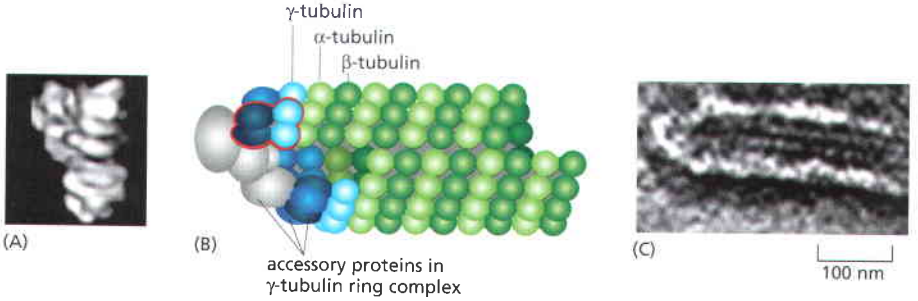
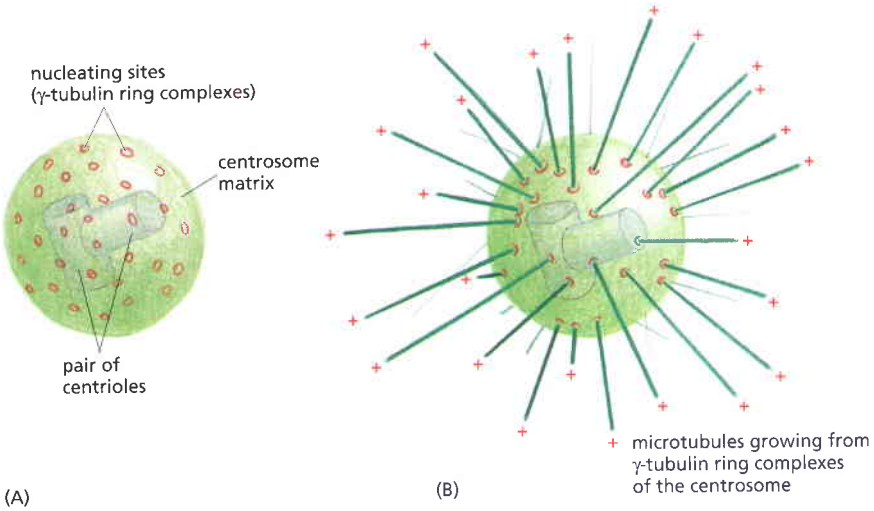


Figure 16–29 Polymerization of tubulin nucleated by γ-tubulin ring complexes. (A) Structure of the γ-tubulin ring complex, reconstructed from averaging electron micrographs of individual purified complexes. (B) Model for the nucleation of microtubule growth by the γ-TuRC. The red outline indicates a pair of proteins bound to two molecules of γ-tubulin; this group can be isolated as a separate subcomplex of the larger ring. Note the longitudinal discontinuity between two protofilaments. Microtubules generally have one such "seam" breaking the otherwise uniform helical packing of the protofilaments. (C) Electron micrograph of a single microtubule nucleated from the purified γ-tubulin ring complex. (A and C, from M. Moritz et al., *Nat. Cell Biol.* 2:365–370, 2000. With permission from Macmillan Publishers Ltd.)



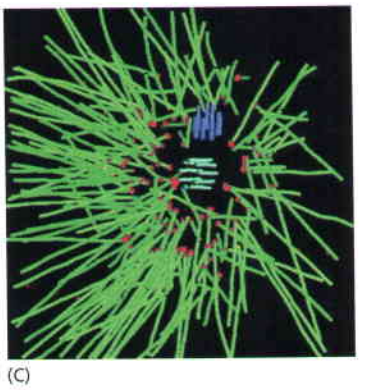


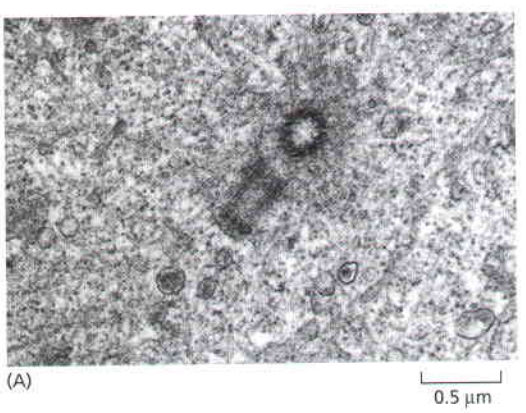
Figure 16–30 The centrosome. (A) The centrosome is the major MTOC of animal cells. Located in the cytoplasm next to the nucleus, it consists of an amorphous matrix of fibrous proteins to which the γ-tubulin ring complexes that nucleate microtubule growth are attached. This matrix is organized by a pair 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 Rockefeller University Press.)

matrix that contains more than fifty copies of γ-TuRC. Most of the proteins that form this matrix remain to be discovered, and it is not yet known how they recruit and activate the γ-TuRC.

Embedded in the centrosome is a pair of somewhat mysterious cylindrical structures arranged at right angles to each other in an L-shaped configuration (**Figure 16–30**). These are the **centrioles**, which become the basal bodies of cilia and flagella in motile cells (described later). The centrioles organize the centrosome matrix (also called the pericentriolar material), ensuring its duplication during each cell cycle as the centrioles themselves duplicate (**Figure 16–31**). As described in Chapter 17, the centrosome duplicates and splits into two equal parts during interphase, each half containing a duplicated centriole pair. These two daughter 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, pp. 1072–1073). A centriole consists of a short cylinder of modified microtubules, plus a large number of accessory proteins. The molecular basis for its duplication is not well-understood.

In fungi and diatoms, microtubules are nucleated at an MTOC that is embedded in the nuclear envelope as a small plaque called the *spindle pole body*. Higher-plant cells seem to nucleate microtubules at sites distributed all around the nuclear envelope. Neither fungi nor most plant cells contain centrioles. Despite these differences, all these cells contain γ-tubulin and seem to use it to nucleate their microtubules.

In animal cells, the astral configuration of microtubules is very 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 the centrosome acts as a device to survey the outlying regions of the cell and to position the centrosome at its center, and it does this even in artificial enclosures



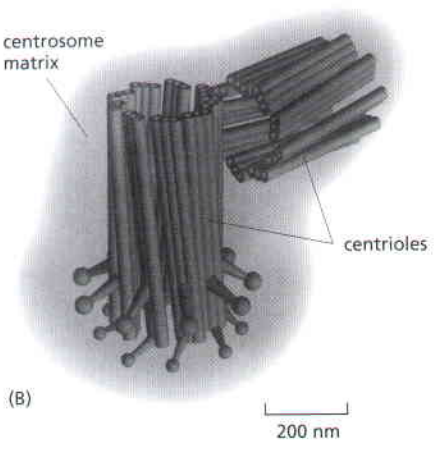
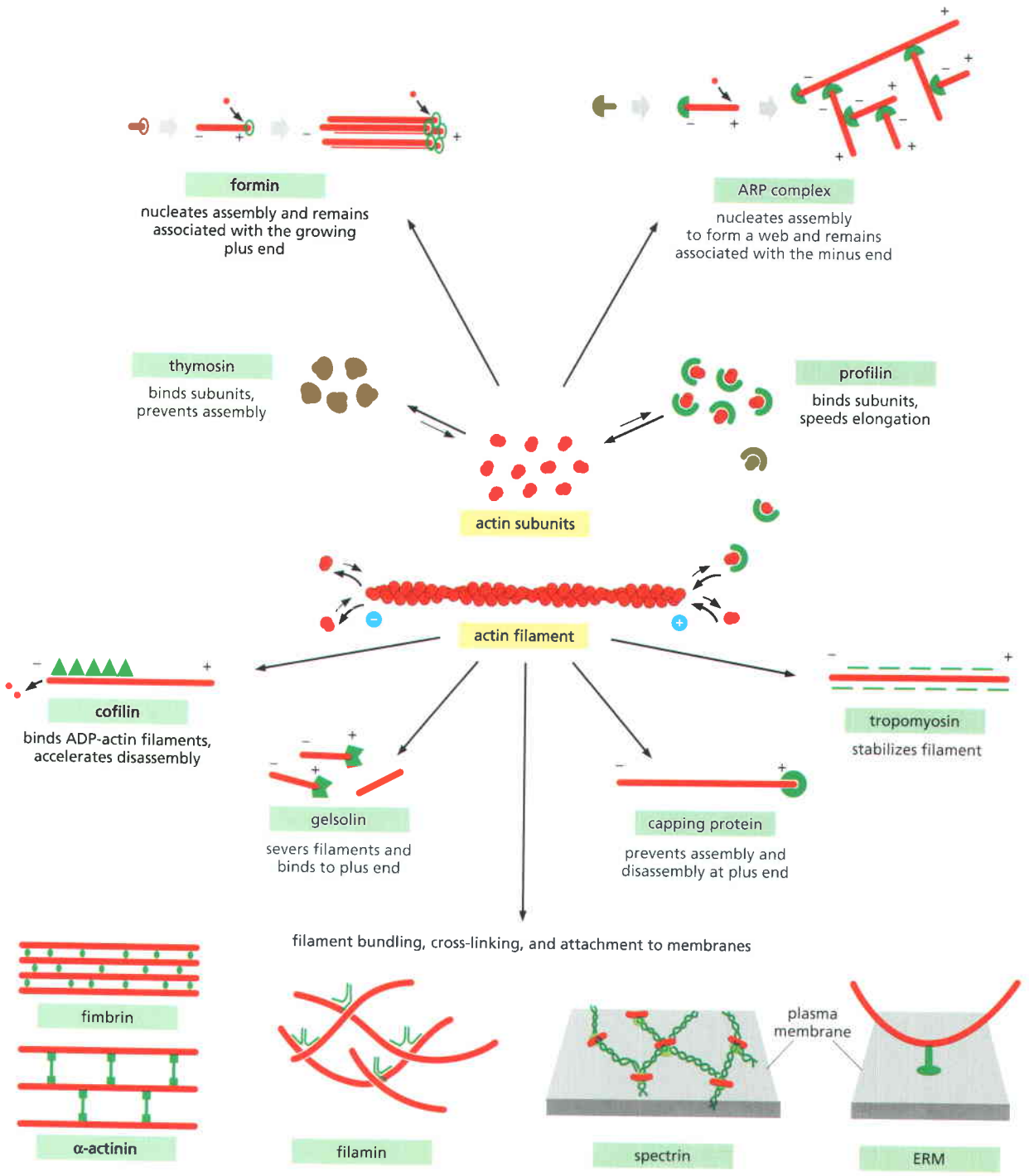


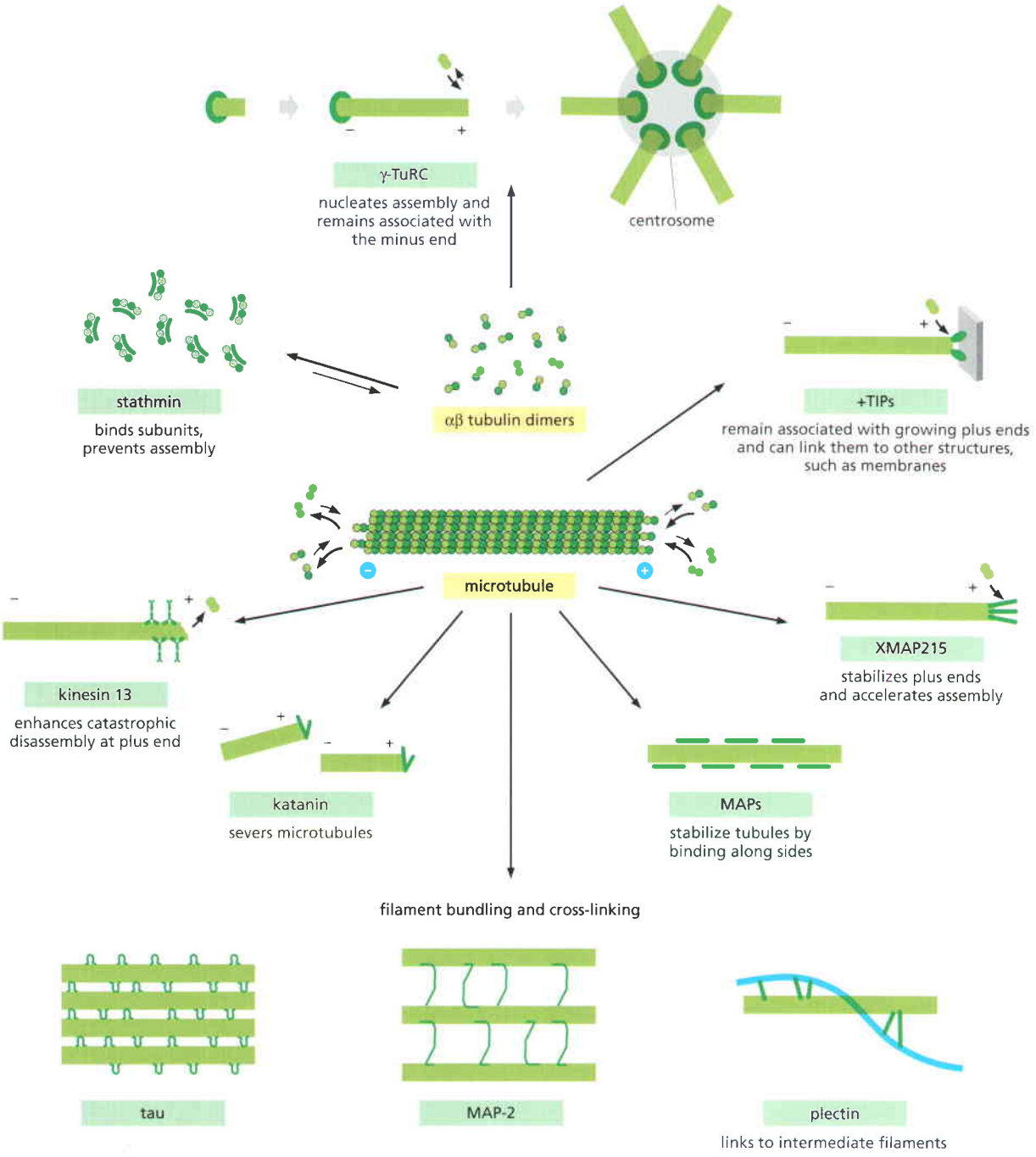
Figure 16–31 A centriole in the centrosome. (A) An electron micrograph of a thin section of a centrosome showing an end-on view of the mother centriole and a longitudinal section of the daughter centriole. Numerous microtubules are seen nearby. (B) Structure of the centriole pair. (A, from G.J. Mack, Y. Ou and J.B. Rattner, *Microsc. Res. Tech.* 49:409–419, 2000. With permission from John Wiley & Sons. B, adapted from D. Chrétien et al., *J. Struct. Biol.* 120:117–133, 1997. With permission from Elsevier.)

ACTIN FILAMENTS



Some of the major accessory proteins of the actin cytoskeleton. Except for the myosin motor proteins, to be discussed in a later section, 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.

MICROTUBULES



Some of the major accessory proteins of the microtubule cytoskeleton. Except for two classes of motor proteins, to be discussed in a later section, 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.

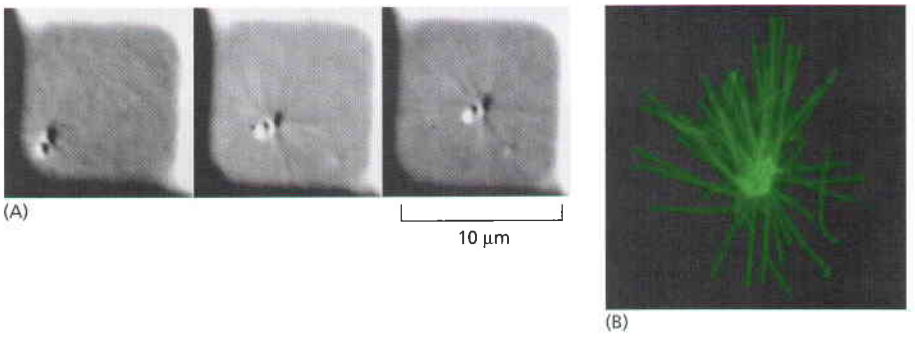


Figure 16–32 The center-seeking behavior of a centrosome. (A) Small square wells were micromachined into a plastic substrate. A single centrosome was placed into one of these wells, along with tubulin subunits in solution. As the microtubules polymerize, nucleated by the centrosome, they push against the walls of the well. The requirement for equal pushing in all directions to stabilize the position forces the centrosome to the center of the well. These pictures were taken at three-minute intervals. (B) A self-centered centrosome, fixed and stained to show the distribution of the microtubules pushing on all four walls of the enclosure. (From T.E. Holy et al., *Proc. Natl Acad. Sci. U.S.A.* 94:6228–6231, 1997. With permission from National Academy of Sciences.)

(**Figure 16–32**). Even in an isolated cell fragment lacking the centrosome, dynamic microtubules interacting with membraneous organelles arrange themselves into a star-shaped array with the microtubule minus ends clustered at the center, although this process may involve more components than just the simple pushing mechanism used by the isolated centrosome (**Figure 16–33**). 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 apical plasma membrane. From this asymmetric location, an array of nearly parallel microtubules forms along the long axis of the cell, with plus ends extending as far as the basal surface (see Figure 16–5).

Actin Filaments Are Often Nucleated at the Plasma Membrane

In contrast to microtubule nucleation, which occurs primarily deep within the cytoplasm near the nucleus, actin filament nucleation most frequently occurs at or near the plasma membrane. Consequently, 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 it determine the shape and movement of the cell surface. For example, depending on their attachments to one another and to the plasma membrane, actin structures can form many strikingly different types of cell surface projections. These include spiky bundles such as *microvilli* or *filopodia*, flat protrusive veils called *lamellipodia* that help move cells over solid substrates, and the phagocytic cups in macrophages.

External signals frequently regulate the nucleation of actin filaments at the plasma membrane, allowing the cell to change its shape and stiffness rapidly in response to changes in its external environment. This nucleation can be catalyzed

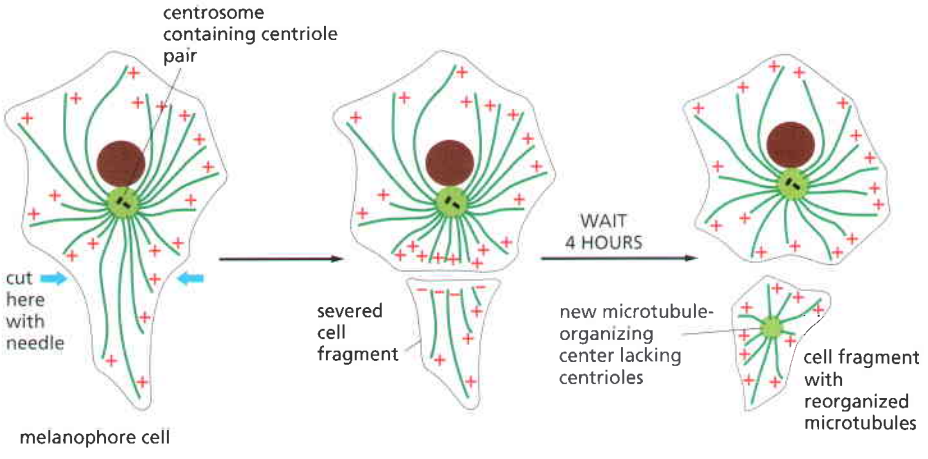


Figure 16–33 A microtubule array can find the center of a cell. After the arm of a fish pigment cell is cut off with a needle, the microtubules in the detached cell fragment reorganize so that their minus ends end up near the center of the fragment, buried in a new microtubule-organizing center.

by two different types of regulated factors, the ARP complex and the formins (discussed below). 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. Analogous to the function of the γ-TuRC, the **ARP complex** (also known as the *Arp 2/3 complex*) nucleates actin filament growth from the minus end, allowing rapid elongation at the plus end (**Figure 16–34**A and B). The complex can also

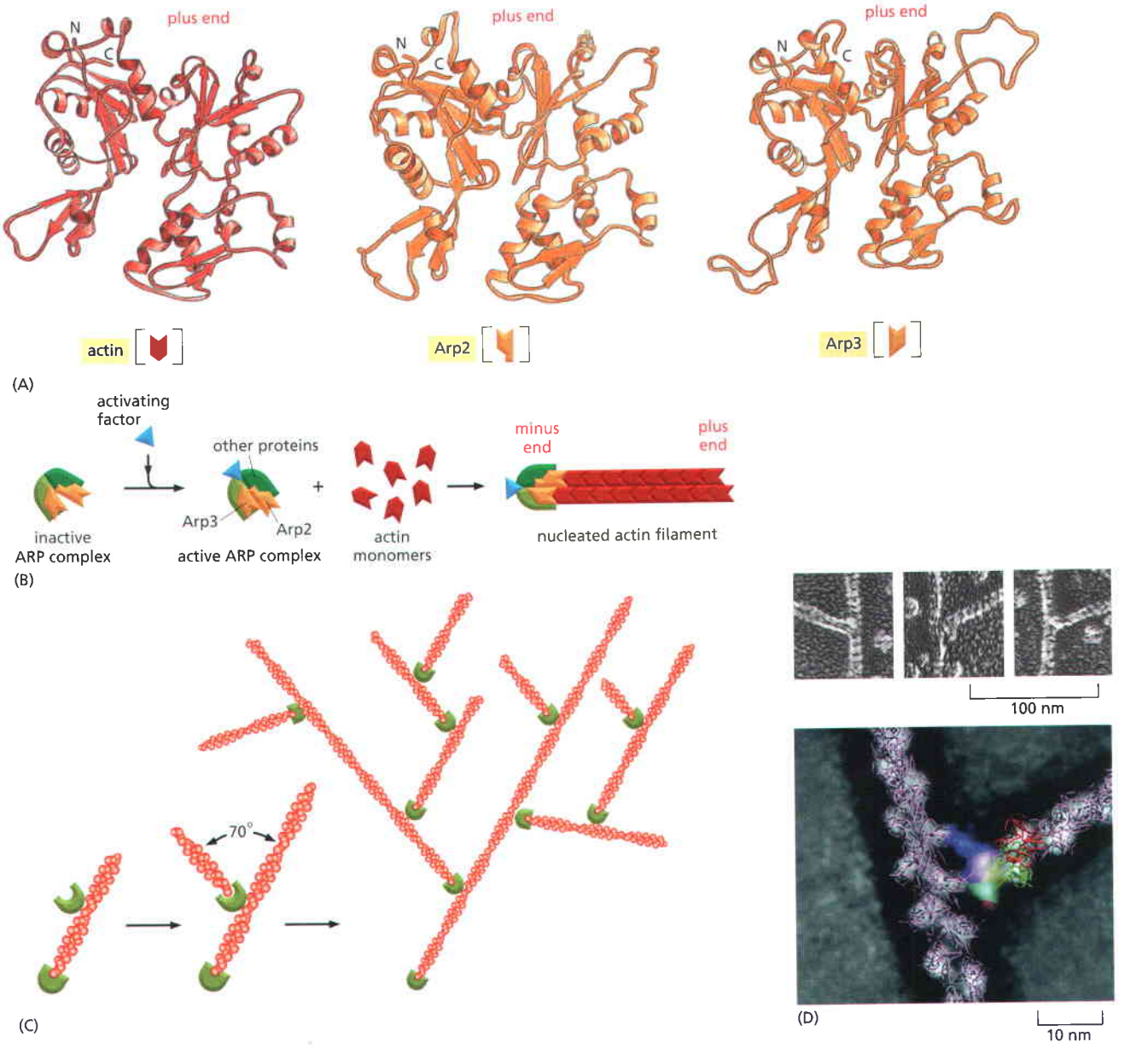


Figure 16–34 Nucleation and actin web formation by the ARP complex. (A)The structures of Arp2 and Arp3, compared to the structure of actin. Although the face of the molecule equivalent to the plus end *(top)* in both Arp2 and Arp3 is very similar to the plus end of actin itself, differences on the sides and minus end *(bottom)* 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 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 (see Figure 16–10). (C) The ARP 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 complexes. Bottom, reconstructed image of a branch where the crystal structures of actin and the ARP 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 complex binds to three actin subunits in the mother filament (D, from R.D. Mullins et al., *Proc. Natl Acad. Sci. U.S.A.* 95:6181–6186, 1998. With permission from National Academy of Sciences, and from N. Volkmann et al., *Science* 293:2456–2459, 2001. With permission from Macmillan Publishers Ltd.)

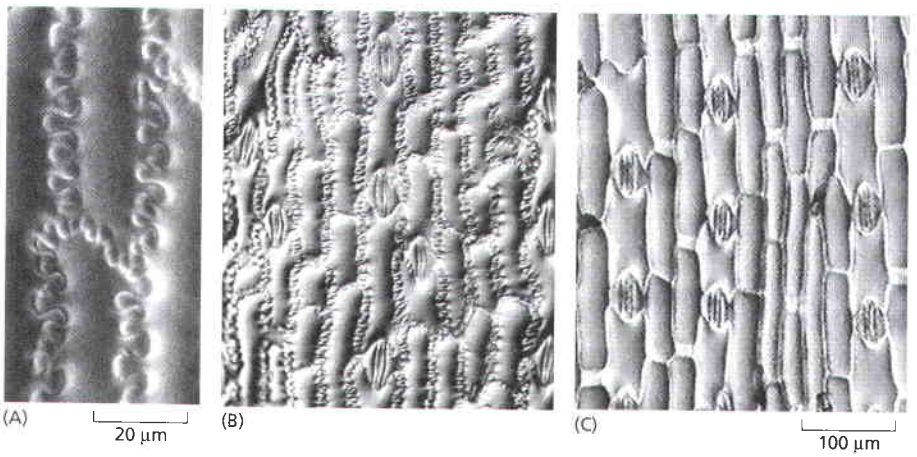


Figure 16–35 Function of the ARP complex in plant cells. (A) Cells in the maize leaf epidermis form small, actin-rich lobes that lock neighboring cells together like pieces of a jigsaw puzzle. (B) The regular pattern of interlocking cells covers the leaf surface. (C) Epidermal cells in a mutant plant lacking the ARP complex do not form the interlocking lobes. The brick-shaped cells are normal in size and spacing, but form leaves that appear too shiny to the naked eye. (From M.J. Frank, H.N. Cartwright and L.G. Smith, *Development* 130:753–762, 2003. With permission from the Company of Biologists.)

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 16–34C and D).

In animals, the ARP complex is associated with structures at the leading edge of migrating cells. The complex is localized in regions of rapid actin filament growth such as lamellipodia, and intracellular signaling molecules and components at the cytosolic face of the plasma membrane regulate its nucleating activity. This conserved complex is also involved in actin filament nucleation near the plasma membrane in yeast, where it is required to form cortical actin patches (see Figure 16–6), and in plant cells, where it directs the formation of actin bundles at the surface that are required for the growth of complex cell shapes in a variety of different tissues (**Figure 16–35**).

Both γ-tubulin and ARPs are evolutionarily ancient, and they are conserved among a wide variety of eucaryotic species. Their genes seem to have arisen by early duplication of the gene for the microtubule or actin filament subunit, respectively, followed by divergence and specialization of the gene copies so that they encode proteins with a special nucleating function. Thus, a similar strategy has evolved for two separate cytoskeletal systems. This underlines the central importance of regulated filament nucleation as a general organizing principle in cells.

The Mechanism of Nucleation Influences Large-Scale Filament Organization

Because the ARP complex nucleates the growth of a new actin filament most efficiently when it is bound to the side of an old actin filament, regulated activation of the ARP complex in animal cells tends to lead to the assembly of gel-like branched actin networks. However, many of the large-scale actin structures seen in cells are made up of parallel bundles of unbranched actin filaments, including the cleavage furrow found at the equator of dividing cells (see Figure 16–2) and the actin cables that point toward the site of bud growth in yeast (see Figure 16–6). The formation of many of these actin bundles is induced by a different set of nucleating proteins, the *formins*, which are able to nucleate the growth of straight, unbranched filaments that can be cross-linked by other proteins to form parallel bundles.

Formins are a large family of dimeric proteins (about 15 distinct formins are encoded in the mouse genome). Each formin subunit has a binding site for monomeric actin, and the formin dimer appears to nucleate actin filament polymerization by capturing two monomers. As the newly nucleated filament grows, the formin dimer remains associated with the rapidly growing plus end, while still allowing the binding of new subunits at that end to elongate the filament (**Figure 16–36**). This is very different from the behavior of the ARP complex or the γ-TuRC, which remain stably bound to the minus end of the actin filament or microtubule and prevent both subunit addition and subunit loss at this end.

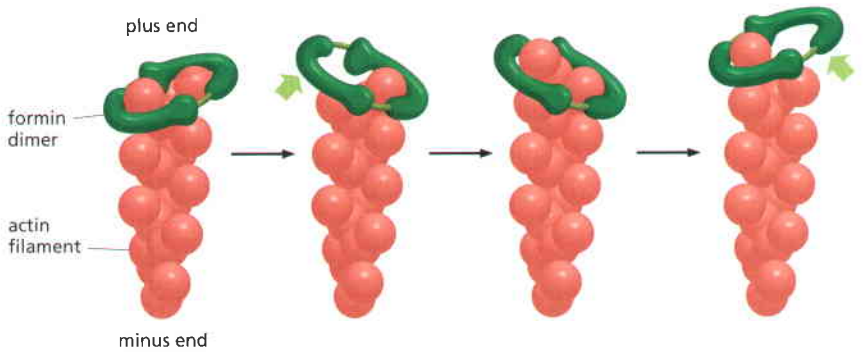


Figure 16–36 Actin elongation mediated by formins. Formin proteins (*green*) form a dimeric complex that can nucleate the formation of a new actin filament (*red*) and remain associated with the rapidly-growing plus end as it elongates. The formin protein maintains its binding to one of the two actin subunits exposed at the plus end as it allows each new subunit to assemble. Only part of the large 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.

Proteins That Bind to the Free Subunits Modify Filament Elongation

Once nucleated, cytoskeletal filaments generally elongate by the addition of soluble subunits. In most nonmuscle vertebrate cells, approximately 50% of the actin is in filaments and 50% is soluble, although this ratio can change rapidly in response to external signals. The soluble monomer concentration is typically 50–200 μM (2–8 mg/ml); this is surprisingly high, given the critical concentration of less than 1 μM observed for pure actin in a test tube. Why does so much of the actin remain soluble, rather than polymerizing into filaments? The reason is that the subunit pool contains special proteins that bind to the actin monomers, thereby making 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 filament 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? It might seem as if signal transduction pathways such as those discussed in Chapter 15 could regulate thymosin, but this has not been found to be the case. Instead, recruitment depends on another monomer-binding protein, *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 16–37**). The profilin–actin complex can readily add onto a free plus end. This addition induces a conformational change in the actin that reduces its affinity for profilin, so the profilin falls off, leaving the actin filament one subunit longer. Because profilin competes with thymosin in binding to individual actin monomers, the net result of a local activation of profilin molecules is a movement of actin subunits from the sequestered thymosin-bound pool onto filament plus ends. Actin filament growth depends even more strongly on profilin activation for those filaments whose plus end is associated with certain formins (the family of actin-nucleating proteins discussed above); in these cases, actin filament elongation can require that the monomeric actin be bound to profilin (**Figure 16–38**).

Several intracellular 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's ability to move sequestered actin subunits onto the growing ends of filaments is critical for filament assembly at the plasma membrane. Profilin is localized at the cytosolic face of the plasma membrane because it binds to acidic membrane phospholipids there. At this location, extracellular signals can activate profilin to produce explosive local actin polymerization and the extension of actin-rich

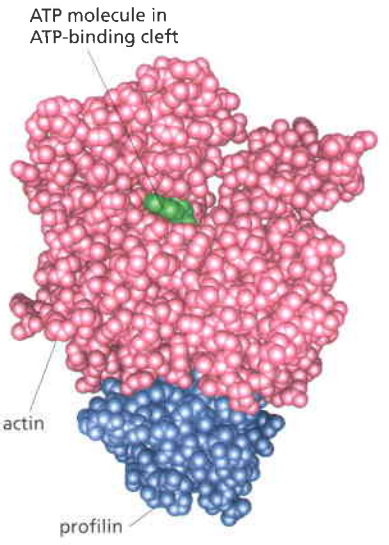


Figure 16–37 Profilin bound to an actin monomer. The profilin protein molecule is shown in *blue*, and the actin in *red*. ATP is shown in *green*. Profilin binds to the face of actin opposite the ATP-binding cleft. This profilin–actin heterodimer can therefore bind to and elongate the plus end of an actin filament, but it is sterically prevented from binding to the minus end. (Courtesy of Michael Rozycki and Clarence E. Schutt.)

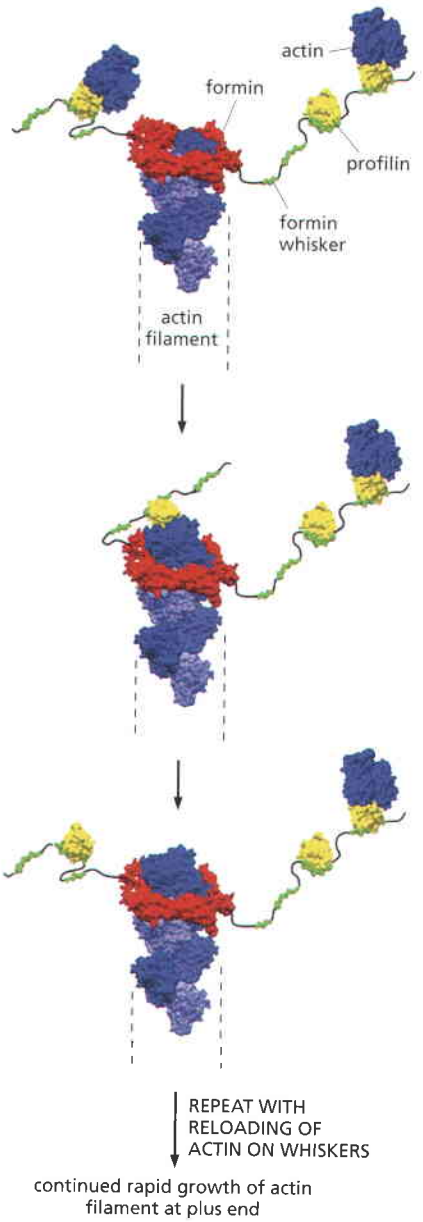


Figure 16–38 Profilin and formins. Some members of the formin protein family have unstructured domains or "whiskers" that contain several binding sites for profilin or the profilin-actin complex. These flexible domains serve as a staging area for addition of actin to the growing plus end of the actin 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 3–80C).

motile structures such as filopodia and lamellipodia (see below). Besides binding to actin and phospholipids, profilin also binds to various other intracellular proteins that have domains rich in proline; these proteins can also help to localize profilin to sites that require rapid actin assembly.

As it does with actin monomers, the cell sequesters unpolymerized tubulin subunits to maintain the subunit pool at a level substantially higher than the critical concentration. One molecule of the small protein *stathmin* binds to two tubulin heterodimers and prevents their addition onto the ends of microtubules. Stathmin thus decreases the effective concentration of tubulin subunits that are available for polymerization (an action analogous to that of the drug colchicine). Furthermore, stathmin enhances the likelihood that a growing microtubule will undergo the catastrophic transition 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. Cancer cells frequently overexpress stathmin, and the increased rate of microtubule turnover that results is thought to contribute to the characteristic change in cell shape associated with malignant transformation.

Severing Proteins Regulate the Length and Kinetic Behavior of Actin Filaments and Microtubules

In some situations, a cell may break an existing long filament into many smaller filaments. This generates a large number of new filament ends: one long filament with just one plus end and one minus end might be broken into dozens of short filaments, each with its own minus end and plus end. Under some intracellular conditions, these newly formed ends nucleate filament elongation, and in this case severing accelerates 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 filaments changes the physical and mechanical properties of the cytoplasm: stiff, large bundles and gels become more fluid when the filaments are severed.

To sever a microtubule, thirteen longitudinal bonds must be broken, one for each protofilament. The protein *katanin,* named after the Japanese word for "sword," accomplishes this demanding task (**Figure 16–39**). 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 it is thought to have an important role in the rapid microtubule depolymerization observed at the poles of spindles during meiosis and mitosis. It may also be involved in microtubule release and depolymerization in proliferating cells in interphase and in postmitotic cells such as neurons.

In contrast to microtubule severing by katanin, which requires ATP, the severing of actin filaments does not require an extra energy input. Most actin-severing proteins are members of the *gelsolin superfamily,* whose severing activity is activated by high levels of cytosolic Ca^{2+}. Gelsolin has subdomains that bind to two different sites on the actin subunit, one exposed on the surface of the filament and one that is normally hidden in the longitudinal bond to the next subunit in the protofilament. According to one model for gelsolin severing, gelsolin binds on the side of an actin filament and waits until a thermal fluctuation happens to create a small gap between neighboring subunits in the protofilament; gelsolin then insinuates its subdomain into the gap, breaking the filament.

Proteins That Bind Along the Sides of Filaments Can Either Stabilize or Destabilize Them

Once a cytoskeletal filament is formed by nucleation and elongated from the subunit pool, a set of proteins that bind along the sides of the polymer may alter the filament's stability and mechanical properties. Different filament-associated proteins use their binding energy to either lower or raise the free energy of the polymer state, and they thereby either stabilize or destabilize the polymer, respectively.

Proteins that bind along the sides of microtubules are collectively called **microtubule-associated proteins**, or **MAPs**. Like the drug taxol, 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 16–40**). 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 16–41**). Tau binding to filaments can also regulate the transport of membrane-enclosed organelles driven by molecular motors, which we will discuss later.

MAPs are the targets of several protein kinases, and the resulting phosphorylation of a MAP can have a primary role in controlling both its activity and localization inside cells. Among the important protein kinases that can regulate MAPs are those that are turned on and off as cells progress through the cell cycle (discussed in Chapter 17). In particular, MAP activities regulate the changes in microtubule dynamics that occur as the cell rearranges its microtubule cytoskeleton to form the mitotic spindle in preparation for chromosome segregation (see Figure 16–2).

In addition to binding along the sides of microtubules, tau protein forms its own helical filaments when present at sufficiently high concentrations. The nerve cell cytoplasm in the brains of people with Alzheimer's disease contains large aggregates of tau filaments, called neurofibrillary tangles. It is not yet clear whether these tangles of tau are a cause or a consequence of the neurodegeneration associated with this disease.

The binding of accessory proteins along their sides also affects actin filaments. Selected actin filaments in most cells are stabilized by the binding of *tropomyosin*, an elongated protein that binds simultaneously to seven adjacent actin subunits in one protofilament. The binding of tropomyosin along an actin filament can prevent the filament from interacting with other proteins; for this reason, the regulation of tropomyosin binding is an important step in muscle contraction, as we discuss later (see Figure 16–78).

Another important actin-filament binding protein present in all eucaryotic cells is *cofilin*, which destabilizes actin filaments. Also called *actin depolymerizing factor*, cofilin is unusual in that it binds to actin in both the filament and free subunit forms. Cofilin binds along the length of the actin filament, forcing the filament to twist a little more tightly (**Figure 16–42**). This mechanical stress

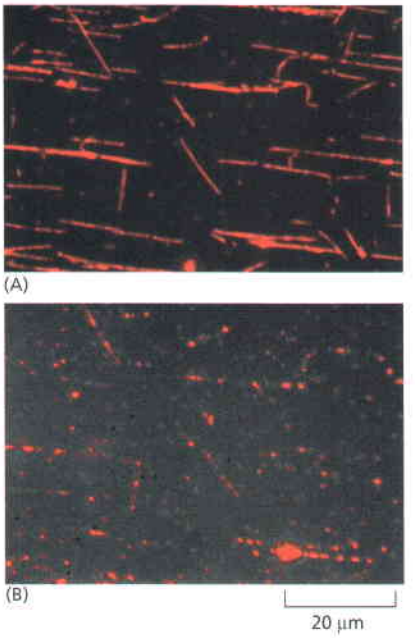


Figure 16–39 Microtubule severing by katanin. Taxol-stabilized, rhodamine-labeled microtubules were adsorbed on the surface of a glass slide, and purified katanin was added along with ATP. (A) There are a few breaks in the microtubules 30 seconds after the addition of katanin. (B) The same field 3 minutes 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 16–40 Localization of MAPs in axon and dendrites of a neuron. This immunofluorescence micrograph shows the distribution of tau staining *(green)* and MAP2 staining *(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.)

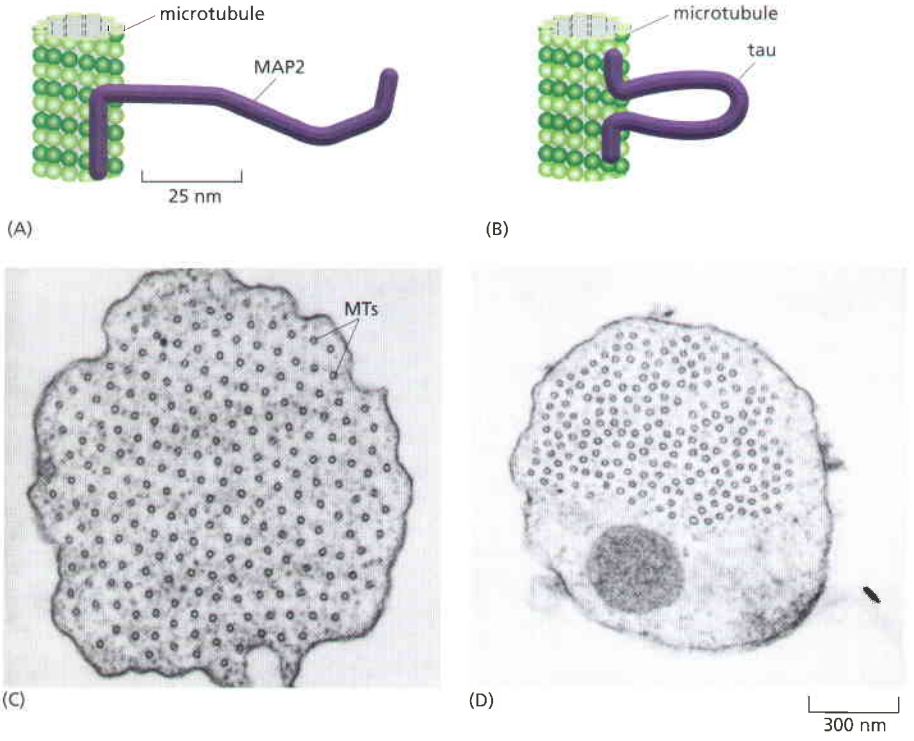


Figure 16–41 Organization of microtubule bundles by MAPs. (A) MAP2 binds along the microtubule lattice at one of its ends and extends a long projecting arm with a second microtubule-binding domain at the other end. (B) Tau binds to the microtubule lattice at both its N- and C-termini, with a short projecting loop. (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 result 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 V. Chen et al., *Nature* 360:674–647, 1992. With permission from Macmillan Publishers Ltd.)

weakens the contacts between actin subunits in the filament, making the filament brittle and more easily severed by thermal motions. In addition, it makes it much easier for an ADP-actin subunit to dissociate from the minus end of the filament. These activities greatly accelerate actin filament disassembly. As a result, most of the actin filaments inside cells are much shorter-lived than are filaments formed from pure actin in a test tube. Actin filaments can be protected from cofilin by tropomyosin binding.

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 efficiently dismantles the older filaments in the cell, ensuring that all actin filaments turn over rapidly. 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 responsible for unidirectional cell crawling.

Proteins That Interact with Filament Ends Can Dramatically Change Filament Dynamics

As we have just seen, proteins that bind along the side of a filament can change the filament's dynamic behavior. For maximum effect, however, these proteins often need to coat the filament completely, and this means they have to be present at fairly high stoichiometries (for example, about one tropomyosin for every

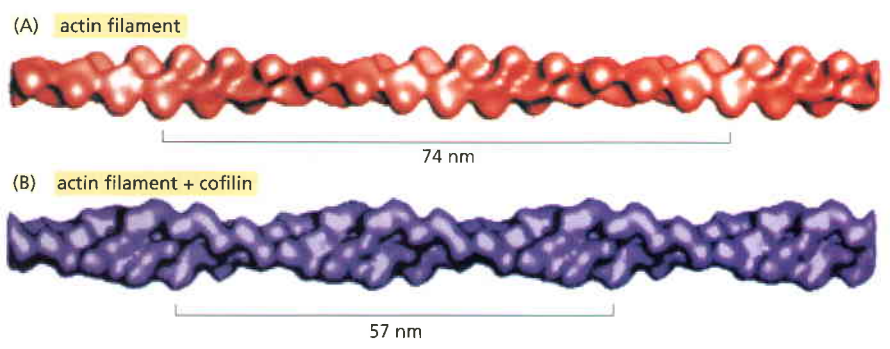


Figure 16–42 Twisting of an actin filament induced by cofilin. (A) Three-dimensional reconstruction from cryo-electron micrographs of filaments made of pure actin. The bracket shows the span of two twists of the actin helix. (B) Reconstruction of an actin filament coated with cofilin, which binds in a 1:1 stoichiometry to actin subunits all along the filament. Cofilin is a small protein (14 kilodaltons) compared to actin (43 kilodaltons), and so the filament appears only slightly thicker. The energy of cofilin binding serves to deform the actin filament lattice, 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 Rockefeller University Press.)

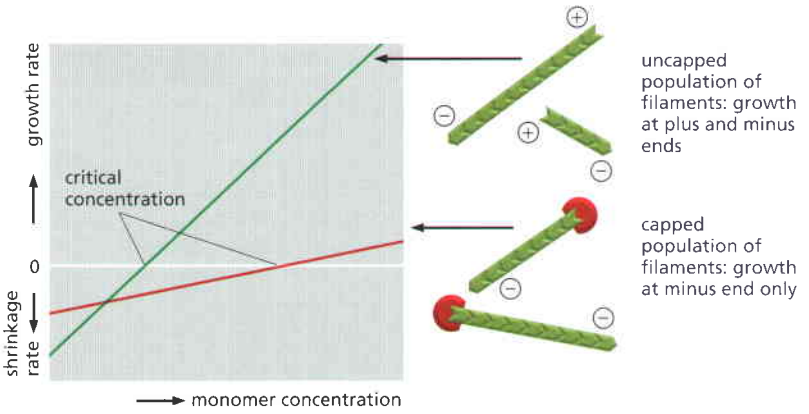


Figure 16–43 Filament capping and its effects on filament dynamics. A population of uncapped filaments adds and loses subunits at both the plus and minus ends, resulting in rapid growth or shrinkage, depending on the concentration of available free monomers (*green* line). In the presence of a protein 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.

seven actin subunits, one tau for every four tubulin subunits, or one cofilin for every actin subunit). In contrast, proteins that bind preferentially to the ends of filaments can have dramatic effects on filament dynamics even when they are present at very low levels. Since subunit addition and loss occur primarily at filament ends, one molecule of such a protein per actin filament (typically one per about 200–500 actin monomers) can be enough to transform the architecture of an actin filament network.

As previously discussed, an actin filament that ceases elongation and is not specifically stabilized by the cell can depolymerize rapidly: it can lose subunits from either its plus or its minus end, once the actin molecules at that end have hydrolyzed their ATP to convert to the D form. The most rapid changes, however, occur at the plus end. The binding of a plus end *capping protein* stabilizes an actin filament at its plus end, which greatly slows the rates of both filament growth and filament depolymerization by making the plus end inactive (**Figure 16–43**). Indeed, most of the actin filaments in a cell are capped at their plus end by proteins such as *CapZ* (named for its location in the muscle Z band, see below; it is also called Capping Protein). At the minus end, an actin filament may be capped by remaining bound to the ARP complex that was responsible for its nucleation, although it is possible that many of the actin filament minus ends in typical cells are released from the ARP complex and are uncapped.

In muscle cells, where actin filaments are exceptionally long-lived, the filaments are specially capped at both ends—by CapZ at the plus end and by *tropomodulin* at the minus end. Tropomodulin binds only to the minus end of actin filaments that have been coated by tropomyosin and have thereby already been somewhat stabilized.

Different Kinds of Proteins Alter the Properties of Rapidly Growing Microtubule Ends

The end of a microtubule, with thirteen protofilaments in a hollow ring (see Figure 16–11), is a much larger and more complex structure than the end of an actin filament, with many more possibilities for accessory protein action. We have already discussed an important microtubule capper: the γ-tubulin ring complex (γ-TuRC), which both nucleates the growth of microtubules at an organizing center and caps their minus ends. Another true capping protein for microtubules is the special protein complex found at the ends of the microtubules in cilia (discussed later), where microtubules are both stable and uniform in length.

Some proteins that act at the ends of microtubules have crucial roles beyond those expected for a simple capping protein. In particular, they can have dramatic effects on the dynamic instability of microtubules (see Figure 16–16). They can influence the 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, a family of kinesin-related proteins known as *catastrophe factors* significantly increases the catastrophe rate

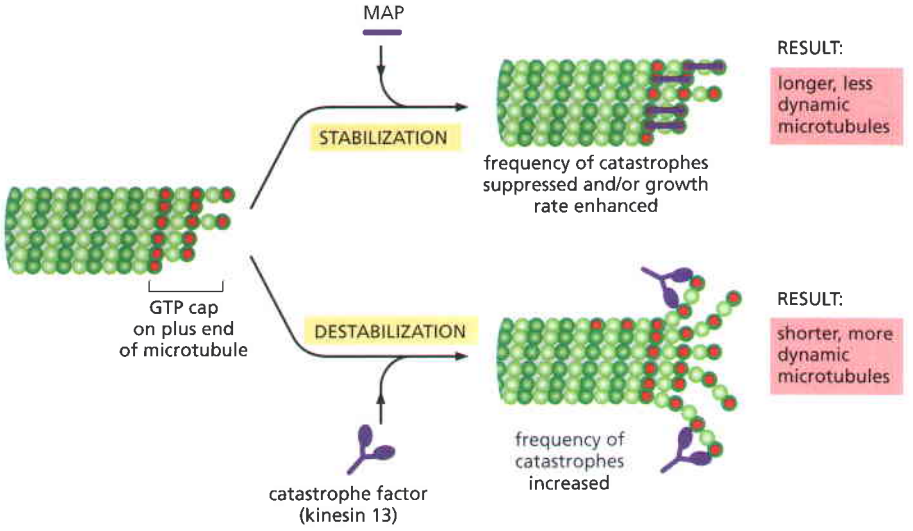


Figure 16–44 The effects of proteins that bind to microtubule ends. The transition between microtubule growth and microtubule shrinking is controlled in cells by special proteins. A MAP such as XMAP215 stabilizes the end of a growing microtubule by its preferential binding there. Opposing its action are catastrophe factors such as kinesin-13, a member of the kinesin motor protein superfamily (discussed later).

(these proteins are members of the kinesin-13 family; see Figure 16–58). They bind specifically to microtubule ends and seem to pry protofilaments apart, lowering the normal activation energy barrier that prevents a microtubule from springing apart into the curved protofilament characteristic of the shrinking state (see Figure 16–16C). Opposing their actions are MAPs such as the ubiquitous *XMAP215* that has close homologs in organisms that range from yeast to humans (XMAP stands for *Xenopus* microtubule-associated protein, and the number refers to its molecular mass in kilodaltons). This protein has a special ability to stabilize free microtubule ends and inhibit their switch from a growing to a shrinking state. The phosphorylation of XMAP215 during mitosis inhibits its activity and shifts the balance of its competition with catastrophe factors (**Figure 16–44**). The shift results in a tenfold increase in the dynamic instability of microtubules observed during mitosis, a transition that is critical for the efficient construction of the mitotic spindle (see Figure 17–33).

In many cells, the minus ends of microtubules are stabilized by association with the centrosome, or else 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 16–45**).

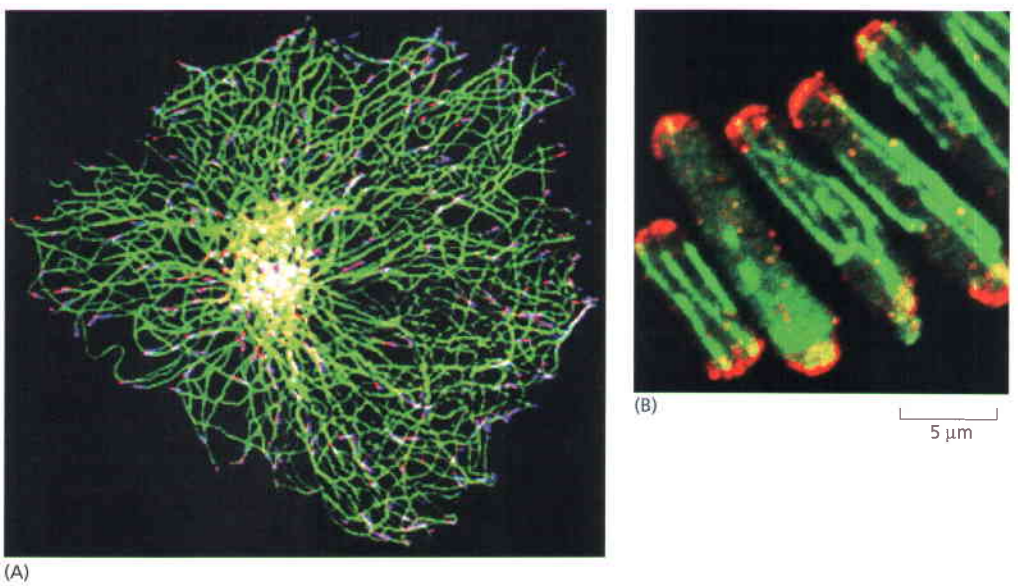


Figure 16–45 +TIP proteins found at the growing plus ends of microtubules. <TAAT> (A) In an epithelial cell grown in tissue culture, each microtubule (*green*) has a growing plus end which is associated with the +TIP protein EB1 (*red*). (B) In the rod-shaped fission yeast *Schizosaccharomyces pombe*, 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, from A. Akhmanova and C.C. Hoogenraad, *Curr. Opin. Cell Biol.* 17:47–54, 2005. With permission from Elsevier; B, courtesy of Ken Sawin.)

Some of the +TIPs, such as the kinesin-related catastrophe factors and XMAP215 mentioned above, modulate the growth and shrinkage of the microtubule end to which they are attached. Others control microtubule positioning by helping to capture and stabilize the growing microtubule end at the location of specific target proteins in the cell cortex. EB1, a +TIP present in both yeasts and humans, for example, is essential for yeast mitotic spindle positioning, directing the growing plus ends of yeast spindle microtubules to a specific docking region in the yeast bud and then helping to anchor them there.

Filaments Are Organized into Higher-Order Structures in Cells

So far, we have described how cells use accessory proteins to regulate the location and dynamic behavior of cytoskeletal filaments. These proteins can nucleate filament assembly, bind to the ends or sides of the filaments, or bind to the free subunits of filaments. But in order for the cytoskeletal filaments to form a useful intracellular scaffold that gives the cell mechanical integrity and determines its shape, the individual filaments must be organized and attached to one another in larger-scale structures. The centrosome is one example of such a cytoskeletal organizer; in addition to nucleating the growth of microtubules, it holds them together in a defined geometry, with all of the minus ends buried in the centrosome and the plus ends pointing outward. In this way, the centrosome creates the astral array of microtubules that is able to find the center of each cell (see Figure 16–32).

Another mechanism that cells use to organize filaments into large structures is filament cross-linking. As described earlier, some MAPs can bundle microtubules together: they have two domains—one that binds along the microtubule side (and thereby stabilizes the filament) and another that projects outward to contact other MAP-coated microtubules. In the actin cytoskeleton, the stabilizing and cross-linking functions are separated. Tropomyosin binds along the sides of actin filaments, but it does not have an outward projecting domain. As we shall see shortly, filament cross-linking is instead mediated by a second group of actin-binding proteins that have only this function. Intermediate filaments are different yet again; they are organized both by a lateral self-association of the filaments themselves and by the cross-linking activity of accessory proteins, as we describe next.

Intermediate Filaments Are Cross-Linked and Bundled Into Strong Arrays

Each individual intermediate filament forms as a long bundle of tetrameric subunits (see Figure 16–19). Many intermediate filaments further bundle themselves by self-association; for example, the neurofilament proteins NF-M and NF-H (see Table 16–1, p. 985) contain a C-terminal domain that extends outward from the surface of the assembled intermediate filament and binds to a neighboring filament. Thus groups of neurofilaments form robust parallel arrays that are held together by multiple lateral contacts, giving strength and stability to the long cell processes of neurons (see Figure 16–22).

Other types of intermediate filament bundles are held together by accessory proteins, such as *filaggrin*, which bundles keratin filaments in differentiating cells of the epidermis to give the outermost layers of the skin their special toughness. *Plectin* is a particularly interesting cross-linking protein. Besides bundling intermediate filaments, it also links the intermediate filaments to microtubules, actin filament bundles, and filaments of the motor protein myosin II (discussed below), as well as helping to attach intermediate filament bundles to adhesive structures at the plasma membrane (**Figure 16–46**).

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

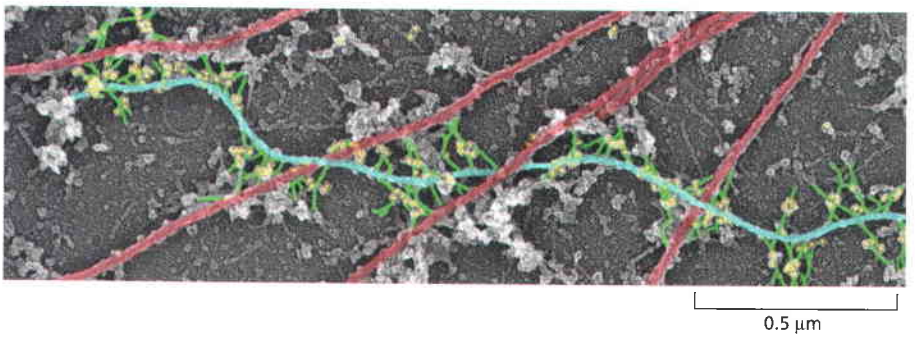


Figure 16–46 Plectin cross-linking of diverse cytoskeletal elements. Plectin *(green)* is seen here making cross-links from intermediate filaments *(blue)* to microtubules *(red)*. In this electron micrograph, the dots *(yellow)* are gold particles linked to anti-plectin antibodies. The entire actin filament network was removed to reveal these proteins. (From T.M. Svitkina and G.G.Borisy, *J. Cell Biol.* 135:991–1007, 1996. With permission from The Rockefeller University Press.)

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.

Cross-linking Proteins with Distinct Properties Organize Different Assemblies of Actin Filaments

Actin filaments in animal cells are organized into two types of arrays: bundles and weblike (gel-like) networks (**Figure 16–47**). As described earlier, these different structures are initiated by the action of distinct nucleating proteins: the long straight filaments produced by formins make bundles and the ARP complex makes webs. The actin filament cross-linking proteins that help to stabilize and maintain these distinct structures are divided into two classes: *bundling proteins* and *gel-forming proteins.* Bundling proteins cross-link actin filaments into a parallel array, while gel-forming proteins hold two actin filaments together at a large angle to each other, thereby creating a looser meshwork. Both types of cross-linking protein 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 16–48**). The spacing and arrangement of these two filament-binding domains determines 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 an actin filament. Myosin II (discussed later) is the motor protein in stress fibers and other contractile arrays that enables them to contract. The

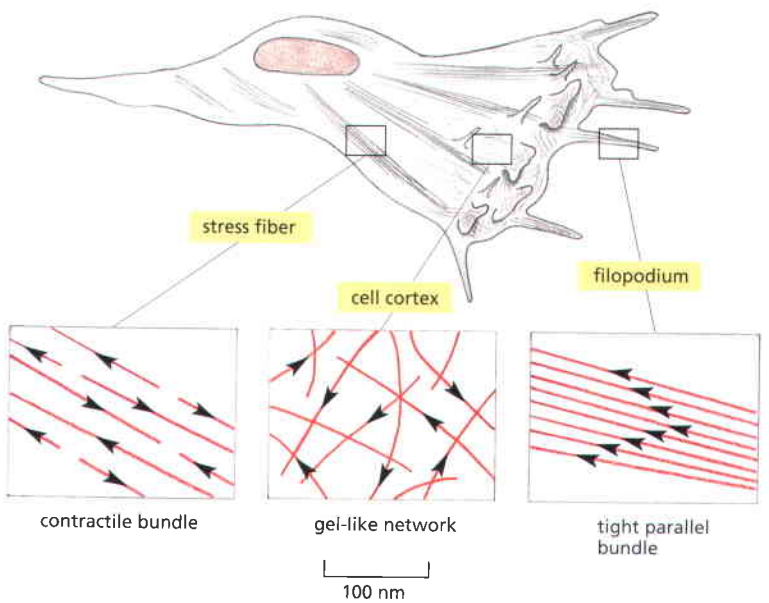


Figure 16–47 Actin arrays in a cell. A fibroblast crawling in a tissue culture dish is shown with three areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in *red,* with arrowheads pointing toward the minus end. Stress fibers are contractile and exert tension. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environment. The cortex underlies the plasma membrane.

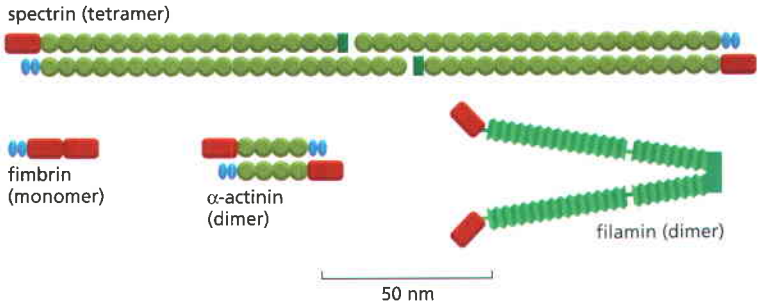


Figure 16–48 The modular structures of four actin-cross-linking proteins. Each of the proteins shown has two actin-binding sites *(red)* that are related in sequence. Fimbrin has two directly adjacent actin-binding sites, so that it holds its two actin filaments very close together (14 nm apart), aligned with the same polarity (see Figure 16–49A). 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 16–49A). 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 16–51). 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 10–41).

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, the looser packing caused by the larger dimeric bundling protein *α-actinin* allows myosin molecules to enter, making stress fibers contractile (**Figure 16–49**). Because of the very different spacing between the actin filaments, bundling by fimbrin automatically discourages bundling by α-actinin, and vice-versa, so that the two types of bundling protein are themselves mutually exclusive.

Villin is another bundling protein that, like fimbrin, has two actin-filament-binding sites very close together in a single polypeptide chain. Villin (together with fimbrin) helps cross-link the 20 to 30 tightly bundled actin filaments found in microvilli, the finger-like extensions of the plasma membrane on the surface of many epithelial cells (**Figure 16–50**). A single absorptive epithelial cell in the human small intestine, for example, has several thousand microvilli on its apical surface. Each is about 0.08 μm wide and 1 μm long, making the cell's absorptive surface area about 20 times greater than it would be without microvilli. When villin is introduced into cultured fibroblasts, which do not normally contain villin and have only a few small microvilli, the existing microvilli become greatly elongated and stabilized, and new ones are induced. The actin filament core of the microvillus is attached to the plasma membrane along its sides by lateral sidearms made of *myosin I* (discussed later), which has a binding site for filamentous actin on one end and a domain that binds lipids on the other end. These two types of cross-linkers, one binding actin filaments to each other and the other binding these filaments to the membrane, seem to be sufficient to form microvilli on cells. Interestingly, when the gene for villin is disrupted in a mouse, the intestinal microvilli form with apparently normal morphology, indicating that other bundling proteins provide sufficient redundant function for this purpose. However, the remodeling of intestinal microvilli in response to certain kinds of stress or starvation is impaired.

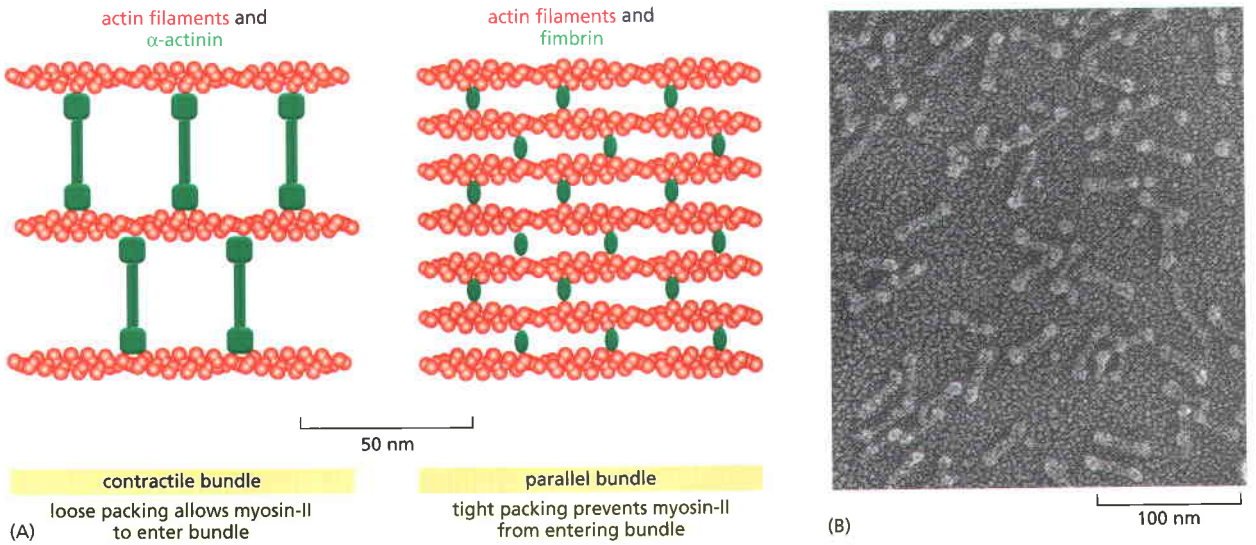


Figure 16–49 The formation of two types of actin filament bundles. (A) α-actinin, which is a homodimer, cross-links actin filaments into loose bundles, which allow the motor protein myosin II (not shown) to participate in the assembly. Fimbrin cross-links actin filaments into tight bundles, which exclude myosin. 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.)

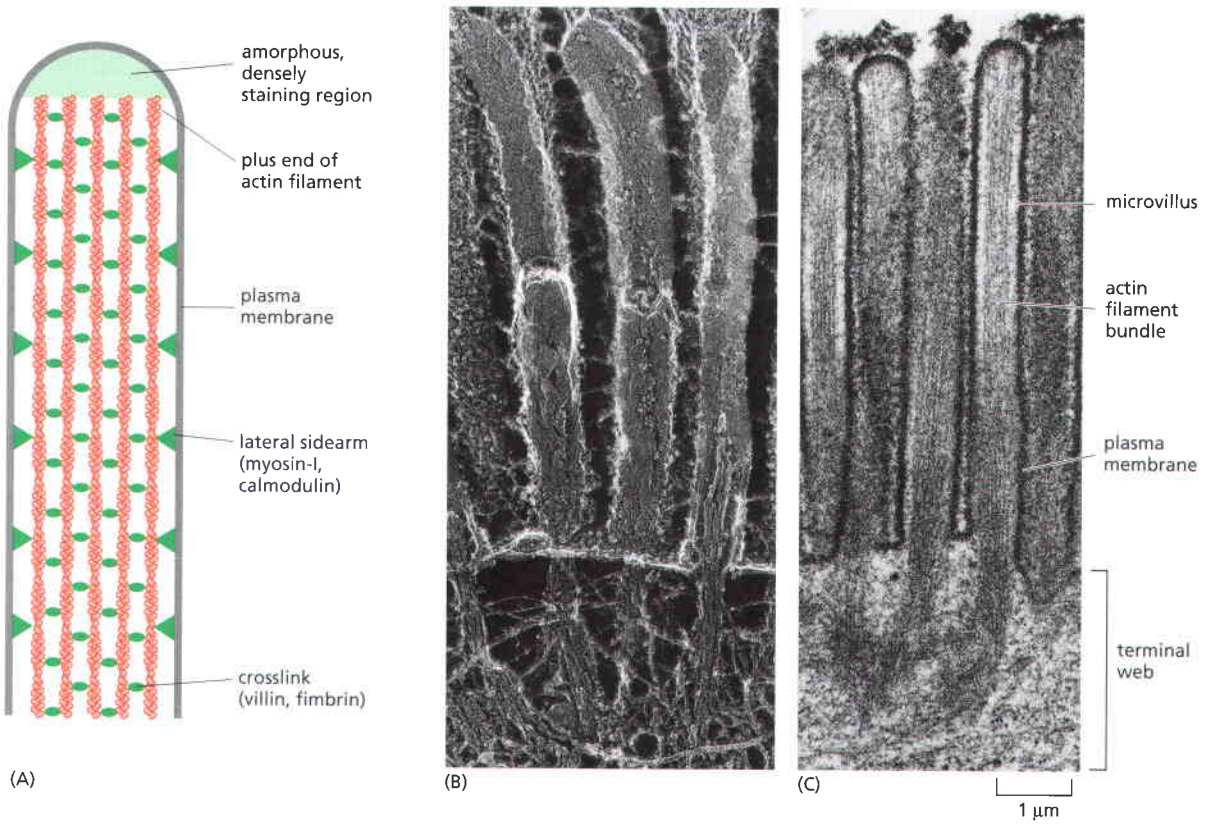


Figure 16–50 **A microvillus.** (A) A bundle of parallel actin filaments cross-linked by the actin-bundling proteins villin and fimbrin forms the core of a microvillus. Lateral sidearms (composed of myosin I and the Ca^{2+}-binding protein calmodulin) connect the sides of the actin filament bundle to the overlying plasma membrane. All the plus ends of the actin filaments are at the tip of the microvillus, where they are embedded in an amorphous, densely staining substance of unknown composition. (B) Freeze-fracture electron micrograph of the apical surface of an intestinal epithelial cell, showing microvilli. Actin bundles from the microvilli extend down into the cell and are rooted in the terminal web, where they are linked together by a complex set of proteins that includes spectrin and myosin II. Below the terminal web is a layer of intermediate filaments. (C) Thin section electron micrograph of microvilli. (B, courtesy of John Heuser; C, from P.T. Matsudaira and D.R. Burgess, *Cold Spring Harb. Symp. Quant. Biol.* 46:845–854, 1985. With permission from Cold Spring Harbor Laboratory Press.)

Filamin and Spectrin Form Actin Filament Webs

The various bundling proteins that we have discussed so far have straight, stiff connections between their two actin-filament-binding domains, and they tend to align filaments in parallel bundles. In contrast, those actin cross-linking proteins that have either a flexible or a stiff, bent connection between their two binding domains form actin filament webs or gels, rather than actin bundles.

Any cross-linking protein that has its two actin-binding domains joined by a long bent linkage can form three-dimensional actin gels. *Filamin* (see Figure 16–48) promotes the formation of a loose and highly viscous gel by clamping together two actin filaments roughly at right angles (**Figure 16–51**). Cells require the actin gels formed by filamin in order to extend the thin sheet-like membrane projections called *lamellipodia* that help them to crawl across solid surfaces. Filamin is lacking in some types of cancer cells, especially some malignant melanomas (pigment-cell cancers). These cells cannot crawl properly, and instead they protrude disorganized membrane blebs (**Figure 16–52**). Losing filamin is bad for the melanoma cells but good for the melanoma patient; because of the cells' inability to crawl, melanoma cells that have lost filamin expression are less invasive than similar melanoma cells that still express filamin, and, as a result, the cancer is much less likely to metastasize.

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

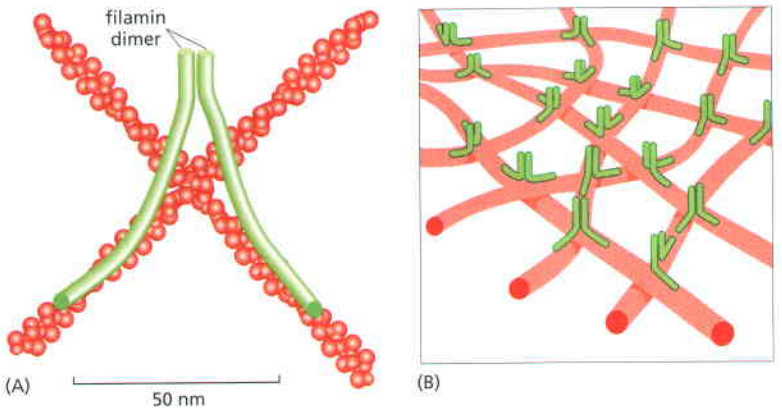


Figure 16–51 Filamin cross-links actin filaments into a three-dimensional network with the physical properties of a gel. (A) Each filamin homodimer is about 160 nm long when fully extended and forms a flexible, high-angle link between two adjacent actin filaments. (B) A set of actin filaments cross-linked by filamin forms a mechanically strong web or gel.

with 14 nm for fimbrin and about 30 nm for α-actinin, see Figure 16–48). In the red blood cell, spectrin is concentrated just beneath the plasma membrane, where it forms a two-dimensional web held together by short actin filaments; 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 10–41). The resulting network creates a stiff 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.

Cytoskeletal Elements Make Many Attachments to Membranes

Actin cytoskeletal structures both stiffen and change the shape of the plasma membrane. We have already discussed two examples: the spectrin-actin web that underlies the plasma membranes and the villin-actin bundles in microvilli that enlarge the absorptive surface area of epithelial cells. The effectiveness of these structures requires specific attachments between the actin filament network and proteins or lipids of the plasma membrane.

The connections of the cortical actin cytoskeleton to the plasma membrane are only partially understood. A widespread family of closely related intracellular proteins, the *ERM* family (named for its first three members, ezrin, radixin, and moesin), contains members that are required for the maintenance of cell polarity and involved in exocytosis and endocytosis. The C-terminal domain of an ERM protein binds directly to the sides of actin filaments. The N-terminal domain binds to the cytoplasmic face of one or more transmembrane glycoproteins, such as CD44, the receptor for the extracellular matrix component hyaluronan.

The attachments between actin and the plasma membrane mediated by ERM proteins are regulated by both intracellular and extracellular signals. ERM proteins can exist in two conformations, an active extended conformation that oligomerizes and binds to both actin and a transmembrane protein, and an inactive folded conformation, in which the N- and C-termini are held together by an intramolecular interaction. Switching to the active conformation can be

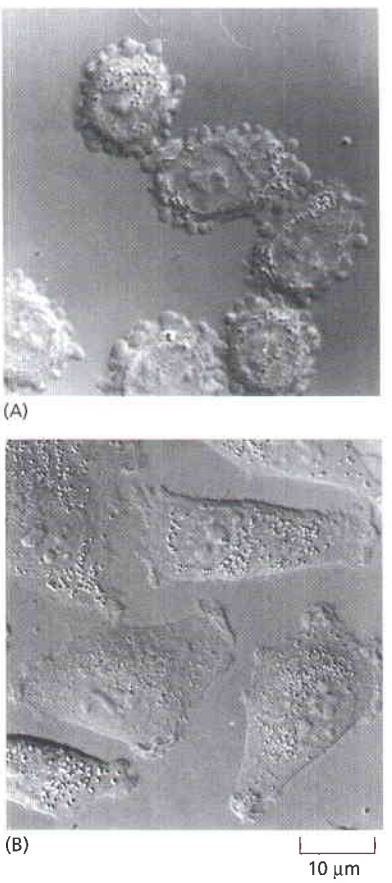


Figure 16–52 Loss of filamin causes abnormal cell motility. (A) A group of melanoma cells that have an abnormally low level of filamin. These cells are not able to make normal lamellipodia and instead are covered with membrane "blebs." As a result, they crawl poorly and tend not to metastasize. (B) The same melanoma cells in which filamin expression has been artificially restored. The cells now make normal lamellipodia and are highly metastatic. This example is one of many demonstrating the profound effect that the presence or absence of a single structural protein can have on cell morphology and motility. (From C. Cunningham et al., *J. Cell Biol.* 136:845–857, 1997. With permission The Rockefeller University Press.)

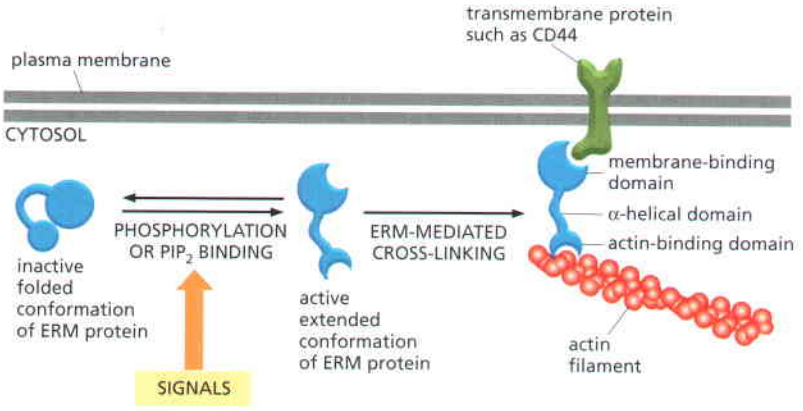


Figure 16–53 The role of ERM-family proteins in attaching actin filaments to the plasma membrane. Regulated unfolding of an ERM-family protein, caused by phosphorylation or by binding to PIP_2, exposes two binding sites, one for an actin filament and one for a transmembrane protein. Activation of ERM-family proteins can thereby generate and stabilize cell-surface protrusions that form in response to extracellular signals.

triggered by protein phosphorylation or by binding to PIP_2, either of which can occur, for example, in response to extracellular signals (the ERM proteins are direct targets of several receptor tyrosine kinases). In this way, the properties of the cell cortex become sensitive to a variety of signals received by the cell (**Figure 16–53**).

The loss of one of the members of the ERM family, called merlin, results in a form of the human genetic disease called *neurofibromatosis,* in which multiple benign tumors develop in the auditory nerves and certain other parts of the nervous system. This is one of many indications of a feedback system that connects cell structural elements to the control of cell growth (see Chapter 17).

The proteins, discussed in this section, that control the assembly and position of actin filaments and microtubules are reviewed in Panel 16–3 (pp. 994–995). Some of these proteins have the additional function of helping to connect the internal structure of a cell to other cells or to an extracellular basement membrane. Both actin filaments and intermediate filaments are critical for these connections, which require the specialized cell-cell junctions and cell-matrix junctions that we will discuss in Chapter 19.

Summary

The varied forms and functions of cytoskeletal filament structures in eucaryotic cells depend on a versatile repertoire of accessory proteins. Each of the three major filament classes (microtubules, intermediate filaments, and actin filaments) has a large dedicated subset of such accessory proteins.

A primary determinant of the sites of cytoskeletal structures is the regulation of the processes that initiate the nucleation of new filaments. In most animal cells, microtubules are nucleated at the centrosome, a complex assembly located near the center of the cell. In contrast, most actin filaments are nucleated near the plasma membrane.

The kinetics of filament assembly and disassembly can be either slowed or accelerated by accessory proteins that bind to either the free subunits or the filaments themselves. Some of these proteins alter filament dynamics by binding to the ends of filaments or by severing the filaments into smaller fragments. Another class of accessory proteins assembles the filaments into larger ordered structures by cross-linking them to one another in geometrically defined ways. Yet other accessory proteins determine the shape and adhesive properties of cells by attaching filaments to the plasma membrane.

MOLECULAR MOTORS

Among the most fascinating proteins that associate with the cytoskeleton are the molecular motors called **motor proteins**. These remarkable proteins bind to a polarized cytoskeletal filament and use the energy derived from repeated cycles of ATP hydrolysis to move steadily along it. Dozens of different motor proteins coexist in every eucaryotic 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.

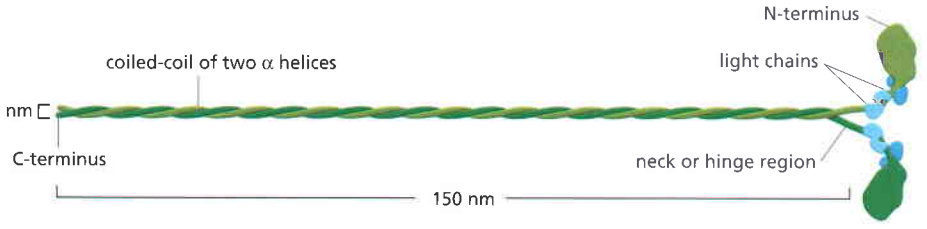
Cytoskeletal motor proteins that move unidirectionally along an oriented polymer track are reminiscent of some other proteins and protein complexes discussed elsewhere in this book, such as DNA and RNA polymerases, helicases, and ribosomes. All of these have the ability to use chemical energy to propel 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 in a protein, as explained in Chapter 3 (see Figure 3–77).

The cytoskeletal motor proteins associate with their filament tracks through a "head" region, or *motor domain*, that binds and hydrolyzes ATP. Driven by cycles of nucleotide hydrolysis that produce conformational changes, the proteins cycle between states in which they are bound strongly to their filament tracks and states in which they are unbound. Through a mechanochemical cycle of filament binding, conformational change, filament release, conformational relaxation, and filament rebinding, the motor protein and its associated cargo move one step at a time along the filament (typically a distance of a few nanometers). The motor domain (head) determines the identity of the track and the direction of movement along it, whereas the tail of the motor protein determines the identity of the cargo (and therefore the biological function of the individual motor protein).

In this section, we begin by describing the three groups of cytoskeletal motor proteins: myosins, kinesins, and dyneins. We then describe how they can work to transport membrane-enclosed organelles and mRNAs or to change the shape of structures built from cytoskeletal filaments. In the final section of this chapter, we will examine how a collaboration between motor proteins and the dynamic cytoskeletal filaments described previously generates complex cell behaviors.

Actin-Based Motor Proteins Are Members of the Myosin Superfamily

The first motor protein identified was skeletal muscle **myosin**, which generates the force for muscle contraction. This myosin, called *myosin II* (see below) is an elongated protein that is 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 16–54**). The two light chains bind close to the N-terminal



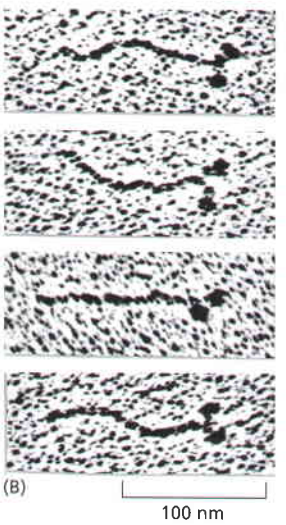


Figure 16–54 Myosin II. (A) A myosin II molecule is composed of two heavy chains (each about 2000 amino acids long *(green)* and four light chains *(blue)*. The light chains are of two distinct types, and one copy of each type is present on each myosin head. Dimerization occurs when the two α helices 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 is called the tail. (B) The two globular heads and the tail can be clearly seen in electron micrographs of myosin molecules shadowed with platinum. (B, courtesy of David Shotton.)

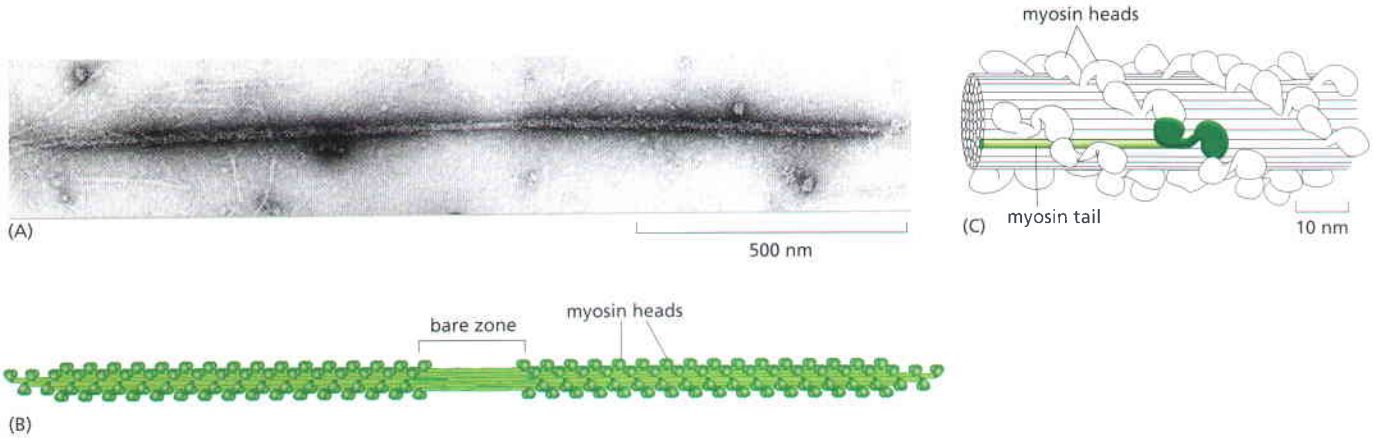


Figure 16–55 The myosin II bipolar thick filament in muscle. (A) Electron micrograph of a myosin II thick filament isolated from frog muscle. Note the central bare zone, which is free of head domains. (B) Schematic diagram, not drawn to scale. The myosin II molecules aggregate by means of their tail 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 16–72). (A, courtesy of Murray Stewart; C, based on R.A. Crowther, R. Padron and R. Craig, *J. Mol. Biol.* 184:429–439, 1985. With permission from Academic Press.)

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 16–55**).

Each myosin head binds and hydrolyzes ATP, using the energy of ATP hydrolysis to walk toward the plus end of an actin filament. The opposing orientation of the heads in the thick filament makes the filament efficient at sliding pairs of oppositely oriented actin filaments past each other. 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 muscle contraction (discussed later). Cardiac and smooth muscle contain myosin II molecules that are similarly arranged, although different genes encode them.

When a muscle myosin is digested by chymotrypsin and papain, the head domain is released as an intact fragment (called S1). The S1 fragment alone can generate filament sliding *in vitro*, proving that the motor activity is contained completely within the head (**Figure 16–56**).

It was initially thought that myosin was present only in muscle, but in the 1970s, researchers found that a similar two-headed myosin protein was also

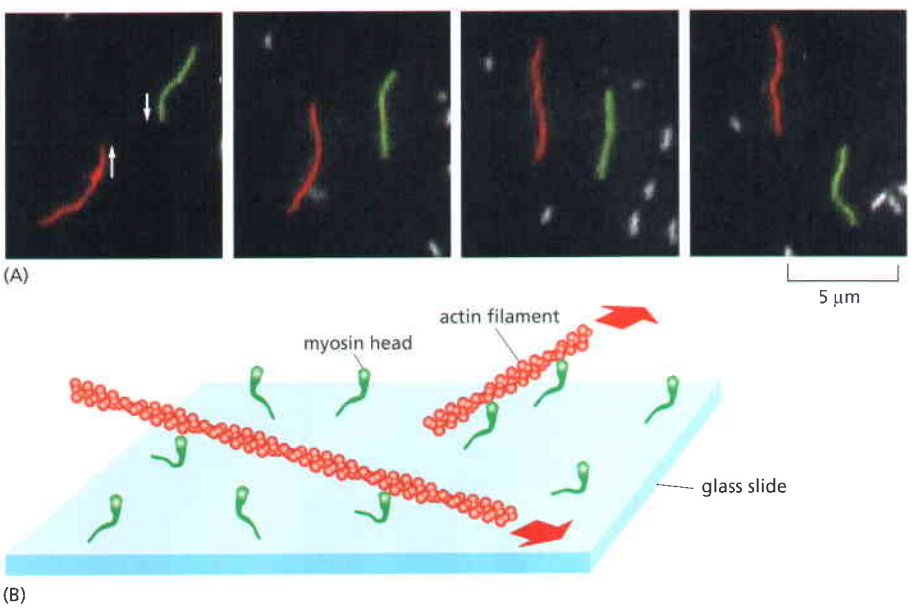


Figure 16–56 Direct evidence for the motor activity of the myosin head. <TTAT> In this experiment, purified S1 myosin heads were attached to a glass slide, and then actin filaments labeled with fluorescent phalloidin were added and allowed to bind to the myosin heads. (A) When ATP was added, the actin 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. (A, courtesy of James Spudich.)

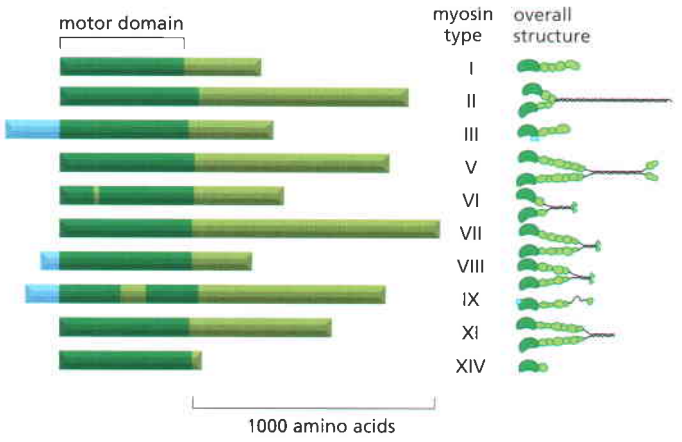


Figure 16–57 Myosin superfamily members. Comparison of the domain structure of the heavy chains of some myosin types. All myosins share similar motor domains (shown in *dark green),* but their C-terminal tails *(light green)* and N-terminal extensions *(light blue)* are very diverse. On the right are 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, IX, 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.

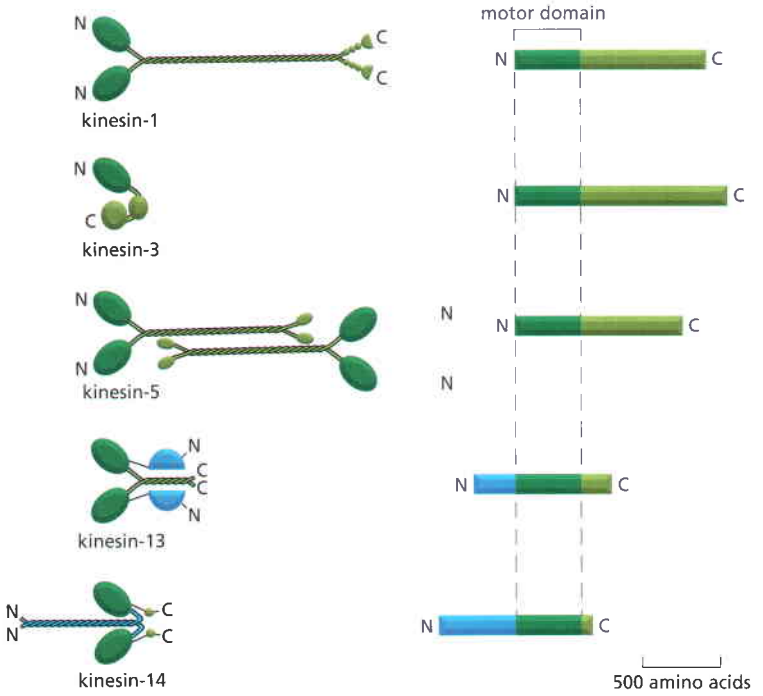
present in nonmuscle cells, including protozoan cells. At about the same time, other researchers found a myosin in the freshwater amoeba *Acanthamoeba castellanii* that was unconventional in having a motor domain similar to the head of muscle myosin but a completely different tail. This molecule seemed to function as a monomer and was named *myosin I* (for one-headed); the conventional 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 16–57**). The newly identified types of myosins include a number of one-headed and two-headed varieties that are approximately 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 eucaryotes indicate that there are at least 37 distinct myosin families in the superfamily. 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 (such as VIII and XI) have been found only in plants, and some have been found only in vertebrates (IX). Most, however, are found in all eucaryotes, suggesting that myosins arose early in eucaryotic evolution. The yeast *Saccharomyces cerevisiae* contains five myosins: two myosin Is, one myosin II, and two myosin Vs. It is tempting to speculate that these three types of myosins are necessary for a eucaryotic cell to survive and that other myosins perform more specialized functions, particularly in multicellular organisms. The nematode *C. elegans*, for example, has at least 15 myosin genes, representing at least seven structural classes; 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-rich stereocilia that are found on the apical surface of these cells (see Figure 9–50); these tilt in response to sound and convert sound waves into electrical signals (discussed in Chapter 23).

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 exact functions for most of the myosins remain to be determined. Myosin V is involved in vesicle and organelle transport. Myosin II is associated with contractile activity in both muscle and nonmuscle cells. It is generally required for cytokinesis (the pinching apart of a dividing cell into two daughters), as well as for the forward translocation of the body of a cell during cell migration. The myosin I proteins often contain 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, as discussed earlier for the construction of microvilli (see Figure 16–50).



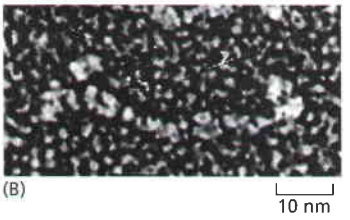


Figure 16–58 Kinesin and kinesin-related proteins. (A) Structures of five kinesin superfamily members. As in the myosin superfamily, only the motor domains are conserved. Kinesin-1 has the motor domain at the N-terminus of the heavy chain. The middle domain forms a long coiled-coil, mediating dimerization. The C-terminal domain forms a tail that attaches to cargo, such as a membrane-enclosed organelle. Kinesin-3 represents an unusual class of kinesins that seem to function as monomers and move membrane-enclosed organelles along microtubules. 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 increase dynamic instability of microtubules; they are therefore called catastrophe factors (see p. 1003). 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. (B) Freeze-etch electron micrograph of a kinesin molecule with the head domains on the left. (B, courtesy of John Heuser.)

There Are Two Types of Microtubule Motor Proteins: Kinesins and Dyneins

Kinesin is a motor protein that moves along microtubules. It was first identified in the giant axon of the squid, where it carries membrane-enclosed organelles away from the neuronal cell body toward the axon terminal by walking toward the plus end of microtubules. Kinesin is similar structurally to myosin II in having two heavy chains and two light chains per active motor; these form two globular head motor domains and an elongated coiled-coil tail responsible for heavy chain dimerization. Like myosin, kinesin is a member of a large protein superfamily, for which the motor domain is the only common element (**Figure 16–58**). The yeast *Saccharomyces cerevisiae* has six distinct kinesins. The nematode *C. elegans* has 16 kinesins, and humans have about 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. A particularly interesting family has the motor domain at the C-terminus and walks in the opposite direction, toward the minus end of the microtubule. Some kinesin heavy chains lack a coiled-coil sequence and seem to function as monomers, analogous to myosin I. Some others are homodimers, and yet others are heterodimers. Members of the kinesin-5 family can self-associate through the tail domain, forming a bipolar motor that slides oppositely oriented microtubules past one another, much as a myosin II thick filament does for actin filaments. Most kinesins carry a binding site in the tail for either a membrane-enclosed organelle or another microtubule. Many of the kinesin superfamily members have specific roles in mitotic and meiotic spindle formation, and in chromosome separation during cell division.

The **dyneins** are a family of minus-end-directed microtubule motors unrelated to the kinesin superfamily. They are composed of two or three heavy chains (that include the motor domain) and a large and variable number of associated intermediate chains and light chains. The dynein family has two major branches (**Figure 16–59**). The most ancient branch contains the *cytoplasmic dyneins*, which are typically heavy-chain homodimers, with two large motor domains as heads. Cytoplasmic dyneins are probably found in all eucaryotic cells, and they

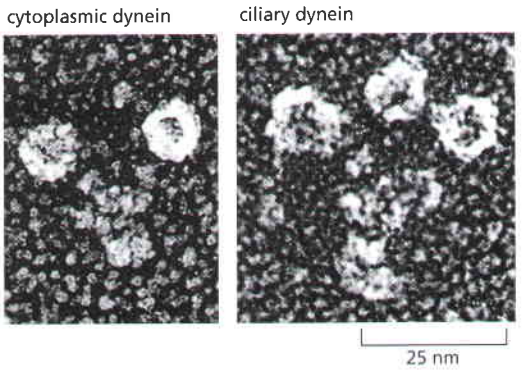


Figure 16–59 Dyneins. Freeze-etch electron micrographs of a molecule of cytoplasmic dynein and a molecule of ciliary (axonemal) dynein. Like myosin II and kinesin, cytoplasmic dynein is a two-headed molecule. The ciliary dynein shown is from a protozoan and has three heads; ciliary dynein from animals has two heads. Note that the dynein head is very large compared with the head of either myosin or kinesin. (Courtesy of John Heuser.)

are important for vesicle trafficking, as well as for localization of the Golgi apparatus near the center of the cell. *Axonemal dyneins,* the other large branch, include heterodimers and heterotrimers, with two or three motor-domain heads, respectively. They are highly specialized for the rapid and efficient sliding movements of microtubules that drive the beating of cilia and flagella (discussed later). A third, minor, branch shares greater sequence similarity with cytoplasmic than with axonemal dyneins but seems to be involved in the beating of cilia.

Dyneins are the largest of the known molecular motors, and they are also among the fastest: axonemal dyneins can move microtubules in a test tube at the remarkable rate of 14 μm/sec. In comparison, the fastest kinesins can move their microtubules at about 2–3 μm/sec. We shall discuss how they work below.

The Structural Similarity of Myosin and Kinesin Indicates a Common Evolutionary Origin

The motor domain of myosins is substantially larger than that of kinesins, about 850 amino acids compared with about 350. The two classes of motor proteins track along different filaments and have different kinetic properties, and they have no identifiable amino acid sequence similarities. However, determination of the three-dimensional structure of the motor domains of myosin and kinesin has revealed that these two motor domains are built around nearly identical cores (**Figure 16–60**). The central force-generating element that the two types of motor proteins have in common includes the site of ATP binding and the machinery necessary to translate ATP hydrolysis into an allosteric conformational change. Large loops extending outward from the central core cause the

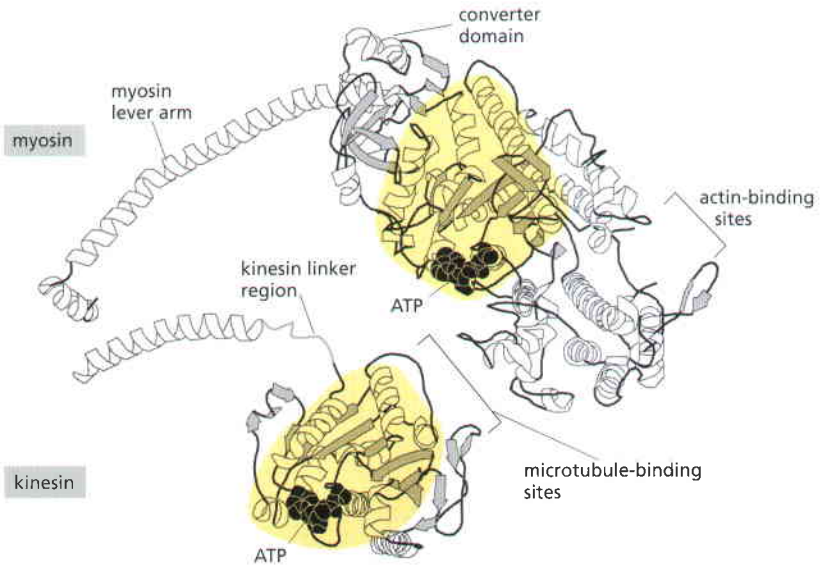


Figure 16–60 X-ray crystal structures of myosin and kinesin heads. The central nucleotide-binding domains of myosin and kinesin (shaded in *yellow)* are structurally very similar. The very different sizes and functions of the two motors are due to major differences in the polymer-binding and force-transduction portions of the motor domain. (Adapted from L.A. Amos and R.A. Cross, *Curr. Opin. Struct. Biol.* 7:239–246, 1997. With permission from Elsevier.)

difference in domain size and are responsible for the choice of track. These loops include the actin-binding and microtubule-binding sites in the myosin and kinesin, respectively. It is thought that both the kinesins and the myosins are descended from a common ancestral motor protein precursor, and that their various specialized functions arose via gene duplication and modification through evolution of the loops coming out from the central core.

An important clue to how the central core is involved in force generation has come from the observation that the motor core also bears some structural resemblance to the nucleotide binding site of the small GTPases of the Ras superfamily. As discussed in Chapter 3 (see Figure 3–72), these proteins exhibit distinct conformations in their GTP-bound (active) and GDP-bound (inactive) forms: mobile "switch" loops in the nucleotide-binding site are in close contact with the γ-phosphate in the GTP-bound state, but these loops swing out when the hydrolyzed γ-phosphate (the terminal phosphate) is released. Although the details of the movement differ for the two motor proteins, and ATP rather than GTP is hydrolyzed, a relatively small structural change in the active site—the presence or absence of a terminal phosphate—is similarly amplified to cause a rotation of a different part of the protein.

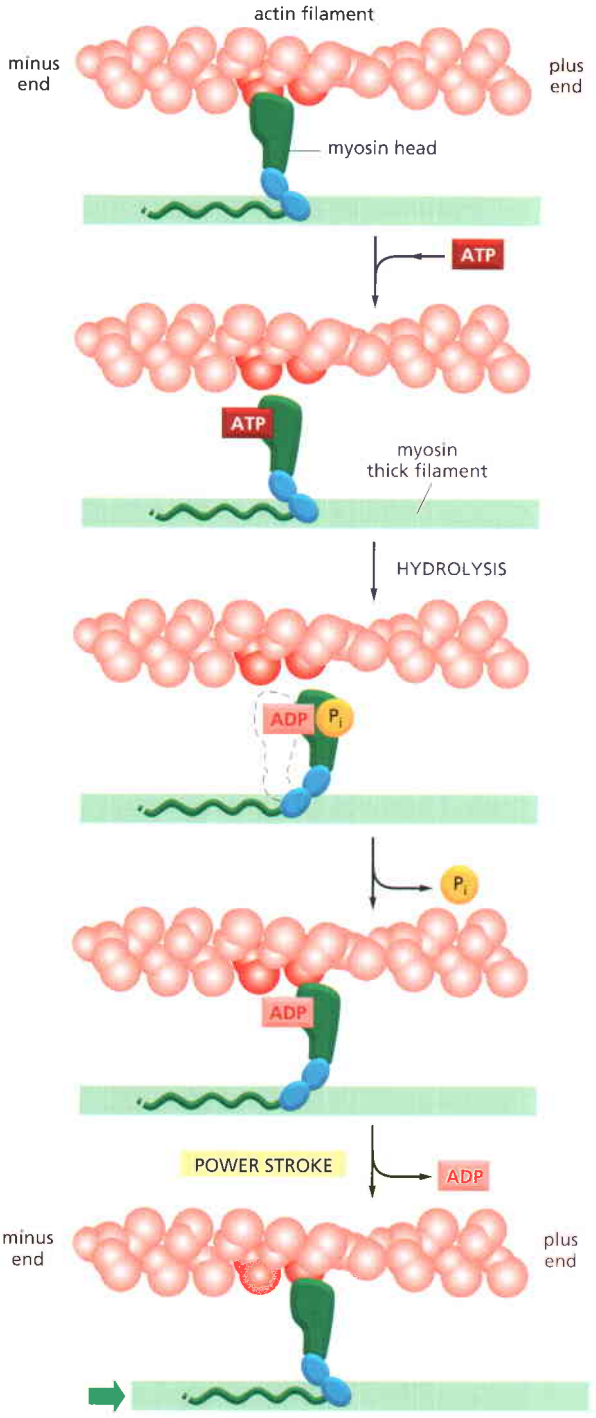
In kinesin and myosin, a switch loop interacts extensively with those regions of the protein involved in microtubule and actin binding, respectively, allowing the structural transitions caused by the ATP hydrolysis cycle to be relayed to the polymer-binding interface. The relay of structural changes between the polymer-binding site and the nucleotide hydrolysis site seems to work in both directions, since the ATPase activity of motor proteins is strongly activated by binding to their filament tracks.

Motor Proteins Generate Force by Coupling ATP Hydrolysis to Conformational Changes

Although the cytoskeletal motor proteins and GTP-binding proteins both use structural changes in their nucleoside-triphosphate-binding sites to produce cyclic interactions with a partner protein, the motor proteins have a further requirement: each cycle of binding and release must propel them forward in a single direction along a filament to a new binding site on the filament. For such unidirectional motion, a motor protein must use the energy derived from ATP binding and hydrolysis to force a large movement in part of the protein molecule. For myosin, 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 piston-like helix that connects movements at the ATP-binding cleft in the head to small rotations of the so-called converter domain (see Figure 16–60). 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 16–61**).

In kinesin, instead of the rocking of a lever arm, the small movements of switch loops at the nucleotide-binding site regulate the docking and undocking of the motor head domain to a long linker region that connects this motor head at one end to the coiled-coil dimerization domain at the other end (see Figure 16–61). When the front (leading) kinesin head is bound to a microtubule before the power stroke, its linker region is relatively unstructured. On the binding of ATP to this bound head, its linker region docks along the side of the head; this throws the second head forward to a position where it will be able to bind a new attachment site on the protofilament, 8 nm closer to the microtubule plus end than the binding site for the first head. The nucleotide hydrolysis cycles in the two heads are closely coordinated, so that this cycle of linker docking and undocking allows



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 domains that make up the actin-binding site. This reduces the affinity of the head for actin and allows it to move along the filament. (The space drawn here between the head and actin emphasizes this change, although in reality the head probably remains very close to the actin.)

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

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

ATTACHED At the end of the cycle, the myosin head is again 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 16–61 The cycle of structural changes used by myosin II to walk along an actin filament. <ATAT> 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. (Based on I. Rayment et al., *Science* 261:50–58, 1993. With permission from AAAS.)

the two-headed motor to move in a hand-over-hand (or head-over-head) stepwise manner (**Figure 16–62**), each time taking a discrete 8-nm step.

The coiled-coil domain seems both to coordinate the mechanochemical cycles of the two heads (motor domains) of the kinesin dimer and to determine its directionality of movement. Recall that whereas most members of the kinesin superfamily, with their motor domains at the N-terminus, move toward the plus end of the microtubule, a few superfamily members have their motor domains at the C-terminus and move toward the minus end. Since the motor domains of these two types of kinesins are essentially identical, how can they move in opposite directions? The answer seems to lie in the way in which the heads are connected. In high-resolution images of forward-walking and backward-walking members of the kinesin superfamily bound to microtubules, the heads that are attached to the microtubule are essentially indistinguishable, but the second, unattached heads are oriented differently. This difference in tilt apparently

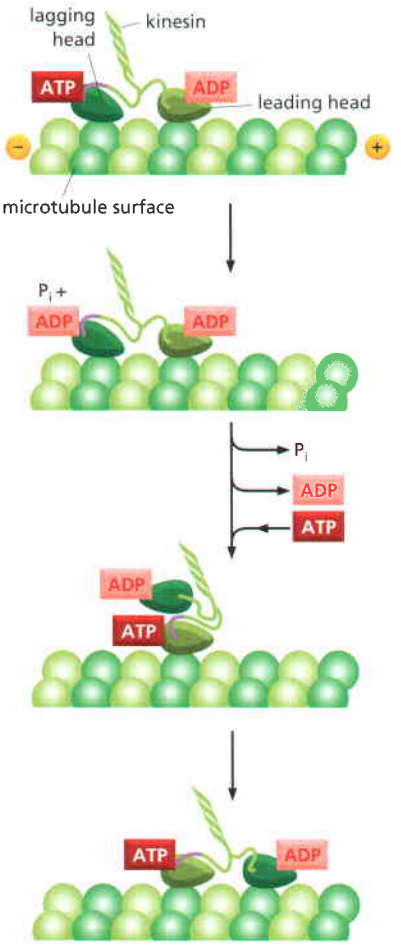


Figure 16–62 The mechanochemical cycle of kinesin. <GAAT> Kinesin-1 is a dimer of two nucleotide-binding motor domains (heads) that are connected through a long coiled-coil tail (see Figure 16–58). The two kinesin motor domains work in a coordinated manner; during a kinesin "step," the rear head detaches 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 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 an exchange of ATP for ADP in the front motor domain (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 connecting line between the motor domain and the intertwined coiled coil). This shift pulls the rear motor domain 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.

biases the next binding site for the second head, and thereby determines the directionality of motor movement (**Figure 16–63**).

The dynein motor is structurally unrelated to myosins and kinesins, but still follows the general rule of coupling the nucleotide hydrolysis to microtubule binding and unbinding as well as to a force-generating conformational change. A giant heavy chain of more than 500,000 daltons forms the basic structure that creates the movement. Its N-terminal portion forms a tail that binds a set of light chains and connects to the other heavy chains in the dynein molecule, while the major portion of the heavy chain is used to form an elaborate, ring-shaped head. The head consists of a planar ring formed from seven domains: six AAA domains plus the heavy-chain C-terminal domain; it is therefore a more complex relative of the hexameric ATPase discussed in Chapter 6 (see Figure 6–91). A hook-

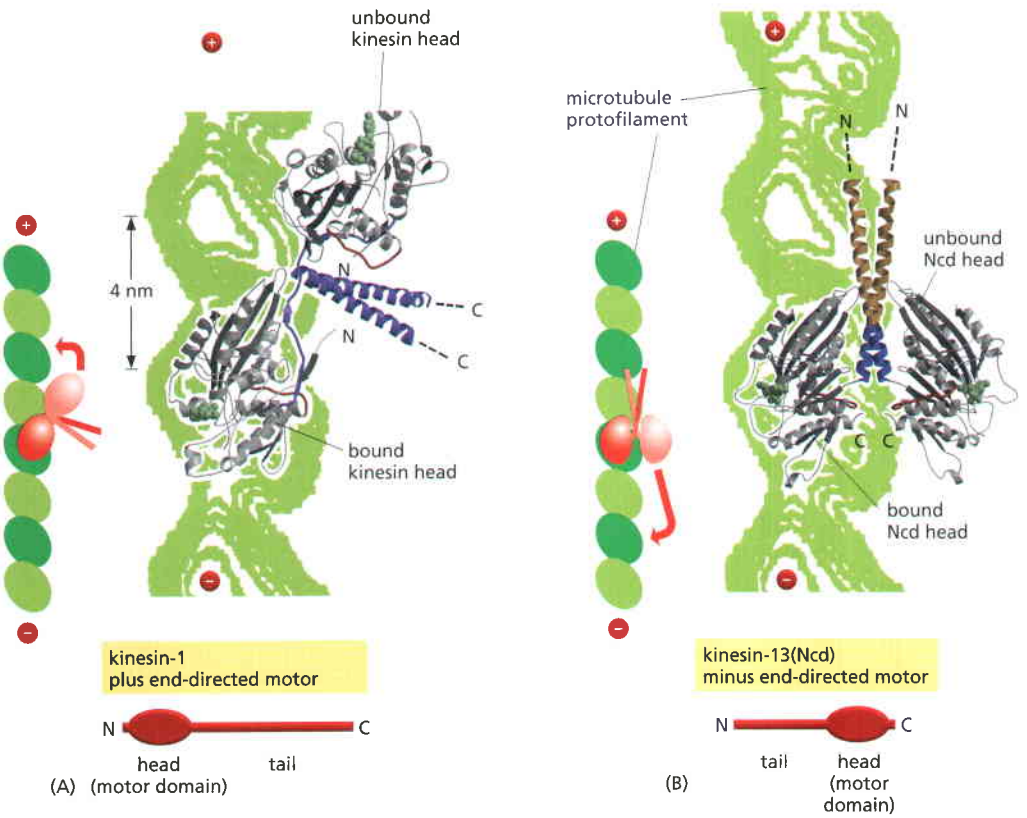


Figure 16–63 Orientation of forward- and backward-walking kinesin superfamily proteins bound to microtubules. These images were generated by fitting the structures of the free motor-protein dimers (determined by x-ray crystallography) onto a lower resolution image of the dimers attached to microtubules (determined by cryoelectron microscopy). (A) Kinesin-1 (conventional kinesin) has its motor domain at the protein's N-terminus and moves toward the plus end of the microtubule. When one head of the dimer is bound to the microtubule in a post-stroke state (with ATP in the nucleotide binding site), the second, unbound head is pointing toward the microtubule plus end, poised to take the next step. (B) Kinesin-13 (called Ncd in *Drosophila*), a minus-end-directed motor with the motor domain at the C-terminus, forms dimers with the opposite orientation. (From E. Sablin et al., *Nature* 395:813–816, 1998. With permission from Macmillan Publishers Ltd.)

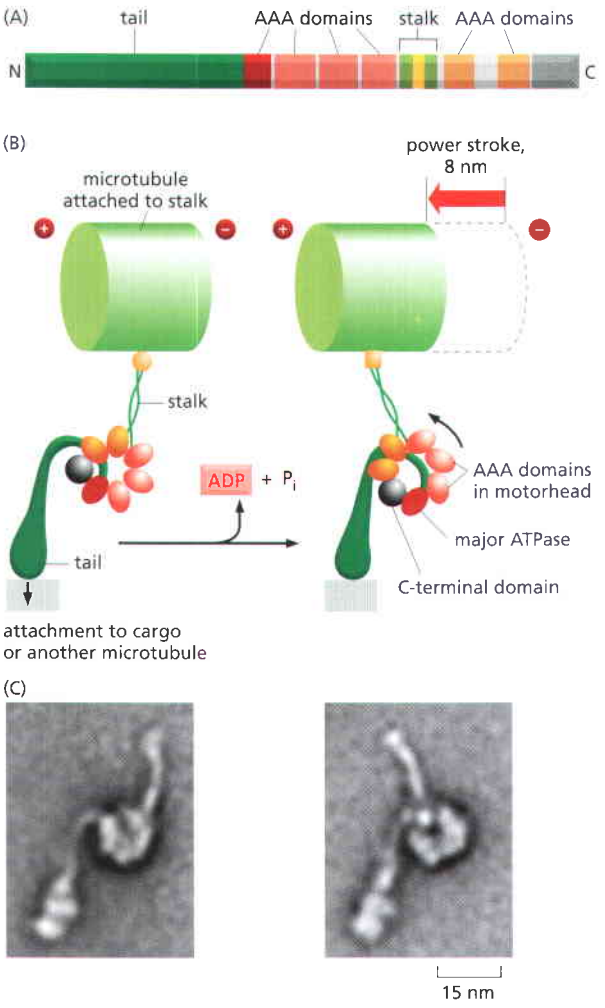


Figure 16–64 The power stroke of dynein. (A) The organization of the domains in each dynein heavy chain. This is a huge molecule, containing nearly 5000 amino acids. The number of heavy chains in a dynein is equal to its number of motor heads. (B) Dynein c is a monomeric flagella dynein found in unicellular 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 with a cargo-attachment site. In the ATP-bound state, the stalk is detached from the microtubule, but ATP hydrolysis causes stalk-microtubule attachment. Subsequent release of ADP and Pi then leads to a large conformational "power stroke" involving rotation of the head and stalk relative to the tail. Each cycle generates a step of about 8 nm along the microtubule towards its minus end. (C) Electron micrographs of purified dyneins in two different conformations representing different steps in the mechanochemical cycle. (B, from S.A. Burgess et al., *Nature* 421:715–718, 2003. With permission from Macmillan Publishers Ltd.)

shaped linker region connects the heavy-chain tail to the AAA domain that is most active as an ATPase. Between the fourth and fifth AAA domains is a heavy chain domain that forms a long anti-parallel coiled-coil stalk. This stalk extends from the top of the ring, with an ATP hydrolysis-regulated microtubule-binding site at its tip. Dynein's "power stroke" is driven by the release of ADP and inorganic phosphate, and it causes the ring to rotate relative to the tail (**Figure 16–64**).

Although kinesin, myosin, and dynein all undergo analogous mechanochemical cycles, the exact nature of the coupling between the mechanical and chemical cycles differs in the three cases. For example, myosin without any nucleotide is tightly bound to its actin track, in a so-called "rigor" state, and it is released from this track by the association of ATP. In contrast, kinesin forms a rigor-like tight association with a microtubule when ATP is bound to the kinesin, and it is hydrolysis of ATP that promotes release of the motor from its track. The mechanochemical cycle of dynein is more similar to myosin than to kinesin, in that nucleotide-free dynein is tightly bound to the microtubule and it is released by binding ATP. However, for dynein the inorganic phosphate and ADP appear to be released at the same time, causing the conformational change driving the power stroke, while for myosin the phosphate is released first and the power stroke does not occur until the ADP subsequently dissociates from the motor head.

Thus, cytoskeletal motor proteins work in a manner highly analogous to GTP-binding proteins, except that in motor proteins the small protein conformational changes (a few tenths of a nanometer) associated with nucleotide hydrolysis are amplified by special protein domains—the lever arm in the case of myosin, the linker in the case of kinesin, and the ring and stalk in the case of dynein—to generate large-scale (several nanometers) conformational changes that move the motor proteins stepwise along their filament tracks. The analogy

between the GTPases and the cytoskeletal motor proteins has recently been extended by the observation that one of the GTP-binding proteins—the bacterial elongation factor G—translates the chemical energy of GTP hydrolysis into directional movement of the mRNA molecule on the ribosome.

Motor Protein Kinetics Are Adapted to Cell Functions

The motor proteins in the myosin and kinesin superfamilies exhibit a remarkable diversity of motile properties, well beyond their choice of different polymer tracks. Most strikingly, a single dimer of kinesin-1 moves in a highly *processive* fashion, traveling for hundreds of ATPase cycles along a microtubule without dissociating. Skeletal muscle myosin II, in contrast, cannot move processively and makes just one or a few steps along an actin filament before letting go. These differences are critical for the motors' various biological roles. A small number of kinesin-1 molecules must be able to transport an organelle all the way down a nerve cell axon, and therefore require a high level of processivity. Skeletal muscle myosin, in contrast, never operates as a single molecule but rather as part of a huge array of myosin II molecules in a thick filament. Here processivity would actually inhibit biological function, since efficient muscle contraction requires that each myosin head perform its power stroke and then quickly get out of the way—in order to avoid interfering with the actions of the other heads attached to the same actin filament.

There are two reasons for the high degree of processivity of kinesin-1 movement. The first is that the mechanochemical cycles of the two motor heads in a kinesin-1 dimer are coordinated with each other, so that one kinesin head does not let go until the other is poised to bind. This coordination allows the motor protein to operate in a hand-over-hand fashion, never allowing the organelle cargo to diffuse away from the microtubule track. In contrast, there is no apparent coordination between the myosin heads in a myosin II dimer. The second reason for the high processivity of kinesin-1 movement is that kinesin-1 spends a relatively large fraction of its ATPase cycle tightly bound to the microtubule. For both kinesin-1 and myosin II, the conformational change that produces the force-generating working stroke must occur while the motor protein is tightly bound to its polymer, and the recovery stroke in preparation for the next step must occur while the motor is unbound. But myosin II spends only about 5% of its ATPase cycle in the tightly bound state, and it is unbound the rest of the time.

What myosin loses in processivity it gains in speed; in an array in which many motor heads are interacting with the same actin filament, a set of linked myosins can move its filament a total distance equivalent to 20 steps during a single cycle time, while kinesins can move only two. Thus, myosin II can typically drive filament sliding much more rapidly than kinesin-1, even though the two different motor proteins hydrolyze ATP at comparable rates and take molecular steps of comparable length. This property is particularly important in the rapid contraction of skeletal muscle, as we will discuss later.

Within each motor protein class, movement speeds vary widely, from about 0.2 to 60 μm/sec for myosins, and from about 0.02 to 2 μm/sec for kinesins. These differences arise from a fine-tuning of the mechanochemical cycle. The number of steps that an individual motor molecule can take in a given time, and thereby the velocity, can be decreased by either decreasing the motor protein's intrinsic ATPase rate or by increasing the proportion of cycle time spent bound to the filament track. For example, myosin V (which acts as a processive vesicle motor) spends up to 90% of its nucleotide cycle tightly bound to the actin filament, in contrast to 5% for myosin II. Moreover, a motor protein can evolve to change the size of each step by either changing the length of the lever arm (for example, the lever arm of myosin V is about three times longer than the lever arm of myosin II) or the angle through which the helix swings (**Figure 16–65**). Each of these parameters varies slightly among different members of the myosin and kinesin families, corresponding to slightly different protein sequences and structures.

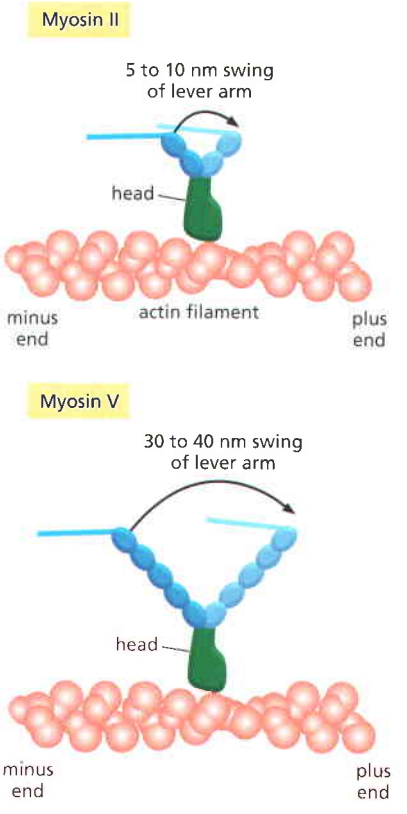


Figure 16–65 The effect of lever arm length on the step size for a motor protein. The lever arm of myosin II is much shorter than the lever arm of myosin V. The power stroke in the head swings their lever arms through the same angle, so myosin V is able to take a bigger step than myosin II.

It is assumed that evolution has fine-tuned the behavior of each motor protein, whose function is determined by the identity of the cargo attached through its tail domain, for speed and processivity according to the specific needs of the cell for the function of that particular family member. Whereas there are many different myosin and kinesin family members found in a typical eucaryotic cell, there is usually only one form of cytoplasmic dynein. It is not yet clear how or whether the mechanical properties of cytoplasmic dynein can be modified in response to differing needs of the cell.

Motor Proteins Mediate the Intracellular Transport of Membrane-Enclosed Organelles <CAAT> <AAAT>

A major function of cytoskeletal motors in interphase cells is the transport and positioning of membrane-enclosed organelles. Kinesin was originally identified as the protein responsible for fast 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. 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 motor proteins such as cytoplasmic dynein, whereas centrifugal movements toward the periphery require plus-end-directed motors such as kinesins.

The clearest 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, 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 (**Figure 16–66**). *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 very much like the ER in cells. Likewise, in living cells the outward movement of ER tubules toward the cell periphery is associated with microtubule growth. Conversely, dyneins are required for positioning the Golgi apparatus near the cell center, moving Golgi vesicles along microtubule tracks toward 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. One of these receptors seems to be the amyloid precursor protein, APP, which binds directly to a light chain on the tail of kinesin-1 and is proposed to be a transmembrane motor receptor in nerve-cell axons. The abnormal processing of this protein gives rise to Alzheimer's disease by producing large, stable

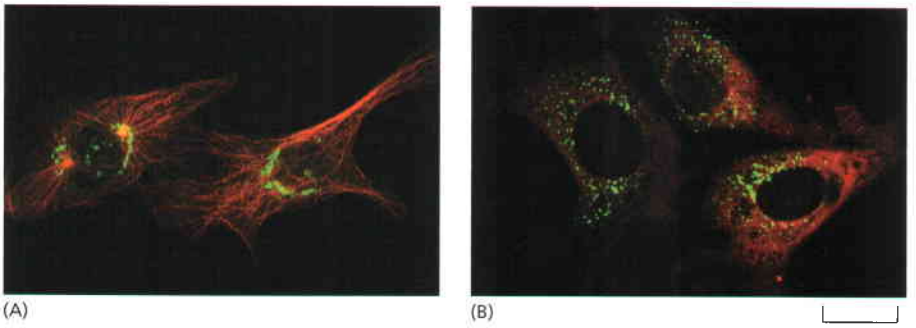


Figure 16–66 Effect of depolymerizing microtubules on the Golgi apparatus. (A) In this endothelial cell, the microtubules are labeled in *red*, and the Golgi apparatus is labeled in *green* (using an antibody against a Golgi protein). As long as the system of microtubules remains intact, the Golgi is localized near the centrosome, close to the nucleus at the center of the cell. The cell on the *right* is in interphase, with a single centrosome. The cell on the *left* is in prophase, and the duplicated centrosomes have moved to opposite sides of the nucleus. (B) After exposure to nocodazole, which causes microtubules to depolymerize (see Table 16–2), the Golgi apparatus fragments and is dispersed throughout the cell cytoplasm. (Courtesy of David Shima.)

protein aggregates in nerve cells of the brain (see Figure 6–95). Other receptors for specific kinesins have been identified on the endoplasmic reticulum, as well as on various other membrane-bound organelles that rely on microtubule-based transport for their localization. The JIPs (JNK-interacting proteins), are scaffold proteins associated with cell signaling. These kinesin receptors may provide a link between transport and cell signaling.

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 complex involved in the nucleation of conventional actin filaments). Membranes of the Golgi apparatus are coated with the proteins ankyrin and spectrin, which have been proposed to associate with the Arp1 filament in the dynactin complex to form a planar cytoskeletal array reminiscent of the erythrocyte membrane cytoskeleton (see Figure 10–41). The spectrin array probably gives structural stability to the Golgi membrane, and—via the Arp1 filament—it may mediate the regulated attachment of dynein to the organelle (**Figure 16–67**). In other cases, cytoplasmic dynein motors may interact directly with their cargo. The cytoplasmic tail of rhodopsin, the light-detecting protein found in the rod cells of the eye, binds directly to one of the dynein light chains, and this interaction is required for normal trafficking of rhodopsin in the rod cell.

Motor proteins also have a significant role in organelle transport along actin filaments. The first myosin shown to mediate organelle motility was myosin V, a two-headed myosin with a large step size (see Figure 16–65). In mice and humans, membrane-enclosed pigment granules, called *melanosomes*, are synthesized in cells called *melanocytes* beneath the skin surface. These melanosomes move out to the ends of dendritic processes in the melanocytes, where they are delivered to the overlying keratinocytes that form the skin (and the fur in mice). Myosin V is associated with the surface of melanosomes (**Figure 16–68**) and is able to mediate their actin-based movement in a test tube. In mice, mutations in the myosin V gene result in a "dilute" phenotype, in which fur color looks faded because the melanosomes are not delivered to the keratinocytes efficiently. Other myosins, including myosin I, are associated with endosomes and a variety of other organelles.

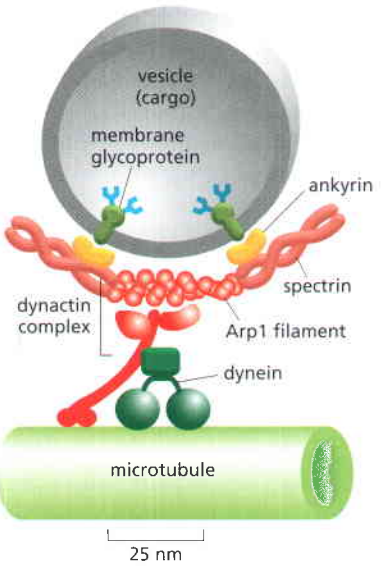


Figure 16–67 A model for the attachment of dynein to a membrane-enclosed organelle. Dynein requires the presence of a large number of accessory proteins to associate with membrane-enclosed organelles. Dynactin is a large complex *(red)* that includes components that bind weakly to microtubules, components that bind to dynein itself, and components that form a small actin-like filament made of the actin-related protein Arp1. It is thought that the Arp1 filament may mediate attachment of this large complex to membrane-enclosed organelles through a network of spectrin and ankyrin, similar to the membrane-associated cytoskeleton of the red blood cell (see Figure 10–41).

The Cytoskeleton Localizes Specific RNA Molecules

In order to concentrate proteins at their site of function, cells often restrict the synthesis of a particular protein by localizing its mRNA molecules, a process that establishes cellular asymmetries. This is particularly important when a parent cell divides to generate two daughters with distinct fates. As another example, several mRNAs encoding proteins involved in synapse function are specifically localized close to synapses in many neurons, and there is evidence that mRNA localization and translation regulation at the synaptic sites play important roles in regulating long-term memory and synaptic plasticity. Not surprisingly, the cytoskeleton and cytoskeletal motor proteins transport and position mRNA molecules in these types of situations.

The giant *Drosophila* oocyte localizes a large number of maternally encoded mRNAs to specific sites within the cell in anticipation of the rapid cell specification events in early embryogenesis (discussed in Chapter 22). A group of mRNAs that encode proteins necessary for proper development of the posterior region of the embryo, including development of the germ cells, is localized posteriorly in the oocyte, and a distinct group of mRNAs encoding proteins necessary for specification of anterior structures in the embyro is localized in the anterior region of the oocyte.

The oocyte takes advantage of its polarized microtubule cytoskeleton, where most microtubule minus ends are clustered in the anterior part of the cell and plus ends near the posterior, to establish these specialized mRNA distributions. For example, the mRNA encoding Bicoid, a transcription factor critical for

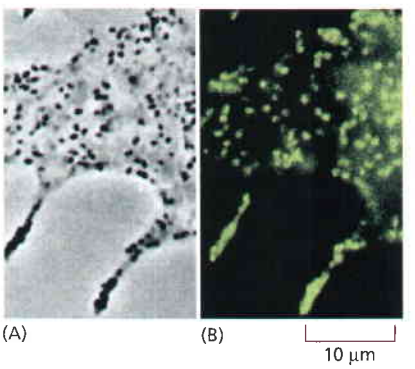
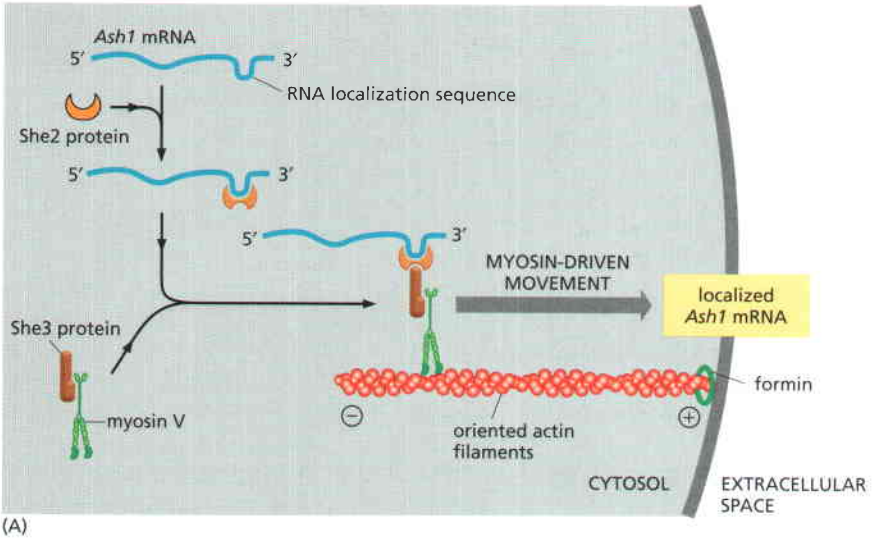


Figure 16–68 Myosin V on melanosomes. (A) Phase-contrast image of a portion of a melanocyte isolated from a mouse. The black spots are melanosomes, which are membrane-enclosed organelles filled with the skin pigment melanin. (B) The same cell labeled with a fluorescent antibody against myosin V. Every melanosome is associated with a large number of copies of this motor protein. (From X. Wu et al., *J. Cell Sci.* 110:847–859, 1997. With permission from The Company of Biologists.)



(A)

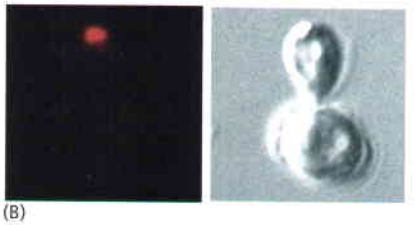

(B)

Figure 16–69 Polarized mRNA localization in the yeast bud tip. (A) The molecular mechanism of *Ash1* mRNA localization, as determined by genetics and biochemistry. (B) Fluorescent *in situ* hybridization (FISH) was used to localize the *Ash1* mRNA (*red*) in this dividing yeast cell. The mRNA is confined to the far tip of the daughter cell (here, still a large bud). Ash1 protein, transcribed from this localized mRNA, is also confined to the daughter cell. (B, courtesy of Peter Takizawa and Ron Vale.)

anterior development, has a structure within the 3′ UTR that binds a protein called Swallow, which in turn binds to a cytoplasmic dynein light chain, presumably enabling its transport to the microtubule minus ends at the cell anterior. Conversely, the transport of mRNA encoding Oskar, a protein necessary for germ cell development in the posterior of the embryo, requires kinesin-1 for its transport to the microtubule plus ends. The anchoring of the mRNAs to their appropriate locations after delivery via microtubules appears to involve the cortical actin cytoskeleton. The mRNA encoding Oskar, for example, binds directly to an actin-binding protein called moesin, a member of the ERM family.

In some cells, mRNA transport as well as anchoring is actin-dependent. The yeast mother and daughter cells retain distinct identities, as revealed by major differences in their subsequent ability to undergo mating-type switching (discussed in Chapter 7) and in the choice of their next bud site. Many of these differences are caused by a gene regulatory protein called *Ash1*. Both *Ash1* mRNA and protein are localized exclusively to the growing bud and therefore end up only in the daughter cell. One of the two type V myosins found in yeast, Myo4p, is required for this asymmetric distribution of *Ash1* mRNA. A genetic screen for other mutations that disrupt the mother/daughter difference has revealed that at least six other gene products that are associated with the cytoskeleton are required for normal polarity; these include one of the formins, tropomyosin, profilin, and actin itself, as well as a complex of two proteins that form a direct link between a specific sequence in the *Ash1* mRNA and the myosin V protein (**Figure 16–69**).

Cells Regulate Motor Protein Function

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 16–70**). The pigment granules aggregate or disperse by moving along an extensive network of microtubules. The centrosome nucleates these microtubules, localizing their minus ends in the center of the cell, while the plus ends are distributed around the cell periphery.

The tracking of individual pigment granules (**Figure 16–71**) reveals that the inward movement is rapid and smooth, while the outward movement is jerky, with frequent backward steps. Both the microtubule motors dynein and kinesin are associated with the pigment granules, as well as the actin motor myosin V. The jerky outward movements apparently result from a tug-of-war between the two microtubule motor proteins, with the stronger kinesin winning out overall.

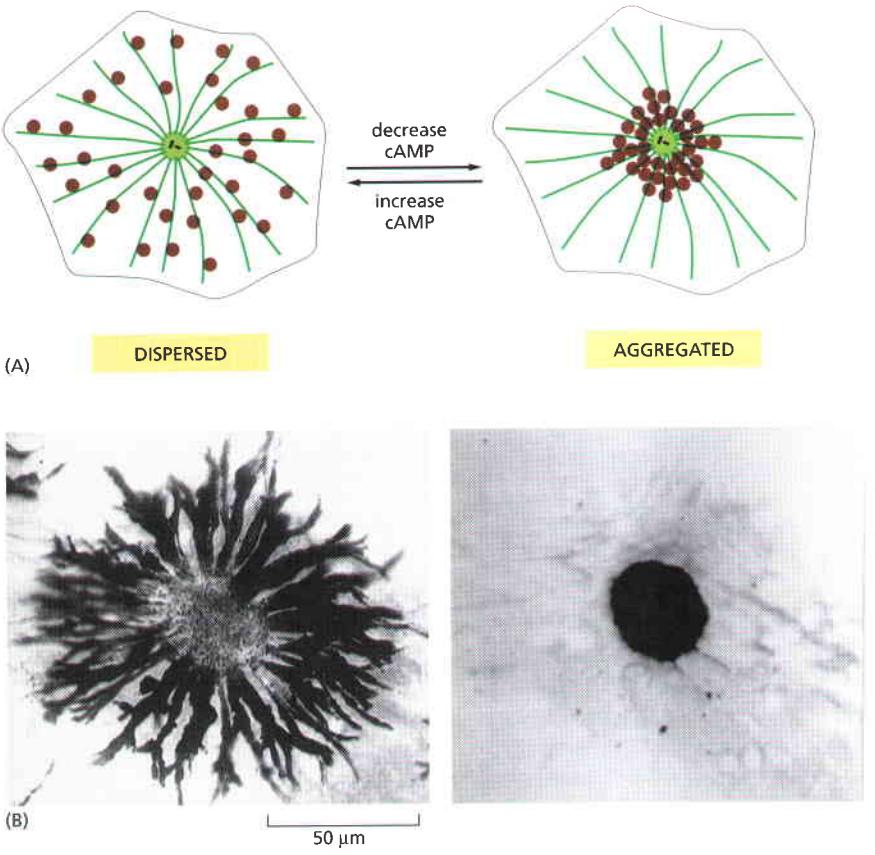
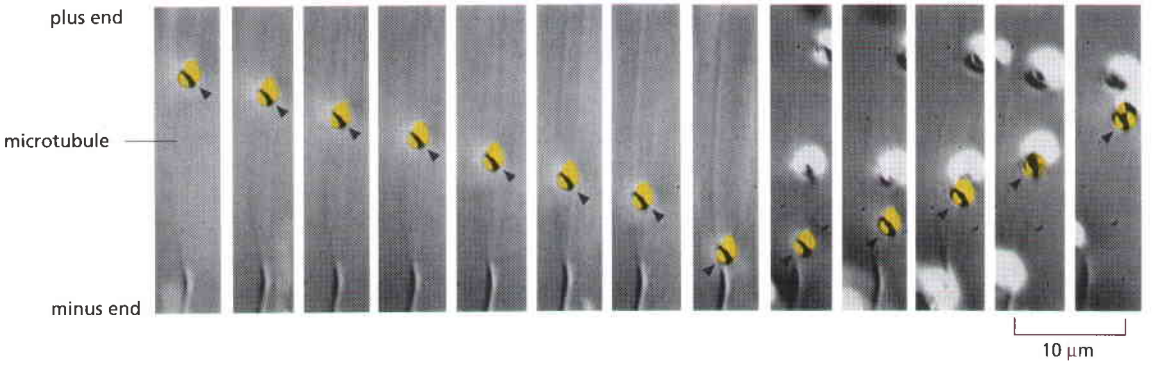


Figure 16–70 Regulated melanosome movements in fish pigment cells. These giant cells, which are responsible for changes in skin coloration in several species of fish, contain large pigment granules, or melanosomes *(brown)*. The melanosomes can change their location in the cell in response to a hormonal 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.)

When the kinesin light chains become phosphorylated after a hormonal stimulation that signals skin color change, 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.

The cell can also use phosphorylation to regulate myosin activity. In nonmuscle cells, myosin II can be phosphorylated on a variety of sites on both heavy and light chains, affecting both motor activity and thick filament assembly. The myosin II can exist in two different conformational states in such cells, an extended state that can form bipolar filaments, and a bent state in which the tail domain apparently interacts with the motor head. Phosphorylation of the regulatory light chain by the calcium-dependent *myosin light-chain kinase (MLCK)* causes the myosin II to preferentially assume the extended state, which promotes its assembly into a bipolar filament and leads to cell contraction (**Figure 16–72**). MLCK is also activated during mitosis, causing myosin II to assemble into the actin-based contractile ring that pinches the mitotic cell into two. As we will discuss below, myosin phosphorylation is also an important component of the control of contraction in smooth muscle cells. Regulation of other members

Figure 16–71 Bidirectional movement of a melanosome on a microtubule. An isolated melanosome *(yellow)* moves along a microtubule on a glass slide, from the plus end toward the minus end. Halfway through the video sequence, it abruptly switches direction and moves from the minus end toward the plus end. (From S.L. Rogers et al., *Proc. Natl Acad. Sci. U.S.A.* 94:3720–3725, 1997. With permission from National Academy of Sciences.)



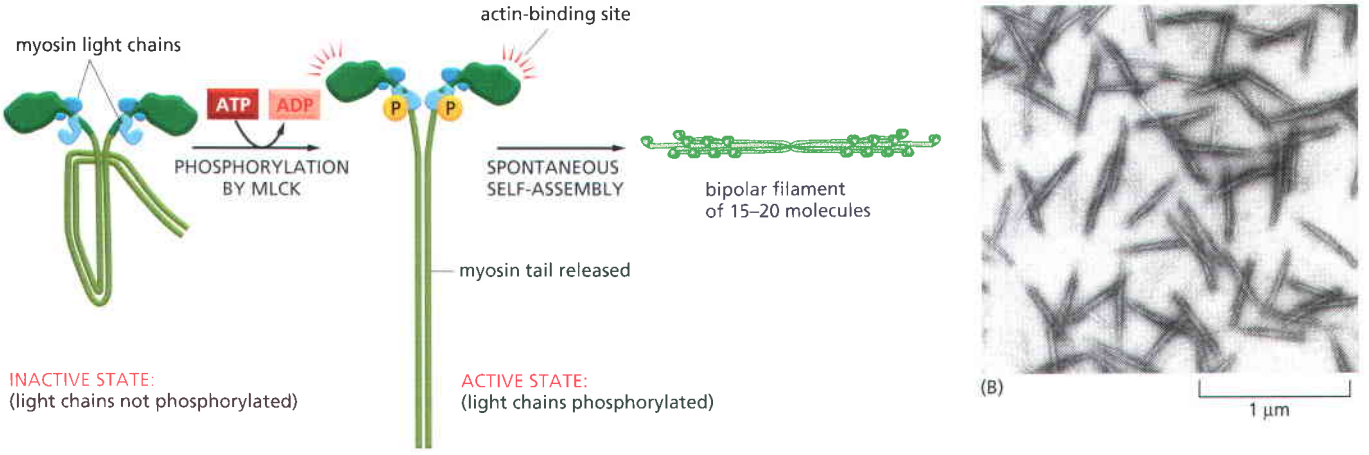


Figure 16–72 Light-chain phosphorylation and the regulation of the assembly of myosin II into thick filaments. (A) The controlled phosphorylation by the enzyme myosin light-chain kinase (MLCK) of one of the two light chains (the so-called regulatory light chain, shown in *light blue)* on nonmuscle myosin 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. (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 16–55). (B, courtesy of John Kendrick-Jones.)

of the myosin superfamily is not as well understood, but the control of these myosins is likewise thought to involve site-specific phosphorylations.

Summary

Motor proteins use the energy of ATP hydrolysis to move along microtubules or actin filaments. They mediate the sliding of filaments relative to one another and the transport of cargo along filament tracks. All known motor proteins that move on actin filaments are members of the myosin superfamily. The motor proteins that move on microtubules are either members of the kinesin superfamily or the dynein family. The myosin and kinesin superfamilies are diverse, with about 40 genes encoding each type of protein in humans. The only structural element shared among all members of each superfamily is the motor "head" domain. These heads are fused to a wide variety of different "tails," which attach to different types of cargo and enable the various family members to perform different functions in the cell. These functions include the transportation and localization of specific proteins, membrane-enclosed organelles, and mRNAs.

Although myosin and kinesin walk along different tracks and use different mechanisms to produce force and movement by ATP hydrolysis, they share a common structural core, suggesting that they are derived from a common ancestor. The dynein motor protein has independently evolved, and it has a distinct structure and mechanism of action.

THE CYTOSKELETON AND CELL BEHAVIOR

A central challenge in all areas of cell biology is to understand how the functions of many individual molecular components combine to produce complex cell behaviors. The cell behaviors that we describe in this final section all rely on a coordinated deployment of the components and processes that we have explored in the first three sections of the 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. How does the cell coordinate all these activities to define its shape, to enable it to crawl, or to divide it neatly into two at mitosis? These problems of cytoskeletal coordination will challenge scientists for many years to come.

To provide a sense of our present understanding, we first discuss examples where specialized cells build stable arrays of filaments and use highly ordered arrays of motor proteins sliding them relative to each other to generate the large-scale movements of muscle, cilia, and eucaryotic flagella. Next, we consider two important instances where filament dynamics collude with motor protein activity to generate complex, self-organized dynamic structures: the microtubule-based mitotic spindle and the actin arrays involved in cell crawling. Finally, we consider the extraordinary organization and behavior of the neuronal cytoskeleton.

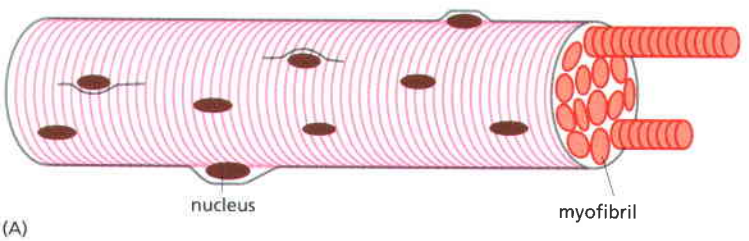
Sliding of Myosin II and Actin Filaments Causes Muscles to Contract

Muscle contraction is the most familiar and the 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 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 development by the fusion of many separate cells, as discussed in Chapter 22. The large muscle cell retains the many nuclei of the contributing cells. These nuclei lie just beneath the plasma membrane (**Figure 16–73**). 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 giant 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 16–74**).

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 16–55). When this region of overlap is examined in cross section by electron microscopy, the myosin filaments are seen to be arranged in a regular hexagonal lattice, with the actin filaments evenly spaced between them (**Figure 16–75**). Cardiac muscle and smooth muscle also contain sarcomeres, although the organization is not as regular as that in skeletal muscle.

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 (Figure 16–74 C 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



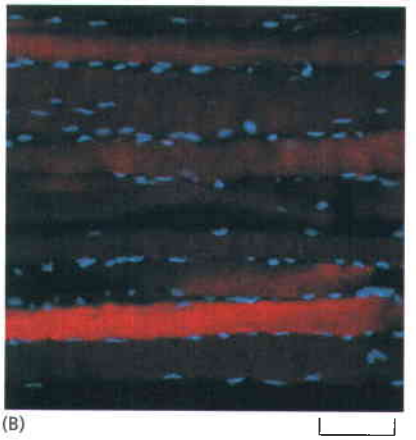


Figure 16–73 Skeletal muscle cells (also called muscle fibers). (A) These huge multinucleated cells form by the fusion of many muscle cell precursors, called myoblasts. In an adult human, a muscle cell is typically 50 μm in diameter and can be up to several centimeters long. (B) Fluorescence micrograph of rat muscle, showing the peripherally located nuclei *(blue)* in these giant cells. Myofibrils are stained *red*; see also Figure 23–46B. (B, courtesy of Nancy L. Kedersha.)

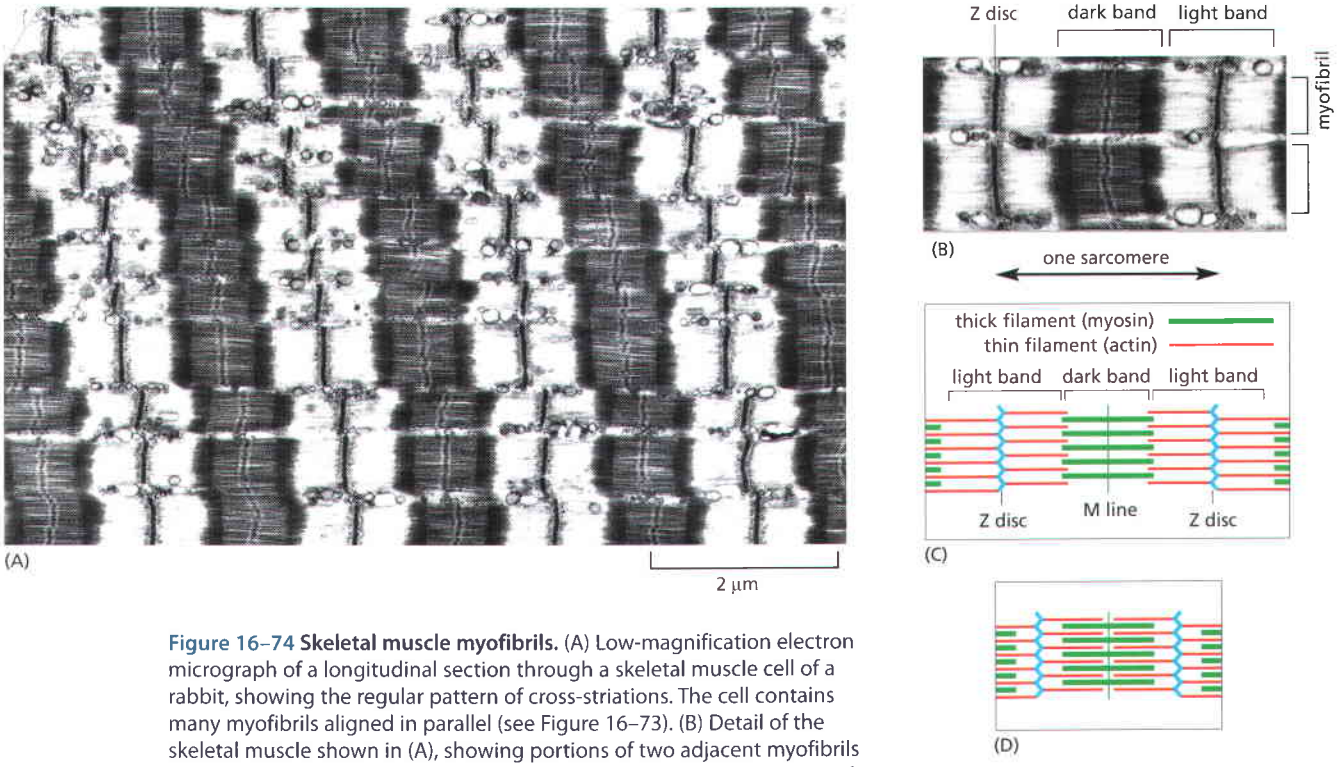


Figure 16–74 Skeletal muscle myofibrils. (A) Low-magnification electron micrograph of a longitudinal section through a skeletal muscle cell of a rabbit, showing the regular pattern of cross-striations. The cell contains many myofibrils aligned in parallel (see Figure 16–73). (B) Detail of the skeletal 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. The dark bands, which mark the location of the thick filaments, are sometimes called A bands because they appear anisotropic in polarized light (that is, their refractive index changes with the plane of polarization). The light bands, which contain only thin filaments and therefore have a lower density of protein, are relatively isotropic in polarized light and are sometimes called I bands. (D) When the sarcomere contracts, the actin and myosin filaments slide past one another without shortening. (A and B, courtesy of Roger Craig.)

that they operate with a low processivity, remaining 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

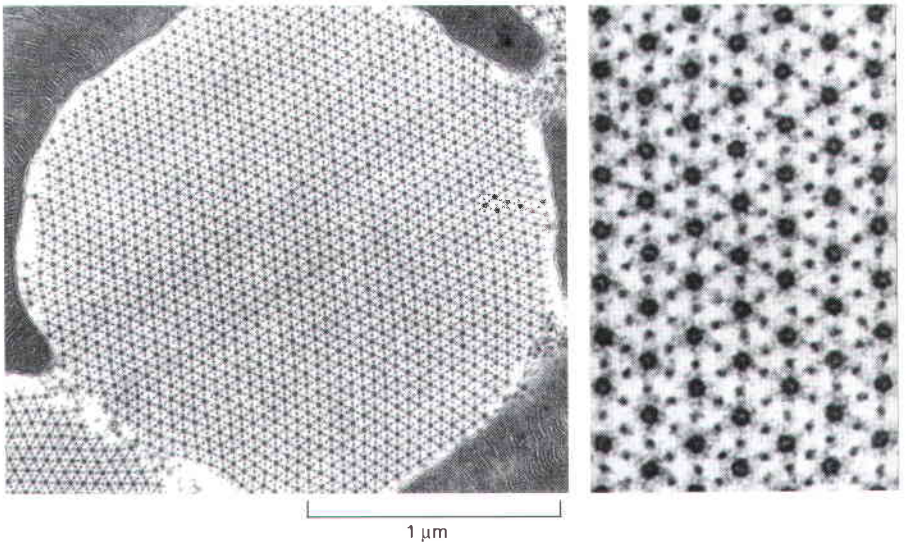
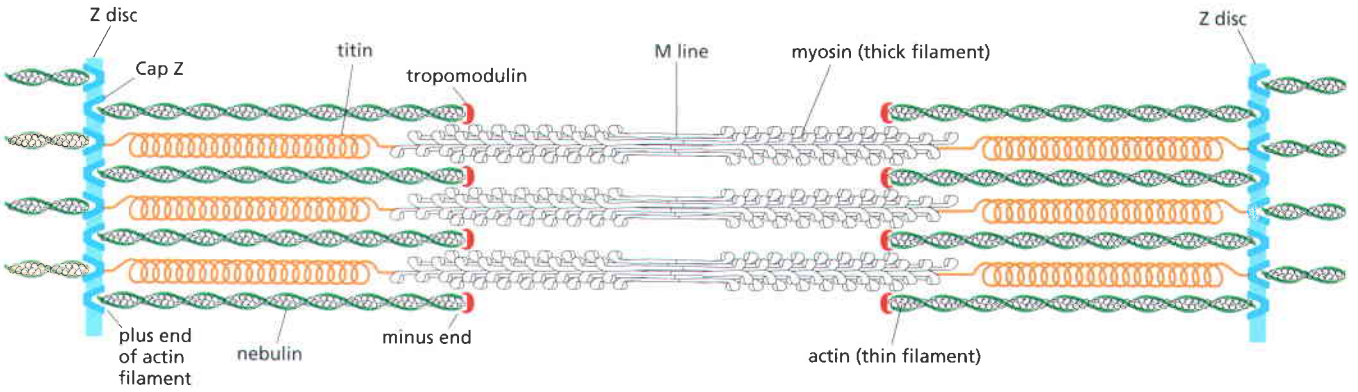


Figure 16–75 Electron micrographs of an insect flight muscle viewed in cross section. The myosin and actin filaments are packed together with almost crystalline regularity. Unlike their vertebrate counterparts, these myosin filaments have a hollow center, as seen in the enlargement on the right. The 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.)



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 1/50th 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 16–76**). 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 determined by a template 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 to the minus end of each thin filament and acts as a "molecular ruler" to dictate the length of the filament. The minus ends of the thin filaments are 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 to the dynamic actin filaments characteristic of most other cell types that turn over with half-lives of a few minutes or less.

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 also longer, suggesting that it too serves as a molecular ruler, determining in this case the overall length of each sarcomere (see Figure 3–33).

Figure 16–76 Organization of accessory proteins in a sarcomere. <CTGC> 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 16–78) 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.

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

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 its motor nerve. 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 are required for extremely rapid contraction. 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

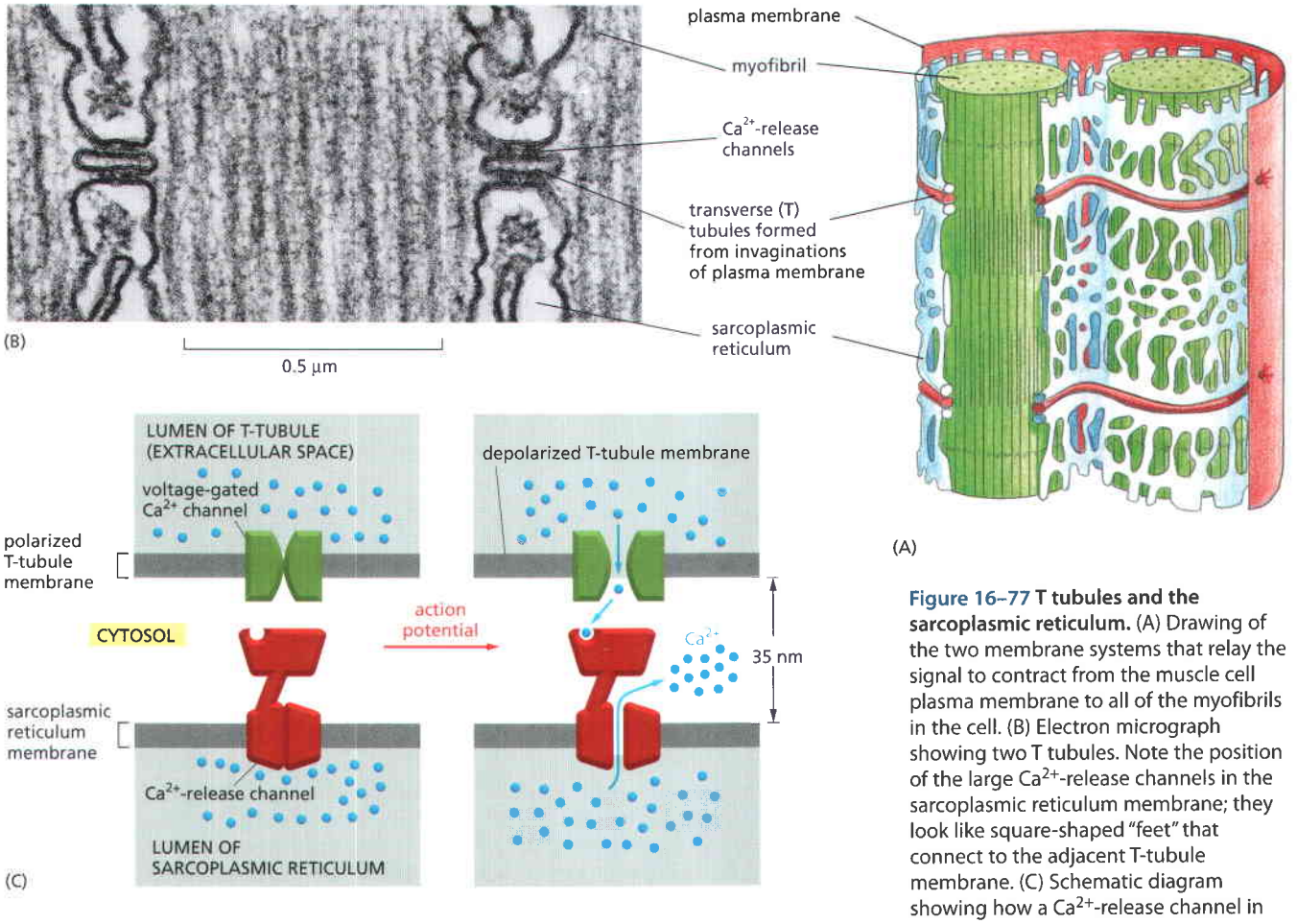


Figure 16–77 T tubules and the sarcoplasmic reticulum. (A) Drawing of the two membrane systems that relay the signal to contract from the muscle cell plasma membrane to all of the myofibrils in the cell. (B) Electron micrograph showing two T tubules. Note the position of the large Ca^{2+}-release channels in the sarcoplasmic reticulum membrane; they look like square-shaped "feet" 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. (B, courtesy of Clara Franzini-Armstrong.)

this electrical excitation spreads rapidly into a series of membraneous 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 web-like sheath of modified endoplasmic reticulum that surrounds each myofibril like a net stocking (**Figure 16–77**A and B).

When the incoming action potential activates a Ca^{2+} channel in the T-tubule membrane, a Ca^{2+} influx triggers the opening of Ca^{2+}-release channels in the sarcoplasmic reticulum (Figure 16–77C). 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.

The Ca^{2+} dependence of vertebrate skeletal muscle contraction, and hence its dependence on motor 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*, an elongated molecule that binds along the groove of the actin 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

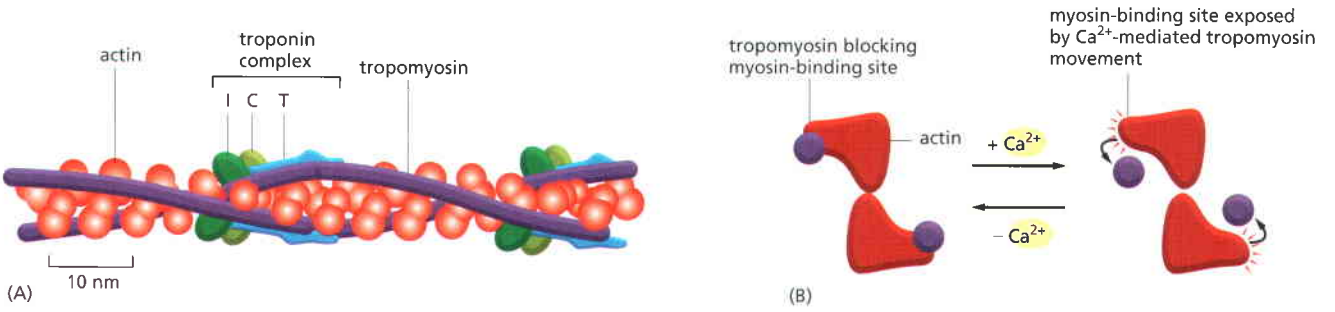


Figure 16–78 The control of skeletal muscle contraction by troponin. (A) A skeletal muscle cell thin filament, showing the positions of tropomyosin and troponin along the actin filament. Each tropomyosin molecule has seven evenly spaced regions with similar amino acid sequences, each of which is thought to bind to an actin subunit in the filament. (B) A thin filament shown end-on, illustrating how Ca^{2+} (binding to troponin) is thought to relieve the tropomyosin blockage of the interaction between actin and the myosin head. (A, adapted from G.N. Phillips, J.P. Fillers and C. Cohen, *J. Mol. Biol.* 192:111–131, 1986. With permission from Academic Press.)

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 16–78**). Troponin C is closely related to the ubiquitous Ca^{2+}-binding protein calmodulin (see Figure 15–44); 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, the walls of arteries, and many other structures requiring slow and sustained contractions. Smooth muscle is composed of sheets of highly elongated spindle-shaped cells, each with a single nucleus. Smooth muscle cells do not express the troponins. Instead, Ca^{2+} influx into the cell regulates contraction by two mechanisms that depend on the ubiquitous calcium binding protein calmodulin.

First, Ca^{2+}-bound calmodulin binds to an actin-binding protein, caldesmon, which blocks the actin sites where the myosin motor heads would normally bind. This causes the caldesmon to fall off of the actin filaments, preparing the filaments for contraction. Second, smooth muscle myosin is phosphorylated on one of its two light chains by myosin light chain kinase (MLCK), as described previously for regulation of nonmuscle myosin II (see Figure 16–72). 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 (in contrast to nonmuscle myosin II, light chain dephosphorylation does not cause thick filament disassembly in smooth muscle cells). MLCK requires bound Ca^{2+}/calmodulin to be fully active.

External signaling molecules such as adrenaline (epinephrine) can also regulate the contractile activity of smooth muscle. Adrenaline binding to its G-protein-coupled cell surface receptor causes an increase in the intracellular level of cyclic AMP, which in turn activates cyclic-AMP-dependent protein kinase (PKA) (see Figure 15–35). PKA phosphorylates and inactivates MLCK, thereby causing the smooth muscle cell to relax.

The phosphorylation events that regulate contraction in smooth muscle cells occur realtively 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 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 <AGGT>

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. This number is about the same as the average number of revolutions in the lifetime of an automobile's internal combustion engine. Heart cells express several specific isoforms of cardiac muscle myosin and cardiac muscle actin. Even subtle changes in these contractile proteins expressed in the heart—changes that would not cause any noticeable consequences in other tissues—can cause serious heart disease (**Figure 16–79**).

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 frequent 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*, that also frequently results in early heart failure.

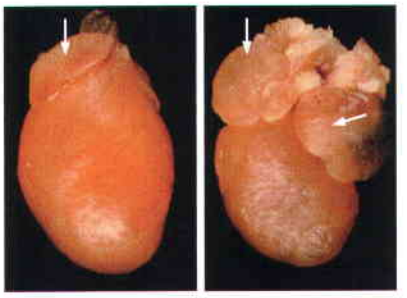


Figure 16–79 Effect on the heart of a subtle mutation in cardiac myosin. *Left*, normal heart from a 6-day old mouse pup. *Right*, heart from a pup with a point mutation in both copies of its cardiac myosin gene, changing Arg 403 to Gln. The arrows indicate the atria. In the heart from the pup with the 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, 1999. With permission from The Rockefeller University Press.)

Cilia and Flagella Are Motile Structures 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 hair-like 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 (**Figure 16–80**A). **Cilia** tend to be shorter than flagella and are organized in a similar fashion, but they beat with a whip-like motion that resembles the breast stroke in swimming (Figure 16–80B). The cycles of adjacent cilia are almost but not quite in synchrony, creating the wave-like patterns that can be seen in fields of beating cilia under the microscope. 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

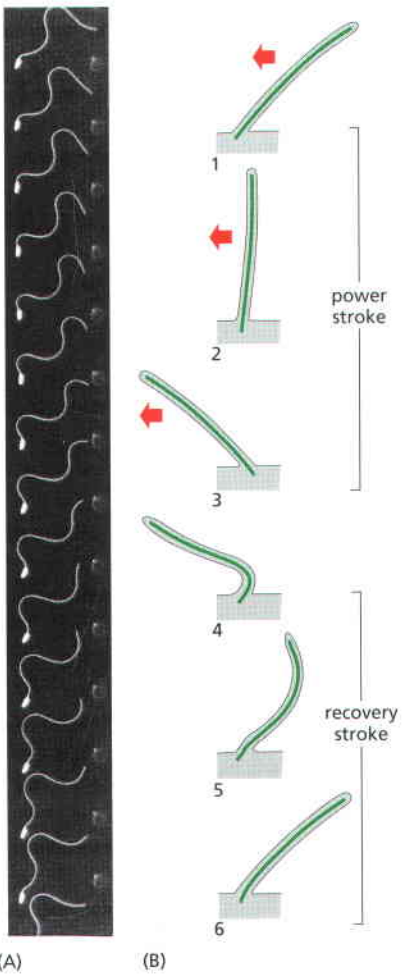


Figure 16–80 The contrasting motions of flagella and cilia. (A) The wave-like motion of the flagellum of a sperm cell from a tunicate. The cell was photographed with stroboscopic illumination at 400 flashes per second. Note that waves of constant amplitude move continuously from the base to the tip of the flagellum. (B) The beat of a cilium, which resembles the breast stroke in swimming. A fast power stroke (*red* arrows), in which fluid is driven over the surface of the cell, is followed by a slow recovery stroke. Each cycle typically requires 0.1–0.2 sec and generates a force perpendicular to the axis of the axoneme (the ciliary core). (A, courtesy of C.J. Brokaw.)

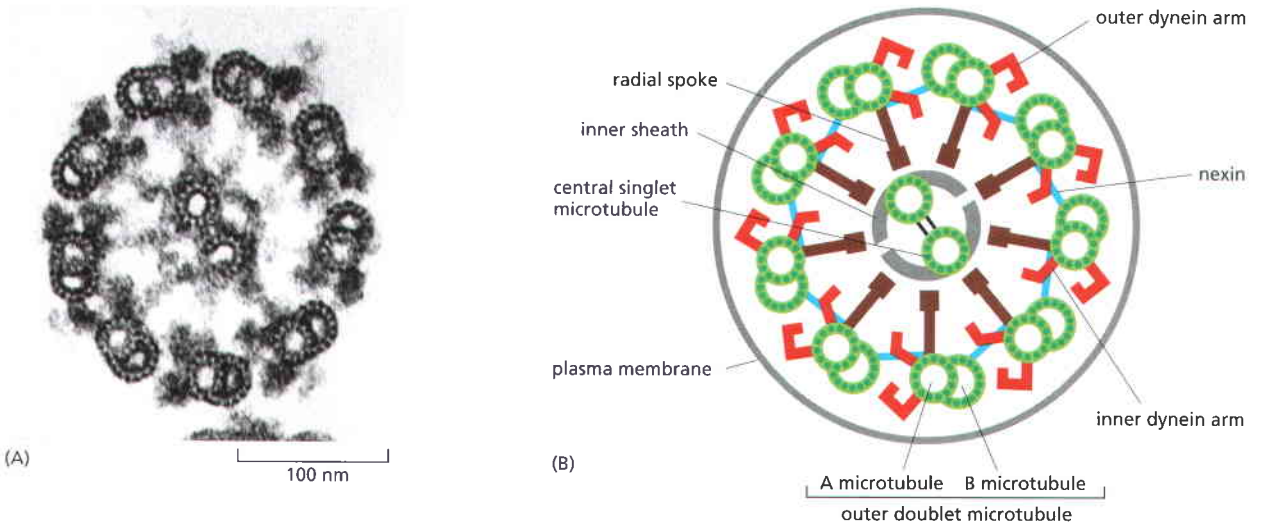


Figure 16–81 The arrangement of microtubules in a flagellum or cilium. (A) Electron micrograph of the flagellum of a green-alga cell *(Chlamydomonas)* shown in cross section, illustrating the distinctive "9 + 2" arrangement of microtubules. (B) Diagram of the parts of a flagellum or cilium. The various projections from the microtubules link the microtubules together and occur at regular intervals along the length of the axoneme. (A, courtesy of Lewis Tilney.)

a ring around a pair of single microtubules (**Figure 16–81**). Almost all forms of eucaryotic 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.

Molecules of *ciliary dynein* form bridges between the neighboring doublet microtubules around the circumference of the axoneme (**Figure 16–82**). When the motor domain of this dynein is activated, the dynein molecules attached to one microtubule doublet (see Figure 16–64) 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 16–83**).

The length of flagella is carefully regulated. If one of the two flagella on a *Chlamydomonas* cell is amputated, the remaining one will transiently shrink as the stump regrows until they reach the same length, and then the two shortened flagella will continue to elongate until both are as long as they were on the unperturbed cell. New flagellar components including tubulin and dynein are incorporated into the growing flagella at the distal tips. Thus, even in these

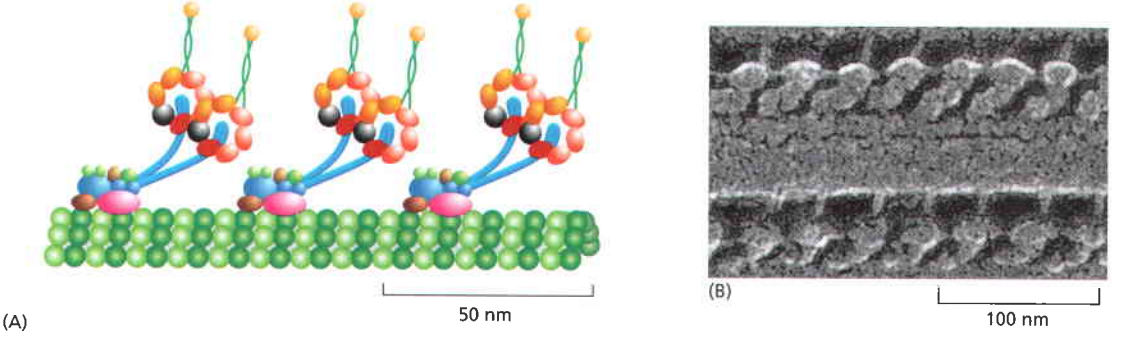


Figure 16–82 Ciliary dynein. Ciliary (axonemal) dynein is a large protein assembly (nearly 2 million daltons) composed of 9–12 polypeptide chains, the largest of which is the heavy chain of more than 500,000 daltons. (A) The heavy chains form the major portion of the globular head and stem domains, and many of the smaller chains are clustered around the base of the stem. There are two heads in the outer dynein in metazoans, but three heads in protozoa, each formed from their own heavy chain (see Figure 16–59B for a view of an isolated molecule). The tail of the molecule binds tightly to an A microtubule in an ATP-independent manner, while the large globular heads have an ATP-dependent binding site for a B microtubule (see Figure 16–81). 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. For details, see Figure 16–64. (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.)

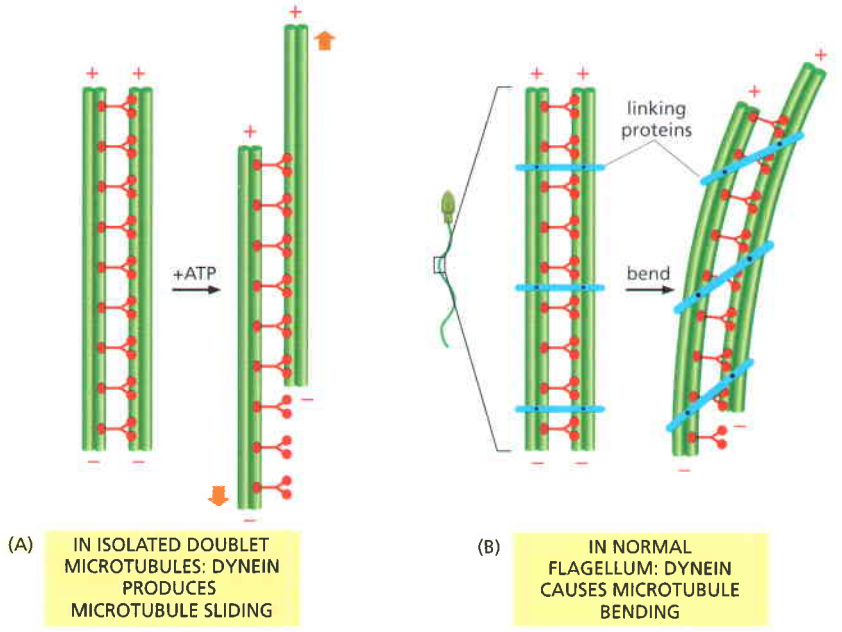


Figure 16–83 The bending of an axoneme. (A) When axonemes are exposed to the proteolytic enzyme trypsin, the linkages holding neighboring doublet microtubules together are broken. In this case, the addition of ATP allows the motor action of the dynein heads to slide one pair of doublet microtubules against 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, as seen in Figure 16–80.

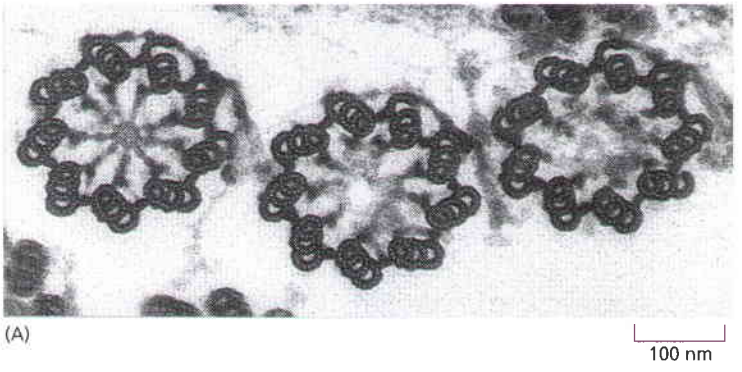
highly ordered and stable filament-motor arrays, cells use the intrinsic flexibility and adaptability of the cytoskeleton to respond rapidly and dynamically to the changes they experience.

In humans, hereditary defects in ciliary dynein cause Kartagener's syndrome. The syndrome is characterized by male sterility due to immotile sperm, a high susceptibility to lung infections owing to the paralyzed cilia in the respiratory tract that fail to clear debris and bacteria, and defects in determination of the left–right axis of the body during early embryonic development (discussed in Chapter 22).

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 (see Figure 15–71). The use of the same name to denote these two very different types of swimming apparatus is an unfortunate historical accident.

Structures called *basal bodies* firmly root eucaryotic cilia and flagella at the cell surface. The basal bodies have the same form as the centrioles that are found embedded at the center of animal centrosomes, with nine groups of fused triplet microtubules arranged in a cartwheel (**Figure 16–84**). Indeed, in some organisms, basal bodies and centrioles are functionally interconvertible: during each mitosis in the unicellular alga *Chlamydomonas*, for example, the flagella are resorbed, and the basal bodies move into the cell interior and become part of the spindle poles. New centrioles and basal bodies arise by a curious replication process, in which a smaller daughter is formed perpendicular to the original structure by a still mysterious mechanism (see Figure 17–31).

Figure 16–84 Basal bodies. (A) Electron micrograph of a cross section through three basal bodies in the cortex of a protozoan. (B) Diagram of a basal body viewed from the side. Each basal body forms the lower portion of a ciliary axoneme and is composed of nine sets of triplet microtubules, each triplet containing one complete microtubule (the A microtubule) fused to two incomplete microtubules (the B and C microtubules). Other proteins (shown in *red* in B) form links that hold the cylindrical array of microtubules together. The arrangement of microtubules in a centriole is essentially the same (see Figure 16–31). (A, courtesy of D.T. Woodrow and R.W. Linck.)



Even in animal cells that lack fully developed beating cilia or flagella, centrioles frequently nucleate the growth of a non-motile, microtubule-rich surface projection called a *primary cilium*. Primary cilia are usually only a few micrometers in length and lack dynein. They are found on the surface of many different cell types including fibroblasts, epithelial cells, neurons, bone cells, and chondrocytes (cartilage cells). Many signaling proteins are concentrated in the primary cilium, including proteins involved in the Hedgehog signaling pathway (see p. 950), and receptors for neurotransmitters on neurons in the central nervous system. On kidney epithelial cells, primary cilia act as flow sensors that detect the movement of fluid through the kidney tubules. Mechanosensitive calcium channels are opened when the fluid flow bends the primary cilia, regulating kidney cell growth and proliferation. Loss of the calcium channel or other structural components of the primary cilium in the kidney cells causes polycystic kidney disease, a common genetic disorder that causes overproliferation of the kidney epithelial cells—resulting in the formation of large fluid-filled cysts throughout the organs and eventually in kidney failure. Another specialized kind of primary cilium that is unusual in being able to beat is required for establishing left-right asymmetry in the developing embryo (see Figure 22–87).

Construction of the Mitotic Spindle Requires Microtubule Dynamics and the Interactions of Many Motor Proteins

Myofibrils and cilia are relatively permanent structures specialized to produce repetitive movement. But most cell movements depend on labile structures that appear at specific stages of the cell cycle or in response to external signals and then disappear once they complete their jobs. The most familiar of these are the mitotic spindle and the contractile ring that form during cell division. In Chapter 17, we will describe in detail both the process of mitosis and the cell cycle control system that determines the timing of the events of cell division. Here, we briefly discuss a few of the cytoskeletal mechanisms that contribute to the construction and mechanical function of the mitotic spindle.

The construction of the mitotic spindle is a particularly important and fascinating example of the power of self-organization by teams of motor proteins interacting with dynamic cytoskeletal filaments. It also features the active participation of the chromosomes. In a rapid sequence of events that typically takes less than an hour in animal cells, the interphase array of microtubules is completely disassembled and reorganized to form the bipolar spindle structure that is responsible for segregating the replicated chromosomes with perfect fidelity to the two daughter cells. Because of the central importance of reliability in transmission of the genetic material, the construction and functioning of the mitotic spindle feature a tremendous degree of redundancy, so that if one set of mechanisms fails for any reason, there are backup mechanisms in place to ensure reliable chromosome partitioning.

In early mitosis, there are dramatic changes in the dynamic behavior and average length of the microtubules. In the interphase array the microtubules are typically long and undergo rare catastrophes, but during mitosis the microtubules are shorter and much more dynamic. Microtubule nucleation and assembly are enhanced in the regions around the condensed chromosomes. As microtubules assemble on condensed chromatin pointing in random directions, the coordinated actions of several motor proteins build a coherent bipolar spindle from the disorganized microtuble mass. First, the bipolar kinesin-5 (see Figure 16–58) bundles the microtubules into a parallel array and slides microtubules that are oriented in opposite directions away from each other. Next, another kinesin that is bound to chromosome arms, kinesin-4, walks toward the plus ends of chromosome-associated microtubules and pushes their minus ends away from the chromosome mass. Finally, the minus-end directed motors cytoplasmic dynein and kinesin-14 form oligomeric complexes with scaffold proteins that gather the microtubule minus ends together to form the spindle poles. In most animal cells, these processes are guided by a pair of centrosomes that help to nucleate and organise these microtubule minus ends. The final result is the elegantly balanced bipolar mitotic spindle (**Figure 16–85**).

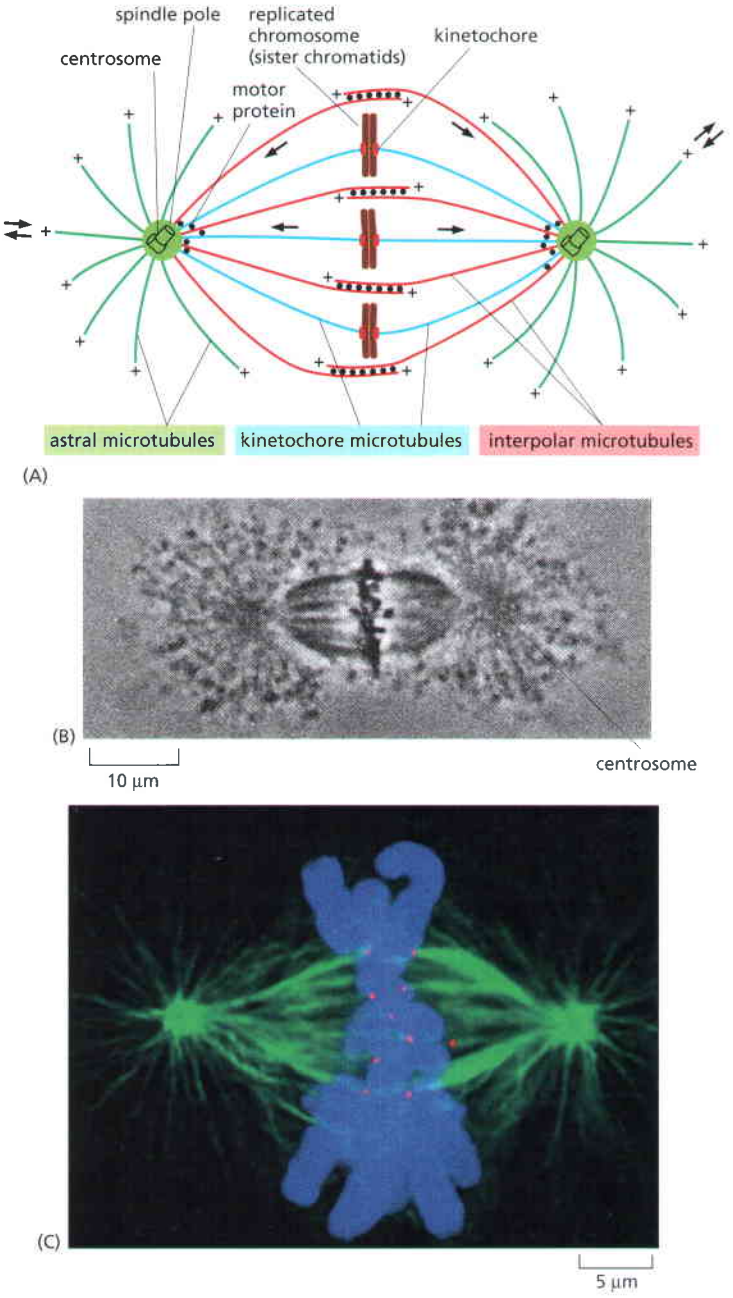


Figure 16–85 The mitotic spindle in animal cells. <GTCT> (A) There are three classes of dynamic microtubules in the mitotic spindle at metaphase: kinetochore microtubules (*blue*) that attach each chromosome to the spindle pole, interpolar microtubules (*red*) that hold the two halves of the spindle together, and astral microtubules (*green*) that can interact with the cell cortex. All of the microtubules are oriented with their minus ends at the spindle poles where the centrosomes reside, and their plus ends projecting away. As indicated by the arrows, the astral microtubules undergo dynamic instability, growing and shrinking at their plus ends, while the kinetochore microtubules and interpolar microtubules both undergo continuous flux toward the spindle poles. (B) A phase-contrast micrograph of an isolated mitotic spindle at metaphase, with the chromosomes aligned at the spindle equator. (C) This fluorescence micrograph shows the microtubules of the spindle in *green* and the chromosomes in *blue*. The *red* spots mark the kinetochores, specialized structures that connect the microtubules to the chromosomes. (B, from E.D. Salmon and R.R. Segall, *J. Cell Biol.* 86:355–365, 1980. With permission from The Rockefeller University Press; C, from A. Desai, *Curr. Biol.* 10:R508, 2000. With permission from Elsevier.)

After the bipolar mitotic spindle has assembled, it can appear stable and quiescent for long periods of time. In many animals, the unfertilized egg arrests its cell cycle in meiotic metaphase, and the spindle waits for days or months until fertilization triggers the progression of the cell cycle (see Chapter 21). This steady appearance is deceptive, because the spindle is actually an extremely dynamic structure, tensed for action that will begin when the chromosomes suddenly begin to separate in anaphase. For example, many of the spindle microtubules exhibit a behavior called *poleward flux*, with a net addition of tubulin subunits at their plus ends, balancing a net loss at their minus ends near the spindle pole. Poleward flux is driven by the action of minus end-directed motor proteins at the spindle pole that are constantly reeling in the microtubules, and the bipolar plus end-directed kinesin-5 motors on the interpolar microtubules that are constantly pushing them apart (see Figure 16–85). As will be discussed in Chapter 17, the delicate balance between these two types of motor protein activities in the spindle also determines its length. Overall the mitotic spindle represents a collaborative effort combining the dynamic properties of microtubules with the individual actions of dozens of molecular motors and other organizing components.

Many Cells Can Crawl Across a Solid Substratum

The process of cell crawling provides another instance where we can appreciate the dynamic integration of cytoskeletal filaments, filament regulators, and motor proteins. 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. 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 23). 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. These cells have diverse fates, becoming skin pigment cells, sensory and sympathetic neurons and glia, and various structures of the face. 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 can 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 crawling is a highly complex integrated process, dependent on the actin-rich cortex beneath the plasma membrane. Three distinct activities are involved: *protrusion,* in which actin-rich structures are 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 16–86**). In some crawling cells, such as

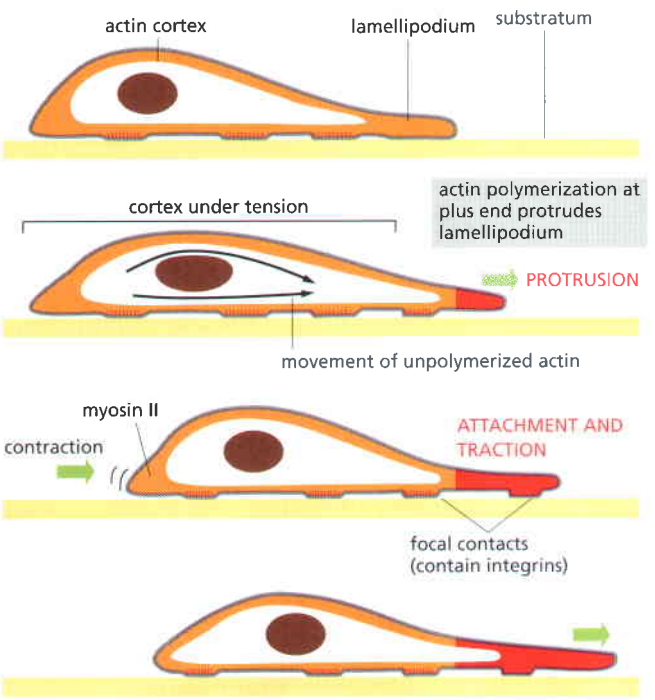


Figure 16–86 A model of how forces generated in the actin-rich cortex move a cell forward. The actin-polymerization-dependent protrusion and firm attachment of a lamellipodium at the leading edge of the cell moves the edge forward *(green* arrows at front) and stretches the actin cortex. Contraction 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.*

keratocytes from the fish epidermis, these activities are closely coordinated, 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, seems to rely primarily 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), lamellipodia, and pseudopodia. All are filled with a dense core of filamentous actin, which excludes membrane-enclosed organelles. The three structures differ primarily in the way in which the actin is organized—in one, two, or three dimensions, respectively—and we have already discussed how this results from the presence of different actin-associated proteins.

Filopodia, formed by migrating growth cones 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, sheet-like structures. They contain an orthogonally cross-linked mesh of actin filaments, most of which lie in a plane parallel to the solid substratum. **Pseudopodia**, formed by amoebae and neutrophils, are stubby three-dimensional projections filled with an actin-filament gel. <ATGG> Perhaps because their two-dimensional geometry is most convenient for examination with the light microscope, we have more information about the dynamic organization and protrusion mechanism of lamellipodia than we have for either filopodia or pseudopodia.

Lamellipodia contain all of the machinery that is required for cell motility. They have been especially well studied in the epithelial cells of the epidermis of fish and frogs, which 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 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 16–87**). 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 can be studied in keratocyte lamellipodia by marking a small patch of actin and examining its fate. This reveals

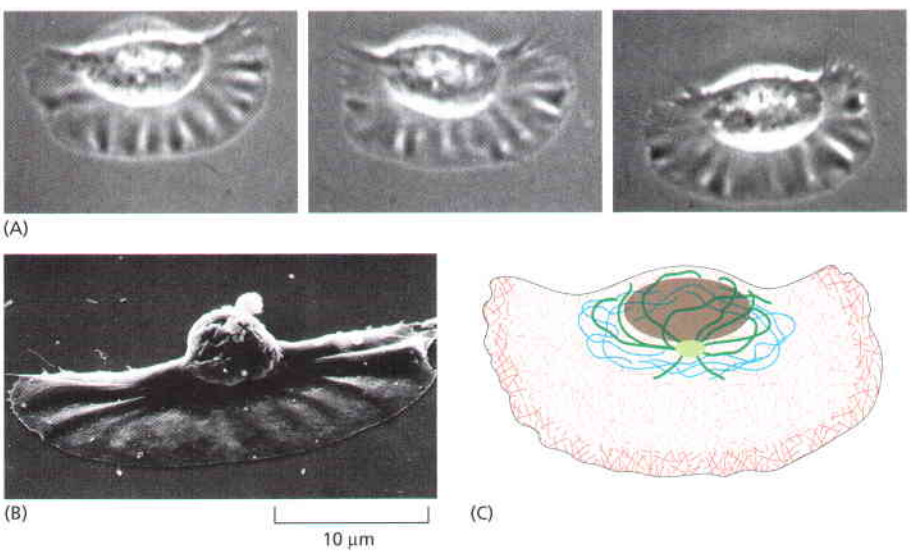


Figure 16–87 Migratory keratocytes from a fish epidermis. <GTTA> (A) Light micrographs of a keratocyte in culture, taken about 15 sec apart. This cell is moving at about 15 μm/sec. (B) Keratocyte seen by scanning electron microscopy, showing its broad, flat lamellipodium and small cell body, including 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.)

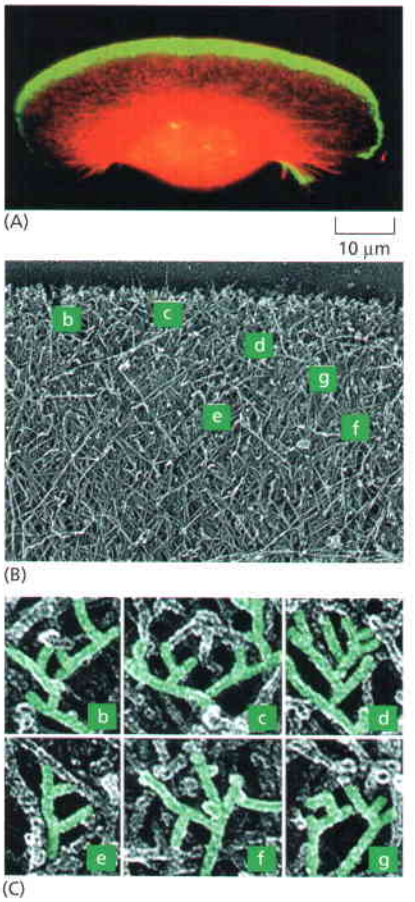


Figure 16–88 Actin filament nucleation and web formation by the ARP complex in lamellipodia. (A) A keratocyte with actin filaments labeled in *red* by fluorescent phalloidin, and the ARP complex labeled in *green* with an antibody raised against one of its component proteins. The regions where the two overlap appear *yellow*. The ARP 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 complex nucleates a new actin filament off the side of a preexisting filament (see Figure 16–34). (From T. Svitkina and G. Borisy, *J. Cell Biol.* 145:1009–1026, 1999. With permission from The Rockefeller University Press.)

that, while the lamellipodia crawl forward, the actin filaments remain stationary with respect to the substrate. 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 complexes (see Figure 16–34), helping to form the two-dimensional web (**Figure 16–88**). The web as a whole seems to be undergoing treadmilling, assembling at the front and disassembling at the back, reminiscent of the treadmilling that occurs in individual actin filaments and microtubules discussed previously (see Figure 16–14). Treadmilling of a dendritic web built by the ARP complex is only one of several ways that cells can use dynamic actin filaments to drive the protrusion of the leading edge. Some slowly-moving cells including fibroblasts appear to use a mechanism that does not depend on the ARP complex, but still requires coordinated actin filament assembly and disassembly, possibly coordinated by formins.

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 16–42) 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 16–89**). 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 16–90**). Finally, bipolar myosin II filaments seem to associate with the actin filaments in the web and pull them into a new orientation—from nearly perpendicular to the leading edge to an orientation almost parallel to the leading edge. This 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 16–91**).

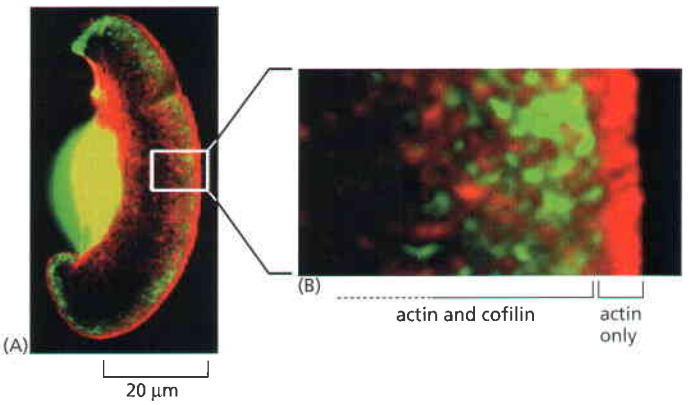


Figure 16–89 Cofilin in lamellipodia. (A) A keratocyte with actin filaments labeled in *red* by fluorescent phalloidin and cofilin labeled in *green* with a fluorescent antibody. The regions where the two overlap appear *yellow*. Although the dense actin meshwork reaches all the way through the lamellipodium, 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) in the filament lattice are generally not associated with cofilin. (From T. Svitkina and G. Borisy, *J. Cell Biol.* 145:1009–1026, 1999. With permission from The Rockefeller University Press.)

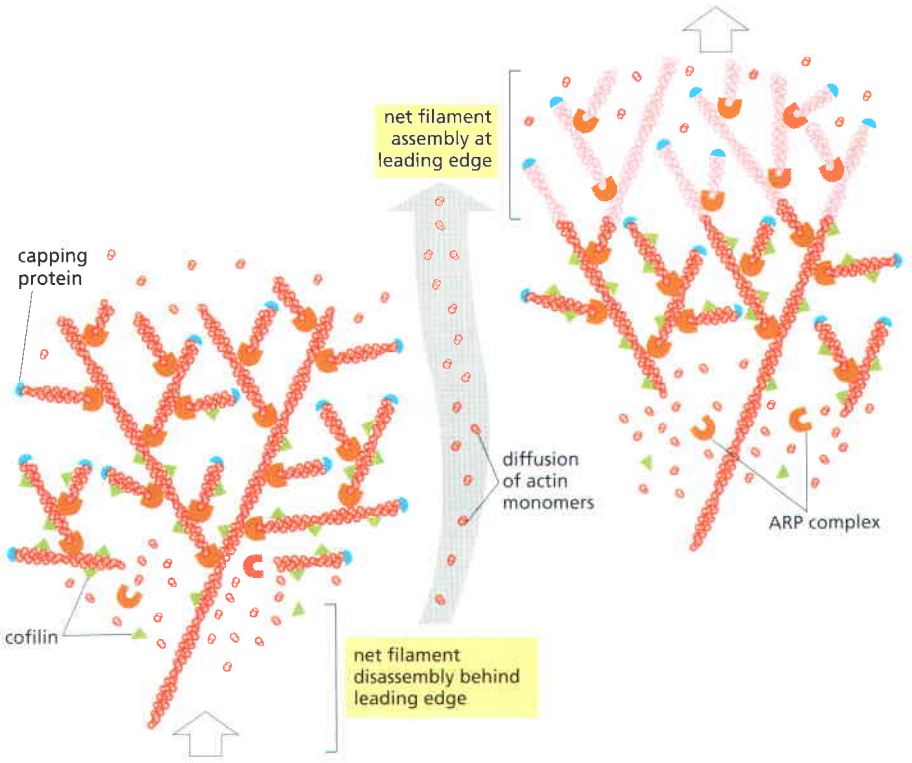
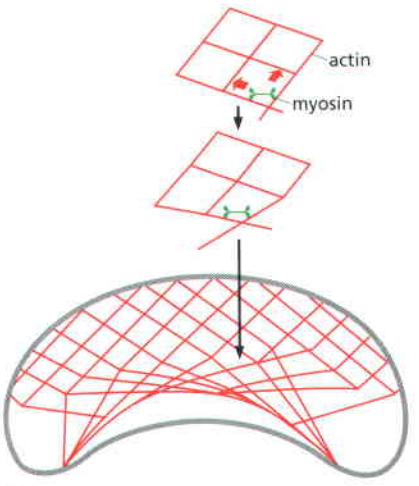


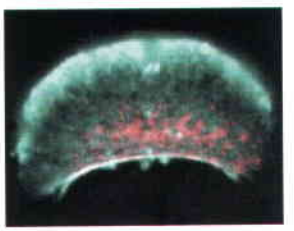
Figure 16–90 A model for protrusion of the actin meshwork at the leading edge. Two time points during advance of the lamellipodium are illustrated, with newly assembled structures at the later time point shown in a lighter color. Nucleation is mediated by the ARP complex at the front. Newly nucleated 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.

The pushing force created by the polymerization of a branched web of actin filaments plays an important role in many cell processes. The polymerization at the plus end can push the plasma membrane outward, as in the example just discussed (see Figure 16–90), or it can propel vesicles or particles through the cell cytoplasm, as in the example of the bacterium *Listeria monocytogenes* discussed in Chapter 24 (see Figure 24–37 <GTAT>). Moreover, when anchored in a more complex way to the membrane, the same type of force drives plasma membrane invaginations, as it does during the endocytotic and phagocytotic processes discussed in Chapter 13.

It is interesting to compare the organization of the actin-rich lamellipodium to the organization of the microtubule-rich mitotic spindle. In both cases, the cell harnesses and amplifies the intrinsic dynamic behavior of the cytoskeletal filament systems to generate large-scale structures that determine the behavior of the whole cell. Both structures feature rapid turnover of their constituent cytoskeletal filaments, even though the structures themselves may remain intact at steady state for long periods of time. The leading edge plasma membrane in the lamellipodium fulfills an organizational role analogous to the condensed chromosomes in organizing and stimulating the dynamics of the mitotic spindle. In both cases, molecular motor proteins help to enhance cytoskeletal filament flux and turnover in the large-scale arrays.

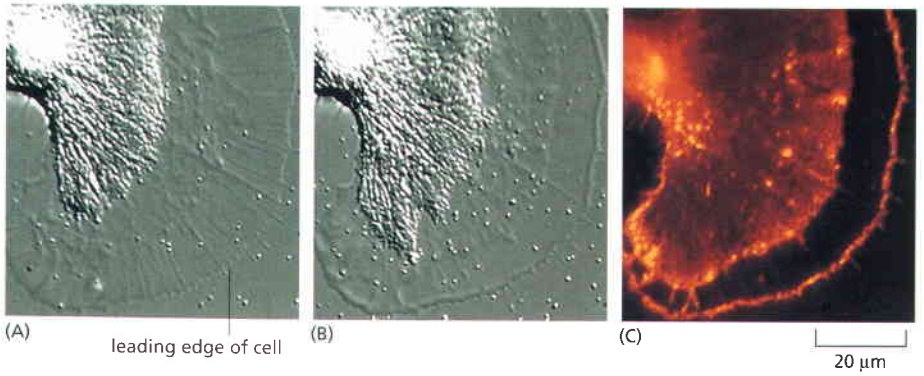


(A)



(B)

Figure 16–91 Contribution of myosin II to polarized cell motility. (A) Myosin II bipolar filaments bind to actin filaments in the dendritic lamellipodial meshwork and cause network contraction. The myosin-driven reorientation of the actin filaments in the dendritic meshwork forms an actin bundle that recruits more myosin II and contributes to generating 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. Verkovsky et al., *Curr. Biol.* 9:11–20, 1999. With permission from Elsevier.)



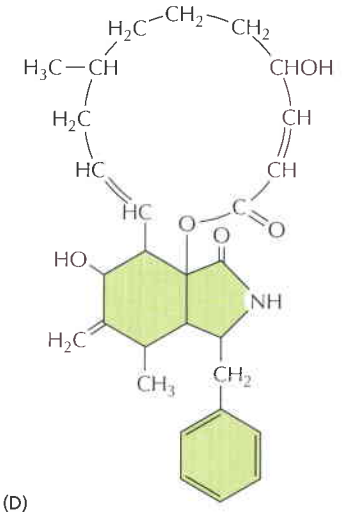


Figure 16–92 **Rearward movement of the actin network in a growth-cone lamellipodium.** (A) A growth cone from a neuron of the sea slug *Aplysia* is cultured on a highly adhesive substratum and viewed by differential-interference-contrast microscopy. Microtubules and membrane-enclosed organelles are confined to the bright, rear area of the growth cone (to the *left),* while a meshwork of actin filaments fills the lamellipodium (on the *right).* (B) After brief treatment with the drug cytochalasin, which caps the plus ends of actin filaments (see Table 16–2, p. 988), the actin meshwork has detached from the front edge of the lamellipodium and has been pulled backward. (C) At the time point shown in B, the cell was fixed and labeled with fluorescent phalloidin to show the distribution of the actin filaments. Some actin filaments persist at the leading edge, but the region behind the leading edge is devoid of filaments. Note the sharp boundary of the rearward-moving actin meshwork. (D) The complex cyclic structure of cytochalasin B. (A–C, courtesy of Paul Forscher.)

Cell Adhesion and Traction Allow Cells to Pull Themselves Forward

Lamellipodia of all cells seem to share a basic, simple type of dynamic organization where actin filament assembly occurs preferentially at the leading edge and actin filament disassembly occurs preferentially at the rear. However, the interactions between the cell and its normal physical environment usually make the situation considerably more complex than for fish keratocytes crawling on a culture dish. Particularly important in locomotion is the intimate crosstalk between the cytoskeleton and the cell adhesion apparatus. Although some degree of adhesion to the substratum is necessary for any form of cell crawling, adhesion and locomotion rate seem generally to be inversely related, with highly adhesive cells moving more slowly than weakly adhesive ones. Keratocytes are so weakly adhesive to the substratum that the force of actin polymerization can push the leading edge forward very rapidly. In contrast, neurons from the sea slug *Aplysia* cultured on a sticky substratum form large lamellipodia that become stuck too tightly to move forward. In these lamellipodia, the same cycle of localized nucleation of new actin filaments, depolymerization of old filaments, and myosin-dependent contraction continues to operate. But because the leading edge is prevented physically from moving forward, the entire actin mesh moves backward toward the cell body instead, pulled by myosins (**Figure 16–92**). The adhesion of most cells lies somewhere between these two extremes, and most lamellipodia exhibit some combination of forward actin filament protrusion (like keratocytes) and rearward actin flux (like the *Aplysia* neurons).

As a lamellipodium, filopodium, or pseudopodium extends forward over a substratum, it can form new attachment sites at the cell front that remain stationary as the cell moves forward over them, persisting until the rear of the cell catches up with them. When an individual lamellipodium fails to adhere to the

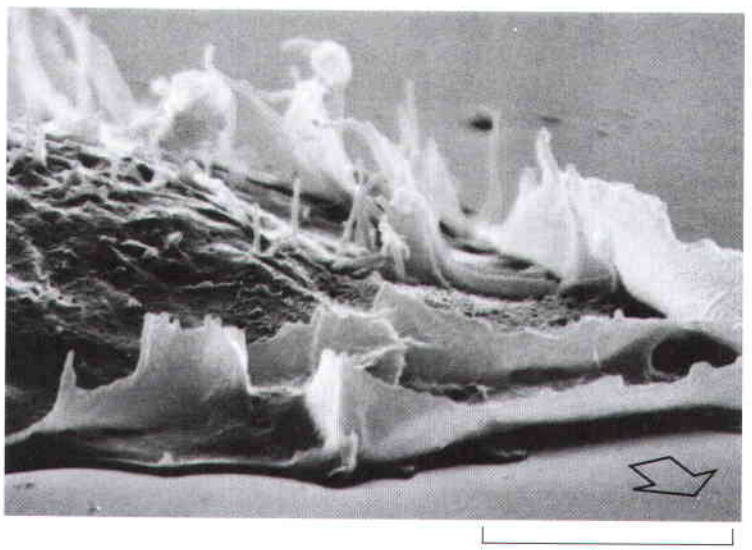


Figure 16–93 **Lamellipodia and ruffles at the leading edge of a human fibroblast migrating in culture.** The *arrow* in this scanning electron micrograph shows the direction of cell movement. As the cell moves forward, lamellipodia that fail to attach to the substratum are swept backward over the dorsal surface of the cell, a movement known as ruffling. (Courtesy of Julian Heath.)

substratum, it is usually lifted up onto the dorsal surface of the cell and rapidly carried backward as a "ruffle" (**Figure 16–93**).

The attachment sites established at the leading edge serve as anchorage points, which allow the cell to generate traction on the substratum and pull its body forward. Myosin motor proteins, especially myosin II, seem to generate traction forces. In many locomoting cells, myosin II is highly concentrated at the posterior of the cell where it may help to push the cell body forward like toothpaste being squeezed out of a tube from the rear (**Figure 16–94**; see also Figure 16–91). *Dictyostelium* amoebae that are deficient in myosin II are able to protrude pseudopodia at normal speeds, but the translocation of their cell body is much slower than that of wild-type amoebae, indicating the importance of myosin II contraction in this part of the cell locomotion cycle. In addition to helping to push the cell body forward, contraction of the actin-rich cortex at the rear of the cell may selectively weaken the older adhesive interactions that tend to hold the cell back. Myosin II may also transport cell body components forward over a polarized array of actin filaments.

The traction forces generated by locomoting cells exert a significant pull on the substratum (**Figure 16–95**). 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. In culture, movement of fibroblasts through a gel of collagen fibrils aligns the collagen, generating an organized extracellular matrix that in turn affects the shape and direction of locomotion of the fibroblasts within it (**Figure 16–96**). 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 be a primary way that vertebrate tissues organize themselves.

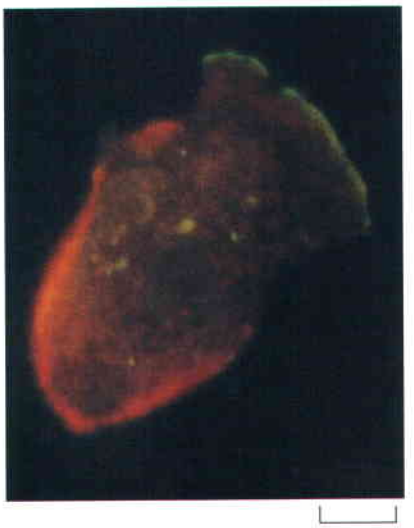


Figure 16–94 The localization of myosin I and myosin II in a normal crawling *Dictyostelium* amoeba. This cell was crawling toward the upper right at the time that it was fixed and labeled with antibodies specific for two myosin isoforms. Myosin I *(green)* is mainly restricted to the leading edge of pseudopodia at the front of the cell. Myosin II *(red)* is highest in the posterior, actin-rich cortex. Contraction of the cortex at the posterior of the cell by myosin II may help to push the cell body forward. (Courtesy of Yoshio Fukui.)

Members of the Rho Protein Family Cause Major Rearrangements of the Actin Cytoskeleton

Cell migration is one example of a process that 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 which is typically several 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 vs. the back, or at the top vs. the

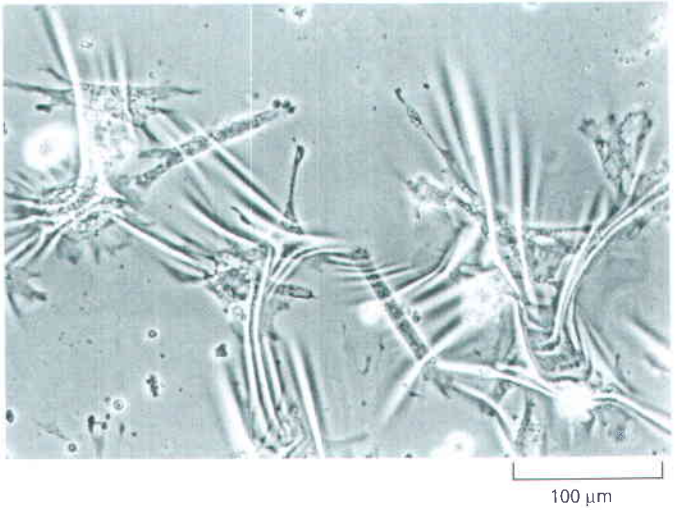


Figure 16–95 Adhesive cells exert traction forces on the substratum. These fibroblasts have been cultured on a very thin sheet of silicon rubber. Attachment of the cells, followed by contraction of their cytoskeleton, has caused the rubber substratum to wrinkle. (From A.K. Harris, P. Wild and D. Stopak, *Science* 208:177–179, 1980. With permission from AAAS.)

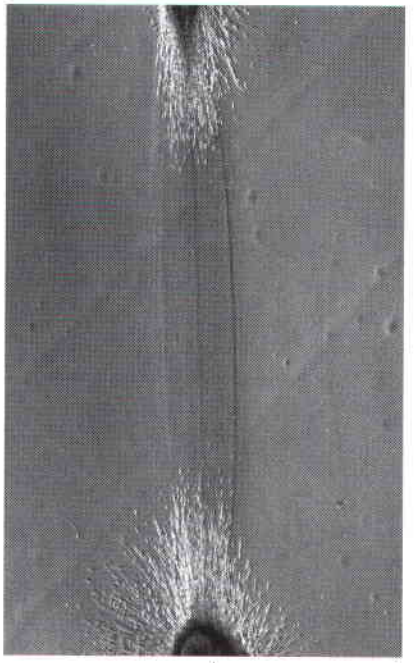


Figure 16–96 Shaping of the extracellular matrix by cell pulling. This micrograph shows a region between two pieces of embryonic chick heart (tissue explants rich in fibroblasts and heart muscle cells) that were grown in culture on a collagen gel for 4 days. A dense tract of aligned collagen fibers has formed between the two explants, apparently as a result of fibroblasts tugging on the collagen. (From D. Stopak and A.K. Harris, *Dev. Biol.* 90:383–398, 1982. With permission from Academic Press.)

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.

For the actin cytoskeleton, diverse cell-surface receptors trigger global structural rearrangements in response to external signals. But all 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*. The same Rho family proteins are also involved in the establishment of many kinds of cell polarity.

Like other members of the Ras superfamily, these Rho proteins act as molecular switches to control cell processes by cycling between an active, GTP-bound state and an inactive, GDP-bound state (see Figure 3–71). Activation of Cdc42 on the plasma membrane triggers actin polymerization and bundling to form either filopodia or shorter cell protrusions called microspikes. Activation of Rac promotes actin polymerization at the cell periphery leading to the formation of sheet-like lamellipodial extensions and membrane ruffles, which are actin-rich protrusions on the cell's dorsal surface (see Figure 16–93). Activation of Rho promotes both the bundling of actin filaments with myosin II filaments into stress fibers and the clustering of integrins and associated proteins to form focal contacts (**Figure 16–97**). 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.

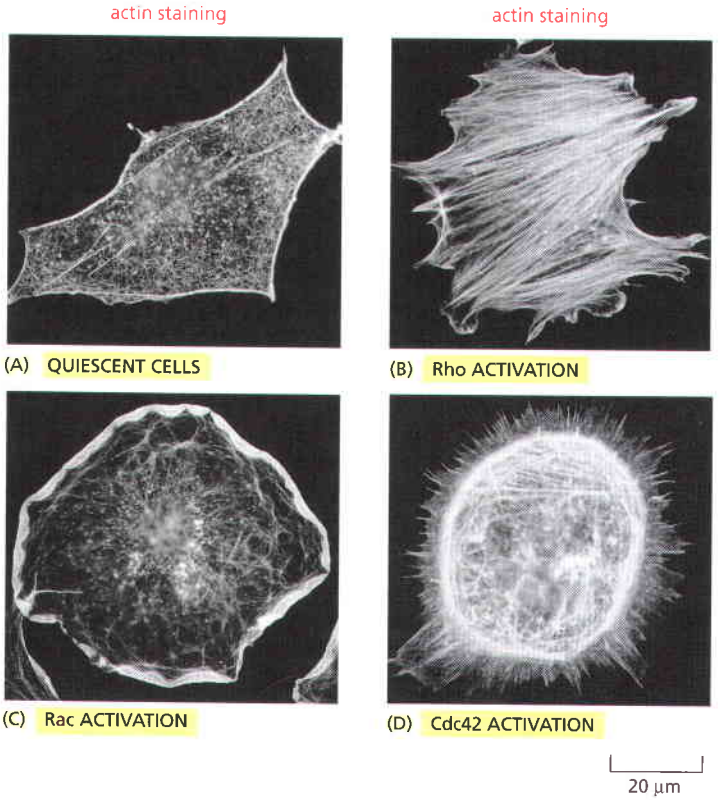


Figure 16–97 The dramatic effects of Rac, Rho, and Cdc42 on actin organization in fibroblasts. In each case, the actin filaments have been labeled with fluorescent phalloidin. (A) Serum-starved fibroblasts have actin filaments primarily in the cortex, and relatively few stress fibers. (B) Microinjection of a constitutively activated form of Rho causes the rapid assembly of many prominent stress fibers. (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 Cdc42, another Rho family member, causes the protrusion of many long filopodia at the cell periphery. The distinct global effects of these three GTPases on the organization of the actin cytoskeleton are mediated by the actions of dozens of other protein molecules that are regulated by the GTPases. These target proteins include some of the various actin-associated proteins that we have discussed in this chapter. (From A. Hall, *Science* 279:509–514, 1998. With permission from AAAS.)

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 where 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 family members are expressed ubiquitously that enable activated Cdc42 to enhance actin polymerization. **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 complex and strongly enhancing this complex's actin-nucleating activity (see Figure 16–34). In this way, activation of Cdc42 increases actin nucleation.

Rac-GTP also activates WASp family members, as well as activating the crosslinking activity of the gel-forming protein filamin, and inhibiting the contractile activity of the motor protein myosin II, stabilizing the lamellipodia and inhibiting the formation of contractile stress fibers (**Figure 16–98**A).

Rho-GTP has a very different set of targets. Instead of activating the ARP 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 16–72). 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 16–98B).

In some cell types, Rac-GTP activates Rho, usually with kinetics that are slow compared to Rac's activation of the ARP complex. This enables cells to use the Rac pathway to build a new actin structure while subsequently activating the Rho pathway to induce a contractility that builds up tension in this structure. This occurs, for example, during the formation and maturation of cell-cell contacts. 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 85 GEFs that have been identified in

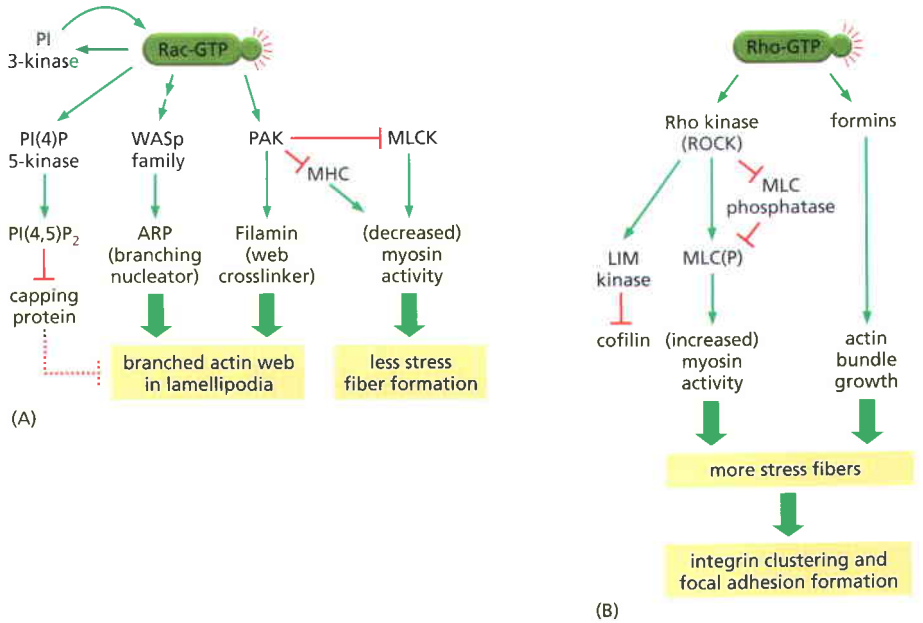


Figure 16–98 The contrasting effects of Rac and Rho activation on actin organization. (A) Activation of the small GTPase Rac leads to actin nucleation by the ARP complex and other alterations in actin accessory proteins that tend to favor the formation of actin networks, as in lamellipodia. Several different 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 complex. In a parallel pathway, Rac-GTP activates a protein kinase, PAK, which has several targets including the web-forming crosslinker filamin, which is activated by phosphorylation, and the myosin light chain kinase (MLCK), which is inhibited by phosphorylation. The resulting decrease in phosphorylation of the myosin regulatory light chain 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). Another set of pathways downstream of Rac activation is mediated by phosphoinositide lipid signals. Local creation of PIP_2 [$PI(4,5)P_2$] may help to reduce the activity of capping protein, to further aid actin polymerization. Activation of PI 3-kinase, which generates PIP_3 from PIP_2, leads to further activation of Rac itself via a positive feedback loop.

(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 17–52).

the human genome, some are specific for an individual Rho family GTPase, whereas others seem to act on all three family members. The number of GEFs exceeds the number of Rho GTPases that they regulate because different GEFs are restricted to specific tissues and even specific subcellular locations, and they are sensitive to distinct kinds of regulatory inputs. Various cell-surface receptors activate GEFs. An example is the Eph receptor tyrosine kinase involved in neurite growth cone guidance, which is discussed in detail in Chapter 15. 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, which is important for the overall integration of cell shape and movement.

The Rho family GTPases are also primary determinants of cell polarity in budding yeast, where extensive genetic analyses have increased our understanding of the general mechanisms involved. On starvation, yeasts, like many other unicellular organisms, sporulate. But sporulation can occur only in diploid budding yeast cells, whereas budding yeasts mainly proliferate as haploid cells. A starving haploid individual must therefore locate a partner of the opposite mating type, woo it, and mate with it before sporulating. Yeast cells are unable to swim and, instead, reach their mates by polarized growth. The haploid form of budding yeast comes in two mating types, **a** and α, which secrete mating factors known as **a**-factor and α-factor, respectively. These secreted signal molecules act by binding to cell-surface receptors that belong to the G-protein-coupled receptor superfamily (discussed in Chapter 15). One consequence of the binding of α-factor to its receptor is to cause the recipient cell to become polarized, adopting a shape known as a "shmoo" (**Figure 16–99**). In the presence of an α-factor gradient, the **a**-cell shmoo tip is directed toward the highest concentration of the signal molecule, which under normal circumstances would direct it toward an amorous α cell located nearby.

This polarized cell growth requires alignment of the actin cytoskeleton in response to the mating factor signal. When the signal binds to its receptor, the receptor activates Cdc42, which in turn induces assembly of actin filaments at the location closest to the source of the signal. Local activation of Cdc42 is further enhanced by a positive feedback loop, requiring actin-dependent transport of Cdc42 itself as well as its GEF and other signaling components along the newly assembled actin structures toward the site of the signal. Subsequently, actin cables are assembled pointing toward the site of Cdc42 accumulation due to the activation of another Rho family GTPase that in turn stimulates a yeast formin. The actin cables serve as tracks for directed transport and exocytosis of new cell wall material, resulting in the polarized growth of the shmoo tip (**Figure 16–100**).

Haploid budding yeast cells use this same polarization machinery during vegetative growth. To form the bud that will grow out to become a daughter cell, the yeast must direct new plasma membrane and cell wall material primarily to a single site. As with shmoo formation, this requires an initial cytoskeletal polarity, with most actin patches in the growing bud and actin cables oriented along the bud axis. In haploid cells, a new bud site is always constructed immediately adjacent to the previous bud site. In this case, the spatial cues that set up cytoskeletal polarity are intrinsic to the cell, left behind from previous rounds of cell division. Cdc42 is once again involved in transducing the signal from the destined bud site to the cytoskeleton, and most of the proteins involved in the upstream and downstream pathways have been identified through genetic

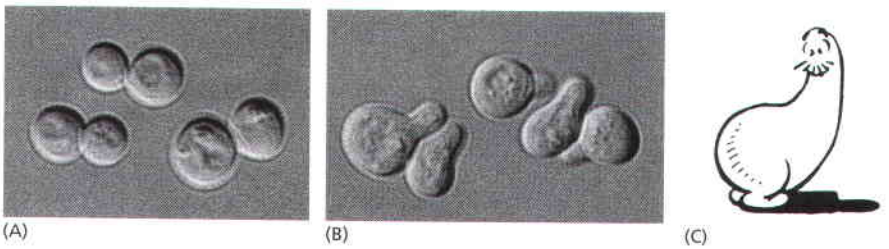


Figure 16–99 Morphological polarization of yeast cells in response to mating factor. (A) Cells of *Saccharomyces cerevisiae* are usually spherical. (B) They become polarized when treated with mating factor from cells of the opposite mating type. The polarized cells are called "shmoos." (C) Al Capp's famous cartoon character, the original Shmoo. (A and B, courtesy of Michael Snyder; C, © 1948 Capp Enterprises, Inc. Used by permission.)

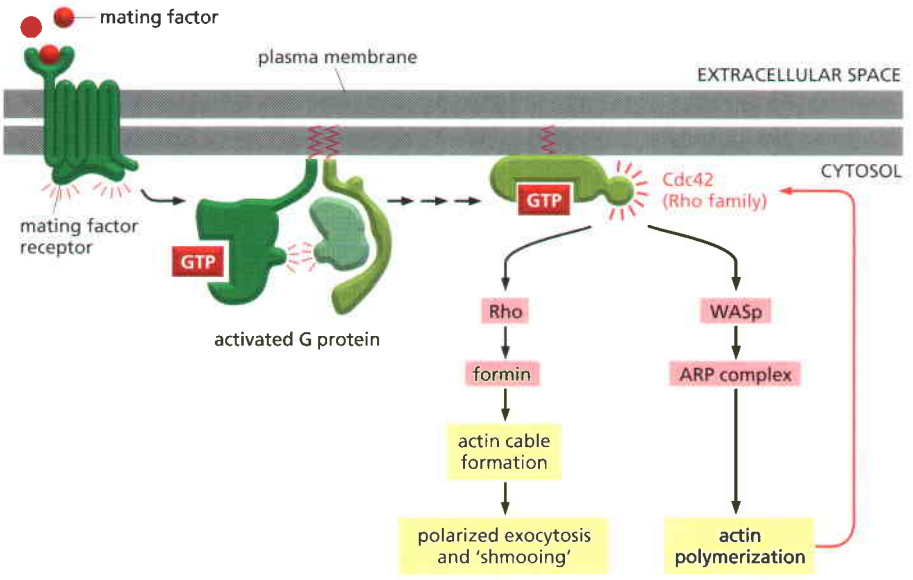


Figure 16–100 The signaling pathway in the yeast mating factor response. The extracellular mating factor binds to a G-protein-coupled receptor in the plasma membrane. Activation of the receptor triggers dissociation of the GTP-bound Gα subunit from a heterotrimeric G-protein (discussed in Chapter 15). This in turn activates the Rho family GTP-binding protein, Cdc42. As in mammalian cells, Cdc42 activates a WASp family protein that activates the ARP complex, leading to local actin nucleation at the site of mating factor binding. The local actin nucleation and filament growth create a positive feedback loop whereby Cdc42 activity is further enhanced. This leads to extensive Rho and formin activation, and finally to actin cable formation, polarized growth, and acquisition of a shmoo shape. In addition, receptor activation triggers other responses through a MAP kinase cascade (discussed in Chapter 15), preparing the haploid cell for mating (not shown).

experiments. Subsequent to their identification in yeast, many of these proteins have been found to have homologs in other organisms, where they are often likewise involved in the establishment of cell polarity.

External Signals Can Dictate the Direction of Cell Migration

Chemotaxis is defined as cell movement in a direction controlled by a gradient of a diffusible chemical. This is a particularly interesting case where external signals trigger the Rho family proteins to set up large-scale cell polarity by influencing the organization of the apparatus required for cell motility, described above. 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 the very low concentrations of the *N*-formylated peptides that are derived from bacterial proteins (only procaryotes 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 16–101**).

Both in this case and in the similar chemotaxis of *Dictyostelium* amoebae toward a source of cyclic AMP, a local polymerization of actin near the receptors is stimulated when the receptors bind their ligands. This actin polymerization response depends on the monomeric Rho-family GTPases discussed earlier. As in the shmooing yeast (see Figure 16–99), the responding cell extends a protrusion toward the signal. For chemotactic cells, binding of the chemoattractant ligand to its G-protein coupled receptor activates phosphoinositide 3′ kinases (PI3Ks), which generates a lipid-based signaling molecule ($PI(3,4,5)P_3$) that in turn activates the Rac GTPase. Rac then activates the ARP complex and lamellipodial protrusion results (see Figure 16–98). Through an unknown mechanism,

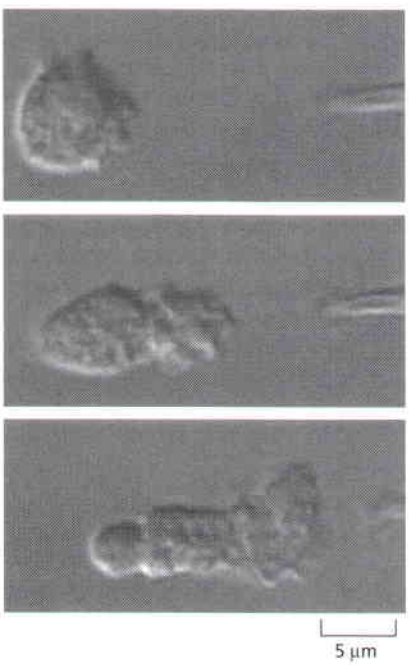


Figure 16–101 Neutrophil polarization and chemotaxis. <GTCG> <TGTA> The pipette tip at the right is leaking a small amount of the peptide formyl-Met-Leu-Phe. Only bacterial proteins have formylated methionine residues, so the human neutrophil recognizes this peptide as the product of a foreign invader (discussed in Chapter 24). 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 this 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 16–4). (From O.D. Weiner et al., *Nat. Cell Biol.* 1:75–81, 1999. With permission from Macmillan Publishers Ltd.)

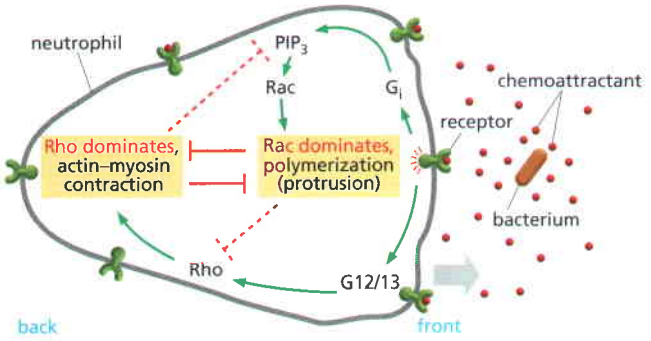


Figure 16–102 Signaling during neutrophil polarization. Bacteria that have invaded the human body secrete molecules that are recognized as foreign by the cells of the immune system, including neutrophils. Binding of the bacterial molecules to G-protein-coupled receptors on the neutrophils stimulate directed 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, close to the source of the bacterial signal, 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 G12 and G13 (denoted G12/13), 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. For a real-life example of the effectiveness of this signaling system, see Figure 16–4.

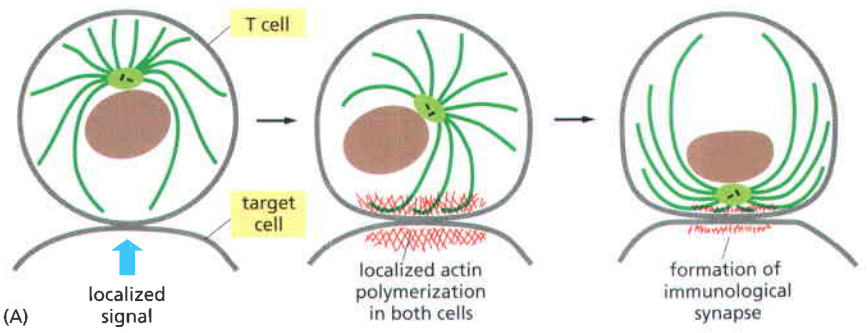
accumulation of the polarized actin web at the leading edge causes further local enhancement of PI3K activity in a positive feedback loop, strengthening the induction of protrusion. The $PI(3,4,5)P_3$ that activates Rac cannot diffuse far from its site of synthesis, since it is rapidly converted back into PIP_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, and as a result, Rac activation dominates in the front of the cell and Rho activation dominates in the rear (**Figure 16–102**). 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 non-diffusible signals to steer the locomoting cells or growth cones to their proper destinations (see Figure 15–62).

Communication Between the Microtubule and Actin Cytoskeletons Coordinates Whole-Cell Polarization and Locomotion

To help organize persistent movement in a particular direction cells use their microtubules along with their actin filaments. In many locomoting cells, the position of the centrosome is influenced by the location of protrusive actin polymerization, being found on the forward side of the nucleus. The mechanism of centrosome reorientation is not clear, although there is evidence that the Rho family protein Cdc42 may be involved. It is thought that the activation of receptors on one edge of a cell might not only stimulate actin polymerization there (and therefore local protrusion) but also locally activate dynein-like 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 therefore inhibit) the tubulin binding protein stathmin (see Panel 16–3, pp. 994–995), destabilizing microtubules, and Rho activation appears to stabilize microtubules.

In turn, microtubule dynamics influence actin rearrangements. The centrosome nucleates a large number of dynamic microtubules, and its repositioning means that many of these microtubules have their plus ends extending from the centrosome into the protrusive region of the cell. The dynamic microtubule plus ends may indirectly modulate local adhesion and also activate the Rac GTPase to further increase actin polymerization in the protrusive region by delivering Rac-GEFs that bind to the +TIPs traveling on growing microtubule ends. The increased concentration of microtubules would thereby encourage further protrusion, creating a positive feedback loop that enables protrusive motility to persist in the same direction for a prolonged period. Regardless of the exact mechanism, the orientation of the centrosome seems to reinforce the polarity information that the actin cytoskeleton receives from the outside world, allowing a sensitive response to weak signals.



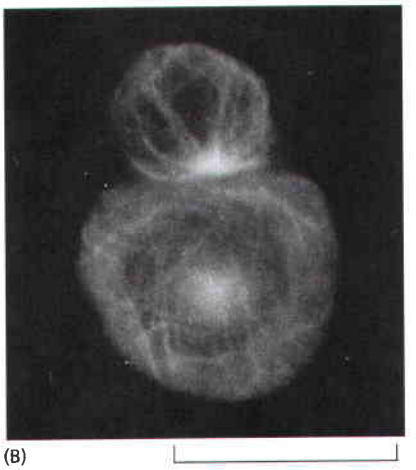


Figure 16–103 The polarization of a cytotoxic T cell after target-cell recognition. (A) Changes in the cytoskeleton of a cytotoxic T cell after it has made contact with a target cell. The initial recognition event results in signals that cause actin polymerization in both cells at the site of contact. In the T cell, interactions between the actin-rich contact zone and microtubules emanating from the centrosome result in reorientation of the centrosome, so that the associated Golgi apparatus is directly apposed to the target cell. (B) Immuno-fluorescence micrograph in which both the T cell *(top)* and its target cell *(bottom)* have been stained with an antibody against microtubules. The centrosome and the microtubules radiating from it in the T cell are oriented toward the point of cell–cell contact. In contrast, the microtubule array in the target cell is not polarized. (B, from B. Geiger, D. Rosen and G. Berke, *J. Cell Biol.* 95:137–143, 1982. With permission from The Rockefeller University Press.)

A similar cooperative feedback loop seems to operate in many other instances of cell polarization. A particularly interesting example is the killing of specific target cells by T lymphocytes. These cells are a critical component of the vertebrate's adaptive immune response to infection by viruses. T cells, like neutrophils, use actin-based motility to crawl through the body's tissue and find infected target cells. When a T cell comes into contact with a virus-infected cell and its receptors recognize foreign viral antigens on the surface of the target cell, the same polarization machinery is engaged in a very different way to facilitate killing of the target cell. Rac is activated at the point of cell–cell contact and causes actin polymerization at this site, creating a specialized region of the cortex. This specialized site causes the centrosome to reorient, moving with its microtubules to the zone of T-cell-target contact (**Figure 16–103**). The microtubules, in turn, position the Golgi apparatus right under the contact zone, focusing the killing machinery onto the target cell. The mechanism of killing is discussed in Chapter 25 (see Figure 25–47).

The Complex Morphological Specialization of Neurons Depends on the Cytoskeleton

For our final case study of the ways that the intrinsic properties of the eucaryotic cytoskeleton enable specific and enormously complicated large-scale cell behaviors, we examine the neuron. Neurons begin life in the embryo as unremarkable cells, which use actin-based motility to migrate to specific locations. Once there, however, they send out a series of long specialized processes that will either receive electrical signals *(dendrites)* or transmit electrical signals *(axons)* to their target cells. <TACC> 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 and often unpredictable 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 forward toward the axon terminals (**Figure 16–104**). The microtubules do not reach

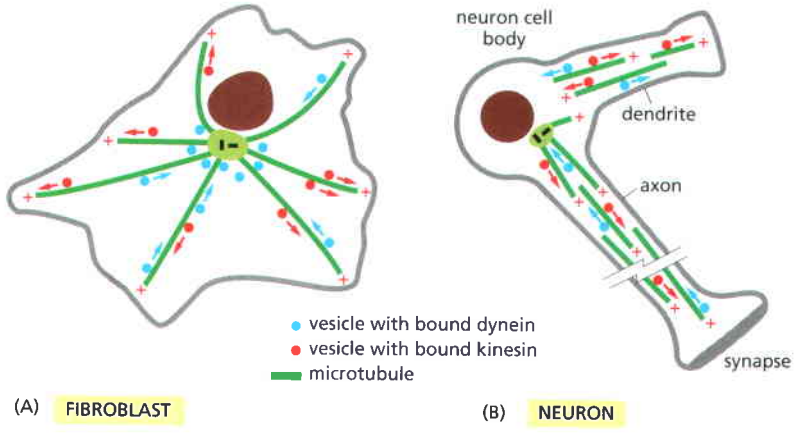


Figure 16–104 Microtubule organization in fibroblasts and neurons. (A) In a fibroblast, microtubules emanate outward from the centrosome in the middle of the cell. Vesicles with plus-end-directed kinesin attached move outward, and vesicles with minus-end-directed dynein attached move inward. (B) In a neuron, microtubule organization is more complex. In the axon, all microtubules share the same polarity, with the plus ends pointing outward toward the axon terminus. No one 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.

from the cell 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. This set of aligned microtubule tracks acts as a highway to transport many specific proteins, protein-containing vesicles, and mRNAs to the axon terminals, where synapses must be constructed and maintained. The longest axon in the human body reaches from the base of the spinal cord to the foot, being up to a meter in length.

Mitochondria, large numbers of specific proteins in transport vesicles, and synaptic vesicle precursors make the long journey in the forward (anterograde) direction. They are carried there by plus-end-directed kinesin-family motor proteins that can move them a meter in as little as two or three days, which is a great improvement over diffusion, which would take approximately several decades to move a mitochondrion this distance. Many members of the kinesin superfamily contribute to this *anterograde axonal transport*, most carrying specific subsets of membrane-enclosed organelles along the microtubules. The great diversity of the kinesin family motor proteins used in axonal transport suggests that they are involved in targeting their cargo to specific structures near the terminus or along the way, as well as in cargo movement. Old components from the axon terminals are carried back to the cell body for degradation and recycling by a *retrograde axonal transport*. This transport occurs along the same set of oriented microtubules, but it relies on cytoplasmic dynein, which is a minus-end-directed motor protein. Retrograde transport is also critical for communicating the presence of growth and survival signals received by the nerve terminus back to the nucleus, in order to influence gene expression.

One form of human peripheral neuropathy, Charcot-Marie-Tooth disease, is caused by a point mutation in a particular kinesin family member that transports synaptic vesicle precursors down the axon. Other kinds of neurodegenerative diseases such as Alzheimer's disease may also be caused in part by disruptions in neuronal trafficking; as pointed out previously, the amyloid precursor protein APP is part of a protein complex that serves as a receptor for kinesin-1 binding to other axonal transport vesicles.

Axonal structure depends on the axonal microtubules, as well as on the contributions of the other two major cytoskeletal systems—actin filaments and intermediate filaments. Actin filaments line the cortex of the axon, just beneath the plasma membrane, and actin-based motor proteins such as myosin V are also abundant in the axon, presumably to help move materials. Neurofilaments, the specialized intermediate filaments of nerve cells, provide the most important structural support in the axon. A disruption in neurofilament structure, or in the cross-linking proteins that attach the neurofilaments to the microtubules and actin filaments distributed along the axon, can result in axonal disorganization and eventually axonal degeneration.

The construction of the elaborate branching architecture of the neuron during embryonic development requires actin-based motility. As mentioned earlier, the tips of growing axons and dendrites extend by means of a *growth cone*, a specialized motile structure rich in actin (**Figure 16–105**). Most neuronal growth cones produce filopodia, and some make lamellipodia as well. The protrusion

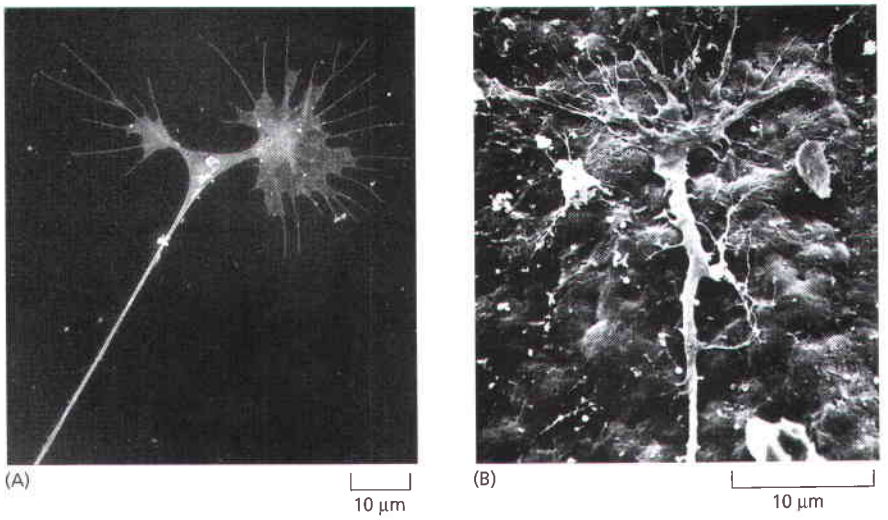


Figure 16–105 Neuronal growth cones. <AAGA> (A) Scanning electron micrograph of two growth cones at the end of a neurite, put out by a chick sympathetic neuron in culture. Here, a previously single growth cone has recently split into two. Note the many filopodia and the large lamellipodia. The taut appearance of the neurite is due to tension generated by the forward movement of the growth cones, which are often the only firm points of attachment of the axon to the substratum. (B) Scanning electron micrograph of the growth cone of a sensory neuron crawling over the inner surface of the epidermis of a *Xenopus* tadpole. (A, from D. Bray, in Cell Behaviour [R. Bellairs, A. Curtis and G. Dunn, eds.]. Cambridge, UK: Cambridge University Press, 1982; B, from A. Roberts, *Brain Res*. 118:526–530, 1976. With permission from Elsevier.)

and stabilization of growth-cone filopodia are exquisitely sensitive to environmental cues. Some cells secrete soluble proteins such as netrin to attract or repel growth cones. These modulate the structure and motility of the growth cone cytoskeleton by altering the balance between Rac activity and Rho activity at the leading edge (see Figure 15–62). In addition, there are fixed guidance markers along the way, attached to the extracellular matrix or to the surfaces of cells. When a filopodium encounters such a "guidepost" in its exploration, it quickly forms adhesive contacts. It is thought that a myosin-dependent collapse of the actin meshwork in the unstabilized part of the growth cone then causes the developing axon to turn toward the guidepost.

Thus, a complex combination of positive and negative signals, both soluble and insoluble, accurately guide the growth cone to its final destination. Microtubules then reinforce the directional decisions made by the actin-rich protrusive structures at the leading edge of the growth cone. Microtubules from the axonal parallel array just behind the growth cone are constantly growing into the growth cone and shrinking back by dynamic instability. Adhesive guidance signals are somehow relayed to the dynamic microtubule ends, so that microtubules growing in the correct direction are stabilized against disassembly. In this way, a microtubule-rich axon is left behind, marking the path that the growth cone has traveled.

Dendrites are generally much shorter projections than axons, and they receive synaptic inputs rather than being specialized for sending signals like axons. The microtubules in dendrites all 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. Nevertheless, dendrites also form as the result of growth-cone activity. Therefore, it is the growth cones at the tips of axons and dendrites that create the intricate, highly individual morphology of each mature neuronal cell (**Figure 16–106**).

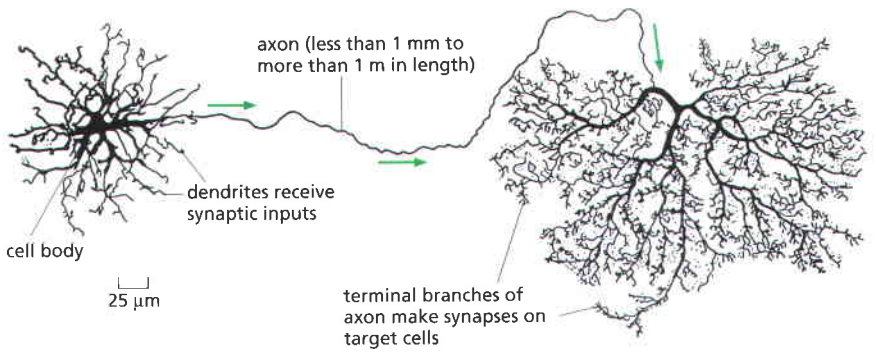


Figure 16–106 The complex architecture of a vertebrate neuron. The neuron shown is from the retina of a monkey. The arrows indicate the direction of travel of the electrical signal along the axon. The longest and largest neurons in the human body extend for a distance of about 1 m (1 million μm), from the base of the spinal cord to the tip of the big toe, and have an axon diameter of 15 μm. (Adapted from B.B. Boycott, in Essays on the Nervous System [R. Bellairs and E.G. Gray, eds.]. Oxford, UK: Clarendon Press, 1974.)

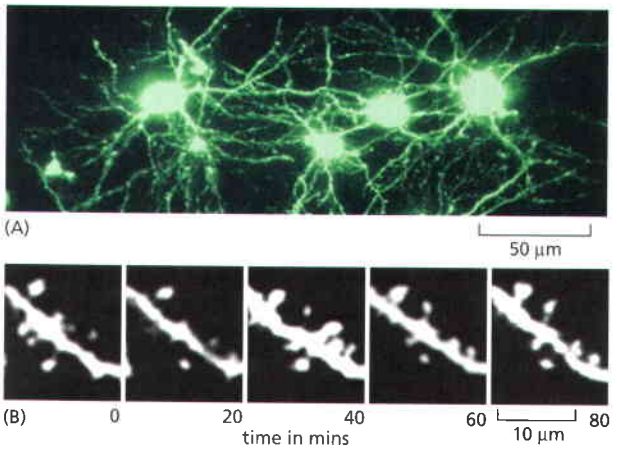


Figure 16–107 Rapid changes in dendrite structure within a living mouse brain. (A) Image of cortical neurons in a transgenic mouse that has been engineered to express green fluorescent protein in a small fraction of its brain cells. Changes in these brain neurons and their projections can be followed for months using highly sensitive fluorescence microscopy. To make this possible, the mouse is subjected to an operation that introduces a small transparent window through its skull, and it is anesthetized each time that an image is recorded. (B) A single dendrite, imaged over the period of 80 minutes, demonstrates that dendrites are constantly sending out and retracting tiny actin-dependent protrusions to create the dendritic spines that receive the vast majority of excitatory synapses from axons in the brain. Those spines that become stabilized and persist for months are thought to be important for brain function, and may be involved in long-term memory. (Courtesy of Karel Svoboda.)

Although the neurons of the central nervous system are long-lived cells, they are by no means static. Synapses are constantly being created, strengthened, weakened, and eliminated as the brain learns, evaluates, and forgets. High-resolution imaging of the structure of neurons in the brains of adult mice has revealed that neuronal morphology is undergoing constant rearrangement as synapses are forged and broken (**Figure 16–107**). These actin-dependent rearrangements are thought to be critical in learning and long-term memory. In this way, the cytoskeleton provides the engine for construction of the entire nervous system, as well as producing the supporting structures that strengthen, stabilize, and maintain its parts.

Summary

Two distinct types of specialized structures in eucaryotic cells are formed from highly ordered arrays of motor proteins that move on stabilized filament tracks. The myosin–actin system of the sarcomere powers the contraction of various types of muscle, including skeletal, smooth, and cardiac muscle. The dynein–microtubule system of the axoneme powers the beating of cilia and the undulations of flagella.

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. During cell division, the functions of the microtubule-based mitotic spindle require spatial and temporal cooperation between dynamic cytoskeletal filaments, active molecular motor proteins, and a wide variety of accessory factors. 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 another 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, forces generated by molecular motors to bring the cell body forward.

Complex cells, such as neurons, require the coordinated assembly of microtubules, neurofilaments (neuronal intermediate filaments), and actin filaments, as well as the actions of dozens of highly specialized molecular motors that transport subcellular components to their appropriate destinations.

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 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.

16–3 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.

Discuss the following problems.

16–4 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?

16–5 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 Q16–1**). How does lateral association promote the subsequent rapid formation of a microtubule?

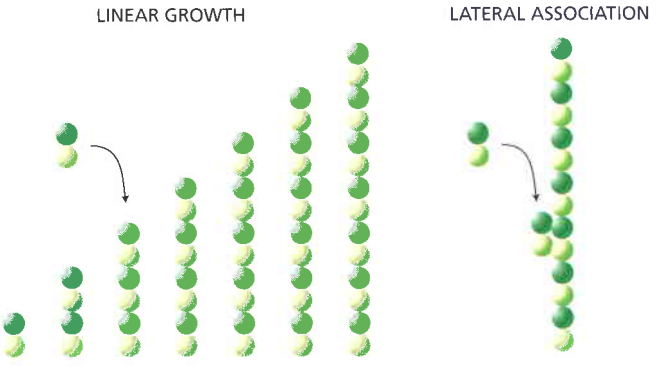


Figure Q16–1 Model for microtubule nucleation by pure αβ-tubulin dimers (Problem 16–5).

16–6 How does a centrosome "know" when it has found the center of the cell?

16–7 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?

16–8 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 Q16–2A**). 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.

Traces of the movements of a kinesin molecule along a microtubule are shown in Figure Q16–2B.

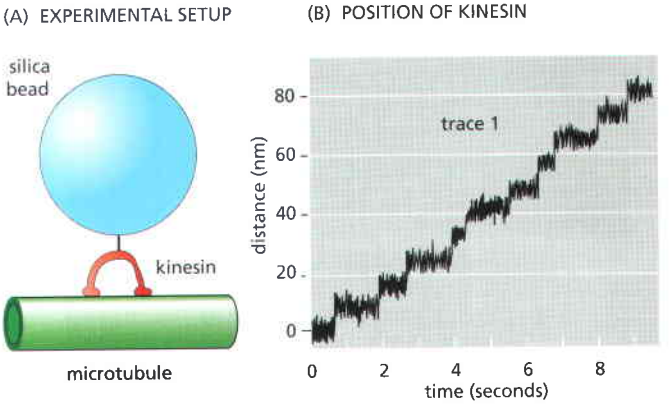


Figure Q16–2 Movement of kinesin along a microtubule (Problem 16–8). (A) Experimental setup with kinesin linked to a silica bead, moving along a microtubule. (B) Position of kinesin (as visualized by position of silica bead) relative to center of interference pattern, as a function of time of movement along the microtubule. The jagged nature of the trace results from Brownian motion of the bead.

A. As shown in Figure Q16–2B, 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 each can 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 Q16–2B that tells you how many ATP molecules are hydrolyzed per step?

16–9 How is the unidirectional motion of a lamellipodium maintained?

16–10 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 Q16–3** 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.)

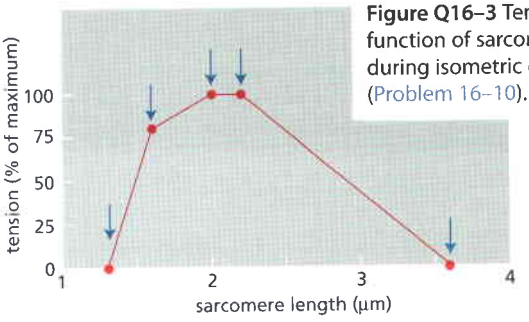


Figure Q16–3 Tension as a function of sarcomere length during isometric contraction (Problem 16–10).

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17

The Cell Cycle

The only way to make a new cell is to duplicate a cell that already exists. This simple fact, first established in the middle of the nineteenth century, carries with it a profound message for the continuity of life. All living organisms, from the unicellular bacterium to the multicellular mammal, are products of repeated rounds of cell growth and division extending back in time to the beginnings of life on Earth over three billion years ago.

A cell reproduces by performing an orderly sequence of events in which it duplicates its contents and then divides in two. This cycle of duplication and division, known as the **cell cycle**, is the essential mechanism by which all living things reproduce. In unicellular species, such as bacteria and yeasts, each cell division produces a complete new organism. In multicellular species, long and complex sequences of cell divisions are required to produce a functioning organism. Even in the adult body, cell division is usually needed to replace cells that die. In fact, each of us must manufacture many millions of cells every second simply to survive: if all cell division were stopped—by exposure to a very large dose of x-rays, for example—we would die within a few days.

The details of the cell cycle vary from organism to organism and at different times in an organism's life. Certain characteristics, however, are universal. The minimum set of processes that a cell has to perform are those that allow it to accomplish its most fundamental task: the passing on of its genetic information to the next generation of cells. To produce two genetically identical daughter cells, the DNA in each chromosome must first be faithfully replicated to produce two complete copies, and the replicated chromosomes must then be accurately distributed *(segregated)* to the two daughter cells, so that each receives a copy of the entire genome (**Figure 17–1**).

Eucaryotic cells have evolved a complex network of regulatory proteins, known as the *cell-cycle control system*, that governs progression through the cell cycle. The core of this system is an ordered series of biochemical switches that initiate the main events of the cycle, including chromosome duplication and segregation. In most cells, additional layers of regulation enhance the fidelity of cell division and allow the control system to respond to various signals from both inside and outside the cell. Inside the cell, the control system monitors progression through the cell cycle and delays later events until earlier events have been completed. It does not permit preparations for the segregation of duplicated chromosomes, for example, until DNA replication is complete. The control system also monitors conditions outside the cell. In a multicellular animal, the system is highly responsive to signals from other cells, stimulating cell division when more cells are needed and blocking it when they are not. The cell-cycle control system therefore has a central role in regulating cell numbers in the tissues of the body. When the system malfunctions, excessive cell divisions can result in cancer.

In addition to duplicating their genome, most cells also duplicate their other organelles and macromolecules; otherwise, daughter cells would get smaller with each division. To maintain their size, dividing cells must coordinate their growth (that is, their increase in cell mass) with their division.

This chapter describes the various events of the cell cycle and how they are controlled and coordinated. We begin with a brief overview of the cell cycle. We then describe the cell-cycle control system and explain how it triggers the

In This Chapter