9.4 | Intermediate Filaments

The second of the three major cytoskeletal elements to be discussed was seen in the electron microscope as solid, unbranched filaments with a diameter of 10–12 nm. They were named **intermediate filaments** (or **IFs**). To date, intermediate filaments have only been identified in animal cells. Intermediate filaments are strong, flexible, ropelike fibers that provide mechanical strength to cells that are subjected to physical stress, including neurons, muscle cells, and the epithelial cells that line the body's cavities. Unlike microfilaments and microtubules, IFs are a chemically heterogeneous group of structures that, in humans, are encoded by approximately 70 different genes. The polypeptide subunits of IFs can be divided into five major classes based on the type of cell in which they are found (Table 9.2) as well as biochemical, genetic, and immunologic criteria. We will restrict the present discussion to classes I-IV, which are found in the construction of cytoplasmic filaments, and consider type V IFs (the lamins), which are present as part of the inner lining of the nuclear envelope, in Section 12.2.

IFs radiate through the cytoplasm of a wide variety of animal cells and are often interconnected to other cytoskeletal filaments by thin, wispy cross-bridges (Figure 9.41). In many cells, these cross-bridges consist of an elongated dimeric protein called *plectin* that can exist in numerous isoforms. Each plectin molecule has a binding site for an intermediate filament at one end and, depending on the isoform, a binding site for another intermediate filament, microfilament, or microtubule at the other end.

Although IF polypeptides have diverse amino acid sequences, all share a similar structural organization that allows them to form similar-looking filaments. Most notably, the polypeptides of IFs all contain a central, rod-shaped, α-helical domain of similar length and homologous amino acid sequence. This long fibrous domain makes the subunits of intermediate filaments very different from the globular tubulin and actin subunits of microtubules and microfilaments. The central fibrous domain is flanked on each side by globular domains of variable size and sequence (step 1, Figure 9.42). Two such polypeptides spontaneously interact as their α-helical rods wrap around each other to form a ropelike dimer approximately 45 nm in length (step 2). Because the two polypeptides are aligned parallel to one another in the same orientation, the dimer has polarity, with one end defined by the C-termini of the polypeptides and the opposite end by their N-termini.

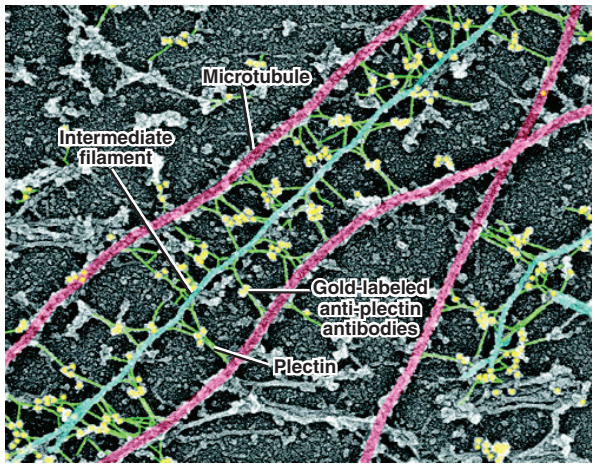


Figure 9.41 **Cytoskeletal elements are connected to one another by protein cross-bridges.** Electron micrograph of a replica of a small portion of the cytoskeleton of a fibroblast after selective removal of actin filaments. Individual components have been digitally colorized to assist visualization. Intermediate filaments (blue) are seen to be connected to microtubules (red) by long wispy cross-bridges consisting of the fibrous protein plectin (green). Plectin is localized by antibodies linked to colloidal gold particles (yellow). (COURTESY OF TATYANA SVITKINA AND GARY BORISY.)

Table 9.2 ***Properties and Distribution of the Major Mammalian Intermediate Filament Proteins***

IF protein	Sequence type	Primary tissue distribution
Keratin (acidic) (28 different polypeptides)	I	Epithelia
Keratin (basic) (26 different polypeptides)	II	Epithelia
Vimentin	III	Mesenchymal cells
Desmin	III	Muscle
Glial fibrillary acidic protein (GFAP)	III	Astrocytes
Peripherin	III	Peripheral neurons
Neurofilament proteins		Neurons of central and peripheral nerves
NF-L	IV	
NF-M	IV	
NF-H	IV	
Nestin	IV	Neuroepithelia
Lamin proteins		All cell types (Nuclear envelopes)
Lamin A	V	
Lamin B	V	
Lamin C	V	

More detailed tables can be found in *Trends Biochem Sci.* 31:384, 2006, *Genes and Development* 21:1582, 2007, and *Trends Cell Biol.* 18:29, 2008.

Intermediate Filament Assembly and Disassembly

The basic building block of IF assembly is thought to be a rodlike tetramer formed by two dimers that become aligned side by side in a staggered fashion with their N- and C-termini pointing in opposite (antiparallel) directions, as shown in Figure 9.42, step 3 and in Figure 9.42*b*. Because the dimers point in opposite directions, the tetramer itself lacks polarity. Recent studies of the self-assembly of IFs in vitro suggest that 8 tetramers associate with one another in a side-by-side (lateral) arrangement to form a filament that is one unit in length (about 60 nm) (step 4). Subsequent growth of the polymer is accomplished as these unit lengths of filaments associate with one another in an end-to-end fashion to form the highly elongated intermediate filament (step 5). None of these assembly steps is thought to require the direct involvement of either ATP or GTP. Because the tetrameric building blocks lack polarity, so too does the assembled filament, which is another important feature that distinguishes IFs from other cytoskeletal elements.

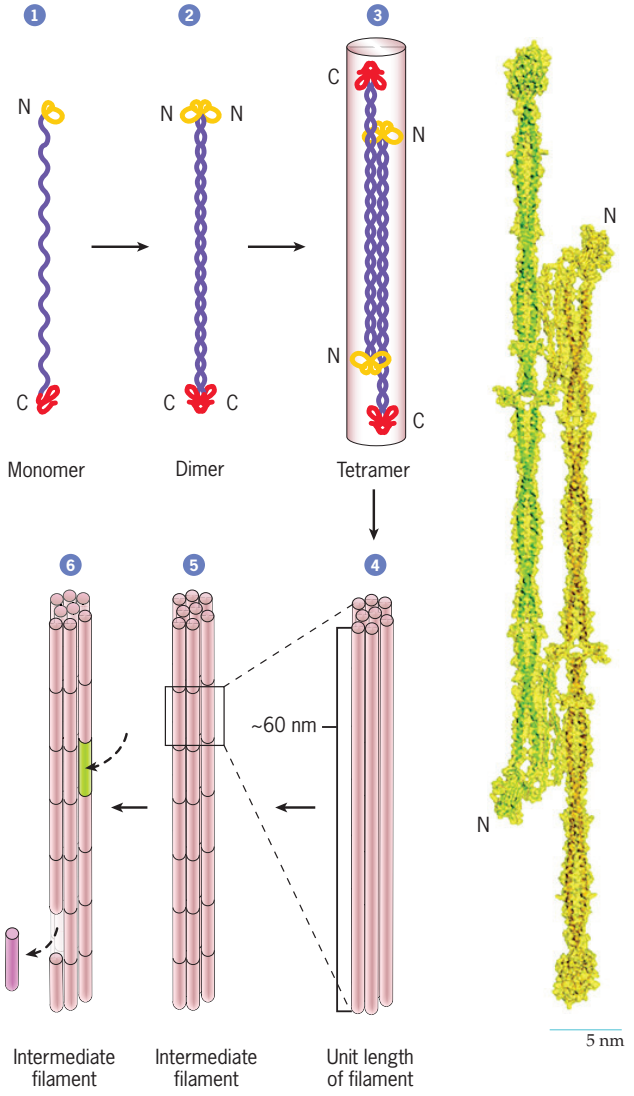


Figure 9.42 **A model of intermediate filament assembly and architecture.** Each monomer has a pair of globular terminal domains (red or yellow) separated by a long α-helical region (step 1). Pairs of monomers associate in parallel orientation with their ends aligned to form dimers (step 2). Depending on the type of intermediate filament, the dimers may be composed of identical monomers (homodimers) or nonidentical monomers (heterodimers). Dimers in turn associate in an antiparallel, staggered fashion to form tetramers (step 3), which are thought to be the basic subunit in the assembly of intermediate filaments. In the model shown here, 8 tetramers associate laterally to form a unit length of the intermediate filament (step 4). Highly elongated intermediate filaments are then formed from the end-to-end association of these unit lengths (step 5). Once formed, intermediate filaments undergo a process of dynamic remodeling that is thought to involve the intercalation of unit lengths of filament into the body of an existing filament (step 6). (*b*) A model of a tetramer of the IF protein vimentin. (B: From Anna V. Sokolova, et al., courtesy of Sergei V. Strelkov, PNAS 103; 16209, 2006, Fig. 3A. © 2006 National Academy of Sciences, U.S.A.)

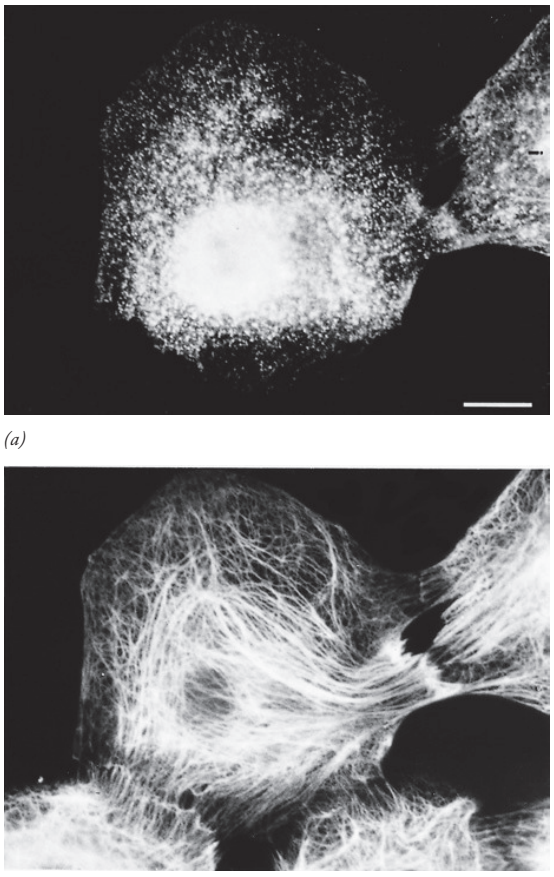


Figure 9.43 **Experimental demonstration of the dynamic character of intermediate filaments.** These photographs show the results of an experiment in which biotin-labeled type I keratin was microinjected into cultured epithelial cells and localized 20 minutes later using immunofluorescence. The photograph in *a* shows the localization of the injected biotinylated keratin (as revealed by fluorescent anti-biotin antibodies) that had become incorporated into filaments during the 20-minute period following injection. The photograph in *b* shows the distribution of intermediate filaments in the cell as revealed by anti-keratin antibodies. The dotlike pattern of fluorescence in *a* indicates that the injected subunits are incorporated into the existing filaments at sites throughout their length, rather than at their ends. (Compare with a similar experiment with labeled tubulin in Figure 9.26.) Bar, 10 μm. (From Rita K. Miller, Karen Vikstrom, and Robert D. Goldman, J. Cell Biol. 113:848, 1991, Fig. 4. Reproduced with permission of The Rockefeller University Press.)

Intermediate filaments tend to be less sensitive to chemical agents than other types of cytoskeletal elements and more difficult to solubilize. In fact, treatment of a cell with ionic detergents extracts just about everything except the cell's intermediate filaments. Because of their insolubility, IFs were initially thought to be permanent, unchanging structures, so it came as a surprise to find that they behave dynamically in vivo. When labeled keratin subunits are injected into cultured skin cells, they are rapidly incorporated into existing IFs. Surprisingly, the subunits are not incorporated at the ends of the filament, as might have been expected by analogy with microtubule and microfilament assembly, but rather into the filament's interior (Figure 9.43). The results depicted in Figure 9.43 might reflect the exchange of unit lengths of filament

directly into an existing IF network (as shown in step 6, Figure 9.42*a*). Unlike the other two major cytoskeletal elements, assembly and disassembly of IFs are controlled primarily by phosphorylation and dephosphorylation of the subunits. For example, phosphorylation of vimentin filaments by protein kinase A leads to their disassembly.

Types and Functions of Intermediate Filaments

Keratin filaments constitute the primary structural proteins of epithelial cells (including epidermal cells, liver hepatocytes, and pancreatic acinar cells). Figure 9.44*a* shows a schematic view of the spatial arrangement of the keratin filaments of a generalized epithelial cell, and Figure 9.44*b* shows the actual arrangement within a pair of cultured epidermal cells. Keratin-containing IFs radiate through the cytoplasm, tethered to the nuclear envelope in the center of the cell and anchored at the outer edge of the cell by connections to the cytoplasmic plaques of desmosomes and hemidesmosomes (pages 257 and 249). Figure 9.44*a* also depicts the interconnections between IFs and the cell's microtubules and microfilaments, which transforms these otherwise separate elements into an integrated cytoskeleton. Because of these various physical connections, the IF network is able to serve as a scaffold for organizing and maintaining cellular architecture and for absorbing mechanical stresses applied by the extracellular environment.

The cytoplasm of neurons contains loosely packed bundles of intermediate filaments whose long axes are oriented parallel to that of the nerve cell axon (see Figure 9.13*b*). These IFs, or **neurofilaments**, as they are called, are composed of three distinct proteins: NF-L, NF-H, and NF-M, all of the type IV group of Table 9.2. Unlike the polypeptides of other IFs, NF-H and NF-M have sidearms that project outward from the neurofilament. These sidearms are thought to maintain the proper spacing between the parallel neurofilaments of the axon (see Figure 9.13*b*). In the early stages of differentiation when the axon is growing toward a target cell, it contains very few neurofilaments but large numbers of supporting microtubules. Once the nerve cell has become fully extended, it becomes filled with neurofilaments that provide support as the axon increases dramatically in diameter. Aggregation of NFs is seen in several human neurodegenerative disorders, including ALS and Parkinson's disease. These NF aggregates may block axonal transport, leading to the death of neurons.

Efforts to probe IF function have relied largely on genetically engineered mice that fail to produce a particular IF polypeptide (a gene knockout) or produce an altered IF polypeptide. These studies have revealed the importance of intermediate filaments in particular cell types. For example, mice carrying deletions in the gene encoding K14, a type I keratin polypeptide normally synthesized by cells of the basal epidermal layer, have serious health problems. These mice are so sensitive to mechanical pressure that even mild trauma, such as that occurring during passage through the birth canal or during nursing by the newborn, can cause severe blistering of the skin or tongue. This phenotype bears strong resemblance to a rare skin-blistering disease in humans, called *epidermolysis bullosa simplex* (*EBS*).[4] Subsequent analysis of EBS patients has shown that they carry mutations in the gene that encodes the homologous K14 polypeptide (or the K5 polypeptide, which forms dimers with K14). These studies confirm the role of IFs in imparting mechanical strength to cells situated in epithelial layers. Similarly, knockout mice that fail to produce the desmin polypeptide exhibit serious cardiac and skeletal muscle abnormalities. Desmin plays a key structural role in maintaining the alignment of the myofibrils of a muscle cell, and the absence of these IFs makes the cells extremely fragile. An inherited human disease, named *desmin-related myopathy*, is caused by mutations in the gene that encodes desmin. Persons with this disorder suffer from skeletal muscle weakness, cardiac arrhythmias, and eventual congestive heart failure. Not all IF polypeptides have such essential functions. For example, mice that lack the vimentin gene, which is expressed in fibroblasts, macrophages, and white blood cells, show relatively minor abnormalities, even though the affected cells lack cytoplasmic IFs. It is evident from these studies that IFs have tissue-specific functions, which are more important in some cells than in others.

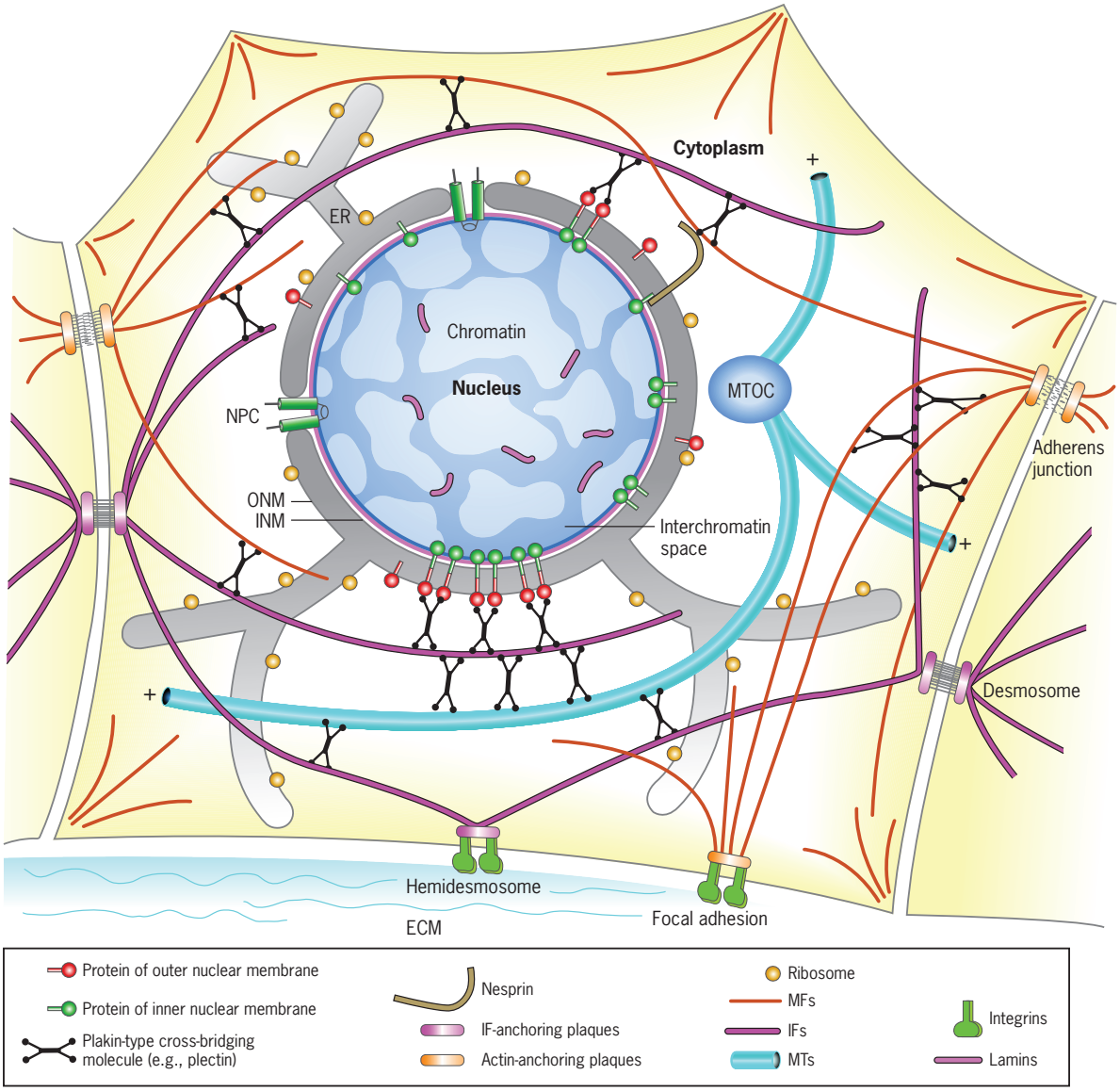
REVIEW

1. Give some examples that reinforce the suggestion that intermediate filaments are important primarily in tissue-specific functions rather than in basic activities that are common to all cells.
2. Compare and contrast microtubule assembly and intermediate filament assembly.

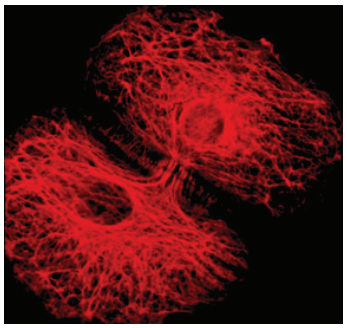
9.5 | Microfilaments

Cells are capable of remarkable motility. The neural crest cells in a vertebrate embryo leave the developing nervous system and migrate across the entire width of the embryo, forming such diverse products as the pigment cells of the skin, teeth, and the cartilage of the jaws (see Figure 7.11). Legions of white blood cells patrol the tissues of the body searching for debris and microorganisms. Certain parts of cells can also be motile; broad projections of epithelial cells at the edge of a wound act as motile devices that pull the sheet of cells over the damaged area, sealing the wound. Similarly, the leading edge of a growing axon sends out microscopic processes that survey the substratum and guide the cell toward a synaptic target. All of these various examples of motility share at least one component: they all depend on microfilaments, the third major type of cytoskeletal element. Microfilaments are also involved in intracellular motile processes, such as the movement of vesicles, phagocytosis, and cytokinesis. In fact, plant cells rely primarily on microfilaments, rather than microtubules, to serve as tracks for the long-distance transport of cytoplasmic vesicles and organelles. This bias toward microfilament-based

[4]As mentioned in Chapter 7, similar types of blistering diseases can be caused by defects in proteins of the hemidesmosome, which holds the basal layer of the epidermis to the basement membrane.



(a)

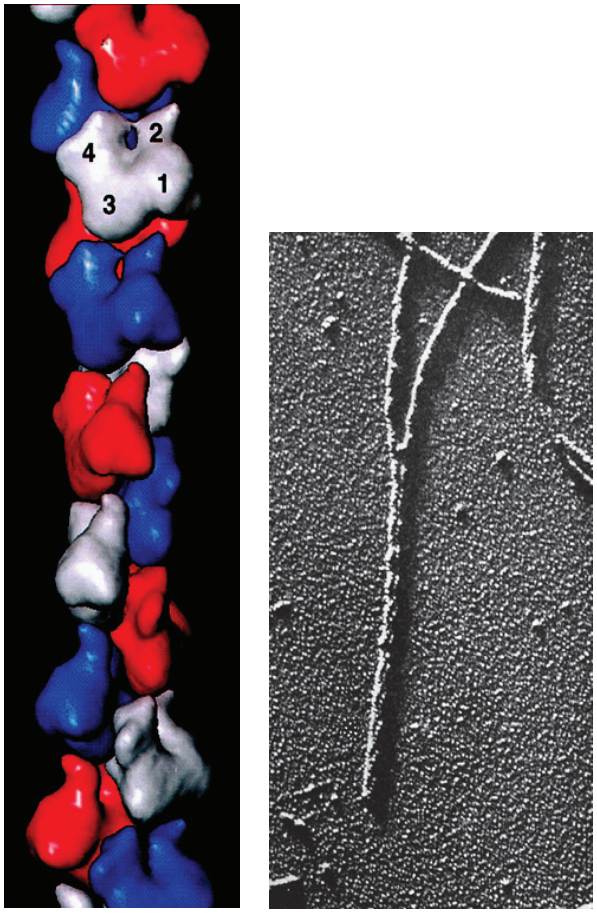


(b)

Figure 9.44 **The organization of intermediate filaments (IFs) within an epithelial cell.** (*a*) In this schematic drawing, IFs are seen to radiate throughout the cell, being anchored at both the outer surface of the nucleus and the inner surface of the plasma membrane. Connections to the nucleus are made via proteins that span both membranes of the nuclear envelope and to the plasma membrane via specialized sites of adhesion such as desmosomes and hemidesmosomes. IFs are also seen to be interconnected to both of the other types of cytoskeletal fibers. Connections to microtubules (MTs) and microfilaments (MFs) are made primarily by members of the plakin family of proteins, such as the dimeric plectin molecule shown in Figure 9.41. (*b*) Distribution of keratin-containing intermediate filaments in cultured skin cells (keratinocytes). The filaments are seen to form a cagelike network around the nucleus and also extend to the cell periphery (A: REPRINTED WITH PERMISSION FROM H. HERRMANN ET AL., NATURE REVS. MOL. CELL BIOL. 8:564, 2007; COPYRIGHT 2007, MACMILLAN MAGAZINES LTD. NATURE REVIEWS MOLECULAR CELL BIOLOGY BY NATURE PUBLISHING GROUP. REPRODUCED WITH PERMISSION OF NATURE PUBLISHING GROUP IN THE FORMAT JOURNAL VIA COPYRIGHT CLEARANCE CENTER. B: FROM PIERRE A. COULOMBE AND M. BISHR OMARY, CURR. OPIN. CELL BIOL. 14:111, 2002, WITH PERMISSION FROM ELSEVIER.)

motility reflects the rather restricted distribution of microtubules in many plant cells (see Figure 9.12). Microfilaments also play an important role in determining the shapes of cells and can provide structural support for various types of cellular projections (as in Figure 9.66).

Microfilaments are approximately 8 nm in diameter and composed of globular subunits of the protein **actin**, which is the most abundant protein in most cells. In the presence of ATP, actin monomers polymerize to form a flexible, helical filament. As a result of its subunit organization (Figure 9.45*a*), an actin filament is essentially a two-stranded structure with two helical grooves running along its length (Figure 9.45*b*). The terms *actin filament*, *F-actin*, and *microfilament* are basically synonyms for this type of filament. Because each actin subunit has polarity and all the subunits of an actin filament are pointed in the same direction, the entire microfilament has polarity. Consequently, the two ends of an actin filament have different structures and dynamic properties. Depending on the type of cell and the activity in which it is engaged, actin filaments can be organized into highly ordered arrays, loose ill-defined networks, or tightly anchored bundles.



(a) *(b)*

Figure 9.45 **Actin filament structure.** (*a*) A model of an actin filament. The actin subunits are represented in three colors to distinguish the consecutive subunits more easily. The subdomains in one of the actin subunits are labeled 1, 2, 3, and 4 and the ATP-binding cleft in each subunit is evident. Actin filaments have polarity, which is denoted as a plus and minus end. The cleft (in the upper red subunit) is present at the minus end of the filament. (*b*) Electron micrograph of a replica of an actin filament showing its double-helical architecture. (A: From Michael F. Schmid et al., courtesy of Wah Chiu, J. Cell Biol. 124:346, 1994, Fig. 4. Reproduced with permission of The Rockefeller University Press; B: from Robert H. Depue, Jr. and Robert V. Rice, J. Mol. Biol. 12:302, 1965. Reproduced with permission of Elsevier.)

The identification of actin filaments in a given cell can be made with certainty using a cytochemical test that takes advantage of the fact that actin filaments, regardless of their source, will interact in a highly specific manner with the protein myosin. To facilitate the interaction, purified myosin (obtained from muscle tissue) is cleaved into fragments by a proteolytic enzyme. One of these fragments, called S1 (see Figure 9.49), binds to the actin molecules all along the microfilament. In addition to identifying the filaments as actin, the bound S1 fragments reveal the filament's polarity. When S1 fragments are bound, one end of the microfilament appears *pointed* like an arrowhead, while the other end looks *barbed*. An example of this arrowhead "decoration" is shown in the microvilli of intestinal epithelial cells of Figure 9.46. The orientation of the arrowheads formed by the S1–actin complex provides information as to the direction in which the microfilaments are likely to be moved by a myosin motor protein. Actin can also be localized by light microscopy using fluorescently labeled phalloidin (Figure 9.74*a*), which binds to actin filaments, or by fluorescently labeled anti-actin antibodies.

Actin was identified more than 50 years ago as one of the major contractile proteins of muscle cells. Since then, it has been identified as a major protein in virtually every type of eukaryotic cell that has been examined. Higher plant and animal species possess a number of actin-coding genes whose encoded products are specialized for different types of motile processes. Actins have been remarkably conserved during the evolution of eukaryotes. For example, the amino acid sequences of actin molecules from a yeast cell and from rabbit skeletal muscle are 88 percent identical. In fact, actin molecules from diverse sources can copolymerize to form hybrid filaments. Although actin filaments can generate forces on their own (page 374), most processes involving actin require the activity of motor proteins, specifically those of the myosin superfamily.

Microfilament Assembly and Disassembly

Before it is incorporated into a filament, an actin monomer binds a molecule of ATP. Actin is an ATPase, just as tubulin is a GTPase, and the role of ATP in actin assembly is similar to that of GTP in microtubule assembly (page 342). The ATP associated with the actin monomer is hydrolyzed to ADP at some time after it is incorporated into the growing actin filament. As a consequence, the bulk of an actin filament consists of ADP-actin subunits.

Actin polymerization is readily demonstrated in vitro in solutions containing ATP-actin monomers. As in the case of microtubules, the initial stage in filament formation (i.e., *nucleation*) occurs slowly in vitro, whereas the subsequent stage of filament *elongation* occurs much more rapidly. The

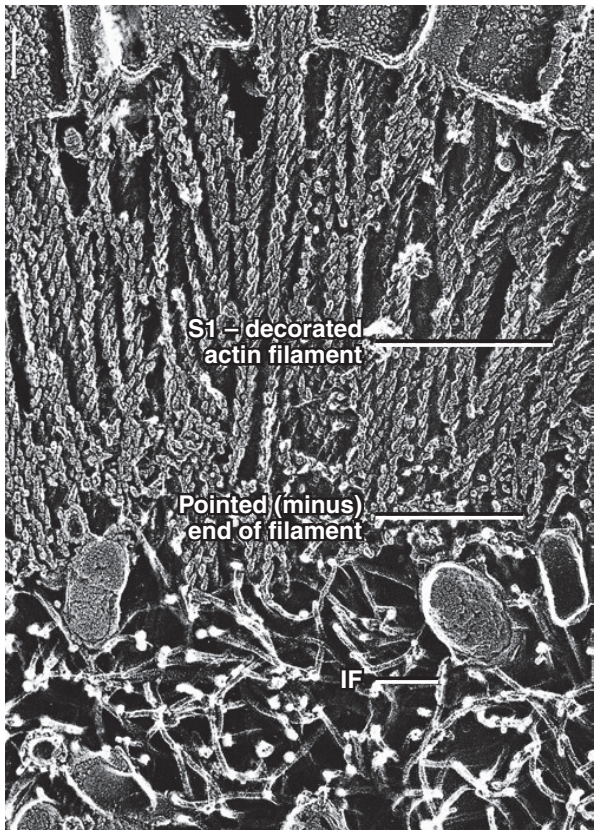
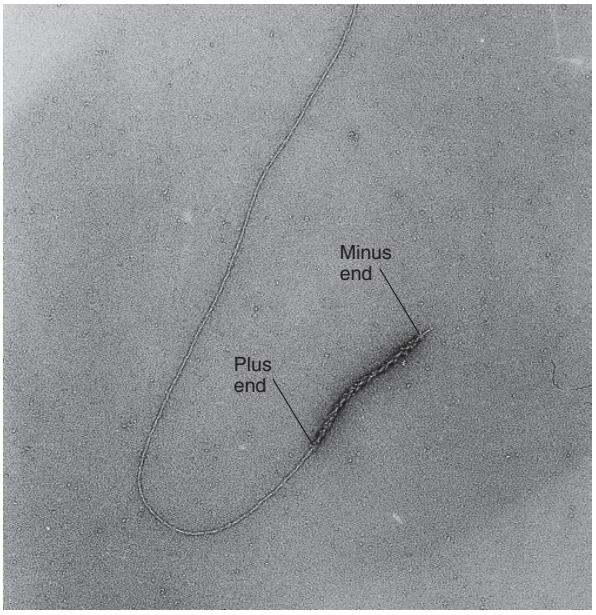


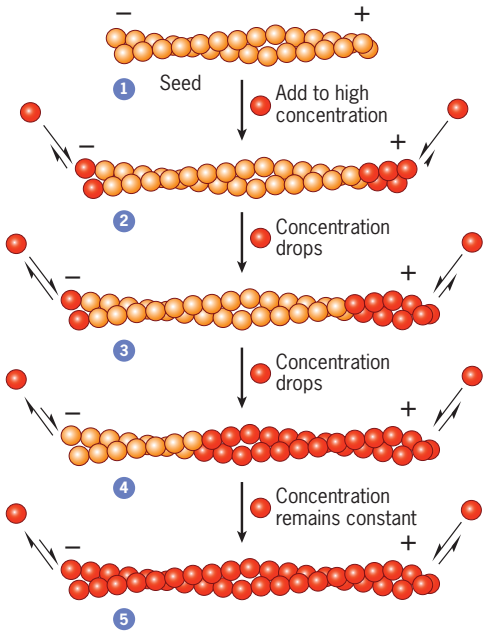
Figure 9.46 **Determining the location and polarity of actin filaments using the S1 subunit of myosin.** Electron micrograph of a replica showing the microfilament bundles in the core of the microvilli of an intestinal epithelial cell. The cell had been fixed, treated with S1 myosin fragments, freeze fractured, and deep etched to expose the filamentous components of the cytoplasm. The intermediate filaments (IFs) at the bottom of the micrograph do not contain actin and therefore do not bind the S1 myosin fragments. These intermediate filaments originate at the desmosomes of the lateral surfaces of the cell. (From N. Hirokawa, L. G. Tilney, K. Fujiwara, and J. E. Heuser, J. Cell Biol. 94:430, 1982, Fig. 3. Reproduced with permission of The Rockefeller University Press.)

nucleation stage of filament formation can be bypassed by including preformed actin filaments in the reaction mixture. When preformed actin filaments are incubated with a high concentration of labeled ATP-actin monomers, both ends of the microfilament become labeled, but one end has a higher affinity for monomers and incorporates them at a rate approximately 10 times that of the other end. Decoration with the S1 myosin fragment reveals that the barbed (or plus) end of the microfilament is the fast-growing end, while the pointed (or minus) end is the slow-growing tip (Figure 9.47*a*).

Figure 9.47*b* illustrates how the events that occur during actin assembly/disassembly in vitro depend on the concentration of actin monomers. Suppose we were to begin by adding preformed actin filaments (seeds) to a solution of actin in the presence of ATP (step 1). As long as the concentration of ATP-actin monomers remains high, subunits will continue to



(a)



(b)

Figure 9.47 **Actin assembly in vitro.** (*a*) Electron micrograph of a short actin filament that was labeled with S1 myosin and then used to nucleate actin polymerization. The addition of actin subunits occurs much more rapidly at the barbed (plus) end than at the pointed (minus) end of the existing filament. (*b*) Schematic diagram of the kinetics of actin-filament assembly in vitro. All of the orange subunits are part of the original seed; red subunits were present in solution at the beginning of the incubation. The steps are described in the text. Once a steady-state concentration of monomers is reached, subunits are added to the plus end at the same rate they are released from the minus end. As a result, subunits treadmill through the filament in vitro. *Note:* No attempt is made to distinguish between subunits with a bound ATP versus ADP. (A: Courtesy of M. S. Runge and T. D. Pollard.)

be added at both ends of the filament (step 2, Figure 9.47*b*). As the monomers in the reaction mixture are consumed by addition to the ends of the filaments, the concentration of free ATP-actin continues to drop until a point is reached where net addition of monomers continues at the plus end, which has a higher affinity for ATP-actin, but stops at the minus end, which has a lower affinity for ATP-actin (step 3). As filament elongation continues, the free monomer concentration drops further. At this point, monomers continue to be added to the plus ends of the filaments, but a net loss of subunits occurs at their minus end. As the free monomer concentration falls, a point is reached where the two reactions at opposite ends of the filaments are balanced so that both the lengths of the filaments and the concentration of free monomers remain constant (step 4). This type of balance between two opposing activities is an example of *steady state* (page 94) and occurs when the ATP-actin concentration is approximately 0.3 μM. Because subunits are being added to the plus ends and removed from the minus ends of each filament at steady state, the relative position of individual subunits within each filament is continually moving—a process known as "treadmilling" (steps 4–5). Studies on living cells containing fluorescently labeled actin subunits have supported the occurrence of treadmilling in vivo (see Figure 9.54*c*).

As discussed on page 372, the rate of assembly and disassembly of actin filaments in the cell can be influenced by a number of different "accessory" proteins. Changes in the local conditions in a particular part of the cell can push events there toward either the assembly or the disassembly of microfilaments. By controlling this dynamic behavior, the cell can reorganize its microfilament cytoskeleton. Such reorganization is required for dynamic processes such as cell locomotion, changes in cell shape, phagocytosis, and cytokinesis.

As noted above, actin filaments play a role in nearly all of a cell's motile processes. The involvement of these filaments is most readily demonstrated by treating the cells with one of the following drugs that disrupt dynamic microfilament-based activities: cytochalasin, obtained from a mold, which blocks the plus ends of actin filaments, allowing depolymerization at the minus end; phalloidin, obtained from a poisonous mushroom, which binds to intact actin filaments and prevents their turnover; and latrunculin, obtained from a sponge, which binds to free monomers and blocks their incorporation into the polymer. Microfilament-mediated processes rapidly grind to a halt when cells contain one of these compounds.

Myosin: The Molecular Motor of Actin Filaments

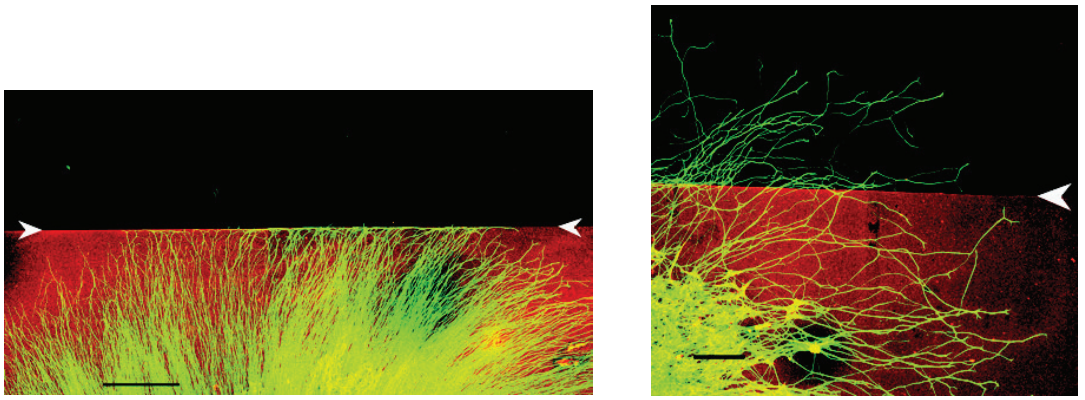
We have previously examined the structure and actions of two molecular motors—kinesin and dynein—that operate in opposite directions over tracks of microtubules. To date, all of the motors known to operate in conjunction with actin filaments are members of the myosin superfamily. Myosins—with the major exception of myosin VI, which is discussed below—move toward the plus end of an actin filament.

Myosin was first isolated from mammalian skeletal muscle tissue and subsequently from a wide variety of eukaryotic cells, including protists, plants, nonmuscle cells of animals, and vertebrate cardiac and smooth muscle tissues. All myosins share a characteristic motor (head) domain. The head contains a site that binds an actin filament and a site that binds and hydrolyzes ATP to drive the myosin motor. Whereas the head domains of various myosins are similar, the tail domains are highly divergent. Myosins also contain a variety of low-molecular-weight (light) chains. Myosins are generally divided into two broad groups: the **conventional** (or **type II**) **myosins**, which were first identified in muscle tissue, and the **unconventional myosins**. The unconventional myosins are subdivided on the basis of amino acid sequence into at least 17 different classes (type I and types III–XVIII). Some of these classes are expressed widely among eukaryotes, whereas others are restricted. Myosin X, for example, is found only in vertebrates, and myosins VIII and XI are present only in plants. Humans contain about 40 different myosins from at least 12 classes, each presumed to have its own specialized function(s). Of the various myosins, type II molecules are best understood.

Conventional (Type II) Myosins Proteins of the myosin II class are the primary motors for muscle contraction but are also found in a variety of nonmuscle cells. The human genome encodes 16 different myosin II heavy chains, 3 of which function in nonmuscle cells. All myosin IIs move toward the plus (barbed) end of an actin filament. Among their nonmuscle activities, type II myosins are required for splitting a cell in two during cell division, generating tension at focal adhesions, cell migration, and the turning behavior of growth cones (see Figure 9.76). The effect of inhibiting myosin II activity in an advancing growth cone is demonstrated in Figure 9.48.

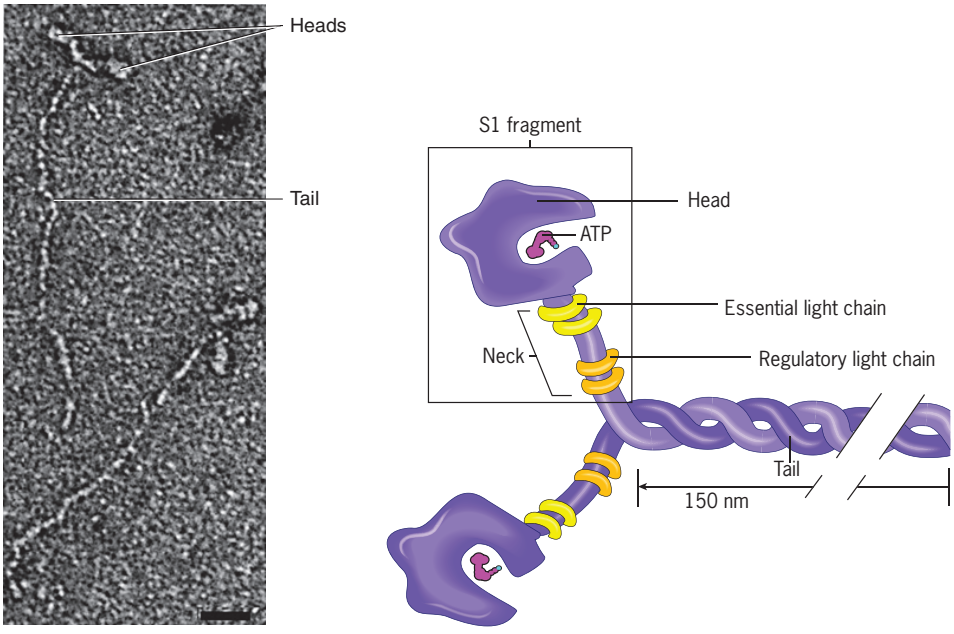
An electron micrograph of a pair of myosin II molecules is shown in Figure 9.49*a*. Each myosin II molecule is composed of six polypeptide chains—one pair of heavy chains and two pairs of light chains—organized in such a way as to produce a highly asymmetric protein (Figure 9.49*a*). Examination of the molecule in Figure 9.49*b* shows it to consist of (1) a pair of globular heads that contain the catalytic site of the molecule; (2) a pair of necks, each consisting of a single, uninterrupted α helix and two associated light chains; and (3) a single, long, rod-shaped tail formed by the intertwining of long α-helical sections of the two heavy chains.

Isolated myosin heads (S1 fragments of Figure 9.49*b*) that have been immobilized on the surface of a glass coverslip are capable of sliding attached actin filaments in an in vitro assay such as that shown in Figure 9.50. Thus, all of the machinery required for motor activity is contained in a single head. The mechanism of action of the myosin head, and the key role played by the neck, are discussed on page 369. The fibrous tail portion of a myosin II molecule plays a structural role, allowing the protein to form filaments. Myosin II molecules assemble so that the ends of the tails point toward the center of the filament and the globular heads point away from the center (Figures 9.51 and 9.57). As a result, the filament is described as *bipolar*, indicating a reversal of polarity at the filament's center. Because they are bipolar, the myosin heads at the opposite ends of a myosin filament have the ability to pull actin filaments toward one another, as occurs in a muscle cell. As described in the following section, the myosin II filaments that assemble in



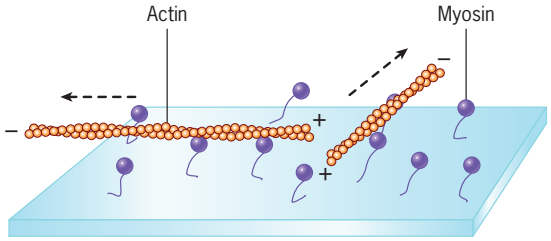
(a) (b)

Figure 9.48 **Experimental demonstration of a role for myosin II in the directional movement of growth cones.** (*a*) Fluorescence micrograph showing fine processes (neurites) growing out from a microscopic fragment of mouse embryonic nervous tissue. The neurites (stained green) are growing outward on a glass coverslip coated with strips of laminin (stained red). Laminin is a common component of the extracellular matrix (page 243). The tip of each nerve process contains a motile growth cone. When the growth cones reach the border of the laminin strip (indicated by the line with arrowheads), they turn sharply and continue to grow over the laminin-coated surface. Bar, 500 μm. (*b*) The tissue in this micrograph was obtained from a mouse embryo lacking myosin IIB. The growth cones no longer turn when they reach the edge of the laminin-coated surface, causing the neurites to grow forward onto a surface (black) lacking laminin. Bar, 80 μm. (From Stephen G. Turney and Paul C. Bridgman, Nature Neurosci. 8:717, 2005; © 2005, reprinted by permission of Macmillan Publishers Ltd.)



(a) (b)

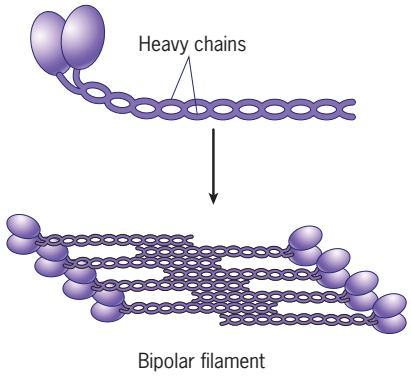
Figure 9.49 **Structure of a myosin II molecule.** (*a*) Electron micrograph of negatively stained myosin molecules. The two heads and tail of each molecule are clearly visible. (*b*) A highly schematic drawing of a myosin II molecule (molecular mass of 520,000 daltons). The molecule consists of one pair of heavy chains (purple) and two pairs of light chains, which are named as indicated. The paired heavy chains consist of a rod-shaped tail in which portions of the two polypeptide chains wrap around one another to form a coiled coil and a pair of globular heads. When treated with a protease under mild conditions, the molecule is cleaved at the junction between the neck and tail, which generates the S1 fragment. (A: From S. A. Burgess, M. L. Walker, H. D. White, and J. Trinick, J. Cell Biol. 139:676, 1997, Fig. 1. Reproduced with permission of The Rockefeller University Press.)



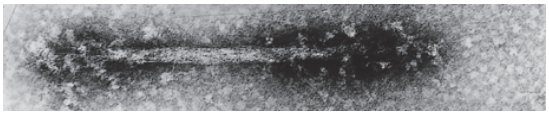
(a)

(b)

Figure 9.50 **In vitro motility assay for myosin.** (*a*) Schematic drawing in which myosin heads are bound to a silicone-coated coverslip, which is then incubated with a preparation of actin filaments. (*b*) Results of the experiment depicted in *a*. Two images were taken 1.5 seconds apart and photographed as a double exposure on the same frame of film. The dashed lines with arrowheads show the sliding movement of the actin filaments over the myosin heads during the brief period between exposures. (BASED ON WORK BY T. YANAGIDA.)



(a)



(b)

Figure 9.51 **Structure of a bipolar myosin II filament.** (*a*) Schematic diagram of the staggered arrangement of the individual myosin molecules in a myosin II filament. (*b*) Electron micrograph of a bipolar myosin filament formed in vitro. The heads of the filament are seen at both ends, leaving a smooth section in the center of the filament. (B: COURTESY OF HUGH HUXLEY.)

skeletal muscle cells are highly stable components of the contractile apparatus. However, the smaller myosin II mini-filaments that form in most nonmuscle cells often display transient construction, assembling when and where they are needed and then disassembling after they have acted.

Unconventional Myosins In 1973, Thomas Pollard and Edward Korn of the National Institutes of Health described a unique myosin-like protein that was extracted from the protist *Acanthamoeba*. Unlike muscle myosin, this smaller unconventional myosin had only a single head and was unable to assemble into filaments in vitro; the protein became known as myosin I. As reflected in the drawing of a microvillus in Figure 9.66, myosin I often serves as a cross-link between actin filaments of the cytoskeleton and the lipid bilayer of the plasma membrane. It has been suggested that myosin I can exert tension on the plasma membrane, which could play a role in processes that require movement or deformation of the membrane.

None of the unconventional myosins are capable of filament formation and instead appear to operate primarily as individual protein molecules. The best-studied unconventional myosins are capable of moving processively along actin filaments in a manner analogous to the way that kinesins and cytoplasmic dynein move along microtubules. The steps taken by one of these myosins—myosin V—have been revealed in a remarkable series of atomic force micrographs that capture a single molecule as it moves rapidly along an actin filament in vitro (Figure 9.52*a*). In order to visualize the various steps in the protein's mechanical cycle, these researchers placed molecular obstacles in the path of the moving motor protein, which slowed the rate at which the protein was able to travel. Figure 9.52*b* shows a series of images that reveal the movements of the molecule during a single mechanical cycle. (Keep in mind that myosin V normally has a sizeable tail segment, as de-

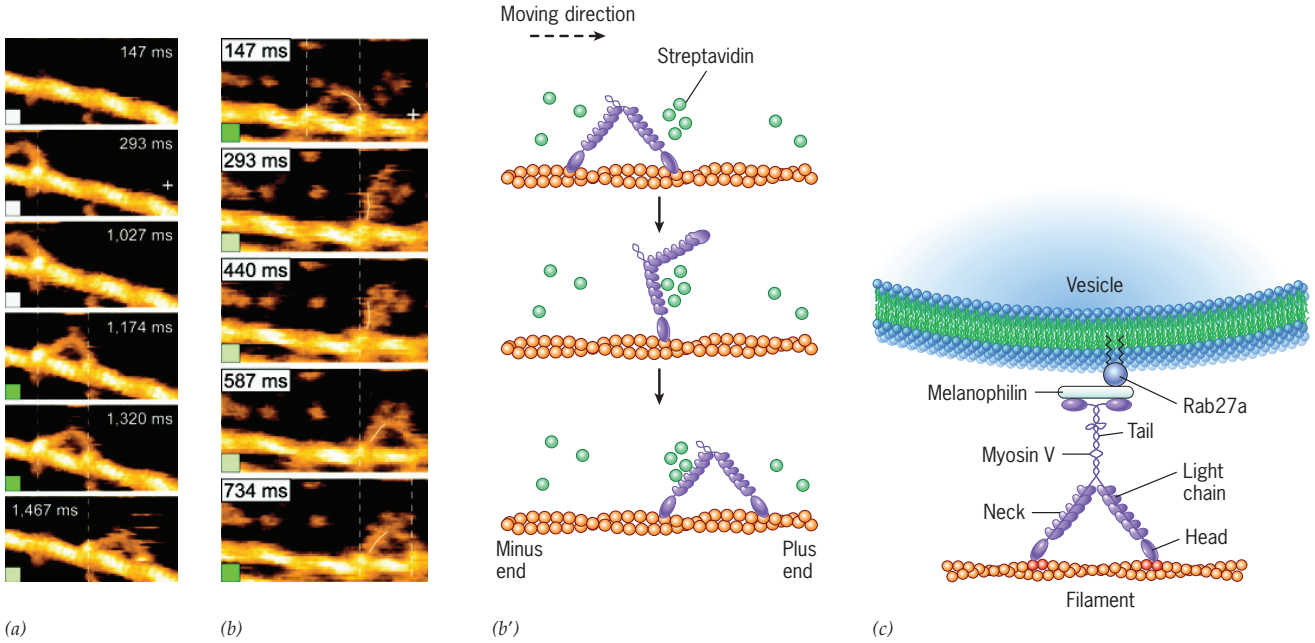


Figure 9.52 **Myosin V—a two-headed unconventional myosin involved in organelle transport.** (*a*) Direct visualization of a single myosin V molecule (one that is lacking its normal tail domain) as it moves along an actin filament in vitro in the presence of ATP. Using high-speed atomic force microscopy (HS-AFM), this series of images shows the movement of the molecule over a period of about one second. (*b*) Succesive HS-AFM images showing the hand-over-hand movement of a single myosin V molecule as it passes through a cluster of obstacles (consisting of streptavidin protein molecules). The swinging neck (or lever arm) is highlighted with a thin white line. Interpretive drawings (*b′*) depict the motor protein as seen in several of the corresponding HS-AFM images. A continuous movie of the protein's movement can be seen in the supplement to this article. (*c*) Schematic drawing of a complete dimeric myosin V molecule, including its numerous light chains, with both heads bound to an actin filament and its tail domain bound to a vesicle. Rab27a and melanophilin serve as adaptors that link the globular ends of the tail to the vesicle membrane. The long neck of myosin V binds 6 light chains. (A-B: FROM NORIYUKI KODERA, ET AL., FROM NATURE 468: 73, 2010; © 2010, REPRINTED BY PERMISSION OF MACMILLAN PUBLISHERS LTD. COURTESY TOSHIO ANDO.)

picted in Figure 9.52*c*, that has been removed for these particular experiments.)

In the first frame of Figure 9.52*b*, both heads of the dimeric protein are seen to be attached to the underlying filament, and a cluster of obstacles are seen in the path of the motor. In the second frame, the trailing head of the myosin V molecule has detached from the filament and is in the process of moving forward through the roadblocks in its path. By the fourth frame, this swinging head has made contact with the filament at a forward position along the filament and thus has become the new leading head. These images provide visual confirmation that myosin V moves in a hand-over-hand fashion similar to that of the kinesin molecule in the drawing of Figure 9.15*b*. To accomplish this type of movement, at least one of the two heads must be bound to its polarized track at all times. Figure 9.52*c* shows an illustration of an intact myosin V molecule. A seen in this illustration, myosin V is noteworthy for the length of each neck, which at 23 nm is about three times that of myosin II.

Because of its long necks, which act as swinging arms (or levers) during movement, myosin V can take very large steps. This is very important for a motor protein that moves processively along an actin filament made up of *helical* strands of subunits. The actin helix repeats itself about every 13 subunits (36 nm), which is just about the step size of a myosin V molecule (Figure 9.52*b*). To accomplish this type of movement, each myosin head must move a distance of 72 nm, twice the distance between two successive binding sites on the actin filament (Figure 9.52*b*). As a result of its giant strides, myosin V can walk along the filament in a straight path even though the underlying "roadway" spirals 360° between its "feet."

Several unconventional myosins (including myosin I, V, and VI) are associated with various types of cytoplasmic vesicles and organelles. In some cases these myosins may act primarily as organelle tethers and in other cases as organelle transporters. Some vesicles have been shown to contain microtubule-based motors (kinesins and/or cytoplasmic dynein) *and* microfilament-based motors (unconventional myosins) and, in fact, the two types of motors may be physically linked to one another. The movement of vesicles and other membranous carriers over long distances within animal cells occurs on microtubules, as previously described. However, once they approach the end of the microtubule, these membranous vesicles are often thought to switch over to microfilament tracks for

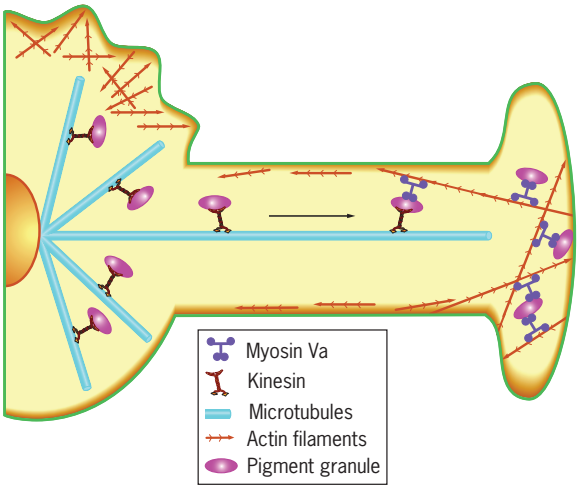


Figure 9.53 **The contrasting roles of microtubule- and microfilament-based motors in intracellular transport.** Most vesicle transport is thought to be mediated by members of the kinesin and dynein families, which carry their cargo over relatively long distances. It is thought that some vesicles also carry myosin motors, such as myosin Va, which transport their cargo over microfilaments, including those present in the peripheral (cortical) regions of the cell. The two types of motors may act in a cooperative manner, as illustrated here in the case of a frog pigment cell in which pigment granules move back and forth within extended cellular processes. (After X, Wu, et al., copyright 1998, Rockefeller University Press. Originally published in The Journal of Cell Biology 143:1915.)

the local movement through the actin-rich periphery of the cell, which is mediated by myosins (Figure 9.53).

Cooperation between microtubules and microfilaments has been best studied in pigment cells (Figure 9.53). In mammals, pigment granules (melanosomes) are transported into fine peripheral processes of the pigment cell by one of the myosin V isoforms called Va. Melanosomes are then transferred to hair follicles where they become incorporated into a developing hair. Mice lacking myosin Va activity are unable to transfer melanosomes into hair follicles, causing their coat to have a much lighter color. Humans lacking a normal gene encoding myosin Va suffer from a rare disorder called Griscelli syndrome; these individuals exhibit partial albinism (lack of skin coloration) and suffer other symptoms thought to be related to defects in vesicle transport. In 2000 it was discovered that a subset of Griscelli patients had a normal myosin Va gene, but lacked a functional gene encoding a peripheral membrane protein called Rab27a. The Rab family of proteins was discussed on page 302 as molecules that regulate vesicle trafficking. Rabs are also involved in the attachment of myosin (and kinesin) motors to membrane surfaces (Figure 9.52*c*).

Hair cells, whose structure is shown in Figure 9.54*a* have been a particularly good system for studying the functions of unconventional myosins. Hair cells are named for the bundle of stiff, hairlike stereocilia that project from the apical surface of the cell into the fluid-filled cavity of the inner ear. Displacement of the stereocilia by mechanical stimuli leads to the opening of Ca^{2+} channels in the plasma membrane and the subsequent generation of nerve impulses that we perceive as sound. Stereocilia have no relation to the true cilia discussed earlier. Instead of containing microtubules, each stereocilium is supported by a bundle of parallel actin filaments (Figure 9.54*b*) whose barbed ends are located at the outer tip of the projection and pointed ends at the base. Stereocilia have provided some of the most striking images of the dynamic nature of the actin cytoskeleton. While the stereocilia themselves are permanent structures, the actin bundles are in constant flux. Actin monomers continually bind to the barbed end of each filament, treadmill through the body of the filament, and dissociate from the pointed end. This process is captured in the fluorescence micrograph of Figure 9.54*c*, which shows the incorporation of GFP-labeled actin subunits at the barbed end of each filament. Several unconventional myosins (I, III, V, VI, VII, and XV) are localized at various sites within the hair cells of the inner ear; two of these are shown in Figure 9.54*d* and *e*. Mutations in myosin VIIa are the cause of Usher 1B syndrome, which is characterized by both deafness and blindness. The morphologic effects of mutations in the myosin VIIa gene on the hair cells of the inner ear of mice are shown in Figure 9.54*f,g*. As in humans, mice that are homozygous for the mutant allele encoding this motor protein are deaf.

Mysoin VI, a processive organelle transporter in the cytoplasm of many cells, is distinguished by its movement in a "reverse direction," that is, toward the pointed (minus) end of an actin filament. Myosin VI is located at the base of the stereocilia where it might connect the cytoskeleton to the overlying plasma membrane. In other types of cells, myosin VI is thought to be involved in the formation of clathrin-coated vesicles at the plasma membrane, the movement of uncoated vesicles to early endosomes, and the maintenance of Golgi morphology. Mutations in myosin VI are the cause of several inherited diseases.

Now that we have described the basics of actin and myosin structure and function, we can see how these two proteins interact to mediate a complex cellular activity.

REVIEW

1. Compare and contrast the characteristics of microtubule assembly versus actin filament assembly.
2. Compare the structure of a fully assembled microtubule, actin filament, and intermediate filament.
3. Describe three functions of actin filaments.
4. Contrast the structure and function of conventional myosin II and the unconventional myosin V.
5. How is it possible for the same vesicle to be transported along both microtubules and microfilaments?

9.6 | Muscle Contractility

Skeletal muscles derive their name from the fact that most of them are anchored to bones that they move. They are under voluntary control and can be consciously commanded to contract. Skeletal muscle cells have a highly unorthodox structure. A single, cylindrically shaped muscle cell is typically 10 to 100 μm thick, over 100 mm long, and contains hundreds of nuclei.

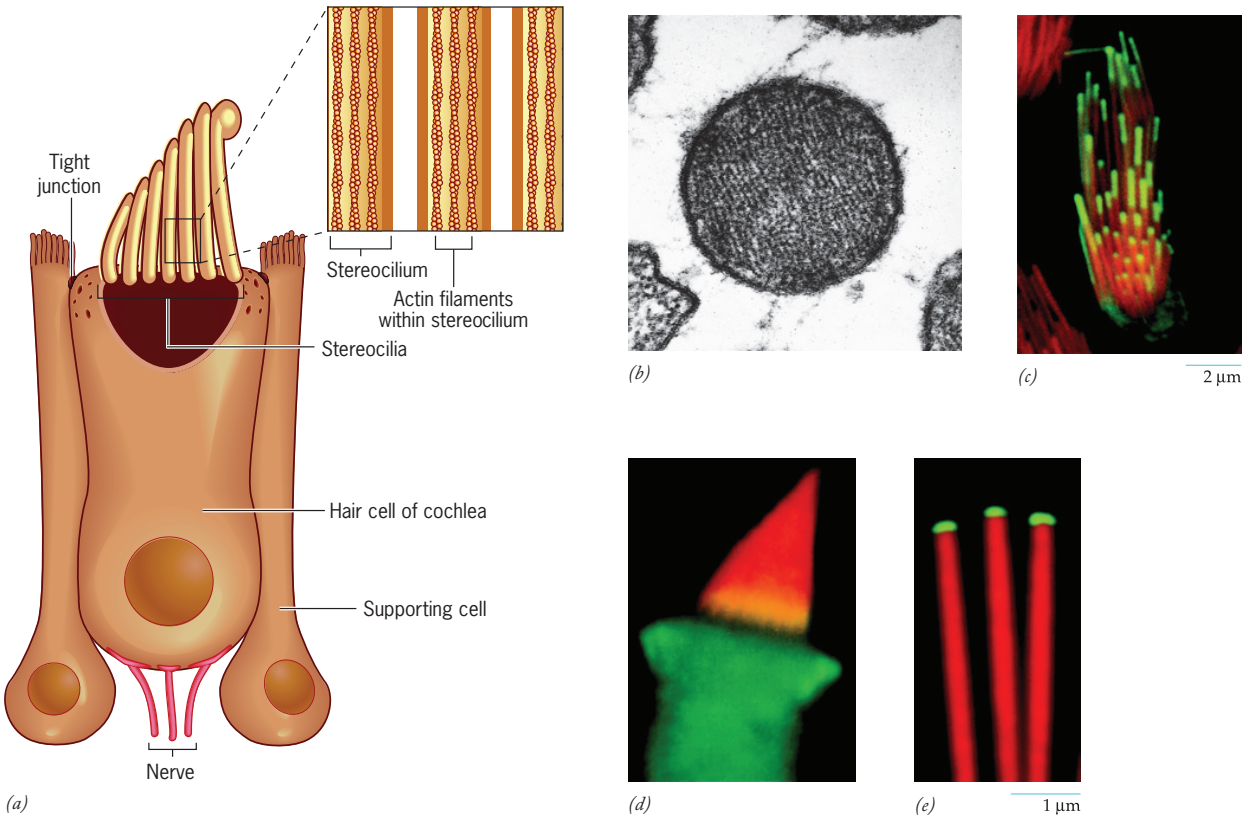


Figure 9.54 **Hair cells, actin bundles, and unconventional myosins.** (*a*) Drawing of a hair cell of the cochlea. The inset shows a portion of several of the stereocilia, which are composed of a tightly grouped bundle of actin filaments. (*b*) Transmission electron micrograph of a cross section of a stereocilium showing it is composed of a dense bundle of actin filaments. (*c*) Fluorescence micrograph of a hair cell from the vestibule of a rat inner ear. The tips of the stereocilia are labeled green due to the incorporation of GFP-actin monomers at their barbed ends. Taller stereocilia contain a longer column of GFP-labeled subunits, which reflects a more rapid incorporation of actin monomers. The stereocilia appear red due to labeling by rhodamine-labeled phalloidin, which binds to actin filaments. (*d*) A hair cell from the bullfrog inner ear. The localization of myosin VIIa is indicated in green. The orange bands near the bases of the stereocilia (due to red and green overlap) indicate a concentration of myosin VIIa. (*e*) Myosin XVa (green) is localized at the tips of the stereocilia of a rat auditory hair cell. (*f*) Scanning electron micrograph of the hair cells of the cochlea of a control mouse. The stereocilia are arranged in V-shaped rows. (*g*) A corresponding micrograph of the hair cells of a mouse with mutations in the gene encoding myosin VIIa, which causes deafness. The stereocilia of the hair cells exhibit a disorganized arrangement. (A: T. Hasson, Curr Biology 9:R839, 1999; with permission from Elsevier Science. B: courtesy of A. J. Hudspeth, R. A. Jacobs, and P. G. Gillespie; C,E: from A. K. Rzadzinska, et al., courtesy of B. Kachar, J. Cell Biol. vol. 164, 891, 892, 2004, Figs. 4, 5; D: from Peter Gillespie and Tama Hasson, J. Cell Biol. vol. 137, Cover #6, 1997; C–E, reproduced with permission of The Rockefeller University Press; F–G: From Tim Self, et al., courtesy of Karen P. Steel, Develop. 125:560, 1998; reproduced by permission of The Company of Biologists, Ltd. http://dev.biologists.org/content/125/4/557.full.pdf+html?sid=1524ec24-e6c0-413f-9c05-ac2111083a85)

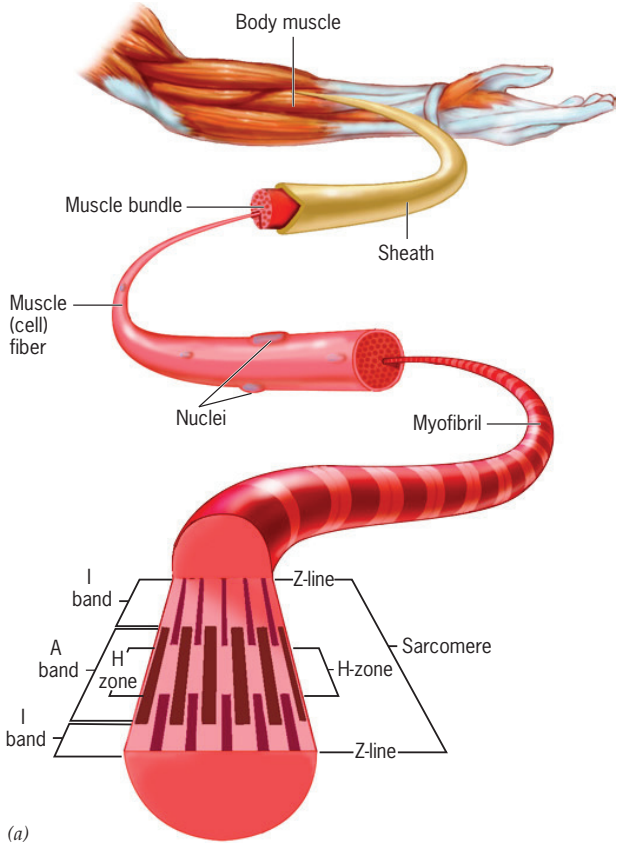
Because of these properties, a skeletal muscle cell is more appropriately called a **muscle fiber**. Muscle fibers have multiple nuclei because each fiber is a product of the fusion of large numbers of mononucleated *myoblasts* (premuscle cells) in the embryo.

Skeletal muscle cells may have the most highly ordered internal structure of any cell in the body. A longitudinal section of a muscle fiber (Figure 9.55) reveals a cable made up of hundreds of thinner, cylindrical strands, called **myofibrils**. Each myofibril consists of a repeating linear array of contractile units, called **sarcomeres**. Each sarcomere in turn exhibits a characteristic banding pattern, which gives the muscle fiber a striped or *striated* appearance. Examination of stained muscle fibers in the electron microscope shows the banding pattern to be the result of the partial overlap of two distinct types of filaments, **thin filaments** and **thick filaments** (Figure 9.56*a*). Each sarcomere extends from one *Z line* to the next Z line and contains several dark bands and light zones. A sarcomere has a pair of lightly staining *I bands* located at its outer edges, a more densely staining *A band* located between the outer I bands, and a lightly staining *H zone* located in the center of the A band. A densely staining *M line* lies in the center of the H zone. The I band contains only thin filaments, the H zone only thick filaments, and that part of the A band on either side of the H zone represents the region of overlap and contains both types of filaments.

Cross sections through the region of overlap show that the thin filaments are organized in a hexagonal array around each thick filament, and that each thin filament is situated between two thick filaments (Figure 9.56*b*). Longitudinal sections show the presence of projections from the thick filaments at regularly spaced intervals. The projections represent cross-bridges capable of forming attachments with neighboring thin filaments.

The Sliding Filament Model of Muscle Contraction

All skeletal muscles operate by shortening; there is no other way they can perform work. The units of shortening are the sarcomeres, whose combined decrease in length accounts for the decrease in length of the entire muscle. The most important clue to the mechanism underlying muscle contraction came from observations of the banding pattern of the sarcomeres at different stages in the contractile process. As a muscle fiber shortened, the A band in each sarcomere remained essentially constant in length, while the H band and I bands decreased in width and then disappeared altogether. As shortening progressed, the Z lines on both ends of the sarcomere moved inward until they contacted the outer edges of the A band (Figure 9.57).



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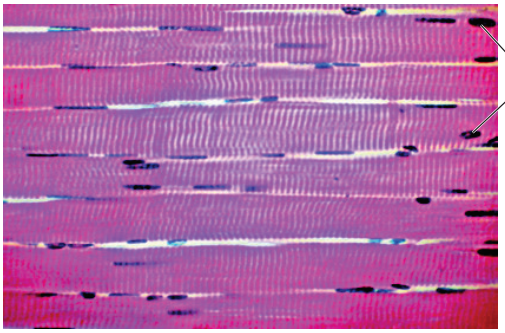

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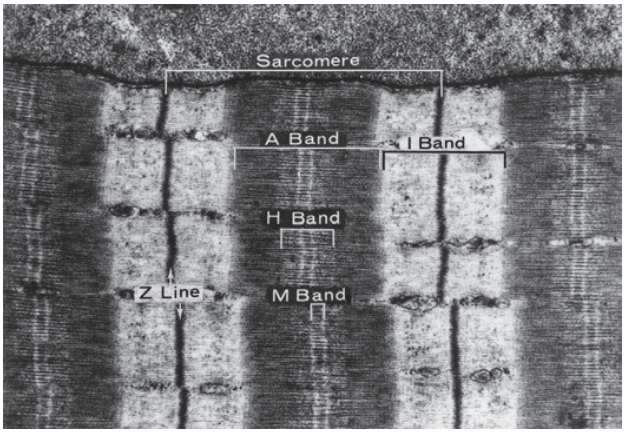


(*c*)

Figure 9.55 **The structure of skeletal muscle.** (*a*) Levels of organization of a skeletal muscle. (*b*) Light micrograph of a multinucleated muscle fiber. (*c*) Electron micrograph of a sarcomere with the bands lettered. (B: Eric Grave/Photo Researchers, Inc.; C: Don W. Fawcett/Photo Researchers, Inc.)

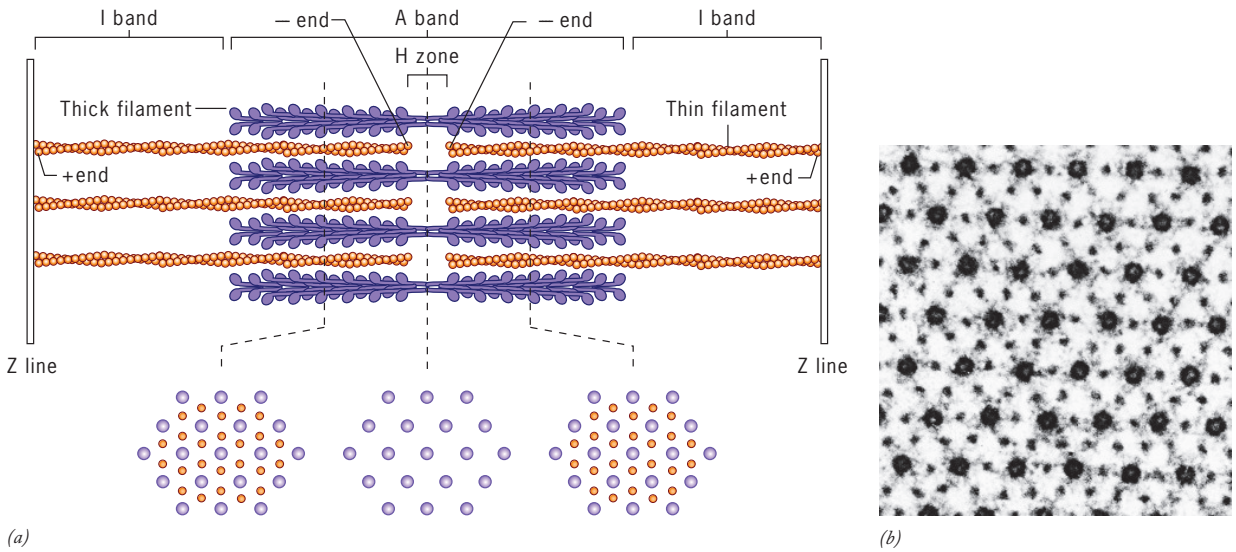


Figure 9.56 **The contractile machinery of a sarcomere.** (*a*) Diagram of a sarcomere showing the overlapping array of thin actin-containing (orange) and thick myosin-containing (purple) filaments. The small transverse projections on the myosin fiber represent the myosin heads (cross-bridges). (*b*) Electron micrograph of a cross section through an insect flight muscle showing the hexagonal arrangement of the thin filaments around each thick filament. (J. AUBER/PHOTO RESEARCHERS, INC.)

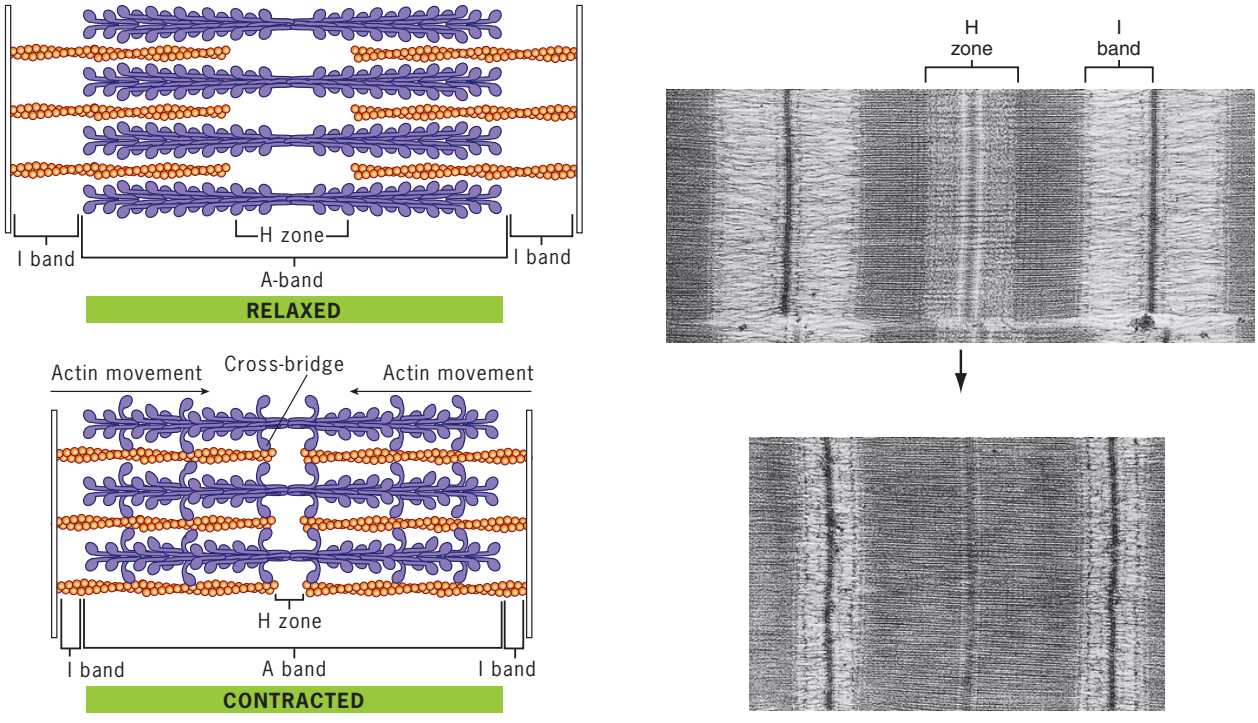


Figure 9.57 **The shortening of the sarcomere during muscle contraction.** (*a*) Schematic diagram showing the difference in the structure of the sarcomere in a relaxed and contracted muscle. During contraction, the myosin cross-bridges make contact with the surrounding thin filaments, and the thin filaments are forced to slide toward the center of the sarcomere. Cross-bridges work asynchronously, so that only a fraction are active at any given instant. (*b*) Electron micrographs of longitudinal sections through a relaxed (*top*) and contracted (*bottom*) sarcomere. The micrographs show the disappearance of the H zone as the result of the sliding of the thin filaments toward the center of the sarcomere. (B: TOP AND BOTTOM IMAGES FROM JAMES E. DENNIS/PHOTOTAKE.)

Based on these observations, two groups of British investigators, Andrew Huxley and Rolf Niedergerke, and Hugh Huxley and Jean Hanson, proposed a far-reaching model in 1954 to account for muscle contraction. They proposed that the shortening of individual sarcomeres did not result from the shortening of the filaments, but rather from their sliding over one another. The sliding of the thin filaments toward the center of the sarcomere would result in the observed increase in overlap between the filaments and the decreased width of the I and H bands (Figure 9.57). The *sliding-filament model* was rapidly accepted, and evidence in its favor continues to accumulate.

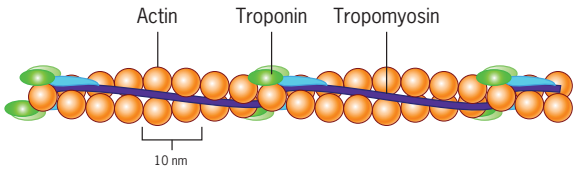


Figure 9.58 **The molecular organization of the thin filaments.** Each thin filament consists of a helical array of actin subunits with rod-shaped tropomyosin molecules situated in the grooves and troponin molecules spaced at defined intervals, as described in the text. The positional changes in these proteins that trigger contraction are shown in Figure 9.63.

The Composition and Organization of Thick and Thin Filaments In addition to actin, the thin filaments of a skeletal muscle contain two other proteins, *tropomyosin* and *troponin* (Figure 9.58). Tropomyosin is an elongated molecule (approximately 40 nm long) that fits securely into the grooves within the thin filament. Each rod-shaped tropomyosin molecule is associated with seven actin subunits along the thin filament (Figure 9.58). Troponin is a globular protein complex composed of three subunits, each having an important and distinct role in the overall function of the molecule. Troponin molecules are spaced approximately 40 nm apart along the thin filament and contact both the actin and tropomyosin components of the filament. The actin filaments of each half sarcomere are aligned with their barbed ends linked to the Z line.

Each thick filament is composed of several hundred myosin II molecules together with small amounts of other proteins. Like the filaments that form in vitro (see Figure 9.51), the polarity of the thick filaments of muscle cells is reversed at the center of the sarcomere. The center of the filament is composed of the opposing tail regions of the myosin molecules and is devoid of heads. The myosin heads project from each thick filament along the remainder of its length due to the staggered position of the myosin molecules that make up the body of the filament (Figure 9.51).

The third most abundant protein of vertebrate skeletal muscles is *titin*, which is the largest protein yet to be discovered in any organism. The entire titin gene (which can give rise to isoforms of different length) encodes a polypeptide more than 3.5 million daltons in molecular mass and containing more than 38,000 amino acids. Titin molecules originate at the M line in the center of each sarcomere and extend along the myosin filament, continuing past the A band and terminating at the Z line (Figure 9.59). Titin is a highly elastic protein that stretches like a molecular spring as certain regions within the molecule become uncoiled. Titin is thought to prevent the sarcomere from becoming pulled apart during muscle stretching. Titin also maintains myosin filaments in their proper position within the center of the sarcomere during muscle contraction.

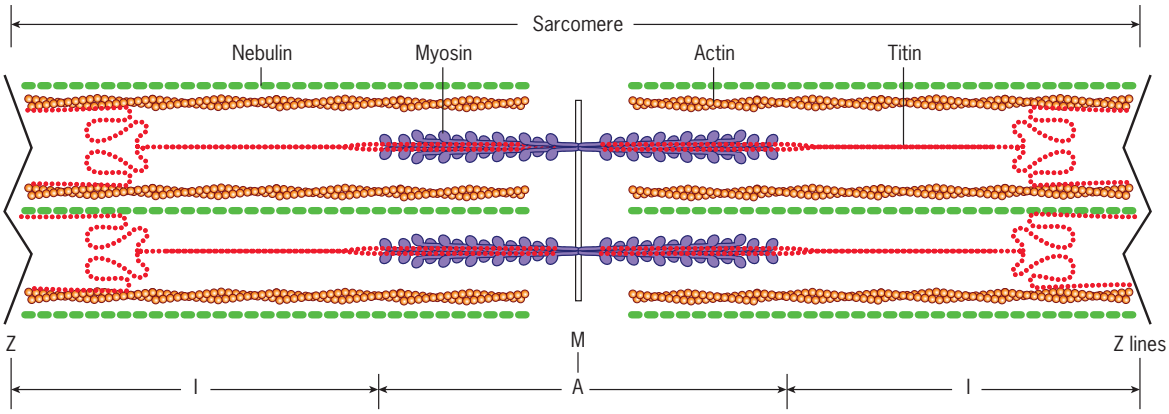


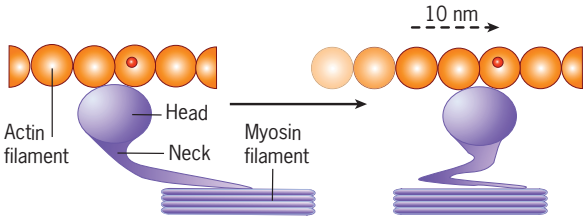
Figure 9.59 **The arrangement of titin molecules within the sarcomere.** These huge elastic molecules stretch from the end of the sarcomere at the Z line to the M band in the sarcomere center. Titin molecules are thought to maintain the thick filaments in the center of the sarcomere during contraction. The I-band portion of the titin molecule contains spring-like domains and is capable of great elasticity. The nebulin molecules (which are not discussed in the text) are thought to act like a "molecular ruler" by regulating the number of actin monomers that are allowed to assemble into a thin filament. (T. C. S. KELLER, CURR. OPIN CELL BIOL. 7:33, 1995. CURRENT OPINION IN CELL BIOLOGY BY ELSEVIER LTD., CURRENT OPINION JOURNALS. REPRODUCED WITH PERMISSION OF ELSEVIER LTD., CURRENT OPINION JOURNALS IN THE FORMAT JOURNAL VIA COPYRIGHT CLEARANCE CENTER.)

The Molecular Basis of Contraction Following the formulation of the sliding-filament hypothesis, attention turned to the heads of the myosin molecules as the force-generating components of the muscle fiber. During a contraction, each myosin head extends outward and binds tightly to a thin filament, forming the cross-bridges seen between the two types of filaments (Figure 9.57). The heads from a single myosin filament interact with six surrounding actin filaments. While it is bound tightly to the actin filament, the myosin head undergoes a conformational change (described below) that moves the thin actin filament approximately 10 nm toward the center of the sarcomere. Unlike myosin V (depicted in Figure 9.52), muscle myosin (i.e., myosin II) is a *nonprocessive motor*. Muscle myosin remains in contact with its track, in this case a thin filament, for only a small fraction (less than 5 percent) of the overall cycle. However, each thin filament is contacted by a team of a hundred or so myosin heads that beat out of synchrony with one another (Figure 9.57*a*). Consequently, the thin filament undergoes continuous motion during each contractile cycle. It is estimated that a single thin filament in a muscle cell can be moved several hundred nanometers during a period as short as 50 milliseconds.

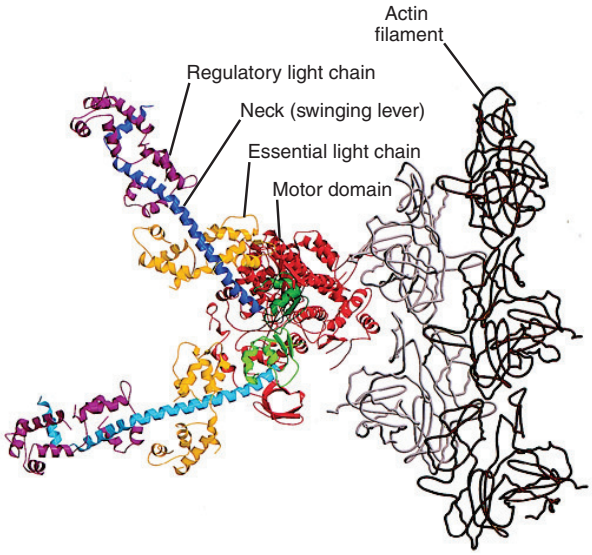
Muscle physiologists have long sought to understand how a single myosin molecule can move an actin filament approximately 10 nm in a single power stroke. The publication of the first atomic structure of the S1 myosin II fragment in 1993 by Ivan Rayment, Hazel Holden, and their colleagues at the University of Wisconsin focused attention on a proposal to explain its mechanism of action. In this proposal, the energy released by ATP hydrolysis induces a small (0.5 nm) conformational change within the head while the head is tightly bound to the actin filament. The small movement within the head is then amplified approximately 20-fold by the swinging movement of an adjoining α-helical neck (Figure 9.60). According to this hypothesis, the elongated myosin II neck acts as a rigid "lever arm" causing the attached actin filament to slide a much greater distance than would otherwise be possible. The two light chains, which are wrapped around the neck, are thought to provide rigidity for the lever.

Support for the "lever-arm" proposal was initially obtained through a series of experiments by James Spudich and colleagues at Stanford University. These researchers constructed genes that encoded altered versions of the myosin II molecule, which contained necks of different length. The genetically engineered myosin molecules were then tested in an in vitro motility assay with actin filaments, similar to that depicted in Figure 9.50. As predicted by the lever-arm hypothesis, the apparent length of the power stroke of the myosin molecules was directly proportional to the length of their necks. Myosin molecules with shorter necks generated smaller displacements, whereas those with longer necks generated greater displacements. Not all studies have supported the correlation between step size and neck length, so the role of the lever-arm remains a matter of controversy.

The Energetics of Filament Sliding Like the other motor proteins kinesin and dynein, myosin converts the chemical energy of ATP into the mechanical energy of filament sliding. Each cycle of mechanical activity of the myosin cross-bridge takes about 50 msec and is accompanied by a cycle of ATPase activity, as illustrated in the model shown in Figure 9.61. According to this model, the cycle begins as a molecule of ATP binds to the myosin head, an event that induces the dissociation of the cross-bridge from the actin filament (Figure 9.61, step 1). Binding of ATP is followed by its hydrolysis, which occurs before the myosin head makes contact with the actin



(a)



(b)

Figure 9.60 **Model of the swinging lever arm of a myosin II molecule.** (*a*) During the power stroke, the neck of the myosin molecule moves through a rotation of approximately 70°, which would produce a movement of the actin filament of approximately 10 nm. (*b*) A model of the power stroke of the myosin motor domain consisting of the motor domain (or head) and adjoining neck (or lever arm). An attached actin filament is shown in gray/brown. The long helical neck is shown in two positions, both before and after the power stroke (depicted as the upper dark blue and lower light blue necks, respectively). It is this displacement of the neck region that is thought to power muscle movement. The essential and regulatory light chains, which wrap around the neck, are shown in yellow and magenta, respectively. (B: From Malcolm Irving et al., Nature Struct. Biol. 7:482, 2000; Reprinted by permission from Macmillan Publishers Ltd. Courtesy of Malcolm Irving, Ivan Rayment, and Carolyn Cohen.)

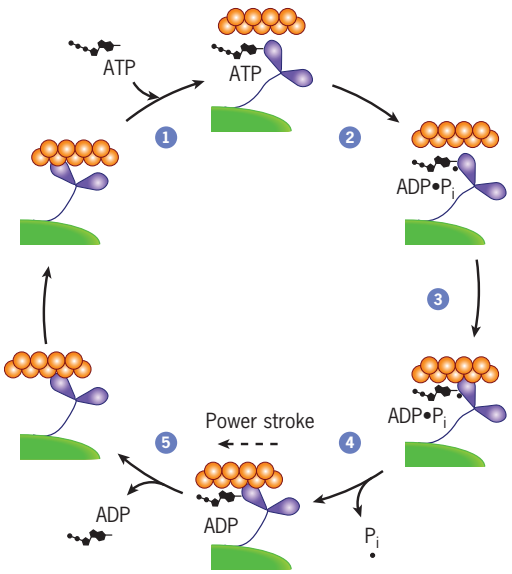


Figure 9.61 **A schematic model of the actinomyosin contractile cycle.** The movement of the thin filament by the force-generating myosin head occurs as the result of a linkage between a mechanical cycle involving the attachment, movement, and detachment of the head and a chemical cycle involving the binding, hydrolysis, and release of ATP, ADP, and P_i. In this model, the two cycles begin in step 1 with the binding of ATP to a cleft in the myosin head, causing the detachment of the head from the actin filament. The hydrolysis of the bound ATP (step 2) energizes the head, causing it to bind weakly to the actin filament (step 3). The release of P_i causes a tighter attachment of the myosin head to the thin filament and the power stroke (step 4) that moves the thin filament toward the center of the sarcomere. The release of the ADP (step 5) sets the stage for another cycle. (M. Y. JIANG & M. P. SHEETZ, BIOESSAYS 16:532, 1994.)

filament. The products of ATP hydrolysis, namely, ADP and P_i, remain bound to the active site of the enzyme, while the energy released by hydrolysis is absorbed by the protein as a whole (Figure 9.61, step 2). At this point, the cross-bridge is in an energized state, analogous to a stretched spring capable of spontaneous movement. The energized myosin then attaches to the actin molecule (step 3) and releases its bound phosphate, which triggers a large conformational change driven by the stored free energy (step 4). This conformational change shifts the actin filament toward the center of the sarcomere. This movement represents the power stroke of the myosin head as shown in Figure 9.60. The release of the bound ADP (step 5) is followed by the binding of a new ATP molecule so that a new cycle can begin. In the absence of ATP, the myosin head remains tightly bound to the actin filament. The inability of myosin cross-bridges to detach in the absence of ATP is the basis for the condition of *rigor mortis*, the stiffening of muscles that ensues following death.

Excitation–Contraction Coupling Muscle fibers are organized into groups termed *motor units*. All the fibers of a motor unit are jointly innervated by branches from a single motor neuron and contract simultaneously when stimulated by an impulse transmitted along that neuron. The point of contact of a terminus of an axon with a muscle fiber is called a **neuromuscular junction** (Figure 9.62; see also Figure 4.56 for a closer view of the structure of the synapse). The neuromuscular junction is a site of transmission of the nerve impulse from the axon across a synaptic cleft to the muscle fiber, whose plasma membrane is also excitable and capable of conducting an action potential.

The steps that link the arrival of a nerve impulse at the muscle plasma membrane to the shortening of the sarcomeres deep within the muscle fiber constitute a process referred to as **excitation–contraction coupling**. Unlike a neuron, where an action potential remains at the cell surface, the impulse generated in a skeletal muscle cell is propagated into the interior of the cell along membranous folds called **transverse (T) tubules** (Figure 9.62). The T tubules terminate in very close proximity to a system of cytoplasmic membranes that make up the **sarcoplasmic reticulum (SR)**, which forms a membranous sleeve around the myofibril. Approximately 80 percent of the integral protein of the SR membrane consists of Ca^{2+}-ATPase molecules whose function is to transport Ca^{2+} out of the cytosol and into the lumen of the SR, where it is stored until needed.

The importance of calcium in muscle contraction was first shown in 1882 by Sydney Ringer, an English physician. Ringer found that an isolated frog heart would contract in a saline solution made with London tap water, but failed to contract in a solution made with distilled water. Ringer determined that calcium ions present in tap water were an essential factor in muscle contraction. In the relaxed state, the Ca^{2+} levels within the cytoplasm of a muscle fiber are very low (approximately 2×10^{-7} M)—below the threshold concentration required for contraction. With the arrival of an action potential by way of the transverse tubules, calcium channels in the SR membrane are opened, and calcium diffuses out of the SR compartment and over the short distance to the myofibrils. As a result, the intracellular Ca^{2+} levels rise to about 5×10^{-5} M. To understand how elevated calcium levels trigger contraction in a skeletal muscle fiber, it is necessary to reconsider the protein makeup of the thin filaments.

When the sarcomere is relaxed, the tropomyosin molecules of the thin filaments (see Figure 9.58) block the myosin-binding sites on the actin molecules. The position of the tropomyosin within the groove is under the control of the attached troponin molecule. When Ca^{2+} levels rise, these ions bind to one of the subunits of troponin (troponin C), causing a conformational change in another subunit of the troponin molecule. Like the collapse of a row of dominoes, the movement of troponin is transmitted to the adjacent tropomyosin, which moves approximately 1.5 nm closer to the center of the filament's groove (from position b to a in Figure 9.63). This shift in position of the tropomyosin exposes the myosin-binding sites on the adjacent actin molecules, allowing the myosin heads to attach to the thin filaments. Each troponin molecule controls the position of one tropomyosin molecule, which in turn controls the binding capacity of seven actin subunits in the thin filament.

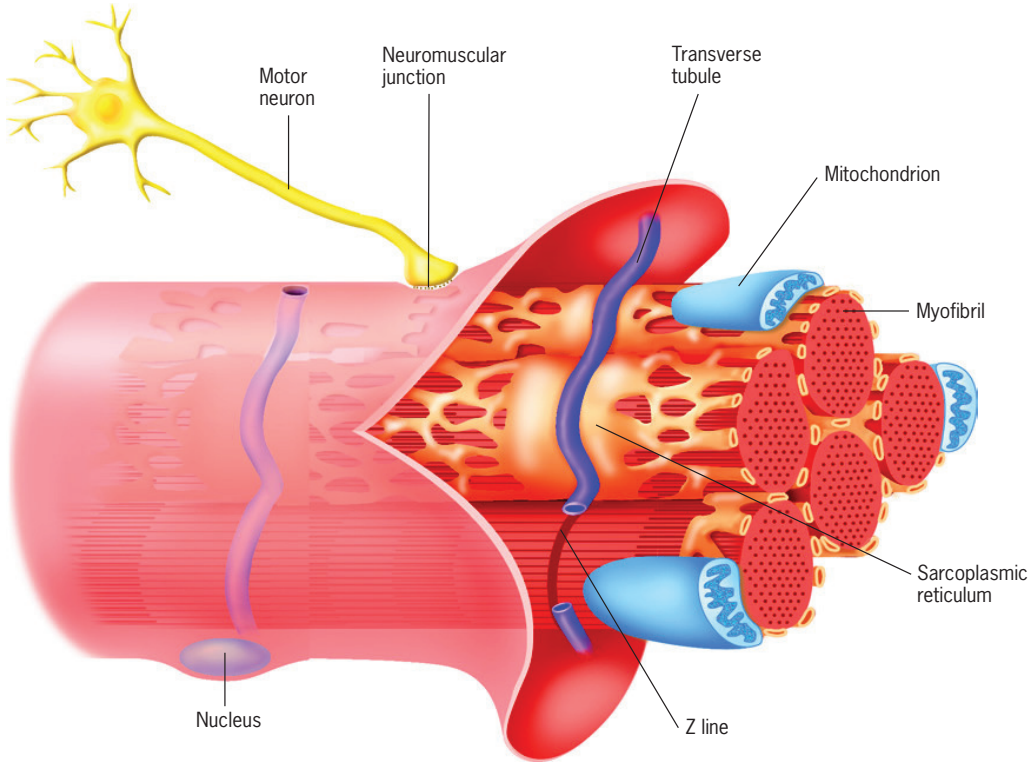


Figure 9.62 **The functional anatomy of a muscle fiber.** Calcium is housed in the elaborate network of internal membranes that make up the sarcoplasmic reticulum (SR). When an impulse arrives by means of a motor neuron, it is carried into the interior of the fiber along the membrane of the transverse tubule to the SR. The calcium gates of the SR open, releasing calcium into the cytosol. The binding of calcium ions to troponin molecules of the thin filaments leads to the events described in the following figure and the contraction of the fiber.

Once stimulation from the innervating motor nerve fiber ceases, the Ca^{2+} channels in the SR membrane close, and the Ca^{2+}-ATPase molecules in that membrane remove excess calcium from the cytosol. As the Ca^{2+} concentration decreases, these ions dissociate from their binding sites on troponin, which causes the tropomyosin molecules to move back to a position where they block the actin–myosin interaction. The process of relaxation can be thought of as a competition for calcium between the transport protein of the SR membrane and troponin. The transport protein has a greater affinity for the ion, so it preferentially removes it from the cytosol, leaving the troponin molecules without bound calcium.

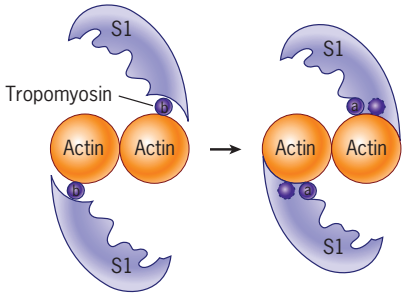


Figure 9.63 **The role of tropomyosin in muscle contraction.** Schematic diagram of the steric hindrance model in which the myosin-binding site on the thin actin filaments is controlled by the position of the tropomyosin molecule. When calcium levels rise, the interaction between calcium and troponin (not shown) leads to a movement of the tropomyosin from position b to position a, which exposes the myosin-binding site on the thin filament to the myosin head.

REVIEW

1. Describe the structure of the sarcomere of a skeletal muscle myofibril and the changes that occur during its contraction.
2. Describe the steps that occur between the time that a nerve impulse is transmitted across a neuromuscular junction to the time that the muscle fiber begins to shorten. What is the role of calcium ions in this process?

9.7 | Nonmuscle Motility

Skeletal muscle cells are an ideal system for the study of contractility and movement because the interacting contractile proteins are present in high concentration and are part of defined cellular structures. The study of nonmuscle

motility is more challenging because the critical components tend to be present in less ordered, more labile, transient arrangements. Moreover, they are typically restricted to a thin *cortex* just beneath the plasma membrane. The cortex is an active region of the cell, responsible for such processes as the ingestion of extracellular materials, the extension of processes during cell movement, and the constriction of a single animal cell into two cells during cell division. All of these processes are dependent on the assembly of microfilaments in the cortex.

In the following pages, we will consider a number of examples of nonmuscle contractility and motility that depend on actin filaments and, in some cases, members of the myosin superfamily. First, however, it is important to survey the factors that govern the rates of assembly, numbers, lengths, and spatial patterns of actin filaments.

Actin-Binding Proteins

Purified actin can polymerize in vitro to form actin filaments, but such filaments cannot interact with one another or perform useful activities. Under the microscope, they resemble the floor of a barn covered by straw. In contrast, actin filaments in living cells are organized into a variety of patterns, including various types of bundles, thin (two-dimensional) networks, and complex three-dimensional gels (Figure 9.64). The organization and behavior of actin filaments inside cells are determined by interaction of actin with a remarkable variety of **actin-binding proteins.** These proteins affect the localized assembly or disassembly of the actin filaments, their physical properties, and their interactions with one another and with cellular organelles. More than 100 different actin-binding proteins belonging to numerous families have been isolated from different cell types. Actin-binding proteins can be divided into several categories based on their presumed function in the cell (Figure 9.65).[5]

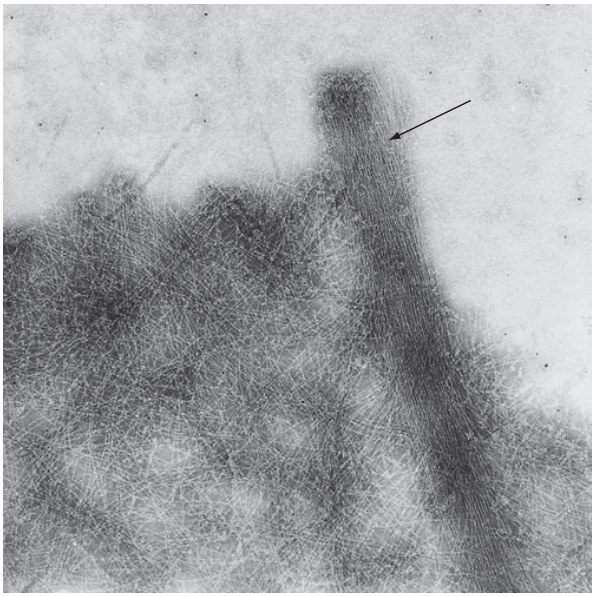


Figure 9.64 **Two different arrangements of actin filaments within a cell.** As described later in the chapter, cells move across a substratum by extending various types of processes. This electron micrograph of the leading edge of a motile fibroblast shows the high density of actin filaments. These filaments are seen to be organized into two distinct arrays: as bundles in which the filaments are arranged parallel to one another (arrow) and as a cross-linked meshwork in which the filaments are arranged in various directions. (COURTESY OF J. VICTOR SMALL.)

1. ***Nucleating proteins.*** The slowest step in the formation of an actin filament is the first step, nucleation, which requires that at least two or three actin monomers come together in the proper orientation to begin formation of the polymer. This is a very unfavorable process for actin molecules left on their own. As noted earlier, the formation of an actin filament is accelerated by the presence of a preexisting seed or nucleus to which monomers can be added (as in Figure 9.47*a*). Several proteins have been identified that promote nucleation of actin filaments. The best studied is the *Arp2/3 complex*, which contains two "actin-related proteins," that is, proteins that share considerable sequence homology with actins but are not considered "true" actins. Once the complex is activated, the two Arps adopt a conformation that provides a template to which actin monomers can be added, analogous to the way that γ-tubulin is proposed to form a template for microtubule nucleation (Figure 9.20*c*). As discussed on page 378, the Arp2/3 complex generates networks of short, branched actin filaments. Another family of nucleating proteins, the *formins*, generate unbranched filaments, such as those found at focal adhesions (page 248) and the contractile rings of dividing cells (Section 14.2). Unlike Arp2/3, which remains at the pointed end of the newly formed filament, formins track with the barbed end even as new subunits are inserted at that site. Thus not only do formins nucleate actin filaments, they can promote very rapid elongation of the filaments that they helped create.
2. ***Monomer-sequestering proteins.*** Thymosins (e.g., thymosin β_4) are proteins that bind to actin-ATP monomers (often called *G-actin*) and prevent them from polymerizing. Proteins with this activity are described as actin monomer-sequestering proteins. These proteins are believed responsible for the relatively high concentration of G-actin in nonmuscle cells (50–200 mM). Without monomer-sequestering proteins, conditions within the cytoplasm would favor the near complete polymerization of soluble actin monomers into filaments. Because of their ability to bind G-actin and stabilize the monomer pool, changes in the concentration or activity of monomer-sequestering proteins can shift the monomer–polymer equilibrium in a certain region of a cell and determine whether polymerization or depolymerization is favored at the time.

[5]It should be noted that some of these proteins can carry out more than one of the types of activities listed, depending on the concentration of the actin-binding protein and the prevailing conditions (e.g., the concentration of Ca^{2+} and H^+). Most studies of these proteins are conducted in vitro, and it is often difficult to extend the results to activities within the cell.

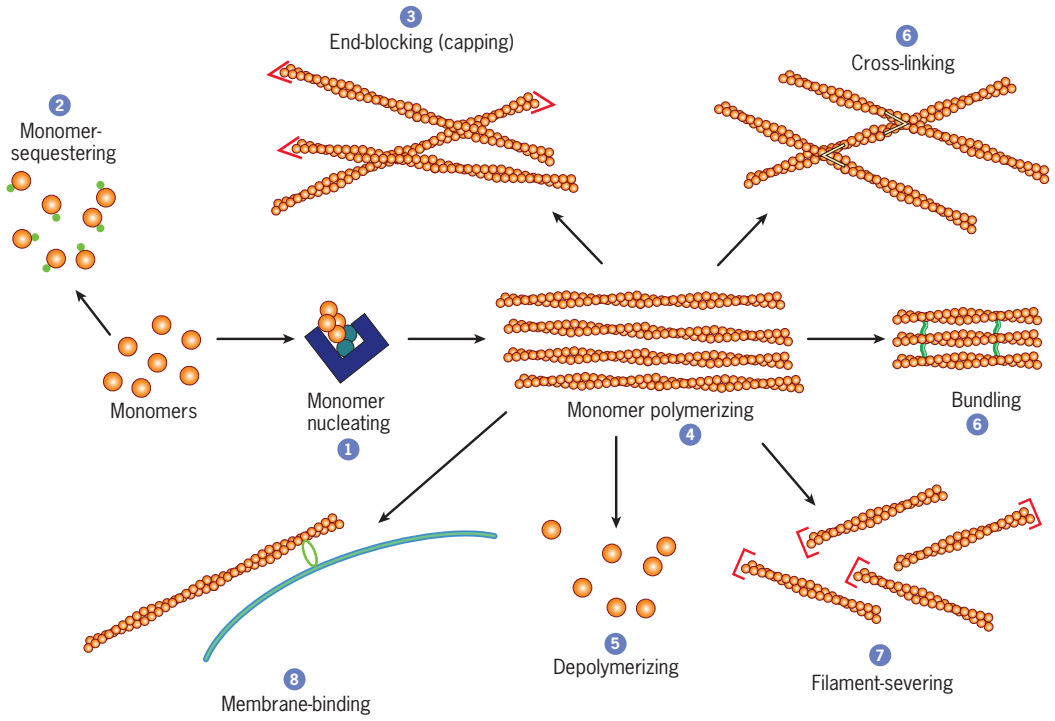


Figure 9.65 **The roles of actin-binding proteins.**

3. ***End-blocking (capping) proteins.*** Proteins of this group regulate the length of actin filaments by binding to one or the other end of the filaments, forming a cap that blocks both loss and gain of subunits. If the fast-growing, barbed end of a filament is capped, depolymerization may proceed at the opposite end, resulting in the disassembly of the filament. If the pointed end is also capped, depolymerization is blocked. The thin filaments of striated muscle are capped at their barbed end at the Z line by a protein called capZ and at their pointed end by the protein tropomodulin. If the tropomodulin cap is disturbed by microinjection of antibodies into a muscle cell, the thin filaments add more actin subunits at their newly exposed pointed end and undergo dramatic elongation into the middle of the sarcomere.
4. ***Monomer-polymerizing proteins.*** Profilin is a small protein that binds to the same site on an actin monomer as does thymosin. However, rather than inhibiting polymerization, profilin promotes the growth of actin filaments. Profilin does this by attaching to an actin monomer and catalyzing the dissociation of its bound ADP, which is rapidly replaced with ATP. The profilin-ATP-actin monomer can then assemble onto the free barbed end of a growing actin filament, which leads to the release of profilin. Filament elongation at the barbed end is further promoted by members of the Ena/VASP protein family.
5. ***Actin filament-depolymerizing proteins.*** Members of the cofilin family of proteins (including cofilin, ADF, and depactin) bind to actin-ADP subunits present within the body and at the pointed end of actin filaments (see Figure 18.33). Cofilin has two apparent activities: it can fragment actin filaments, and it can promote their depolymerization at the pointed end. These proteins play a role in the rapid turnover of actin filaments at sites of dynamic changes in cytoskeletal structure. They are essential for cell locomotion, phagocytosis, and cytokinesis.
6. ***Cross-linking proteins.*** Proteins of this group are able to alter the three-dimensional organization of a population of actin filaments. Each of these proteins has two or more actin-binding sites and therefore can cross-link two or more separate actin filaments. Certain of these proteins (e.g., filamin) have the shape of a long, flexible rod and promote the formation of loose networks of filaments interconnected at near right angles to one another (as in Figure 9.64). Regions of the cytoplasm containing such networks have the properties of a three-dimensional elastic gel that resists local mechanical pressures. Other cross-linking proteins (e.g., villin and fimbrin) have a more globular shape and promote the bundling of actin filaments into tightly knit, parallel arrays. Such arrays are found in the microvilli that project from certain epithelial cells (Figure 9.66) and in the hairlike stereocilia (Figure 9.54) that project from receptor cells of the inner ear. Bundling filaments together adds to their rigidity, allowing them to act as a supportive internal skeleton for these cytoplasmic projections.
7. ***Filament-severing proteins.*** Proteins of this class have the ability to bind to the side of an existing filament and

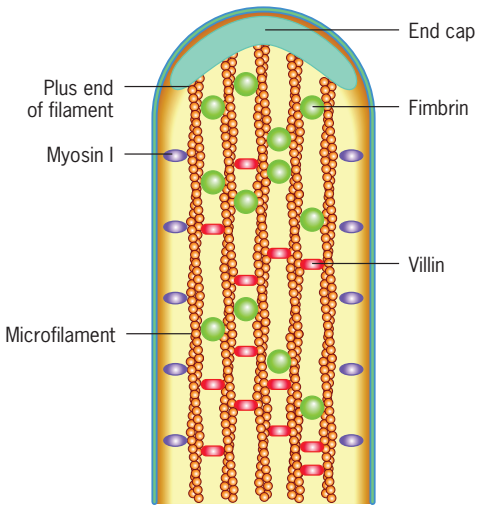


Figure 9.66 **Actin filaments and actin-binding proteins in a microvillus.** Microvilli are present on the apical surface of epithelia that function in absorption of solutes, such as the lining of the intestine and wall of the kidney tubule. Actin filaments are maintained in a highly ordered arrangement by the bundling proteins villin and fimbrin. The role of myosin I, which is present between the plasma membrane of the microvillus and the peripheral actin filaments, remains unclear.

break it in two. Severing proteins (e.g., gelsolin) may also promote the incorporation of actin monomers by creating additional free barbed ends, or they may cap the fragments they generate. As indicated in Figure 9.71, cofilin is also capable of severing filaments.

8. ***Membrane-binding proteins.*** Much of the contractile machinery of nonmuscle cells lies just beneath the plasma membrane. During numerous activities, the forces generated by the contractile proteins act on the plasma membrane, causing it to protrude outward (as occurs, for example, during cell locomotion) or to invaginate inward (as occurs, for example, during phagocytosis or cytokinesis). These activities are generally facilitated by linking the actin filaments to the plasma membrane indirectly, by means of attachment to a peripheral membrane protein. Two examples were described in previous chapters: the inclusion of short actin polymers into the membrane skeleton of erythrocytes (see Figure 4.32*d*), and the attachment of actin filaments to the membrane at focal adhesions and adherens junctions (see Figures 7.17 and 7.26). Proteins that link membranes to actin include vinculin, members of the ERM family (ezrin, radixin, and moesin), and members of the spectrin family (including dystrophin, the protein responsible for muscular dystrophy).

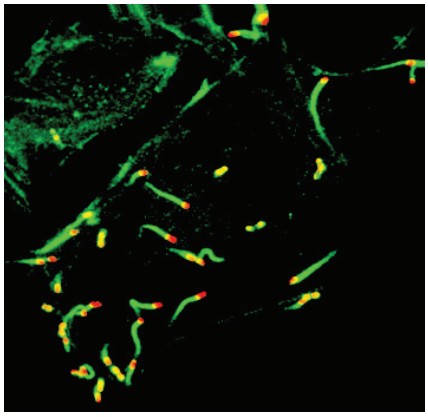
Examples of Nonmuscle Motility and Contractility

Actin filaments, often working in conjunction with myosin motors, are responsible for a variety of dynamic activities in nonmuscle cells, including cytokinesis, phagocytosis, cytoplasmic streaming (the directed bulk flow of cytoplasm that occurs in certain large plant cells), vesicle trafficking, blood platelet activation, lateral movements of integral proteins within membranes, cell–substratum interactions, cell locomotion, axonal outgrowth, and changes in cell shape. Nonmuscle motility and contractility are illustrated by the following examples.

Actin Polymerization as a Force-Generating Mechanism Some types of cell motility occur solely as the result of actin polymerization and do not involve the activity of myosin. Consider the example of *Listeria monocytogenes*, a bacterium that infects macrophages and can cause encephalitis or food poisoning. *Listeria* is propelled like a rocket through the cytoplasm of an infected cell by the polymerization of actin monomers just behind the bacterium (Figure 9.67). How is the bacterial cell able to induce the formation of actin filaments at a particular site on its surface? Questions about location are important in the study of any type of motile process because motility depends on a cell being able to assemble the necessary machinery at a particular site and a particular time. *Listeria* can accomplish this feat because it contains a surface protein called ActA that is present only at one end of the bacterium. When ActA is exposed within the host cytoplasm, it recruits and activates a number of host proteins (including the Arp2/3 complex, discussed below) that work together to direct the process of actin polymerization. The process of *Listeria* propulsion has been reconstituted in vitro, which has allowed researchers to demonstrate conclusively that actin polymerization by itself, that is, without the participation of myosin motors, is capable of providing the force required for motility.

The same events that occur during *Listeria* propulsion are utilized for normal cellular activities, ranging from the propulsion of cytoplasmic vesicles and organelles to the movement of cells themselves, which is the subject of the following section.

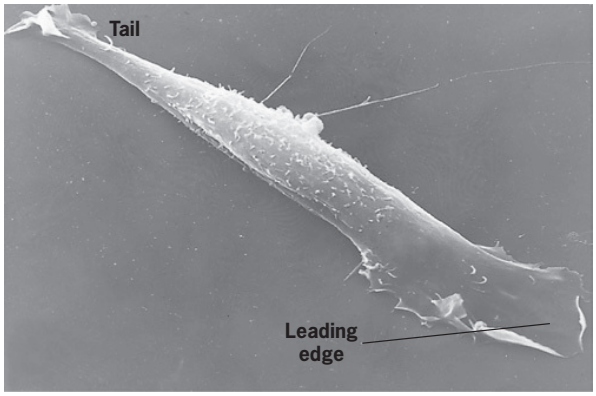
Cell Locomotion Cell locomotion is required for many activities in higher vertebrates, including tissue and organ development, formation of blood vessels, development of axons, wound healing, and protection against infection. Cell locomotion also contributes to the spread of cancerous tumors. In the following discussion, we will concentrate on studies of cultured cells moving over a flat (i.e., two-dimensional) substrate because these are the experimental conditions that have dominated this field of research. Keep in mind that cells in the body don't move over bare, flat substrates, and there is increasing evidence that some of the findings from these studies may not apply to cells traversing more complicated terrain. Researchers have recently begun to develop more complex substrata, including various types of three-dimensional extracellular matrices (see Figure 18.22), which may lead to



(a) 5 µm

(b)

Figure 9.67 **Cell motility can be driven by actin polymerization.** (*a*) Fluorescence micrograph of a portion of a cell infected with the bacterium *L. monocytogenes*. The bacteria appear as red-stained objects just in front of the green-stained filamentous actin tails. (*b*) Electron micrograph of a cell infected with the same bacterium as in *a*, showing the actin filaments that form behind the bacterial cell and push it through the cytoplasm. The actin filaments have a bristly appearance because they have been decorated with myosin heads. Bar at the upper left, 0.1 µm. (A: COURTESY OF PASCALE COSSART; B: FROM LEWIS G. TILNEY ET AL., J. CELL BIOL. 118:77, 1992, FIG. 8. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)



15 µm

Figure 9.68 **Scanning electron micrograph of a mouse fibroblast crawling over the surface of a culture dish.** The leading edge of the cell is spread into a flattened lamellipodium whose structure and function are discussed later in the chapter. (FROM GUENTER ALBRECHT-BUEHLER, INT. REV. CYTOL. 120:194, 1990.)

revision of some aspects of the mechanism of cell locomotion discussed below.

Figure 9.68 shows a single fibroblast that was in the process of moving toward the lower right corner of the field when it was prepared for microscopy. Cell locomotion, as exhibited by the fibroblast in Figure 9.68, shares properties with other types of locomotion, for example, walking. As you walk, your body performs a series of repetitive activities: first, a leg is extended in the direction of locomotion; second, the bottom of your foot makes contact with the ground, which acts as a point of temporary adhesion; third, force is generated by the muscles of your legs that moves your entire body forward past the stationary foot, all the while generating traction against the point of adhesion; fourth, your foot—which is now behind your body rather than in front of it—is lifted from the ground in anticipation of your next step. Even though motile cells may assume very different shapes as they crawl over a substra-

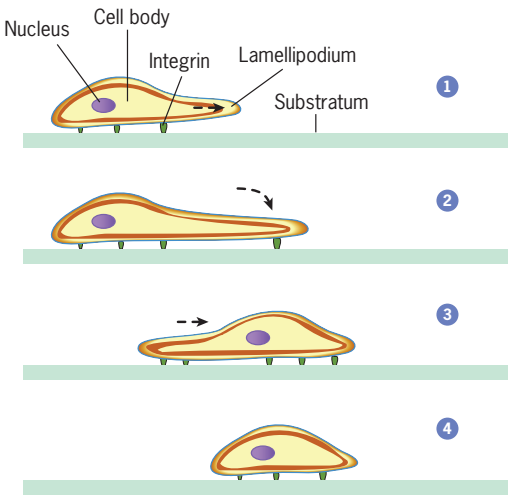
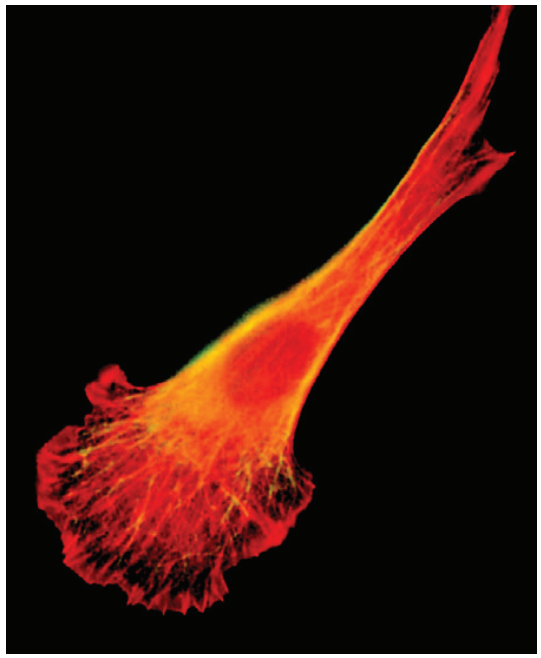


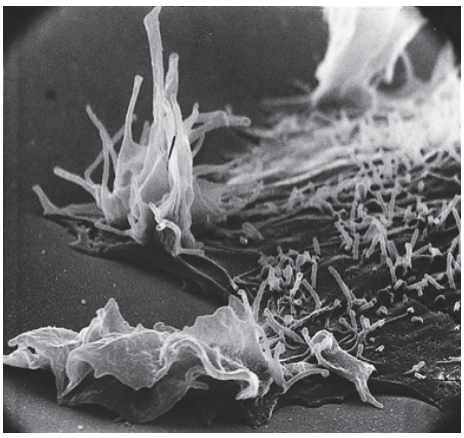
Figure 9.69 **The repetitive sequence of activities that occurs as a cell crawls over the substratum.** Step 1 shows the protrusion of the leading edge of the cell in the form of a lamellipodium. Step 2 shows the adhesion of the lower surface of the lamellipodium to the substratum, an attachment that is mediated by integrins residing in the plasma membrane. The cell uses this attachment to grip the substratum. Step 3 shows the movement of the bulk of the cell forward over the site of attachment, which remains relatively stationary. This movement is accomplished by a contractile (traction) force exerted against the substratum. Step 4 shows the cell after the rear attachments with the substratum have been severed and the trailing portion of the cell has been pulled forward.

tum, they display a similar sequence of activities (Figure 9.69). (1) Movement is initiated by the protrusion of a part of the cell surface in the direction in which the cell is to move. (2) A portion of the lower surface of the protrusion attaches to the substratum, forming temporary sites of anchorage. (3) The bulk of the cell is pulled forward over the adhesive contacts, which eventually become part of the rear of the cell. (4) The cell breaks its rear contacts with the substratum, causing retraction of the trailing edge, or "tail."

Cells that Crawl over the Substratum When a small piece of living tissue, such as skin or liver, is placed in a culture dish in an appropriate culture medium, individual cells migrate out of the specimen and onto the surface of the dish. Examination of these cells under the microscope typically shows them to be fibroblasts, which are the predominant cells present in connective tissue (see Figure 7.1). As it moves, a fibroblast flattens itself close to the substratum and becomes fan-shaped, with a broadened frontal end and a narrow "tail" (as in Figure 9.68). Its movement is erratic and jerky, sometimes advancing and other times withdrawing. On a good day, a fibroblast may move a distance of about 1 mm. The key to the fibroblast's locomotion is seen by examining its leading edge, which is extended out from the cell as a broad, flattened, veil-like protrusion, called a **lamellipodium** (Figure 9.70*a*). Lamellipodia are typically devoid of cytoplasmic vesicles and other



(a)



(b)

Figure 9.70 **The leading edge of a motile cell.** (*a*) The leading edge of this motile fibroblast is flattened against the substratum and spread out into a veil-like lamellipodium. (*b*) Scanning electron micrograph of the leading edge of a cultured cell, showing the ruffled membranes of the lamellipodium. (A: COURTESY OF J. VICTOR SMALL; B: FROM JEAN PAUL REVEL, SYMP. SOC. EXP. BIOL. 28:447, 1974.)

particulate structures, and the outer edge often exhibits an undulating motion, giving it a ruffled appearance (Figure 9.70*b*). As a lamellipodium is extended from the cell, it adheres to the underlying substratum at specific points, providing temporary sites of anchorage for the cell to pull itself forward.

We saw on page 374 how the polymerization of actin monomers can provide the force that propels a *Listeria* bacterium through the cytoplasm. This type of intracellular movement is accomplished without the involvement of mo-

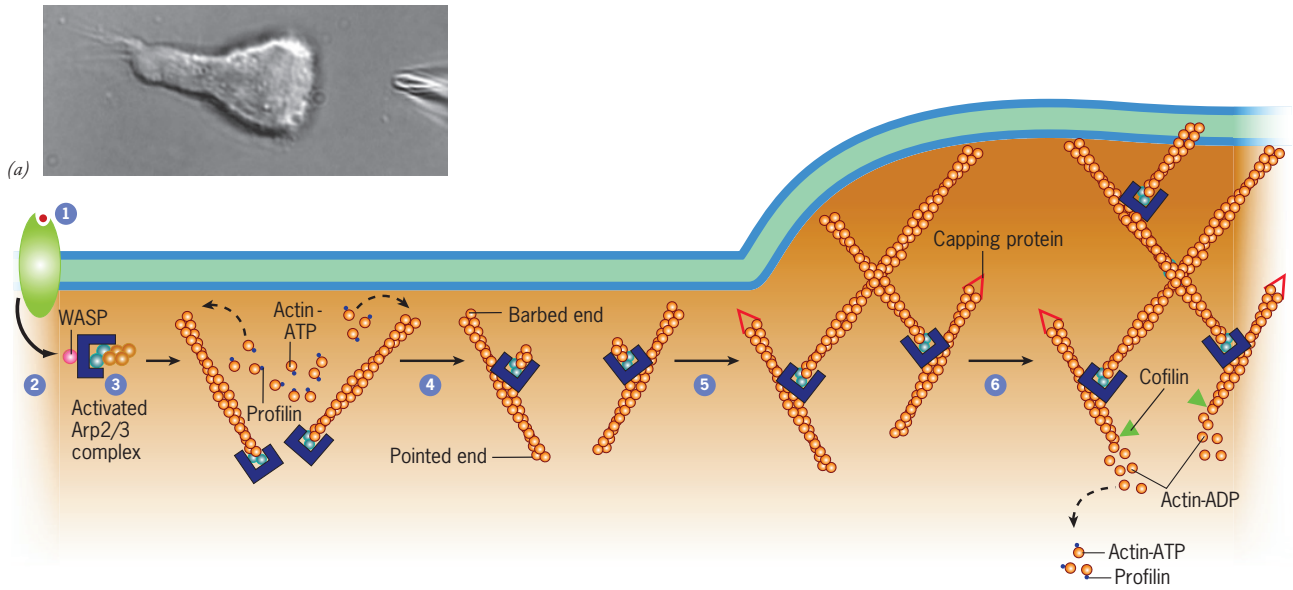


Figure 9.71 **Directed cell motility.** (*a*) Micrograph of a white blood cell (a neutrophil) that has responded to a chemoattractant being delivered by a pipette (seen on the right). The cell has become polarized and is moving toward the source of the stimulus. (*b*) A proposed mechanism for the movement of a cell in a directed manner. A stimulus is received at the cell surface (step 1) that leads to the activation of Arp2/3 complex by a member of the WASP/WAVE family (step 2). Activated Arp2/3 complexes serve as nucleating sites for the formation of new actin filaments (step 3). Once filaments have formed, Arp2/3 complexes attach to the sides of the filaments (step 4), which stimulates their nucleating activity. As a result, the bound Arp2/3 complexes initiate side branches that extend outward (step 5) at an angle of about 70° relative to the existing filaments to which they are anchored. As these filaments polymerize, they are thought to push the plasma membrane outward, resulting in extension of the leading edge of the lamellipodium. Meanwhile, the barbed end of previously formed filaments are bound by a capping protein, which prevents further growth of these filaments, keeping them short and rigid. Eventually, the pointed ends of the older, preexisting actin filaments undergo depolymerization, releasing ADP-actin subunits (step 6). Depolymerization is promoted by cofilin, which binds to ADP-actin subunits within the filament and stimulates dissociation of the subunits from the pointed end of the filament. Released subunits bind profilin and become recharged by ATP/ADP exchange, which makes them ready to engage in actin polymerization (as in step 3). (A discussion of the validity of this model can be found in *Trends Cell Biol.* 21:2, 2011 and *Nature Cell Biol,* 13: 1012, 2011.) (A: From Carole A. Parent, Curr. Opin. Cell Biol. 16:5, 2004; © 2004, with permission from Elsevier.)

lecular motors. A similar type of actin-polymerization mechanism is thought to provide the motile force required for the protrusion of the leading edge of a lamellipodium. This type of nonmuscle motility also demonstrates the importance of actin-binding proteins (depicted in Figure 9.65) in orchestrating the assembly and disassembly of actin-filament networks at a particular site within the cell at a particular time.

Suppose we begin with a rounded white blood cell that receives a chemical signal coming from one particular direction where the body has been wounded. Once the stimulus is received at the plasma membrane, it triggers the localized polymerization of actin, which leads to the polarization of the cell and its movement toward the source of the stimulus (Figure 9.71*a*).[6] Just as *Listeria* has a protein (ActA) that activates polymerization at the bacterial cell surface, mammalian cells have a family of proteins (the WASP/WAVE family) that activates the Arp2/3 complex at the site of stimulation near the plasma membrane. WASP, the founding member of the family, was discovered as the product of a gene responsible for Wiskott-Aldrich syndrome. Patients with this disorder have a crippled immune system because their white blood cells lack a functional WASP protein and consequently fail to respond to chemotactic signals.

Figure 9.71*b* depicts a model for the major steps in formation of a lamellipodium that would guide a cell in a particular direction. A stimulus is received at one end of the cell (step 1, Figure 9.71*b*), which leads to the activation of Arp2/3 protein complexes by activated WASP proteins (step 2). In its activated state, the Arp2/3 complex adopts a conformation that resembles the free surface at the barbed end of an actin filament. As a result, free ATP-actin monomers bind to the Arp2/3 template, leading to the nucleation of actin filaments (step 3). Polymerization of ATP-bound actin monomers onto the free barbed ends of the growing filaments is promoted by

[6]This type of response can be seen in a remarkable film on the Web showing a neutrophil chasing a bacterium. It can be found using the search words: "neutrophil crawling."

profilin molecules (page 373). Once new actin filaments have formed, Arp2/3 complexes bind to the sides of these filaments (step 4) and nucleate the formation of additional actin filaments that form as branches (step 5). The Arp2/3 complexes remain at the pointed ends, which are situated at the branch-points. Meanwhile, growth of the barbed ends of older filaments is blocked by the addition of capping protein (step 5). In contrast, addition of actin subunits to the barbed ends of more recently formed filaments of the network pushes the membrane of the lamellipodium outward in the direction of the attractive stimulus (steps 5 and 6). As newer filaments are growing by addition of subunits to their barbed ends, the older capped filaments undergo disassembly from their pointed ends (step 6). Disassembly is promoted by cofilin, which binds to actin-ADP subunits along the filaments (step 6). Actin-ADP subunits released from the disassembling filaments are recharged by conversion into profilin-ATP-actin monomers, which can be reutilized in the assembly of actin filaments at the leading edge.

Figure 9.72 illustrates some of the major structural features of cell locomotion. The electron micrograph of Figure 9.72 shows the branched, cross-linked nature of the filamentous actin network that resides just beneath the plasma membrane of an advancing lamellipodium. The circular insets in Figure 9.72 show a succession of short actin-filament branches, with Arp2/3 complexes highlighted by immunogold labeling. The Arp2/3 complexes are seen to reside at the Y-shaped junctions where the newly polymerized filaments have branched off of preexisting filaments.

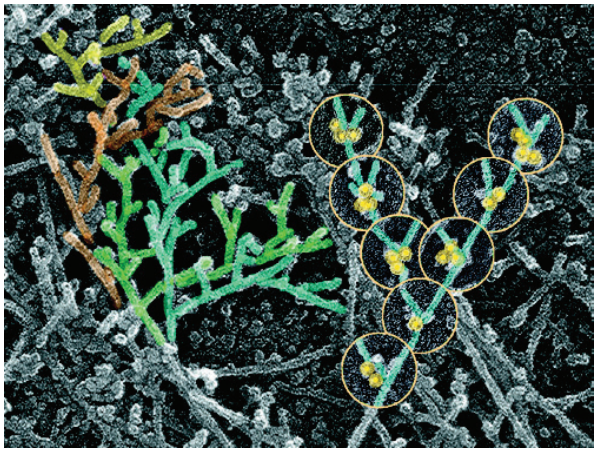


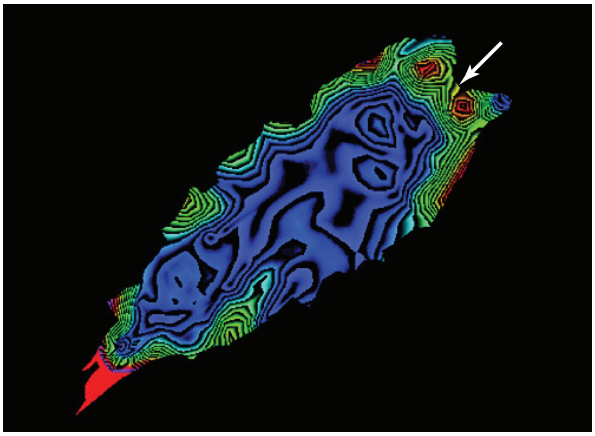
Figure 9.72 **The structural basis of lamellipodial extension.** Electron micrograph of a replica of the cytoskeleton at the leading edge of a motile mouse fibroblast. The actin filaments are seen to be arranged in a branched network, which has been colorized to indicate individual "trees." The circular insets show a succession of Y-shaped junctions between branched actin filaments. Arp2/3 complexes are localized at the base of each branch by antibodies linked to colloidal gold particles (yellow). (From Tatyana M. Svitkina and Gary G. Borisy, from J. Cell Biol., vol. 145, #5, 1999; reproduced with permission of The Rockefeller University Press.)

Lamellipodial movement is a dynamic process. As actin filament polymerization and branching continue at the very front edge of the lamellipodium, assembled actin filaments flow in a rearward direction and then depolymerize toward the back of the lamellipodium (step 6, Figure 9.71). Thus taken as a whole, the entire actin-filament array undergoes a type of treadmilling (page 360) in which actin subunits are added to barbed ends of the array at its front end and lost from pointed ends of the array toward the rear.

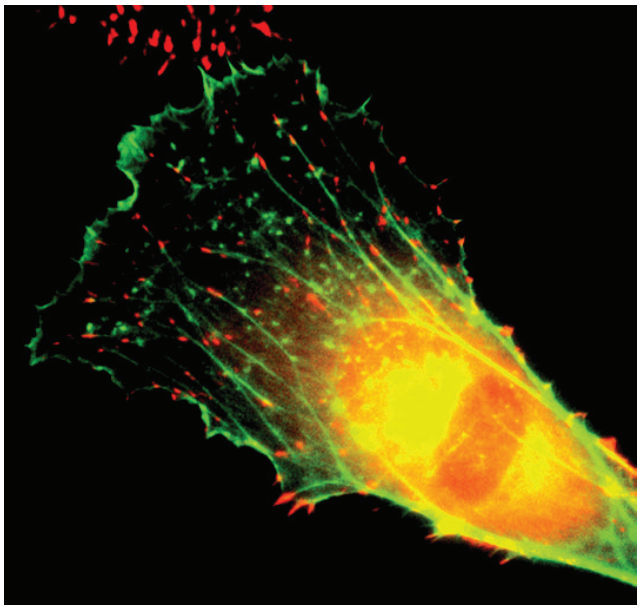
According to the sequence of events depicted in Figure 9.69, protrusion of the leading edge is followed by movement of the bulk of the cell. The major forces involved in cell locomotion are those generated at sites of adhesion that are required to pull or "tow" the main body of the cell forward (step 3 of Figure 9.69). They are often described as "traction forces" because they occur at sites where the cell grips the substrate.

When cells are allowed to migrate over a thin sheet of elastic material, movements of the cell are accompanied by deformation of the underlying substratum (see Figure 7.18). The magnitude of the traction forces exerted at various locations within a live migrating cell can be calculated from the dynamic patterns of substrate deformation and portrayed as shown in Figure 9.73*a*. As seen by examination of this computerized image of a migrating fibroblast, the greatest traction forces are exerted just behind the cell's leading edge where the cell adheres strongly to the underlying substratum. The presence of these sites of attachment is best revealed by following the localization of fluorescently labeled vinculin within a living cell. This allows investigators to specifically visualize structures where the cell makes contact with the underlying substratum. Figure 9.73*b* is a fluorescence micrograph showing the presence of red fluorescent vinculin molecules concentrated just behind the leading edge of a migrating cell. Vinculin is a major component of focal adhesions, the actin-containing sites illustrated in Figure 7.17. The vinculin-containing sites where the leading edge of a migrating cell adheres to the substratum tend to be smaller and simpler than the mature focal adhesions seen in highly spread, stationary cultured cells and are often referred to as *focal complexes*. Traction forces are thought to be generated in the actin cytoskeleton associated with these adhesion sites and then transmitted to the extracellular substrate by way of the transmembrane integrin molecules that connect the inside and outside of the cell. The focal complexes that form near the leading edge of a motile cell either disassemble as the cell moves forward or mature into larger, more contractile focal adhesions. Maturation of focal complexes is probably stimulated by tension exerted on these adhesion sites.

A large body of evidence indicates that actin polymerization is responsible for pushing the leading edge of a cell outward (step 1, Figure 9.69), whereas myosin (in conjunction with actin filaments) is responsible for pulling the remainder of the cell forward (step 3, Figure 9.69). These contrasting roles of actin and myosin are best illustrated in studies on fish keratocytes, which are cells derived from the epidermis that covers the fish's scales. Keratocytes have been a favored system for studying locomotion because their rapid gliding movement depends on the formation of a very broad, thin lamel-



(*a*)



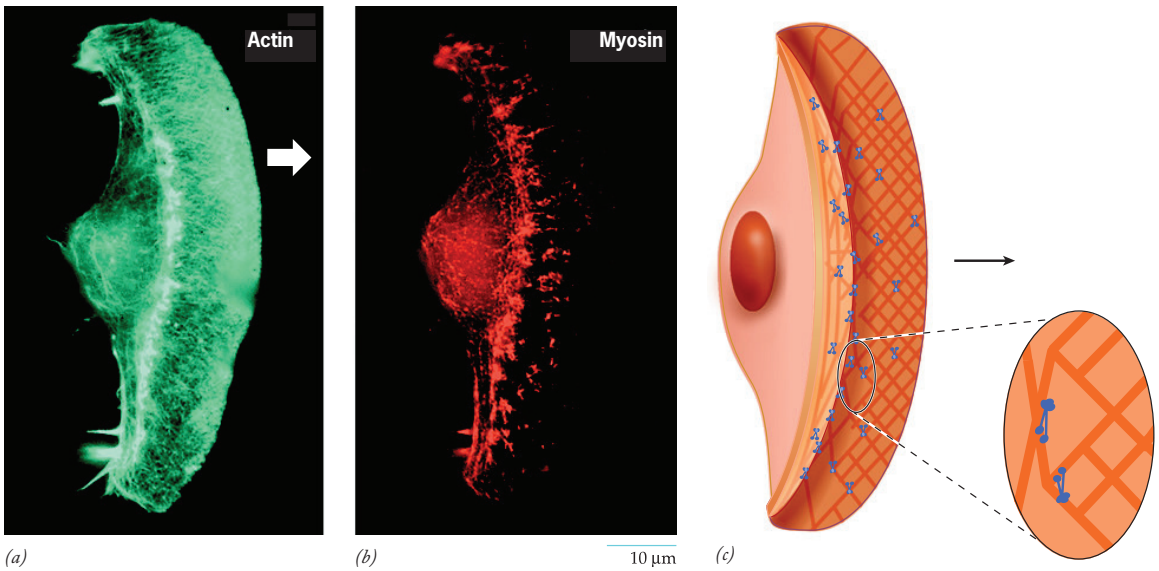
(*b*)

Figure 9.73 **Distribution of traction forces within a migrating fibroblast.** (*a*) As a cell migrates it generates traction (pulling) forces against its substrate. The present image shows the traction forces generated per unit area by the surface of a migrating fibroblast. Traction forces were calculated at different sites on the surface based on the degree of substrate deformation (see Figure 7.18). The magnitude of the traction forces are expressed by varying colors with red representing the strongest forces. The largest forces are generated at sites of small focal complexes that form transiently behind the leading edge of the cell where the lamellipodium is being extended (arrow). Deformation at the rear of the cell (shown in red) occurs as the front end actively pulls against the tail, which is passively anchored. (*b*) A living, migrating fibroblast exhibiting a well-developed lamellipodium that is adhering to the underlying substratum at numerous sites (red). This cell is expressing GFP-actin (green) and had been injected with rhodamine-tagged vinculin (red). The fluorescently labeled vinculin is incorporated into dot-like focal complexes near the leading edge of the cell. Some of these focal complexes disassemble, whereas others mature into focal adhesions, which are situated farther from the advancing edge. (A: From Karen A. Beningo, Micah Dembo, and Yu-Li Wang, J. Cell Biol. 153:885, 2001, Fig. 3. Reproduced with permission of The Rockefeller University Press; B: From J. Victor Small, et al., image courtesy of Olga Krylyshkina, Nature Revs. Mol. Cell Biol. 3:957, 2002; © 2002, reprinted with permission from Macmilian Publishers Ltd.)

lipodium. Figure 9.74 shows a moving keratocyte that has been fixed and stained for actin (Figure 9.74*a*) and myosin II (Figure 9.74*b*). As expected from the previous discussion, the advancing edge of the lamellipodium is filled with actin. Myosin, on the other hand, is concentrated in a band where the rear of the lamellipodium joins the remainder of the cell. Electron micrographs of this region show the presence of clusters of small, bipolar myosin II filaments bound to the actin network (Figure 9.74*c*). Contractile forces generated by these myosin molecules are presumed to pull the bulk of the cell along behind the advancing lamellipodium. Myosin I and other unconventional myosins are also thought to generate forces for cell locomotion in some organisms.

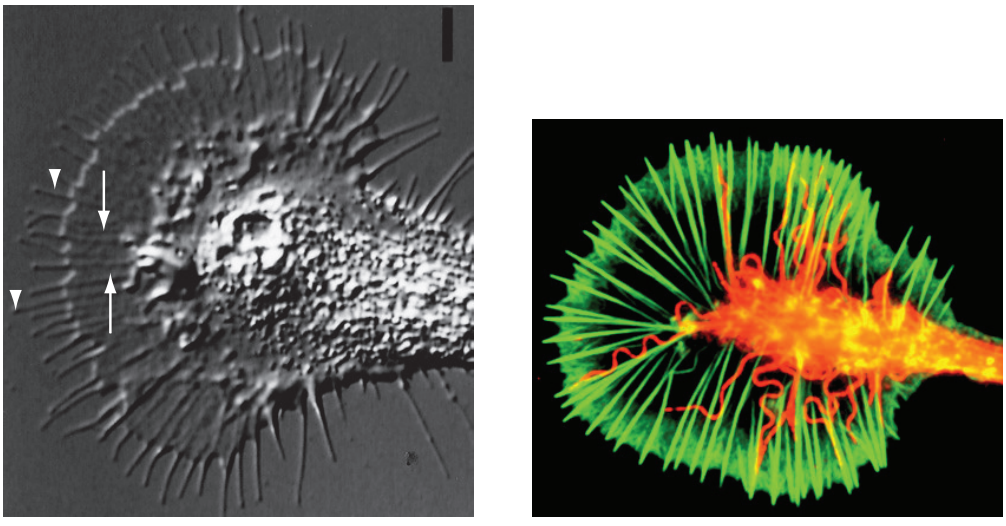
Axonal Outgrowth In 1907, Ross Harrison of Yale University performed one of the classic experiments in biology. Harrison removed a small piece of tissue from the developing nervous system of a frog embryo and placed the fragment into a tiny drop of lymphatic fluid. Harrison watched the tissue under a microscope over the next few days and found that the nerve cells not only remained healthy, but many of them sprouted processes that grew out into the surrounding medium. Not only was this the first time that cells had been kept alive in tissue culture, the experiment provided strong evidence that axons develop by a process of active outgrowth and elongation.

The tip of an elongating axon is very different from the remainder of the cell (see Figure 9.1*b*). Although the bulk of the axon shows little outward evidence of motile activity, the tip, or **growth cone**, resembles a highly motile, crawling fibroblast. Close examination of a living growth cone reveals several types of locomotor protrusions: a broad, flattened lamellipodium that creeps outward over the substratum; short, stiff *microspikes* (Figure 9.75*a*) that point outward to the edge of the lamellipodium; and highly elongated *filopodia* that extend and retract in a continuous display of motile activity. Fluorescence microscopy shows all of these structures in the peripheral domain of the growth cone to be filled with actin filaments (shown in green, Figure 9.75*b*). These actin filaments are presumed to be responsible for the motile activities



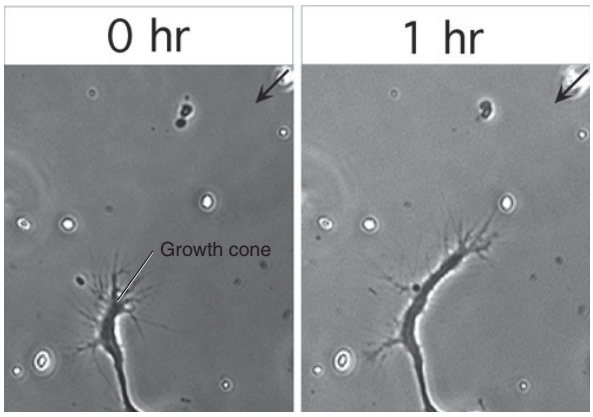
(a) (b) (c)

Figure 9.74 The roles of actin and myosin in the lamellipodial-based movement of fish keratocytes. (*a,b*) Fluorescence micrographs of a fish keratocyte moving over a culture dish by means of a broad, flattened lamellipodium. The arrow shows the direction of movement, which can occur at rates of 10 µm/min. The distribution of filamentous actin is revealed in part *a*, which shows the localization of fluorescently labeled phalloidin, which binds only to actin filaments. The distribution of myosin in the same cell is revealed in part *b*, which shows the localization of fluorescent antimyosin antibodies. It is evident that the body of the lamellipodium contains actin filaments but is virtually devoid of myosin. Myosin is concentrated, instead, in a band that lies just behind the lamellipodium, where it merges with the body of the cell. (*c*) A schematic drawing depicting the filamentous actin network of the lamellipodium and the actin–myosin interactions toward the rear of the lamellipodium. The actin network is shown in red, myosin molecules in blue. (By Alexander B. Verkhovsky, from Tatyana M. Svitkina et al., J. Cell Biol. 139:397, 1997, Fig. 1. Reproduced with permission of The Rockefeller University Press.)

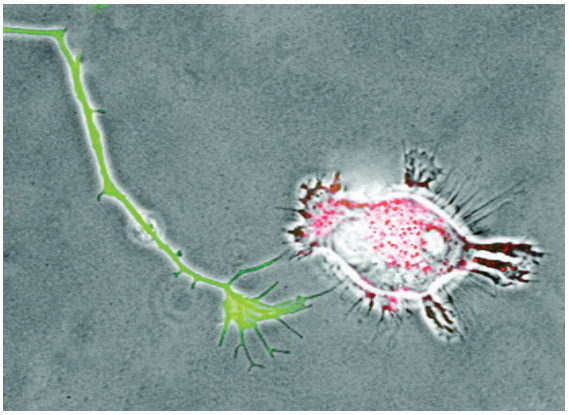


(a) (b)

Figure 9.75 The structure of a growth cone: the motile tip of a growing axon. (*a*) A video image of a live growth cone. The terminus is spread into a flattened lamellipodium that creeps forward over the substratum. Rodlike microspikes (arrows) can be seen within the transparent veil of the lamellipodium, and fine processes called filopodia (arrowheads) can be seen projecting ahead of the leading edge of the lamellipodium. Bar, 5 µm. (*b*) Fluorescence micrograph of the growth cone of a neuron showing the actin filaments (green) concentrated in the peripheral domain and the microtubules (orange) concentrated in the central domain. A number of microtubules can be seen to invade the peripheral domain, where they interact with actin-filament bundles. (A: from Paul Forscher and Stephen J. Smith, J. Cell Biol. 107:1508, 1988, Fig. 2; B: from Feng-Quan Zhou, Clare M. Waterman-Storer, and Christopher S. Cohan, J. Cell Biol. vol. 157, #5, cover, 2002. Both reproduced with permission of The Rockefeller University Press.)



(a)



(b)

Figure 9.76 **The directed movements of a growth cone.** (*a*) A video image of a live growth cone of a *Xenopus* neuron that has turned toward a diffusible protein (netrin-1) released from a pipette whose position is indicated by the arrow. (*b*) The growth cone (green) at the tip of a motor axon has made contact by means of its filopodia with a target cell that is expressing the neuronal guidance factor ephrin (red). (A: From Elke Stein and Marc Tessier-Lavigne, Science 291:1929, 2001. Reprinted with permission from AAAS; B: courtesy of Irina Dudanova.)

of the growth cone. Microtubules, on the other hand, fill the axon and the central domain of the growth cone, providing support for the thin, elongating axon. A number of individual microtubules are seen to penetrate into the actin-rich periphery (shown in orange, Figure 9.75*b*). These penetrating microtubules are highly dynamic and are thought to play an important role in steering the growth cone in the appropriate direction.

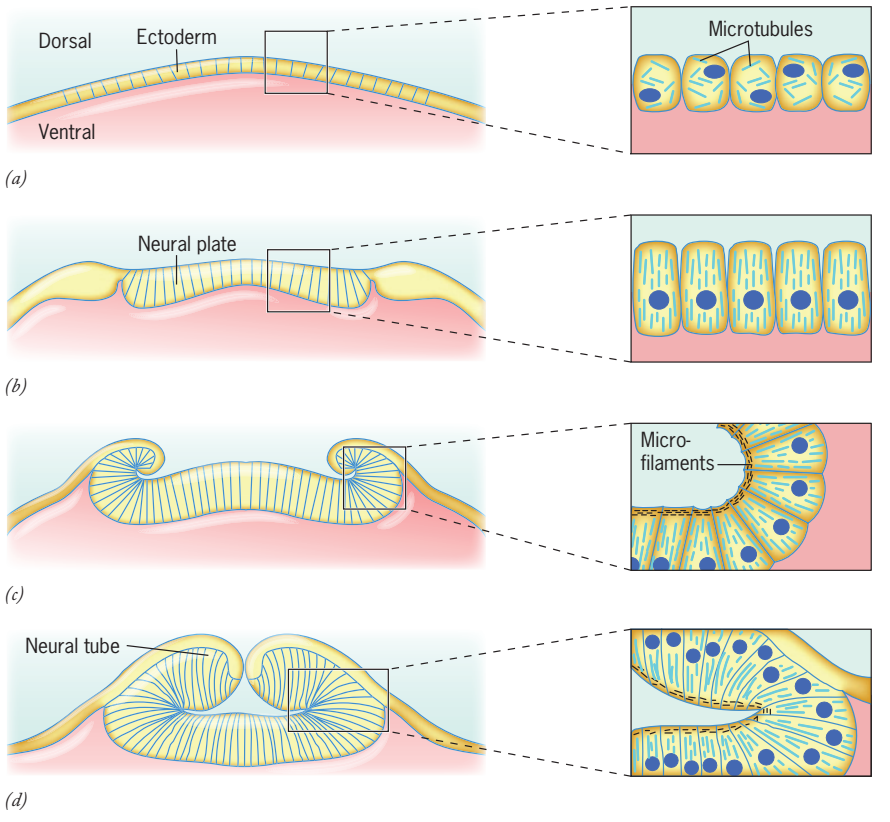
The growth cone is a highly motile region of the cell that explores its environment and elongates the axon. Within the embryo, the axons of developing neurons grow along defined paths, following certain topographical features of the substratum or responding to the presence of certain chemicals that diffuse into their path. The lamellipodia and filopodia from the growth cone respond to the presence of these physical and chemical stimuli, causing the pathfinding axons to turn toward attractive factors and away from repulsive factors. Figure 9.76*a* shows a cultured neuron whose advancing tip has made a direct turn toward a diffusible protein called netrin, which acts as an attractant for axons growing within the early embryo. Figure 9.76*b* shows a growth cone (green) making contact with a cell that is expressing another protein called ephrin (red) that also acts as a neuronal guidance factor. Unlike netrin, ephrin is a non-diffusible, integral protein of the plasma membrane that binds to an ephrin receptor on the surface of the growth cone. The growth cone is making contact with the ephrin-expressing cell by means of its long filopodia, which serve a sensory function. Ultimately, the correct wiring of the entire nervous system depends on the uncanny ability of embryonic growth cones to make the proper steering "decisions" that lead them to the target organ they must innervate.

Changes in Cell Shape during Embryonic Development Each part of the body has a characteristic shape and internal architecture that arises during embryonic development: the spinal cord is basically a hollow tube, the kidney consists of microscopic tubules, each lung is composed of microscopic air spaces, and so forth. Numerous cellular activities are necessary for the development of the characteristic morphology of an organ, including programmed changes in cell shape. Changes in cell shape are brought about largely by changes in the orientation of cytoskeletal elements within the cells. One of the best examples of this phenomenon is seen in the early stages of the development of the nervous system.

Toward the end of gastrulation in vertebrates, the outer (ectodermal) cells situated along the embryo's dorsal surface elongate and form a tall epithelial layer called the *neural plate* (Figure 9.77*a*,*b*). The cells of the neural plate elongate as microtubules become oriented with their long axes parallel to that of the cell (inset, Figure 9.77*b*). Following elongation, the cells of the neural epithelium become constricted at one end, causing them to become wedge shaped and the entire layer of cells to curve inward (Figure 9.77*c*). This latter change in cell shape is brought about by the contraction of a band of microfilaments that assemble in the cortical region of the cells just beneath the apical cell membrane (inset, Figure 9.77*c*). Eventually, the curvature of the neural tube causes the outer edges to contact one another, forming a cylindrical, hollow tube (Figure 9.77*d*,*e*) that will give rise to the animal's entire nervous system.

REVIEW

1. List the various types of actin-binding proteins and one function of each type.
2. Describe the steps taken by a mammalian cell crawling over a substratum.
3. Describe the role of actin filaments in the activities of the growth cone of a neuron.



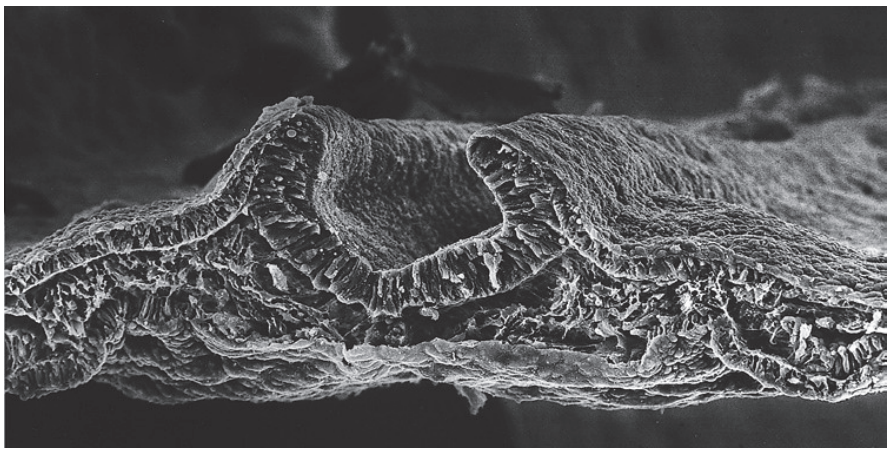

(e)

Figure 9.77 **Early stages in the development of the vertebrate nervous system.** (*a–d*) Schematic drawings of the changes in cell shape that cause a layer of flattened ectodermal cells at the mid-dorsal region of the embryo to roll into a neural tube. The initial change in height of the cells is thought to be driven by the orientation and elongation of microtubules, whereas the rolling of the plate into a tube is thought to be driven by contractile forces generated by actin filaments at the apical ends of the cells. (*e*) Scanning electron micrograph of the dorsal surface of a chick embryo as its neural plate is being folded to form a tube. (E: Courtesy of Kathryn W. Tosney.)

Synopsis

The cytoskeleton is composed of three distinct types of fibrous structures: microtubules, intermediate filaments, and microfilaments (actin filaments), which participate in a number of cellular activities. Collectively, the elements of the cytoskeleton function as a structural support that helps maintain the shape of the cell; as an internal framework responsible for positioning the various organelles within the cell interior; as part of the machinery required for the movement of materials and organelles within cells; and as force-generating elements responsible for the movement of cells from one place to another. (p. 324)

Microtubules are hollow, tubular structures 25 nm in diameter that are assembled from the protein tubulin and, in addition to the cytoskeleton, form part of the mitotic spindle, centrioles, and the core of cilia and flagella. Microtubules are polymers assembled from αβ-tubulin heterodimers that are arranged in rows, or protofilaments. Many of the properties of microtubules, including their stability and capabilities for interaction, are influenced by members of a group of microtubule-associated proteins (MAPs). Because of their stiffness, microtubules often act in a supportive capacity not unlike the way that steel girders provide support for a tall building. The structural role of