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Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.

Molecular Motors

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

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

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

In this section, we begin by describing the three groups of cytoskeletal motor proteins. We then describe how they work to transport membrane-enclosed organelles or to change the shape of structures built from cytoskeletal filaments. We end by describing their action in muscle contraction and in powering the whiplike motion of structures formed from microtubules.

Actin-based Motor Proteins Are Members of the Myosin Superfamily

The first motor protein identified was skeletal muscle **myosin**, which is responsible for generating the force for muscle contraction. This myosin, called *myosin II* (see below) is an elongated protein that is formed from two heavy chains and two copies of each of two light chains. Each of the heavy chains has a globular head domain at its N-terminus that contains the force-generating machinery, followed by a very long amino acid sequence that forms an extended coiled-coil that mediates heavy chain dimerization (Figure 16-51). The two light chains bind close to the N-terminal head domain, while the long coiled-coil tail bundles itself with the tails of other myosin molecules. These tail-tail interactions result in the formation of large bipolar “thick filaments” that have several hundred myosin heads, oriented in opposite directions at the two ends of the thick filament (Figure 16-52).

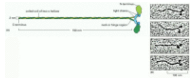


Figure 16-51

Myosin II. (A) A myosin II molecule is composed of two heavy chains (each about 2000 amino acids long *(green)* and four light chains *(blue)*. The light chains are of two distinct types, and one copy of each type is present on each myosin head. Dimerization (more...)

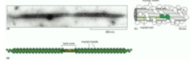


Figure 16-52

The myosin II bipolar thick filament. (A) Electron micrograph of a myosin II thick filament isolated from frog muscle. Note the central bare zone, which is free of head domains. (B) Schematic diagram, not drawn to scale. The myosin II molecules aggregate (more...)

Each myosin head binds and hydrolyses ATP, using the energy of ATP hydrolysis to walk toward the plus end of an actin filament. The opposing orientation of the heads in the thick filament makes the filament efficient at sliding pairs of oppositely oriented actin filaments past each other. In skeletal muscle, in which carefully arranged actin filaments are aligned in "thin filament" arrays surrounding the myosin thick filaments, the ATP-driven sliding of actin filaments results in muscle contraction (discussed later). Cardiac and smooth muscle contain myosins that are similarly arranged, although they are encoded by different genes.

When a muscle myosin is digested by chymotrypsin and papain, the head domain is released as an intact fragment (called S1). The S1 fragment alone can generate filament sliding *in vitro,* proving that the motor activity is contained completely within the head (Figure 16-53).

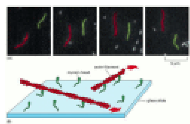


Figure 16-53

Direct evidence for the motor activity of the myosin head. In this experiment, purified S1 myosin heads were attached to a glass slide, and then actin filaments labeled with fluorescent phalloidin were added and allowed to bind to the myosin heads. (A) (more...)

It was initially thought that myosin was present only in muscle, but in the 1970's, researchers found that a similar two-headed myosin protein was also present in nonmuscle cells, including protozoan cells. At about the same time, other researchers found a myosin in the freshwater amoeba *Acanthamoeba castellanii* that was unconventional in having a motor domain similar to the head of muscle myosin but a completely different tail. This molecule seemed to function as a monomer and was named *myosin I* (for one-headed); the conventional myosin was renamed *myosin II* (for two-headed).

Subsequently, many other myosin types were discovered. The heavy chains generally start with a recognizable myosin motor domain at the N-terminus, and then diverge widely with a variety of C-terminal tail domains (Figure 16-54). The new types of myosins include a number of one-headed and two-headed varieties that are approximately equally related to myosin I and myosin II, and the nomenclature now reflects their approximate order of discovery (myosin III through at least myosin XVIII). The myosin tails (and the tails of motor proteins generally) have apparently diversified during evolution to permit the proteins to dimerize with other subunits and to interact with different cargoes.

Figure 16-54

Myosin superfamily tree. (A) A family tree for a few of the many known members of the myosin superfamily. The length of the lines separating individual family members indicates the amount of difference in the amino acid sequence of the motor domain. Groups (more...)

Some myosins (such as VIII and XI) have been found only in plants, and some have been found only in vertebrates (IX). Most, however, are found in all eucaryotes, suggesting that myosins arose early in eucaryotic evolution. The yeast *Saccharomyces cerevisiae* contains five myosins: two myosin Is, one myosin II, and two myosin Vs. One can speculate that these three types of myosins are necessary for a eucaryotic cell to survive and that other myosins perform more specialized functions in multicellular organisms. The nematode *C. elegans*, for example, has at least 15 myosin genes, representing at least seven structural classes; the human genome includes about 40 myosin genes.

All of the myosins except one move toward the plus end of an actin filament, although they do so at different speeds. The exception is myosin VI, which moves toward the minus end.

The exact functions for most of the myosins remain to be determined. Myosin II is always associated with contractile activity in muscle and nonmuscle cells. It is also generally required for cytokinesis, the pinching apart of a dividing cell into two daughters (discussed in Chapter 18), as well as for the forward translocation of the body of a cell during cell migration. The myosin I proteins contain a second actin-binding site or a membrane-binding site in their tails, and they are generally involved in intracellular organization and the protrusion of actin-rich structures at the cell surface. Myosin V is involved in vesicle and organelle transport. Myosin VII is found in the inner ear in vertebrates, and certain mutations in the gene coding for myosin VII cause deafness in mice and humans.

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

Kinesin is a motor protein that moves along microtubules. It was first identified in the giant axon of the squid, where it carries membrane-enclosed organelles away from the neuronal cell body toward the axon terminal by walking toward the plus end of microtubules. Kinesin is similar structurally to myosin II in having two heavy chains and two light chains per active motor, two globular head motor domains, and an elongated coiled-coil responsible for heavy chain dimerization. Like myosin, kinesin is a member of a large protein superfamily, for which the motor domain is the only common element (Figure 16-55). The yeast *Saccharomyces cerevisiae* has six distinct kinesins. The nematode *C. elegans* has 16 kinesins, and humans have about 40.

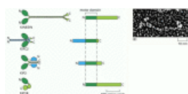


Figure 16-55

Kinesin and kinesin-related proteins. (A) Structures of four kinesin superfamily members. As in the myosin superfamily, only the motor domains are conserved. Conventional kinesin has the motor domain at the N-terminus of the heavy chain. The middle domain (more...)

There are at least ten families of **kinesin-related proteins**, or **KRPs**, in the kinesin superfamily. Most of them have the motor domain at the N-terminus of the heavy chain and walk toward the plus end of the microtubule. A particularly interesting family has the motor domain at the C-

terminus and walks in the opposite direction, toward the minus end of the microtubule. Some KRP heavy chains lack a coiled-coil sequence and seem to function as monomers, analogous to myosin I. Some others are homodimers, and yet others are heterodimers. At least one KRP (BimC) can self-associate through the tail domain, forming a bipolar motor that slides oppositely oriented microtubules past one another, much as a myosin II thick filament does for actin filaments. Most kinesins carry a binding site in the tail for either a membrane-enclosed organelle or another microtubule. Many of the kinesin superfamily members have specific roles in mitotic and meiotic spindle formation and chromosome separation during cell division.

The dyneins are a family of minus-end-directed microtubule motors, but they are unrelated to the kinesin superfamily. They are composed of two or three heavy chains (that include the motor domain) and a large and variable number of associated light chains. The dynein family has two major branches (Figure 16-56). The most ancient branch contains the *cytoplasmic dyneins,* which are typically heavy-chain homodimers, with two large motor domains as heads. Cytoplasmic dyneins are probably found in all eucaryotic cells, and they are important for vesicle trafficking, as well as for localization of the Golgi apparatus near the center of the cell. *Axonemal dyneins,* the other large branch, include heterodimers and heterotrimers, with two or three motor-domain heads, respectively. They are highly specialized for the rapid and efficient sliding movements of microtubules that drive the beating of cilia and flagella (discussed later). A third, minor, branch shares greater sequence similarity with cytoplasmic than with axonemal dyneins but seems to be involved in the beating of cilia.

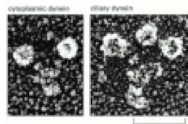


Figure 16-56

Dyneins. Freeze-etch electron micrographs of a molecule of cytoplasmic dynein and a molecule of ciliary (axonemal) dynein. Like myosin II and kinesin, cytoplasmic dynein is a two-headed molecule. The ciliary dynein shown has three heads. Note that the (more...)

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

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

The motor domain of myosins is substantially larger than that of kinesins, about 850 amino acids compared with about 350. The two classes of motor proteins track along different filaments and have different kinetic properties, and they have no identifiable amino acid sequence similarities. However, determination of the three-dimensional structure of the motor domains of myosin and kinesin has revealed that these two motor domains are built around nearly identical cores (Figure 16-57). The central force-generating element that the two types of motor proteins have in common includes the site of ATP binding and the machinery necessary to translate ATP hydrolysis into an allosteric conformational change. The differences in domain size and in the choice of track can be attributed to large loops extending outward from this central core. These loops include the actin-binding and microtubule-binding sites, respectively.

Figure 16-57

X-ray crystal structures of myosin and kinesin heads. The central nucleotide-binding domains of myosin and kinesin

(shaded in *yellow)* are structurally very similar. The very different sizes and functions of the two motors are due to major differences (more...)

An important clue to how the central core is involved in force generation has come from the observation that the motor core also bears some structural resemblance to the nucleotide binding site of the small GTPases of the Ras superfamily. As discussed in Chapter 3 (see Figure 3-74), these proteins exhibit distinct conformations in their GTP-bound (active) and GDP-bound (inactive) forms: mobile "switch" loops in the nucleotide-binding site are in close contact with the γ-phosphate in the GTP-bound state, but these loops swing out when the hydrolyzed γ-phosphate is released. Although the details of the movement are different for the two motor proteins, and ATP rather than GTP is hydrolyzed, the relatively small structural change in the active site—the presence or absence of a terminal phosphate—is similarly amplified to cause a rotation of a different part of the protein. In kinesin and myosin, a switch loop interacts extensively with those regions of the protein involved in microtubule and actin binding, respectively, allowing the structural transitions caused by the ATP hydrolysis cycle to be relayed to the polymer-binding interface. The relay of structural changes between the polymer-binding site and the nucleotide hydrolysis site seems to work in both directions, since the ATPase activity of motor proteins is strongly activated by binding to their filament tracks.

Motor Proteins Generate Force by Coupling ATP Hydrolysis to Conformational Changes

Although the cytoskeletal motor proteins and GTP-binding proteins both use structural changes in their nucleoside-triphosphate-binding sites to produce cyclic interactions with a partner protein, the motor proteins have a further requirement: each cycle of binding and release must propel them forward in a single direction along a filament to a new binding site on the filament. For such unidirectional motion, a motor protein must use the energy derived from ATP binding and hydrolysis to force a large movement in part of the protein molecule. For myosin, each step of the movement along actin is generated by the swinging of an 8.5-nm-long α helix, or *lever arm* (see Figure 16-57), which is structurally stabilized by the binding of light chains. At the base of this lever arm next to the head, there is a piston-like helix that connects movements at the ATP-binding cleft in the head to small rotations of the so-called converter domain. A small change at this point can swing the helix like a long lever, causing the far end of the helix to move by about 5.0 nm. These changes in the conformation of the myosin are coupled to changes in its binding affinity for actin, allowing the myosin head to release its grip on the actin filament at one point and snatch hold of it again at another. The full mechanochemical cycle of nucleotide binding, nucleotide hydrolysis, and phosphate release (which causes the "power stroke") produces a single step of movement (Figure 16-58). In the myosin VI subfamily of myosins, which move backward (toward the minus end of the actin filament), the converter domain probably lies in a different orientation, so that the same piston-like movement of the small helix causes the lever arm to rotate in the opposite direction.

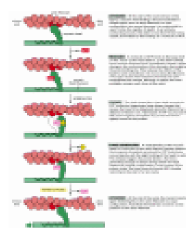


Figure 16-58

The cycle of structural changes used by myosin to walk along an actin filament. (Based on I. Rayment et al., *Science* 261:50–58, 1993. © AAAS.)

In kinesin, instead of the rocking of a lever arm, the small movements of switch loops at the nucleotide-binding site regulate the docking and undocking of the motor head domain to a long linker region that connects this motor head at one end to the coiled-coil dimerization domain at the other end. When the front (leading) kinesin head is bound to a microtubule before the power stroke, its linker region is relatively unstructured. On the binding of ATP to this bound head, its linker region docks along the side of the head, which throws the second head forward to a position where it will be able to bind a new attachment site on the protofilament, 8 nm closer to the microtubule plus end than the binding site for the first head. The nucleotide hydrolysis cycles in the two heads are closely coordinated, so that this cycle of linker docking and undocking can allow the two-headed motor to move in a hand-over-hand (or head-over-head) stepwise manner (Figure 16-59A).

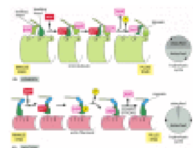


Figure 16-59

Comparison of the mechanochemical cycles of kinesin and myosin II. The shading in the two circles representing the hydrolysis cycle indicates the proportion of the cycle spent in attached and detached states for each motor protein. (A) Summary of the (more...)

The coiled-coil domain seems both to coordinate the mechanochemical cycles of the two heads (motor domains) of the kinesin dimer and to determine its directionality of movement. Recall that whereas most members of the kinesin superfamily, with their motor domains at the N-terminus, move toward the plus end of the microtubule, a few superfamily members have their motor domains at the C-terminus and move toward the minus end. Since the motor domains of these two types of kinesins are essentially identical, how can they move in opposite directions? The answer seems to lie in the way in which the heads are connected. In high-resolution images of forward-walking and backward-walking members of the kinesin superfamily bound to microtubules, the heads that are attached to the microtubule are essentially indistinguishable, but the second, unattached heads are oriented very differently. This difference in tilt apparently biases the next binding site for the second head, and thereby determines the directionality of motor movement (Figure 16-60).

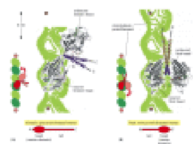


Figure 16-60

Orientation of forward- and backward-walking kinesin superfamily proteins bound to microtubules. These images were generated by fitting the structures of the free motor-protein dimers (determined by x-ray crystallography) onto a lower resolution image (more...)

Although both myosin and kinesin undergo analogous mechanochemical cycles, the exact nature of the coupling between the mechanical and chemical cycles is different in the two cases (see Figure 16-60). For example, myosin without any nucleotide is tightly bound to its actin track, in a so-called “rigor” state, and it is released from this track by the association of ATP. In contrast, kinesin forms a rigor-like tight association with a microtubule when ATP is bound to the kinesin, and it is hydrolysis of ATP that promotes release of the motor from its track.

Thus, cytoskeletal motor proteins work in a manner highly analogous to GTP-binding proteins, except that in motor proteins the small protein conformational changes (a few tenths of a nanometer) associated with nucleotide hydrolysis are amplified by special protein domains—the

lever arm in the case of myosin and the linker in the case of kinesin—to generate large-scale (several nanometers) conformational changes that move the motor proteins stepwise along their filament tracks. The analogy between the GTPases and the cytoskeletal motor proteins has recently been extended by the observation that one of the GTP-binding proteins—the bacterial elongation factor G—translates the chemical energy of GTP hydrolysis into directional movement of the mRNA molecule on the ribosome.

Motor Protein Kinetics Are Adapted to Cell Functions

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

There are two reasons for the high degree of processivity of kinesin movement. The first is that the mechanochemical cycles of the two motor heads in a kinesin dimer are coordinated with each other, so that one kinesin head does not let go until the other is poised to bind. This coordination allows the motor protein to operate in a hand-over-hand fashion, never allowing the organelle cargo to diffuse away from the microtubule track. There is no apparent coordination between the myosin heads in a myosin II dimer. The second reason for the high processivity of kinesin movement is that kinesin spends a relatively large fraction of its ATPase cycle tightly bound to the microtubule. For both kinesin and myosin, the conformational change that produces the force-generating working stroke must occur while the motor protein is tightly bound to its polymer, and the recovery stroke in preparation for the next step must occur while the motor is unbound. But as we have seen in Figure 16-59, myosin spends only about 5% of its ATPase cycle in the tightly bound state and is unbound the rest of the time.

What myosin loses in processivity it gains in speed; in an array in which many motor heads are interacting with the same actin filament, a set of linked myosins can move their filament a total distance equivalent to 20 steps during a single cycle time, while kinesins can move only two. Thus, myosins can typically drive filament sliding much more rapidly than kinesins, even though they hydrolyze ATP at comparable rates and take molecular steps of comparable length.

Within each motor protein class, movement speeds vary widely, from about 0.2 to 60 μm/sec for myosins, and from about 0.02 to 2 μm/sec for kinesins. These differences arise from a fine-tuning of the mechanochemical cycle. The number of steps that an individual motor molecule can take in a given time, and thereby the velocity, can be increased by either increasing the motor protein's intrinsic ATPase rate or decreasing the proportion of cycle time spent bound to the filament track. Moreover, the size of each step can be changed by either changing the length of the lever arm (for example, the lever arm of myosin V is about three times longer than the lever arm of myosin II) or the angle through which the helix swings (Figure 16-61). Each of these parameters varies slightly among different members of the myosin and kinesin families, corresponding to slightly different protein sequences and structures. It is assumed that the behavior of each motor protein, whose function is determined by the identity of the cargo

attached through its tail-domain, has been fine-tuned during evolution for speed and processivity according to the specific needs of the cell.

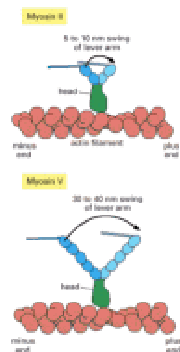


Figure 16-61

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

Motor Proteins Mediate the Intracellular Transport of Membrane-enclosed Organelles

A major function of cytoskeletal motors in interphase cells is the transport and positioning of membrane-enclosed organelles. Kinesin was originally identified as the protein responsible for fast axonal transport, the rapid movement of mitochondria, secretory vesicle precursors, and various synapse components down the microtubule highways of the axon to the distant nerve terminals. Although organelles in most cells need not cover such long distances, their polarized transport is equally necessary. A typical microtubule array in an interphase cell is oriented with the minus ends near the center of the cell at the centrosome, and the plus ends extending to the cell periphery. Thus, centripetal movements of organelles toward the cell center require the action of minus-end-directed motor proteins such as cytoplasmic dynein, whereas centrifugal movements toward the periphery require plus-end-directed motors such as kinesins.

The role of microtubules and microtubule motors in the behavior of intracellular membranes is best exemplified by the part they play in organizing the endoplasmic reticulum (ER) and the Golgi apparatus. The network of ER membrane tubules aligns with microtubules and extends almost to the edge of the cell, whereas the Golgi apparatus is located near the centrosome. When cells are treated with a drug that depolymerizes microtubules, such as colchicine or nocodazole, the ER collapses to the center of the cell, while the Golgi apparatus fragments and disperses throughout the cytoplasm (Figure 16-62). *In vitro*, kinesins can tether ER-derived membranes to preformed microtubule tracks, and walk toward the microtubule plus ends, dragging the ER membranes out into tubular protrusions and forming a membranous web very much like the ER in cells. Likewise, the outward movement of ER tubules toward the cell periphery is associated with microtubule growth in living cells. Conversely, dyneins are required for positioning the Golgi apparatus near the cell center, moving Golgi vesicles along microtubule tracks toward minus ends at the centrosome.

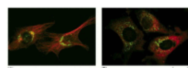


Figure 16-62

Effect of depolymerizing microtubules on the Golgi apparatus. (A) In this endothelial cell, the microtubules are labeled in *red,* and the Golgi apparatus is labeled in *green* (using an antibody against a Golgi protein). As long as the system of microtubules (more...)

The different tails and their associated light chains on specific motor proteins allow the motors to attach to their appropriate organelle cargo. For example, there is evidence for membrane-

associated motor receptors, sorted to specific membrane-enclosed compartments, that interact directly or indirectly with the tails of the appropriate kinesin family members. One of these receptors seems to be the amyloid precursor protein, APP, which binds directly to a light chain on the tail of kinesin-I and is proposed to be a transmembrane motor protein receptor molecule in nerve-cell axons. It is the abnormal processing of this protein that gives rise to Alzheimer's disease, as discussed in Chapter 15.

For dynein, attachment to membranes is known to be mediated by a large macromolecular assembly. Cytoplasmic dynein is itself a huge protein complex, and it requires association with a second large protein complex called *dynactin* to translocate organelles effectively. The dynactin complex includes a short actinlike filament that is made of the actin-related protein Arp1 (distinct from Arp2 and Arp3, the components of the ARP complex involved in the nucleation of conventional actin filaments). Membranes of the Golgi apparatus are coated with the proteins ankyrin and spectrin, which have been proposed to associate with the Arp1 filament in the dynactin complex to form a planar cytoskeletal array reminiscent of the erythrocyte membrane cytoskeleton (see Figure 10-31). The spectrin array probably gives structural stability to the Golgi membrane, and—via the Arp1 filament—it may mediate the regulatable attachment of dynein to the organelle (Figure 16-63).

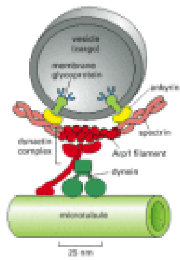


Figure 16-63

A model for the attachment of dynein to a membrane-enclosed organelle. Dynein requires the presence of a large number of accessory proteins to associate with membrane-enclosed organelles. Dynactin is a large complex *(red)* that includes components that (more...)

Motor proteins also have a significant role in organelle transport along actin filaments. The first myosin shown to mediate organelle motility was myosin V, a two-headed myosin with a large step size (see Figure 16-61). In mice, mutations in the myosin V gene result in a “dilute” phenotype, in which fur color looks faded. In mice (and humans), membrane-enclosed pigment granules, called *melanosomes,* are synthesized in cells called *melanocytes* beneath the skin surface. These melanosomes move out to the ends of dendritic processes in the melanocytes, from where they are delivered to the overlying keratinocytes that form the skin and fur. Myosin V is associated with the surface of melanosomes, and it is able to mediate their actin-based movement in a test tube (Figure 16-64). In dilute mutant mice, the melanosomes are not delivered to the keratinocytes efficiently, and pigmentation is defective. Other myosins, including myosin I, are associated with endosomes and a variety of other organelles.

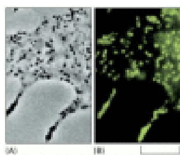


Figure 16-64

Myosin V on melanosomes. (A) Phase-contrast image of a portion of a melanocyte isolated from a mouse. The black spots are melanosomes, which are membrane-enclosed organelles filled with the skin pigment melanin. (B) The same cell labeled with a fluorescent (more...)

Motor Protein Function Can Be Regulated

The cell can regulate the activity of motor proteins, allowing it to change either the positioning of its membrane-enclosed organelles or its whole-cell movements. One of the most dramatic examples is provided by fish melanocytes. These giant cells, which are responsible for rapid

changes in skin coloration in several species of fish, contain large pigment granules that can alter their location in response to neuronal or hormonal stimulation (Figure 16-65). These pigment granules aggregate or disperse by moving along an extensive network of microtubules. The minus ends of these microtubules are nucleated by the centrosome and are located in the center of the cell, while the plus ends are distributed around the cell periphery. The tracking of individual pigment granules reveals that the inward movement is rapid and smooth, while the outward movement is jerky, with frequent backward steps (Figure 16-66). Both dynein and kinesin are associated with the pigment granules. The jerky outward movements apparently result from a tug-of-war between the two motor proteins, with the stronger kinesin winning out overall. When the kinesin light chains become phosphorylated after a hormonal stimulation that signals skin color change, kinesin is inactivated, leaving dynein free to drag the pigment granules rapidly toward the cell center, changing the fish's color. In a similar way, the movement of other membrane organelles coated with particular motor proteins is controlled by a complex balance of competing signals that regulate both motor protein attachment and activity.

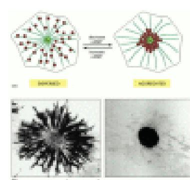


Figure 16-65

Regulated melanosome movements in fish pigment cells. These giant cells, which are responsible for changes in skin coloration in several species of fish, contain large pigment granules, or melanosomes *(brown)*. The melanosomes can change their location (more...)

Figure 16-66

Bidirectional movement of a melanosome on a microtubule. An isolated melanosome *(yellow)* moves along a microtubule on a glass slide, from the plus end toward the minus end. Halfway through the video sequence, it abruptly switches direction and moves from (more...)

Myosin activity can also be regulated by phosphorylation. In nonmuscle cells, myosin II can be phosphorylated on a variety of sites on both heavy and light chains, affecting both motor activity and thick filament assembly. The myosin II can exist in two different conformational states in such cells, an extended state that is capable of forming bipolar filaments, and a bent state in which the tail domain apparently interacts with the motor head. Phosphorylation of the regulatory light chain by the calcium-dependent *myosin light-chain kinase (MLCK)* causes the myosin II to preferentially assume the extended state, which promotes its assembly into a bipolar filament and leads to cell contraction (Figure 16-67). Myosin light-chain phosphorylation is an indirect target of activated Rho, the small GTPase discussed previously whose activation causes a reorganization of the actin cytoskeleton into contractile stress fibers. The MLCK is also activated during mitosis, causing myosin II to assemble into the contractile ring that is responsible for dividing the mitotic cell into two. Regulation of other members of the myosin superfamily is not as well understood, but control of these myosins is also likely to involve site-specific phosphorylations.

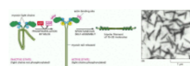


Figure 16-67

Light-chain phosphorylation and the regulation of the assembly of myosin II into thick filaments. (A) The controlled phosphorylation by the enzyme myosin light-chain

kinase (MLCK) of one of the two light chains (the so-called regulatory light chain, shown (more...)

Muscle Contraction Depends on the Sliding of Myosin II and Actin Filaments

Muscle contraction is the most familiar and the best understood form of movement in animals. In vertebrates, running, walking, swimming, and flying all depend on the rapid contraction of skeletal muscle on its scaffolding of bone, while involuntary movements such as heart pumping and gut peristalsis depend on the contraction of cardiac muscle and smooth muscle, respectively. All these forms of muscle contraction depend on the ATP-driven sliding of highly organized arrays of actin filaments against arrays of myosin II filaments.

Muscle was a relatively late evolutionary development, and muscle cells are highly specialized for rapid and efficient contraction. The long thin muscle fibers of skeletal muscle are actually huge single cells that form during development by the fusion of many separate cells, as discussed in Chapter 22. The many nuclei of the contributing cells are retained in this large cell and lie just beneath the plasma membrane, but the bulk of the cytoplasm inside is made up of myofibrils, which is the name given to the basic contractile elements of the muscle cell (Figure 16-68). A myofibril is a cylindrical structure 1–2 μm in diameter that is often as long as the muscle cell itself. It consists of a long repeated chain of tiny contractile units—called *sarcomeres*, each about 2.2 μm long, which give the vertebrate myofibril its striated appearance (Figure 16-69).

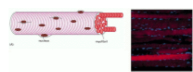


Figure 16-68

Skeletal muscle cells (also called muscle fibers). (A) These huge multinucleated cells form by the fusion of many muscle cell precursors, called myoblasts. In an adult human, a muscle cell is typically 50 μm in diameter and can be up to several (more...)

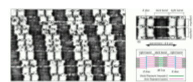
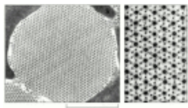


Figure 16-69

Skeletal muscle myofibrils. (A) Low-magnification electron micrograph of a longitudinal section through a skeletal muscle cell of a rabbit, showing the regular pattern of cross-striations. The cell contains many myofibrils aligned in parallel (see Figure (more...)

Each sarcomere is formed from a miniature, precisely ordered array of parallel and partly overlapping thin and thick filaments. The *thin filaments* are composed of actin and associated proteins, and they are attached at their plus ends to a *Z disc* at each end of the sarcomere. The capped minus ends of the actin filaments extend in toward the middle of the sarcomere, where they overlap with *thick filaments*, the bipolar assemblies formed from specific muscle isoforms of myosin II (see Figure 16-52). When this region of overlap is examined in cross section by electron microscopy, the myosin filaments are seen to be arranged in a regular hexagonal lattice, with the actin filaments evenly spaced between them (Figure 16-70). Cardiac muscle and smooth muscle also contain sarcomeres, although the organization is not as regular as that in skeletal muscle.

Figure 16-70



Electron micrographs of an insect flight muscle viewed in cros
myosin and actin filaments are packed together with almost cr
regularity. Unlike their vertebrate counterparts, these myosin f
hollow center, as seen (more...)

Sarcomere shortening is caused by the myosin filaments sliding past the actin thin filaments, with no change in the length of either type of filament (Figure 16-71). Bipolar thick filaments walk toward the plus ends of two sets of thin filaments of opposite orientations, driven by dozens of independent myosin heads that are positioned to interact with each thin filament. There is no coordination among the movements of the myosin heads, so it is critical that they operate with a low processivity, remaining tightly bound to the actin filament for only a small fraction of each ATPase cycle so that they do not hold one another back. Each myosin thick filament has about 300 heads (294 in frog muscle), and each head cycles about five times per second in the course of a rapid contraction—sliding the myosin and actin filaments past one another at rates of up to 15 μm/sec and enabling the sarcomere to shorten by 10% of its length in less than 1/50th of a second. The rapid synchronized shortening of the thousands of sarcomeres lying end-to-end in each myofibril gives skeletal muscle the ability to contract rapidly enough for running and flying, and even for playing the piano.

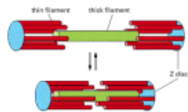


Figure 16-71

The sliding-filament model of muscle contraction. The actin *(red)* and myosin *(green)* filaments in a sarcomere slide past one another without shortening.

Accessory proteins govern the remarkable uniformity in filament organization, length, and spacing in the sarcomere (Figure 16-72). As mentioned previously, the actin filament plus ends are anchored in the Z disc, which is built from CapZ and α-actinin; the Z disc caps the filaments (preventing depolymerization), while holding them together in a regularly spaced bundle. The precise length of each filament is determined by a template protein of enormous size, called *nebulin*, which consists almost entirely of a repeating 35-amino-acid actin-binding motif. Nebulin stretches from the Z disc to the minus end of each thin filament and acts as a "molecular ruler" to dictate the length of the filament. The minus ends of the thin filaments are capped and stabilized by tropomodulin. Thus, the actin filaments in sarcomeres are remarkably stable, unlike the dynamic actin filaments characteristic of most other cell types.

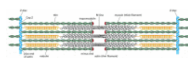


Figure 16-72

Organization of accessory proteins in a sarcomere. Each giant titin molecule extends from the Z disc to the M line—a distance of over 1 μm. Part of each titin molecule is closely associated with a myosin thick filament (which switches (more...)

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

that it too serves as a moleculer ruler, determining in this case the overall length of each sarcomere (see Figure 3-34).

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

The force-generating molecular interaction between myosin thick filaments and actin thin filaments takes place only when a signal passes to the skeletal muscle from its motor nerve. The signal from the nerve triggers an action potential in the muscle cell plasma membrane (discussed in Chapter 11), and this electrical excitation spreads rapidly into a series of membraneous folds, the transverse tubules, or *T tubules*, that extend inward from the plasma membrane around each myofibril. The signal is then relayed across a small gap to the *sarcoplasmic reticulum*, an adjacent web-like sheath of modified endoplasmic reticulum that surrounds each myofibril like a net stocking (Figure 16-73A, B).

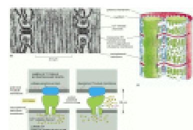


Figure 16-73

T tubules and the sarcoplasmic reticulum. (A) Drawing of the two membrane systems that relay the signal to contract from the muscle cell plasma membrane to all of the myofibrils in the cell. (B) Electron micrograph showing two T tubules. Note the position (more...)

When voltage-sensitive proteins in the T-tubule membrane are activated by the incoming action potential, they trigger the opening of Ca^{2+}-release channels in the sarcoplasmic reticulum (Figure 16-73C). Ca^{2+} flooding into the cytosol then initiates the contraction of each myofibril. Because the signal from the muscle-cell plasma membrane is passed within milliseconds (via the T tubules and sarcoplasmic reticulum) to every sarcomere in the cell, all of the myofibrils in the cell contract at the same time. The increase in Ca^{2+} concentration is transient because the Ca^{2+} is rapidly pumped back into the sarcoplasmic reticulum by an abundant, ATP-dependent Ca^{2+}-pump (also called a Ca^{2+}-ATPase), in its membrane (see Figure 3-77). Typically, the cytoplasmic Ca^{2+} concentration is restored to resting levels within 30 msec, allowing the myofibrils to relax. Thus, muscle contraction depends on two processes that consume enormous amounts of ATP: filament sliding, driven by the ATPase of the myosin motor domain, and Ca^{2+} pumping, driven by the Ca^{2+}-pump.

The Ca^{2+} dependence of vertebrate skeletal muscle contraction, and hence its dependence on motor commands transmitted via nerves, is due entirely to a set of specialized accessory proteins that are closely associated with the actin thin filaments. One of these accessory proteins is a muscle form of *tropomyosin*, an elongated molecule that binds along the groove of the actin helix. The other is *troponin*, a complex of three polypeptides, troponins T, I, and C (named for their tropomyosin-binding, inhibitory, and Ca^{2+}-binding activities, respectively). Troponin I binds to actin as well as to troponin T. In a resting muscle, the troponin I-T complex pulls the tropomyosin out of its normal binding groove into a position along the actin filament that interferes with the binding of myosin heads, thereby preventing any force-generating interaction. When the level of Ca^{2+} is raised, troponin C—which binds up to four molecules of Ca^{2+}—causes troponin I to release its hold on actin. This allows the tropomyosin molecules to slip back into their normal position so that the myosin heads can walk along the actin filaments (Figure 16-74). Troponin C is closely related to the ubiquitous Ca^{2+}-binding protein calmodulin (see Figure 15-40); it can be thought of as a specialized form of calmodulin that has acquired binding sites

for troponin I and troponin T, thereby ensuring that the myofibril responds extremely rapidly to an increase in Ca^{2+} concentration.

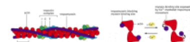


Figure 16-74

The control of skeletal muscle contraction by troponin. (A) A skeletal muscle cell thin filament, showing the positions of tropomyosin and troponin along the actin filament. Each tropomyosin molecule has seven evenly spaced regions with similar amino (more...)

Heart Muscle Is a Precisely Engineered Machine

The heart is the most heavily worked muscle in the body, contracting about 3 billion (3×10^9) times during the course of a human lifetime. This number is about the same as the average number of revolutions in the lifetime of an automobile's internal combustion engine. Several specific isoforms of cardiac muscle myosin and cardiac muscle actin are expressed in heart cells. Even subtle changes in contractile proteins expressed in the heart—changes that would not cause any noticeable consequences in other tissues—can cause serious heart disease (Figure 16-75).

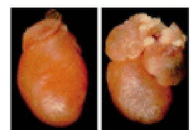


Figure 16-75

Effect on the heart of a subtle mutation in cardiac myosin. *Left*, normal heart from a 6-day old mouse pup. *Right*, heart from a pup with a point mutation in both copies of its cardiac myosin gene, changing Arg 403 to Gln. Both atria are greatly enlarged (more...)

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

Cilia and Flagella Are Motile Structures Built from Microtubules and Dyneins

Just as myofibrils are highly specialized and efficient motility machines built from actin and myosin filaments, cilia and flagella are highly specialized and efficient motility structures built from microtubules and dynein. Both cilia and flagella are hair-like cellular appendages that have a bundle of microtubules at their core. **Flagella** are found on sperm and many protozoa. By their undulating motion, they enable the cells to which they are attached to swim through liquid media (Figure 16-76A). **Cilia** tend to be shorter than flagella and are organized in a similar fashion, but they beat with a whip-like motion that resembles the breast stroke in swimming (Figure 16-76B). The cycles of adjacent cilia are almost but not quite in synchrony, creating the wave-like patterns that can be seen in fields of beating cilia under the microscope. Ciliary beating can either propel

single cells through a fluid (as in the swimming of the protozoan *Paramecium*) or can move fluid over the surface of a group of cells in a tissue. In the human body, huge numbers of cilia ($10^9/cm^2$ or more) line our respiratory tract, sweeping layers of mucus, trapped particles of dust, and bacteria up to the mouth where they are swallowed and ultimately eliminated. Likewise, cilia along the oviduct help to sweep eggs toward the uterus.

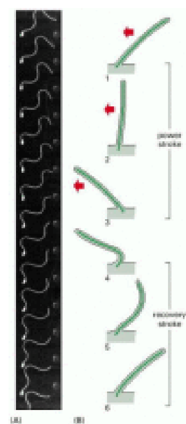


Figure 16-76

The contrasting motions of flagella and cilia. (A) The wave-like motion of the flagellum of a sperm cell from a tunicate. The cell was photographed with stroboscopic illumination at 400 flashes per second. Note that waves of constant amplitude move continuously (more...)

The movement of a cilium or a flagellum is produced by the bending of its core, which is called the axoneme. The axoneme is composed of microtubules and their associated proteins, arranged in a distinctive and regular pattern. Nine special doublet microtubules (comprising one complete and one partial microtubule fused together so that they share a common tubule wall) are arranged in a ring around a pair of single microtubules (Figure 16-77). This characteristic arrangement is found in almost all forms of eucaryotic flagella and cilia from protozoans to humans. The microtubules extend continuously for the length of the axoneme, which can be 10–200 µm. At regular positions along the length of the microtubules, accessory proteins cross-link the mircotubules together. Molecules of *ciliary dynein* form bridges between the neighboring doublet microtubules around the circumference of the axoneme (Figure 16-78). When the motor domain of this dynein is activated, the dynein molecules attached to one microtubule doublet attempt to walk along the adjacent microtubule doublet, tending to force the adjacent doublets to slide relative to one another, much as actin thin filaments slide during muscle contraction. However, the presence of other links between the microtubule doublets prevents this sliding, and the dynein force is instead converted into a bending motion (Figure 16-79).

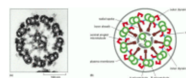


Figure 16-77

The arrangement of microtubules in a flagellum or cilium. (A) Electron micrograph of the flagellum of a green-alga cell *(Chlamydomonas)* shown in cross section, illustrating the distinctive “9 + 2” arrangement of microtubules. (B) Diagram (more...)

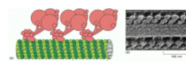
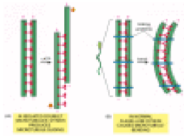


Figure 16-78

Ciliary dynein. Ciliary (axonemal) dynein is a large protein assembly (nearly 2 million daltons) composed of 9–12 polypeptide chains, the largest of which is the heavy chain of more than 500,000 daltons. (A) The heavy chains are believed to form (more...)

Figure 16-79



The bending of an axoneme. (A) When axonemes are exposed to the proteolytic enzyme trypsin, the linkages holding neighboring doublet microtubules together are broken. In this case, the addition of ATP allows the motor action of the dynein heads to slide (more...)

Bacteria also swim using cell surface structures called flagella, but these do not contain microtubules or dynein and do not wave or beat. Instead, *bacterial flagella* are long, rigid helical filaments, made up of repeating subunits of the protein flagellin. The flagella rotate like propellers, driven by a special rotary motor embedded in the bacterial cell wall (see Figure 15-67). The use of the same name to denote these two very different types of swimming apparatus is an unfortunate historical accident.

Structures called *basal bodies* firmly root eucaryotic cilia and flagella at the cell surface. The basal bodies have the same form as the centrioles that are found embedded at the center of animal centrosomes, with nine groups of fused triplet microtubules arranged in a cartwheel (Figure 16-80). Indeed, in some organisms, basal bodies and centrioles are functionally interconvertible: during each mitosis in the unicellular alga *Chlamydomonas*, for example, the flagella are resorbed, and the basal bodies move into the cell interior and become part of the spindle poles. New centrioles and basal bodies arise by a curious replication process, in which a smaller daughter is formed perpendicular to the original structure by a still mysterious mechanism (see Figure 18-6).

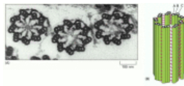


Figure 16-80

Basal bodies. (A) Electron micrograph of a cross section through three basal bodies in the cortex of a protozoan. (B) Diagram of a basal body viewed from the side. Each basal body forms the lower portion of a ciliary axoneme and is composed of nine sets (more...)

In humans, hereditary defects in ciliary dynein cause Kartagener's syndrome. The syndrome is characterized by male sterility due to immotile sperm, a high susceptibility to lung infections owing to the paralyzed cilia in the respiratory tract that fail to clear debris and bacteria, and defects in determination of the left-right axis of the body during early embryonic development (discussed in Chapter 21).

Summary

Motor proteins use the energy of ATP hydrolysis to move along microtubules or actin filaments. They mediate the sliding of filaments relative to one another and the transport of membrane-enclosed organelles along filament tracks. All known motor proteins that move on actin filaments are members of the myosin superfamily. The motor proteins that move on microtubules are members of either the kinesin superfamily or the dynein family. The myosin and kinesin superfamilies are diverse, with about 40 genes encoding each type of protein in humans. The only structural element shared among all members of each superfamily is the motor “head” domain. These heads can be attached to a wide variety of “tails,” which attach to different types of cargo and enable the various family members to perform different functions in the cell. Although myosin and kinesin walk along different tracks and use different mechanisms to produce force and movement by ATP hydrolysis, they share a common structural core, suggesting that they are derived from a common ancestor.

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

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