or promote cytosolic microtubule depolymerization; other MAPs organize microtubules into bundles or cross-link them to membranes and intermediate filaments or both (see Table 20-1).

- Various drugs, including colchicine and taxol, disrupt microtubule dynamics and have an antimitotic effect. Some of these drugs are useful in the treatment of certain cancers.
- Cell polarity including the organization of cell organelles, direction of membrane trafficking, and orientation of microtubules is determined by microtubule-organizing centers (MTOCs). Most interphase animal cells contain a single, perinuclear MTOC from which cytosolic microtubules radiate (see Figure 20-13).
- Because microtubule assembly is nucleated from MTOCs, the (−) end of most microtubules is adjacent to the MTOC and the (+) end is distal (see Figure 20-14).
- A γ-tubulin–containing complex is a major component of the pericentriolar material and is able to nucleate the polymerization of tubulin subunits to form microtubules in vitro.

20.2 Kinesin- and Dynein-Powered Movements

Within cells, proteins, organelles, and other membrane-limited vesicles, organelles, and proteins are frequently transported distances of many micrometers along well-defined routes in the cytosol and delivered to particular addresses. Diffusion alone cannot account for the rate, directionality, and destinations of such transport processes. Findings from early experiments with fish-scale pigment cells and nerve cells first demonstrated that microtubules function as tracks in the intracellular transport of various types of "cargo." Eventually, two families of motor proteins—**kinesins** and **dyneins**—were found to mediate transport along microtubules.

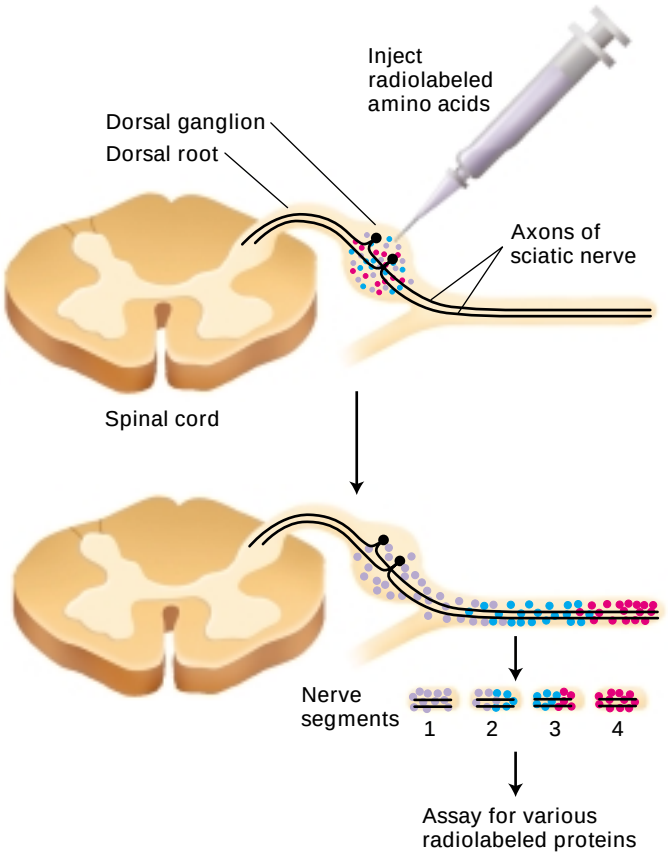
A second type of movement that depends on microtubule motor proteins is the beating of cilia and flagella. Huge numbers of cilia (more than $10^7/mm^2$) cover the surfaces of mammalian respiratory passages where their beating dislodges and expels particulate matter that collects in the mucus secretions of these tissues. In the oviduct, cilia help transport eggs down the fallopian tube. In contrast, sperm cells and many unicellular organisms have a single flagellum, which propels the cells forward at velocities approaching 1 mm/s.

In this section, we first consider the transport of materials in axons. Studies of such *axonal transport*, a process first discovered more than 50 years ago, have contributed greatly to our understanding of microtubule-associated intracellular transport. We then consider the structure and function of the microtubule motor proteins. A description of the unique microtubule-based structures and motor proteins responsible for the movement of cilia and flagella concludes this section.

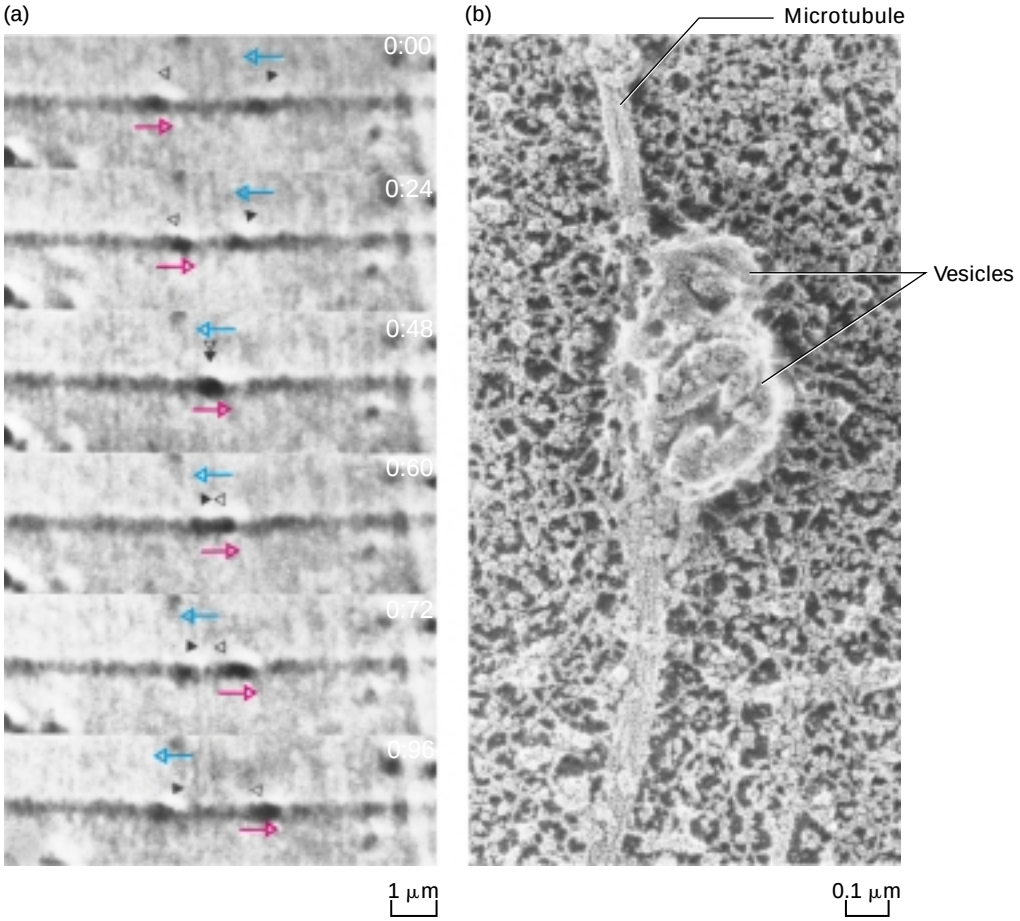
Axonal Transport Along Microtubules Is in Both Directions

A neuron must constantly supply new materials—proteins and membranes—to an axon terminal to replenish those lost in the exocytosis of neurotransmitters at the junction (synapse) with another cell. Because proteins and membranes are synthesized only in the cell body, these materials must be transported down the axon, which can be as much as a meter in length, to the synaptic region. This movement of materials is accomplished on microtubules, which are all oriented with their (+) ends toward the terminal (see Figure 20-14c).

The results of classic pulse-chase experiments in which radioactive precursors are microinjected into the dorsal-root ganglia near the spinal cord and then tracked along their nerve axons showed that axonal transport is in both directions. *Anterograde* transport proceeds from the cell body to



▶ EXPERIMENTAL FIGURE 20-17 **The rate of axonal transport in vivo can be determined by radiolabeling and gel electrophoresis.** The cell bodies of neurons in the sciatic nerve are located in dorsal-root ganglia. Radioactive amino acids injected into these ganglia in experimental animals are incorporated into newly synthesized proteins, which are then transported down the axon to the synapse. Animals are sacrificed at various times after injection and the dissected sciatic nerve is cut into small segments for analysis with the use of gel electrophoresis. The red, blue, and purple dots represent groups of proteins that are transported down the axon at different rates, red most rapidly, purple least rapidly.

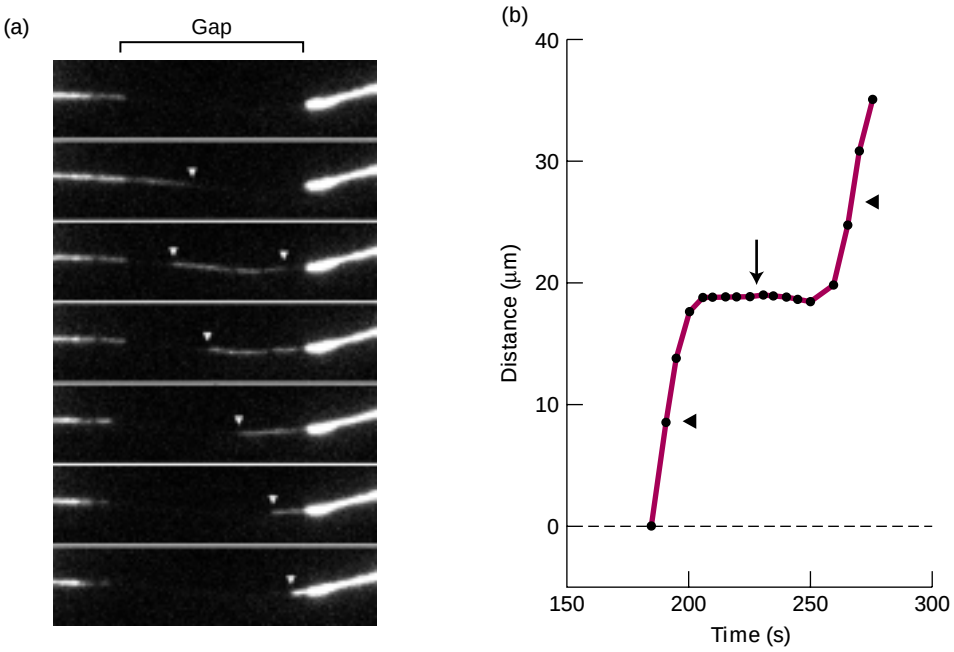


▲ EXPERIMENTAL FIGURE 20-18 **DIC microscopy demonstrates microtubule-based vesicle transport in vitro.** (a) The cytoplasm was squeezed from a squid giant axon with a roller onto a glass coverslip. After buffer containing ATP was added to the preparation, it was viewed in a differential interference contrast microscope, and the images were recorded on videotape. In the sequential images shown, the two organelles indicated by open and solid triangles move in opposite directions (indicated by colored arrows) along the same filament, pass each other, and continue in their original directions. Elapsed time in seconds appears at the upper-right corner of each video frame. (b) A region of cytoplasm similar to that shown in part (a) was freeze dried, rotary shadowed with platinum, and viewed in the electron microscope. Two large structures attached to one microtubule are visible; these structures presumably are small vesicles that were moving along the microtubule when the preparation was frozen. [See B. J. Schnapp et al., 1985, *Cell* **40:**455; courtesy of B. J. Schnapp, R. D. Vale, M. P. Sheetz, and T. S. Reese.]

MEDIA CONNECTIONS
Video: Organelle Movement Along Microtubules in a Squid Axon

the synaptic terminals and is associated with axonal growth and the delivery of synaptic vesicles. In the opposite, *retrograde*, direction, "old" membranes from the synaptic terminals move along the axon rapidly toward the cell body where they will be degraded in lysosomes. Findings from such experiments also revealed that different materials move at different speeds (Figure 20-17). The fastest-moving material, consisting of membrane-limited vesicles, has a velocity of about 250 mm/day, or about 3 μm/s. The slowest-moving material, comprising tubulin subunits and neurofilaments, moves only a fraction of a millimeter per day. Organelles such as mitochondria move down the axon at an intermediate rate.

Axonal transport can be directly observed by video microscopy of cytoplasm extruded from a squid giant axon. The movement of vesicles along microtubules in this cell-free system requires ATP, its rate is similar to that of fast axonal transport in intact cells, and it can proceed in both the anterograde and the retrograde directions (Figure 20-18a). Electron microscopy of the same region of the axon cytoplasm reveals vesicles attached to individual microtubules (Figure 20-18b). These pioneering in vitro experiments established definitely that organelles move along individual microtubules and that their movement requires ATP. As discussed shortly, these two observations led to the identification of microtubule motor proteins, which generate the movements.



▲ EXPERIMENTAL FIGURE 20-19 **Transport of GFP-tagged neurofilaments down axons exhibits periodic pauses.** (a) A segment of an axon is imaged after GFP-labeled neurofilament protein, NF-M, is expressed in a cultured neuronal cell. Bundles of labeled neurofilaments are separated by gaps within an axon. A GFP-labeled neurofilament (arrowhead) is seen to traverse a 15-μm gap between two labeled bundles. In this time series, each frame is taken at 5-second intervals. (b) A plot of the time-dependent distance traveled shows pauses (arrow) in neurofilament transport. Although the peak velocity (arrowheads) is similar to fast axonal transport, the average velocity is much lower. [From L. Wang et al., 2000, *Nature Cell Biol.* **2:**137; courtesy of A. Brown.]

Findings from recent experiments in which neurofilaments tagged with green fluorescent protein (GFP) were injected into cultured cells suggest that neurofilaments pause frequently as they move down an axon (Figure 20-19). Although the peak velocity of neurofilaments is similar to that of fast-moving vesicles, their numerous pauses lower the average rate of transport. These findings suggest that there is no fundamental difference between fast and slow axonal transport, although why neurofilament transport stops periodically is unknown.

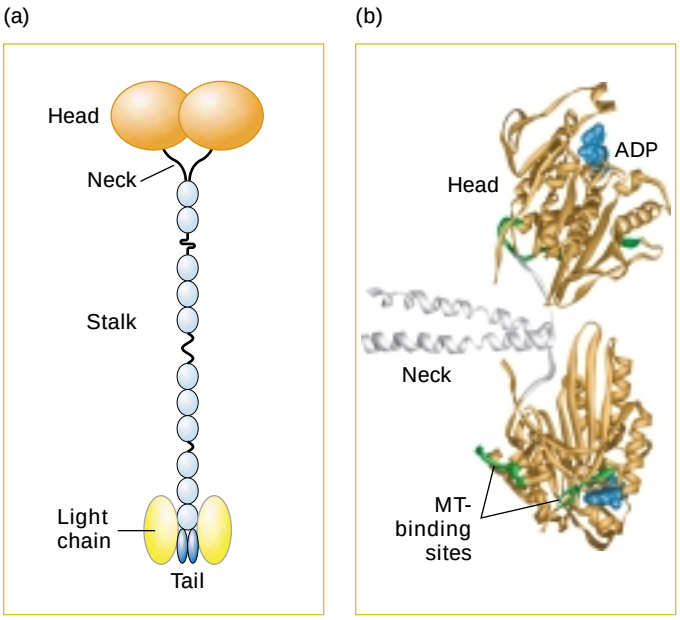
Kinesin I Powers Anterograde Transport of Vesicles in Axons

The first microtubule motor protein was identified by using a simple system consisting of microtubules assembled in vitro from purified tubulin subunits and stabilized by the drug taxol. When synaptic vesicles and ATP were added to these microtubules, the vesicles neither bound to the microtubules nor moved along them. However, the addition of a cytosolic extract of squid giant axon (free of tubulin) caused the vesicles to bind to the microtubules and to move along them, indicating that a soluble protein in the axonal cytosol is required for translocation.

When researchers incubated vesicles, axonal cytosol, and microtubules in the presence of AMPPNP, a nonhydrolyzable analog of ATP, the vesicles bound tightly to the microtubules but did not move. However, the vesicles did move when ATP was added. These results suggested that a motor protein in the cytosol binds to microtubules in the presence of ATP or AMPPNP, but movement requires ATP hydrolysis. To purify the soluble motor protein, scientists incubated a mixture of microtubules, cell or tissue extract, and AMPPNP, with the rationale that AMPPNP would promote tight binding between the microtubules and motor proteins in the extract. After incubation, the microtubules with any bound proteins were collected by centrifugation. Treatment of the microtubule-rich material in the pellet with ATP released one predominant protein back into solution; this protein is now known as kinesin I.

Kinesin I isolated from squid giant axons is a dimer of two heavy chains, each complexed to a light chain, with a total molecular weight of 380,000. The molecule comprises a pair of large globular *head domains* connected by a long *central stalk* to a pair of small globular *tail domains*, which contain the light chains (Figure 20-20). Each domain carries out a particular function: the head domain, which binds microtubules and ATP, is responsible for the motor activity of kinesin, and the tail domain is responsible for binding to the membrane of vesicles, most likely through the kinesin light chain.

Kinesin-dependent movement of vesicles can be tracked by in vitro motility assays similar to those used to study myosin-dependent movements. In one type of assay, a vesicle



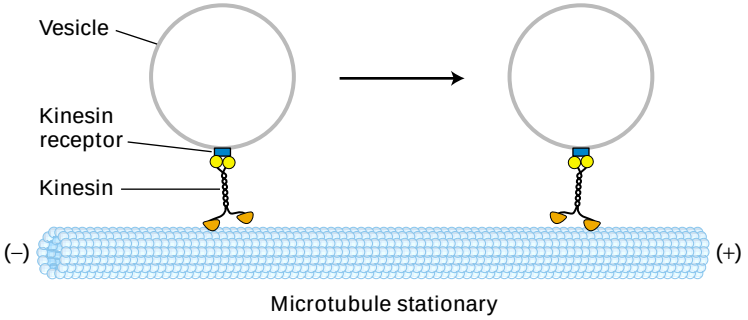
▶ FIGURE 20-20 **Structure of kinesin.** (a) Schematic model of kinesin showing the arrangement of the two heavy chains (each with a molecular weight of 110,000–135,000) and the two light chains (60,000–70,000 MW). (b) Three-dimensional structure of the kinesin dimer based on x-ray crystallography. Each head is attached to an α-helical neck region, which forms a coiled-coil dimer. Microtubules bind to the helix indicated; this interaction is regulated by the nucleotide (orange) bound at the opposite side of the domain. The distance between microtubule binding sites is 5.5 nm. [Part (b) courtesy of E. Mandelkow and E. M. Mandelkow, adapted from M. Thormahlen et al., 1998, *J. Struc. Biol.* **122:**30.]

or a plastic bead coated with kinesin is added to a glass slide along with a preparation of microtubules. In the presence of ATP, the beads can be observed microscopically to move along a microtubule in one direction. By determining the polarity of the microtubules, researchers found that the beads coated with kinesin I always moved from the (−) to the (+) end of a microtubule (Figure 20-21). Thus kinesin I is a (+) end–directed microtubule motor protein. Because this direction corresponds to anterograde transport, kinesin I is implicated as a motor protein that mediates anterograde axonal transport.

Most Kinesins Are Processive (+) End–Directed Motor Proteins

To date, approximately 10 different kinesin subfamilies have been identified. All contain a globular head (motor) domain, but they differ in their tail domains and several other properties. In most kinesins, the motor domain is at the N-terminus (N-type) of the heavy chain but, in others, the motor domain is centrally located (M-type) or at the C-terminus (C-type). Both N- and M-type kinesins are *(+) end–directed motors*, whereas C-type kinesins are *(−) end–directed motors*. Although most kinesins have two heavy chains (e.g., kinesin I), others have a single heavy chain (e.g., KIF1) or four heavy chains (e.g., BimC). Tetrameric BimC has an unusual bipolar arrangement in which pairs of motor domains lie at opposite ends of a central rod segment.

Kinesins can be divided into two broad functional groups— *cytosolic* and *mitotic* kinesins—on the basis of the nature of the cargo that they transport (Table 20-2). The functional differences between kinesins are related to their unique tail domains, which determine their cargoes. Cytosolic kinesins take part in vesicle and organelle transport; they include the classic axonal kinesin I, which has been shown to transport lysosomes and other organelles. Some cytosolic kinesins, however, transport one specific cargo. For example, KIF1B and its close relative KIF1A transport mitochondria and synaptic vesicles, respectively, to nerve terminals. Other cytosolic kinesins mediate the transport of secretory vesicles to the plasma membrane and the radial movement of ER



▲ FIGURE 20-21 **Model of kinesin-catalyzed vesicle transport.** Kinesin molecules, attached to unidentified receptors on the vesicle surface, transport the vesicles from the (−) end to the (+) end of a stationary microtubule. ATP is required for movement. [Adapted from R. D. Vale et al., 1985, *Cell* **40:**559; and T. Schroer et al., 1988, *J. Cell Biol.* **107:**1785.]

MEDIA CONNECTIONS
Video: Kinesin-Driven Transport of Vesicles Along Microtubules in Vitro

TABLE 20-2 Functional Classes of Microtubule Motor Proteins

Class	Common Members	Cargo	Direction of Movement*
Cytosolic motors	Kinesins (I, KIFIA, KIFIB)	Cytosolic vesicles/organelles	(+)
	Cytosolic dynein	Cytosolic vesicles/organelles	(−)
	Kinesin II	Cytosolic vesicles/organelles	(+)
Mitotic motors	Kinesin BimC (bipolar)	Spindle and astral MTs	(+)
	Chromokinesins	Chromosomes (arms)	(+)
	MCAK	Kinetochores	(+)
	CENP-E	Kinetochores	(+)
	Kinesin Ncd	Spindle and astral MTs	(−)
	Cytosolic dynein	Kinetochores, centrosomes, cell cortex near spindle poles	(−)
Axonemal motors	Outer-arm and inner-arm dyneins†	Doublet microtubules in cilia and flagella	(−)

*Movement of motor protein toward the (+) end or (−) end of microtubules.
† Outer-arm dyneins have three heavy chains, and inner-arm dyneins have two heavy chains.

membranes and pigment granules. Mitotic kinesins, in contrast, participate in spindle assembly and chromosome segregation in cell division. This group comprises numerous proteins, including the kinetochore-associated protein CENP-E, the bipolar BimC, and a (−) end–directed motor protein called Ncd. The functions of mitotic kinesins are described in more detail in Section 20.3.

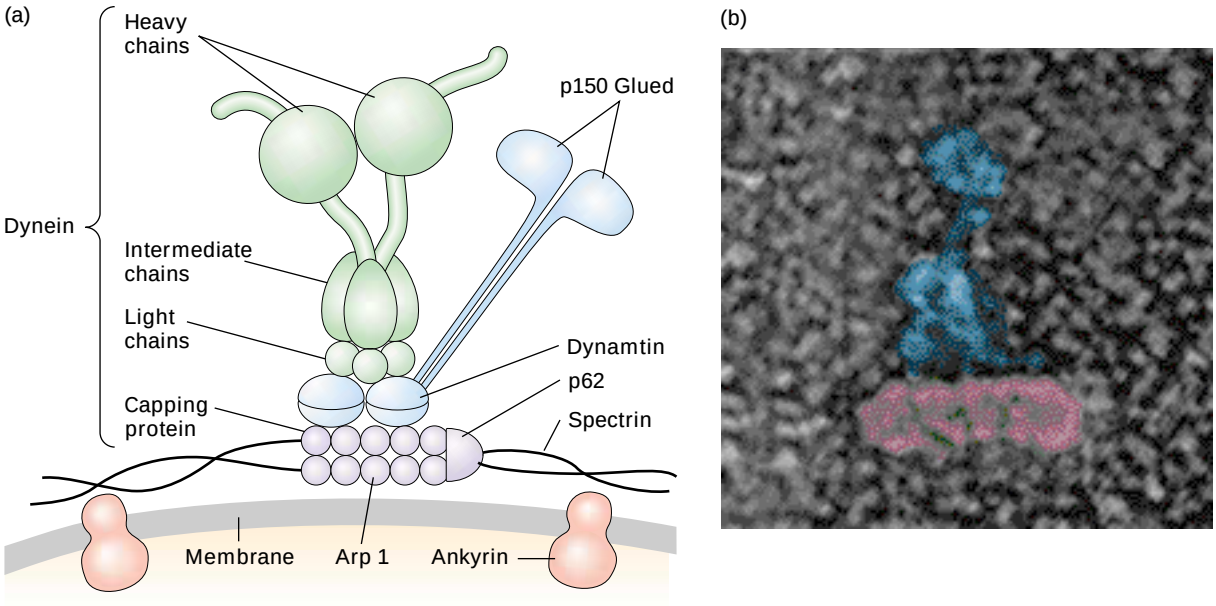
A sequence called the *tetratrico peptide sequence* has been recently identified in the light chains of kinesin I and may interact with receptor proteins in the membrane of various cargoes. Such interactions would tether the cargo organelle or vesicle to kinesin. For instance, the tetratrico peptide sequence has been found to bind to several proteins, including the amyloid precursor protein. Other kinesins may have different interaction sequences that bind to other receptors on membranes.

Two fundamental properties of the kinesin motor—its step size and force—have been determined in optical-trap experiments similar to those performed on myosin (see Figure 19-18). Findings from these studies show that a dimeric kinesin molecule (e.g., kinesin I) exerts a force of 6 piconewtons, which is sufficient to pull a bound vesicle through the viscous cytoplasm. The kinesin step size of 8 nm matches the distance between successive α- or β-tubulin monomers in a protofilament, suggesting that kinesin binds to only one or the other monomer. Electron microscopy reconstructions show that kinesin binds primarily to β-tubulin. In other experiments, researchers have established that a double-headed kinesin molecule moves along a single protofilament, with one head always bound to the microtubule. As a result, a kinesin molecule can move along a microtubule for a long distance without detaching from it, a property referred to as *processivity*. Because of their high processivity, dimeric kinesins are very efficient in transporting cargo from one part of a cell to another.

In Chapter 3, we saw that the neck region of myosin, which acts as a rigid lever arm, is critical in coupling ATP hydrolysis to the movement of myosin along an actin microfilament. In contrast with myosin, kinesin has a flexible neck domain, which links the head domain to the central stalk domain (see Figure 20-20). Current models propose that ATP hydrolysis by kinesin causes movement of the flexible neck, which then positions the head domain into the next step along a microtubule protofilament. According to this model, the direction of kinesin movement depends on neck function, not on the motor domain. This function of the neck is supported by findings from recent domain-replacement experiments. For example, replacing the motor domain in (−) end–directed Ncd with the motor domain from (+) end–directed kinesin I yielded a (−) end–directed chimeric protein. Likewise, swapping the kinesin and Vcd motor domains into kinesin I produced a (+) end–directed protein. These results show that the direction of movement is not an intrinsic property of the motor domain. However, mutations in the neck region of Ncd converted it from a (−) into a (+) end–directed motor protein.

Cytosolic Dyneins Are (−) End–Directed Motor Proteins That Bind Cargo Through Dynactin

The second family of microtubule motor proteins, the dyneins, is responsible for retrograde axonal transport,



▲ FIGURE 20-22 **Cytosolic dynein and the dynactin heterocomplex.** (a) Diagram of dynein (green) bound to the dynactin complex (orange) through interactions between the dynein light chains and the dynamtin subunits of dynactin. The Arp1 subunits of dynactin form a minifilament that associates with spectrin underlying the cell membrane. The Glued subunits bind microtubules and vesicles. (b) Electron micrograph of a metal replica of the dynactin complex isolated from brain cells. The Arp1 minifilament (purple) and the dynamtin/Glued side arm (blue) are visible. [Part (a) adapted from N. Hirokawa, 1998, *Science* **279:**518. Part (b) from D. M. Eckley et al., 1999, *J. Cell Biol.* **147:**307.]

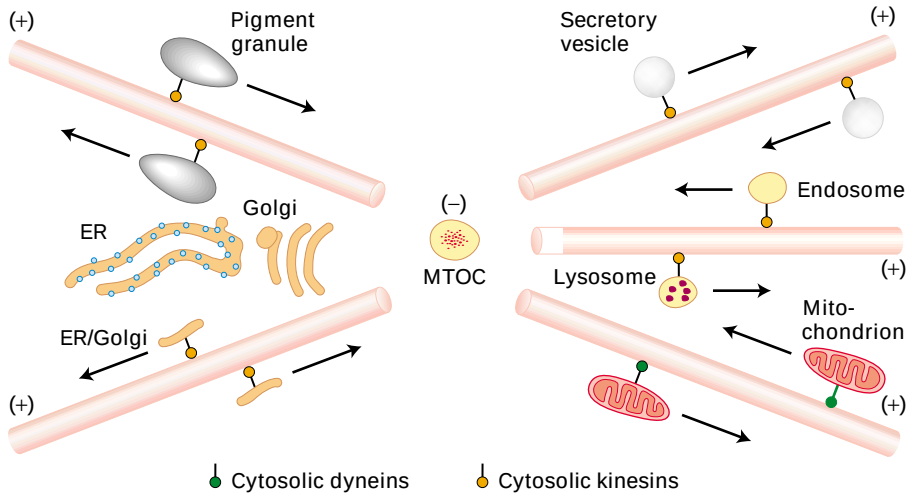
transit of Golgi vesicles to the centrosome, and some other (−) end–directed movements. Dyneins are exceptionally large, multimeric proteins, with molecular weights exceeding 1×10^6. They are composed of two or three heavy chains complexed with a poorly determined number of intermediate and light chains. As summarized in Table 20-2, the dyneins are divided into two functional classes. Here we consider *cytosolic* dynein, which has a role in the movement of vesicles and chromosomes. *Axonemal* dyneins, responsible for the beating of cilia and flagella, are considered later.

Like kinesin I, cytosolic dynein is a two-headed molecule, with two identical or nearly identical heavy chains forming the head domains. However, unlike kinesin, dynein cannot mediate cargo transport by itself. Rather, dynein-related transport requires *dynactin*, a large protein complex that links vesicles and chromosomes to the dynein light chains (Figure 20-22). The results of in vitro binding experiments show that dynactin also binds to microtubules, thereby enhancing the processivity of dynein-dependent movement. Dynactin consists of at least eight subunits, including a protein called Glued, which binds microtubules; Arp1, an actin-related protein that binds spectrin; and dynamtin, which interacts with the light chains of dynein. The microtubule-binding site in Glued contains a 57-residue motif that is also present in CLIP170, a microtubule-associated protein that cross-links microtubules and endocytic vesicles (see Table 20-1). One model proposes that dynein generates the force for vesicle movement but remains tethered to a microtubule through dynactin.

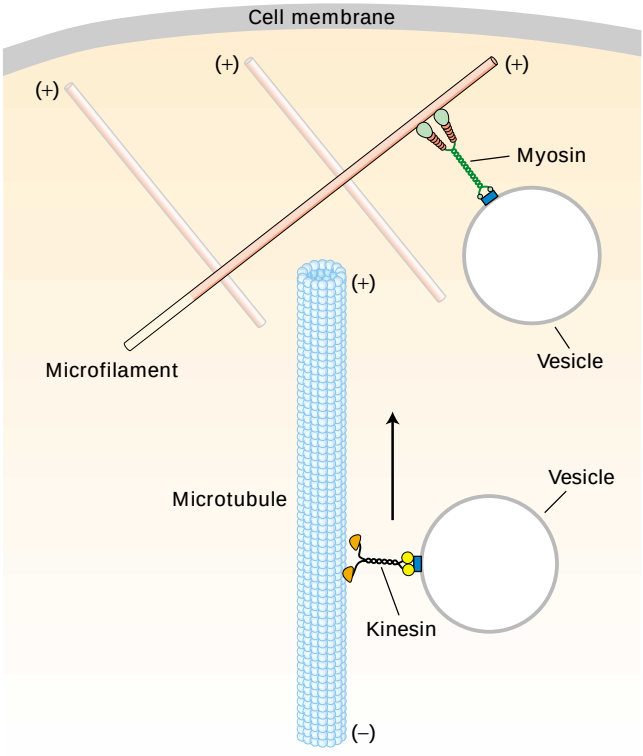
As discussed later, several lines of evidence suggest that the dynein-dynactin complex and another complex, the nuclear/mitotic apparatus (NuMA) protein, mediate the association of microtubules with centrosomes in mitosis. The results of in vitro studies show that truncated NuMA protein binds microtubules if the C-terminal region is retained. As in MAPs, the C-terminal region of NuMA protein is highly acidic, and ionic interactions may mediate its binding to microtubules.

Multiple Motor Proteins Sometimes Move the Same Cargo

Figure 20-23 summarizes the role of kinesins and cytosolic dyneins in intracellular transport along microtubules. Because the orientation of microtubules is fixed by the MTOC, the direction of transport—toward or away from the cell periphery—depends on the motor protein. Some cargoes, such as pigment granules, can alternate their direction of movement along a single microtubule. In this case, both anterograde and retrograde microtubule motor proteins must associate with the same cargo. Recent biochemical experiments have identified dynactin in a complex with kinesin. A model proposes that dynactin is part of the membrane receptor and serves as a common adapter for binding kinesin and cytoplasmic dynein. Thus the direction of movement can be switched by swapping one motor protein for the other.



▲ FIGURE 20-23 **General model of kinesin- and dynein-mediated transport in a typical cell.** The array of microtubules, with their (+) ends pointing toward the cell periphery, radiates from an MTOC in the Golgi region. Kinesin-dependent anterograde transport (red) conveys mitochondria, lysosomes, and an assortment of vesicles to the endoplasmic reticulum (ER) or cell periphery. Cytosolic dynein–dependent retrograde transport (green) conveys mitochondria, elements of the ER, and late endosomes to the cell center. [Adapted from N. Hirokawa, 1998, *Science* **279:**518.]

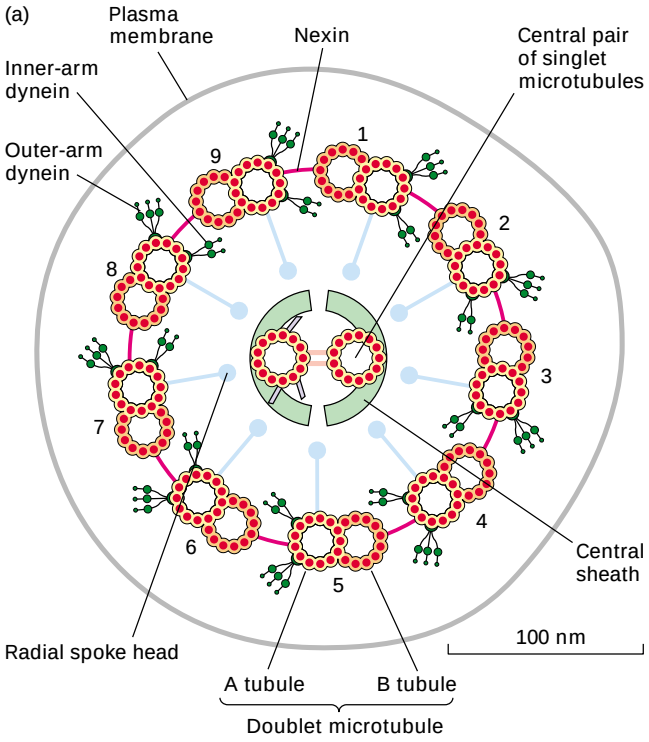


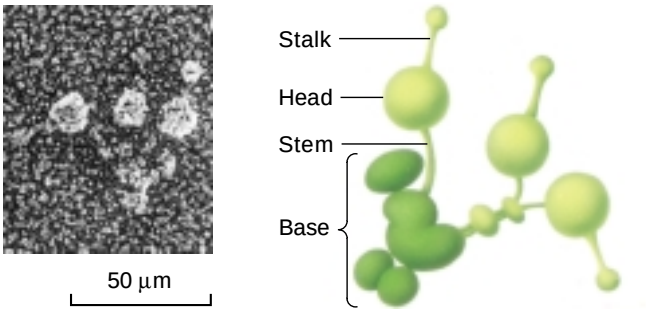
▲ FIGURE 20-24 **Cooperation of myosin and kinesin at the cell cortex.** Microtubules approach the actin-rich cell membrane. Consequently, some cargoes are transported to the cell periphery by kinesin motor proteins on microtubules but complete the journey on microfilaments under the power of myosin motor proteins.

In some cases, a vesicle must traverse microtubule-poor but microfilament-rich regions in the cell. For example, during endocytosis, vesicles from the actin-rich plasma membrane are carried inward, whereas during secretion, vesicles derived from the endoplasmic reticulum and Golgi are moved outward. The results of several complementary experiments imply that microtubule and microfilament motor proteins bind to the same vesicles and cooperate in their transport. One piece of evidence was obtained from microscopy of vesicle movements in extruded cytoplasm from a squid giant axon. As observed many times before, vesicles traveled along microtubule tracks; surprisingly, movement continued at the periphery of the extruded cytoplasm through a region containing microfilaments but no microtubules. Subsequent experiments demonstrated that a given vesicle could move on a microtubule *or* a microfilament. Thus at least two motor proteins, myosin and either kinesin or cytosolic dynein, must be bound to the same vesicle (Figure 20-24). The discovery that a given vesicle can travel along both cytoskeletal systems suggests that, in a neuron, synaptic vesicles are transported at a fast rate by kinesin in the microtubule-rich axon and then travel through the actin-rich cortex at the nerve terminal on a myosin motor.

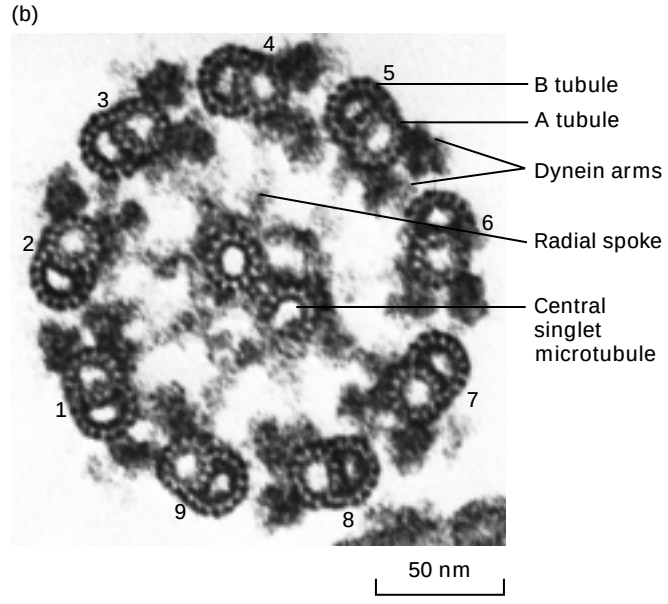
Eukaryotic Cilia and Flagella Contain a Core of Doublet Microtubules Studded with Axonemal Dyneins

Cilia and flagella are flexible membrane extensions that project from certain cells. They range in length from a few





▲ EXPERIMENTAL FIGURE 20-26 **Freeze-etching reveals structure of axonemal dynein.** Electron micrograph of freeze-etched outer-arm dynein from *Tetrahymena* cilia and an artist's interpretation of the structure. The base contains several intermediate and light chains. Attached to the common base are three heavy chains each composed of a long stem, large globular head domain and small globular domain, and short stalk connecting the globular domains. Microtubules bind to the tip of the stalk. All axonemal dyneins are thought to have the general structure shown here, although some outer-arm dyneins contain two heavy chains, and inner-arm dyneins contain one or two heavy chains. [Electron micrograph from U. W. Goodenough and J. E. Heuser, 1984, *J. Mol. Biol.* **18:**1083.]



▲ FIGURE 20-25 **Structure of an axoneme.**
(a) Cross-sectional diagram of a typical flagellum showing its major structures. The dynein arms and radial spokes with attached heads surround a central pair of singlet microtubules. (b) Micrograph of a transverse section through an isolated demembranated cilium. [See U. W. Goodenough and J. E. Heuser, 1985, *J. Cell Biol.* **100:**2008. Part (b) courtesy of L. Tilney.]

micrometers to more than 2 mm for some insect sperm flagella. Virtually all eukaryotic cilia and flagella possess a central bundle of microtubules, called the **axoneme,** which consists of nine doublet microtubules surrounding a central pair of singlet microtubules (Figure 20-25). This characteristic "9 + 2" arrangement of microtubules is seen in cross section with the electron microscope. Each doublet microtubule consists of A and B tubules. The (+) end of axonemal microtubules is at the distal end of the axoneme. At its point of attachment to the cell, the axoneme connects with the **basal body.** Containing nine triplet microtubules (see Figure 20-4), the basal body plays an important role in initiating the growth of the axoneme.

The axoneme is held together by three sets of protein cross-links (see Figure 20-25a). The central pair of singlet microtubules is connected by periodic bridges, like rungs on a ladder, and is surrounded by a fibrous structure termed the *inner sheath.* A second set of linkers, composed of the protein *nexin,* joins adjacent outer doublet microtubules. *Radial spokes,* which radiate from the central singlets to each A tubule of the outer doublets, are proposed to regulate dynein.

Permanently attached periodically along the length of the A tubule of each doublet microtubule are *inner-arm* and *outer-arm* dyneins (see Figure 20-25a). These axonemal dyneins are complex multimers of heavy chains, intermediate chains, and light chains. When isolated axonemal dyneins are slightly denatured and spread out on an electron microscope grid, they are seen as a bouquet of two or three "blossoms" connected to a common base (Figure 20-26). Each blossom consists of a large globular head domain attached to a small globular domain through a short stalk; a stem connects one or more blossoms to a common base. The base is thought to attach a dynein to the A tubule, whereas the globular domains project outward toward the B tubule of the neighboring doublet.

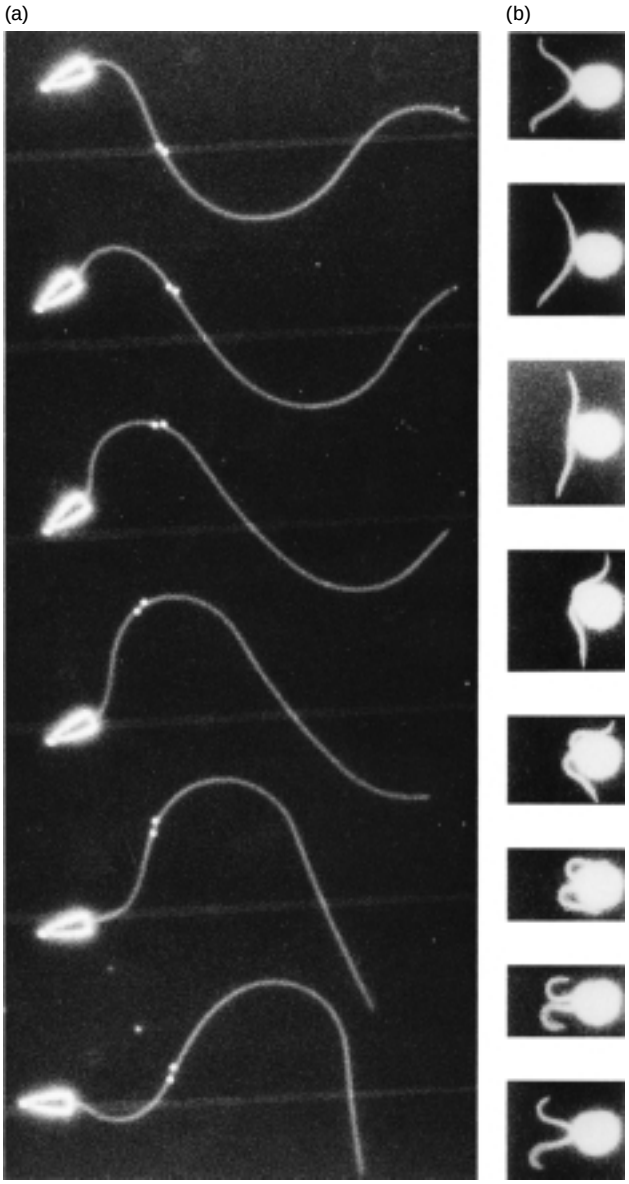
A single dynein heavy chain, which forms each stalk, head, and stem is enormous, approximately 4500 amino acids in length with a molecular weight exceeding 540,000. At least eight or nine different heavy chains have been identified, each capable of hydrolyzing ATP. On the basis of sequence comparisons with the ATP-binding sites in other proteins, the ATP-binding site of axonemal dynein is predicted to lie in the globular head domain of the heavy chain, with the microtubule-binding site being at the tip of the stalk. Inner-arm dyneins are either one- or two-headed structures, containing one or two heavy chains. Outer-arm dyneins contain two heavy chains (e.g., in a sea urchin sperm flagellum) or three heavy chains (e.g., in *Tetrahymena* cilia and *Chlamydomonas* flagella).

The intermediate and light chains in axonemal dynein are thought to form the base region. These chains help mediate the attachment of the dynein arm to the A tubule and may also participate in regulating dynein activity. The base proteins of axonemal dyneins are thus analogous to those composing the dynactin complexes associated with cytosolic dynein.

Ciliary and Flagellar Beating Are Produced by Controlled Sliding of Outer Doublet Microtubules

Ciliary and flagellar beating is characterized by a series of bends, originating at the base of the structure and propagated toward the tip (Figure 20-27). The bends push against the surrounding fluid, propelling the cell forward or moving the fluid across a fixed epithelium. A bend results from the sliding of adjacent doublet microtubules past one another. Because active sliding occurs all along the axoneme, bends can be propagated without damping. Findings from microscopic studies with isolated axonemes from which the cross-linkage proteins (e.g., nexin) are removed have shown that doublet microtubules slide past one another in the presence of ATP but no bending occurs (Figure 20-28a). Thus the ATP-dependent movement of doublet microtubules must be restricted by cross-linking proteins in order for sliding to be converted into the bending of an axoneme.

On the basis of the polarity and direction of sliding of the doublet microtubules and the properties of axonemal dyneins, the small head domains of the dynein arms on the A tubule of one doublet are thought to "walk" along the adjacent doublet's B tubule toward its base, the (−) end (Figure 20-28b). The force producing active sliding requires ATP and probably entails a conformational change in the head and stem that translocates the stalk. Successive binding and hydrolysis of ATP causes the dynein stalks to successively release from and attach to the adjacent doublet. Although this general model is most likely correct, many important details such as the mechanism of force transduction by dynein are still unknown.



▶ EXPERIMENTAL FIGURE 20-27 **Video microscopy shows flagellar movements that propel sperm and *Chlamydomonas* forward.** In both cases, the cells are moving to the left. (a) In the typical sperm flagellum, successive waves of bending originate at the base and are propagated out toward the tip; these waves push against the water and propel the cell forward. Captured in this multiple-exposure sequence, a bend at the base of the sperm in the first (*top*) frame has moved distally halfway along the flagellum by the last frame. A pair of gold beads on the flagellum are seen to slide apart as the bend moves through their region. (b) Beating of the two flagella on *Chlamydomonas* occurs in two stages, called the *effective stroke* (top three frames) and the *recovery stroke* (remaining frames). The effective stroke pulls the organism through the water. During the recovery stroke, a different wave of bending moves outward from the bases of the flagella, pushing the flagella along the surface of the cell until they reach the position to initiate another effective stroke. Beating commonly occurs 5–10 times per second. [Part (a) from C. Brokaw, 1991, *J. Cell Biol.* **114**(6): cover photograph; courtesy of C. Brokaw. Part (b) courtesy of S. Goldstein.]