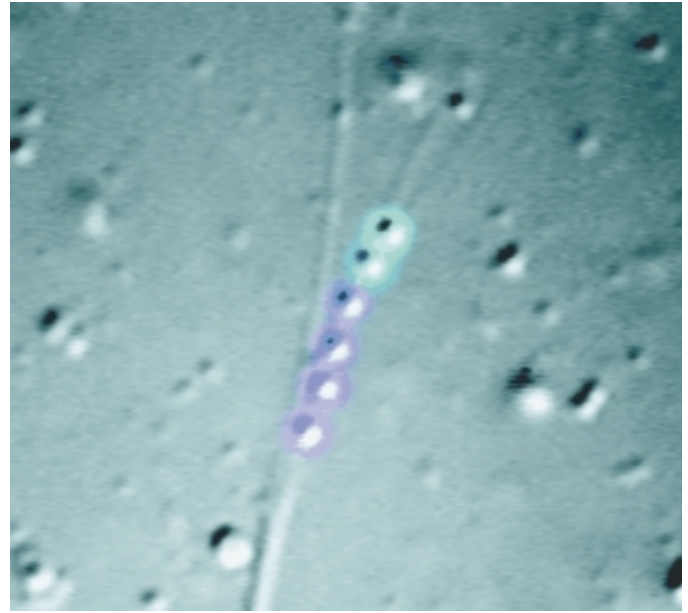


20

MICROTUBULES



A 3-second time lapse movie captures the kinesin-powered movement of a vesicle along a microtubule. [From N. Pollack et al., 1999, *J. Cell Biol.* **147**:493–506; courtesy of R. D. Vale.]

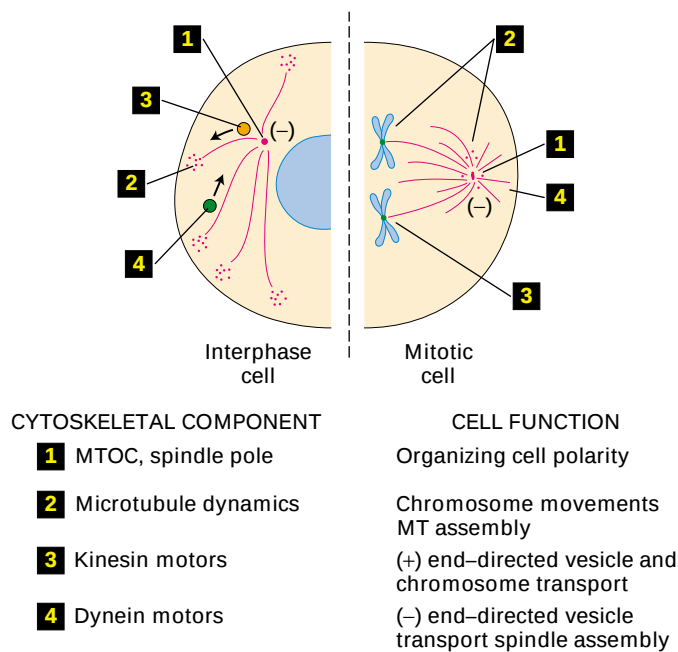
In Chapter 19, we looked at microfilaments and intermediate filaments—two of the three types of cytoskeletal fibers—and their associated proteins. This chapter focuses on the third cytoskeletal system—**microtubules**. Like microfilaments, microtubules take part in certain cell movements, including the beating of cilia and flagella and the transport of vesicles in the cytoplasm. These movements result from the polymerization and depolymerization of microtubules or the actions of microtubule **motor proteins**. Both processes are required for some other cell movements, such as the alignment and separation of chromosomes in meiosis and mitosis (see Figure 9-3). Microtubules also direct the migration of nerve-cell axons by guiding the extension of the neuronal growth cone.

In addition to contributing to cell motility, microtubules play a major role in organizing the cell through a special structure called the **microtubule-organizing center**, or **MTOC**. Located near the nucleus, the MTOC directs the assembly and orientation of microtubules, the direction of vesicle trafficking, and the orientation of organelles. Because organelles and vesicles are transported along microtubules, the MTOC becomes responsible for establishing the polarity of the cell and the direction of cytoplasmic processes in both interphase and mitotic cells (Figure 20-1).

In this chapter, we build on the general principles learned in Chapter 19 about the structure and function of the microfilament cytoskeleton and show how many of the same concepts also apply to microtubules. We begin the chapter by examining the structure and assembly of microtubules and then consider how microtubule assembly and microtubule motor proteins can power cell movements. The discussion of microtubules concludes with a detailed examination of the translocation of chromosomes in mitosis. Although we consider microtubules, microfilaments, and intermediate filaments individually, the three cytoskeletal systems do not act completely independently of one another. An important example of their interdependence can be found in cell division when interaction between actin microfilaments and microtubules determines the plane of cleavage.

OUTLINE

- 20.1 Microtubule Organization and Dynamics
- 20.2 Kinesin- and Dynein-Powered Movements
- 20.3 Microtubule Dynamics and Motor Proteins in Mitosis

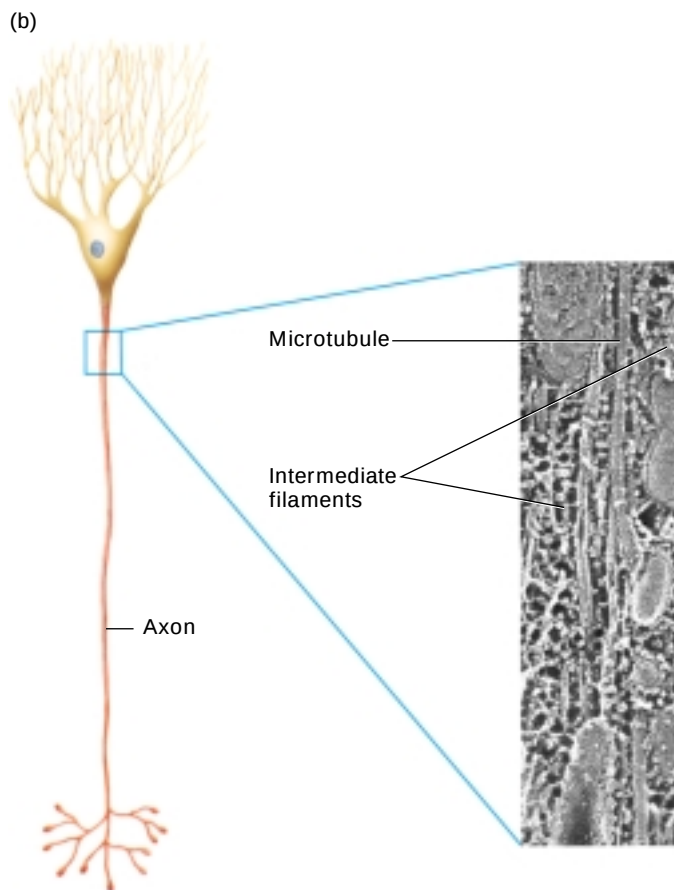
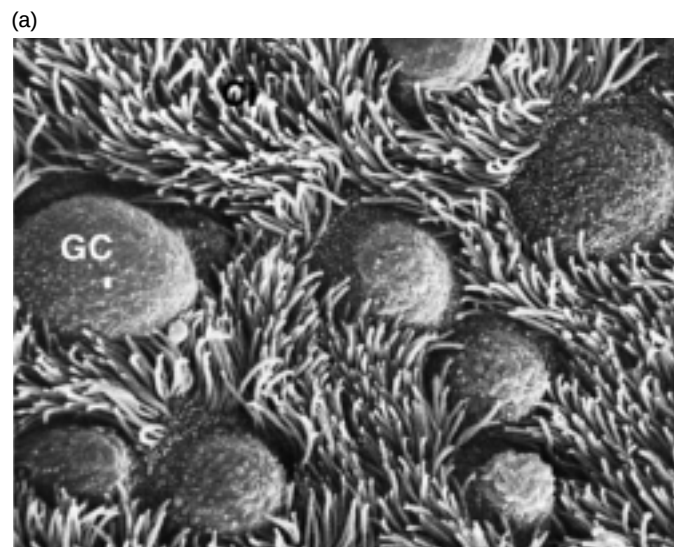


▲ **FIGURE 20-1 Microtubules (blue) organized around the MTOC and spindle poles (1)** establish an internal polarity to movements and structures in the interphase cell (*left*) and the mitotic cell (*right*). Assembly and disassembly (**2**) cause microtubules to probe the cell cytoplasm and are harnessed at mitosis to move chromosomes. Long-distance movement of vesicles (**3** and **4**) are powered by kinesin and dynein motors. Both motors are critical in the assembly of the spindle and the separation of chromosomes in mitosis.

20.1 Microtubule Organization and Dynamics

A microtubule is a polymer of globular **tubulin** subunits, which are arranged in a cylindrical tube measuring 25 nm in diameter—more than twice the width of an intermediate filament and three times the width of a microfilament (see Figure 5-29). Varying in length from a fraction of a micrometer to hundreds of micrometers, microtubules are much stiffer than either microfilaments or intermediate filaments because of their tubelike construction. A consequence of this tubular design is the ability of microtubules to generate pushing forces without buckling, a property that is critical to the movement of chromosomes and the mitotic spindle in mitosis.

Cells contain two populations of microtubules: stable, long-lived microtubules and unstable, short-lived microtubules. Stable microtubules are generally found in nonreplicating cells. They include a central bundle of microtubules in **cilia** and **flagella**, extensions of the plasma membrane that beat rhythmically to propel materials across epithelial surfaces, to enable sperm to swim, or to push an egg through the oviduct (Figure 20-2a). A marginal band of stable microtubules present in some erythrocytes and platelets enables these cells to pass through small blood vessels. Another example exists in nerve cells (neurons), which must maintain long processes



◀ EXPERIMENTAL FIGURE 20-2 Various microscopic techniques are used to visualize stable and transient microtubule structures.

(a) Surface of the ciliated epithelium lining a rabbit oviduct viewed in the scanning electron microscope. Microtubule-containing cilia cover ciliated cells, and actin-containing microvilli populate the surface of secretory cells. Beating cilia propel an egg down the oviduct. (b) Microtubules and intermediate filaments in a quick-frozen frog axon visualized by the deep-etching technique (*right*). Several 24-nm-diameter microtubules and thinner, 10-nm-diameter intermediate filaments can be seen. Both types of fibers are oriented longitudinally; they are cross-linked by various proteins. (c) Isolated mitotic apparatus visualized by differential interference contrast (DIC) microscopy. The spindle and asters, which are critical in pulling the chromosomes to the poles, are composed of transient microtubules that assemble early in mitosis and disassemble at its completion. [Part (a) from R. G. Kessels and R. H. Kardon, 1975, *Tissues and Organs*, W. H. Freeman and Company. Part (b) from N. Hirokawa, 1982, *J. Cell Biol.* **94**:129; courtesy of N. Hirokawa. Part (c) from E. D. Salmon and R. R. Segall, 1980, *J. Cell Biol.* **86**:355.]

called **axons** (see Figure 7-29). An internal core of stable microtubules in axons not only supports their structure but also provides tracks along which vesicles move through the axonal cytoplasm (Figure 20-2b). The disassembly of such stable structures would have catastrophic consequences—sperm would be unable to swim, a red blood cell would lose its springlike pliability, and axons would retract.

In contrast with these permanent, stable structures, unstable microtubules are found in cells that need to assemble and disassemble microtubule-based structures quickly. For example, in mitosis, the cytosolic microtubule network characteristic of interphase cells disassembles, and the tubulin from it is used to form the spindle-shaped apparatus that partitions chromosomes equally to the daughter cells (Figure 20-2c). When mitosis is complete, the spindle disassembles and the interphase microtubule network re-forms.

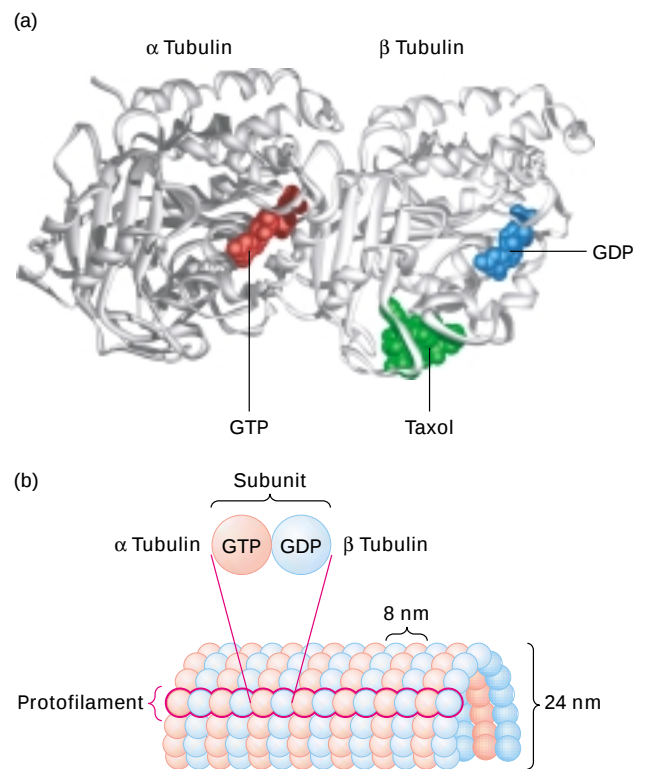
Before proceeding to a discussion of microtubule-based movements, we examine the assembly, disassembly, and polarity of microtubules, as well as a group of proteins that are integrally associated with microtubules. An important property of a microtubule is oscillation between growing and shortening phases. This complex dynamic behavior permits a cell to quickly assemble or disassemble microtubule structures.

Heterodimeric Tubulin Subunits Compose the Wall of a Microtubule

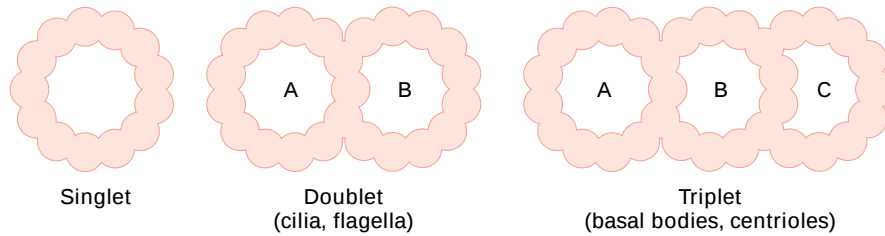
The building block of a microtubule is the tubulin subunit, a heterodimer of α - and β -tubulin. Both of these 55,000-MW monomers are found in all eukaryotes, and their sequences are highly conserved. Although a third tubulin, γ -tubulin, is not part of the tubulin subunit, it probably nucleates the polymerization of subunits to form $\alpha\beta$ -microtubules. Encoded by separate genes, the three tubulins exhibit homology with a 40,000-MW bacterial GTPase, called FtsZ (see Figure 5-30b). Like tubulin, this bacterial protein

has the ability to polymerize and participates in cell division. Perhaps the protein carrying out these ancestral functions in bacteria was modified in the course of evolution to fulfill the diverse roles of microtubules in eukaryotes.

Each tubulin subunit binds two molecules of GTP. One GTP-binding site, located in α -tubulin, binds GTP irreversibly and does not hydrolyze it. The second site, located on β -tubulin, binds GTP reversibly and hydrolyzes it to GDP. Thus, tubulin is a GTPase like bacterial FtsZ protein. In the atomic structure of the tubulin subunit, the GTP bound to α -tubulin is trapped at the interface between the α - and β -tubulin monomers and is thus nonexchangeable. The second GTP lies at the surface of the β -tubulin monomer; this GTP is freely exchangeable with GDP (Figure 20-3a). As discussed later, the



▲ **FIGURE 20-3 Structure of tubulin monomers and their organization in microtubules.** (a) Ribbon diagram of the dimeric tubulin subunit. The GTP (red) bound to the α -tubulin monomer is nonexchangeable, whereas the GDP (blue) bound to the β -tubulin monomer is exchangeable with GTP. The anticancer drug taxol (green) was used in structural studies to stabilize the dimer structure. (b) The organization of tubulin subunits in a microtubule. The subunits are aligned end to end into protofilaments, which pack side by side to form the wall of the microtubule. In this model, the protofilaments are slightly staggered so that α -tubulin in one protofilament is in contact with α -tubulin in the neighboring protofilaments. The microtubule displays a structural polarity in that subunits are added preferentially at the end, designated the (+) end, at which β -tubulin monomers are exposed. [Part (a) modified from E. Nogales et al., 1998, *Nature* **391**:199; courtesy of E. Nogales. Part (b) adapted from Y. H. Song and E. Mandelkow, 1993, *Proc. Nat'l. Acad. Sci. USA* **90**:1671.]



▲ **FIGURE 20-4 Arrangement of protofilaments in singlet, doublet, and triplet microtubules.** In cross section, a typical microtubule, a singlet, is a simple tube built from 13 protofilaments. In a doublet microtubule, an additional set of

10 protofilaments forms a second tubule (B) by fusing to the wall of a singlet (A) microtubule. Attachment of another 10 protofilaments to the B tubule of a doublet microtubule creates a C tubule and a triplet structure.

guanine bound to β -tubulin modulates the addition of tubulin subunits at the ends of a microtubule.

In a microtubule, lateral and longitudinal interactions between the tubulin subunits are responsible for maintaining the tubular form. Longitudinal contacts between the ends of adjacent subunits link the subunits head to tail into a linear *protofilament*. Within each protofilament, the dimeric subunits repeat every 8 nm. Through lateral interactions, protofilaments associate side by side into a sheet or cylinder—a microtubule. In most microtubules, the heterodimers in adjacent protofilaments are staggered only slightly, forming tilted rows of α - and β -tubulin monomers in the microtubule wall. The head-to-tail arrangement of the α - and β -tubulin dimers in a protofilament confers an overall polarity on a microtubule. Because all protofilaments in a microtubule have the same orientation, one end of a microtubule is ringed by α -tubulin, whereas the opposite end is ringed by β -tubulin (Figure 20-3b). As in actin microfilaments, the two ends of a microtubule, designated the (+) and (–) ends, differ in their rates of assembly and critical concentrations (C_c). The (+) end corresponds to the β -tubulin end of a microtubule.

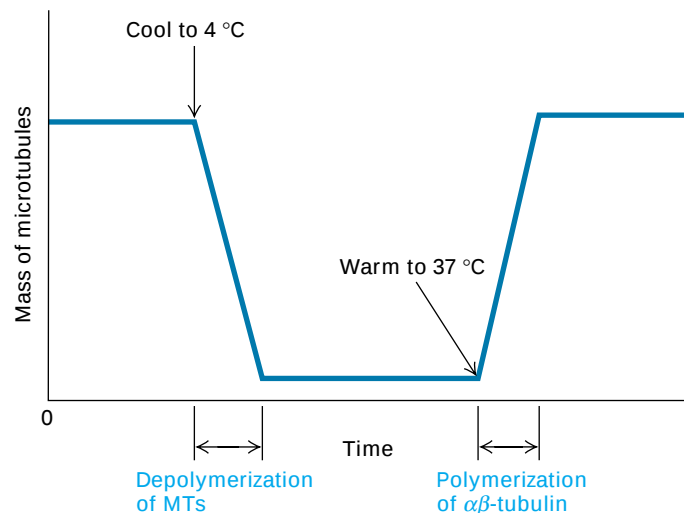
Virtually every microtubule in a cell is a simple tube, a *singlet* microtubule, built from 13 protofilaments. In rare cases, singlet microtubules contain more or fewer protofilaments; for example, certain microtubules in the neurons of nematode worms contain 11 or 15 protofilaments. In addition to the simple singlet structure, *doublet* or *triplet* microtubules are found in specialized structures such as cilia and flagella (doublet microtubules) and centrioles and basal bodies (triplet microtubules). Each doublet or triplet contains one complete 13-protofilament microtubule (A tubule) and one or two additional tubules (B and C) consisting of 10 protofilaments (Figure 20-4).

Microtubule Assembly and Disassembly Take Place Preferentially at the (+) End

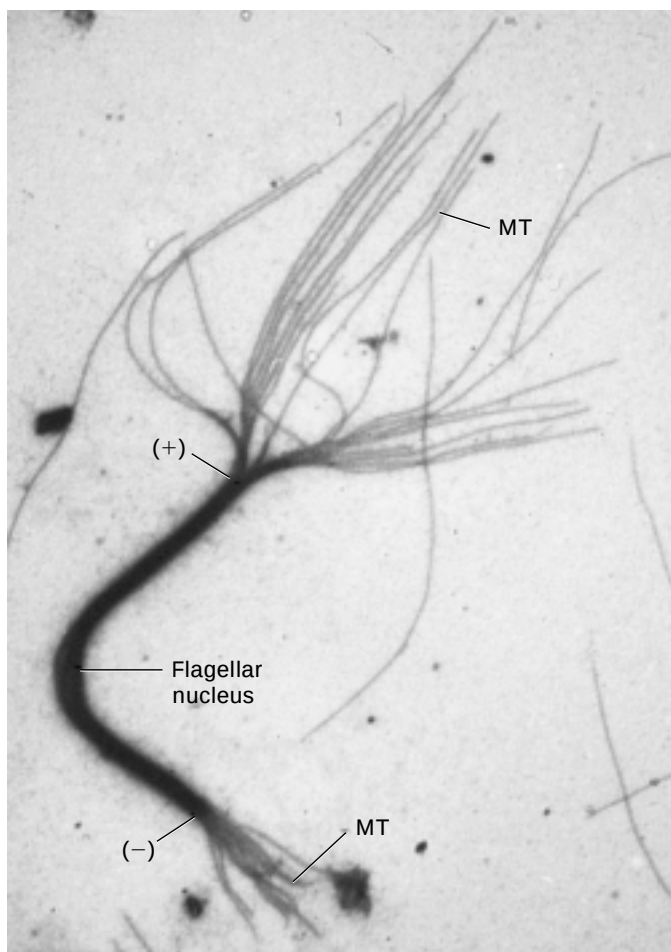
Microtubules assemble by the polymerization of dimeric $\alpha\beta$ -tubulin. Assembly and stability of microtubules are temperature dependent. For instance, if microtubules are cooled

to 4 °C, they depolymerize into $\alpha\beta$ -tubulin dimers (Figure 20-5). When warmed to 37 °C in the presence of GTP, the tubulin dimers polymerize into microtubules. Cycles of heating and cooling are key steps in purifying microtubules and their associated proteins from cell extracts.

Tubulin polymerization has several properties in common with the polymerization of actin to form microfilaments. First, at $\alpha\beta$ -tubulin concentrations above the *critical concentration* (C_c), the dimers polymerize into microtubules, whereas at concentrations below the C_c , microtubules depolymerize, similar to the behavior of G-actin and F-actin (see Figure 19-7). Second, the nucleotide, either GTP or GDP, bound to the β -tubulin causes the critical concentration (C_c) for assembly at the (+) and (–) ends of a microtubule to differ. By analogy with F-actin assembly, the preferred assembly end is designated the (+) end. Third,



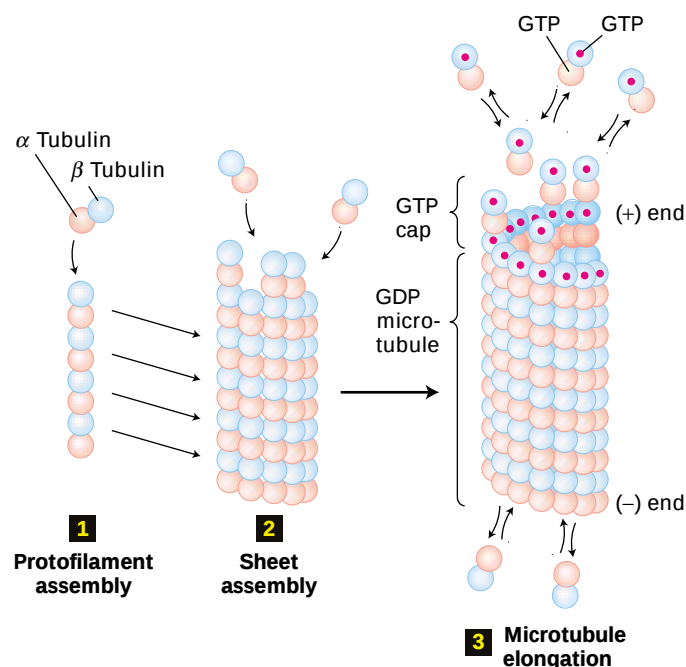
▲ **EXPERIMENTAL FIGURE 20-5 Temperature affects whether microtubules (MTs) assemble or disassemble.** At low temperatures, microtubules depolymerize, releasing $\alpha\beta$ -tubulin, which repolymerizes at higher temperatures in the presence of GTP.



▲ **EXPERIMENTAL FIGURE 20-6 Addition of microtubule fragments demonstrates polarity of tubulin polymerization.**

Fragments of flagellar microtubules act as nuclei for the *in vitro* addition of $\alpha\beta$ -tubulin. The nucleating flagellar fragment can be distinguished in the electron microscope from the newly formed microtubules (MT) seen radiating from the ends of the flagellar fragment. The greater length of the microtubules at one end indicates that tubulin subunits are added preferentially to this end. [Courtesy of G. Borisy.]

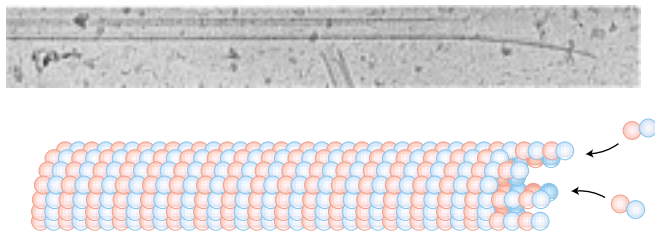
at $\alpha\beta$ -tubulin concentrations higher than the C_c for polymerization, dimers add preferentially to the (+) end. Fourth, when the $\alpha\beta$ -tubulin concentration is higher than the C_c at the (+) end but lower than the C_c at the (-) end, microtubules can *treadmill* by adding subunits to one end and dissociating subunits from the opposite end (see Figure 19-9). Because the intracellular concentration of assembly-competent tubulin (10–20 μM) is much higher than the critical concentration (C_c) for assembly (0.03 μM), polymerization is highly favored in a cell. Finally, the initial rate of tubulin polymerization is accelerated in the presence of nuclei—that is, microtubule-based structures or fragments (Figure 20-6).



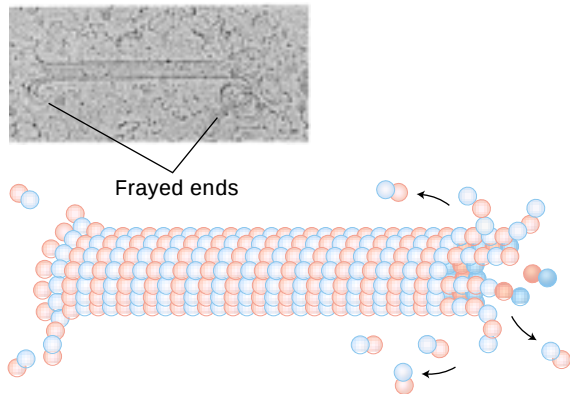
▲ **FIGURE 20-7 Stages in assembly of microtubules.** Free $\alpha\beta$ -tubulin dimers associate longitudinally to form short protofilaments (1). These protofilaments are probably unstable and quickly associate laterally into more stable curved sheets (2). Eventually, a sheet wraps around into a microtubule with 13 protofilaments. The microtubule then grows by the addition of subunits to the ends of protofilaments composing the microtubule wall (3). The free tubulin dimers have GTP (red dot) bound to the exchangeable nucleotide-binding site on the β -tubulin monomer. After incorporation of a dimeric subunit into a microtubule, the GTP on the β -tubulin (but not on the α -tubulin) is hydrolyzed to GDP. If the rate of polymerization is faster than the rate of GTP hydrolysis, then a cap of GTP-bound subunits is generated at the (+) end, although the bulk of β -tubulin in a microtubule will contain GDP. The rate of polymerization is twice as fast at the (+) end as at the (-) end.

Microtubule assembly comprises three steps: (1) protofilaments assemble from $\alpha\beta$ -tubulin subunits, (2) protofilaments associate to form the wall of the microtubule, and (3) the addition of more subunits to the ends of the protofilaments elongates the microtubule (Figure 20-7). In the electron microscope, the ends of growing microtubules frequently appear uneven because some protofilaments elongate faster than other protofilaments. The appearance of microtubules undergoing shortening is quite different, suggesting that the mechanism of disassembly differs from that of assembly (Figure 20-8). Under shortening conditions, the microtubule ends are splayed, as if the lateral interactions between protofilaments have been broken. When frayed apart and freed from lateral stabilizing interactions, the protofilaments may depolymerize by endwise dissociation of tubulin subunits. The splayed appearance of a shortening microtubule provided clues about the potential instability of a microtubule.

(a) Assembly (elongation)

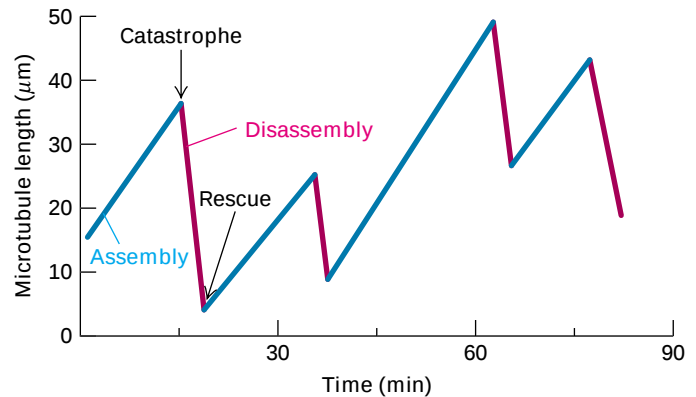


(b) Disassembly (shrinkage)



▲ **EXPERIMENTAL FIGURE 20-8 Cryoelectron microscopy allows observation of disassembled microtubules.**

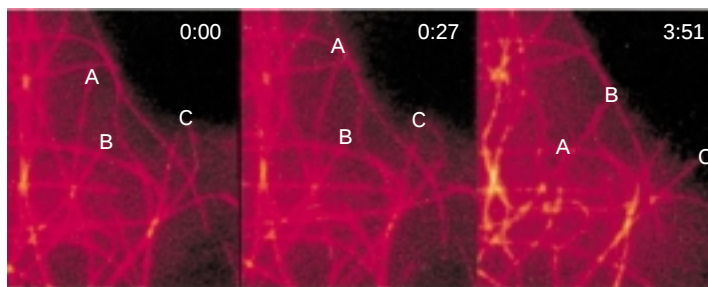
Microtubules undergoing assembly (a) or disassembly (b) can be quickly frozen in liquid ethane and examined in the frozen state in a cryoelectron microscope. In assembly conditions, microtubule ends are relatively smooth; occasionally a short protofilament is seen to extend from one end. In disassembly conditions, the protofilaments splay at the microtubule ends, giving the ends a frayed appearance. Splaying of protofilaments probably promotes the loss of tubulin subunits from their ends, leading to shrinkage of the microtubule. [Micrographs courtesy of E. Mandelkow and E. M. Mandelkow.]



▲ **EXPERIMENTAL FIGURE 20-9 Rate of microtubule growth in vitro is much slower than shrinkage.** Individual microtubules can be observed in the light microscope, and their lengths can be plotted at different times during assembly and disassembly. Assembly and disassembly each proceed at uniform rates, but there is a large difference between the rate of assembly and that of disassembly, as seen in the different slopes of the lines. Shortening of a microtubule is much more rapid ($7 \mu\text{m}/\text{min}$) than growth ($1 \mu\text{m}/\text{min}$). Notice the abrupt transitions to the shrinkage stage (catastrophe) and to the elongation stage (rescue). [Adapted from P. M. Bayley, K. K. Sharma, and S. R. Martin, 1994, in *Microtubules*, Wiley-Liss, p. 118.]

Dynamic Instability Is an Intrinsic Property of Microtubules

Under appropriate in vitro conditions, some individual microtubules oscillate between growth and shortening phases (Figure 20-9). In all cases, the rate of microtubule growth is much slower than the rate of shortening. When first discovered, this behavior of microtubules, termed *dynamic instability*, was surprising to researchers because they expected that under any condition all the microtubules in a solution or the same cytosol would behave identically.



▲ **EXPERIMENTAL FIGURE 20-10 Fluorescence microscopy reveals in vivo growth and shrinkage of individual microtubules.** Fluorescently-labeled tubulin was microinjected into cultured human fibroblasts. The cells were chilled to depolymerize preexisting microtubules into tubulin dimers and were then incubated at 37°C to allow repolymerization,

thus incorporating the fluorescent tubulin into all the cell's microtubules. A region of the cell periphery was viewed in the fluorescence microscope at 0 second, 27 seconds later, and 3 minutes 51 seconds later (left to right panels). In this period, several microtubules elongate and shorten. The letters mark the position of ends of three microtubules. [From P. J. Sammak and G. Borisy, 1988, *Nature* **332**:724.]

The results of subsequent *in vivo* studies showed that individual cytosolic and mitotic microtubules display dynamic instability. In one set of experiments, fluorescent $\alpha\beta$ -tubulin subunits were microinjected into live cultured cells. The cells were chilled to depolymerize preexisting microtubules into tubulin dimers and then incubated at 37 °C to allow repolymerization, thus incorporating the fluorescent tubulin into all the cellular microtubules. Video recordings of a small region in labeled cells showed that some microtubules became longer, others became shorter, and some appeared alternately to grow and to shrink over a period of several minutes (Figure 20-10). Because most microtubules in a cell associate by their (–) ends with MTOCs, their instability is largely limited to the (+) ends.

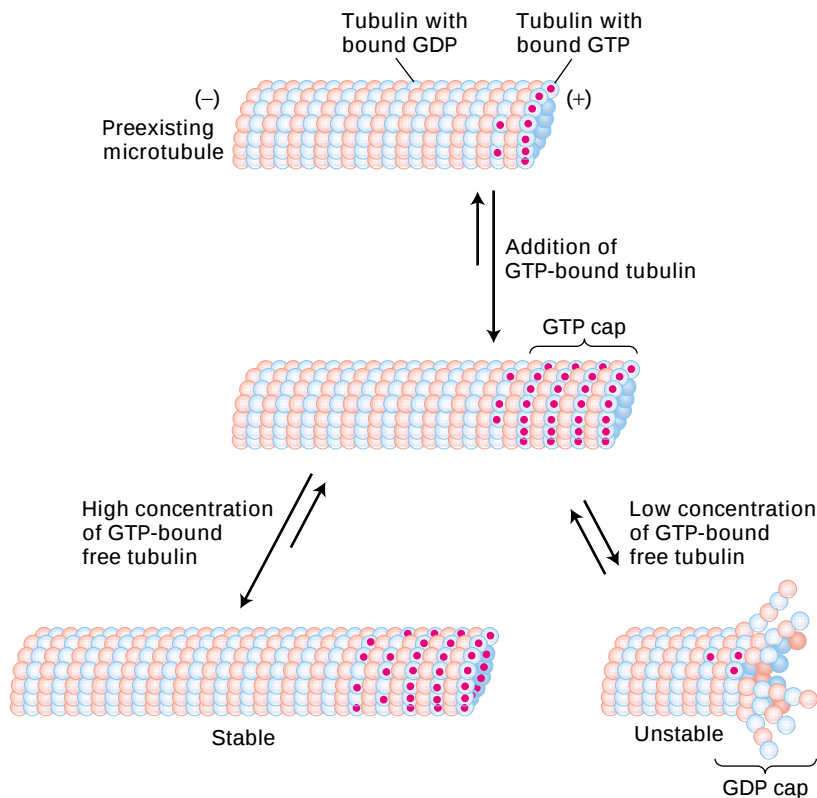
Two conditions influence the stability of microtubules. First, the oscillations between growth and shrinkage *in vitro* occur at tubulin concentrations near the C_c . As already stated, at tubulin concentrations above the C_c , the entire population of microtubules grows and, at concentrations below the C_c , all microtubules shrink. At concentrations near the C_c , however, some microtubules grow, whereas others shrink. The second condition affecting microtubule stability is whether GTP or GDP occupies the exchangeable nucleotide-binding site on β -tubulin at the (+) end of a microtubule (Figure 20-11). Because dissociation (“off” rate) of a GDP-tubulin dimer is four orders of magnitude as fast as that of a GTP-tubulin dimer, a microtubule is destabilized and depolymerizes rapidly if the (+) end becomes capped with subunits containing GDP- β -tubulin rather than GTP- β -tubulin. This situation can arise

when a microtubule shrinks rapidly, exposing GDP- β -tubulin in the walls of the microtubule, or when a microtubule grows so slowly that the hydrolysis of β -tubulin-bound GTP converts it into GDP before additional subunits can be added to the (+) end of the microtubule. Before a shortening microtubule vanishes entirely, it can be “rescued” and start to grow if tubulin subunits with bound GTP add to the (+) end before the bound GTP hydrolyzes. Thus the parameters that determine the stability of a microtubule are the growth rate, the shrinkage rate, the catastrophe frequency, and the rescue frequency.

Numerous Proteins Regulate Microtubule Dynamics and Cross-Linkage to Other Structures

A large number of proteins influence the assembly and stability of microtubules and their association with other cell structures (Table 20-1). These proteins are collectively called **microtubule-associated proteins (MAPs)** because most copurify with microtubules isolated from cells. The results of immunofluorescence localization studies also have shown a parallel distribution of MAPs and microtubules in cells—strong evidence for their interaction *in vivo*.

MAPs are classified into two groups on the basis of their function. One group stabilizes microtubules. The structure of a stabilizing MAP consists of two domains—a basic microtubule-binding domain and an acidic projection domain. In the electron microscope, the projection domain appears as a filamentous arm that extends from the wall of the microtubule. This arm can bind to membranes, intermediate



◀ **FIGURE 20-11 Dynamic instability model of microtubule growth and shrinkage.** GTP-bound $\alpha\beta$ -tubulin subunits (red) add preferentially to the (+) end of a preexisting microtubule. After incorporation of a subunit, the GTP (red dot) bound to the β -tubulin monomer is hydrolyzed to GDP. Only microtubules whose (+) ends are associated with GTP-tubulin (those with a GTP cap) are stable and can serve as primers for the polymerization of additional tubulin. Microtubules with GDP-tubulin (blue) at the (+) end (those with a GDP cap) are rapidly depolymerized and may disappear within 1 minute. At high concentrations of unpolymerized GTP-tubulin, the rate of addition of tubulin is faster than the rate of hydrolysis of the GTP bound in the microtubule or the rate of dissociation of GTP-tubulin from microtubule ends; thus the microtubule grows. At low concentrations of unpolymerized GTP-tubulin, the rate of addition of tubulin is decreased; consequently, the rate of GTP hydrolysis exceeds the rate of addition of tubulin subunits and a GDP cap forms. Because the GDP cap is unstable, the microtubule end peels apart to release tubulin subunits. [See T. Mitchison and M. Kirschner, 1984, *Nature* **312**:237; M. Kirschner and T. Mitchison, 1986, *Cell* **45**:329; and R. A. Walker et al., 1988, *J. Cell Biol.* **107**:1437.]

TABLE 20-1 Proteins That Modulate Microtubule (MT) Dynamics

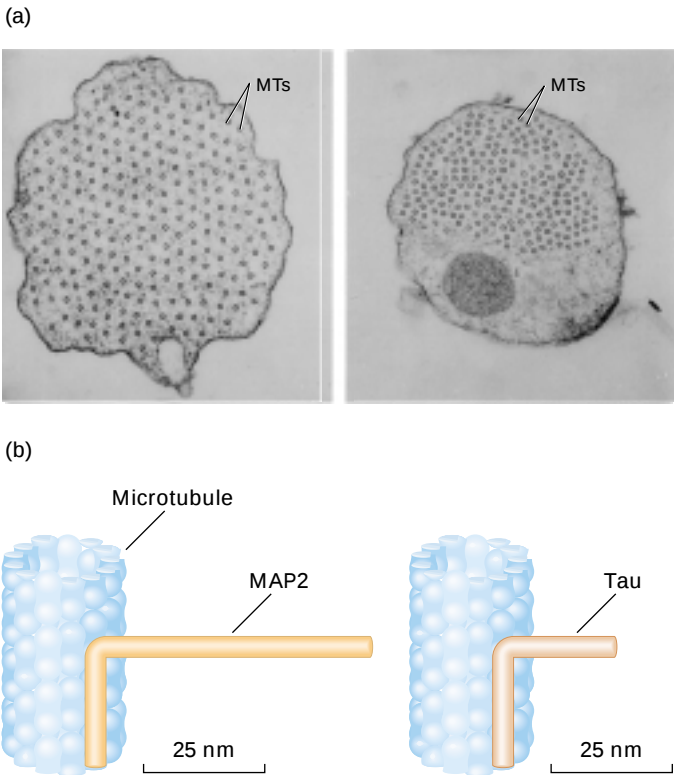
Protein	MW	Location	Function
MICROTUBULE-STABILIZING PROTEINS			
MAP1	250,000–300,000 (heavy chain)	Dendrites and axons; non-neuronal cells	Assembles and stabilizes MTs
MAP2	42,000 and 200,000	Dendrites	Assembles and cross-links MTs to one another and to intermediate filaments
MAP4	210,000	Most cell types	Stabilizes MTs
Tau	55,000–62,000	Dendrites and axons	Assembles, stabilizes, and cross-links MTs
CLIP170	170,000	Most cell types	Cross-links MTs to endosomes and chromosomes
MICROTUBULE-DESTABILIZING PROTEINS			
Katanin	84,000	Most cell types	Microtubule severing
Op18 (stathmin)	18,000	Most cell types	Binds tubulin dimers

filaments, or other microtubules, and its length controls how far apart microtubules are spaced (Figure 20-12).

The microtubule-binding domain contains several repeats of a conserved, positively charged four-residue amino acid sequence that binds the negatively charged C-terminal part of tubulin. This binding is postulated to neu-

tralize the charge repulsion between tubulin subunits within a microtubule, thereby stabilizing the polymer. MAP1A and MAP1B are large, filamentous molecules found in axons and dendrites of neurons as well as in non-neuronal cells. Each of these MAPs is derived from a single precursor polypeptide, which is proteolytically processed in a cell to generate one light chain and one heavy chain.

Other stabilizing MAPs include MAP2, MAP4, Tau, and CLIP170. MAP4, the most widespread of all the MAPs, is found in neuronal and non-neuronal cells. In mitosis, MAP4 regulates microtubule stability, and CLIP170 cross-links microtubules to chromosomes. MAP2 is found only in dendrites, where it forms fibrous cross-bridges between microtubules and links microtubules to intermediate filaments. Tau, which is much smaller than most other MAPs, is present in both axons and dendrites. This protein exists in several iso-



◀ **EXPERIMENTAL FIGURE 20-12 Spacing of microtubules depends on length of projection domain in bound microtubule-associated proteins.** Insect cells transfected with DNA expressing either long-armed MAP2 protein or short-armed Tau protein grow long axonlike processes. (a) Electron micrographs of cross sections through the processes induced by the expression of MAP2 (left) or Tau (right) in transfected cells. Note that the spacing between microtubules (MTs) in MAP2-containing cells is larger than in Tau-containing cells. Both cell types contain approximately the same number of microtubules, but the effect of MAP2 is to enlarge the caliber of the axonlike process. (b) Diagrams of association between microtubules and MAPs. Note the difference in the lengths of the projection arms in MAP2 and Tau. [Part (a) from J. Chen et al., 1992, *Nature* **360**:674.]

forms derived from alternative splicing of a *tau* mRNA. The ability of Tau to cross-link microtubules into thick bundles may contribute to the stability of axonal microtubules. Findings from gene transfection experiments implicate Tau in axonal elongation. Deletion of the genes encoding Tau and MAP1B leads to more severe phenotypes of axonal dysgenesis and lethality. Furthermore, aberrant polymerization of Tau into filaments is linked to neurodegenerative diseases such as human dementia in Alzheimer's patients.

When stabilizing MAPs coat the outer wall of a microtubule, tubulin subunits are unable to dissociate from the ends of that microtubule. Although bound MAPs generally dampen the rate of microtubule disassembly, the assembly of microtubules is affected to varying degrees: some MAPs, such as Tau and MAP4, stabilize microtubules, whereas other MAPs do not. Because of the effect of assembly MAPs on microtubule dynamics, modulating the binding of MAPs can control the length of microtubules. In most cases, this control is accomplished by the reversible phosphorylation of the MAP projection domain. Phosphorylated MAPs are unable to bind to microtubules; thus they promote microtubule disassembly. **MAP kinase**, a key enzyme for phosphorylating MAPs, is a participant in many signal-transduction pathways (Chapter 14), indicating that MAPs are targets of many extracellular signals. MAPs, especially MAP4, are also phosphorylated by a **cyclin-dependent kinase (CDK)** that plays a major role in controlling the activities of various proteins in the course of the cell cycle (Chapter 21).

A second group of MAPs directly destabilizes microtubules in many cell types. One of this group, called katanin, severs intact cytosolic microtubules by an ATP-dependent process. Internal bonds between tubulin subunits in the microtubule wall are broken, causing microtubules to fragment. This activity may release microtubules at the MTOC. Another protein, called Op18 or stathmin, increases the frequency of rapid disassembly of microtubules in the mitotic spindle. This protein may act by binding tubulin dimers, thereby reducing the pool of dimers available for polymerization. Phosphorylation inactivates Op18 and inhibits its destabilizing effect.

Colchicine and Other Drugs Disrupt Microtubule Dynamics

Some of the earliest studies of microtubules employed several drugs that inhibit mitosis, a cell process that depends on microtubule assembly and disassembly. Two such drugs isolated from plants, *colchicine* and *taxol*, have proved to be very powerful tools for probing microtubule function, partly because they bind only to $\alpha\beta$ -tubulin or microtubules and not to other proteins and because their concentrations in cells can be easily controlled.

Colchicine and a synthetic relative, colcemid, have long been used as mitotic inhibitors. In cells exposed to high concentrations of colcemid, cytosolic microtubules depolymerize, leaving an MTOC. However, when plant or animal cells are exposed to low concentrations of colcemid, the microtubules remain and the cells become “blocked” at **meta-**

phase, the mitotic stage at which the duplicated chromosomes are fully condensed (see Figure 9-3). When the treated cells are washed with a colcemid-free solution, colcemid diffuses from the cell and mitosis resumes normally. Thus experimenters commonly use colcemid to accumulate metaphase cells for cytogenetic studies; removal of the colcemid leaves a population of cells whose cell cycle is in synchrony. Such synchronous populations are advantageous for studies of the cell cycle (Chapter 21).

The interface between α -tubulin and β -tubulin monomers in dimeric tubulin contains a high-affinity but reversible binding site for colchicine. Colchicine-bearing tubulin dimers, at concentrations much less than the concentration of free tubulin subunits, can add to the end of a growing microtubule. However, the presence of one or two colchicine-bearing tubulins at the end of a microtubule prevents the subsequent addition or loss of other tubulin subunits. Thus colchicine “poisons” the end of a microtubule and alters the steady-state balance between assembly and disassembly. As a result of this disruption of microtubule dynamics, mitosis is inhibited in cells treated with low concentrations of colchicine.

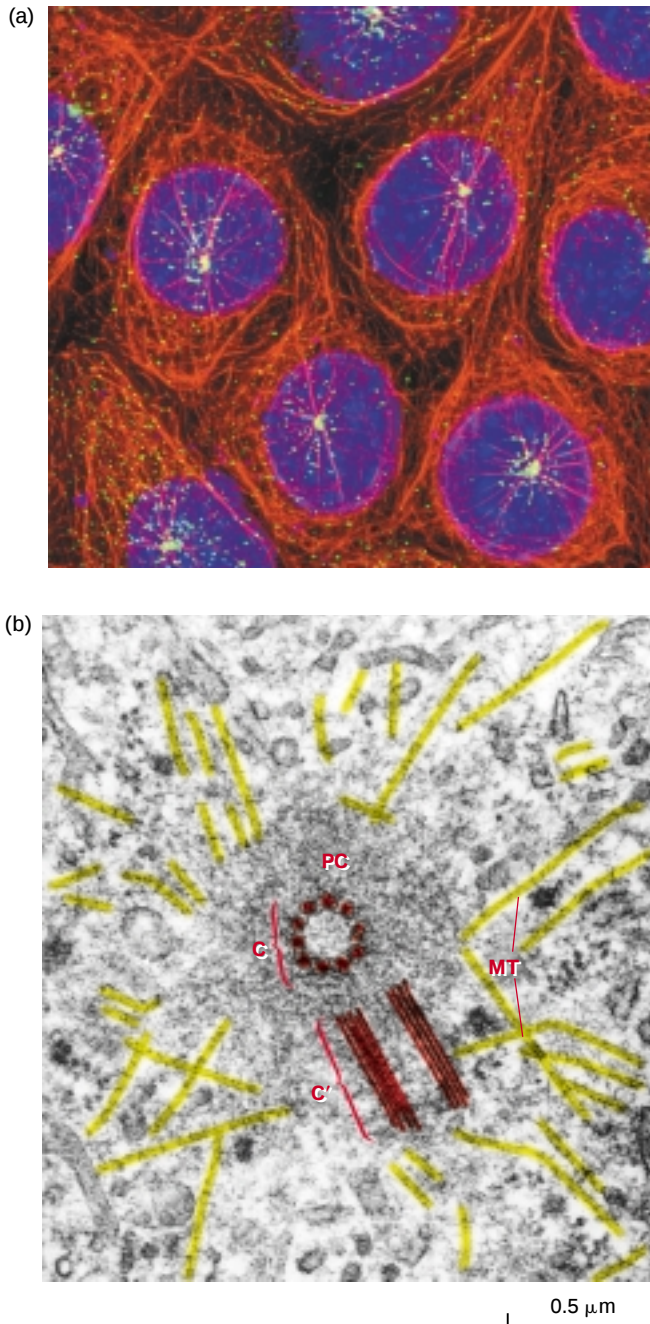
Other drugs bind to different sites on tubulin dimers or to microtubules and therefore affect microtubule stability through different mechanisms. For example, at low concentrations, taxol binds to microtubules and stabilizes them by inhibiting their shortening.



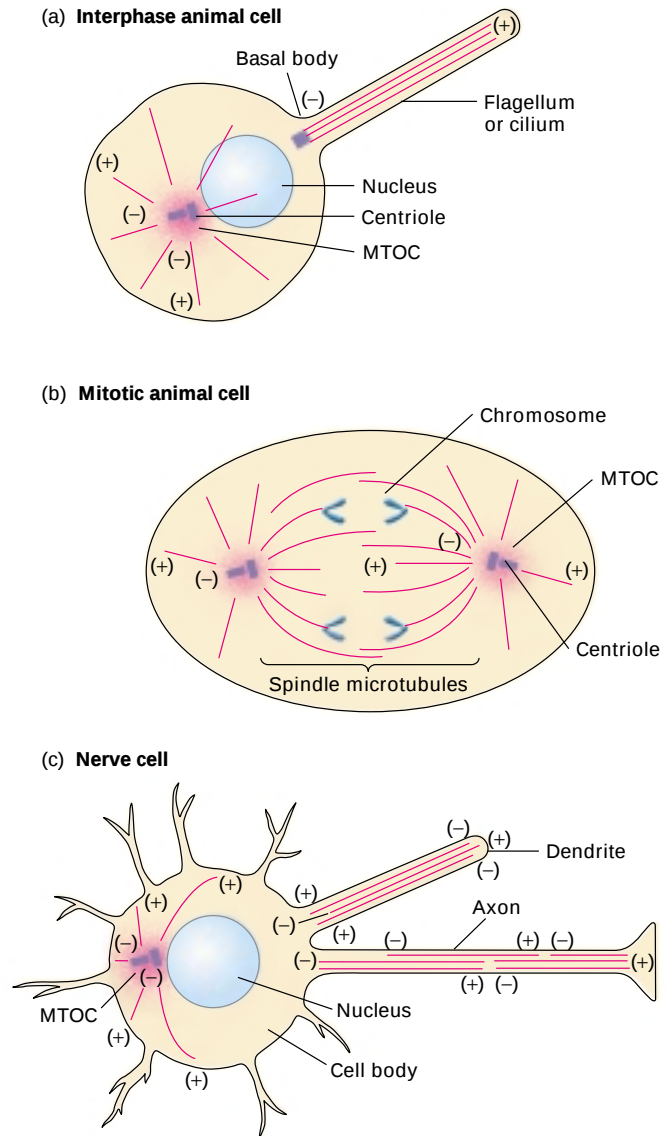
Drugs that disturb the assembly and disassembly of microtubules have been widely used to treat various diseases. Indeed, more than 2500 years ago, the ancient Egyptians treated heart problems with colchicine. Nowadays, this drug is used primarily in the treatment of gout and certain other diseases affecting the joints and skin. Other inhibitors of microtubule dynamics, including taxol, are effective anticancer agents and are used in the treatment of ovarian cancer. ■

MTOCs Orient Most Microtubules and Determine Cell Polarity

In an interphase fibroblast cell, cytosolic microtubules are arranged in a distinctive hub-and-spoke array that lies at the center of a cell (Figure 20-13a). The microtubule spokes radiate from a central site occupied by the **centrosome**, which is the primary microtubule-organizing center in many interphase cells. We will use the term **MTOC** to refer to any of the structures used by cells to nucleate and organize microtubules. In animal cells, the MTOC is usually a centrosome, a collection of microtubule-associated proteins that sometimes but not always contains a pair of **centrioles** (Figure 20-13b). The centrioles, each a pinwheel array of triplet microtubules, lie in the center of the MTOC but do not make direct contact with the (–) ends of the cytosolic microtubules. Centrioles are not present in the MTOCs of plants and fungi; moreover, some epithelial cells and newly fertilized eggs from animals also lack centrioles. Thus, it is the associated



▲ **EXPERIMENTAL FIGURE 20-13 The centrosome, which functions as a microtubule-organizing center, contains a pair of orthogonal centrioles in most animal cells.** (a) Micrograph showing several cells each with an MTOC identified by fluorescently labeled antibodies against PCM1, a centrosomal protein. (b) Electron micrograph of the MTOC in an animal cell. The pair of centrioles (red), C and C', in the center are oriented at right angles; thus one centriole is seen in cross section, and the other longitudinally. Surrounding the centrioles is a cloud of material, the pericentriolar (PC) matrix, which contains γ -tubulin and pericentrin. Embedded within the MTOC, but not contacting the centrioles, are the (-) ends of microtubules (MT; yellow). [Part (a) from A. Kubo and S. Tsukita, 2003, *J. Cell Sci.* **116**:919. Part (b) from B. R. Brinkley, 1987, in *Encyclopedia of Neuroscience*, vol. 2, Birkhauser Press, p. 665; courtesy of B. R. Brinkley.]



▲ **FIGURE 20-14 Orientation of cellular microtubules.** (a) In interphase animal cells, the (-) ends of most microtubules are proximal to the MTOC. Similarly, the microtubules in flagella and cilia have their (-) ends continuous with the basal body, which acts as the MTOC for these structures. (b) As cells enter mitosis, the microtubule network rearranges, forming a mitotic spindle. The (-) ends of all spindle microtubules point toward one of the two MTOCs, or poles, as they are called in mitotic cells. (c) In nerve cells, the (-) ends of all axonal microtubules are oriented toward the base of the axon, but dendritic microtubules have mixed polarities.

proteins in an MTOC that have the capacity to organize cytosolic microtubules.

Because microtubules assemble from the MTOC, microtubule polarity becomes fixed in a characteristic orientation (Figure 20-14). In most interphase animal cells, for instance, the (-) ends of microtubules are closest to the MTOC. In mitosis, the centrosome duplicates and migrates to new positions flanking the nucleus. There the centrosome becomes

the organizing center for microtubules forming the mitotic apparatus, which will separate the chromosomes into the daughter cells in mitosis. The microtubules in the axon of a nerve cell, which help stabilize the long process, are all oriented in the same direction.

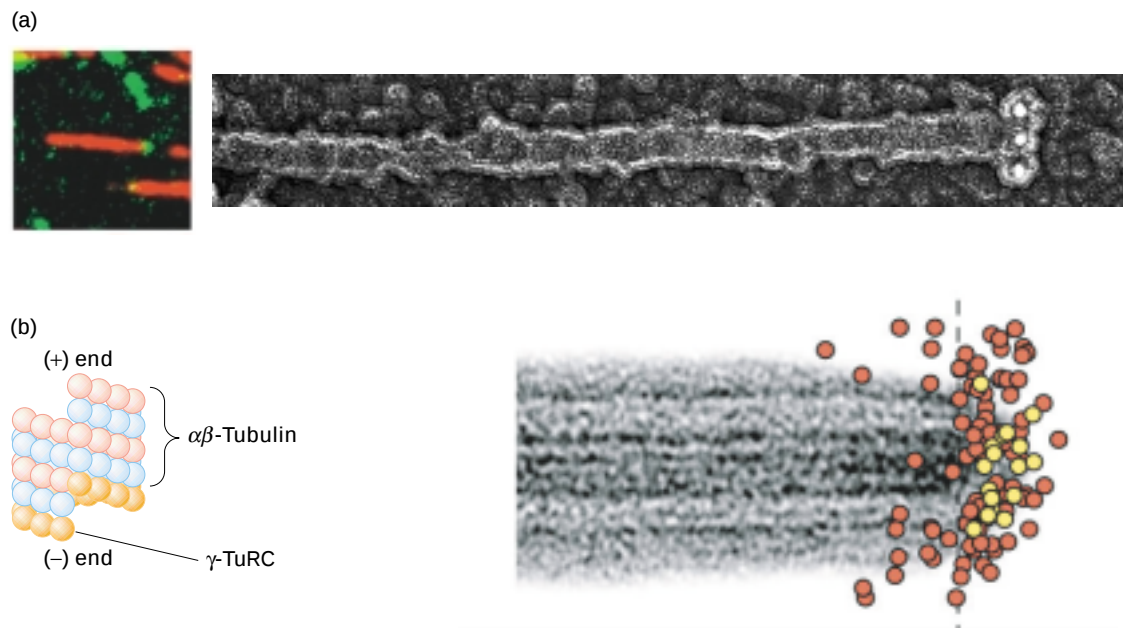
In contrast with the single perinuclear MTOC present in most interphase animal cells, plant cells, polarized epithelial cells, and embryonic cells contain hundreds of MTOCs, which are distributed throughout the cell, often near the cell cortex. In plant cells and polarized epithelial cells, a cortical array of microtubules aligns with the cell axis. In both cell types, the polarity of the cell is linked to the orientation of the microtubules.

The γ -Tubulin Ring Complex Nucleates Polymerization of Tubulin Subunits

The MTOC organizes cytosolic microtubules by first nucleating microtubule assembly and then anchoring and releasing microtubules. Despite its amorphous appearance, the pericentriolar material of an MTOC is an ordered lattice that contains many proteins that are necessary for initiating the assembly of microtubules (see Figure 20-13). One of these proteins, γ -tubulin, was first identified in genetic studies designed

to discover proteins that interact with β -tubulin. The results of subsequent studies demonstrated that γ -tubulin and the lattice protein pericentrin are part of the pericentriolar material of centrosomes; these proteins have also been detected in MTOCs that lack a centriole. The finding that the introduction of antibodies against γ -tubulin into cells blocks microtubule assembly implicates γ -tubulin as a necessary factor in nucleating the polymerization of tubulin subunits.

Approximately 80 percent of the γ -tubulin in cells is part of a 25S complex, which has been isolated from extracts of frog oocytes and fly embryos. Named the *γ -tubulin ring complex* (γ -TuRC) for its ringlike appearance in the electron microscope, the complex comprises eight polypeptides and measures 25 nm in diameter. Findings from in vitro experiments show that the γ -TuRC can directly nucleate microtubule assembly at subcritical tubulin concentrations—that is, at concentrations below which polymerization would not take place in the absence of the γ -TuRC. To investigate how γ -TuRC associates with microtubules, scientists performed immunolabeling experiments with the use of gold-conjugated antibodies specific for γ -TuRC components, either γ -tubulin or XGRIP. The results of these studies reveal that complexes are localized to one end of a microtubule and are not present along the sides (Figure 20-15a). This location is consistent with a role for



▲ EXPERIMENTAL FIGURE 20-15 The γ -tubulin ring complex (γ -TuRC) is localized to one end of the microtubule.

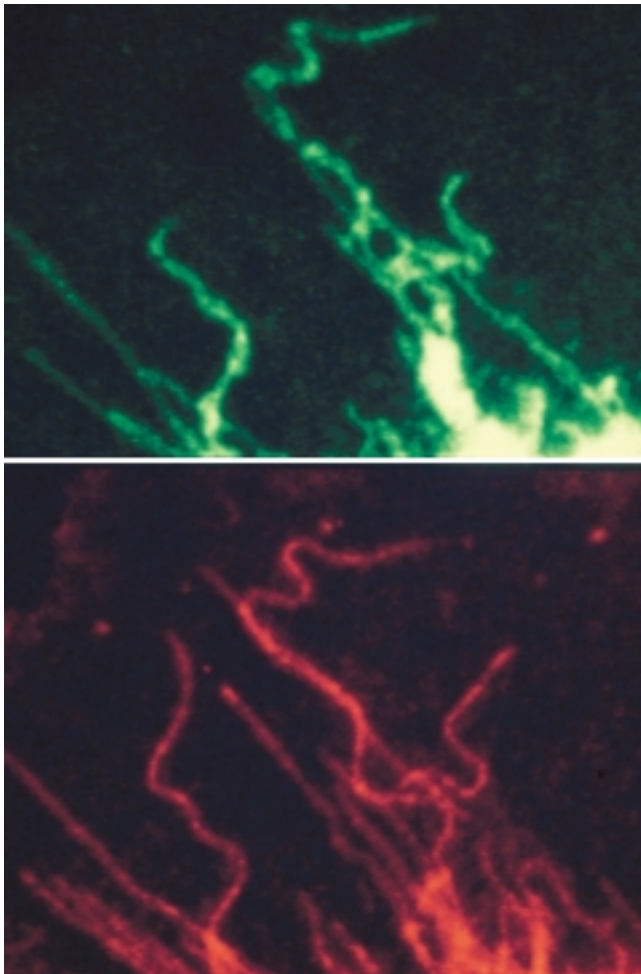
(a) A fluorescence micrograph (left) and an electron micrograph (right) of microtubules stained with gold-labeled antibodies to γ -tubulin or XGRIP, a microtubule-binding protein. Both proteins are components of the γ -TuRC. The labeled proteins are localized to one end of the microtubules. (b) A model of the γ -TuRC. This complex is thought to nucleate microtubule assembly by

presenting a row of γ -tubulin subunits, which can directly bind α -tubulin subunits. This model is supported by plotting the positions of gold-labeled antibodies to either γ -tubulin (red) or XGRIP109 (yellow) from several experiments onto a microtubule end. [Parts (a) and (b, right) from T. J. Keating and G. G. Borisy, 2000, *Nature Cell Biol.* **2**:352; courtesy of T. J. Keating and G. G. Borisy. Part (b, left) modified from C. Wiese and Y. Zheng, 1999, *Curr. Opin. Struc. Biol.* **9**:250.]

γ -TuRC in nucleating microtubule assembly. A model of γ -TuRC based on electron microscopy shows γ -tubulin in contact with the (–) end of a microtubule (Figure 20-15b).

Cytoplasmic Organelles and Vesicles Are Organized by Microtubules

Fluorescence microscopy reveals that membrane-limited organelles such as the endoplasmic reticulum (ER), Golgi, endosomes, and mitochondria are associated with microtubules. For instance, in cultured fibroblasts stained with anti-tubulin antibodies and DiOC₆, a fluorescent dye specific for the ER, the anastomosing ER network in the cytosol is seen to colocalize with microtubules (Figure 20-16). If cells are treated with a microtubule-depolymerizing drug, the ER



▲ **EXPERIMENTAL FIGURE 20-16** Fluorescence microscopy reveals colocalization of endoplasmic reticulum membranes and cytosolic microtubules. DiOC₆, an ER-binding fluorescent dye (green), and fluorescently-labeled anti-tubulin antibodies (red) were used to stain a cultured frog fibroblast. The alignment of the ER network and microtubules in many but not all regions of the cytoplasm is evident because the cell has sparse microtubules. [Courtesy of M. Terasaki.]

loses its networklike organization. After the drug has been washed from the cells, tubular fingers of ER grow as new microtubules assemble. In cell-free systems, the ER can be reconstituted with microtubules and an ER-rich cell extract. Even under this cell-free regime, ER membranes elongate along microtubules. This close association between the ER and intact microtubules suggests that proteins bind ER membranes to microtubules.

The role of microtubules in organizing the Golgi complex also has been studied extensively. In interphase fibroblasts, the Golgi complex is concentrated near the MTOC. In mitosis (or after the depolymerization of microtubules by colcemid), the Golgi complex breaks into small vesicles that are dispersed throughout the cytosol. When the cytosolic microtubules re-form during interphase (or after removal of the colcemid), the Golgi vesicles move along these microtubule tracks toward the MTOC, where they reaggregate to form large membrane complexes.

These observations were among the first to suggest that microtubules play a role in the intracellular transport of membrane-limited organelles and vesicles. Other examples of such transport and the motor proteins that power them are described in Section 20.2.

KEY CONCEPTS OF SECTION 20.1

Microtubule Organization and Dynamics

- Tubulins belong to an ancient family of GTPases that polymerize to form microtubules, hollow cylindrical structures 25 nm in diameter.
- Microtubules, like actin microfilaments, exhibit both structural and functional polarity.
- Dimeric $\alpha\beta$ -tubulin subunits interact end-to-end to form protofilaments, which associate laterally into microtubules (see Figure 20-7).
- Microtubules exhibit structural polarity. Subunits are added and lost preferentially at one end, the (+) end.
- Assembly and disassembly of microtubules depends on the critical concentration, C_c , of $\alpha\beta$ -tubulin subunits. Above the C_c , microtubules assemble; below the C_c , microtubules disassemble.
- Microtubules exhibit two dynamic phenomena that are pronounced at tubulin concentrations near the C_c : (1) treadmilling, the addition of subunits at one end and their loss at the other end, and (2) dynamic instability, the oscillation between lengthening and shortening (see Figure 20-9).
- The balance between growth and shrinkage of unstable microtubules depends on whether the exchangeable GTP bound to β -tubulin is present on the (+) end or whether it has been hydrolyzed to GDP (see Figure 20-11).
- Microtubule-associated proteins (MAPs) organize microtubules and affect their stability. Some MAPs prevent

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 $10^7/\text{mm}^2$) 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 unicel-

lular organisms have a single flagellum, which propels the cells forward at velocities approaching 1 mm/s.

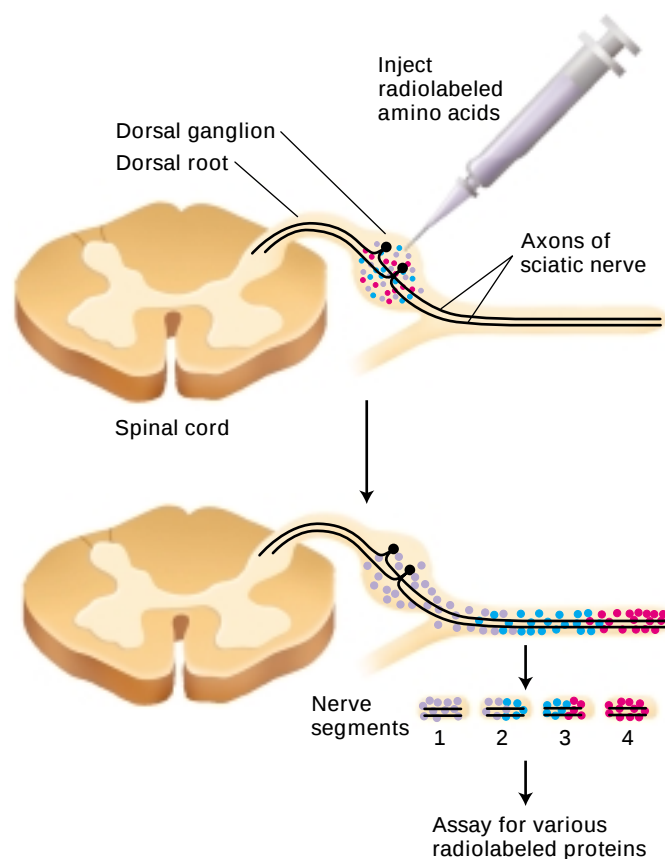
In this section, we first consider the transport of materials in axons. Studies of such *axonal transport*, a process first discovered more than 50 years ago, have contributed greatly to our understanding of microtubule-associated intracellular transport. We then consider the structure and function of 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.

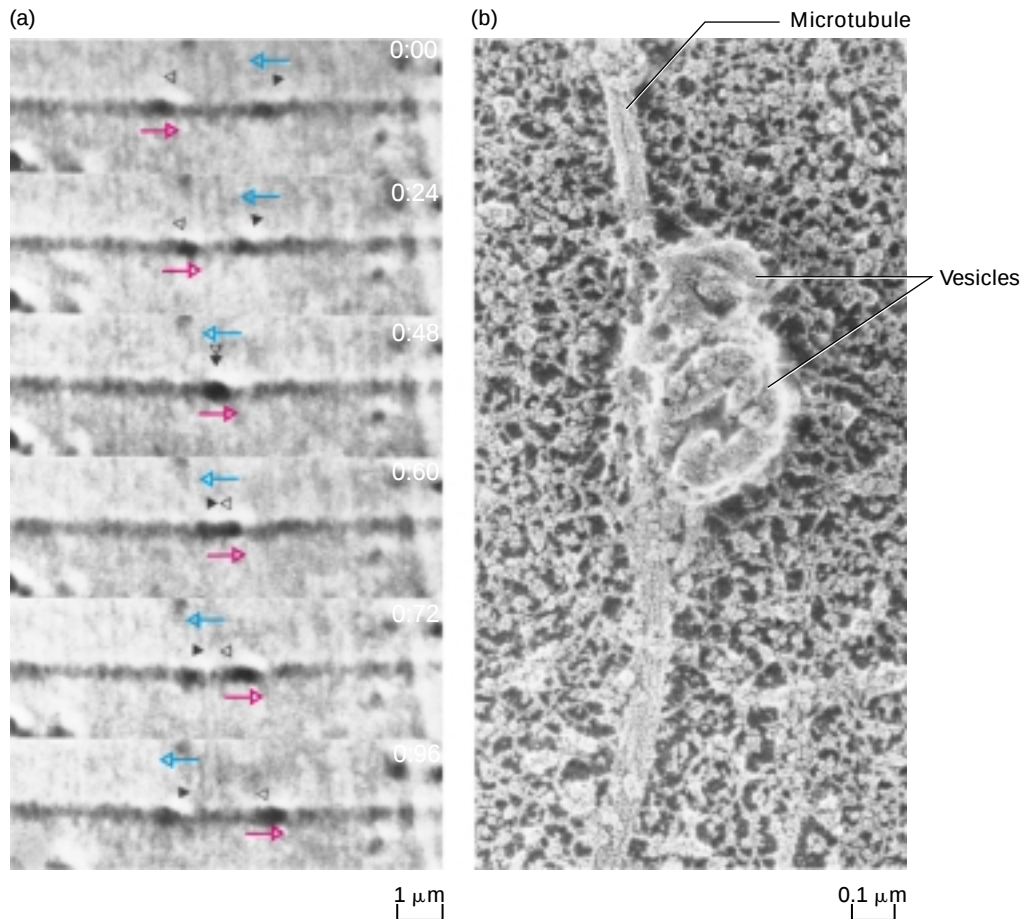
Axonal Transport Along Microtubules Is in Both Directions

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.



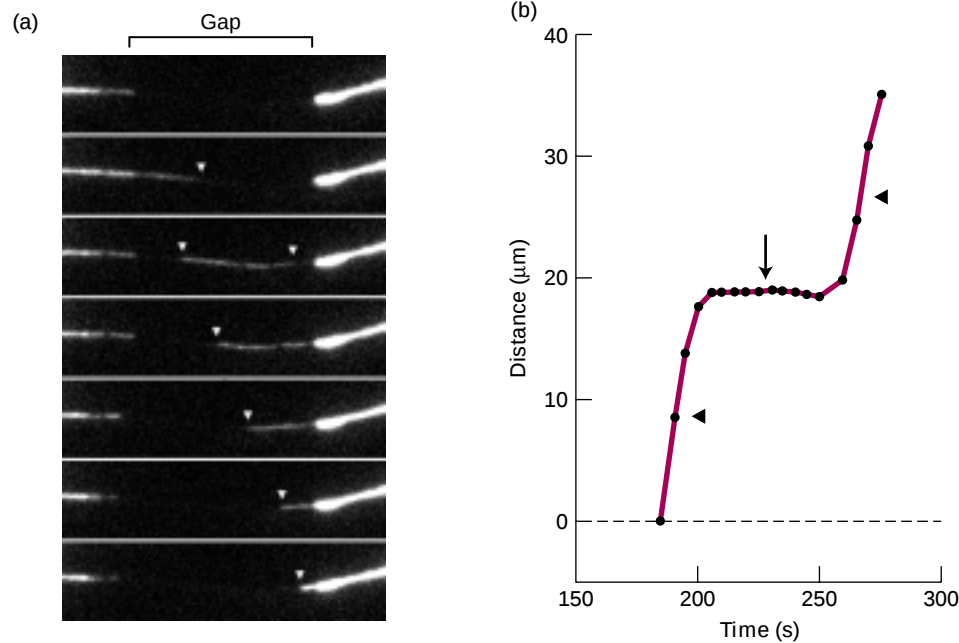


▲ **EXPERIMENTAL FIGURE 20-18 DIC microscopy demonstrates microtubule-based vesicle transport in vitro.** (a) The cytoplasm was squeezed from a squid giant axon with a roller onto a glass coverslip. After buffer containing ATP was added to the preparation, it was viewed in a differential interference 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.]

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 $\mu\text{m/s}$. 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.

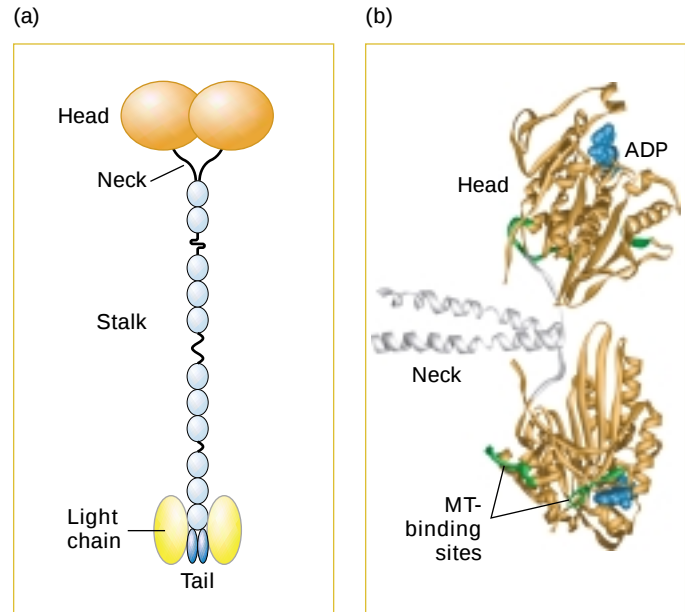
Kinesin-dependent movement of vesicles can be tracked by *in vitro* motility assays similar to those used to study myosin-dependent movements. In one type of assay, a vesicle

► **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. Struct. 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.

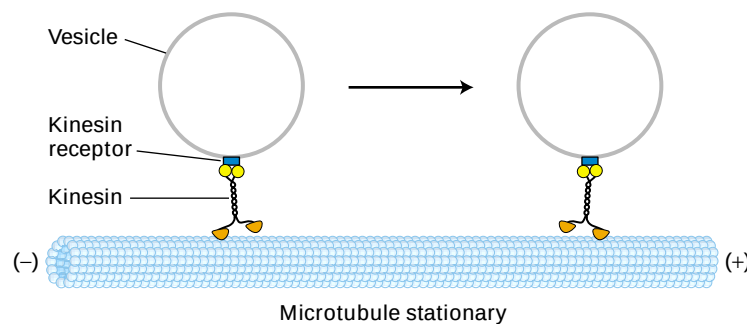
Most Kinesins Are Processive (+) End-Directed Motor Proteins

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.]



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 *tetratricopeptide 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 tetratricopeptide 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.

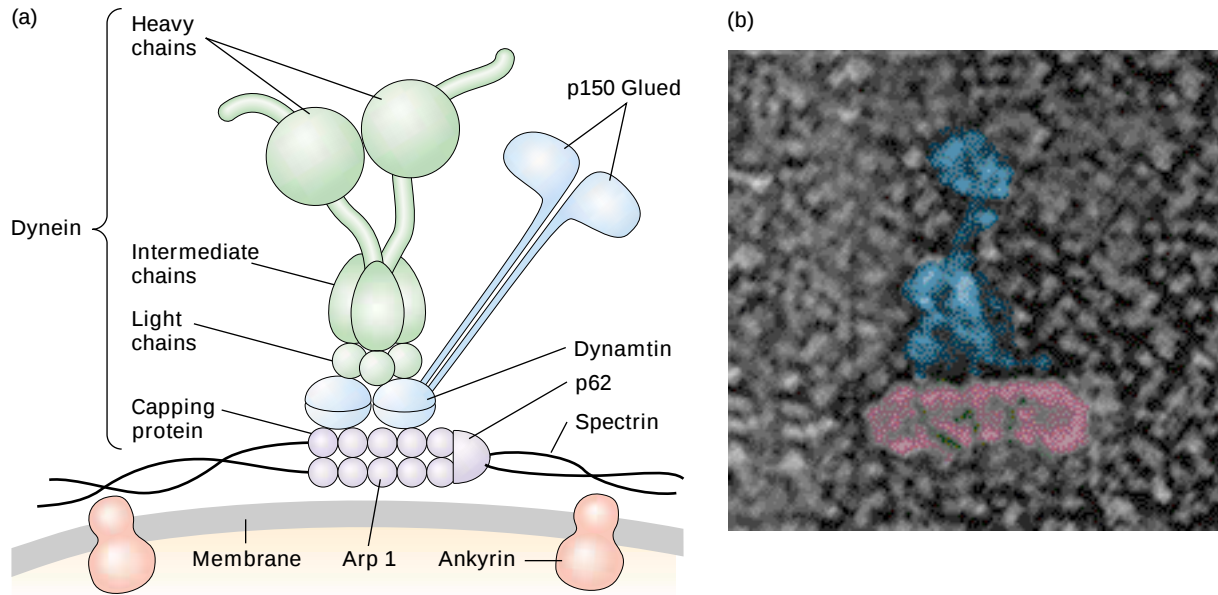
Two fundamental properties of the kinesin motor—its step size and force—have been determined in optical-trap experiments similar to those performed on myosin (see Figure 19-18). Findings from these studies show that a dimeric kinesin molecule (e.g., kinesin I) exerts a force of 6 piconewtons, which is 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 mi-

cro-tubule 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 dynamitin 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 dynamitin/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.

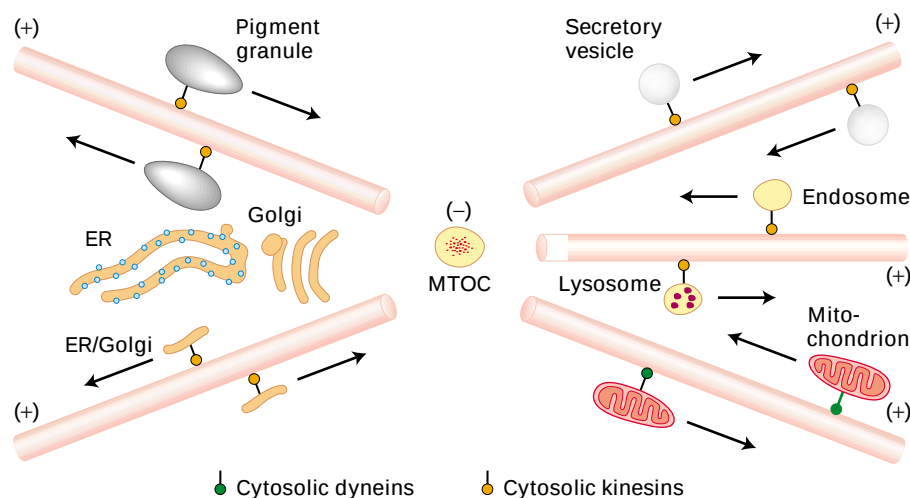
Like kinesin I, cytosolic dynein is a two-headed molecule, with two identical or nearly identical heavy chains forming the head domains. However, unlike kinesin, dynein cannot mediate cargo transport by itself. Rather, dynein-related transport requires *dynactin*, a large protein complex that links vesicles 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 dynamitin, 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.

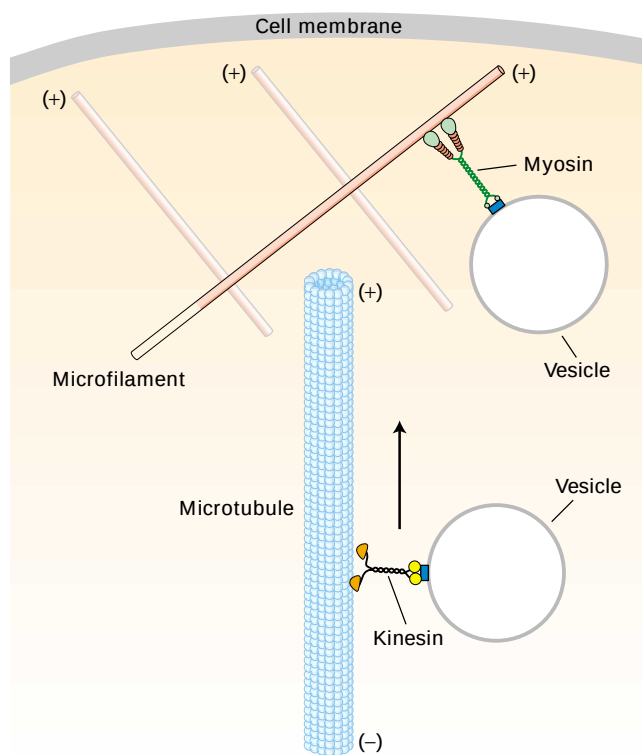
Multiple Motor Proteins Sometimes Move the Same Cargo

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.]

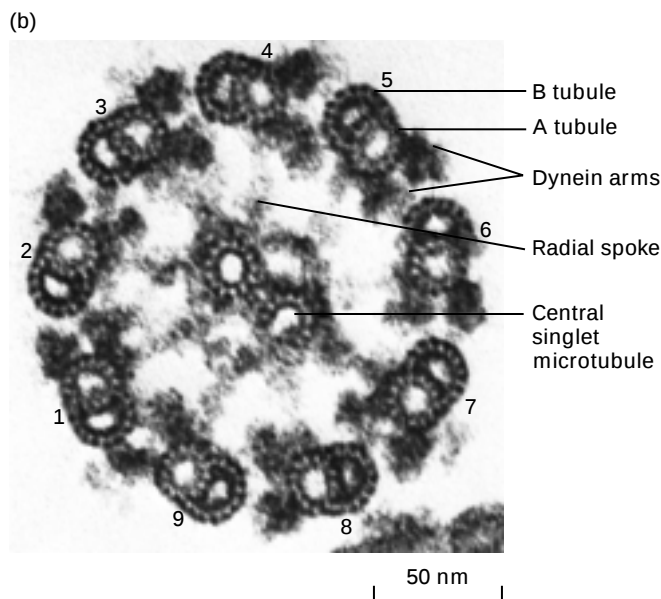
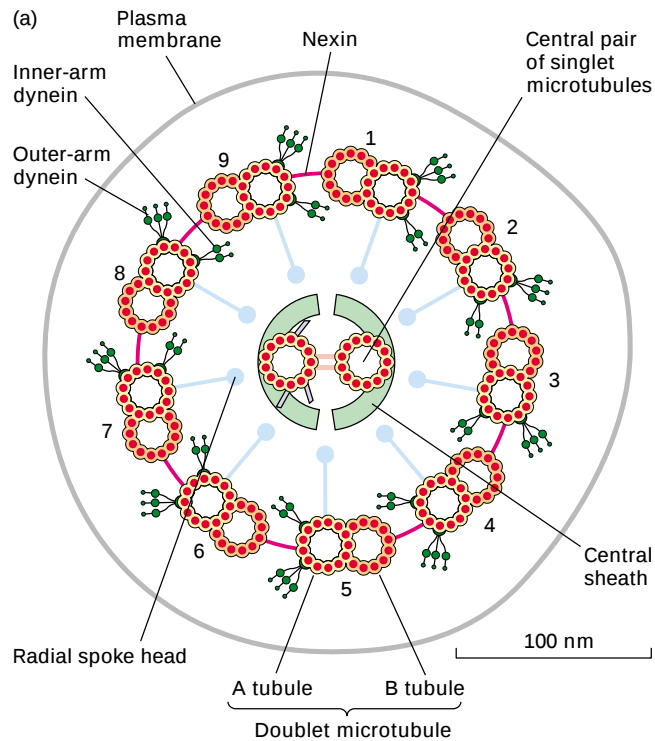


▲ FIGURE 20-24 Cooperation of myosin and kinesin at the cell cortex. Microtubules approach the actin-rich cell membrane. Consequently, some cargoes are transported to the cell periphery by kinesin motor proteins on microtubules but complete the journey on microfilaments under the power of myosin motor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

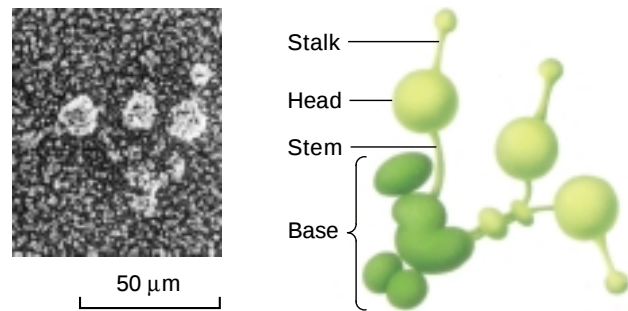
Cilia and flagella are flexible membrane extensions that project from certain cells. They range in length from a few



▲ 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 demembrated 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



▲ 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.]

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

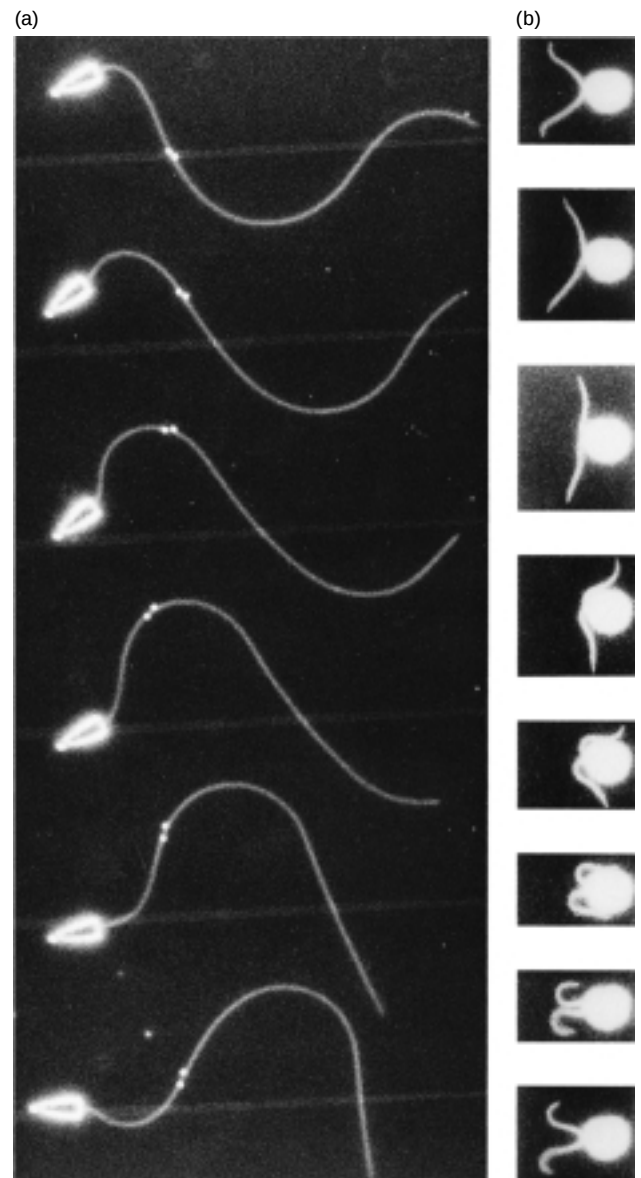
Ciliary and flagellar beating is characterized by a series of bends, originating at the base of the structure and propagated toward the tip (Figure 20-27). The bends push against the surrounding fluid, propelling the cell forward or moving the fluid across a fixed epithelium. A bend results from the 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

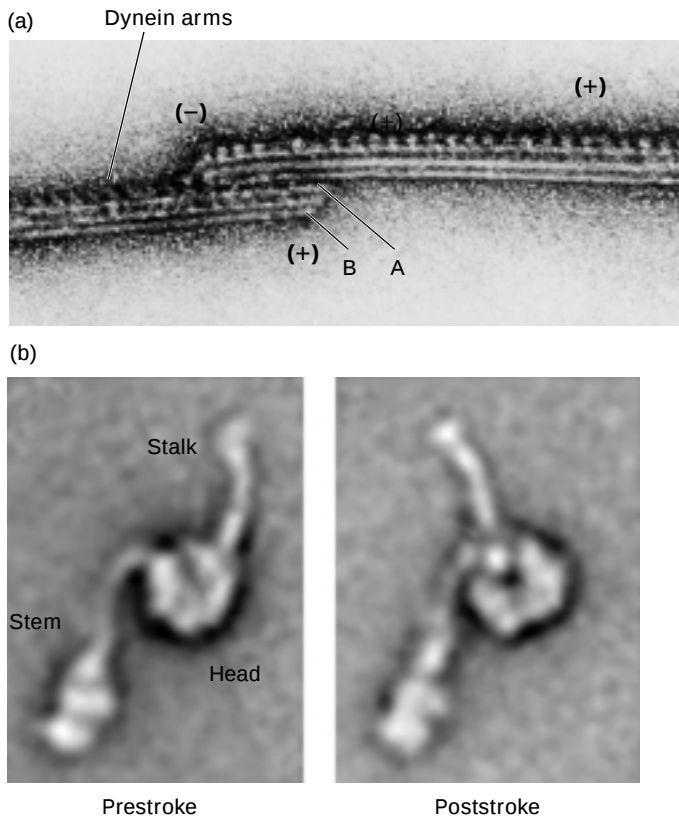
ATP-dependent movement of doublet microtubules must be restricted by cross-linking proteins in order for sliding to be converted into the bending of an axoneme.

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.]





▲ **EXPERIMENTAL FIGURE 20-28** In vitro dynein-mediated sliding of doublet microtubules requires ATP.

(a) Electron micrograph of two doublet microtubules in a protease-treated axoneme incubated with ATP. In the absence of cross-linking proteins, which are removed by the protease, doublet microtubules slide excessively. The dynein arms can be seen projecting from A tubules and interacting with B tubule of the top microtubule. (b) Single-headed dynein molecules in their prestroke and poststroke states. Thousands of images of purified inner-arm dynein were recorded in an electron microscope and then averaged. A comparison of dynein containing ADP and vanadate, a state mimicking the ADP-Pi state, with dynein absent of any bound nucleotide, suggests that the difference in structure may be related to the conformational changes taking place in the ATP cycle. A model of the force-generation mechanism suggests that the head changes orientation relative to the stem, causing a movement of the microtubule-binding stalk. [Part (a) courtesy of P. Satir. Part (b) from S. A. Burgess et al., 2003, *Nature* **421**:715; courtesy of S. A. Burgess.]

KEY CONCEPTS OF SECTION 20.2

Kinesin- and Dynein-Powered Movements

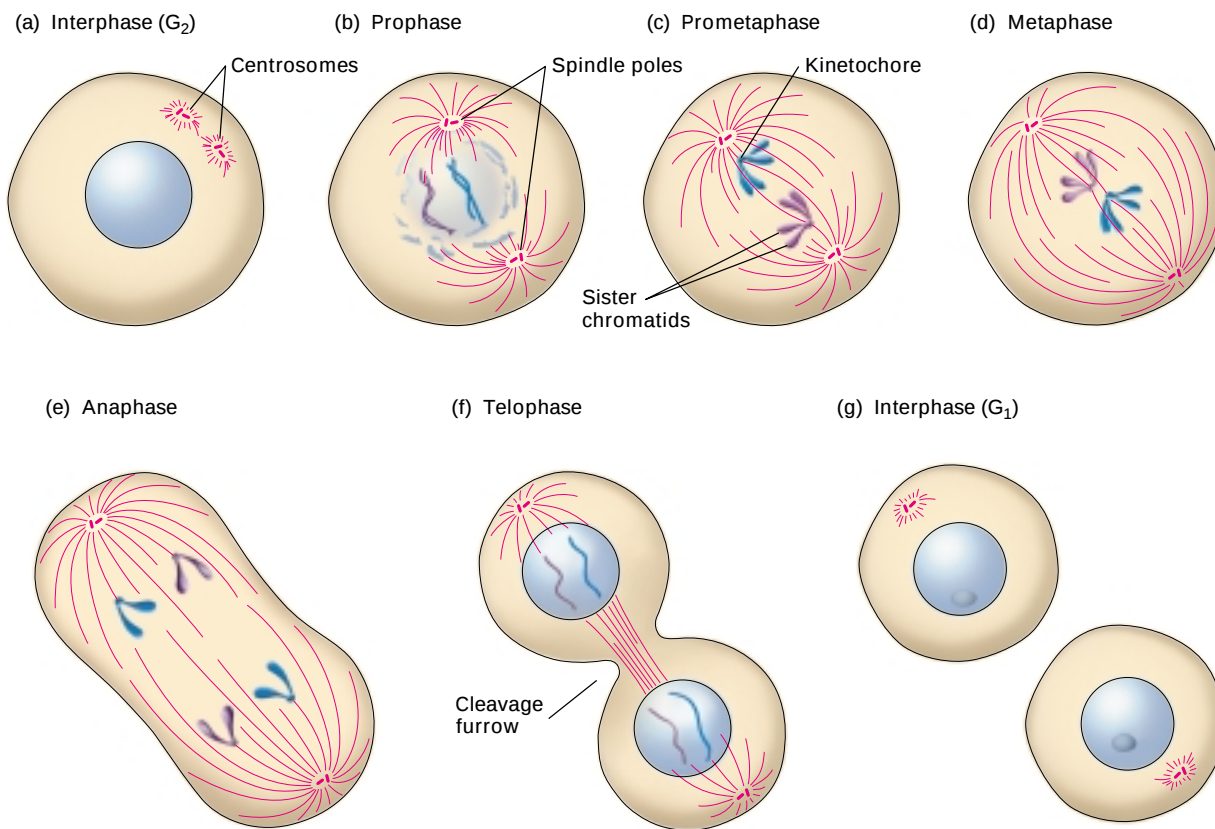
- Two families of motor proteins, kinesin and dynein, transport membrane-limited vesicles, proteins, and organelles along microtubules (see Table 20-2).
- Nearly all kinesins move cargo toward the (+) end of microtubules (anterograde transport), whereas dyneins transport cargo toward the (–) end (retrograde transport).

- Most kinesins are dimers with a head domain that binds microtubules and ATP and a tail domain that binds vesicles or other cargo (see Figure 20-20). The flexible neck region determines the direction of kinesin movement, and the tail domain determines cargo specificity.
- Cytosolic dyneins are linked to their cargoes (vesicles and chromosomes) by dynactin, a large multiprotein complex (see Figure 20-22). Dynactin also binds to microtubules, thereby increasing the processivity of dynein-mediated transport.
- In microtubule-poor regions of the cell, vesicles are probably transported along microfilaments powered by a myosin motor.
- Flagellar beating propels cells forward, and ciliary beating sweeps materials across tissues.
- The axoneme in both flagella and cilia contains nine outer doublet microtubules arranged in a circle around two central singlet microtubules (see Figure 20-25).
- Axonemal dyneins, which are larger and more complex than cytosolic dyneins, are permanently attached to doublet microtubules in axonemes. The dynein arms with their small globular heads project toward the adjacent doublet.
- Walking of dynein arms extending from one doublet toward the (–) end of a neighboring doublet generates a sliding force in the axoneme (see Figure 20-27). This linear force is converted into a bend by regions that resist sliding.

20.3 Microtubule Dynamics and Motor Proteins in Mitosis

Mitosis is the process that partitions newly replicated chromosomes equally into separate parts of a cell. The last step in the cell cycle, mitosis takes about 1 hour in an actively dividing animal cell (see Figure 1-17). In that period, the cell builds and then disassembles a specialized microtubule structure, the **mitotic apparatus**. Larger than the nucleus, the mitotic apparatus is designed to attach and capture chromosomes, align the chromosomes, and then separate them so that the genetic material is evenly partitioned to each daughter cell. Fifteen hours later, the whole process is repeated by the two daughter cells.

Figure 20-29 depicts the characteristic series of events that can be observed by light microscopy in mitosis in a eukaryotic cell. Although the events unfold continuously, they are conventionally divided into four substages: **prophase**, **metaphase**, **anaphase**, and **telophase**. The beginning of mitosis is signaled by the appearance of condensing chromosomes, first visible as thin threads inside the nucleus. By late prophase, each chromosome appears as two identical filaments, the **chromatids** (often called *sister chromatids*), held together at a constricted region, the **centromere**. Each chromatid contains one of the two new daughter DNA molecules produced in the preceding S phase of the cell cycle; thus each cell that enters mitosis has four copies of each chromosomal DNA, designated $4n$.



▲ **FIGURE 20-29 The stages of mitosis and cytokinesis in an animal cell.** For simplicity, only two sets of duplicated chromosomes, distinguished by color, are depicted.

(a) *Interphase (G₂): DNA and centrosome replication.* After DNA replication during the S phase, the chromosomes, each containing a sister chromatid, are decondensed and not visible as distinct structures. By G₂ the centrioles have replicated to form daughter centrosomes.

(b) *Prophase: centrosome migration.* The centrosomes, each with a daughter centriole, begin moving toward opposite poles of the cell. The chromosomes begin to condense, appearing as long threads.

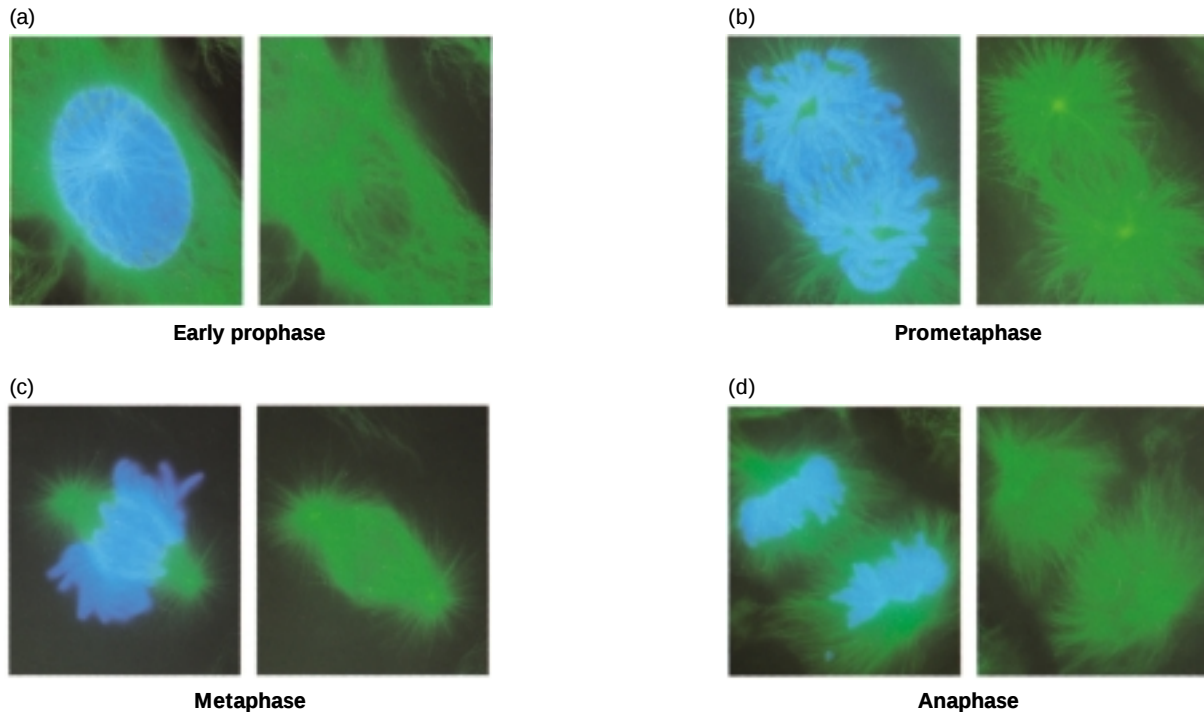
(c) *Prometaphase: spindle formation.* The nuclear envelope fragments into small vesicles and spindle microtubules enter the nuclear region. Chromosome condensation is completed; each visible chromosome is composed of two chromatids held together at their centromeres. Kinetochore at centromeres attach

chromosomes to spindle microtubules. (d) *Metaphase: chromosome alignment.* The chromosomes move toward the equator of the cell, where they become aligned in the equatorial plane. (e) *Anaphase: chromosome separation.* The two sister chromatids separate into independent chromosomes. Each chromosome, attached to a kinetochore microtubule, moves toward one pole. Simultaneously, the poles move apart. (f) *Telophase and cytokinesis.* Nuclear membranes re-form around the daughter nuclei; the chromosomes decondense and become less distinct. The spindle disappears as the microtubules depolymerize, and cell cleavage proceeds. (g) *Interphase (G₁):* Following cleavage, the daughter cells enter G₁ of interphase.

In Chapter 21, we consider in detail how progression through the cell cycle, and hence cell replication, is regulated. In this section, we focus on the mechanics of mitosis in a “typical” animal cell. Mistakes in mitosis can lead to missing or extra chromosomes, causing abnormal patterns of development when they occur during embryogenesis and pathologies when they occur after birth. To ensure that mitosis proceeds without errors in the trillions of cell divisions that take place in the life span of an organism, a highly redundant mechanism has evolved in which each crucial step is carried out concurrently by microtubule motor proteins and microtubule assembly dynamics.

The Mitotic Apparatus Is a Microtubule Machine for Separating Chromosomes

The structure of the mitotic apparatus changes constantly during the course of mitosis (Figure 20-30). For one brief moment at metaphase, however, the chromosomes are aligned at the equator of the cell. We begin our discussion by examining the structure of the mitotic apparatus at metaphase and then describe how it captures and organizes chromosomes during prophase, how it separates chromosomes during anaphase, and how it determines where cells divide during telophase.



▲ **EXPERIMENTAL FIGURE 20-30** Fluorescence microscopy reveals changes in the organization of chromosomes and microtubules at four mitotic stages.

Cultured fibroblasts were stained with a fluorescent anti-tubulin antibody (green) and the DNA-binding dye ethidium (purple). Thus in these fluorescence micrographs, green reveals microtubules; purple, chromatin; and blue, regions with both structures. (a) During early prophase, the nucleus is surrounded by an array of interphase microtubules and the chromatin is diffuse. (b) By prometaphase, the nuclear

membrane has broken down and the replicated centrosomes (centrioles) have migrated to the poles from which microtubules radiate. (c) At metaphase, the fully condensed chromosomes have aligned midway between the poles to form the metaphase plate. Dense bundles of microtubules connect the chromosomes to the poles. (d) In late anaphase, the chromosomes are pulled to the poles along the radiating microtubules. [From J. C. Waters, R. W. Cole, and C. L. Rieder, 1993, *J. Cell Biol.* **122**:361; courtesy of C. L. Rieder.]

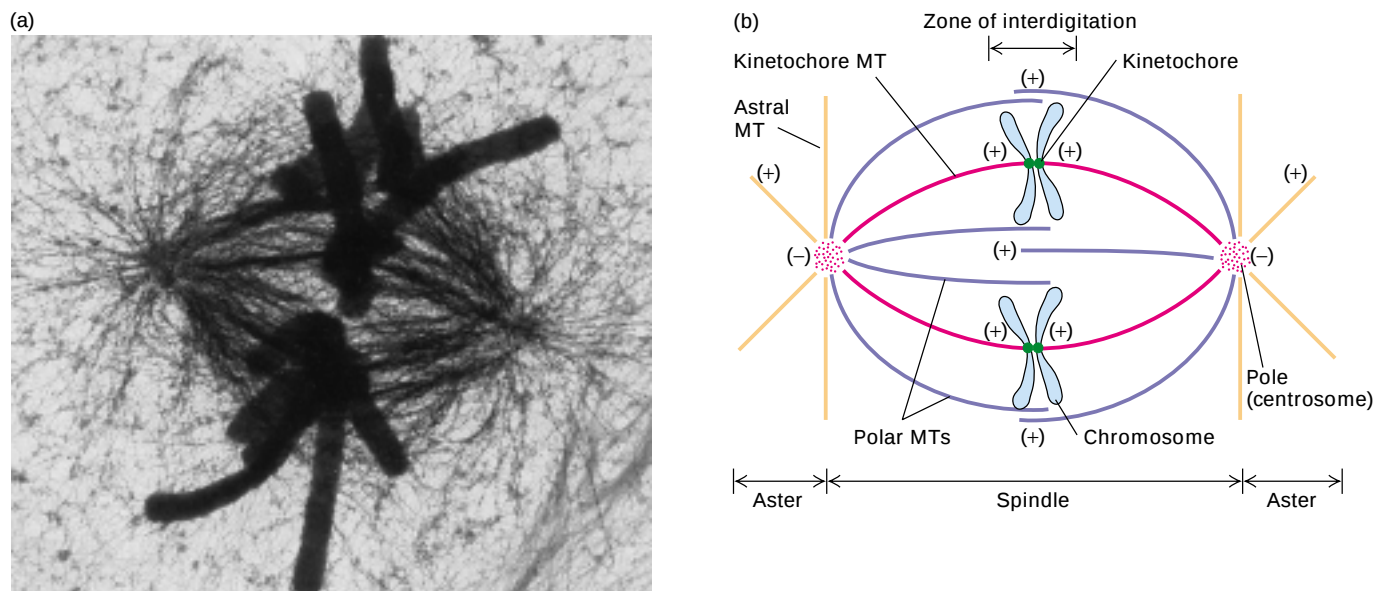
At metaphase, the mitotic apparatus is organized into two parts: a central **mitotic spindle** and a pair of **asters** (Figure 20-31a; see also Figure 20-2c). The spindle is a bilaterally symmetric bundle of microtubules and associated proteins with the overall shape of a football; it is divided into opposing halves at the equator of the cell by the metaphase chromosomes. An aster is a radial array of microtubules at each pole of the spindle.

In each half of the spindle, a single centrosome at the pole organizes three distinct sets of microtubules whose (–) ends all point toward the centrosome (Figure 20-31b). One set, the **astral microtubules**, forms the aster; they radiate outward from the centrosome toward the cortex of the cell, where they help position the mitotic apparatus and later help to determine the cleavage plane in cytokinesis. The other two sets of microtubules compose the spindle. The **kinetochore microtubules** attach to chromosomes at specialized attachment sites on the chromosomes called **kinetochores**. **Polar microtubules** do not interact with chromosomes but instead overlap with polar microtubules from the opposite pole. Two types of interactions hold the spindle halves together to form

the bilaterally symmetric mitotic apparatus: (1) lateral interactions between the overlapping (+) ends of the polar microtubules and (2) end-on interactions between the kinetochore microtubules and the kinetochores of the sister chromatids. The large protein complexes, called **cohesins**, that link sister chromatids together are discussed in Chapter 21.

The mitotic apparatus is basic to mitosis in all organisms, but its appearance and components can vary widely. In the budding yeast *Saccharomyces cerevisiae*, for instance, the mitotic apparatus consists of just a spindle, which itself is constructed from a minimal number of kinetochore and polar microtubules. These microtubules are organized by **spindle pole bodies**, trilaminated structures located in the nuclear membrane, which do not break down during mitosis. Furthermore, because a yeast cell is small, it does not require well-developed asters to assist in mitosis. Although the spindle pole body and centrosome differ structurally, they have proteins such as γ -tubulin in common that act to organize the mitotic spindle. Like yeast cells, most plant cells do not contain visible centrosomes. We consider the unique features of the mitotic apparatus in plant cells at the end of this section.





▲ **EXPERIMENTAL FIGURE 20-31 High-voltage electron microscopy visualizes components of the mitotic apparatus in a metaphase mammalian cell.** (a) Microtubules were stained with biotin-tagged anti-tubulin antibodies to increase their size in this electron micrograph. The large cylindrical objects are chromosomes. (b) Schematic diagram corresponding to the metaphase cell in (a). Three sets of microtubules (MTs) make up

the mitotic apparatus. All the microtubules have their (–) ends at the poles (centrosomes). Astral microtubules project toward the cortex and are linked to it. Kinetochore microtubules are connected to chromosomes (blue). Polar microtubules project toward the cell center with their distal (+) ends overlapping. [Part (a) courtesy of J. R. McIntosh.]

The Kinetochore Is a Centromere-Based Protein Complex That Captures and Helps Transport Chromosomes

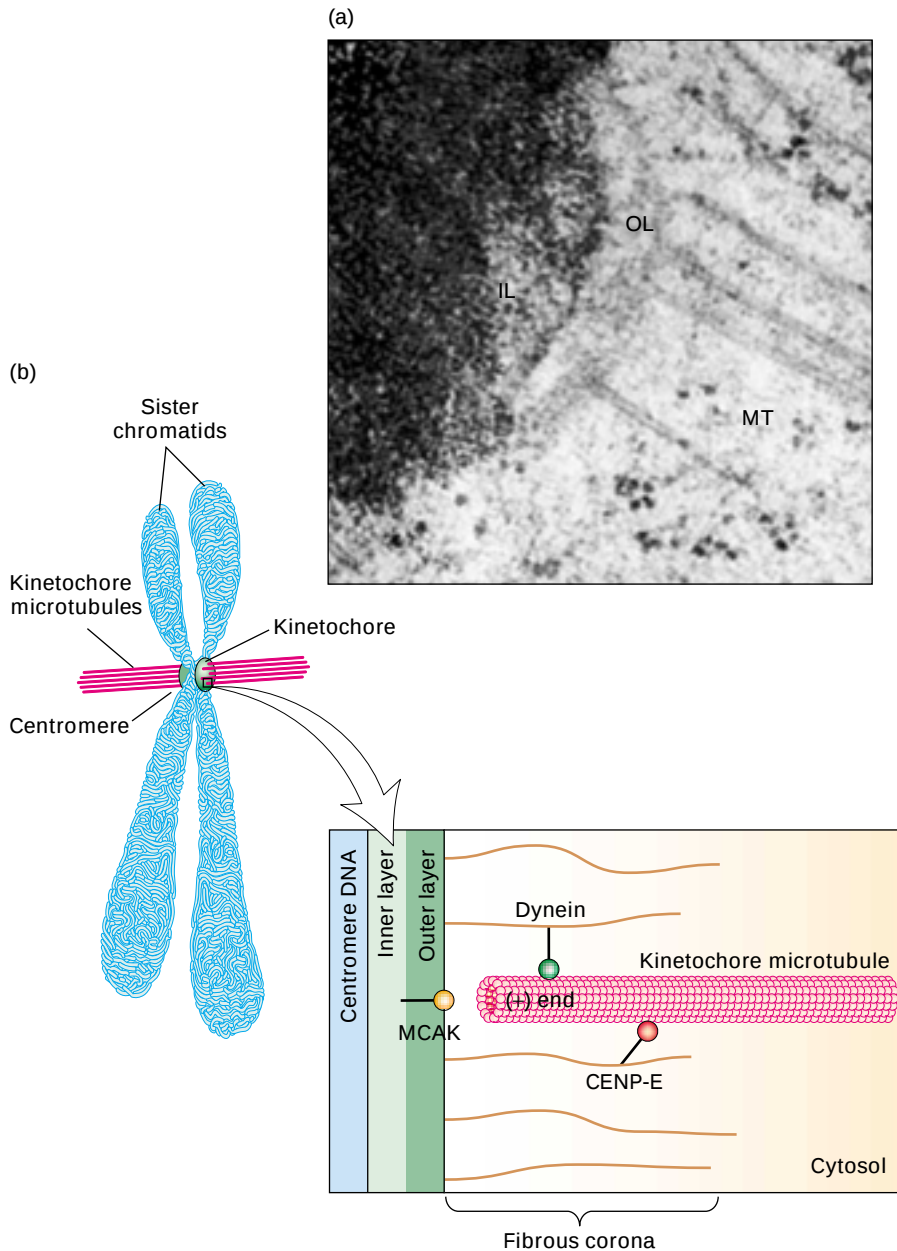
The sister chromatids of a metaphase chromosome are transported to each pole bound to kinetochore microtubules. In regard to their attachment to microtubules and movement, chromosomes differ substantially from the vesicle and organelle cargoes transported along cytosolic microtubules. The linkage of metaphase chromosomes to the (+) ends of kinetochore microtubules is mediated by a large protein complex, the kinetochore, which has several functions: to trap and attach microtubule ends to the chromosomes, to generate force to move chromosomes along microtubules, and to regulate chromosome separation and translocation to the poles. In an animal cell, the kinetochore forms at the centromere and is organized into an inner and outer layer embedded within a fibrous corona (Figure 20-32).

In all eukaryotes, three components participate in attaching chromosomes to microtubules: the centromere, kinetochore and spindle proteins, and the cell-cycle machinery. The location of the centromere and hence that of the kinetochore is directly controlled by a specific sequence of chromosomal DNA termed *centromeric DNA* (Chapter 10). Although the sequences of centromeric DNA and of DNA-binding proteins in the kinetochore are not well conserved through evolution, the cell-cycle proteins and many of the

proteins that link the kinetochore to the spindle are homologous in humans and yeast. Microtubule-binding proteins (e.g., CLIP170, CENP-E) and microtubule motor proteins (e.g., the mitotic kinesin MCAK and cytosolic dynein) cooperate in attaching the kinetochore to a microtubule end while tubulin subunits are added or released. The presence of these motor proteins indicates that kinetochores play a role in transporting chromosomes to opposite ends of the cell in mitosis.

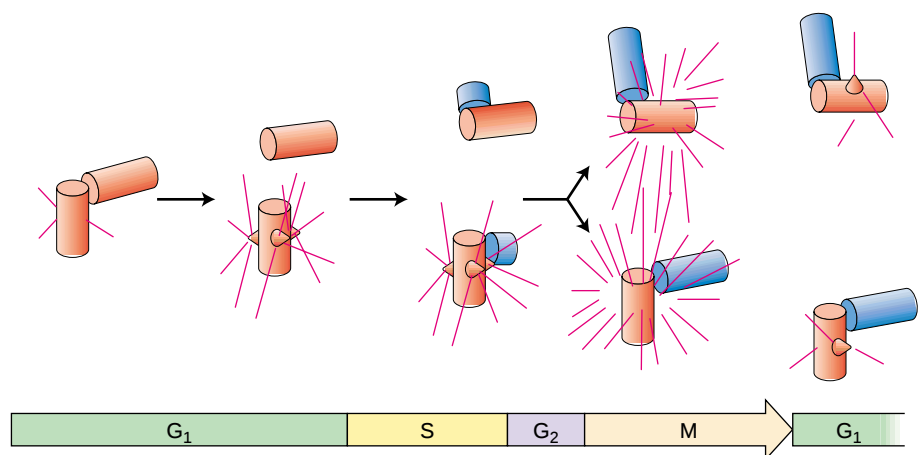
Duplicated Centrosomes Align and Begin Separating in Prophase

Because each half of the metaphase mitotic apparatus emanates from a polar centrosome, its assembly depends on duplication of the centrosome and movement of the daughter centrosomes to opposite halves of the cell. This process, known as the *centriole cycle* (or *centrosome cycle*) marks the first steps in mitosis, beginning during G_1 when the centrioles and other centrosome components are duplicated (Figure 20-33). By G_2 , the two “daughter” centrioles have reached full length, but the duplicated centrioles are still present within a single centrosome. Early in mitosis, the two pairs of centrioles separate and migrate to opposite sides of the nucleus, establishing the bipolarity of the dividing cell. In some respects, then, mitosis can be understood as the migration of duplicated centrosomes, which along their journey pick up chromosomes, pause in metaphase, and during



◀ **EXPERIMENTAL FIGURE 20-32 Kinetochore proteins mediate attachment of chromosomes to microtubules.** (a) Electron micrograph section through a kinetochore reveals the microtubules (MT), inner and outer kinetochore layers (IL and OL), and chromosome. (b) In animal cells, the kinetochore consists of an inner layer containing proteins that bind centromeric DNA and an outer layer connected to the (+) ends of kinetochore microtubules. The microtubules embedded in the outer layer extend toward one of the two poles of the cell. The outer layer and fibrous corona around the microtubule ends contain microtubule-binding proteins and motor proteins, including CLIP170, cytosolic dynein, and the kinesins CENP-E and MCAK. [Part (a) from B. McEwen et al., 1998, *Chromosoma* **107**:366; courtesy of B. McEwen.]

► **FIGURE 20-33 Relation of centrosome duplication to the cell cycle.** After the pair of parent centrioles (red) separates slightly, a daughter centriole (blue) buds from each and elongates. By G_2 , growth of the daughter centrioles is complete, but the two pairs remain within a single centrosomal complex. Early in mitosis, the centrosome splits, and each centriole pair migrates to opposite ends of the cell.



anaphase continue their movement to new locations in the daughter cells, where they release the chromosomes and organize the cytosolic microtubules.

Microtubule dynamics change drastically at the onset of mitosis, when long interphase microtubules disappear and are replaced by astral and spindle microtubules. These mitotic microtubules, which are nucleated from the newly duplicated centrosomes, are more numerous, shorter, and less stable than interphase microtubules. The average lifetime of a microtubule decreases from 10 minutes in interphase cells to 60–90 seconds in the mitotic apparatus. This increase in dynamic instability enables microtubules to assemble and disassemble quickly in mitosis.

The results of genetic and cell biological studies, primarily in yeast and flies, have implicated several kinesins in organ-

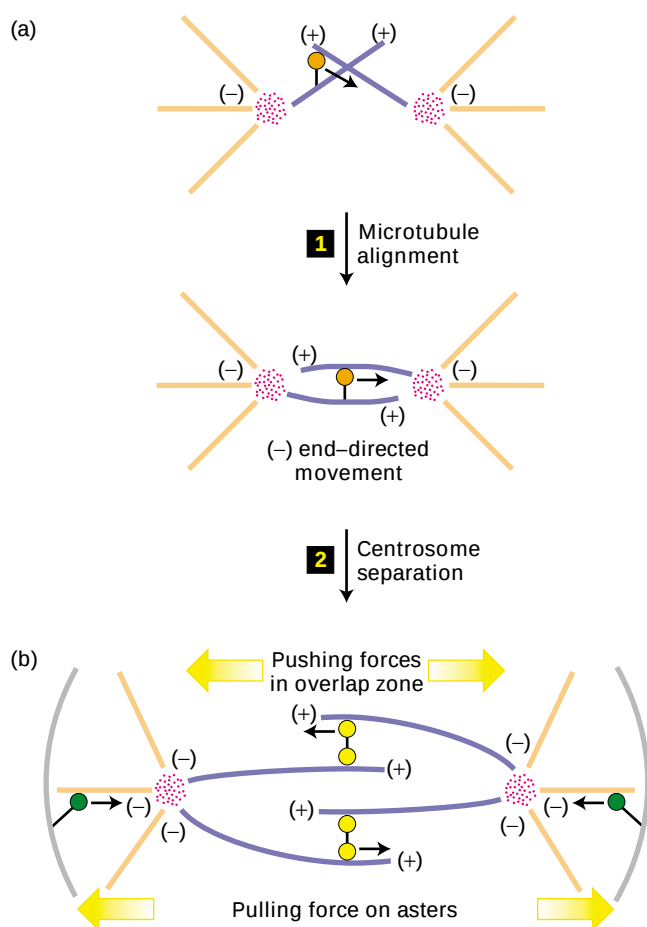
izing polar microtubules into a bipolar array, thereby orienting assembly of the spindle and spindle asters. For instance, antibodies against either a (+) or a (–) end-directed kinesin inhibit the formation of a bipolar spindle when they are microinjected into a cell before but not after prophase. A (–) end-directed kinesin protein, such as Kin-C, is thought to help align the oppositely oriented polar microtubules extending from each centrosome. Then a (+) end-directed kinesin, most likely the bipolar BimC, cross-links antiparallel microtubules and pushes them apart. In addition, findings from localization experiments with anti-dynein antibodies have demonstrated the presence of cytosolic dynein in the centrosomes and cortex of dividing animal cells. The results of other studies with yeast mutants lacking cytosolic dynein suggest that dynein at the cortex simultaneously helps tether the astral microtubules and orient the poles of the spindle. Thus the alignment and initial separation of centrosomes at prophase depend on the growth of polar and astral microtubules and on the action of several motor proteins, as depicted in Figure 20-34.

Although centrosomes facilitate the formation of the spindle poles, recent findings show that polar microtubules can be assembled and organized into antiparallel bundles in the absence of centrosomes. For instance, the Ran GTPase that functions in nuclear import and export (Chapter 12) appears to promote the polymerization of tubulin subunits. Ran acts with other proteins, possibly motor proteins, to stabilize microtubules by increasing their frequency of rescue.

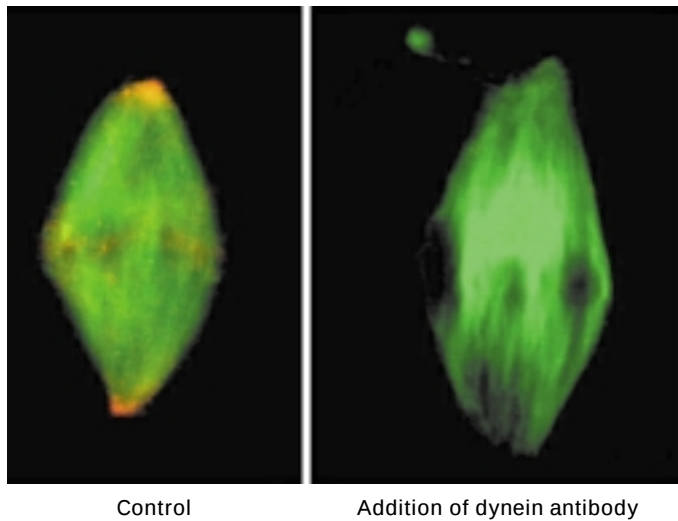
Formation of the Metaphase Mitotic Spindle Requires Motor Proteins and Dynamic Microtubules

When the duplicated centrosomes have become aligned, formation of the spindle proceeds, driven by simultaneous events at centrosomes and chromosomes. As just discussed, the centrosome facilitates spindle formation by nucleating the assembly of the spindle microtubules. In addition, the (–) ends of microtubules are gathered and stabilized at the pole by dynein-dynactin working with the nuclear/mitotic apparatus protein. The role of dynein in spindle pole formation has been demonstrated by reconstitution studies in which bipolar spindles form in *Xenopus* egg extracts in the presence of centrosomes, microtubules, and sperm nuclei. The addition of antibodies against cytosolic dynein to this in vitro system releases and splays the spindle microtubules but leaves the centrosomal astral microtubules in position (Figure 20-35).

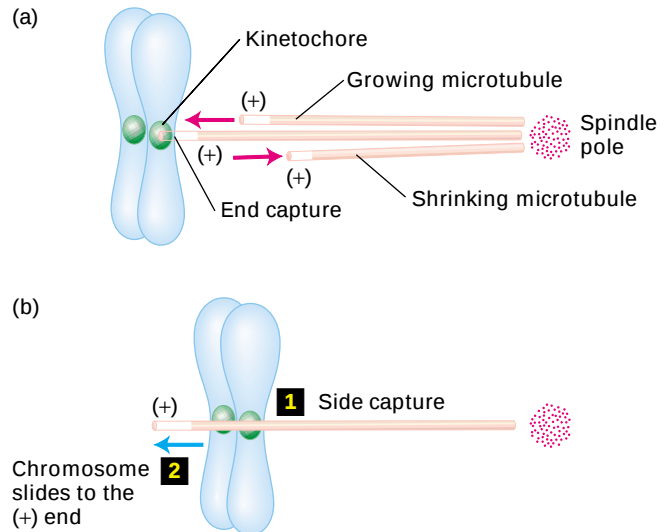
The dynamic instability of spindle microtubules at the other end, the (+) end, is critical to their capture of chromosomes during late prophase as the nuclear membrane begins to break down. By quickly lengthening and shortening at its (+) end, a dynamic microtubule probes into the chromosome-rich environment of the cell. Sometimes the (+) end of a microtubule directly contacts a kinetochore, scoring a “bull’s-eye.” More commonly, a kinetochore contacts the side of a microtubule and then slides along the microtubule to the (+) end in a process that includes cytosolic dynein and mitotic kinesins



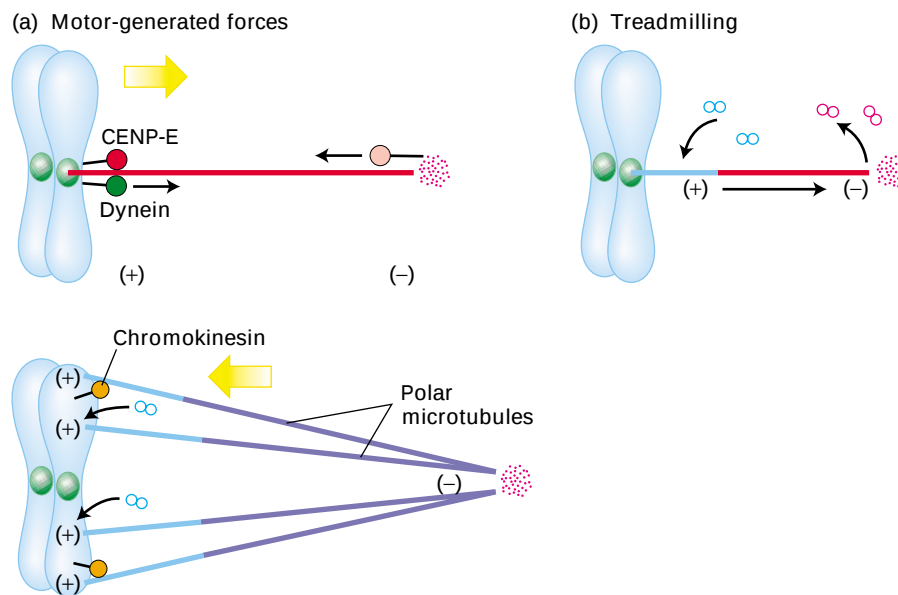
▲ **FIGURE 20-34 Model for participation of microtubule motor proteins in centrosome movements at prophase.** (a) At prophase, polar microtubules growing randomly from opposite poles are aligned with the aid of (–) end-directed motors (orange). (b) After alignment, (+) end-directed mitotic kinesins (yellow), including the bipolar kinesin BimC, generate pushing forces that separate the poles. In addition, a (–) end-directed force exerted by cytosolic dynein (green) located at the cortex may pull asters toward the poles. Similar forces act later at anaphase.



▲ **EXPERIMENTAL FIGURE 20-35 Cytosolic dynein participates in the formation and stabilization of mitotic spindle poles.** In vitro reconstituted spindles were stained with fluorescently-labeled polyclonal antibodies to tubulin (green) and dynein (red) and examined with a fluorescence microscope. In the control spindle (*left*), cytosolic dynein is present at each tapered end, as well as at the equator. Addition of a dynein monoclonal antibody after the formation of the spindle (*right*) disrupts dynein localization and causes the poles to splay. [From R. Heald et al., 1997, *J. Cell Biol.* **138**:615; courtesy of R. Heald.]

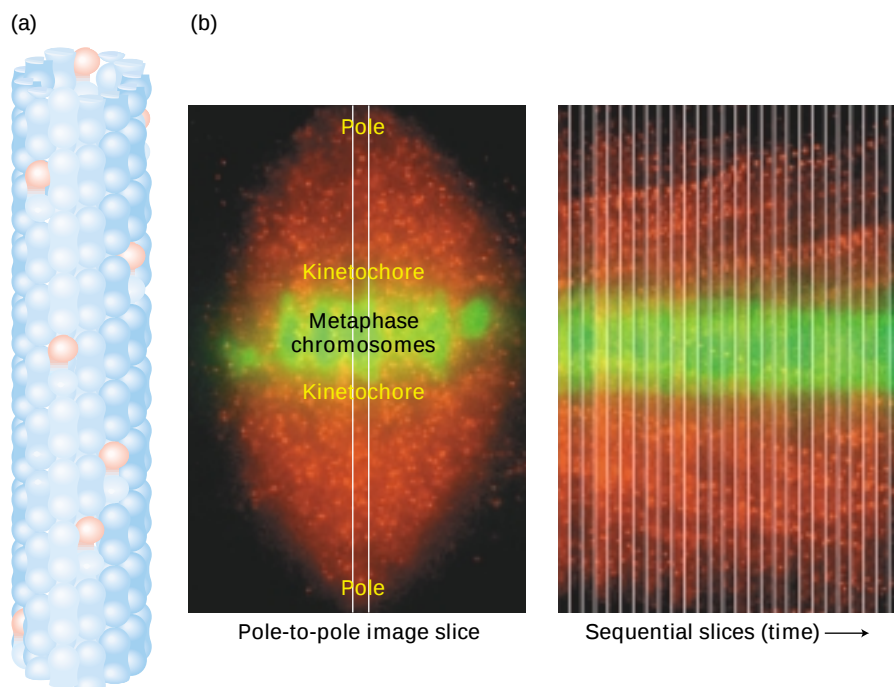


▲ **FIGURE 20-36 Capture of chromosomes by microtubules in prometaphase.** (a) In late prophase, spindle microtubules probe randomly for chromosomes by alternately growing and shrinking at their distal (+) ends. (b) Some chromosomes first encounter the side (1), not the end, of a microtubule, interacting with the microtubule through proteins at the kinetochore. Kinetochore-associated (+) end-directed motor proteins (e.g., MCAK) then move the chromosome to the (+) end (2), thereby stabilizing the microtubule.



▲ **FIGURE 20-37 Model of the forces stabilizing metaphase chromosomes at the equatorial plate.** (a) Cytoplasmic dynein, a (-) end-directed motor (light green) at the kinetochore and a (+) end-directed motor (pink) at the spindle pole pull chromosomes toward the pole. Chromokinesin, a nonkinetochore (+) end-directed motor on the chromatid arms, exerts an opposite force on

polar microtubules, pushing chromosomes away from the pole. CENP-E, a kinesin that does not mediate movement, keeps the kinetochore tethered to the kinetochore microtubule. (b) Treadmilling of tubulin subunits briefly stabilizes the lengths of spindle microtubules at metaphase by balancing assembly at the kinetochore with disassembly at the poles.



◀ **EXPERIMENTAL FIGURE 20-38**
Poleward flux of tubulin subunits during metaphase is visualized by fluorescence speckle microscopy.

(a) When a substoichiometric amount of fluorescent tubulin subunits is assembled into a microtubule, the microtubule appears speckled. (b) In a live cell at metaphase, the speckled microtubules do not have the fibrous appearance observed in conventional fluorescence microscopy. However, the dynamics of subunit translocation is revealed by taking a slice through the same position from each frame of a time-lapse series (boxed area). If the labeled tubulin subunits move, then the slope of the line formed by a speckle in consecutive images corresponds to the translocation rate. Here, the speckles on both sides of the spindle travel toward the poles at a rate of $0.75 \mu\text{m/s}$. [Part (b) from T. J. Mitchison and E. D. Salmon, 2001, *Nature Cell Biol.* 3:E17; courtesy of A. Desai.]

Video: Fluorescent Speckle Microscopy of Metaphase Dynamics

MEDIA CONNECTIONS
 Video: Fluorescent Speckle Microscopy of Metaphase Dynamics

on the kinetochore (Figure 20-36). Whether a chromosome attaches to the (+) end of a spindle microtubule by a direct hit or by the side capture/sliding process, the kinetochore “caps” the (+) end of the microtubule. Eventually, the kinetochore of each sister chromatid in a chromosome is captured by microtubules arising from the nearest spindle poles. Each chromosome arm becomes attached to additional microtubules as mitosis progresses toward metaphase.

During late prophase (prometaphase), the newly condensed chromosomes attached to the (+) ends of kinetochore microtubules move to the equator of the spindle. Along the way, the chromosomes exhibit *saltatory behavior*, oscillating between movements toward and then away from the pole or equator. These oscillations result from alternating depolymerization and polymerization at the (+) ends of kinetochore microtubules. In addition, motor proteins associated with both ends of kinetochore microtubules and with the distal ends of polar microtubules on chromosome arms generate opposing forces that are thought to position captured chromosomes equally between the two spindle poles (Figure 20-37a).

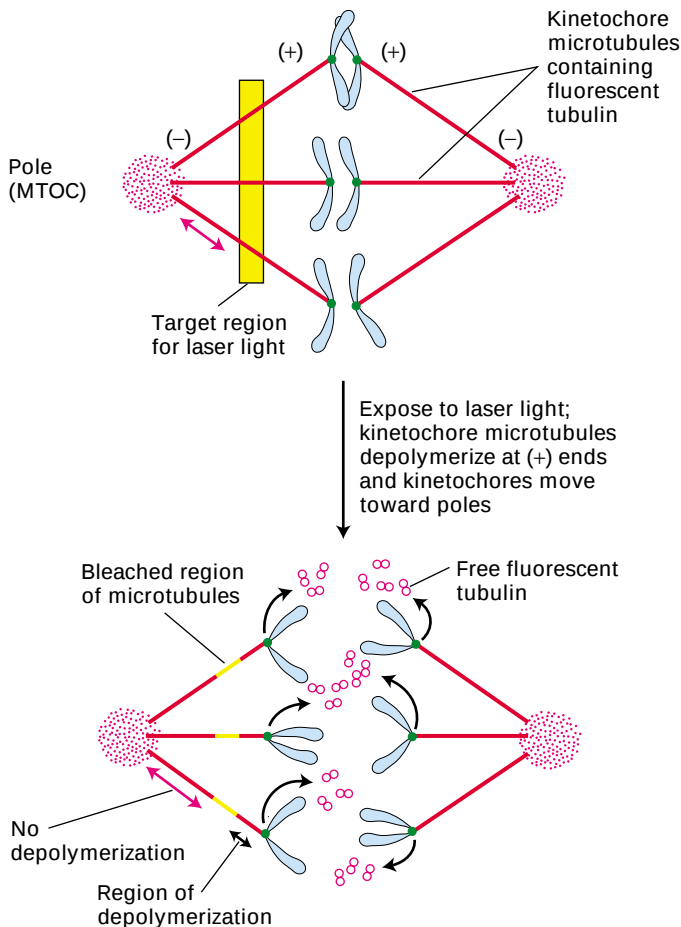
Although the lengths of kinetochore and polar microtubules eventually become stable, there continues to be a flow, or treadmilling, of subunits through the microtubules toward the poles. At metaphase, the loss of tubulin subunits at the (–) ends of spindle microtubules is balanced by the addition of subunits at the (+) ends (Figure 20-37b). The flow of tubulin subunits from kinetochores to the poles can be visualized after a very small “pulse” of fluorescently labeled tubulin subunits has been microinjected into a cell (Figure 20-38). Microtubules appear speckled because very few of the subunits are fluores-

cent. By comparing the positions of each speckle, one can see whether the tubulin subunits are moving in a specific direction. The images show that the mitotic spindle at metaphase is a finely balanced, yet dynamic, structure that holds chromosomes at the equatorial plate. By mechanisms discussed in Chapter 21, the cell cycle is held in check until all chromosomes have been captured and aligned. A single unattached kinetochore is sufficient to prevent entry into anaphase.

Anaphase Chromosomes Separate and the Spindle Elongates

The same forces that form the spindle during prophase and metaphase also direct the separation of chromosomes toward opposite poles at anaphase. Anaphase is divided into two distinct stages, *anaphase A* and *anaphase B* (early and late anaphase). Anaphase A is characterized by the shortening of kinetochore microtubules at their (+) ends, which pulls the chromosomes toward the poles. In anaphase B, the two poles move farther apart, bringing the attached chromosomes with them into what will become the two daughter cells.

Microtubule Shortening in Anaphase A The results of *in vitro* studies have indicated that the depolymerization of microtubules in *Xenopus* eggs is sufficient to move chromosomes toward the poles. In one such study, purified microtubules were mixed with purified anaphase chromosomes; as expected, the kinetochores bound preferentially to the (+) ends of the microtubules. To induce depolymerization of the



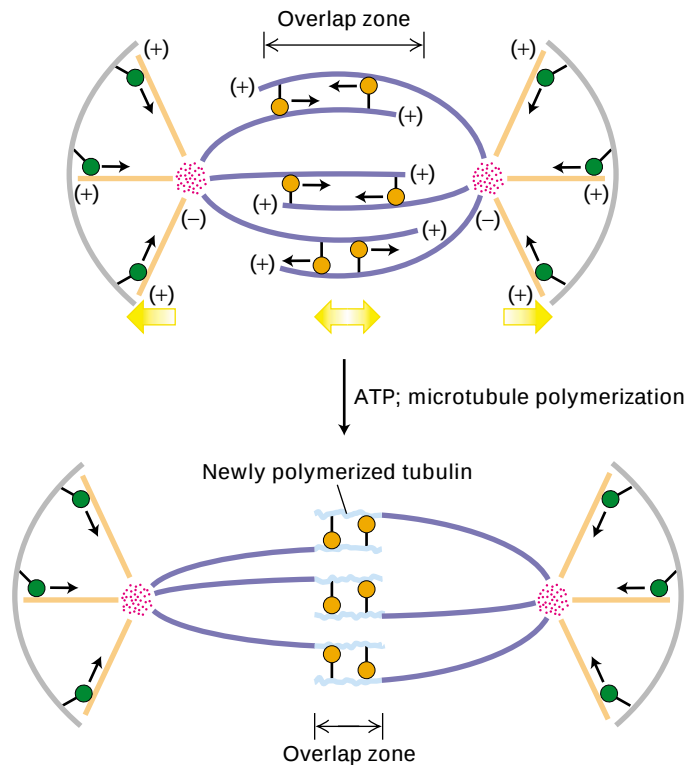
▲ EXPERIMENTAL FIGURE 20-39 Shortening at the (+) end of kinetochore microtubules moves chromosomes poleward in anaphase A. Fibroblasts are injected with fluorescent tubulin and then allowed to enter metaphase so that all the microtubules are fluorescent. Only the kinetochore microtubules are shown. In early anaphase, a band of microtubules (yellow box) is subjected to a laser light, which bleaches the fluorescence but leaves the microtubules continuous and functional across the bleached region. The bleached segment of each microtubule thus provides a marker for the position of that part of the microtubule. In anaphase, the distance between the bleached zone and the adjacent pole (marked by red double-headed arrows) does not change, whereas the distance to the adjacent chromatid (marked by black double-headed arrows) becomes shorter. This finding indicates that during anaphase microtubules disassemble at the (+) end just behind the kinetochores, not at the poles. [Adapted from G. J. Gorbisky et al., 1987, *J. Cell Biol.* **104**:9, and 1988, *J. Cell Biol.* **106**:1185.]

microtubules, the reaction mixture was diluted, thus lowering the concentration of free tubulin dimers. Video microscopy analysis then showed that the chromosomes moved toward the (–) end, at a rate similar to that of chromosome movement during anaphase in intact cells. Because no ATP (or any other energy source) was present in these experiments, chromosome movement toward the (–) end must have been

powered, in some way, by microtubule disassembly and must not have been powered by microtubule motor proteins.

The *in vivo* fluorescence-tagging experiment depicted in Figure 20-39 provides evidence that shortening of kinetochore microtubules at their (+) ends moves chromosomes toward the poles in mammalian cells. A kinetochore-associated kinesin, MCAK, promotes disassembly at the (+) end while CENP-E, also at kinetochores, binds to the progressively shortening end. According to this model, microtubule disassembly drives poleward movement of chromosomes. Although kinetochore kinesins promote this disassembly or keep chromosomes attached to the shortening (+) end of kinetochore microtubules, they do not actually move chromosomes along the microtubules.

Spindle Elongation in Anaphase B Findings from several types of experiments have implicated three processes in the separation of the poles in anaphase B: a pushing force gen-



▲ FIGURE 20-40 Model of spindle elongation and movement of poles during anaphase B. One or more (+) end-directed spindle kinesins (orange) bind to antiparallel polar microtubules in the overlap region and then “walk” along a microtubule in the other half-spindle toward its (+) end. In cells that assemble an aster, cytoplasmic dynein, a (–) end-directed motor protein (green) anchored in the cortex of the plasma membrane, walks along astral microtubules, pulling the poles outward. Tubulin subunits are simultaneously added to the plus ends of all polar microtubules, thereby lengthening the spindle. [Adapted from H. Masuda and W. Z. Cande, 1987, *Cell* **49**:193.]

erated by kinesin-mediated sliding of polar microtubules past one another, a pulling force generated by cortex-associated cytosolic dynein, and lengthening of polar microtubules at their (+) ends. The coordinated effect of these processes is depicted in Figure 20-40.

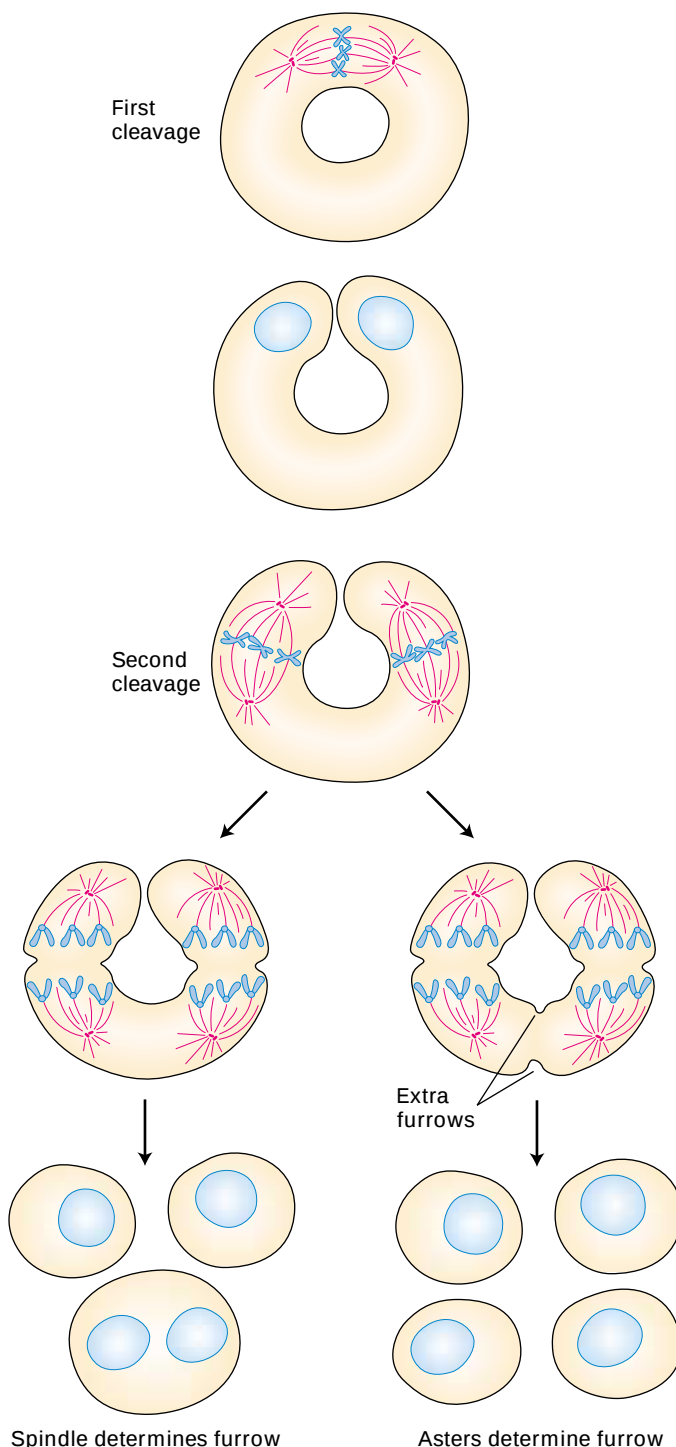
When metaphase cells are depleted of ATP by mild detergent treatment, poleward chromosome movement (anaphase A) proceeds but the spindle poles do not separate (anaphase B). This finding indicates that microtubule motor proteins take part in separating the spindle poles, as they do in centrosome movement in prometaphase (see Figure 20-34). Experiments with artificial spindles assembled from frog egg extracts were sources of further insight into anaphase B movements. In the presence of calcium, the spindle is activated and elongates, simulating anaphase B, and the zone of overlap between the two halves of the spindle decreases in length. The observation that adjacent antiparallel microtubules in this system migrate in the direction of their pole-facing (–) ends suggests that a (+) end-directed kinesin separates spindle poles in anaphase B. In one model, the bipolar kinesin BimC attached to a microtubule in the overlap region walks toward the (+) end of a neighboring but antiparallel microtubule, thus pushing the adjacent microtubule in the direction of its (–) end (see Figure 20-40). Involvement of a kinesin motor is supported by experiments in which antibodies raised against a conserved region of the kinesin superfamily inhibit ATP-induced elongation of diatom spindles *in vitro*.

In the presence of $\alpha\beta$ -tubulin, reactivated frog spindles and isolated diatom spindles add tubulin subunits to the (+) end of polar microtubules, thus lengthening them. The third process taking place in anaphase B can be demonstrated by cutting the spindle in half with a microneedle at anaphase; the resulting half-spindles move quickly to the poles, at a rate faster than usual during anaphase. This observation suggests that cytosolic dynein, a (–) end-directed motor protein associated with the cortex, pulls on astral microtubules, thereby moving the poles farther apart (see Figure 20-40).

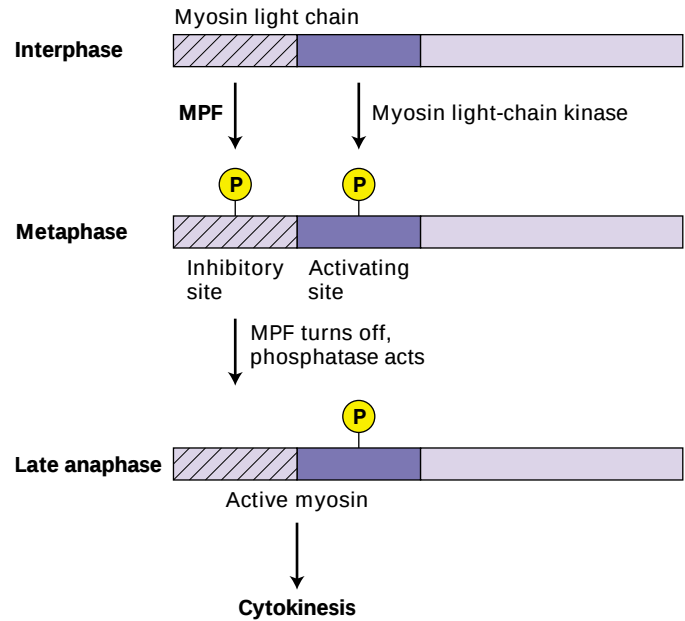
► **EXPERIMENTAL FIGURE 20-41 Micromanipulation experiments can determine whether the spindle or the asters control location of the cleavage plane during cytokinesis.** A small glass ball is pressed against a fertilized egg until membranes from opposite sides of the cell touch and fuse, thus changing the spherical egg into a doughnut shape. In the first cell division, a normal spindle develops and the doughnut-shaped cell divides to produce a single C-shaped cell with two normal nuclei. This result is expected whether the asters or the spindle determines the cleavage plane. In the second cell division, the two nuclei each produce a normal spindle. If the spindle determined the cleavage plane, then cleavage of the C-shaped cell would yield three cells, one of them with two nuclei (*lower left*). If the asters determined the cleavage plane, then a third cleavage plane would form between asters from two different spindles (*lower right*). Cell division produced four cells, indicating an extra furrow formed between a pair of asters.

Microtubules and Microfilaments Work Cooperatively During Cytokinesis

After the chromosomes have migrated to opposite ends of the cell during anaphase, two closely related events proceed (see Figure 20-29): the nuclear envelope re-forms around each complete set of chromosomes, marking the end of mitosis (telophase), and the cytoplasm divides, a process termed



► **FIGURE 20-42 Regulation of myosin light chain by mitosis-promoting factor.** The mitosis-promoting factor (MPF) is an activated complex of a cyclin-dependent kinase (CDK1) and a mitotic cyclin protein. Phosphorylation of inhibitory sites on the myosin light chain by the kinase activity of MPF early in mitosis prevents active myosin heavy chains from interacting with actin filaments and sliding along them, a process required for cytokinesis. When MPF activity falls at anaphase, a constitutive phosphatase dephosphorylates the inhibitory sites, permitting cytokinesis to proceed. Another enzyme, myosin light-chain kinase, phosphorylates a different residue, activating myosin to interact with actin filaments. [See L. L. Satterwhite et al., 1992, *J. Cell Biol.* **118**:595; adapted from A. Murray and T. Hunt, 1993, *The Cell Cycle: An Introduction*, W. H. Freeman and Company.]



cytokinesis. With completion of cytokinesis, two daughter cells are formed. In Chapter 19, we saw how the contractile ring of actin and myosin II constricts the cell during cytokinesis but does not constrict the mechanism that determines the plane of cleavage through the cell. It is clear that the contractile ring and hence the cleavage furrow always develop where the chromosomes line up during metaphase, but which component of the mitotic apparatus dictates where the contractile ring will assemble is not clear.

Findings from micromanipulation experiments have shown that different cells rely on either the spindle or the asters to determine the cleavage plane (Figure 20-41). For instance, the presence of two asters determines where cleavage occurs in fertilized sand dollar eggs, whereas the spindle determines the cleavage plane in animal cells.

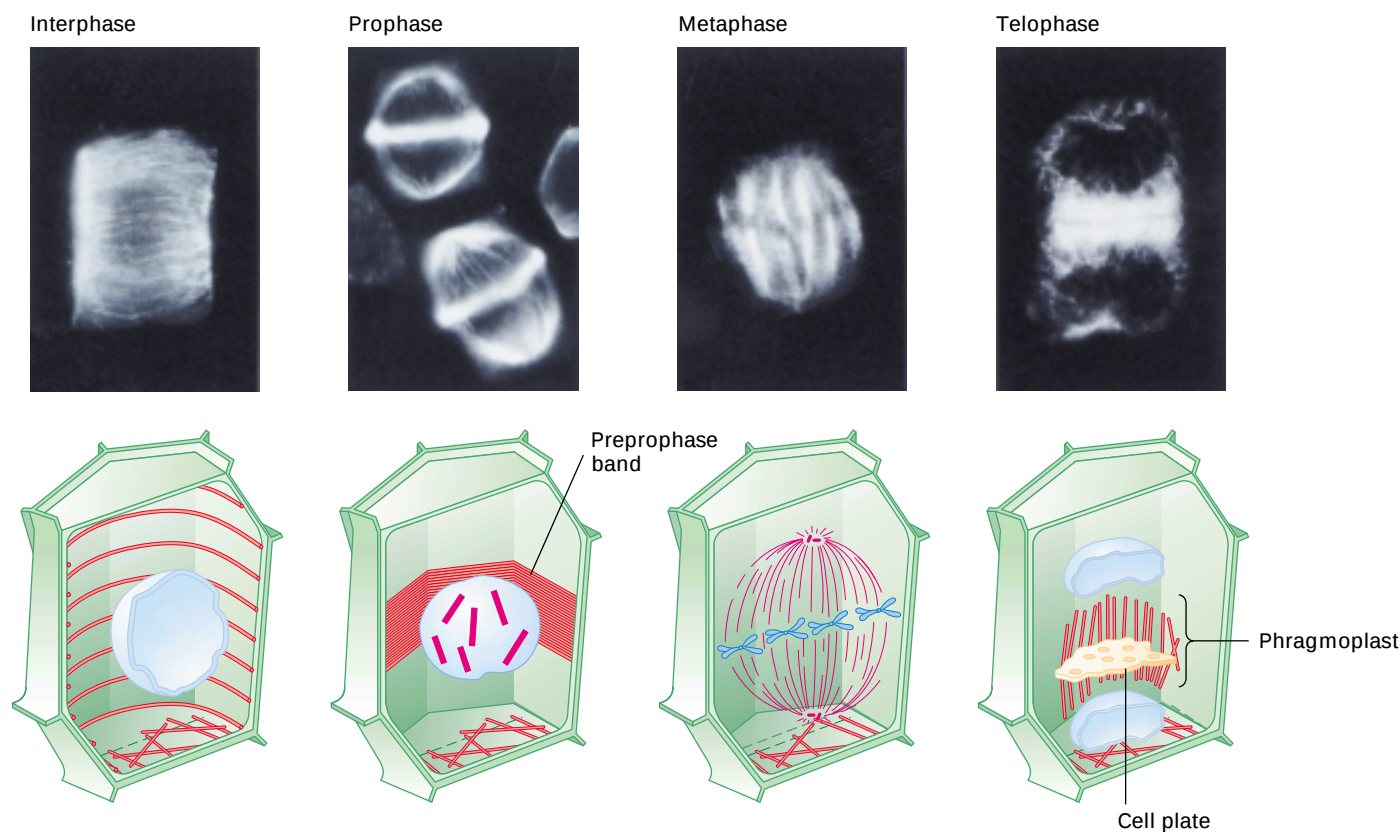
One hypothesis is that astral or spindle microtubules send a signal to the region of the cortex midway between asters. This signal promotes the interaction of actin microfilaments and myosin II, resulting in the formation of the contractile ring followed by the development of the cleavage furrow. The signal is unidentified as yet, but an alluring candidate is CDK1. Not only does activation of this cyclin-dependent kinase trigger entry into mitosis, but the myosin light chain is one of its known substrates. Phosphorylation of the regulatory light chains in myosin II by CDK1 early in mitosis inhibits interaction of myosin with actin filaments, thus preventing premature formation of the contractile ring (Figure 20-42). The decrease in CDK1 activity in late anaphase relieves this inhibition, leading to assembly of the contractile ring and its contraction to form the cleavage furrow (see Figure 19-20). In Chapter 21, we examine how the activity of CDK1 and other cyclin-dependent kinases waxes and wanes in replicating cells.

Plant Cells Reorganize Their Microtubules and Build a New Cell Wall in Mitosis



As noted previously, interphase plant cells lack a single perinuclear microtubule-organizing center that organizes microtubules into the radiating interphase array typical of animal cells. Instead, numerous MTOCs line the cortex of plant cells and nucleate the assembly of transverse bands of microtubules below the cell wall (Figure 20-43, *left*). These cortical microtubules, which are cross-linked by plant-specific MAPs, aid in laying down extracellular cellulose microfibrils, the main component of the rigid cell wall (see Figure 6-33).

Although mitotic events in plant cells are generally similar to those in animal cells, formation of the spindle and cytokinesis have unique features in plants (see Figure 20-43). Plant cells bundle their cortical microtubules and reorganize them into a spindle at prophase without the aid of centrosomes. At metaphase, the mitotic apparatus appears much the same in plant and animal cells. Golgi-derived vesicles, which appear at metaphase, are transported into the mitotic apparatus along microtubules that radiate from each end of the spindle. At telophase, these vesicles line up near the center of the dividing cell and then fuse to form the *phragmoplast*, a membrane structure that replaces the animal-cell contractile ring. The membranes of the vesicles forming the phragmoplast become the plasma membranes of the daughter cells. The contents of these vesicles, such as polysaccharide precursors of cellulose and pectin, form the early cell plate, which develops into the new cell wall between the daughter cells.



▲ **FIGURE 20-43 Mitosis in a higher plant cell.**

Immunofluorescence micrographs (*top*) and corresponding diagrams (*bottom*) showing arrangement of microtubules in interphase and mitotic plant cells. A cortical array of microtubules girdles a cell during interphase. Webs of microtubules cap the growing ends of plant cells and remain intact during cell division. As a cell enters prophase, the microtubules are bundled around the nucleus and reorganized into a spindle that appears similar

to that in metaphase animal cells. By late telophase, the nuclear membrane has re-formed around the daughter nuclei, and the Golgi-derived phragmoplast has assembled at the equatorial plate. Additional small vesicles derived from the Golgi complex accumulate at the equatorial plate and fuse with the phragmoplast to form the new cell plate. [Adapted from R. H. Goddard et al., 1994, *Plant Physiol.* **104**:1; micrographs courtesy of Susan M. Wick.]

KEY CONCEPTS OF SECTION 20.3

Microtubule Dynamics and Motor Proteins in Mitosis

- In mitosis, the replicated chromosomes are separated and evenly partitioned to two daughter chromosomes. This part of the cell cycle is commonly divided into four sub-stages: prophase, metaphase, anaphase, and telophase (see Figure 20-29).
- The mitotic apparatus of animal cells comprises the astral microtubules forming the asters, the polar and kinetochore microtubules forming the football-shaped spindle, the spindle poles derived from the duplicated centrosomes, and chromosomes attached to the kinetochore microtubules (see Figure 20-31).
- A simple set of microtubule motor proteins—BimC, CENP-E, and cytosolic dynein—are conserved in all spindles. Additional motor proteins are present in more complex organisms.

- Motor protein-mediated interactions between opposing polar microtubules and between asters and the cell cortex align and separate the centrosomes at prophase, establishing the bipolar orientation of the spindle (see Figure 20-34).
- Kinetochore microtubules capture chromosomes during prophase (see Figure 20-32). Opposing forces generated by motor proteins at both ends of spindle microtubules center the chromosomes at metaphase (see Figure 20-37).
- The combined action of microtubule dynamics (polymerization and depolymerization) and microtubule motors are responsible for the transport of chromosomes to the opposite poles at anaphase (see Figures 20-39 and 20-40).
- Cleavage during cytokinesis generates two daughter cells. The signal for cleavage is unknown, but it probably relieves the inhibition on interaction between actin filaments and myosin II that prevents premature formation of the contractile ring.

PERSPECTIVES FOR THE FUTURE

Dynein is an enigmatic molecular motor for several reasons. First, the head domain is based on distinctive repeats of a β -propeller motif. The β -propeller-based design suggests that the catalytic mechanism may differ from those of kinesin and myosin. Equally distinctive is the microtubule-binding stalk that extends from the catalytic head domain. Electron micrographs suggest that conformation changes in the head are amplified into large motions of the stalk. Clues to the mechanism will emerge from an atomic-level structure of the entire dynein molecule. As in previous studies of myosin, scientists will take a two-pronged approach employing microscopy and crystallography to create a composite model of dynein structure. Three-dimensional reconstructions from cryoelectron micrographs will reveal the overall organization of the stalk, head, and stem domains under conditions that simulate different stages in force generation. High-resolution information about the catalytic core and coupling to the stalk will be obtained by x-ray crystallography. Structures determined by this approach will be fitted into the electron densities from cryoelectron microscopy.

Dynein seems to be a complementary partner of kinesin and myosin in trafficking vesicles and chromosomes. Although an emerging theme is that dynactin couples a membrane cargo to dynein or kinesin, how microtubule-based movements are coordinated with myosin-based movements remains an unresolved question. Several lines of investigation suggest that dynein could play a central role in coordination. Because the (+) ends of actin filaments and microtubules tend to point toward the cell periphery, dynein is the only major class of motors that moves toward the cell interior. The location of cytoplasmic dynein in the actin-rich cortex is similar to that of myosin and distinct from that of kinesin. Whether dynactin also couples myosin to membrane trafficking is unknown.

The most impressive microtubule machine is the mitotic apparatus. In the course of the cell cycle, it assembles and disassembles and faithfully carries out its core function—the separation of chromosomes. The mitotic apparatus does not exist as a program hardwired in the genome. Instead, the instructions for structure are a consequence of chemistry, the assembly encoded in the reversible interactions between proteins and the timing controlled by signaling circuits. Particularly interesting questions concern the tension-sensing mechanisms that center chromosomes at the metaphase plate and translocate chromosomes at anaphase, the positioning mechanisms of the spindle pole body and the contractile ring, and the force generation mechanism at cytokinesis. A combination of biophysical force-measuring methods and molecular biological mutagenesis methods will be employed to extract the answers to these questions.

KEY TERMS

anaphase 838	kinetochores 840
asters 840	metaphase 838
axonal transport 829	microtubule-associated proteins (MAPs) 823
axoneme 836	microtubule-organizing center (MTOC) 817
basal body 836	microtubules 817
centriole cycle 841	mitosis 838
centromere 838	mitotic spindle 840
centrosome 825	prophase 838
cytokinesis 848	telophase 838
dynamic instability 822	tubulin 818
dyneins 829	
kinesins 829	

REVIEW THE CONCEPTS

1. Microtubules are polar filaments; that is, one end is different from the other. What is the basis for this polarity, how is polarity related to microtubule organization within the cell, and how is polarity related to the intracellular movements powered by microtubule-dependent motors?
2. Microtubules both in vitro and in vivo undergo dynamic instability, and this type of assembly is thought to be intrinsic to the microtubule. What is the current model to account for dynamic instability?
3. In cells, microtubule assembly depends on other proteins as well as tubulin concentration and temperature. What types of proteins influence microtubule assembly in vivo, and how does each type affect assembly?
4. Microtubules within a cell appear to be arranged in specific arrays. What cellular structure is responsible for determining the arrangement of microtubules within a cell? How many of these structures are found in a typical cell? Describe how such structures serve to nucleate microtubule assembly.
5. Many drugs that inhibit mitosis bind specifically to tubulin, microtubules, or both. What diseases are such drugs used to treat? Functionally speaking, these drugs can be divided into two groups based on their effect on microtubule assembly. What are the two mechanisms by which such drugs alter microtubule assembly?
6. Kinesin I was the first member of the kinesin motor family to be identified and therefore is perhaps the best-characterized family member. Describe how kinesin I was first isolated and identified. What fundamental property of microtubule-dependent motors was taken advantage of in this procedure?
7. Certain cellular components appear to move bidirectionally on microtubules. Describe how this is possible given

that microtubule orientation is fixed by the MTOC. What types of regulatory processes are thought to control which direction a given cellular cargo will move? Is it possible for certain cellular components to move on both microtubules and actin filaments? Explain your answer.

8. The motile properties of kinesin motor proteins involve both the motor domain and the neck domain. Describe the role of each domain in kinesin movement, direction of movement, or both. What is the experimental evidence for the importance of the neck in kinesin motility?

9. Cell swimming depends on appendages containing microtubules. What is the underlying structure of these appendages, and how do these structures generate the force required to produce swimming?

10. The mitotic spindle is often described as a microtubule-based cellular machine. The microtubules that constitute the mitotic spindle can be classified into three distinct types. What are the three types of spindle microtubules, and what is the function of each?

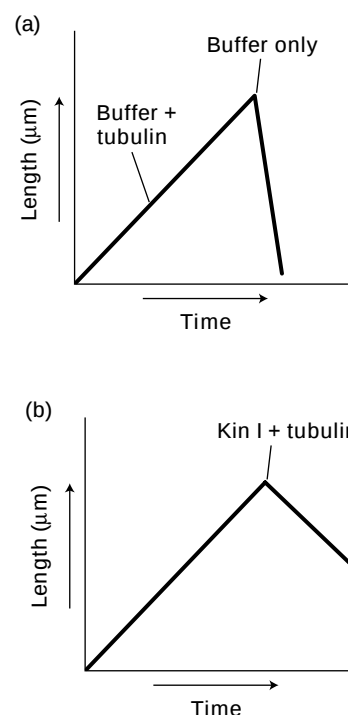
11. Mitotic spindle function relies heavily on microtubule motors. For each of the following motor proteins, predict the effect on spindle formation, function, or both of adding a drug that specifically inhibits only that motor: Kin-C, BimC, cytosolic dynein, and CENP-E.

12. The poleward movement of kinetochores, and hence chromatids, during anaphase A requires that kinetochores maintain a hold on the shortening microtubules. How does a kinetochore hold onto shortening microtubules?

13. Anaphase B involves the separation of spindle poles. What forces have been proposed to drive this separation? What underlying molecular mechanisms are thought to provide these forces?

14. Cytokinesis, the process of cytoplasmic division, occurs shortly after the separated sister chromatids have neared the opposite spindle poles. How is the plane of cytokinesis determined? What are the respective roles of microtubules and actin filaments in cytokinesis?

buffer alone. Part (b) depicts the results when the tubulin and buffer solution in the chamber was replaced with a solution containing tubulin and the M-type kinesin motor protein Kin I. What conclusions can you draw from these experiments?

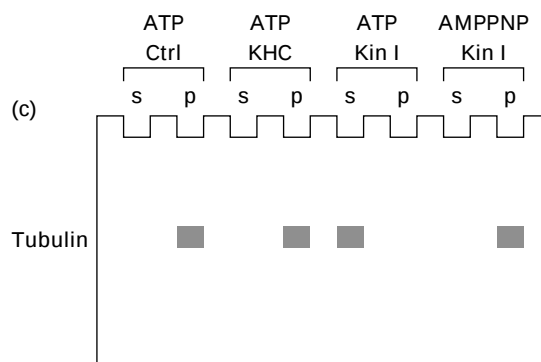


b. In another assay, taxol-stabilized microtubules were incubated alone (Ctrl), with kinesin heavy chain (KHC) and ATP, with Kin I and ATP, or with Kin I and AMPPNP, and then centrifuged to pellet polymerized tubulin. Supernatant (s) and pellet (p) fractions were then separated by SDS-PAGE, and the gel was stained to reveal the position of tubulin, as shown in part (c). What conclusions can you draw from these experiments? What is the significance of the nucleotide present for Kin I activity? Would you expect ATP to be present in the experiments depicted in part (b) of the figure? Why or why not?

ANALYZE THE DATA

Motor proteins are generally considered to move cellular components along microtubules. However, some kinesin family motors may have other functions as described below.

a. In one set of experiments, microtubules were observed in a microscope chamber in which the solution could be rapidly exchanged. Part (a) of the accompanying figure depicts the effect on microtubule length as a function of time when the tubulin and buffer in the chamber were replaced first by an identical tubulin and buffer solution and then by



c. Similar results to those shown in part (c) were also obtained when GMPCPP, a nonhydrolyzable GTP analog, was used to assemble stabilized microtubules. What does this result suggest about the action of Kin I?

d. There is additional evidence that Kin I can produce similar results even at low ratios relative to tubulin. Given this fact and what you know about microtubule assembly, where might you expect to find the motor on microtubules?

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Kinesin- and Dynein-Powered Movements

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