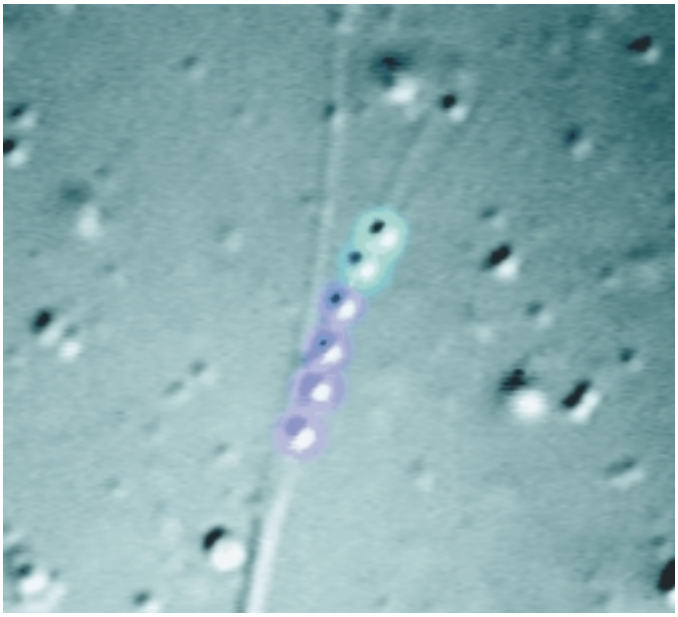
20

MICROTUBULES



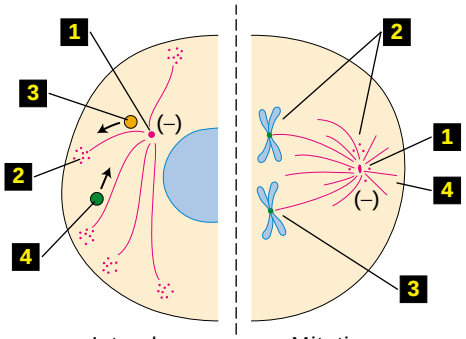
A 3-second time lapse movie captures the kinesin-powered movement of a vesicle along a microtubule. [From N. Pollack et al., 1999, *J. Cell Biol.* **147:**493–506; courtesy of R. D. Vale.]

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



CYTOSKELETAL COMPONENT	CELL FUNCTION
1 MTOC, spindle pole	Organizing cell polarity
2 Microtubule dynamics	Chromosome movements MT assembly
3 Kinesin motors	(+) end–directed vesicle and chromosome transport
4 Dynein motors	(–) end–directed vesicle transport spindle assembly

▲ 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

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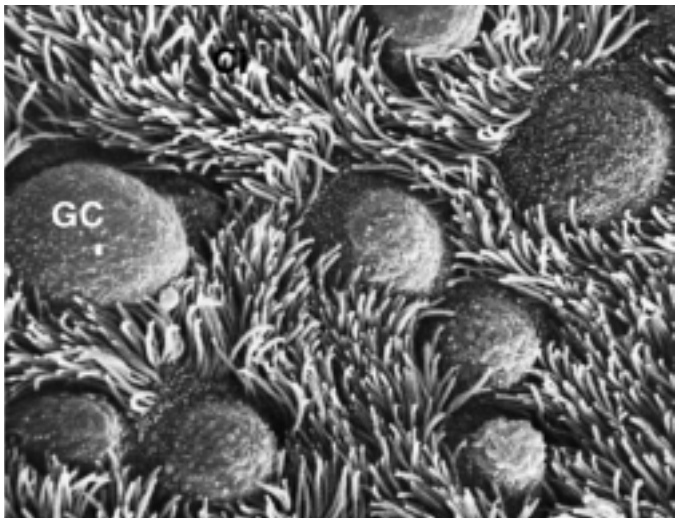

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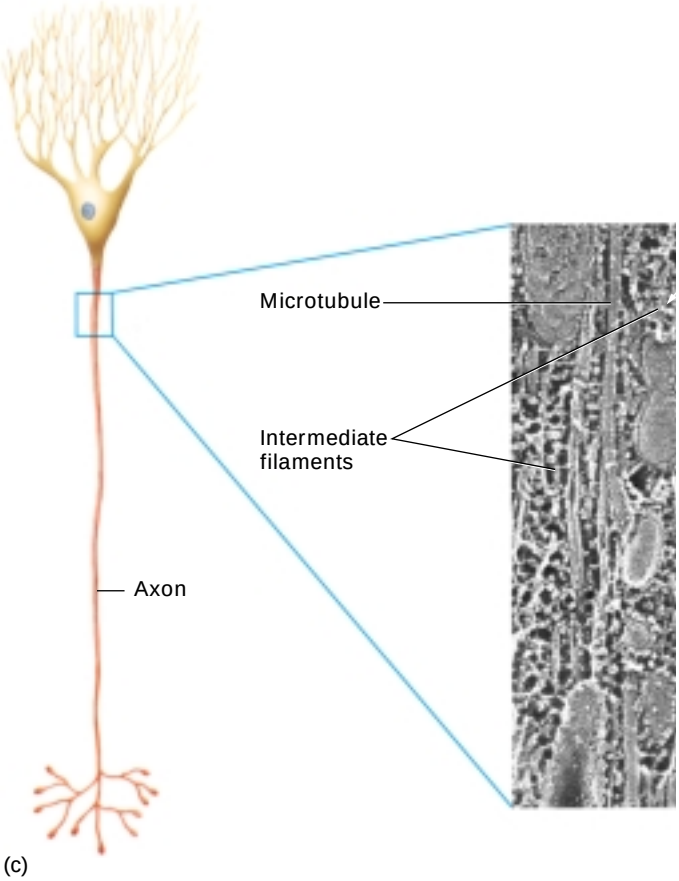

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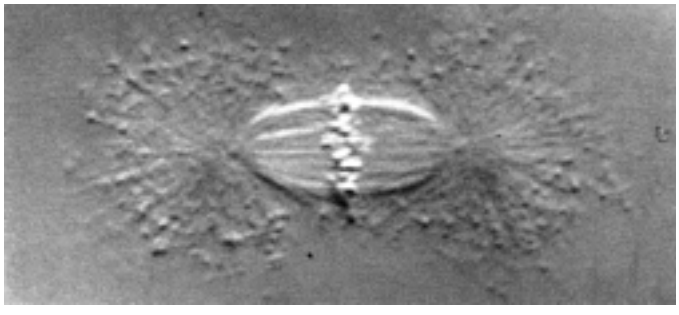


◀ EXPERIMENTAL FIGURE 20-2 **Various microscopic techniques are used to visualize stable and transient microtubule structures.** (a) Surface of the ciliated epithelium lining a rabbit oviduct viewed in the scanning electron microscope. Microtubule-containing cilia cover ciliated cells, and actin-containing 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.

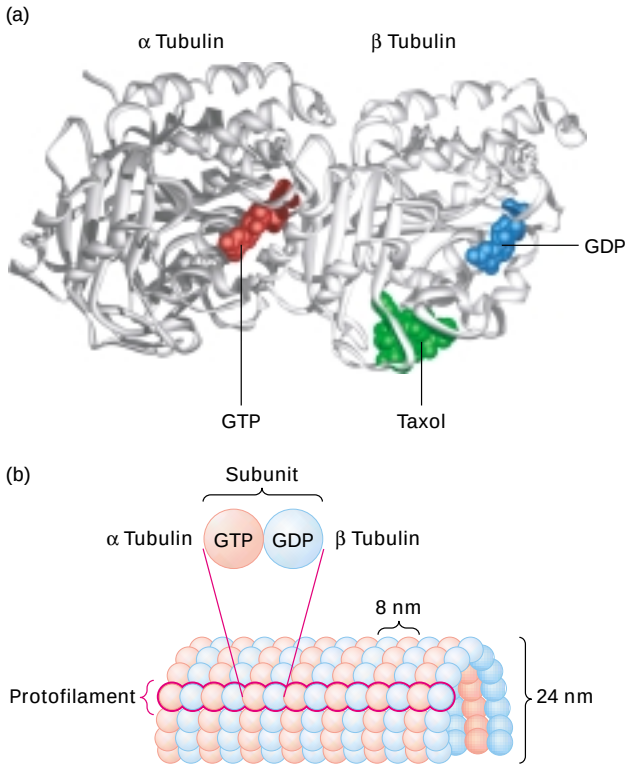
In contrast with these permanent, stable structures, unstable microtubules are found in cells that need to assemble and disassemble microtubule-based structures quickly. For example, in mitosis, the cytosolic microtubule network characteristic of interphase cells disassembles, and the tubulin from it 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.

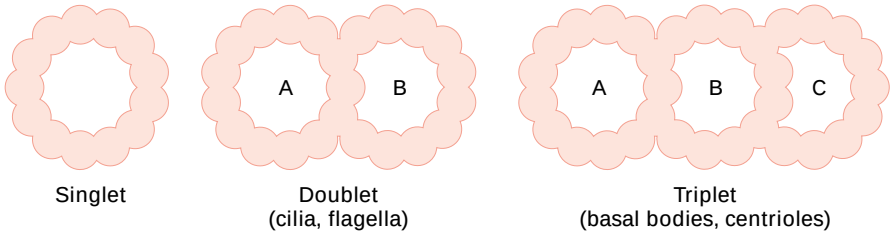
Heterodimeric Tubulin Subunits Compose the Wall of a Microtubule

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 αβ–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.

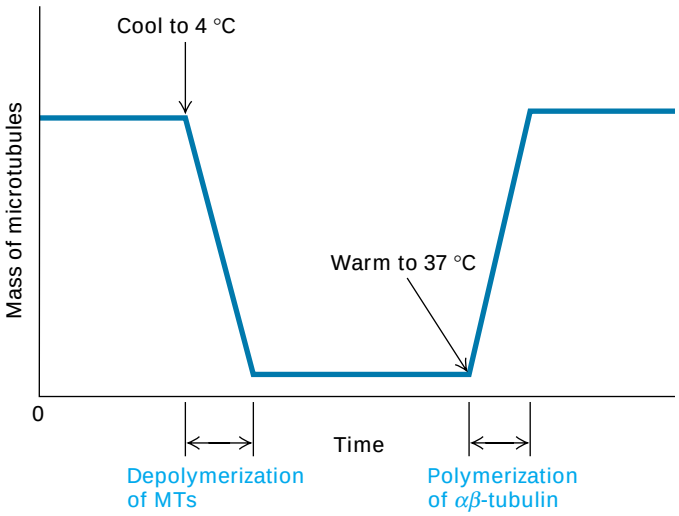
In a microtubule, lateral and longitudinal interactions between the tubulin subunits are responsible for maintaining the tubular form. Longitudinal contacts between the ends of adjacent subunits link the subunits head to tail into a linear *protofilament*. Within each protofilament, the dimeric subunits 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).

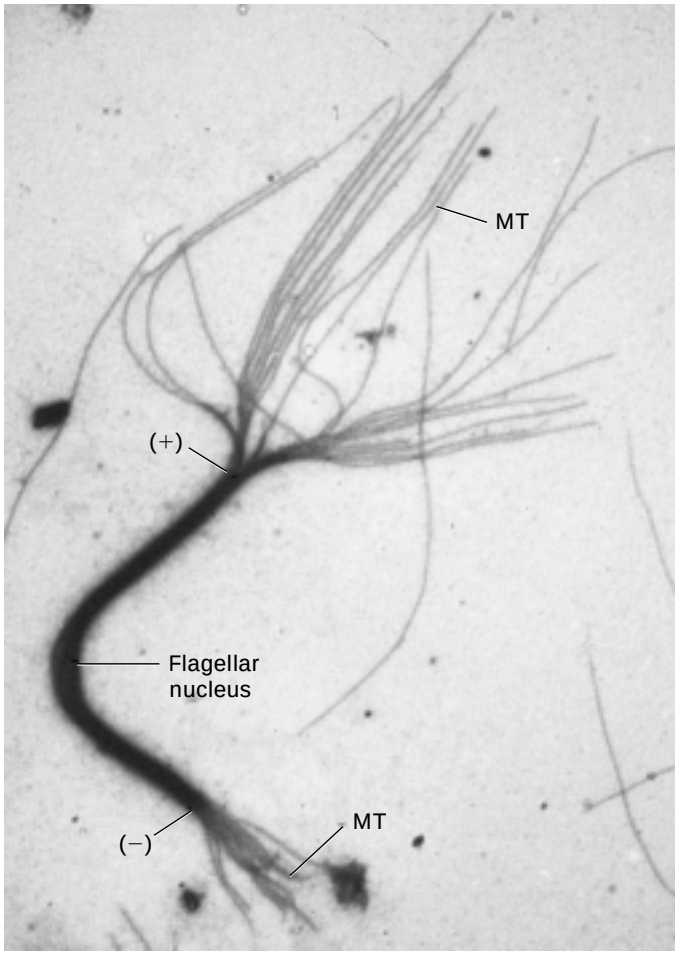
Microtubule Assembly and Disassembly Take Place Preferentially at the (+) End

Microtubules assemble by the polymerization of dimeric αβ-tubulin. Assembly and stability of microtubules are temperature dependent. For instance, if microtubules are cooled to 4 °C, they depolymerize into αβ-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.

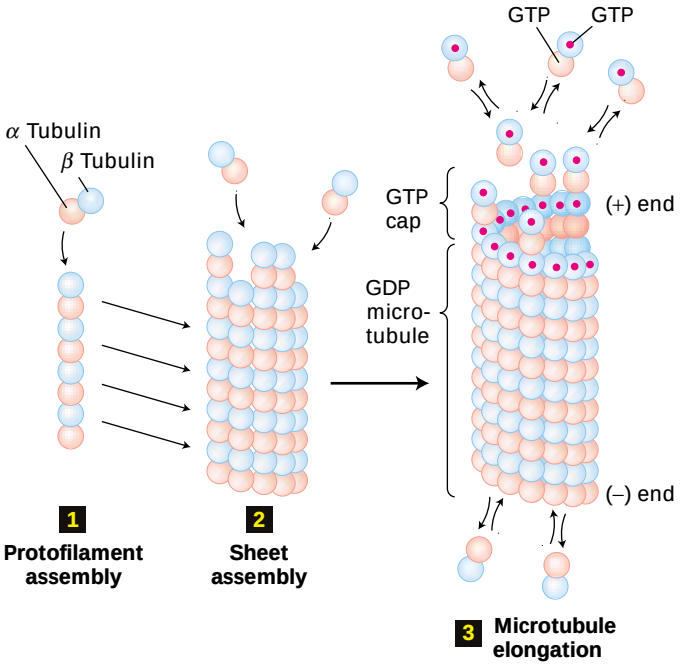
Tubulin polymerization has several properties in common with the polymerization of actin to form microfilaments. First, at αβ-tubulin concentrations above the *critical concentration* (C_c), the dimers polymerize into microtubules, whereas at concentrations below the C_c, microtubules depolymerize, similar 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 αβ-tubulin, which repolymerizes at higher temperatures in the presence of GTP.



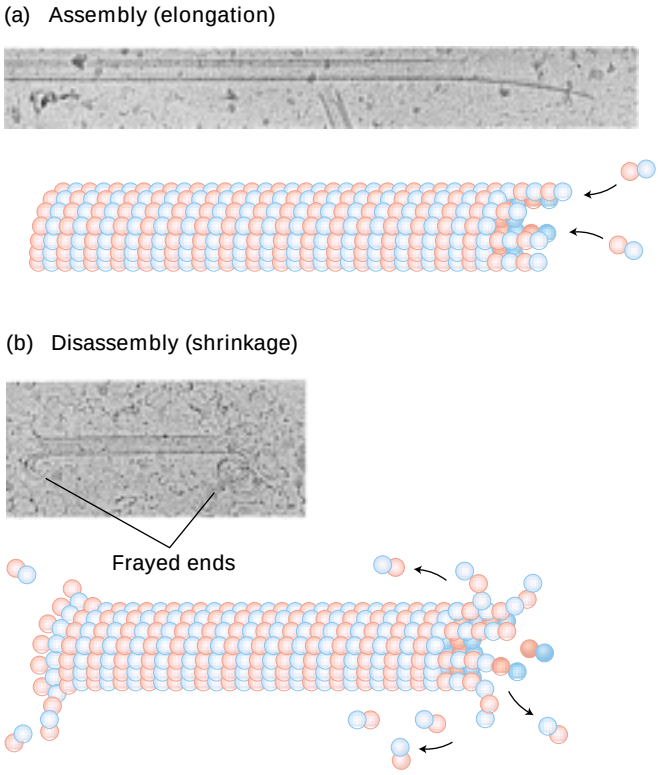
▲ EXPERIMENTAL FIGURE 20-6 **Addition of microtubule fragments demonstrates polarity of tubulin polymerization.** Fragments of flagellar microtubules act as nuclei for the in vitro addition of αβ-tubulin. The nucleating flagellar fragment can be distinguished in the electron microscope from the newly 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.]



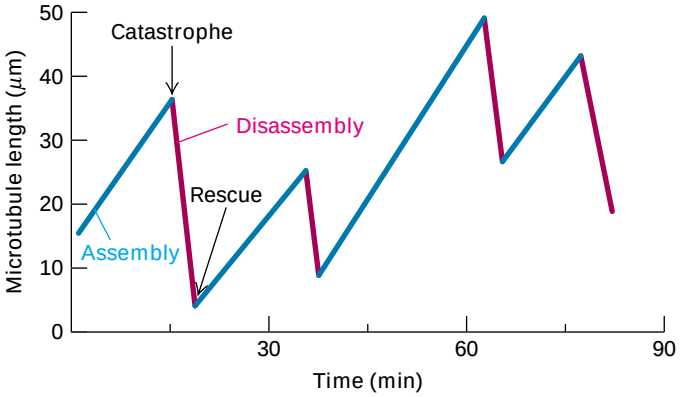
▲ FIGURE 20-7 **Stages in assembly of microtubules.** Free αβ−tubulin dimers associate longitudinally to form short protofilaments (1). These protofilaments are probably unstable and quickly associate laterally into more stable curved sheets (2). Eventually, a sheet wraps around into a microtubule with 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.

at αβ–tubulin concentrations higher than the C_c for polymerization, dimers add preferentially to the (+) end. Fourth, when the αβ–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).

Microtubule assembly comprises three steps: (1) protofilaments assemble from αβ–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.



▲ 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.]

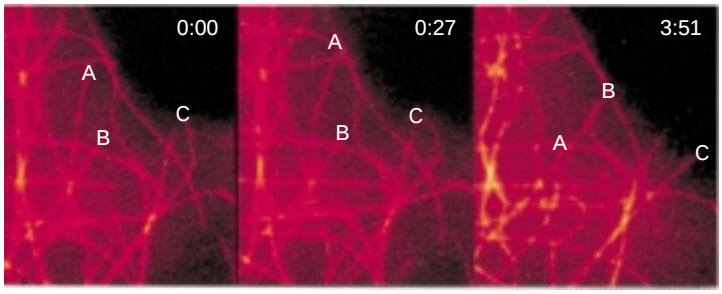


▲ EXPERIMENTAL FIGURE 20-9 **Rate of microtubule growth in vitro is much slower than shrinkage.** Individual microtubules can be observed in the light microscope, and their lengths can be plotted at different times during assembly and disassembly. Assembly and disassembly each proceed at uniform rates, 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 μm/min) than growth (1 μm/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.

MEDIA CONNECTIONS
Video: Cytoplasmic Assembly of Microtubules in Cultured Cells



▲ 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.]