Microtubules

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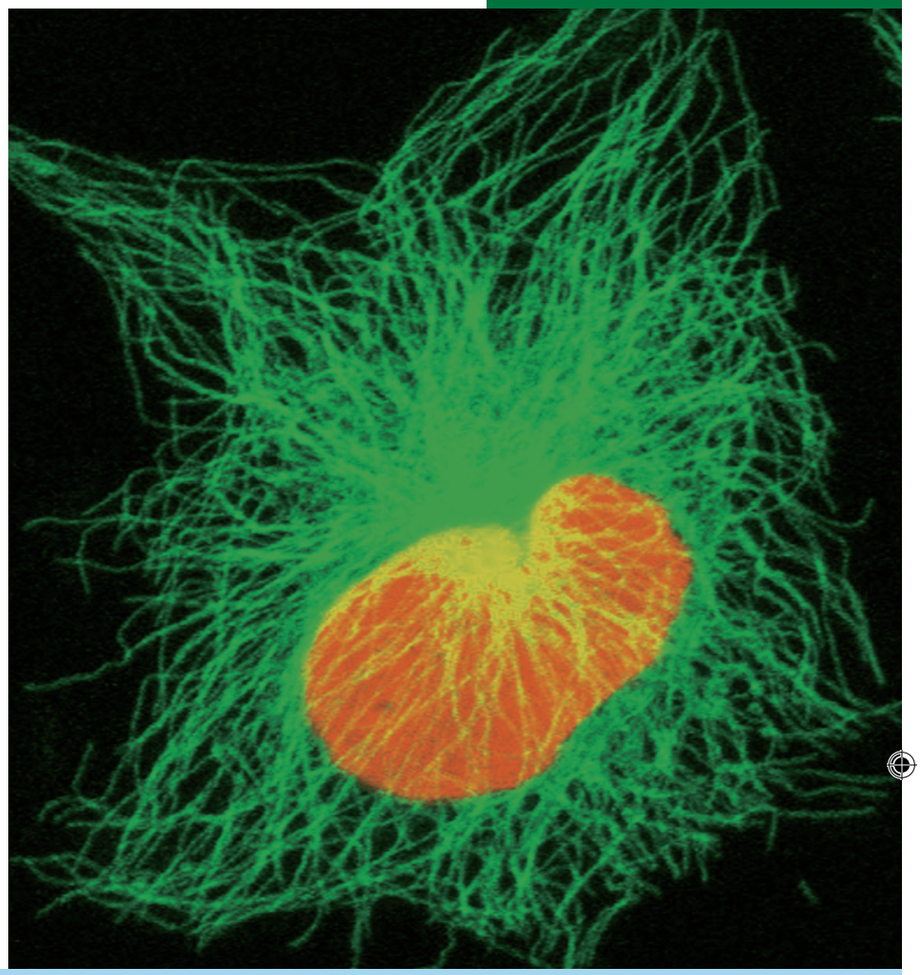


HUMAN EPITHELIAL CELL showing microtubules (green) and DNA (red). Photo courtesy of Lynne Cassimeris, Lehigh University.

CHAPTER OUTLINE

11.1 Introduction

Key concepts

- The cytoskeleton is made up of protein polymers. Each polymer contains many thousands of identical subunits that are strung together to make a filament.
- The cytoskeleton generates cell movements and provides mechanical support for the cell.
- Cells have three types of cytoskeletal polymers: actin filaments, intermediate filaments, and microtubules.
- All cytoskeletal polymers are dynamic; they continually gain and lose subunits.
- Microtubules are polymers of tubulin subunits.
- Microtubules almost always function in concert with molecular motors that generate force and move vesicles and other complexes along the microtubule surface.
- Cilia and flagella are specialized organelles composed of microtubules and motor proteins that propel a cell through fluid or move fluid over the surface of a cell.
- Drugs that disrupt microtubules have medicinal and agricultural uses.

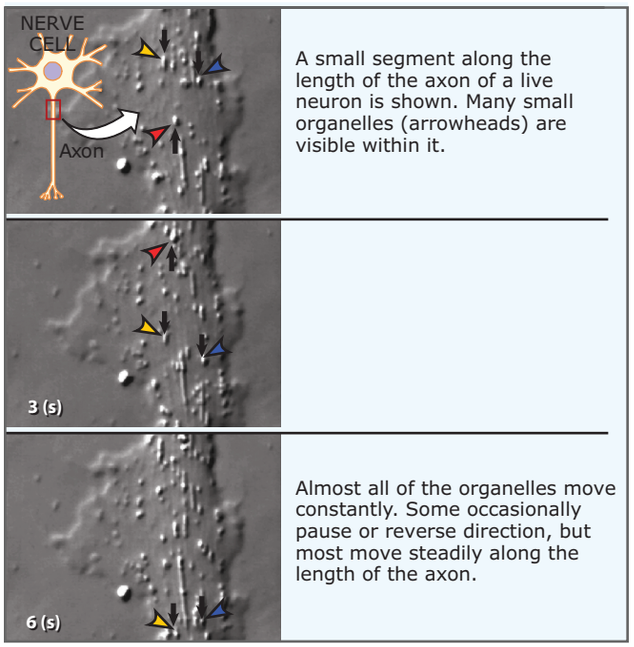


FIGURE 11.1 Three images, from a video, show an axon from a living neuron. An outline of the entire cell is shown in the sketch in the top panel. Three vesicles, marked by red, yellow, and blue arrowheads are followed over a 6 second interval. Two vesicles move toward the tip of the axon and one moves toward the cell body. Photos courtesy of Paul Forscher, Yale University.

The cytoplasm of eukaryotic cells is in constant motion as organelles are continually transported from place to place. Such motion is particularly clear in the cytoplasm of a large, elongated cell such as the neuron shown in **FIGURE 11.1**. Motion occurs in all other types of cells as well. Movement of organelles serves many purposes. Secretory vesicles leave the Golgi apparatus near the center of the cell and are transported to the plasma membrane, where they empty their contents into the extracellular space. At the same time, vesicles internalized at the plasma membrane are transported to endosomes. Mitochondria constantly move about, and the ER stretches and reorganizes. In mitotic cells, chromosomes first align and then move to opposite sides of the cell. Movement of organelles and chromosomes to the right place, at the right time, is accomplished by the **cytoskeleton**, a collection of proteins that form the roadways of the cell's transportation system and the motors that run on them.

The cytoskeleton also has several other important functions: it powers the movements of motile cells and provides the organization and structural support that define the shapes of all cells. Many types of cells move themselves, either within the body (animal cells) or through the environment (single-celled organisms and some gametes). Cells like the white blood cells that track and destroy bacterial invaders crawl across a surface. Others, like sperm cells, swim through fluid to reach their destination. The cytoskeleton powers and guides both these forms of cell locomotion. In addition to its role in motility, the cytoskeleton organizes the internal structure of the cell and defines up from down, left from right and front from back. By determining the general organization of the cytoplasm, the cytoskeleton determines the overall shape of the cell, making it possible to have rectangular epithelial cells or neurons with long, thin axons and dendrites that can stretch for up to a meter in humans.

The cytoskeleton is composed of three major types of structural proteins: **microtubules**, microfilaments (see *12 Actin*), and intermediate filaments (see *13 Intermediate filaments*). These three types of proteins, shown in **FIGURE 11.2**, share several very general features. Each functions not as a single protein molecule, but as a polymer composed of many identical subunit proteins. Like stringing beads together to make a necklace, the cytoskeletal

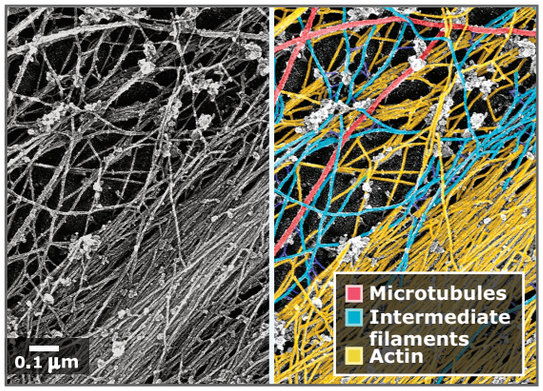


FIGURE 11.2 A small region of a fibroblast cell viewed by electron microscopy (left panel). Numerous filaments are visible. In the right panel, the three types of cytoskeletal polymers present in eukaryotic cells have been colored so that they can be easily distinguished from one another. Reprinted from *J. Struct. Biol.*, vol. 115, M. T. Svitkina, A. B. Verkhovsky, and G. G. Borisy, Improved Procedures for Electron Microsopic Visualization..., pp. 290–303, Copyright (1995) with permission from Elsevier. [http://www.sciencedirect.com/science/journal/10478477]. Photos courtesy of Tatyana Svitkina, University of Pennsylvania.

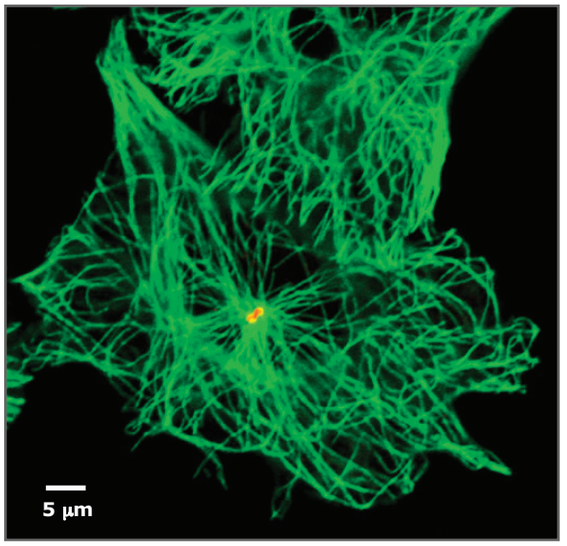


FIGURE 11.3 The microtubules in a fibroblast cell, viewed after labeling them with a fluorescent dye so that they appear green. The microtubules are organized around a point (red) near the center of the cell and extend throughout the cytoplasm. Most of the microtubules are long enough to run from one part of the cell to another. Photo courtesy of Lynne Cassimeris, Lehigh Universitys.

polymers are built in the cytoplasm by stringing together thousands of subunit proteins. A general feature of all cytoskeletal polymers is that they constantly gain and lose subunits, rather than form static structures. The dynamic turnover of the cytoskeletal polymers allows the cytoskeleton to reorganize itself, building new roadways for transport or new struts for support as needs change within a cell.

While the three cytoskeletal polymers share general features, each also has unique properties, making each polymer best suited to carry out specific tasks within the cell. The three polymer systems will be considered separately, although they often work together.

Here, we focus on microtubules. The basic subunit that forms a microtubule is the protein tubulin. Tubulin molecules assemble with one another to form a hollow tube about 25 nm in diameter, giving microtubules their name. A single microtubule can contain tens or hundreds of thousands of tubulin molecules and stretch for many microns, allowing it to extend over half the length of most eukaryotic cells. Interphase cells often contain hundreds of long microtubules that run throughout the cytoplasm and connect one area of a cell to another, as shown in **FIGURE 11.3**.

Microtubules almost always function in concert with the molecular motors that move on them (see *11.12 How motor proteins work*). These motor proteins attach to various cargo, including organelles and vesicles, and pull them along the surface of the microtubule, much like trucks move cargo on a highway. Microtubules and motor proteins also work together to separate replicated chromosomes at mitosis (see *14 Mitosis*), and they form the core of motile structures used by cells to swim or move fluid over their surface (see *11.16 Cilia and flagella are motile structures*). Microtubules and motor proteins are even used by viruses, such as HIV and adenovirus, allowing the viruses to rapidly reach the nucleus and replicate.

Small organic molecules that disrupt microtubule polymerization have medicinal and agricultural uses. Drugs that make microtubules more or less stable block mitosis and are used to treat cancers. One such drug is paclitaxel (Taxol™) shown in **FIGURE 11.4**, which is used to treat ovarian and breast cancers. Taxol™ binds to microtubules and makes them more stable by preventing tubulin subunits from dis-

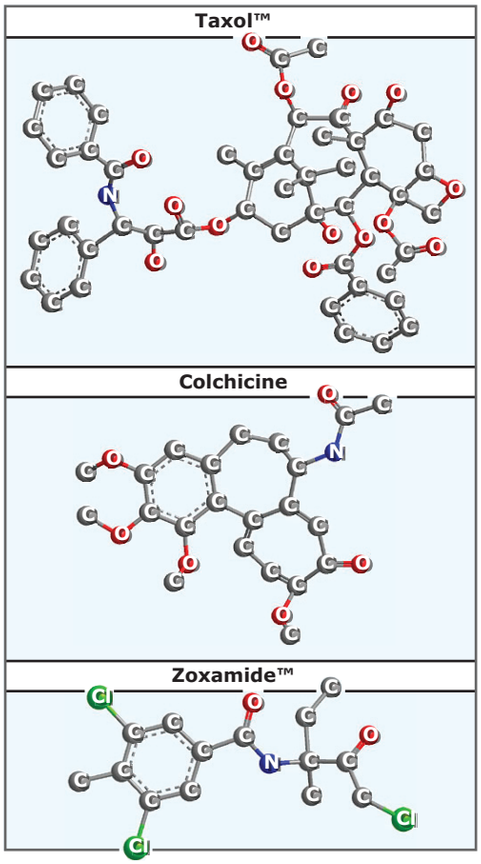


FIGURE 11.4 The structures of three small organic molecules that disrupt microtubule assembly or disassembly. Paclitaxel (Taxol™) and colchicine are natural products produced by specific plants (the Pacific yew tree and the meadow saffron, respectively). Zoxamide™ is a man-made molecule discovered by screening a large collection of small molecules for those with the ability to interfere with microtubules.

sociating. Colchicine (see Figure 11.4), another tubulin poison, has the opposite effect, causing a cell's microtubules to disappear. It is used to treat gout because disruption of the microtubule cytoskeleton blocks migration of the white blood cells responsible for inflammation in this disease. Small molecules targeted to tubulin also have important applications in agriculture. For example, Zoxamide™ (see Figure 11.4), a fungicide, binds specifically to fungal tubulins and prevents fungal growth. Zoxamide™ is used to control late blight in potatoes, the fungal disease responsible for the Irish potato famine of 1850. The search for new tubulin-binding drugs with medical or agricultural uses is an active area of research today.

11.2 General functions of microtubules

Key concepts

- Cells use microtubules to provide structural support because microtubules are the strongest of the cytoskeletal polymers. Microtubules resist compression.
- Cells also rely on the dynamic assembly and disassembly of microtubules to allow them to quickly reorganize the microtubule cytoskeleton.
- Cells can make their microtubules more or less dynamic, allowing them to take advantage of the adaptability of microtubules (when dynamic) or the strength of microtubules (when stable).
- Different cells can have unique organizations of microtubules to suit specific needs.

A simple experiment, to disrupt the cell's microtubules, illustrates the functions of this component of the cytoskeleton. The tubulin subunits strung together to make a microtubule can be depolymerized into individual subunits by treating cells with drugs like colchicine (see *11.1 Introduction*). These drugs block the formation of new microtubules, causing an imbalance in the continual formation and dissolution of the microtubule cytoskeleton. Microtubules that depolymerize cannot be replaced, soon leading to the loss of all microtubules from the cytoplasm. In general, most cells lose their shape and form round balls when their microtubules are depolymerized. The internal organization of the cell is also disrupted. The Golgi complex, which is normally present as a single structure located near the nucleus, fragments into pieces and disperses throughout the cell. The ER, normally a network that extends throughout the cytoplasm, collapses around the nucleus because it is connected to the nuclear envelope. All of these changes are reversed when the microtubule depolymerizing drug is subsequently removed: microtubules re-form into their original pattern, the cell regains its shape, and the ER and Golgi return to their normal positions. This simple experiment illustrates the broad functions of microtubules in cell organization, structure, and movement.

The functions of microtubules in cells depend upon two seemingly contradictory properties: microtubules can both act as stiff structural elements and they can easily fall apart. The tubular structure and relatively large diameter of microtubules make them relatively stiff and allow them to resist compression. Microtubules can be thought of as similar to a garden hose,

which can bend over long distances but cannot be compressed. Unlike a hose, however, microtubules are exceptionally dynamic. If left to themselves, microtubules constantly either elongate or shorten by the addition or loss of subunits. The shortening of a microtubule is particularly dramatic, frequently covering most of its length and often making it fall apart and disappear altogether. Microtubules are inherently prone to falling apart once they have assembled, and cells often use other proteins to stabilize them and prevent their disassembly. Although a structural element designed to fall apart might seem odd, such instability has the great advantage of allowing the microtubules within a cell to be disassembled and reorganized within minutes when necessary. A common example of this, shown in **FIGURE 11.5**, is the dramatic rearrangement of microtubules that takes place at the beginning of mitosis, a reorganization that occurs within just a few minutes. Another example of microtubule reorganization occurs in some developing oocytes and illustrates how extensive it can be. A single oocyte of the frog *Xenopus laevis*, shown in **FIGURE 11.6**, is about 1 mm in diameter and contains roughly a half million microtubules of 600 μm average length. If all the subunits in these microtubules were present as a single long microtubule, it would be about 300 m in length, close to the length of three football fields. Despite this large quantity of microtubules, the entire microtubule cytoskeleton is depolymerized and reorganized within 30 minutes when the oocyte is stimulated to mature into an egg.

In some cells, the dynamic nature of the microtubule cytoskeleton does more than just allow rapid remodeling from one type of array to another. A fibroblast, for example, must move within the body and be able to change direction. Its microtubules are organized in a starlike pattern, radiating outward in all directions from a single point near the nucleus, as shown in **FIGURE 11.7**. These microtubules are short-lived, most lasting only a fraction of the time it takes the cell to move any significant distance. A fibroblast can continue to move if its microtubules are all depolymerized. Intriguingly, however, without its microtubules it can no longer turn and navigate, suggesting that the dynamic nature of its microtubules is required to allow it to change direction.

A neuron is much different from a fibroblast in both shape (see Figure 11.7) and behavior and uses its microtubules for their strength.

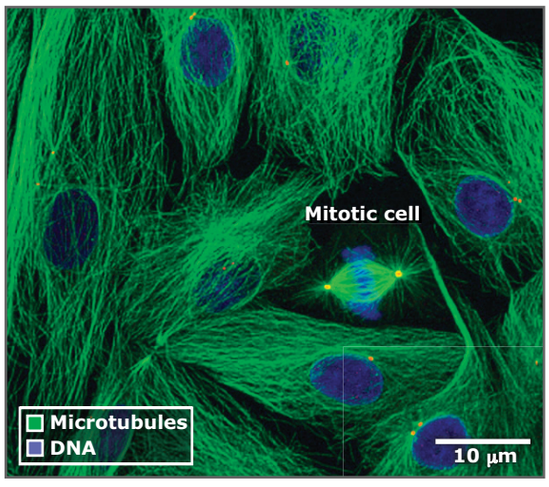


FIGURE 11.5 A field of about a dozen cells is shown, with their microtubules and chromosomes visualized by fluorescence microscopy. One mitotic cell—with its microtubules assembled into a very clear mitotic spindle—is surrounded by interphase cells. The reorganization of microtubules that takes place as a cell enters mitosis is dramatic but requires only a few minutes. Photo courtesy of Lynne Cassimeris, Lehigh University.

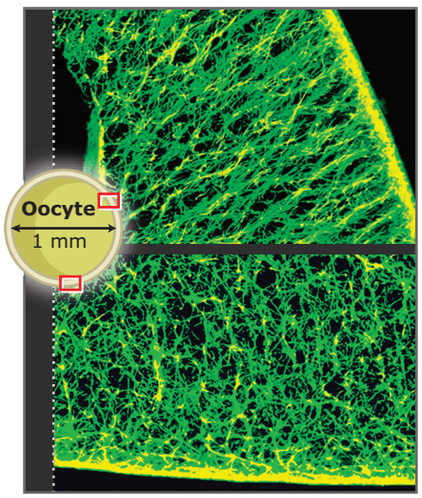


FIGURE 11.6 Mature oocytes of the frog *Xenopus laevis* are huge cells that are densely packed with microtubules. The two photographs show the microtubules at two points around the edge of an oocyte. Despite the enormous number of microtubules present in the oocyte and their great length, they can all be taken apart completely in only a few minutes. Photo courtesy of Dr. David L. Gard, University of Utah.

A neuron is immobile and has a small cell body from which long-lived processes (axons and dendrites) extend great distances. The interiors of the processes are packed with parallel microtubules. These microtubules carry large numbers of vesicles and other material to and from the synapse. Unlike the microtubules of the fibroblast, those in the process of a neuron are stable and are essential for the structure of the cell; if depolymerized, the processes slowly collapse. Neurons, then, take advantage of the strength of microtubules to use them as a stable structural element.

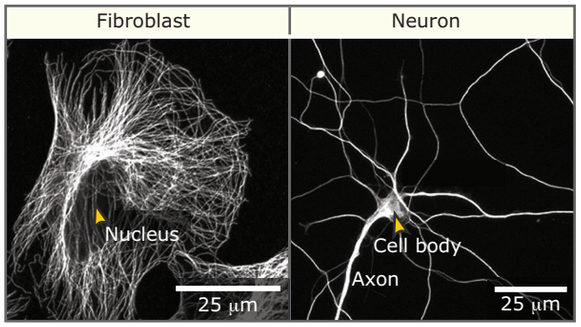


FIGURE 11.7 The extemely different shapes of these two types of cells require that their microtubules be organized differently. In the human fibroblast, individual microtubules are visible and run throughout the cytoplasm from a point near the nucleus. In the neuron, the microtubules are packed together within the long, thin projections that extend from the cell body. Left photo courtesy of Lynne Cassimeris, Lehigh University and right photo courtesy of Ginger Withers, Whitman College.

Although the mature neuron uses its microtubules for their strength, a growing neuron also uses the dynamic properties of microtubules. When neurons are first growing and forming synapses with other neurons, each extends from its cell body thin projections that will become the axon and dendrites. At the tip of each projection is a very active, motile region called a growth cone, which is clearly visible in **FIGURE 11.8**. Growth cones extend the projections by moving over long distances, the projections forming behind them as they travel. To help them move, growth cones have dynamic microtubules that function very much like those in a locomoting fibroblast. Thus, neurons can regulate when and where their microtubules are dynamic and when and where they are stable. The ability to regulate the dynamic turnover of microtubules in time and space is a general feature of all cells.

Microtubules are organized to suit the unique needs of each cell. An example, shown in **FIGURE 11.9**, is for two cells with similar shapes: a single-celled fission yeast, *Schizosaccharomyces pombe*, and a nucleated red blood cell from a nonmammalian vertebrate such as a chicken or a frog. In each case, the cell

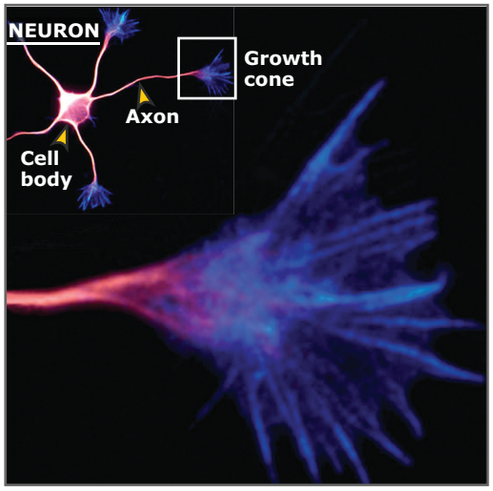


FIGURE 11.8 In the upper left is a picture of an entire neuron with several axons projecting from its cell body. Each axon has a growth cone (blue) at its tip. A single growth cone with its axon leading away to the right is shown enlarged in the main photograph. Microtubules are in red and actin filaments in blue. Photo courtesy of Drs. Leif Dehmelt, The Scripps Research Institute; and Shelly Halpain, University of California, San Diego.

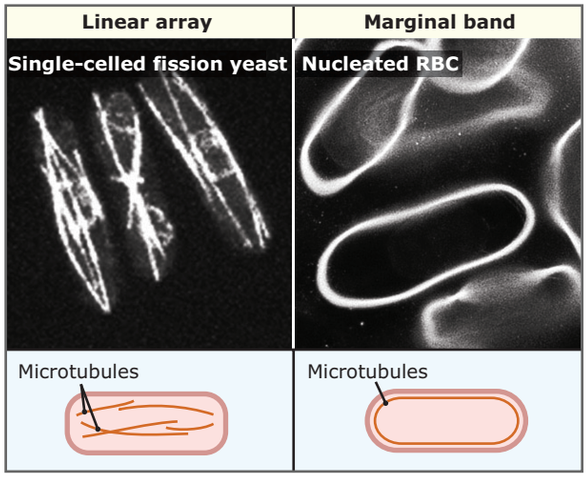


FIGURE 11.9 The yeast *S. pombe* (left) has a relatively small number of bundled microtubules that center the nucleus within the cell and transport factors that regulate growth to its ends. In an amphibian's red blood cells (right), a circular band of microtubules underlies the plasma membrane and helps a cell resist deformation as it sqeezes through the capillaries. Left photo courtesy of Phong T. Tran, University of Pennsylvania and right photo courtesy of Lynne Cassimeris, Lehigh University.

is shaped like a sausage, but the microtubule organizations are very different. In *S. pombe*, bundles of microtubules extend toward the cell tips, where new material is deposited for polarized cell growth. The bundles of microtubules also position the nucleus in the center of the cell. *S. pombe* does not need to use its microtubules to resist compression because it has a cell wall to provide this function. A very different microtubule organization is found in the red blood cells, which, like all animal cells, lack any form of cell wall. These cells have bundles of microtubules associated with the plasma membrane in a structure called a marginal band. The marginal band microtubules provide strength to the cell membrane, much like ankryin and spectrin do in mammalian red blood cells.

These examples illustrate the general functions of the microtubule cytoskeleton and raise many questions. How are microtubules put together and taken apart so rapidly? How do cells regulate the dynamic assembly and disassembly of microtubules? What determines the organization of the microtubules within a cell? How does the microtubule cytoskeleton generate movement? This chapter will answer these questions.

11.3 Microtubules are polar polymers of α- and β-tubulin

Key concepts

- Microtubules are hollow polymers of tubulin heterodimers.
- Thirteen linear chains of subunits, called protofilaments, associate laterally to form the microtubule.
- Lateral bonds between protofilaments stabilize the microtubule and limit subunit addition and subtraction to microtubule ends.
- Microtubules are polarized polymers. The plus end is crowned by β-tubulin and assembles faster. The minus end is crowned by α-tubulin and assembles slower.

Microtubules participate in a wide range of cell functions, yet the underlying microtubule structure, and the subunits from which it is built, are virtually identical from yeast to humans. The next several sections will describe the structure of microtubules and how they assemble (and disassemble) from purified subunits in the test tube. Although how microtubules behave in a test tube may seem a bit remote from what happens in cells, it is critical to understand because it establishes what the basic properties and behaviors of microtubules are. These in turn determine what a cell must do in order to organize its microtubules and how it can put them to use.

The building block of a microtubule is the protein tubulin. Tubulin is a heterodimer, made up of two closely related proteins, α- and β-tubulin. The two proteins share about 40% sequence identity and are never found alone. Instead, one molecule of α-tubulin and one of β-tubulin are always found associated together, making up the 100 kDa tubulin heterodimer. For simplicity, the heterodimer is usually just called "tubulin" in recognition of the fact that it always functions as a single unit. Since both α- and β-tubulin are nearly spherical in shape, the heterodimer resembles a peanut, as illustrated in **FIGURE 11.10**. Its structure is known at atomic resolution: α- and β-tubulin have very similar structures and are arranged "in line" in the dimer such that the front of one is bound to the back of the other.

Each molecule of α- and β-tubulin binds a molecule of GTP. The structure of the tubulin heterodimer shows that the GTP bound to α-tubulin is located near the interface with β-tubulin (see Figure 11.10). This GTP is never hydrolyzed and does not exchange with nucleotides in solution. In contrast, the GTP-bound to β-tubulin is exposed at one end of the heterodimer, where it can exchange

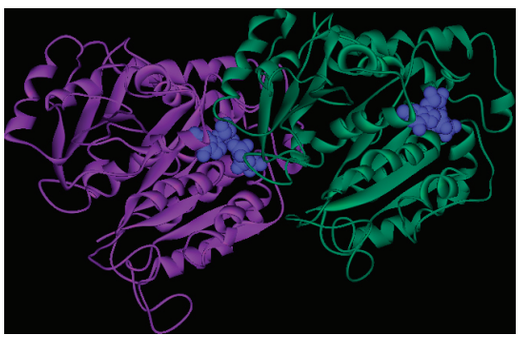


FIGURE 11.10 The three-dimensional structure of the tubulin heterodimer, the basic building block of a microtubule. In purple and green colors are the polypeptide backbones of the two protein subunits, and in blue are the two molecules of GTP that are bound to each heterodimer. Note the similarity in the structures of the two subunits, and that they are arranged head to tail in the complex. At the bottom is a schematic drawing that shows how the dimer will be represented in the figures in the chapter. Structure from Protein Data Bank 1TUB. E. Nogales, S. G. Wolf, and K. H, Downing, *Nature* 391 (1998): 199–203.

with nucleotide in solution (see Figure 11.10). β-tubulin's GTP is hydrolyzed to GDP during microtubule assembly. Hydrolysis of GTP to GDP is thought to result in a change in conformation of the tubulin heterodimers, which plays a significant role in the dynamic turnover of microtubules.

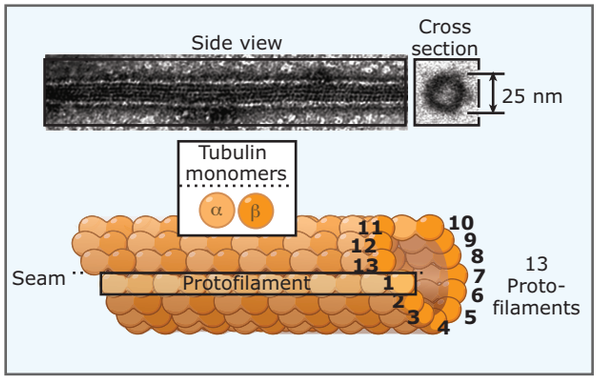


FIGURE 11.11 The structure of a small segment of a microtubule. The individual tubulin heterodimers are aligned end to end in straight protofilaments, and the protofilaments are arranged side by side to form a hollow tube. All of the heterodimers have the same orientation, with the β subunit toward one end of the microtubule and the alpha subunit toward the other. At the top are electron micrographs of microtubules assembled from pure tubulin. Photo (left) courtesy of Lynne Cassimeris, Lehigh University and micrograph (right) provided by Harold Erickson, Duke University School of Medicine.

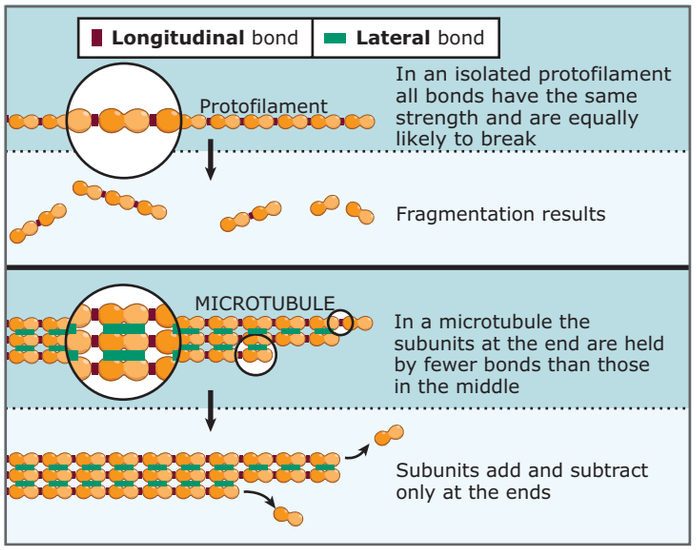


FIGURE 11.12 Tubulin heterodimers form both longitudinal bonds (along the length of the protofilament) and lateral bonds (between subunits in adjacent protofilaments). Single protofilaments are prone to fragmenting because all bonds are of equal strength and are equally likely to break. In a microtubule, the existence of lateral bonds makes it unlikely that the polymer will break in the middle. Subunits add or subtract only at the ends, where each subunit is held by fewer bonds than in the middle of the microtubule. For clarity, only three protofilaments are shown.

Microtubules are protein polymers composed of thousands of tubulin subunits organized into a hollow tube. Typically, a microtubule is made up of 13 linear chains of subunits that run parallel to one another along its length; each linear chain is termed a **protofilament**. These protofilaments, shown in **FIGURE 11.11**, associate laterally to form the microtubule. While it is possible to form a microtubule from anywhere between 11 and 15 protofilaments, the large majority of microtubules found in cells have 13 protofilaments. With 13 protofilaments a microtubule has a diameter of 25 nm, about five times the thickness of its wall.

Tubulin heterodimers bind head to tail along the length of each protofilament (see Figure 11.11). Within most adjacent protofilaments, α-tubulins are next to other α-tubulins and β-tubulins next to other β-tubulins. Because adjacent protofilaments are slightly out of register with one another, there is a single discontinuity, or seam, where the α-tubulins in one protofilament are adjacent to the β-tubulins in the next. It is likely that this seam plays an important role in microtubule assembly.

Within a microtubule, each tubulin heterodimer forms extensive noncovalent bonds with its neighbors. As diagrammed in **FIGURE 11.12**, these noncovalent bonds form longitudinally as well as laterally between dimers in a protofilament, linking adjacent protofilaments. A single longitudinal bond is stronger than a single lateral bond, but it is the presence of numerous lateral bonds that makes the polymer strong. It is easy to illustrate why this is so. Subunits in an isolated protofilament have only longitudinal bonds. The bonds are all of equal strength, so they all have the same probability of breaking. This makes a single protofilament prone to fragmentation (see Figure 11.12). In a microtubule, subunits in the middle of the polymer also form lateral bonds to the subunits in the adjacent protofilaments. For a microtubule to break, all 13 of its protofilaments would have to simultaneously lose their longitudinal bonds at the same point. This is highly unlikely, making it very rare that a microtubule breaks.

The presence of lateral bonds between subunits in a microtubule also makes it unlikely that tubulin dimers in the center of the microtubule will come unbound from the polymer,

since release of a dimer from the middle of the microtubule requires simultaneous breakage of several bonds. Instead, subunits add and subtract only at filament ends, where fewer bonds connect each subunit to the rest of the microtubule (see Figure 11.12).

Purified tubulin heterodimers will spontaneously form the lateral and longitudinal bonds necessary to form a microtubule. Therefore, microtubule assembly is a **self-assembly** process, in which all the information needed to build the final structure is contained within its subunits. Other examples of self-assembly processes include actin filament polymerization and the assembly of some viral capsids.

The arrangement of subunits within a microtubule makes its two ends different (see Figure 11.11). Within each protofilament all the tubulin heterodimers have the same orientation, and within a microtubule, all of the protofilaments run in the same direction. Thus, a cap of β-tubulins is exposed at one end of the microtubule (called the plus end), while at the opposite end a ring of α-tubulins is exposed (called the minus end). This organization gives a microtubule two very important properties. First, its two ends are structurally different and can behave differently. As we shall see, cells often make use of this, for example, by regulating assembly at the two ends independently. Second, each microtubule has a polarity—an inherent directionality—and can be thought of as pointing in one direction or another. This polarity is present throughout the microtubule, not just at its ends. Just by looking at the surface of a microtubule at any point—even far from either end—it is possible to tell which direction leads to the plus end and which leads to the minus end. The polarity of microtubules allows them to act as directional tracks for molecular motor proteins and is essential for the roles played by both the microtubules and the motors in organizing the interior of a cell.

Within cells, microtubules are highly organized with respect to their polarity, as illustrated in **FIGURE 11.13**. In a fibroblast or other type of cell with a radial array of microtubules, for example, all of the microtubules are arranged with their minus ends near the center of the cell and their plus ends near the periphery. In epithelial cells the microtubules are arranged parallel to one another, running from top to bottom of the cell, all with their plus ends at the bottom of the cell and their minus ends at the top. Similarly, all of the microtubules in an axon point in the same direction. In each case the polarity of the microtubules in the array is essential for the organization and function of the cell.

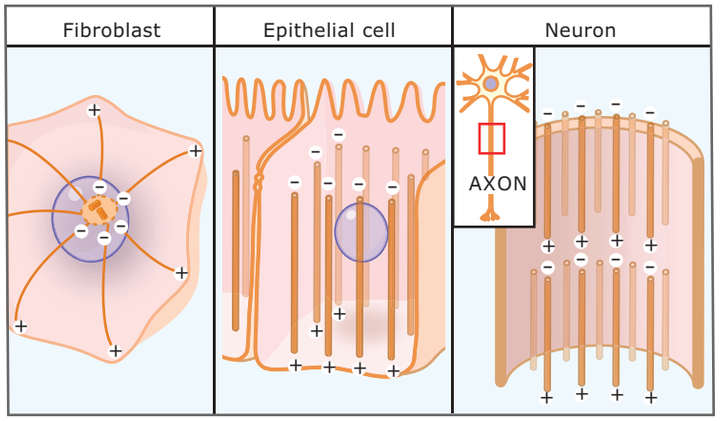


FIGURE 11.13 In each of the three types of cells, all of the microtubules within the cell have the same polarity. This allows the polarity of a microtubule to be used as an accurate indicator of direction in the cytoplasm. In the epithelial cell, for example, the direction + to – is "up." Cell shape and the ability of cells to specialize regions of their surface or interior depend on microtubule polarity.

11.4 Purified tubulin subunits assemble into microtubules

Key concepts

- Microtubule polymerization begins with the formation of a small number of nuclei (small polymers).
- Microtubules polymerize by addition of tubulin subunits to both ends of the polymer.
- A critical concentration of tubulin subunits always remains in solution. The concentration of tubulin must be above the critical concentration for assembly to occur.

Microtubules form by the polymerization of tubulin subunits. This process can be studied *in vitro* using purified tubulin. To form a microtubule, tubulin and GTP are first combined in an appropriate buffer and the solution is then warmed to 37°C to initiate polymerization (for mammalian tubulin). The formation of microtubules is easily detected by light scattering, because the assembled polymers scatter light, whereas individual tubulin molecules do not. The amount of light scattered is proportional to the amount of microtubule polymer.

A plot of the amount of microtubule polymer formed over time is shown in **FIGURE 11.14**. Initially, there is a lag phase during which no polymer is detected. Polymer then begins to

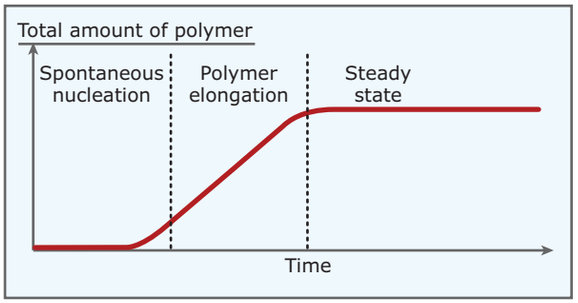


FIGURE 11.14 A graph showing the amount of microtubule polymer formed with time as purified tubulin polymerizes *in vitro*. Initially, no polymer is detected. The amount of polymer then increases linearly with time before leveling off. At each of the three stages different molecular events are occurring.

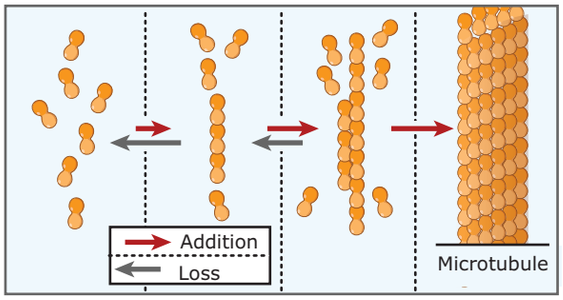


FIGURE 11.15 A simplified view of microtubule nucleation. A small number of dimers can associate, but the complex usually falls apart. In rare instances, however, additional dimers add on and dimers associate side by side. Once a complex of 6-12 dimers is formed, it is likely to continue growing. A small sheet of protofilaments results that will eventually close into a short microtubule, often called a "seed."

form and the amount rises linearly until it reaches a plateau. The initial stage, when no polymer is detected, is called the spontaneous nucleation phase. During this initial lag stage, small nuclei—assemblies of only a few tubulin subunits—begin to form, as shown in **FIGURE 11.15**. These nuclei are unstable because they are more likely to fall apart than they are to gain additional subunits. Some do increase in size, however. Once a sufficient number of subunits associate (6–12), a nucleus is stable because it is more likely to grow than to depolymerize. Because the formation of large enough nuclei is difficult, nucleation is the rate-limiting step in microtubule polymerization. Cells avoid being limited by the slow rate of spontaneous nucleation by having specialized protein complexes that accelerate the nucleation step and determine where it occurs.

During the linear rise in amount of polymer, tubulin subunits add to the ends of the

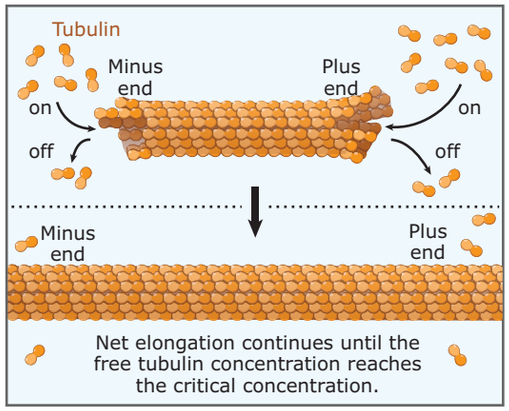


FIGURE 11.16 After a microtubule seed has formed, the microtubule elongates by the addition of subunits at its two ends. One end grows faster than the other. This growth leads to the linear increase in the total amount of polymer that follows the spontaneous nucleation phase.

microtubule seeds (nuclei) that formed during spontaneous nucleation. Each microtubule elongates by the addition of subunits onto both its plus and minus ends. Subunits also dissociate from the ends but do so less frequently than they are added. The net result is the addition of subunits, as illustrated in **FIGURE 11.16**. The net elongation rate is dependent on the rates of subunit addition and loss and is described by Equation 11.1:

$$dP/dt = k_{on}[\text{tubulin}] - k_{off} \qquad (11.1)$$

where dP/dt is the amount of polymer formed per unit time, [tubulin] is the concentration of tubulin molecules in solution, k_{on} is the on rate constant (units of $M^{-1}sec^{-1}$), and k_{off} is the off rate (units of sec^{-1}). The rate constants for the plus and minus ends differ, so Equation 11.1 must be written for each end of the polymer. As shown mathematically in Equation 11.1, microtubules polymerize faster at higher tubulin concentrations.

Eventually, the amount of microtubule polymer reaches a maximum as the system reaches a steady state in which subunit loss and addition are balanced. When the maximum amount of polymer is formed, some tubulin subunits will remain in solution. The concentration of subunits remaining in solution is called the **critical concentration (C_C)**, and this soluble tubulin concentration will be the same no matter what the starting tubulin concentration was. It is easy to see why this is so if we describe the critical concentration mathematically. At steady

state, there is no net assembly of polymer, so dP/dt in Equation 11.1 is equal to zero. As shown in Equation 11.2, we can then solve for [tubulin] when dP/dt is zero:

$$[\text{tubulin}] = C_c = k_{off}/k_{on} \qquad (11.2)$$

For purified tubulin, the critical concentration is about 7 μM. Since this is the concentration of subunits that will always remain in solution at steady state, the concentration of tubulin dimers must be higher than the critical concentration to get assembly of polymer. Similarly, if microtubule polymers at steady state are diluted so that the total concentration of tubulin is reduced, they will begin to disassemble and the amount of polymer will decrease. The decrease will stop when the concentration of soluble tubulin subunits again equals the critical concentration. Thus, the critical concentration can also be thought of as the minimum concentration required for microtubule assembly.

An important question is what is happening to the individual microtubules once steady state is achieved. One possibility—the simplest—is that, like many chemical reactions, microtubule polymerization comes to a true equilibrium, with both ends of every microtubule in equilibrium with the tubulin molecules in solution. Were this the case, there could be only very minor exchange of subunits in and out of a microtubule at steady state, for reasons that will be discussed shortly. Experimental evidence, however, demonstrates just the opposite: at steady state, subunit addition and loss into and out of microtubules are extensive, much greater than that predicted by an equilibrium exchange. This indicates that something much more intriguing than a simple equilibrium is happening. The mechanisms that account for this enhanced exchange and polymer turnover are fundamental to understanding the behavior of microtubules and are discussed in the next section.

Concept and Reasoning Check

Draw curves representing the amount of microtubule polymer over time under the following conditions:

1. You have a sample of microtubules and tubulin dimers at steady state and now dilute the sample such that the tubulin dimer concentration is below the critical concentration.
2. Again, you have a sample of microtubules and tubulin dimers at steady state and you dilute your sample such that the tubulin dimer concentration is now equal to the critical concentration.

11.5 Microtubule assembly and disassembly proceed by a unique process termed dynamic instability

Key concepts

- Microtubules constantly switch between phases of growth and shortening; this process is termed dynamic instability.
- The transition from growing to shortening states is called a catastrophe.
- The transition from shortening to growing states is called a rescue.
- A population of microtubules grows and shortens asynchronously; at any instant in time, most are growing and a few are shortening.
- The structures of growing and shortening microtubule ends are different: growing ends have extensions of protofilaments, whereas shortening ends have curling protofilaments that bend back, away from the microtubule lattice.

In vitro experiments to understand microtubule polymerization dynamics and kinetics initially used methods such as light scattering that could only measure the total amount of polymer present at any time. In the mid-1980s, methods that followed tubulin polymerization by immunofluorescence or electron microscopy were introduced. Unlike light scattering, these techniques allow visualization of the individual microtubules in a polymerization reaction. The microtubules can then be counted, and their lengths measured. The result was completely unexpected and provided an intriguing answer to why exchange of tubulin subunits into and out of the polymer was so much greater than predicted on the basis of equilibrium exchange. Were microtubules equilibrium polymers, they would have shown only tiny changes in length once at steady state, and the total number of microtubules could not have changed significantly with time. Using the new methods, however, it was clear that at steady state (the plateau in Figure 11.14) both the lengths and the number of microtubules changed. Over approximately 40 minutes, some microtubules grew ten times longer, while the total number of microtubules decreased. Additional experiments examining microtubules polymerized from centrosomes (stable nucleation sites; see also *11.7 Cells use microtubule-organizing centers to nucleate microtubule assembly*) also gave unexpected results. Microtubules were first allowed to polymerize from centrosomes, and the sample was then diluted to a concentration just below the criti-

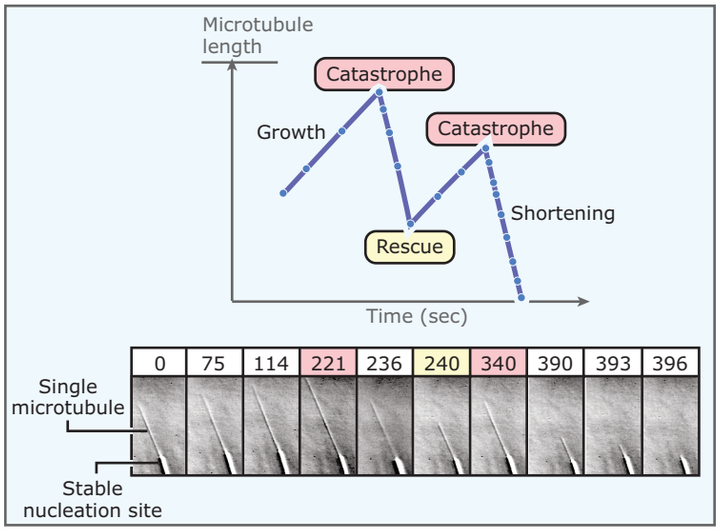


FIGURE 11.17 A single microtubule extends from a stable nucleation site *in vitro*. Its minus end is attached to the nucleating structure and its plus end is free. The length of the microtubule is recorded over time by video microscopy and is graphed at the top. The microtubule first grows, then abruptly begins to shorten. After losing most of its length it begins to grow again. A short while later it undergoes a second catastrophe, and this time depolymerizes completely. Note that the microtubule depolymerizes several times as fast as it polymerizes. The time in seconds is above each frame from the video. Photos courtesy of Lynne Cassimeris, Lehigh University.

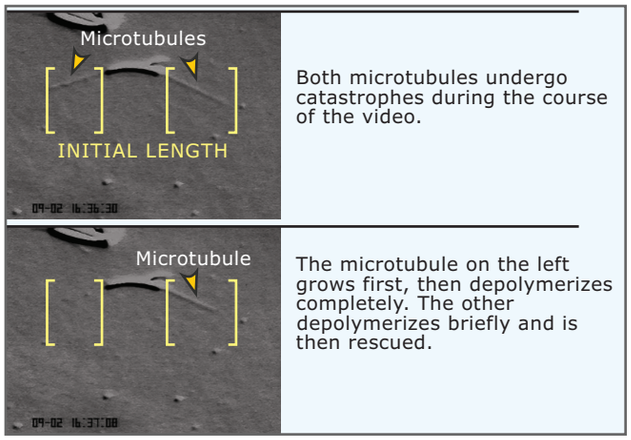


FIGURE 11.18 A small piece of a flagellar axoneme was used as a stable nucleation site for assembly of purified tubulin *in vitro*. Two frames of a video are shown. In the upper panel, a single microtubule has polymerized from each end of the axoneme. The microtubule on the left has its plus end free; the one on the right has its minus end free. In the time between the top and bottom video frames, the microtubule on the left has depolymerized completely; the microtubule on the right has depolymerized a short distance and then grows again. Photos courtesy of Lynne Cassimeris, Lehigh University.

cal concentration. The predicted result for an equilibrium polymer would be that all the microtubules would immediately begin to depolymerize and would continue to depolymerize until the critical concentration was reestablished. Instead, while some microtubules did depolymerize, the rest continued to polymerize, a result that is impossible with an equilibrium polymer. These results and others led to a model of microtubule polymerization called **dynamic instability**, in which microtubules exist in persistent phases of either growth or shortening, with abrupt transitions between them. These abrupt transitions are termed **catastrophe** for the switch from growing to shortening, and **rescue** for the switch from shortening to growth.

The dynamic instability model was confirmed using light microscopy to observe individual microtubules as they grow and shorten. FIGURE 11.17 shows an experiment of this type. These experiments demonstrated that an individual microtubule grows steadily for a distance of several microns, then undergoes a catastrophe and shortens quickly. The microtubule may be rescued and resume growing, or it may depolymerize completely. FIGURE 11.18 shows two images from a video recording of microtubules undergoing transitions between growing and shrinking phases. The two microtubules shown are nucleated from a stable structure (a short fragment of a flagellar axoneme, an organelle composed of stable microtubules), one growing at its plus end and the other at its minus end. This technique makes it possible to follow the polymerization dynamics at both the plus and minus ends of microtubules. Such experiments showed that the plus ends of microtubules grow faster than the minus ends. The plus ends of microtubules also undergo catastrophe more frequently, making them the more dynamic end of the microtubule.

The defining features of dynamic instability are the persistence of the elongation and shortening phases and the abruptness of transitions between them. Because catastrophes and rescues occur at random intervals, the behavior of the individual microtubules in a group of microtubules will be heterogenous and asynchronous. Most microtubules will be slowly growing, but at the same time a few will be shortening rapidly. Individual microtubules do not reach a steady-state length; instead, each is always becoming either longer or shorter.

The structure of microtubule ends is different for growing and shortening microtubules. During polymerization, microtubule ends often have sheetlike extensions in which some protofilaments have grown longer than others, as shown in FIGURE 11.19. Subunits add only to the ends of protofilaments. As each protofilament

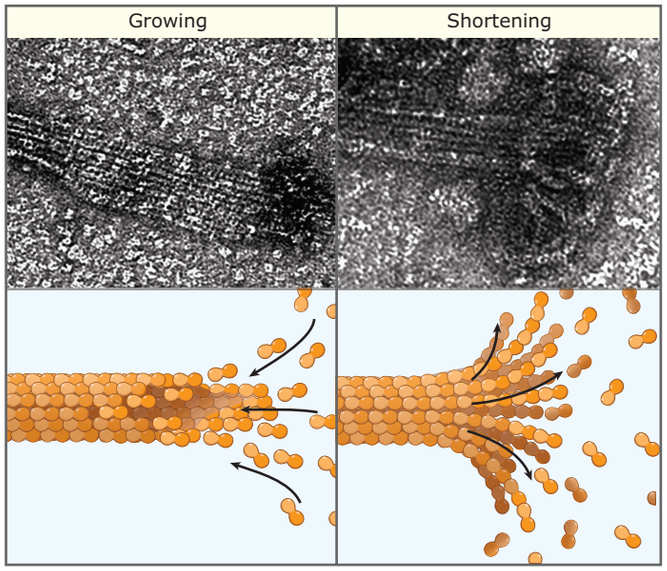


FIGURE 11.19 At the top are electron micrographs of the plus ends of growing and shortening microtubules. Longer protofilaments extend from one side of the end of the growing microtubule. These protofilaments lie flat against the surface onto which the microtubule is attached for electron microscopy. The result is the appearance of a flat sheet of protofilaments at the end. At a shortening end, the protofilaments peel away from the wall of the microtubule and curl backward. Photos courtesy of Lynne Cassimeris, Lehigh University.

elongates, subunits form lateral bonds with their neighbors, eventually closing the sheet into the tubular shape of a microtubule. When microtubules depolymerize, individual protofilaments peel away from the polymer lattice. Subunits within the individual curling protofilaments are held together only by longitudinal bonds. As discussed above (see *11.3 Microtubules are polar polymers of* α- *and* β-*tubulin*), the bonds between subunits in a single protofilament are equally likely to break, leading to rapid disassembly of the peeling protofilaments by both dissociation of subunits from their ends and by breakage at other points.

11.6 A cap of GTP-tubulin subunits regulates the transitions of dynamic instability

Key concepts

- Growing microtubules have a cap of GTP-tubulins at their tip because the GTP associated with β-tubulin is hydrolyzed to GDP shortly after a subunit adds to a microtubule.
- The bulk of the microtubule is made up of GDP-tubulins.
- Hydrolysis of GTP is coupled to a structural change in the tubulin dimers.
- GTP-tubulins form straight protofilaments, which maintain contacts with subunits in adjacent protofilaments and allow these protofilaments to continue growing.
- GDP-tubulins curve away from the microtubule, breaking lateral bonds with adjacent subunits, causing protofilaments to peel apart.

Dynamic instability is possible because tubulin binds and hydrolyzes GTP. Microtubule assembly and disassembly by dynamic instability is a nonequilibrium process and requires energy input. The energy input is provided by GTP hydrolysis. As tubulin heterodimers are incorporated into a microtubule, β-tubulin is stimulated to hydrolyze its bound GTP to GDP. Hydrolysis does not occur immediately but lags behind polymerization slightly. Thus, the core of a growing microtubule is composed of GDP-tubulins, while the ends are capped by GTP-tubulins.

The cap of GTP-tubulins at microtubule ends regulates dynamic instability, determining whether a microtubule will grow or shorten, as shown in **FIGURE 11.20**. Shortening microtubules have lost this cap, exposing GDP-tubulin subunits at the end of the polymer. Whether GTP or GDP is associated with the subunits at the end of a microtubule determines the rate at which they dissociate. GDP-tubulin dissociates from an end approximately 50 times faster than GTP-tubulin. Exposure of GDP-tubulins at the end of a microtubule, therefore, results in rapid depolymerization. Catastrophes are thus the result of a growing microtubule losing its GTP cap, while rescue requires that GTP-tubulins recap the end of a shortening microtubule (see Figure 11.20).

The presence of GTP- or GDP-tubulins at microtubule ends regulates tubulin association and dissociation rates by changing the structure of the microtubule end. Structural studies indicate that GTP-tubulin forms straight protofilaments. GDP-tubulin, however, is most

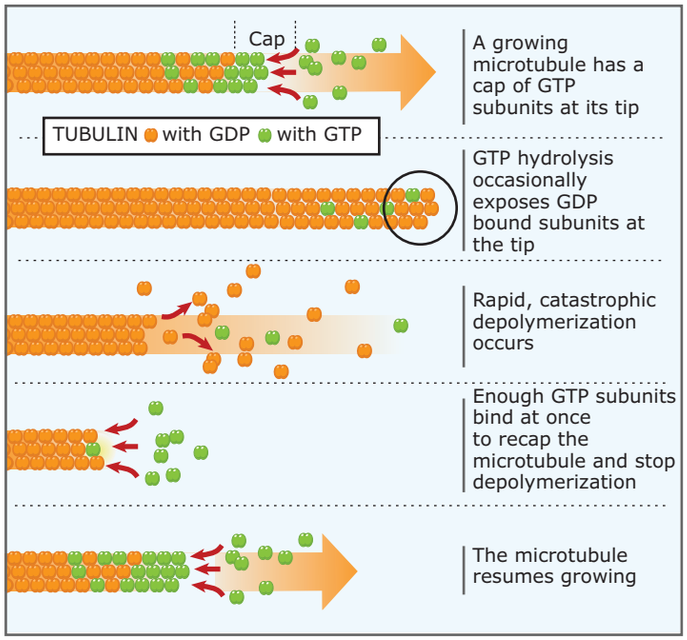


FIGURE 11.20 A schematic view of events at the end of a dynamic microtubule. The GTP bound to β-tubulin is hydrolyzed shortly after a tubulin dimer adds to a microtubule, creating a small cap of GTP-bound subunits at the microtubule's end as it grows. As long as the cap is present the microtubule will grow. However, if GDP-bound subunits ever become exposed at its end, the microtubule will begin to depolymerize very rapidly. The microtubule can be rescued if GTP subunits bind as it is depolymerizing. Depolymerization is fast enough, and rescue rare enough, so that a large fraction of a microtubule can depolymerize before it is rescued.

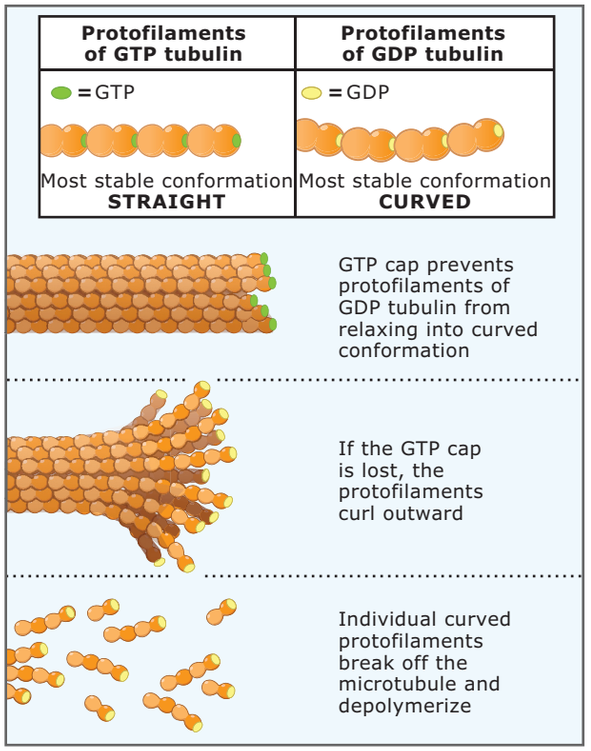


FIGURE 11.21 A GTP cap at the end of a microtubule forces the GDP-bearing protofilaments that make up the rest of the microtubule to lie straight. As soon as the GTP cap is lost, the protofilaments can relax into their more stable curved conformation. As they curl the protofilaments separate from one another, making them prone to breakage. At the top, the nucleotide associated with every β subunit is shown. For simplicity, in the bottom three panels only the nucleotide associated with the subunit at the end of each protofilament is shown.

stable as curved protofilaments, very similar to the curling protofilaments seen at the ends of depolymerizing microtubules, as illustrated in **FIGURE 11.21**. The GTP or GDP status of tubulins at the microtubule tip, therefore, determines the structure of the tip, and the structure of the tip determines whether a microtubule will grow or shorten. A cap of GTP-tubulins holds the protofilaments straight and prevents GDP-tubulins within the core of the microtubule from relaxing to their preferred curved conformation. If the GDP-tubulin core is ever exposed at the end of a microtubule, however, the GDP-tubulins are able to break the lateral bonds to their neighbors, and the protofilaments curl outward away from the core of the microtubule (see Figure 11.21). These curling protofilaments break in random positions and the fragments depolymerize into individual subunits (see Figure 11.21).

How is a GTP cap generated and how can a microtubule lose it? We don't yet know exactly how large a GTP cap is, but do know that it is quite small (less than 200 subunits), perhaps as small as a single layer of subunits in depth. The existence of a cap requires that tubulin hydrolyze GTP shortly after entering the polymer, but only after another tubulin molecule has joined the same protofilament. One possible mechanism that would allow this depends on the structure of tubulin and where it binds GTP. At microtubule plus ends, the GTP of β-tubulin is exposed at the end of the microtubule. **FIGURE 11.22** shows that when a new tubulin heterodimer adds to the end, its α-tubulin makes contact with this GTP;

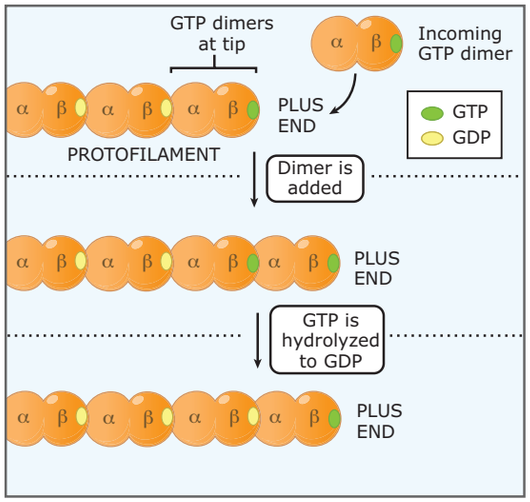


FIGURE 11.22 One possible mechanism that would create a GTP cap at the end of a microtubule. Because the GTP bound to β-tubulin is positioned at one end of the tubulin heterodimer, the GTP on the last dimer is exposed at one end of a protofilament. The α-tubulin of an incoming dimer contacts the GTP and may cause its hydrolysis. This mechanism would maintain a GTP cap of only a single layer of subunits.

this is thought to cause its hydrolysis. In this scenario, the GTP cap always remains one subunit deep, since each new subunit added stimulates hydrolysis by the subunit below it in the microtubule. If this is correct, how can a GTP cap ever be lost? One way would be through dissociation of GTP subunits from the polymer end. Another possibility would be by spontaneous GTP hydrolysis by the subunits at the end, which would occur slowly relative to the rate of hydrolysis when stimulated by another tubulin molecule. It is not yet known how many GDP-capped protofilaments must be exposed before the entire end structure begins peeling apart and shortening.

How can a shortening microtubule, with its protofilaments peeling outward, ever switch back to growth (i.e., be rescued)? GTP-tubulin subunits are likely adding to the ends of protofilaments even as they peel away during shortening. But addition of a GTP-tubulin to a peeling protofilament cannot stabilize the end because it cannot form lateral bonds with its neighbors. A rescue likely requires that some of the peeling protofilaments break off near the microtubule wall, allowing several incoming GTP-tubulins to form lateral bonds to each other and stabilize the end.

The molecular models describing catastrophe and rescue may suggest that these are unlikely events. In fact, they are. Catastrophes and rescues are rare events compared to the addition or loss of subunits from microtubule ends. For a microtubule depolymerizing in a fibroblast cell, approximately 11,000 subunits are lost before a rescue occurs. The infrequency of catastrophes and rescues allows rounds of polymerization and depolymerization to often cover significant fractions of the length of a microtubule and, sometimes, depolymerize it completely. As we shall see, the ability of microtubules to grow or shrink persistently over long distances is essential for the roles they play in cells.

Concept and Reasoning Check

1. You have microtubules growing from a stable nucleation site, as shown in Figure 11.18. What do you suppose would happen to a microtubule if you were able to cut off its end? Why?

11.7 Cells use microtubule-organizing centers to nucleate microtubule assembly

Key concepts

- In cells, microtubule-organizing centers (MTOCs) nucleate microtubules.
- The position of the MTOC determines the organization of microtubules within the cell.
- The centrosome is the most common MTOC in animal cells.
- Centrosomes are made up of a pair of centrioles surrounded by a pericentriolar matrix.
- The pericentriolar matrix contains γ-tubulin; it is γ-tubulin, in complex with several other proteins, that nucleates microtubules.
- Motile animal cells contain a second MTOC, the basal body.

In previous sections we saw how purified tubulin assembles into microtubules: the microtubules are first nucleated and then grow and shorten by adding and subtracting subunits. A similar sequence of events occurs inside cells, but cells start microtubule assembly using a specific organelle, the **MTOC** that functions to nucleate microtubules. Because spontaneous nucleation is very slow, almost all of a cell's

microtubules are nucleated at MTOCs. As their name implies, MTOCs also organize the microtubules in a cell because they often remain associated with the minus ends of the microtubules they nucleate and, thus, dictate their position and orientation.

The most common MTOC in animal cells is the **centrosome**, as shown in FIGURE 11.23. The centrosome is composed of a pair of **centrioles** surrounded by the **pericentriolar material**. The centrioles are small organelles shaped like barrels and are organized at right angles to one another in the center of the centrosome. Centrioles are constructed from unusual microtubule structures called triplet microtubules, nine of which are arranged symmetrically to form the walls of the barrel. Each triplet microtubule contains one complete microtubule (the A tubule) and two partial microtubules (the B and C tubules). In addition to α- and β-tubulin, centrioles also contain two other members of the tubulin superfamily of proteins, δ- and ε-tubulins. Tubulins are not the only components of centrioles and the pericentriolar matrix; at least 100 different types of proteins make up these structures.

The pericentriolar material was originally identified in electron micrographs as a fuzzy region surrounding the centrioles that stains more darkly than the adjacent cytoplasm (see Figure 11.23). It is now clear that it is composed of a large number of different types of proteins somehow gathered together by the centrioles. At least some of the pericentriolar proteins are arranged in a three-dimensional lattice structure. This matrix is partially composed of **γ-tubulin**, another member of the tubulin superfamily. γ-tubulin is found in a complex with several other proteins called the **γ-tubulin ring complex (γTuRC)**.

The γTuRCs of the pericentriolar material bind to α- and β-tubulins and are the component of the centrosome that nucleates microtubules. The mechanism that the complex uses to nucleate microtubules is not yet clear, but its structure is suggestive. The γ-tubulin molecules within each γTuRC are arranged as one turn of a very shallow helix. This gives them the shape of a lock washer as illustrated in FIGURE 11.24. This arrange-

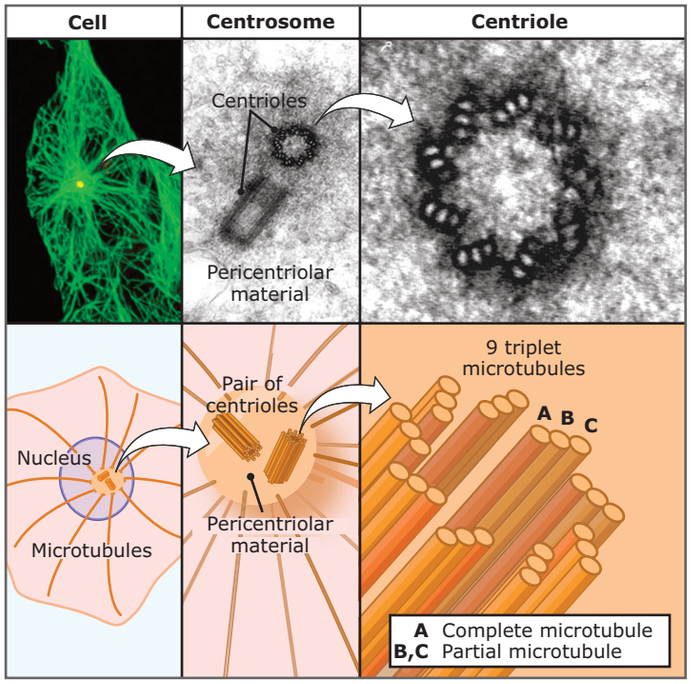


FIGURE 11.23 At the top left is a fluorescence micrograph of a whole cell with its microtubules labeled in green and its centrosome in yellow. Microtubules radiate from the centrosome. In the upper middle and right are electron micrographs of a whole centrosome and one of its two centrioles. The micrograph of the centrosome shows how the centrioles are arranged at right angles to one another. The pericentriolar material appears in the micrograph as a granular material immediately surrounding the two centrioles. Note how much clearer the cytoplasm is at the top and bottom of the picture. Photos courtesy of Lynne Cassimeris, Lehigh University.

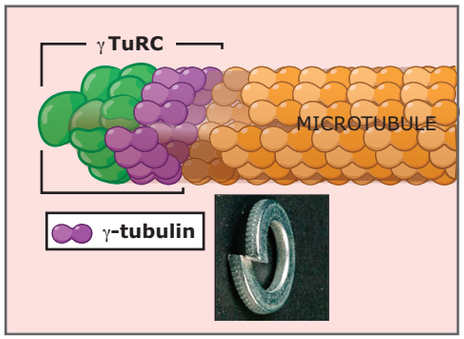


FIGURE 11.24 γ-tubulin (purple) and a number of associated proteins (green) form a large complex that serves as a template for the assembly of microtubules at their minus ends. Within the complex, γ-tubulin subunits are arranged as one turn of a helix, forming a structure that resembles a lock washer (bottom) and has the same diameter as a microtubule. The helical pitch of the ring formed by the γ-tubulins is the same as that of the subunits in a microtubule, suggesting that the complex nucleates a microtubule by positioning the first turn of subunits. Photos courtesy of Lynne Cassimeris, Lehigh University.

ment resembles one turn of the helix that results if tubulin subunits are traced side to side around the surface of a microtubule, suggesting that the γTuRC serves as a template for the formation of the end of a microtubule. However γTuRC nucleates microtubules; it is clear that the γTuRC do this from their minus ends.

The nucleation of microtubules by γTuRCs dictates the orientation of microtubules in many cells. Because γTuRCs associate only with microtubule minus ends, the plus ends of the microtubules nucleated by a centrosome all face away from it, as shown in **FIGURE 11.25**. When the centrosome is in the center of the cell, microtubules are found in a starlike array with all the plus ends located at the cell's edges.

Centrosomes reproduce themselves during each cell cycle in preparation for mitosis. The centrioles duplicate first, at the same time that the DNA is replicated, as shown in Figure 14.20 (see *15 Cell cycle regulation*). During duplication, a new centriole forms at right angles to each of the two original centrioles. As the centrosome splits into two, each new centrosome receives one original centriole and one of the newly formed ones. The two new centrosomes are separated at mitosis (see *14 Mitosis*) so that each daughter cell receives a single centrosome containing a pair of centrioles. Why new centrioles form only next to existing centrioles, why only one new centriole forms for each of the original centrioles, and why centrioles are positioned precisely at right angles to one another are all unclear. It is also not yet clear how centrioles contribute to the formation of the pericentriolar matrix.

The centrosome is a dynamic structure that changes in size during the cell cycle. Once duplicated, the centrosomes grow bigger as cells prepare for mitosis. At the beginning of mitosis, centrosomes increase their rate of microtubule nucleation approximately 5-fold. This large increase in the microtubule "birth rate" is likely to be important during mitosis because a very high density of microtubules is needed to build a mitotic spindle.

Motile animal cells (e.g., sperm cells) contain a second, more specialized MTOC, the basal body. Basal bodies serve as templates for assembly of the axoneme, a structurally complex bundle of microtubules that forms the core of cilia and flagella and is responsible for their movement (see *11.16 Cilia and flagella are motile structures*). Basal bodies are structurally very similar to centrioles, containing the same barrel-shaped arrangement of nine interconnected triplet microtubules. The similarity in structure reflects some overlap in function: in some cells, basal bodies can be converted to centrioles. Unlike centrioles, however, basal bodies need not act as pairs and nucleate microtubules directly rather than from a surrounding matrix. During the formation of a cilium or flagellum, microtubules grow directly from the triplet microtubules within the basal body. Basal bodies remain attached at the minus ends of the microtubules they have formed and are present at the base of cilia and flagella as they function.

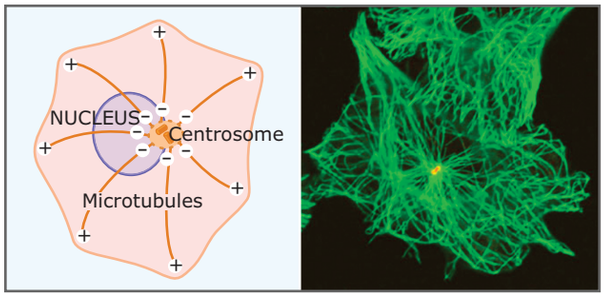


FIGURE 11.25 MTOCs orient microtubules in a cell. Photo courtesy of Lynne Cassimeris, Lehigh University.

Not all cells use centrosomes to nucleate microtubules, but all eukaryotic cells have one or more MTOCs of some sort to nucleate and organize microtubules. In fungi, the equivalent of the centrosome is a structure called the spindle pole body, which is embedded in the nuclear envelope. Plant cells lack a well-defined structure that acts as an MTOC but have a number of microtubule nucleating sites distributed throughout the cell cortex. Many types of differentiated animal cells, including neurons, epithelial cells, and muscle cells, have microtubule arrays that are not attached to the centrosome, suggesting that other, smaller types of MTOCs can be positioned within the cell to create specialized arrangements of microtubules. Epithelial cells, for example, have a number of microtubule nucleation sites located near the apical end of the cell. Microtubule plus ends grow out from the apical MTOCs toward the basal end of the cell. All the MTOCs of plants, animals, or fungi contain γ-tubulin, suggesting that all MTOCs use a similar mechanism to nucleate microtubules.

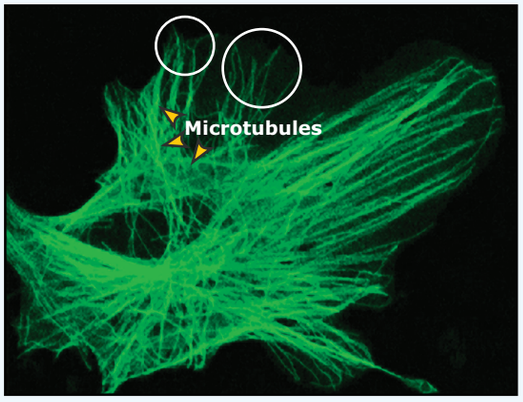
11.8 Microtubule dynamics in cells

Key concepts

- Dynamic instability is the major pathway of microtubule turnover in cells.
- Microtubule plus ends are much more dynamic in cells than they are *in vitro*.
- Free minus ends never grow; they either are stabilized or depolymerize.
- Cells contain a subpopulation of nondynamic, stable microtubules.

Microtubules in cells assemble and disassemble by dynamic instability. Since dynamic instability describes the simultaneous growth of some microtubules and shortening of others, it is necessary to observe individual microtubules in living cells in order to detect this mechanism of turnover. To visualize microtubule assembly in live cells, their tubulin is first made fluorescent by either expressing tubulin fused to a fluorescent protein or by injecting them with purified tubulin that has been covalently tagged with a fluorescent dye. Cells are then observed using a light microscope, and fluorescence images are collected every few seconds. **FIGURE 11.26** was made using these techniques and shows a static image of the microtubules in a living cell. At points around the cell's edges many individual microtubules can be seen repeatedly growing and shrinking, the two types of microtubules often occurring immediately adjacent to one another. Two such microtubules near the edge of a cell—one growing, the other shrinking—are shown in **FIGURE 11.27**.

Images like these can be used to measure microtubule lengths over time and allow the behavior of microtubules in cells and *in vitro* to be compared. They reveal that the dynamic instability of microtubules assembled *in vitro* from purified tubulin and that of microtubules in cells differ in several respects. In living cells, the plus ends of microtubules are much more dynamic than they are in microtubules polymerized from purified tubulin. Microtubule plus ends grow about 5 to 10 times faster in cells than *in vitro*. Microtubules in cells also switch between growth and shortening more frequently, as shown in **FIGURE 11.28**. Pauses,



A live cell expressing α-tubulin fused to the green fluorescent protein. The individual microtubules are visible. Growing and shrinking microtubules are visible at many points around the cell's periphery but are clearest in the indicated regions.

FIGURE 11.26 Image from a video of a cell expressing fluorescent tubulin is shown. Microtubules are shown in green. In the video sequence many microtubules grow and shorten by dynamic instability. Two regions of the cell, marked by circles, have a number of dynamic microtubules. A series of images from one of these regions is shown in Figure 11.27. Photo courtesy of Michelle Piehl, Lehigh University.

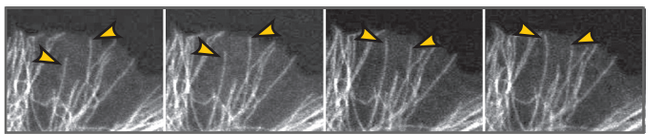


FIGURE 11.27 A small region at the very edge of a live cell expressing flourescent tubulin. The four frames are successive images taken over time. Two individual microtubules are indicated by the arrowheads. The microtubule on the left grows steadily even as the one next to it shrinks. Photos courtesy of Lynne Cassimeris, Lehigh University.

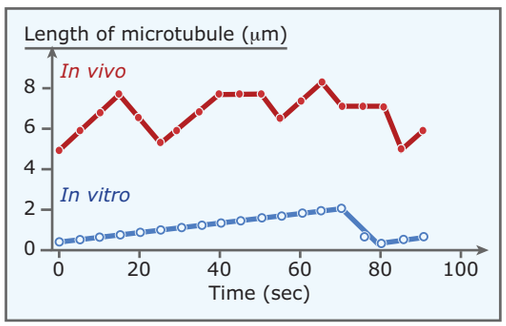


FIGURE 11.28 Each graph shows the length of a single microtubule over time. A microtubule assembled from pure tubulin *in vitro* (blue) grows steadily for more than a minute before depolymerizing almost completely and then beginning to grow again. A typical microtubule within a cell (red) is several times as long and undergoes many more transitions in the same amount of time. Unlike the *in vitro* microtubule, it loses only a fraction of its total length each time it depolymerizes, and sometimes pauses between growing and shrinking.

during which microtubules do not show detectable growth or shortening, are rare *in vitro* but are frequently observed for microtubules in living cells. These differences between microtubule dynamics *in vitro* and *in vivo* indicate that cells modify dynamic instability in order to speed it up or slow it down. As we will see later, this is done by proteins that bind to microtubles.

The ability of cells to regulate microtubule assembly dynamics was first demonstrated by comparing microtubule dynamics during interphase and mitosis. Initially, fluorescence recovery after photobleaching (FRAP)—a technique that measures how quickly new microtubules form in a particular region of the cell (see *11.20 Supplement: Fluorescence recovery after photobleaching*)—was used to follow microtubule turnover in living cells. An image of microtubule turnover detected by FRAP is shown in **FIGURE 11.29**. Photobleaching experiments demonstrated that interphase microtubules depolymerize and are replaced with newly polymerized microtubules with a half-time of approximately 5 to 10 minutes, whereas mitotic microtubules are replaced with a half-time of 0.5 to 1 minute. Direct observation of individual microtubules in mitotic cells—by the techniques described above—demonstrated that the increase in turnover is due to changes in the transition frequencies (e.g., more catastrophes, fewer rescues) and a large reduction in pauses. An example of a change in transition frequencies is shown in the graph in **FIGURE 11.30**.

The changes in microtubule dynamics that occur as a cell enters mitosis occur throughout its cytoplasm. Microtubule dynamics can also be regulated within specific regions of a cell. For example, microtubules near the center of the cell have a low probability of undergoing a catastrophe and show persistent growth toward the cell periphery. Switching between growth and shortening is much more frequent toward the edges of the cell in the regions near the plasma membrane. If the dynamics were not regulated differently in the cell's interior and at its periphery, few microtubules would reach all the way to its edges.

Not all microtubules in a cell show the same dynamics. Many interphase cells have two distinct populations of microtubules that differ in their rates of turnover. One population is dynamic and turns over rapidly (within minutes). The second consists of microtubules that are much more stable and often last for an hour or more. These stable microtubules do not grow or shorten at their plus ends, suggesting that they are somehow capped there. The differences in these populations are apparent in **FIGURE 11.31**.

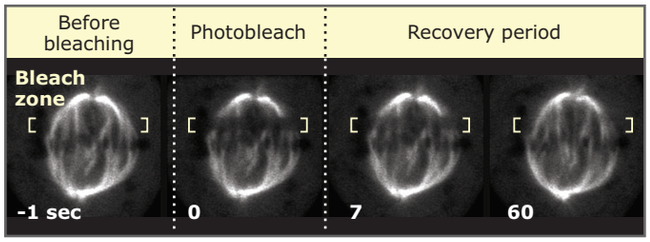


FIGURE 11.29 The rapid turnover of spindle microtubules demonstrated by fluorescence recovery after photobleaching (FRAP). Pictured are the microtubules of the mitotic spindle in a cell expressing fluorescent tubulin. The spindle's poles are at the top and bottom. Between the first two frames, the fluorescent tag on tubulin is locally destroyed (bleached) in a stripe across the spindle (indicated by the brackets). Within 60 seconds the flourescence in the bleached area has regained its original intensity, indicating that new microtubules with fluorescent subunits have grown through it. This is the result of constant assembly and disassembly of microtubules in the spindle. Photos courtesy of Lynne Cassimeris, Lehigh University.

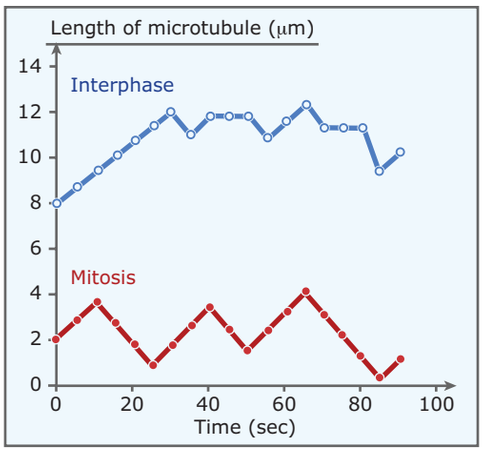


FIGURE 11.30 The two graphs show the length of a typical interphase (blue) or mitotic (red) microtubule over time. The interphase microtubule is much longer and undergoes relatively minor changes in length, sometimes neither growing nor shrinking. The mitotic microtubule never remains at one length and loses most of its length every time it depolymerizes. Because of cell cycle-dependent changes in the parameters of dynamic instability, mitotic microtubules tend to be shorter and more dynamic than interphase microtubules.

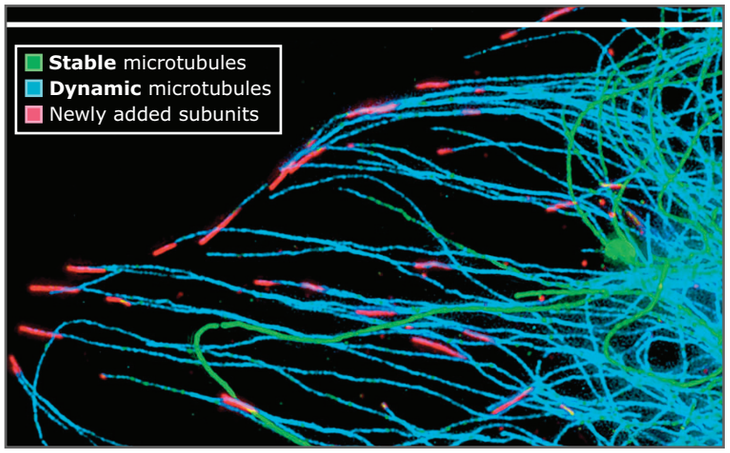


FIGURE 11.31 A fluorescence micrograph of the microtubules in a small region at the edge of a cell. A covalently modified form of tubulin found only in stable microtubules is in green; unmodified tubulin appears blue. Just before the cell was prepared for the photograph it was injected with a form of labeled tubulin that appears red in order to mark the ends of its growing microtubules. The microtubules in the cell are clearly either green or blue, indicating two distinct populations. Only blue microtubules have red caps, showing that stable microtubules do not add subunits. Credit line still to come.

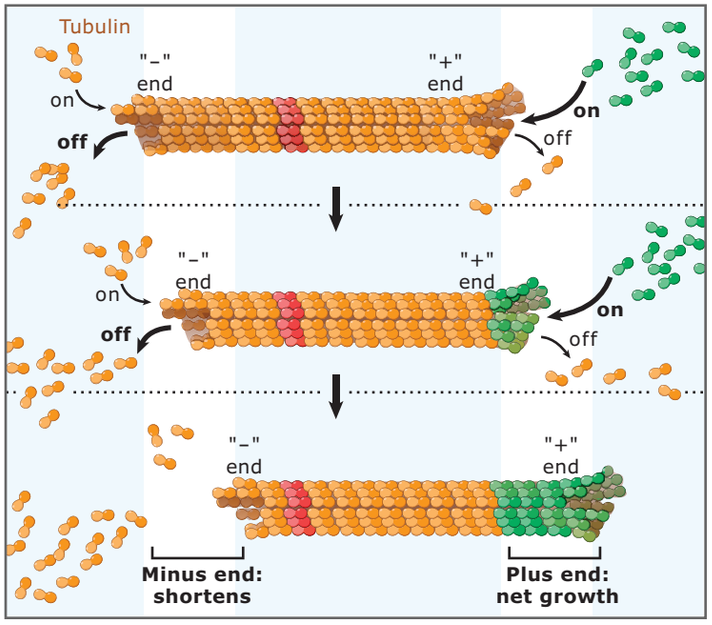


FIGURE 11.32 Under some conditions, microtubules undergo a treadmilling process where tubulin subunits preferentially add to the microtubule plus end and preferentially come off at the minus end. Having addition at one end and loss at the other means that tubulin subunits within the microtubule "treadmill" from the plus end to the minus end, as shown by the subunits marked in red. Treadmilling is a major pathway of microtubule turnover in plant cells.

Stable microtubules arise from dynamic microtubules, although how this occurs is not yet clear. Cells contain several types of enzymes that covalently modify γ-tubulin in different ways, and these may play a role (see *11.21 Supplement: Tubulin synthesis and modification*). Stable microtubules contain much more modified tubulin than other microtubules, suggesting that they are preferred substrates for these enzymes. The functions of stable microtubules are not yet known, but it is clear that the number of stable microtubules varies in different cell types. In nondifferentiated cells, approximately 70% of the microtubules are dynamic and 30% are stable. Stable microtubules are much more abundant in nonmitotic, differentiated cells such as muscle, epithelial, or neuronal cells.

Microtubules are nucleated at the centrosome, but not all microtubules remain tethered there. Microtubules can be released from the centrosome, although the rate at which this occurs varies between cell types and cell cycle stages. Release of microtubules from the centrosome results in microtubules with both plus and minus ends free in the cytoplasm. Microtubules that are not anchored to the centrosome can also arise by breakage of existing centrosomal microtubules. Free microtubules only persist in cells if the minus end is stabilized, although the stabilizing mechanism has not been identified. In fibroblasts, free microtubules rapidly disassemble, and only those microtubules anchored to the centrosome persist. In epithelial cells and neurons, microtubule minus ends are stable and the free microtubules can persist in the cytoplasm. These free microtubules can be transported and organized by molecular motors (see *11.11 Introduction to microtubule-based motor proteins*), allowing cells to organize microtubules in patterns other than the radial array seen in fibroblasts (see Figure 11.7).

In some cells, microtubules that are not anchored to a centrosome undergo a form of turnover called treadmilling. For a treadmilling microtubule, the dynamic instability of the microtubule plus end is biased toward net growth whereas the minus end shortens. By this process, a tubulin subunit enters at the plus end and exits at the minus end, effectively moving the length of the microtubule, as **FIGURE 11.32** shows. Treadmilling is prominent in plant cells, which lack centrosomes (see *21.15 Cortical microtubules are highly dynamic and can change their orientation*). The treadmilling of microtubules observed in cells requires accessory proteins and is not observed in solutions of purified tubulin. (For details on treadmilling of actin filaments, see *12 Actin*.)

11.9 Why do cells have dynamic microtubules?

Key concepts

- Dynamic microtubules can search intracellular space and quickly find targets, regardless of their location.
- Dynamic microtubules are adaptable and can be easily reorganized.
- Growing and shortening microtubules can generate force and can be used to move vesicles or other intracellular components.
- The ability of microtubules to generate force allows the entire array of microtubules to organize itself into a starlike pattern.

Evolution has clearly favored an unstable microtubule cytoskeleton because dynamic microtubules are found in every eukaryotic organism examined so far, suggesting that dynamic microtubules have been a feature of eukaryotic cells for at least 700 million years. Why might selection favor a dynamic polymer that consumes energy (GTP hydrolysis) over a static one that would be made just once? It is likely that cells need dynamic microtubules because they are easily adapted for new purposes. Dynamic microtubules can search intracellular space, they can reorganize, and they can even generate force. These properties make the dynamic microtubule cytoskeleton adaptable to a wide range of cellular functions. Figure 11.5 shows the dynamic remodeling of the microtubule cytoskeleton as cells move from interphase to mitosis; this is just one example of the adaptability of microtubules. (None of this would be possible if microtubules did not hydrolyze GTP. How important GTP hydrolysis is to making microtubules adaptable is emphasized by considering what their properties would be if they did not hydrolyze nucleotide; see *11.19 Supplement: What if tubulin did not hydrolyze GTP?*)

The ability of dynamic microtubules to search the interior of a cell is illustrated during the formation of the mitotic spindle. The formation of a spindle requires that microtubules originating from centrosomes find and connect their plus ends to kinetochores, the regions on each chromosome where microtubules are attached to the mitotic spindle. On the scale of a cell, kinetochores are tiny and the centrosome is a significant distance away, as shown in **FIGURE 11.33**. If cells were scaled up so that each kinetochore was the size of a 1-inch bull's eye in the center of a dartboard, the centrosome would be positioned at the throwing line for a game of darts. If the centrosome had to aim its microtubules, both extraordinary aim as well as some preexisting "knowledge" of the location of the kinetochore would be required. Instead, dynamic instability allows centrosomes and kinetochores to be connected reliably without either. Centrosomes nucleate microtubules in all directions, essentially probing the entire volume of the cytoplasm with the tips of a large number of growing microtubules. Microtubules that do not hit a kinetochore rapidly fall apart, freeing their tubulin subunits to

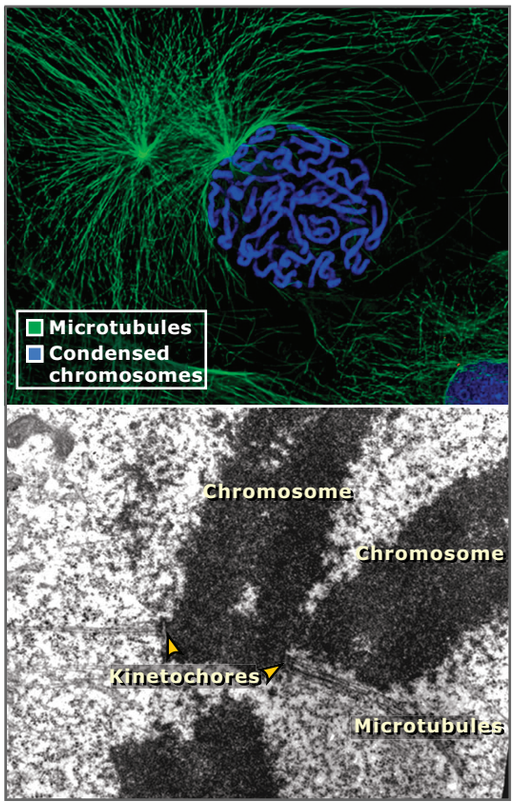


FIGURE 11.33 The upper photograph shows the microtubules and condensed chromosomes in a cell as it enters mitosis. At the bottom is an electron micrograph showing a small fraction of a condensed chromosome around its two kinetochores. Several microtubules can be seen leading into each kinetochore. These two photographs emphasize how randomly arranged the chromosomes are at the start of mitosis and how small the kinetochore is relative to an entire chromosome. The upper photograph illustrates the density of dynamic microtubules nucleated by each centrosome. The density and dynamics of the microtubules allow each of the kineotochores to be found reliably despite their small size and uncertain position. Top photo © Conly L. Rieder, Wadsworth Center and bottom photo courtesy of Lynne Cassimeris, Lehigh University.

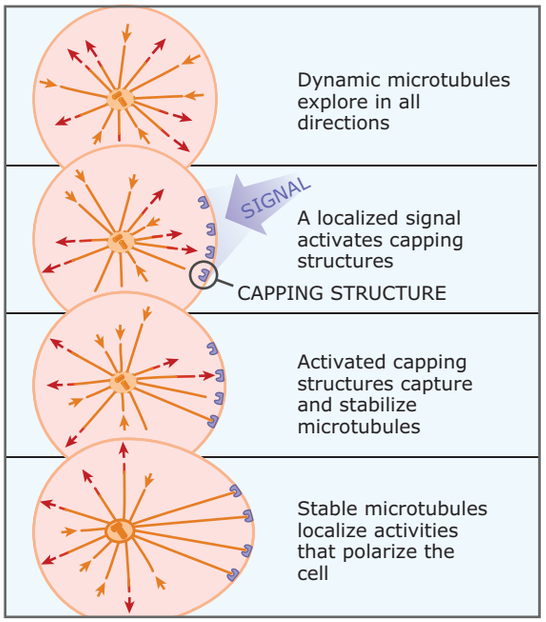


FIGURE 11.34 Localized changes in microtubule stability contribute to cell polarity and changes in cell shape. Here, microtubules are initially arranged radially in a spherical cell. The microtubules in effect explore the cell through their constant turnover by dynamic instability. A localized signal stabilizes a subset of microtubules. The stable microtubules then contribute to cell polarity by initiating the specialization of that region, for example, by causing the insertion of membrane there. This "selective stabilization" mechanism is one way that cells could modify the random assembly and disassembly of microtubules to generate a polarized cell.

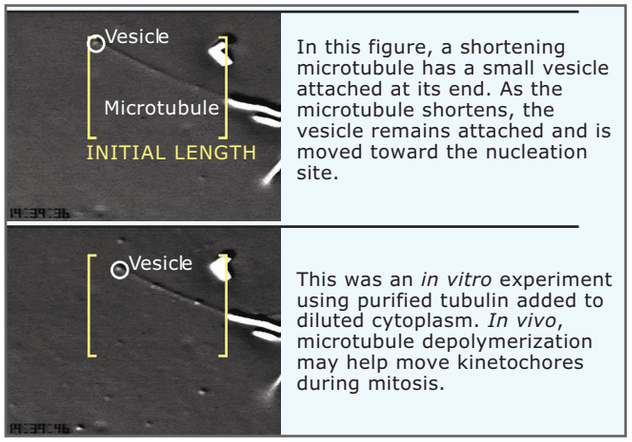


FIGURE 11.35 Two images from a video sequence are shown. In an *in vitro* experiment, a vesicle has bound to the end of a microtubule. As the microtubule shortens toward the nucleation site, the vesicle remains bound to the microtubule's tip and is transported toward the nucleation site. Photos courtesy of Lynne Cassimeris, Lehigh University.

assemble again. The rare microtubules that hit a kinetochore are stabilized, establishing the connections between the poles and the chromosomes. Although only a small fraction of the microtubules nucleated encounter a kinetochore, the continuous, rapid assembly and disassembly of microtubules made possible by dynamic instability allow all of the kinetochores to be found and connected to the centrosome within just a few minutes. Each kinetochore is connected with up to 40 microtubules in this short time frame. This "search-and-capture" mechanism of spindle formation has the advantage that it requires no preset positioning between the centrosomes and the kinetochores for a spindle to be constructed. This flexibility is put to use in every cell division, since the positions of the chromosomes at the start of mitosis are different in every cell and in each division.

FIGURE 11.34 shows how the growing, shrinking, and selective stabilization of microtubules is also useful in other contexts, particularly in allowing cells to respond to changes in their environment. Cells are often required to polarize toward signals that they detect at their plasma membrane, such as contact with another cell. The site at which a signal is received is unpredictable beforehand and may be only a small fraction of the cell's surface. The constant growth and shortening of dynamic microtubules throughout the cytoplasm makes it certain that some of them will encounter the signal, however. If the signal stabilizes these microtubules, they will serve as better highways for transport of vesicles, which will favor vesicle traffic toward that region of the cell. By inserting new membrane in one area, the cell becomes polarized and begins to change shape into a more elongated form. One example of local microtubule stabilization and cell polarization occurs as cells begin to heal an artificial wound caused by clearing cells from part of a petri dish. Healing requires that the cells at the edge of the "wound" move into it and begin to divide. Before the cells move, they first polarize in that direction. Polarization involves the reorientation of the cells' microtubules, caused by local stabilization of those microtubules that face the wound.

Dynamic microtubules may also have been selected during evolution because they can generate force and cause movement. Such movement is possible if an object is able to hold onto the tip of a growing or shrinking microtubule, allowing it to be either pushed or pulled

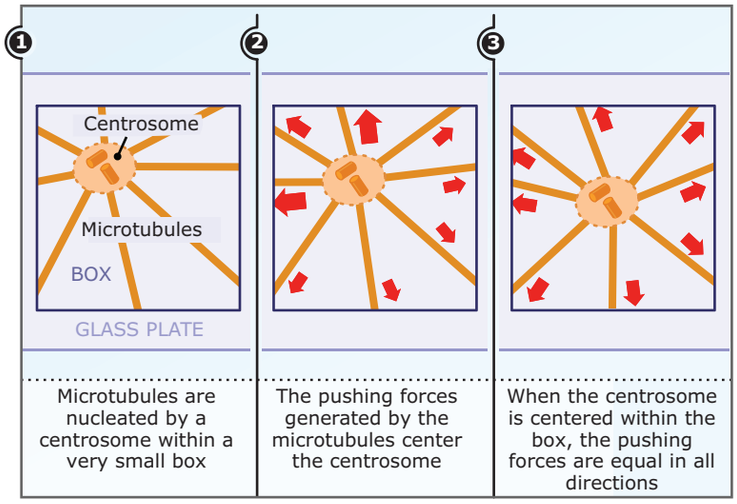


FIGURE 11.36 In this experiment, a centrosome is used to form a radial array of dynamic microtubules in a small chamber about the size of a cell. Over the course of several minutes, the radial array centers itself via pushing forces generated by microtubules polymerizing against the walls of the chamber. The relative magnitude of the forces are indicated by the size of the arrows. The centering or balancing ability of a three-dimensional microtubule array that this experiment demonstrates may sometimes be used to position structures within cells.

as the microtubule changes length. A number of proteins and organelles, including chromosomes and some types of vesicles, have this ability and can be transported on the tips of microtubules. An example of a vesicle being moved on the end of a shortening microtubule is shown in **FIGURE 11.35**. Movement coupled to microtubule shortening uses energy stored in the microtubule from the GTP hydrolysis that occurred as it polymerized. Most movements in cells are generated by molecular motors and not by the growing and shortening of microtubules, but it is interesting to speculate that dynamic microtubules may have evolved first because they allowed rapid reorganization of the cytoskeleton in response to signals, the ability to search for targets within the cytoplasm, and the ability to generate force. (For more on molecular motors see *11.11 Introduction to microtubule-based motor proteins.*)

The ability of dynamic microtubules to generate force can also be used by the microtubule cytoskeleton to position itself. This is true even in the absence of a cell. An experiment with pure centrosomes and tubulin illustrates this property and is shown in **FIGURE 11.36**. If a centrosome is placed in a very small box (formed by photolithography on a glass surface, the same technology that is used to make computer chips) and then stimulated to nucleate microtubules, the centrosome will move to the center of the box, regardless of where it was initially. This occurs because the polymerizing microtubules push against the box's walls. Since the walls are immobile, the centrosome and the microtubules move instead. Pushing forces are equal in all directions when the centrosome is centered within the box. This process is similar to what happens in a cell, where many microtubules grow from the interior out to the plasma membrane. Much like the microtubules in the artificial box, microtubules in the cell polymerize against the plasma membrane in all directions, positioning the centrosome near the center of the cell.

11.10 Cells use several classes of proteins to regulate the stability of their microtubules

Key concepts

- Microtubule-associated proteins (MAPs) regulate microtubule assembly by stabilizing or destabilizing microtubules.
- MAPs determine how likely it is that a microtubule will grow or shorten.
- MAPs can bind to different locations on the microtubule. Some MAPs bind along the sides of the microtubule; others bind only at the tips of microtubules. Still others bind only to the tubulin dimers and prevent them from polymerizing.
- Changing the balance between active stabilizers and destabilizers regulates microtubule turnover.
- The activity of MAPs is regulated by phosphorylation.
- MAPs can also link membranes or protein complexes to microtubules.

Cells use their microtubule cytoskeleton for a wide range of functions. Some of these functions require stable microtubules, whereas others require dynamic microtubules. Cells also

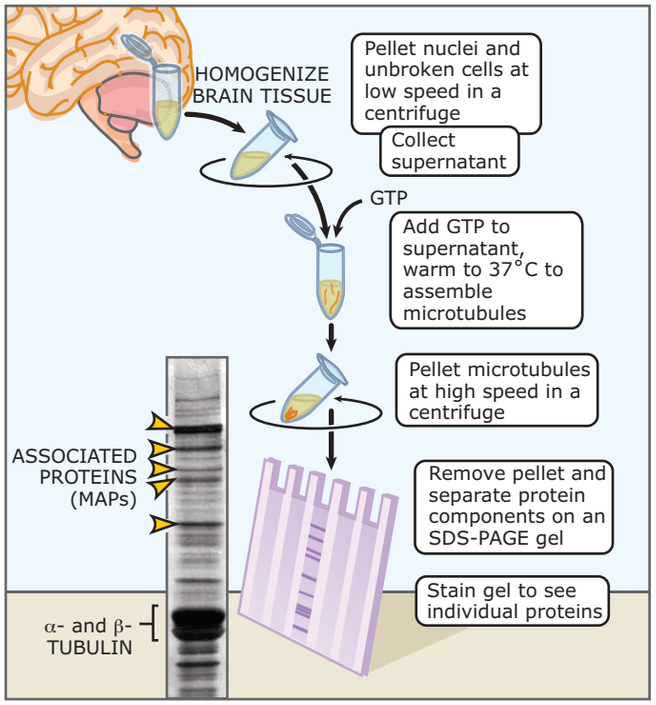


FIGURE 11.37 How the first MAPs were identified. Brain was used because of the large quantity of microtubules in neurons. Brain was homogenized under conditions in which the microtubules would depolymerize. Centrifugation then removed any large material and left only soluble proteins in the supernatant, including a large concentration of tubulin that resulted from all the microtubules in the brain tissue. When the tubulin was polymerized and the microtubules collected with a second centrifugation, many proteins in addition to α- and β-tubulin were also present. Photo courtesy of Lynne Cassimeris, Lehigh University.

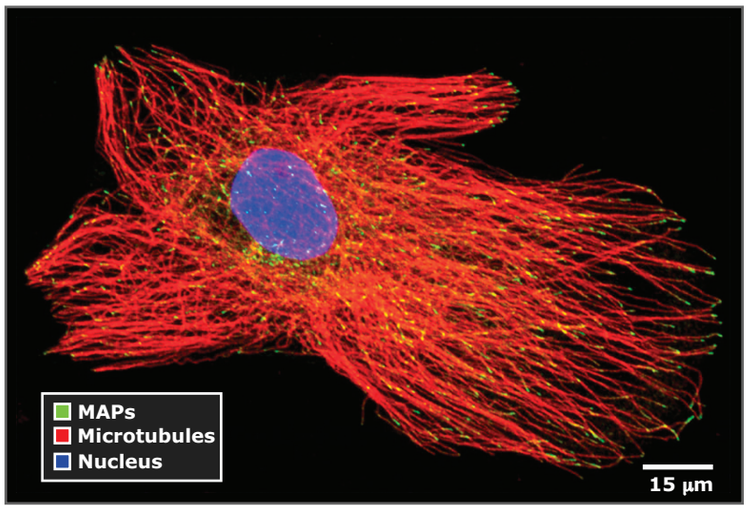
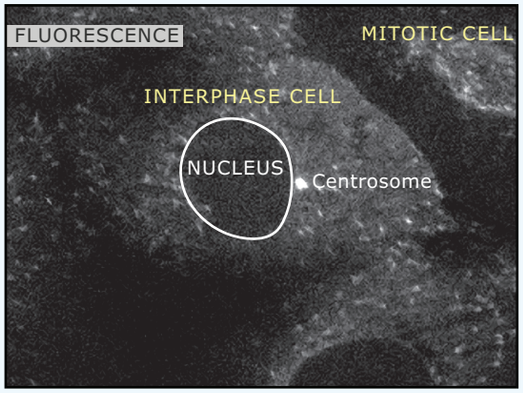


FIGURE 11.38 A fluorescence micrograph with tubulin in red and EB1, a MAP that binds only to the plus ends of growing microtubules, in green. The nucleus is in blue. EB1 appears only in short, elongated segments at the ends of microtubules. Photo courtesy of Lynne Cassimeris, Lehigh University.

often connect organelles or other structures (including other components of the cytoskeleton) to a microtubule's wall or tip. Cells use MAPs, to accomplish these tasks. Some MAPs modify dynamic instability by slowing down or speeding up tubulin addition or subtraction at the microtubule ends. Others function as linkers between the microtubule tip or sides and membrane vesicles or other structures. Some MAPs do both, regulating assembly and linking components to the microtubule.

The first MAPs were identified as proteins that copurified with microtubules isolated from mammalian brain tissue, a process illustrated in **FIGURE 11.37**. Two of these MAPs, called MAP2 and tau, are found only in neuronal cells, and are responsible for creating the very long-lived microtubules essential to the maintenance of axons and dendrites. They do this by binding along the sides of the microtubules. Because they bind to several tubulin subunits at once, they affect how frequently the microtubules undergo the transitions of dynamic instability. Both proteins suppress catastrophes and greatly increase the likelihood of rescue, making it less likely that a microtubule will fall apart. The result is long microtubules that last much longer than would be possible with tubulin alone. Proteins of this sort usually bind all along the length of a microtubule, coating its surface, and can be viewed as braces nailed to the wall of the microtubule that make it much harder to get the subunits apart.

Several proteins bind to microtubules only at their plus ends. These MAPs are referred to as "+TIPs." As shown in **FIGURE 11.38**, fluorescently labeled +TIPs appear as short segments at the plus ends of microtubules. +TIPs bind to a microtubule only when its plus end is growing and appear to ride along on the growing tip. **FIGURE 11.39** shows a still image of a cell expressing a fluorescently labeled +TIP. A video shows the protein appearing as many small comets moving through the cytoplasm, each marking the tip of an individual growing microtubule. For some +TIPs, it is thought that an individual +TIP attaches to new tubulin subunits as they add to the growing end of a microtubule. Each +TIP remains bound for only a short time before falling off the microtubule. While it is bound, the microtubule continues growing and new +TIPs are continuously added. The continuous addition of +TIP proteins at the end of the microtubule and their loss slightly behind it ensure that the concentration of bound +TIP proteins is always



The cell viewed by fluorescence microscopy, showing the location of EB1 +TIP proteins bound to the ends of growing microtubules. Most of the fluorescence appears as many small, slighty elongated spots throughout the cytoplasm. The protein is also present at the centrosome.

FIGURE 11.39 A single image from a video sequence showing a fluorescent +TIP protein, EB1, bound to microtubule ends in an epithelial cell. Because the +TIP proteins bind to the tips of growing microtubule ends, they appear as fluorescent comets that "move" through the cytoplasm. The "movement" actually represents microtubule growth. Photo of EB1-GFP in epithelial cells provided by U. S. Tulu and P. Wadsworth.

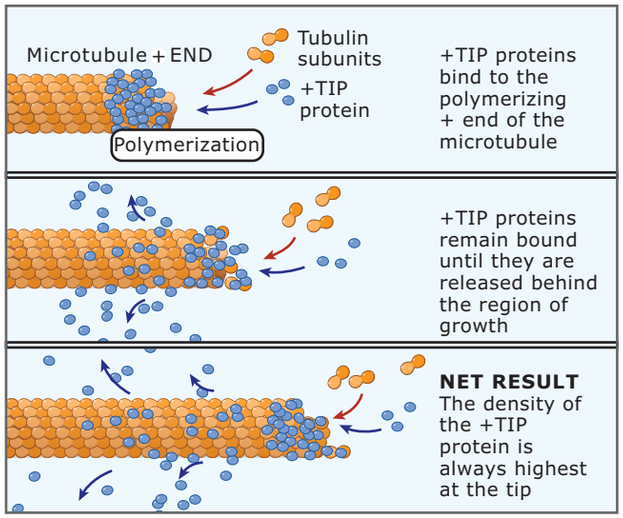


FIGURE 11.40 The binding and release mechanism that allows +TIPs to localize to the growing end of a microtubule.

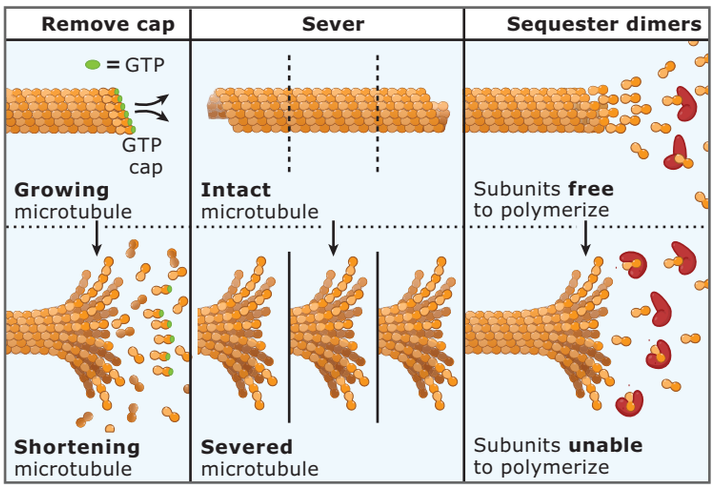


FIGURE 11.41 Three ways to destablize microtubules. Removing a microtubule's GTP cap or severing a microtubule in its interior immediately exposes GDP-bound subunits at an end and causes depolymerization to begin. Severing also increases the number of ends that can depolymerize at once. Sequestering free tubulin subunits slows polymerization and makes it more likely that GTP hydrolysis will occur in the subunits at the end of each protofilament.

highest at the microtubule's end, as shown in **FIGURE 11.40**. Some +TIPs are concentrated at the microtubule plus end through a different mechanism—they are transported to microtubule plus ends by the motor protein kinesin (see Section 11.11). Several different families of proteins show binding to microtubule ends and can regulate microtubule dynamics in different ways. Some +TIPs stabilize microtubules and promote their growth, while others make microtubule ends more prone to catastrophe. Additional proteins that destabilize the microtubule end are discussed next.

Cells can also speed up microtubule turnover when they need very dynamic microtubules, as they do during mitosis. Cells speed up microtubule turnover with MAPs that make microtubules less stable. These destabilizers make it more likely that microtubules will have a catastrophe and shorten. They also make rescues less likely, so that microtubules lose many subunits before they start to grow again. There are three general ways that destabilizers work: they disrupt the GTP cap to stimulate catastrophes; they cut microtubules into pieces to make more ends that can shorten; or they bind free tubulin subunits to decrease the amount of tubulin available for polymerization. These three processes are illustrated in **FIGURE 11.41**.

Microtubules are cut by katanin, a protein named for the Japanese word for sword. Katanin cuts microtubules by binding to their walls and disrupting contacts between tubulin subunits. Several molecules of katanin must bind to a microtubule and hydrolyze ATP in order to break it. Although its cutting mechanism is well understood, it is not yet clear how katanin's microtubule severing ability is put to use in cells. Katanin is present in all cells,

and studies with katanin mutants and inhibitors indicate that it participates in major cellular events. Katanin is, for example, required for assembly of the meiotic spindle in some organisms and for proper organization of the microtubules in plant cells, but in neither case is it understood how it participates. One possible role for katanin in cells is accelerating the depolymerization of long microtubules by breaking them into multiple pieces. This would be particularly useful in very large cells like eggs. Katanin may also have other roles, however. In some cell types it is found at the centrosome, and it is hypothesized that it could release newly polymerized microtubules from MTOCs.

One example of a microtubule-destabilizing protein that functions by disrupting the GTP cap is mitotic centromere associated kinesin (MCAK). MCAK is a member of the kinesin superfamily of molecular motors and plays a significant role in controlling microtubule dynamics during mitosis (see *11.11 Introduction to microtubule-based motor proteins*). Unlike most motors, MCAK does not transport cargo. Instead, MCAK is a +TIP protein; it binds at microtubule ends and destabilizes the microtubule tip structure by favoring formation of protofilaments that curve away from the microtubule wall. Curved protofilaments lose contact with their next-door neighbors, disrupting the GTP cap, and the microtubule begins to shorten. MCAK is then released from the depolymerizing tubulin subunits and is free to bind to the microtubule again.

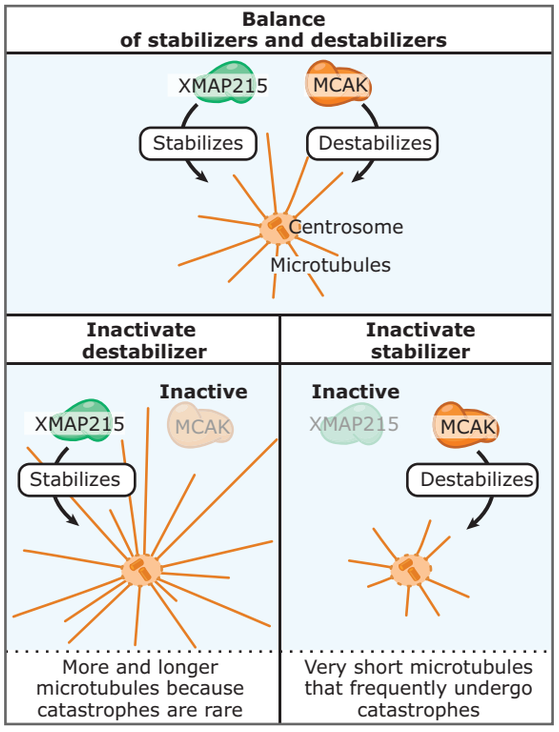


FIGURE 11.42 Microtubule-stabilizing and microtubule-destabilizing MAPs act in pairs to determine the size of a microtubule array. Inactivating the destabilizer makes catastrophes less likely and results in a larger array with more microtubules. Inactivating the stabilizer has the opposite effect. Using a balance of opposing components to determine the size of a microtubule array allows its size to be changed quickly.

How dynamic the microtubules in a cell are at any given time is determined by the balance between stabilizers and destabilizers. Shifting the balance by activating or inactivating various MAPs will lead to either greater microtubule stability or greater microtubule turnover. The idea that microtubule dynamics are regulated by a balance between stabilizing and destabilizing MAPs was first proposed for two MAPs found in frog eggs—XMAP215 (a microtubule stabilizer) and MCAK. Removing XMAP215 from frog cells allows MCAK to dominate; the result is very short microtubules with a high rate of catastrophe, as illustrated in **FIGURE 11.42**. Conversely, removal of MCAK tips the balance in favor of more stable microtubules that grow to longer lengths because catastrophes rarely occur. During mitosis, the proper balance of XMAP215 and MCAK is needed or a proper spindle does not form.

How is MAP activity regulated? In general, changes in microtubule stability occur too rapidly to involve changes in expression of the genes encoding MAPs. Instead, the activity of many MAPs is changed by phosphorylation. For example, phosphorylation of the tau protein reduces its affinity for microtubules and, therefore, reduces its ability to stabilize them. Phosphorylation of MAPs does not necessarily happen throughout the cell. Instead, local activation of a kinase in a small area of the cell can change MAP activity only in that area. By switching microtubule stabilizers and destabilizers between their "on" or "off" states, cells can tip the balance toward more stable or more dynamic microtubules. By locally changing MAP activity, a cell can respond to signals by making microtubules grow longer or disappear within a defined region of the cell. Local regulation of MAP activity occurs during cell locomotion and may happen during the assembly of the mitotic spindle.

Phosphorylation of tau protein is also associated with Alzheimer's disease, although it

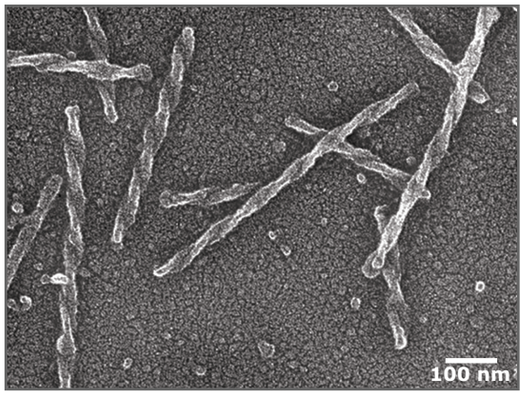


FIGURE 11.43 Tau protein, a MAP present in neuronal tissue, is hyperphosphorylated and aggregates into paired helical filaments during the neural degeneration associated with Alzheimer's disease. Here, purified filaments are viewed by electron microscopy. Photo courtesy of Denah Appelt and Brian Balin, Philadelphia College of Osteopathic Medicine.

is not clear that altered microtubule dynamics play a role in the disease. When tau protein is hyperphosphorylated, it aggregates into the neurofibrillary tangles seen in the brains of patients with the disease, as shown in **FIGURE 11.43**. It is not yet clear whether the tau defects observed in Alzheimer's disease are the cause or are an effect of the neurodegenerative process. Although it is not yet known if tau defects play a causal role in Alzheimer's disease, several other human neuronal diseases result from specific mutations in the tau gene, leading to formation of filamentous tangles of tau proteins and eventual neurodegeneration.

11.11 Introduction to microtubule-based motor proteins

Key concepts

- Almost every cell function that depends on microtubules requires microtubule-based motors.
- Molecular motors are enzymes that generate force and "walk" along microtubules toward the plus or minus ends.
- The motor "head" domain binds microtubules and generates force.
- The "tail" domain typically binds membrane or other cargo.
- Most kinesins "walk" toward the plus ends of microtubules.
- Dyneins "walk" toward the minus ends of microtubules.

One of the main functions of microtubules is to serve as tracks for the movement of material from place to place within a cell. The trucks that move cargo over these intracellular highways are called molecular motors. Molecular motors are microtubule-binding proteins that use repeated cycles of ATP hydrolysis to power continuous movement along the side of a microtubule. These motors deliver secretory vesicles to the plasma membrane, transport internalized vesicles to endosomes, and move mitochondria and the ER throughout the cell. One spectacular and very visible example of the work of molecular motors is the coordinated movement of pigment granules—small vesicles packed with pigment molecules—in cells within the scales of some fish and the skin of some amphibian species. In response to hormones or signals from the nervous system, microtubule-dependent molecular motors alternately collect these vesicles in the center of the cell or disperse them throughout its cytoplasm, allowing the animal to change color and avoid predators. Examples of this are shown in **FIGURE 11.44**.

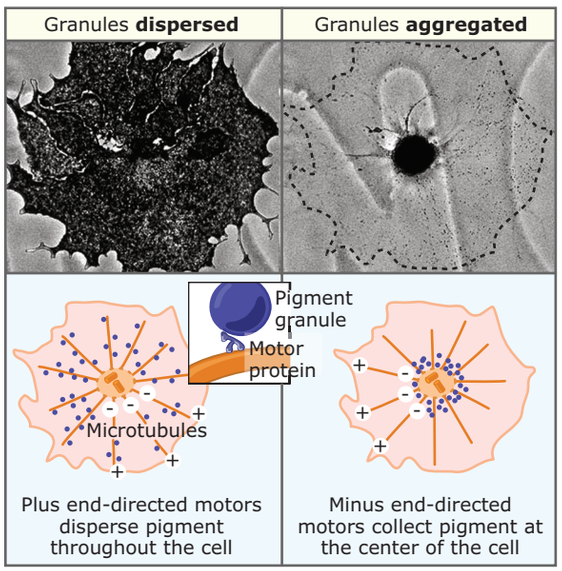


FIGURE 11.44 The photographs show a single cell containing thousands of pigment granules, small particles densely packed with dark pigment. A few individual granules are visible as very small dots in the clear area of the picture on the right. Each granule has both plus end- and minus end-directed motors attached to it. In response to the hormone melatonin, minus end-directed motors move the granules inward along the microtubules, aggregating the pigment in the center of the cell. In the absence of the hormone, other motors move the granules back out along the microtubules so that the pigment is again dispersed throughout the cell. Photos courtesy of Vladimir Rodionov, University of Connecticut Health Center.

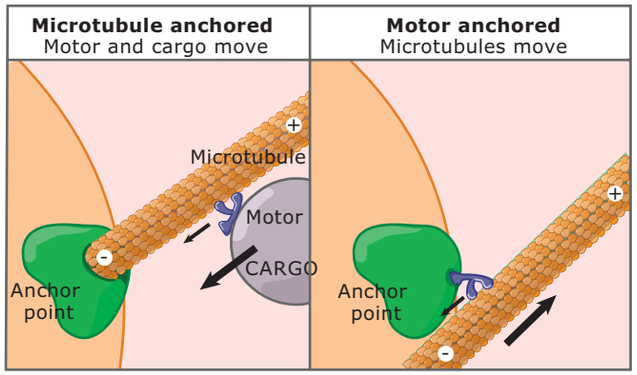


FIGURE 11.45 Whether it is the motor or the microtubule that moves when they interact depends upon which is anchored. Both cases are common in cells. Anchoring the microtubule allows vesicular traffic, whereas anchoring the motor results in rearrangement of the cytoskeleton.

In addition to moving many of the cell's internal membranes, motors move chromosomes during mitosis and position the spindle within the cell. Motors also power the beating of cilia and flagella, allowing specialized cells such as sperm to swim and stationary cells to move material over their surfaces. Some viruses hijack the cell's motors and use them to transport themselves to the nucleus of the cell; HIV is one example of a virus that uses the cell's trucking system.

Transport is not the only function of molecular motors, however. Unlike the trucks that transport cargo on our highway systems, molecular motors can also reshape and organize the microtubule highway system on which they run. It should be obvious from this brief description of some of the things that motors do that these molecular machines are ubiquitous components of all eukaryotic cells and play a major role in almost everything that depends on microtubules.

Cells have two families of molecular motors that move on microtubules: **kinesins**, which usually move toward the plus ends of microtubules, and **dyneins**, which move toward microtubules' minus ends. The organization of the microtubule array, together with the direction moved by a particular motor, provides the navigational information needed to direct cargo to the proper destination in the cell. For the radial array of microtubules found in a typical fibroblast cell (see Figure 11.7), motors that move toward the minus ends of microtubules will transport cargo to the center of the cell (e.g., to the nucleus or Golgi apparatus), while plus end-directed motors will move cargo to its margins (e.g., to the plasma membrane).

Movement in one direction on a polar polymer is an essential feature of all molecular motors, whether it is a motor that moves on microtubules or actin filaments. In this way, the polarity of the polymer contributes to the direction of movement and navigation by the motor. Intermediate filaments lack polarity (see *13 Intermediate filaments*), and no motors have been identified that use intermediate filaments as a roadway for movement.

The cargo pulled by kinesin or dynein can include the microtubules themselves, and motors frequently play a role in organizing and reorganizing the microtubules within a cell. As shown in **FIGURE 11.45**, if the microtubule is anchored (as it would be if attached to the centrosome), the motors move and can transport cargo along the microtubule. If the situation is reversed and the motor protein is anchored (e.g., to the cell cortex), the motor moves the microtubule instead, helping reorganize the microtubule array (see Figure 11.47). When it is the microtubule itself that is moved, its polarity is still important in providing navigational cues; in this case the microtubule's polarity determines the direction of its own movement.

All molecular motors, including the actin-based motor myosin (see *12 Actin*), have a characteristic shape that allows them to perform their task. This shape is clear when individual motor molecules are examined by electron microscopy, as shown in **FIGURE 11.46**. In each case, the motor consists of a pair of identical large globular domains attached to one end of a long rod-shaped domain, giving the motor a long (40–100 nm), thin shape overall. Many motors also have a second pair of smaller globular domains at the other end. The large globular domains contain both the polymer-binding (microtubule or actin) and ATP-binding sites of the motor and are referred to as the "head" or "motor" domains. They are the only parts of the motor that are needed to generate force; the other domains allow the force that they generate to be used for a specific purpose within the cell. Dynein motors are unique in having an extra "stalk" that extends from the globular head. For dyneins, it is the tip of the stalk that binds microtubules. The opposite end of a motor from the two heads is called the "tail" domain; it is here that cargoes, such as vesicles, are bound.

Typically, a single motor molecule contains several polypeptides of different sizes. Most of it

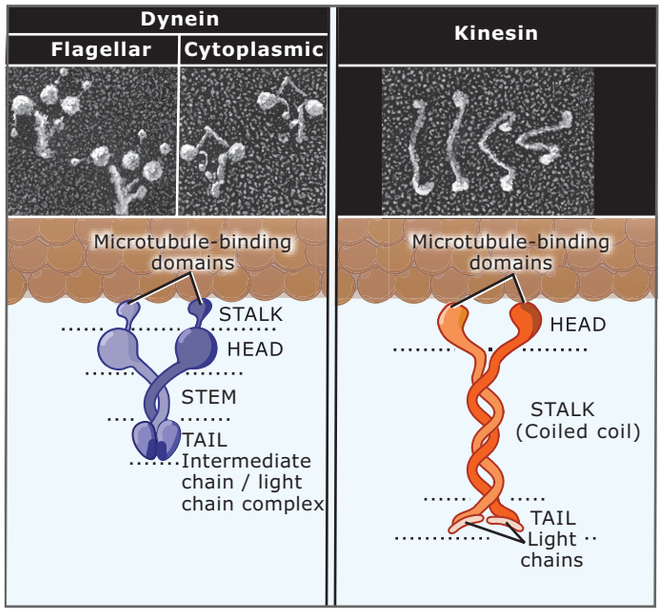


FIGURE 11.46 Structures of microtubule-based motor proteins are shown by rotary shadowing and electron microscopy (top panels). Schematic representations of motors bound to microtubules are shown in the lower panels. Each motor is made up of a two or more large polypeptides (heavy chains) and several smaller polypeptides (intermediate and light chains). Photos courtesy of John Heuser, Washington University, School of Medicine.

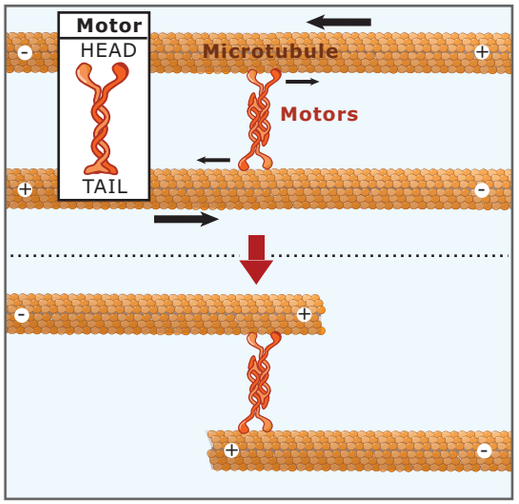


FIGURE 11.47 Some kinesins associate through their tail domains, forming bipolar motors with two motor head domains at each end. Such motors can simultaneously bind and move along two microtubules of opposite polarity. The net effect is to slide the microtubules past one another. Small arrows indicate the direction the motors move; large arrows indicate the direction the microtubules move as a result.

is composed of a dimer of the largest polypeptide, called the "heavy chain." The two heavy chains are connected by coiled-coil interactions over much of their length to form the central, rod-shaped region of the motor. Regions at each end of the coiled-coil form the head and tail domains. In each type of motor one or two different smaller polypeptides—called "light chains"—associate with each of the heavy chains and often play regulatory roles in motor function.

The kinesin family of microtubule-based motors is quite large; humans have about 45 different kinesin motors. This number alone suggests the variety of roles that microtubule-dependent motor proteins play in cells and how specialized some of them are. More than half of the kinesins are responsible for moving cargo to specific destinations in the cell, while the remainder function during mitosis. The kinesin family is defined by a high degree of sequence homology within the motor domain. Outside the motor domain, kinesin family members show much greater diversity in sequence, often being completely different from one another. These variable regions likely enable each kinesin to bind its specific cargo.

The kinesin superfamily can be broadly divided into three groups based upon the position of the motor domain within the heavy chain. The first kinesin identified has its motor domain near the N-terminus. This "conventional" kinesin moves vesicles toward the plus end of a microtubule. Other members of the kinesin superfamily have the motor domain near the C-terminus of the heavy chain. Having the motor domain at the C-terminus is correlated with kinesins that move toward the minus ends of microtubules. A few kinesins have the motor domain near the middle of the heavy chain (e.g., MCAK). Rather than powering movement, kinesins with an internal motor domain regulate microtubule dynamics by using ATP hydrolysis to weaken the structure at the end of a microtubule (see *11.10 Cells use several classes of proteins to regulate the stability of their microtubules*).

The tails of some kinesin motors can associate together to form a four-headed, bipolar motor. As shown in **FIGURE 11.47**, having motor domains facing in opposite directions allows these motors to bind two microtubules at once and slide them past each other. Sliding of microtubules past each other is particularly

important during mitosis (see *14 Mitosis*). Such activities are essential for the formation of both the mitotic spindle and the midbody, a microtubule structure that plays an essential role during cytokinesis. Clearly, the only cargo of this type of motor is microtubules themselves, and their role is strictly to rearrange the microtubule cytoskeleton.

Compared to the large family of kinesin motors, the dynein family is relatively small. Unlike the kinesins, dyneins move only toward the minus ends of microtubules. All cells have a single cytoplasmic form of dynein, which functions in cargo transport and mitosis. Cytoplasmic dynein is a dimer of two identical heavy chains, giving each dynein molecule two motor domains. The other members of the dynein family, the axonemal dyneins, are found specifically in cilia and flagella. In contrast to cytoplasmic dynein, the axonemal dyneins are heterodimers or heterotrimers of different heavy chain subunits, and have either two or three motor domains per molecule. This, as well as how axonemal dyneins power flagellar or ciliary motion, will be discussed in *11.16 Cilia and flagella are motile structures*.

11.12 How motor proteins work

Key concepts

- Motor proteins use ATP hydrolysis to power movement.
- The nucleotide (ATP, ADP, or no nucleotide) bound to a motor's head domain determines how tightly the head binds to the microtubule.
- ATP hydrolysis also changes the shape of the head. This shape change is amplified to generate a larger movement of the motor molecule.
- Cycles of ATP hydrolysis and nucleotide release couple microtubule attachments with changes in the shape of the motor's head domain. By this mechanism the motor steps along the microtubule, taking one step for each ATP hydrolyzed.

Molecular motors use ATP as the fuel to power movement, but how do motor proteins convert the chemical energy stored in ATP into mechanical work? In this section, we will examine how motor proteins move on microtubules. We would know little about this if we did not have ways to watch motors move. We will not go into how microtubule-dependent motors are assayed here, but if you would like to find out, see the *Methods and Techniques* box on pages 32–33.

The most basic requirement for a motor is that it must undergo a very large conformational change between when it has ATP bound and when it has ADP bound. This is accomplished by changes in the motor domain and neighboring parts of the molecule that are analogous to the way we move our arms and legs. For both molecular motors and our limbs, a small change in shape in one place is amplified to produce a much larger change in shape or position in another place. As someone walks, for example, a small contraction of the thigh muscles pulls the leg up and forward, amplifying the small change in the muscle's length into a much larger change in the position of the foot. For motor proteins, the motor's head domain undergoes small changes in shape in the region that binds ATP (the nucleotide-binding pocket) as the result of the hydrolysis of ATP to ADP. These changes within the nucleotide-binding pocket are amplified in another part of the molecule, moving one of the heads forward.

Molecular motors and walkers also share the requirement that they must be able to let go of the surface to which they are bound; otherwise, neither will be able to move forward. Just as a walker must pick her foot up off the ground in order to move her leg forward, a motor protein must release from the microtubule in order to move. To let go of a microtubule, a motor protein must decrease its binding affinity for it. How strongly a motor binds to a microtubule is determined by whether ATP, ADP, or no nucleotide is bound to the motor's nucleotide-binding pocket. For kinesin, binding to a microtubule is tightest when ATP is bound. By changing the strength of kinesin's hold on a microtubule, ATP hydrolysis and nucleotide release regulate the attachment of the motor to the microtubule. Because ATP hydrolysis also causes a shape change in the motor's head domain, the cycle of nucleotide binding, hydrolysis, and release, coordinates changes in the motor's shape with its binding to the microtubule. This allows motors to take one "step"—a cycle of binding to the microtubule, conformational change, and release—along the microtubule for each ATP hydrolyzed.

For a two-headed motor, one can imagine two different ways that steps could be used to produce movement along a microtubule. The two motors could move in a head-over-head motion, as shown in **FIGURE 11.48**, in which the rear head passes the forward head with each step forward. This type of motion is analogous to the way we walk, with each forward step moving one foot past the other. The alternative

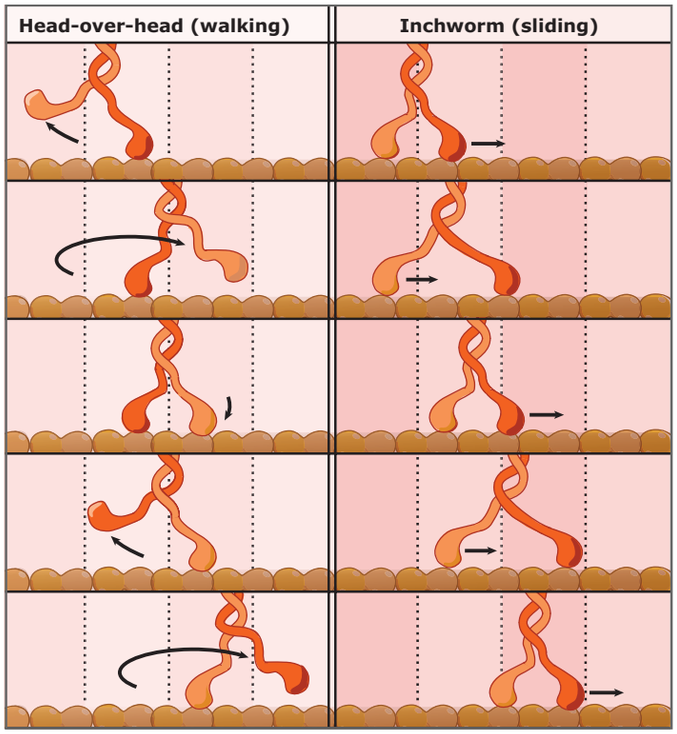


FIGURE 11.48 Two possible ways that a two-headed motor could move along a microtubule. Coordination of the activities of the two heads could result in an "inchworm"-type motility (right), in which the red head steps forward, the orange head catches up, and the cycle repeats itself. The orange head would never be in front of the red head. Although this mechanism is possible, a motor that uses it has yet to be found. Instead, all known two-headed motors move by a mechanism analogous to walking, the two heads stepping past one another and taking turns leading (left).

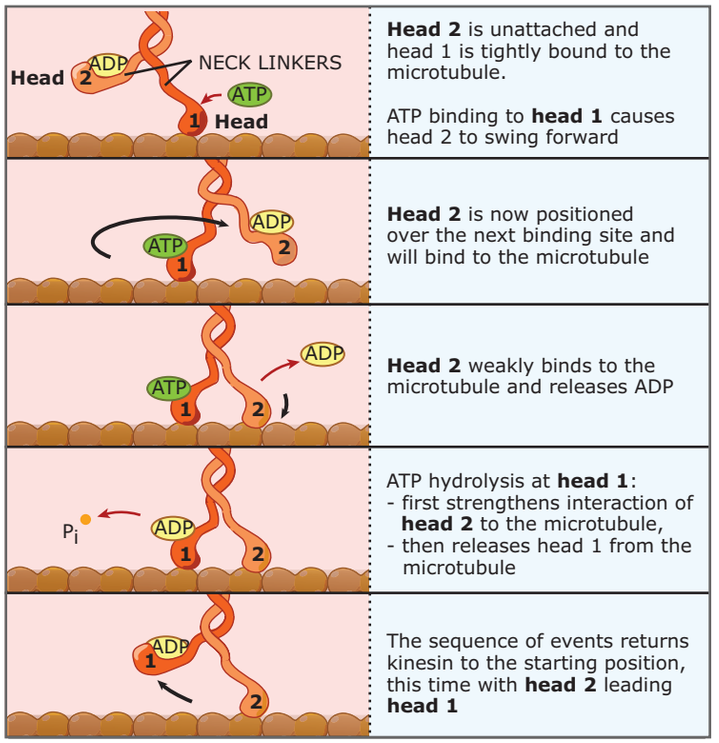


FIGURE 11.49 The sequence of events that allows kinesin to walk along a microtubule. Several times a change occurs in one head in response to an event in the other. Note that kinesin always has at least one of its two heads tightly bound to the microtubule.

mechanism would have the motor domains move in an "inchworm" motion, in which the rear head moves up to the forward head, the forward head advances, and the cycle repeats (see Figure 11.48). All two-headed motors studied to date use a head-over-head motion as they traverse a microtubule. In other words, kinesin and other motors can be viewed as "walking" along a microtubule.

Essential to kinesin's ability to walk is a small domain called the neck linker, an elongated sequence of 15 amino acids that connects the head domain of kinesin with its coiled-coil domain. The neck linker is the part of the kinesin molecule that amplifies small changes of structure in the nucleotide-binding pocket into the large changes necessary to enable kinesin to take steps of significant length. The large changes take the form of swings of the neck linkers back and forth, allowing one to view the neck linkers as the "legs" of the kinesin molecule. As we will now see, walking is achieved by using the ATPase cycles of the two heads to control when the neck linkers swing.

As kinesin moves along a microtubule, the two heads work in tandem. An event in one head often occurs as a result of a change in the other. To understand the cycle that enables a motor to walk along a microtubule, we begin just after kinesin has first landed on the microtubule, as shown in **FIGURE 11.49**. One head is tightly bound to the microtubule and has no nucleotide in its active site. Its neck linker trails behind it. The second head has ADP in its active site and is positioned behind the first, waving in the breeze alongside the microtubule. Kinesin is now ready for its first step and the coordination between the two heads comes into play. ATP binding at the forward head (head 1) causes its neck linker to swing forward toward the plus end of the microtubule. The consequence of this motion associated with head 1 is

METHODS AND TECHNIQUES: MOTILITY ASSAYS FOR MICROTUBULE-BASED MOTOR PROTEINS

The discovery and isolation of microtubule-based motor proteins depended on the development of new assays that allowed scientists to watch motility *in vitro*, either in cell extracts or with purified motor proteins. Discovery of kinesin began when scientists observed that vesicles continued to move in a crude extract—essentially undiluted cytoplasm—prepared from a single particularly large axon found in squid. An axon offered a promising place to search for such molecular motors because of the great volume of microtubule-dependent vesicle traffic in axons (see Figure 11.1). The giant axon was dissected out of the squid, squeezed like a tube of toothpaste, and the extruded cytoplasm (called the axoplasm by neurobiologists) placed on a glass slide to observe motility. By fractionating the axoplasm and testing each fraction for motility, Ron Vale and colleagues isolated the first kinesin motor.

Unlike conventional biochemical assays, which are performed in test tubes, motility assays must be performed under a microscope in order to watch the movement powered by the motor. How can motor activity be viewed under a microscope? To detect movement it is necessary to follow a sample over time, meaning that the proteins must be in their native form. This excludes electron microscopy, which requires extensive preparation of each sample with chemicals that leave the proteins inactive. Light microscopy does not have the resolution of electron microscopy, but it does have the advantage that it usually requires little if any modification of the proteins in a sample. It can, therefore, be used to follow events that require active proteins and last several minutes or more. Microtubules are too small to be seen with a conventional light microscope, however—a basic requirement of any assay for microtubule-dependent motility or the motors that drive it. This problem is solved by making microtubules visible in either of two ways. One way to visualize individual microtubules is to use differential interference contrast (DIC) microscopy (a special form of light microscopy) and enhance the images with video and computer methods. An example of a microtubule viewed by this method is shown in **FIGURE 11.B1**. Alternatively, microtubules can be polymerized from fluorescently tagged tubulin dimers and the fluorescent microtubules viewed using a fluorescence microscope.

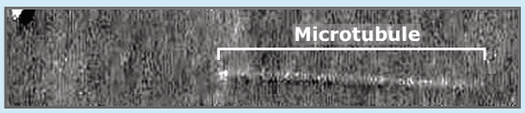


FIGURE 11.B1 A microtubule viewed by differential interference contrast (DIC) microscopy and computer image enhancement. The image is grainy, but the position and length of the microtubule are clear. This form of microscopy can be used to measure how long microtubules are, watch them move, or watch particles move along them. Photo courtesy of Lynne Cassimeris, Lehigh University.

Surprisingly, motility assays to measure movement generated by motors do not require something to serve as cargo. This is because motor proteins bind to glass (the microscope slide and coverslip), whereas microtubules do not. With the motors anchored to glass and the microtubules free, motor activity makes the microtubules glide over the glass surface. Examples of this can be seen in **FIGURE 11.B2** and **FIGURE 11.B3**.

This simple microtubule-gliding assay was used to assay biochemical fractions during purification of the first kinesin and is used routinely in the study of microtubule-based motor proteins. A variation on the gliding assay uses glass or latex beads coated with motor proteins. These coated spheres can be observed to move along microtubules. This "bead assay" is a bit more complicated because both the bead and the microtubule must be viewed by light microscopy. In all cases, the microtubules are first made stable by binding of the drug paclitaxel (see *11.1 Introduction*).

A critical feature of motor proteins is their ability to move in only one direction along a microtubule. To figure

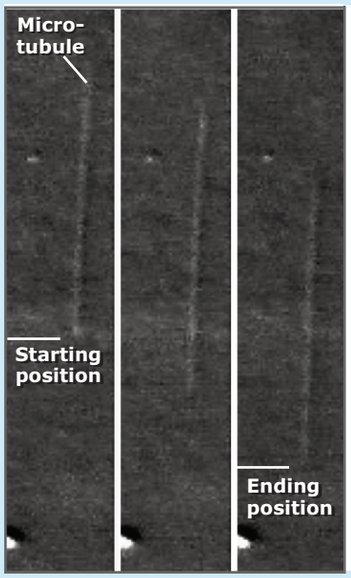
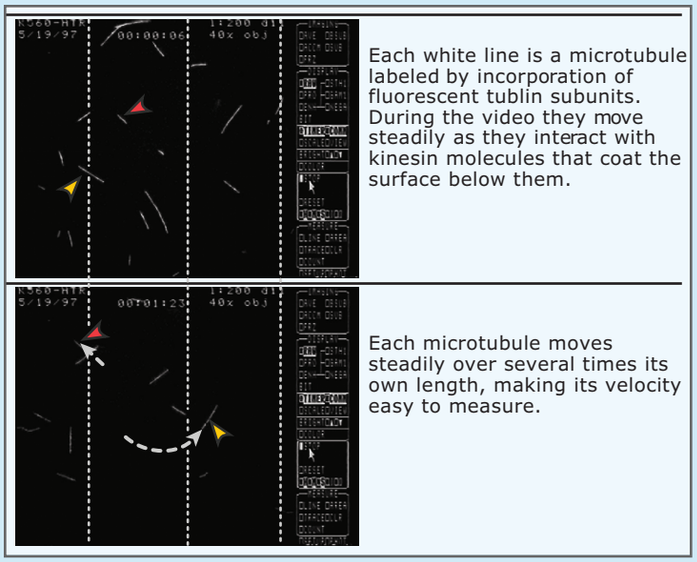


FIGURE 11.B2 A series of images taken from a video sequence show a microtubule gliding over a glass surface in an *in vitro* experiment. The microtubules are detected by differential interference microscopy and image processing. Motor proteins bound to the glass coverslip generate force to transport the microtubule. Photos courtesy of Lynne Cassimeris, Lehigh University.

METHODS AND TECHNIQUES: MOTILITY ASSAYS FOR MICROTUBULE-BASED MOTOR PROTEINS



Each white line is a microtubule labeled by incorporation of fluorescent tublin subunits. During the video they move steadily as they interact with kinesin molecules that coat the surface below them.

Each microtubule moves steadily over several times its own length, making its velocity easy to measure.

FIGURE 11.B3 Two images taken from a video sequence show several microtubules gliding over a glass surface. The microtubules were polymerized from purified tubulin mixed with a small percentage of tubulin modified with a fluorescent group. Kinesin sticks to the glass simply because a positively charged region in its tail domain interacts with the negative charge on surface of the glass. Two microtubules are noted by the red and yellow arrowheads; the paths of their movements are noted by the dashed arrows. Animation courtesy of Ron Milligan, The Scripps Research Institute; Ronald Vale, Howard Hughes Medical Center; and, Graham Johnson, fiVth.com.

out whether a motor moves toward the microtubule's plus or minus end, we need a way to know the polarity of the microtubule. One way to be certain of the polarity of the microtubules in an assay is to assemble a radial array of microtubules from a purified centrosome and then attach it to the microscope slide. Beads coated with the motor protein are then added; beads that move to the center of the aster move to the minus ends while beads that move away from

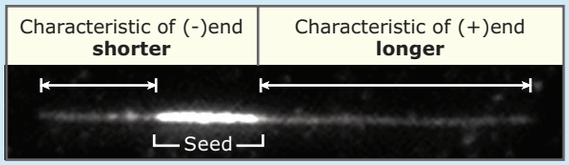


FIGURE 11.B4 A single microtubule grown from a short seed, with a much higher concentration of fluorescent tubulin subunits at its plus end. The difference in length of the extensions at its plus and minus ends is clear. Use of microtubules labeled in this way provides an easy way of determining which way a motor moves along them. Photo courtesy of Arshad Desai, Ludwig Institute for Cancer Research.

the center move to the plus ends. Another way to mark the polarity of microtubules takes advantage of the faster polymerization at the plus end. To do this, short microtubules with a very high percentage of fluorescent subunits are first formed. These bright fluorescent microtubules are then used as seeds to nucleate longer microtubules in a solution in which a much lower percentage of the tubulin is fluorescent. Tubulin molecules add to both of a seed's ends but much more readily to its plus end. The result is a collection of microtubules each with a single short and very bright segment in the middle and dimmer but still clearly visible segments extending from its ends as shown in **FIGURE 11.B4**. The extension from the plus end is much longer than the one from the minus end, allowing the polarity of the microtubule to be determined at a glance. These fluorescent, polarity-marked microtubules can then be used in gliding assays to determine the directionality of a motor. When interpreting such assays, it is important to remember that the motor protein is anchored. A lawn of a plus-end directed motor will make a microtubule glide with its minus end leading.

Almost all motility experiments are some variant of one of these assays. The question being asked and the sophistication and resolution of the detection system may vary, but the basic principle of the assay is the same.

to move head 2 from the trailing to the leading position. There, it is positioned over the next binding site in the microtubule. It binds weakly and releases its ADP. ATP hydrolysis at head 1 then strengthens the interaction between head 2 and the microtubule, resulting in an intermediate with both heads strongly bound to the microtubule. Once head 2—that is now the leading head—is tightly bound, head 1—now trailing—releases the phosphate group generated when it hydrolyzed ATP. Release of phosphate causes head 1 to let go of the microtubule and results in a conformational change in head 2 that reopens its active site. This whole cycle of events returns kinesin to the starting condition, with the essential differences that head 2 is now in front and the kinesin molecule is 8 nm closer to the plus end of the microtubule. A second cycle will begin when head 2 binds ATP, and the two heads will then alternate roles for hundreds or thousands of rounds, each round producing a step and moving the motor along

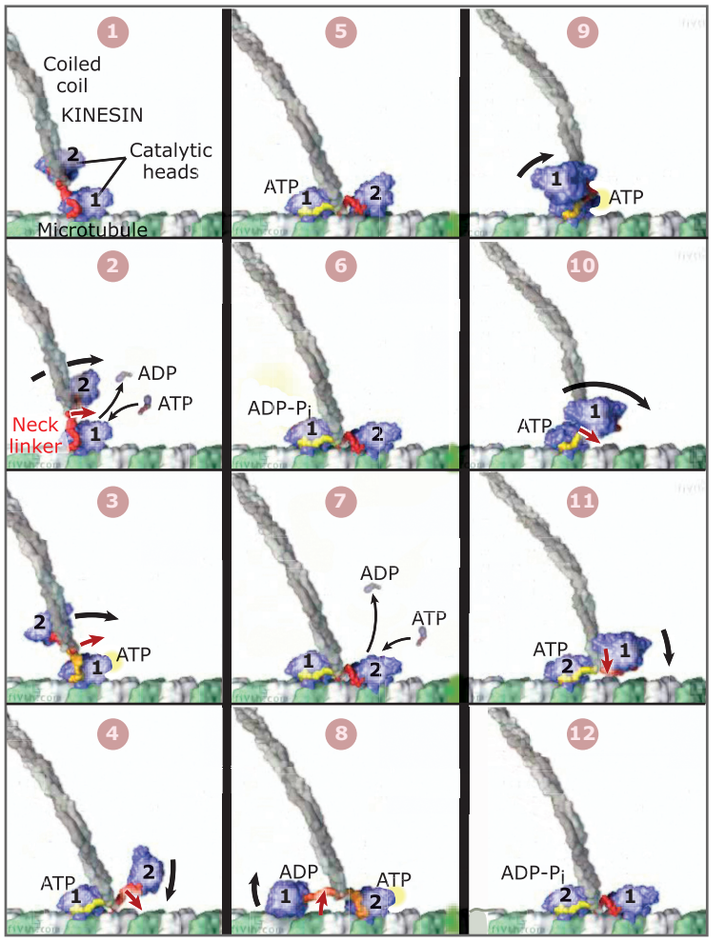


FIGURE 11.50 A series of frames from a video animation show the coordinated movement of kinesin's two heads along a microtuble. The two heads of kinesin (labeled 1 and 2) are shown in blue, the coiled-coil region shown in grey and the neck linker regions are shown in yellow when pointed forward and red when pointed backwards. For simplicity only one protofilament of the microtubule is shown. The α and β subunits of tubulin are shown in white and green, respectively, and the plus end is to the right. Reproduced from R. D. Vale and R. A. Milligan, *Science* 288 (2000): 88–95 [http://www.sciencemag.org]. Reprinted with permission from AAAS. Video courtesy of Ron Milligan, The Scripps Research Institute; Ronald Vale, Howard Hughes Medical Institute; and, Graham Johnson, fiVth.com.

the microtubule toward its plus end. The series of still frames, in **FIGURE 11.50**, of a kinesin molecule taking several successive steps show how the coordination of events in the two heads and their exchange of roles after every step allow it to walk along a microtubule.

The sequence of events that kinesin goes through to perform each step can be thought of as analogous to a tightrope artist taking a step on a rope high above the ground. Kinesin is initially stable and standing balanced on one leg. It then swings the other leg forward (the change in position of the neck linker) and gingerly tests the rope with its foot (the initially weak binding of the forward head). Only when kinesin is convinced that its forward foot is properly positioned on the rope does it shift its weight onto that foot (tight binding of the newly landed forward head). Kinesin can then release its rear foot and lift it off the rope, putting itself in position to take another step. By analogy with the tightrope walker, if either kinesin or the tightrope walker ever moves its rear foot before its front foot is secure, the walk is over. The tightrope artist ends up on the ground, and kinesin ends up floating away from the microtubule. If kinesin frequently released its trailing head from the microtubule before its leading head was bound tightly, continuous motion over long distances would be impossible.

Kinesin's walking mechanism clearly requires very reliable coordination between the two heads. How is that coordination achieved? How does one head "know" what the other is doing? It appears to be the neck linkers that act as the paths of communication between the two heads. When both of kinesin's heads are bound tightly to the microtubule, the neck linkers are stretched and are under mechanical strain. It is apparently this strain that allows the two heads to communicate with one another and coordinate their activities (i.e., their ATPase cycles). The strain indicates to the trailing head that the leading head has bound tightly. The trailing head can, therefore, be safely released from the microtubule. Strain probably accomplishes the coordination of the heads by determining how fast different steps in the ATPase cycle occur. For example, were strain to accelerate phosphate release from a negligible rate to a significant one, release of the trailing head from the microtubule could not occur until the leading head was tightly bound.

Dynein motility is also based on amplification of a conformational change, but the structural change occurs over a much larger distance. Both kinesin and dynein walk along a microtubule in 8-nm steps, which is equal to the length of a single tubulin heterodimer. Kinesin walks "carefully," stepping from one heterodimer to the next along a single protofilament. By comparison, dynein "wanders" as it walks, stepping randomly between protofilaments as it traverses toward the microtubule's minus end.

Kinesin's mechanism of movement gives it the ability to walk continuously along a microtubule (i.e., its movement is highly "processive"). For example, in *in vitro* experiments, a single two-headed kinesin motor attached to a glass bead (which serves as a convenient cargo) can take hundreds or thousands of steps along a microtubule and move the bead for a considerable distance without falling off the microtubule and drifting away. The ability of a single kinesin to move cargo long distances along a microtubule is possible because each kinesin head spends approximately half its time bound to the microtubule, and the activities of the two heads are coordinated so that at least one head is always bound. Having one of the two heads always bound to the microtubule is a property found in motors that work individually or in small numbers, such as those that move vesicles. Because of the way they work, these motors can reliably move cargoes long distances within cells.

Motors that do not always keep one head bound to the microtubule, allowing the motor and its cargo to quickly lose contact with the microtubule surface, also have uses. Motors that act in large arrays, such as those in flagella (see *11.16 Cilia and flagella are motile structures*), spend much less time bound to the microtubule than those that move vesicles. In an array of dyneins (in a flagellum), some heads will be bound and generating force. Those that have completed their steps quickly let go of the microtubule so that their binding does not impede active motors from generating force on the same microtubule.

Many organelles move bidirectionally in cells, moving one direction along a microtubule for some distance and then turning around and traveling an appreciable distance in the opposite direction. These organelles bind both dynein and a member of the kinesin family, raising the question of how it is possible to achieve prolonged movement in either direction. Two possible models, shown in **FIGURE 11.51**, have been suggested to explain the bidirectional movement of organelles. Opposite-polarity motors may compete in a tug-of-war, in which both types of motors are always active and the one able to generate the stronger pulling force (because it is present in greater number) wins the war. Alternatively, motor activities may be coordinated such that one set of motors is turned off while the other is active. It appears that the second mechanism is present in cells, but it is not yet understood how the activities of the motors are coordinated on the vesicle surface.

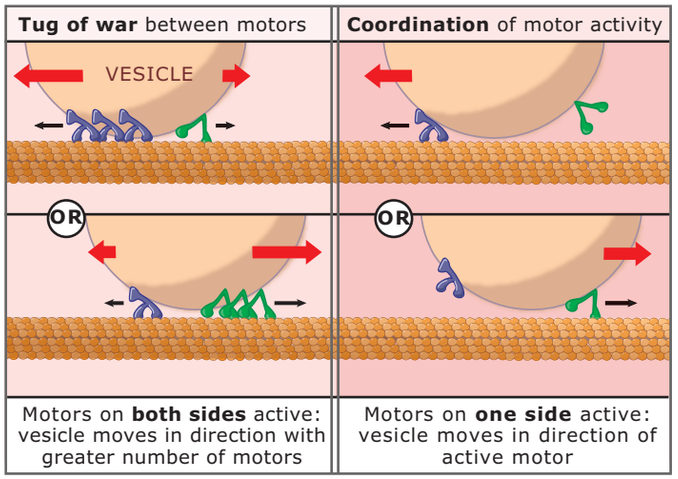


FIGURE 11.51 Possible ways to generate bidirectional movement of a cargo along a microtubule. In each case, both plus end- and minus end-directed motors are bound to the vesicle surface. On the left they are active at the same time, and the one able to generate the larger pulling force (probably because it is present in a larger number) would determine the direction of vesicle movement. On the right the motors are coordinated so that only motors that pull in one direction are active at a time. Current evidence suggests that cells use the mechanism on the right, but how the motors are coordinated is not yet understood.

Concepts and Reasoning Check

1. You have discovered a new molecular motor that walks on microtubules. How would you determine which way this motor transported cargo on a microtubule?
2. Kinesin is described as a processive motor—what does processive mean? How are the motions of kinesin's two heads coordinated to generate processive movement?

11.13 How cargoes are loaded onto the right motor

Key concepts

- Binding of motors to specific cargoes is mediated by the motor tail domain.
- Adaptor proteins associate with motors to regulate motor activity and to link motors to cargo.
- Coordination of plus end- and minus end-directed motor activities is used to generate bidirectional movement of organelles.

Cells need to move many different cargoes to specific locations in the cytoplasm. Specificity

is achieved by matching cargoes and motors. This raises the question of how the correct motor is attached to each cargo to get it delivered where it belongs. Membrane traffic from the interior of the cell to the plasma membrane and back illustrates the need to specify which motor binds to a specific cargo. Plus end-directed kinesins bind vesicles leaving the Golgi apparatus and deliver them to the plasma membrane or the endosomes, while minus end-directed motors pick up internalized vesicles at the cell periphery and deliver them to the center of the cell. Binding cargo to the correct motor is mediated by the motor's tail domain. For the large kinesin family of motors, the tail domains of the family members are very different and distinguish each as a unique motor. The motor domains are much more similar and do not contribute to cargo specificity. In this way, the head domain is the engine common to all trucks, and the tail domains are unique trailers loaded with a select group of cargoes.

In general, the motor's tail domain does not bind directly to cargo. An adaptor protein typically binds to a membrane protein on one end and the motor's tail at the other end, indirectly linking the motor to the vesicle. For example, vesicles leaving the *trans*-Golgi network for the endosome contain the mannose-6-phosphate receptor in their membranes. The cytoplasmic domain of this receptor binds the adaptor complex AP-1, and AP-1 binds to the tail of a kinesin. AP-1 is a familiar adaptor because it also links clathrin to regions of the *trans*-Golgi network where vesicles bud. In this way, AP-1 links vesicle budding to loading of the motor, ensuring that newly budded vesicles are properly equipped for transport. (For more on AP-1 see *4.14 Adaptor complexes link clathrin and transmembrane cargo proteins*.)

Adaptor proteins also link cytoplasmic dynein to membranes. The best-characterized adaptor is the dynactin complex. Dynactin is a complex of seven polypeptides and a short filament composed of Arp1, a protein closely related to actin. A recent model, shown in **FIGURE 11.52**, proposes that the Arp1 filament links dynein to membrane vesicles by binding to spectrin at the cytoplasmic surface of the membrane. This connection would be similar to the interaction between spectrin and actin filaments in the network they form on many membranes and would explain why dynactin includes an actin-like filament. In addition to attaching dynein to membranes, dynactin also helps dynein stay associated with a microtubule so that movement is possible.

Motor proteins are responsible for transporting more than just membrane vesicles. Additional cargoes include some mRNAs and virus particles, although the latter are clearly not among a cell's normal cargoes. Transport of mRNAs allows cells to restrict the synthesis of some proteins to specific locations and to ensure that mRNAs reach distant locations in very large cells. In some neurons, for example, specific mRNAs are sorted to the axons or dendrites, where proteins specific to these highly specialized cellular domains are then made. Because of the great length of axons and dendrites, molecular motors are required to transport the mRNAs. Movement of RNAs by diffusion alone would be both too slow and lack the specificity required to allow cells to construct and maintain such long, specialized structures. Similar to membrane vesicles, adaptor proteins are used to link mRNAs to a motor's tail. Viruses such as HIV, Herpes simplex virus, and adenovirus enter cells as a core particle of nucleic acid surrounded by a protein capsid. To replicate, the virus particles must reach the nucleus where they use the host cell's DNA replication machinery. Viruses hasten their replication process by binding dynein once they have entered the cell, allowing them to move directly from the plasma membrane to the nucleus.

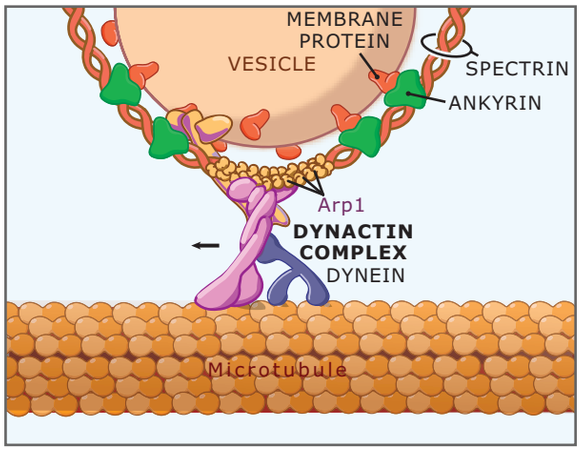


FIGURE 11.52 A model of how the dynactin complex (purple) links cytoplasmic dynein to membrane vesicles. The Arp1 filament of the dynactin complex is thought to associate with spectrin on the membrane in a manner similar to the way that actin filaments and spectrin interact on other membranes. Other components of the dynactin complex associate with both dynein and the microtubule.

Concepts and Reasoning Check

Figure 11.13 shows the organization of microtubules in an epithelial cell.

1. In this type of cell, which motor would move cargo to the apical end of the cell (shown toward the top in Figure 11.13)?
2. Which motor would deliver cargo to the basal end of the cell (shown toward the bottom in Figure 11.13)?

11.14 Microtubule dynamics and motors combine to generate the asymmetric organization of cells

Key concepts

- Dynamic microtubules and motors work together to generate cell asymmetries.
- Microtubules work together with the actin cytoskeleton during processes such as cell locomotion and mitotic spindle positioning.

The positions of organelles within a cell and the overall shape of the cell as a whole often have a definite, intentional asymmetry. The ability of cells to make one end different from the other by arranging and orienting their organelles and specializing regions of cytoplasm is a fundamental and exceptionally important property. Although an isolated individual cell could live without this ability, such cells would be completely incapable of moving or forming any of the many highly shaped and specialized cell types that are necessary to construct and maintain an organism. Fibroblasts and other motile cells, for example, must extend at one end and retract at the other in order to allow them to move through the body and respond to injury or infection. Somehow, the different activities at the two ends of each cell must be set up and then coordinated.

The asymmetric organization of a cell usually depends on the arrangement of its microtubules, their dynamic turnover, and the movement of microtubule-dependent motor proteins along them. Actin and intermediate filaments also participate in organizing the internal architecture of a cell, and the three filament systems interact and control one another's behavior. In this section, we describe several examples of how the components of the microtubule cytoskeleton function to generate cell asymmetries, including cases that illustrate how the microtubule and actin cytoskeletons work together.

When the brain is wired during development, each nerve cell sends out long extensions called axons that make contacts with target neurons to form synapses and establish the appropriate circuits for neuronal communication. At the tip of each axon as it is elongating is a growth cone, a highly motile region rich in actin and microtubules that crawls over the surface (see Figure 11.8). Movement by the growth cone elongates the axon. As it moves, the growth cone explores the area around it and responds to navigational cues by turning. Through a series of turns stimulated by cues, the growth cone is led to its target, where it ceases to move and a synapse is formed.

The microtubule cytoskeleton plays a critical role in the steering process that directs movement of the growth cone. A growth cone is a large, flattened structure that spreads out over the surface across which it moves. In the absence of an external navigational cue, dynamic microtubules are nucleated at the rear of the growth cone, and these microtubules grow and shrink throughout the growth cone in a fanlike array. When a growth cone encounters a cue, a signal is generated only in the small area of the growth cone's plasma membrane that is in contact with the cue. As shown in **FIGURE 11.53**, a spectacular response follows. Microtubules within the growth cone respond by growing toward the source of the signal. Although the MAPs responsible are not known, it is likely that local activation of a stabilizing MAP by the signal keeps microtubules in the growth phase for longer periods of time and causes them to accumulate at the site where the signal originates. **FIGURE 11.54** shows the microtubules in the two growth cones in the video several minutes after the growth cones first come into contact. After the microtubules have reoriented and elongated, plus end-directed kinesins then move vesicles out to the cell periphery along them, causing the vesicles to fuse with the plasma membrane and expand the cell in a small region. A local asymmetry in the structure of the growth cone is created as a result. The actin cytoskeleton then provides the force to drive the membrane forward in the direction marked by the microtubules.

Another process where microtubules and motors contribute to cell asymmetry is in positioning the mitotic spindle in epithelial cells. The generation of new cells to expand or repair an epithelium requires that a dividing cell within the epithelial sheet produce two daugh-

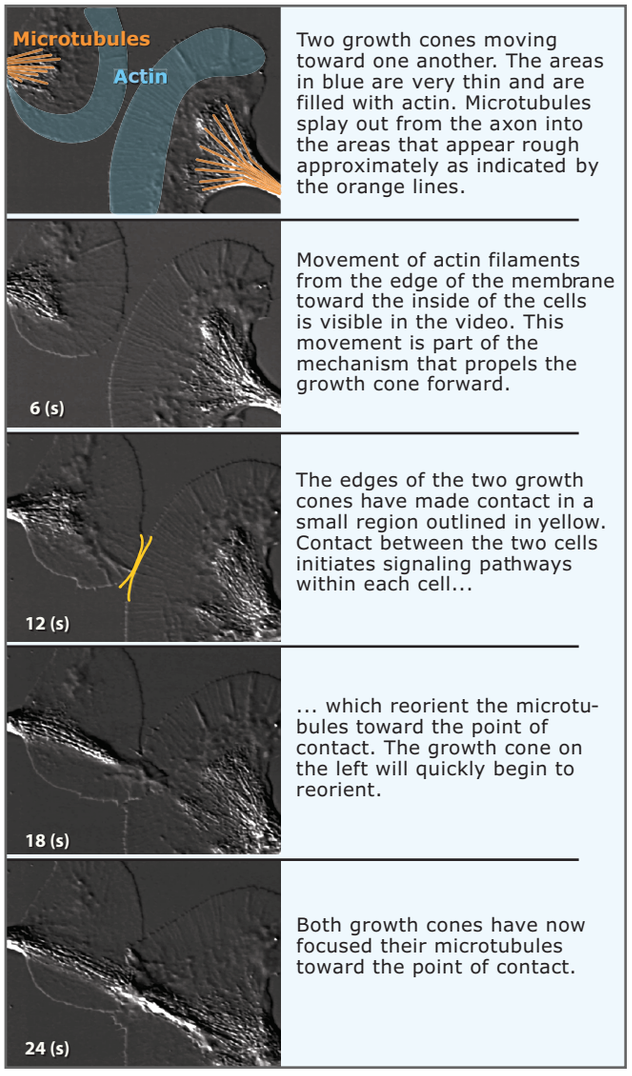


FIGURE 11.53 A series of images from a video sequence shows the growth cones of two neurons cultured *in vitro*. The response within the growth cones when they collide is very similar to the response that occurs *in vivo* when a growth cone encounters a navigational cue on another cell. Each growth cone rapidly extends microtubules toward the point of contact. © Lin and Forscher, 1993. Originally published in *The Journal of Cell Biology*, 121: 1369–1383. Photos courtesy of Paul Forscher, Yale University.

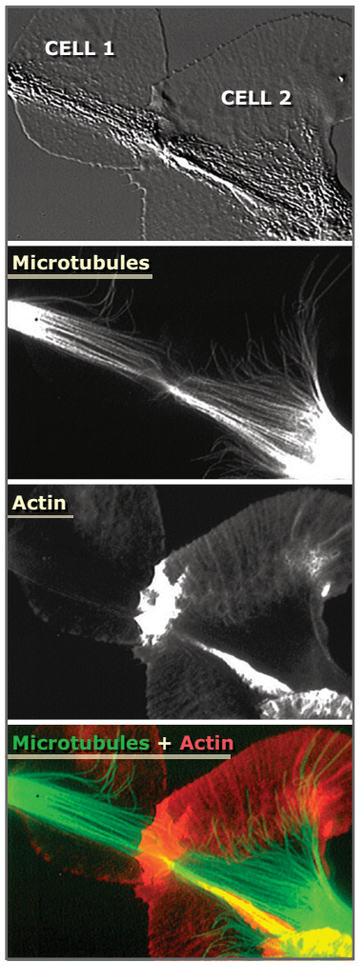


FIGURE 11.54 Actin and microtubules in the two growth cones after they have had several minutes to respond. Note how focused the microtubules are on the point of contact, and how massive polymerization of actin is also centered there. © Lin and Forscher, 1993. Originally published in *The Journal of Cell Biology*, 121: 1369–1383. Photos courtesy of Paul Forscher, Yale University.

ter cells with the same elongated shape and the same orientation as those already present, as shown in **FIGURE 11.55**. Cells always divide perpendicular to the spindle, so for division to occur along the long axis of an epithelial cell, the spindle must be oriented from side to side within the cell before the chromosomes separate. The spindle forms with a random orientation, however. In order to achieve the required orientation, the spindle is rotated by its astral microtubules (the microtubules at each end of the spindle that extend away from the chromosomes). Astral microtubules are highly dynamic and are able to constantly search the cell's periphery. Cytoplasmic dynein is anchored to sites at the plasma membrane in a beltlike array that runs around the middle of the cell. Astral microtubules that encounter the belt become associated with the dynein/dynactin complex, which then generates a pulling force that in turn swings the spindle into the correct orientation.

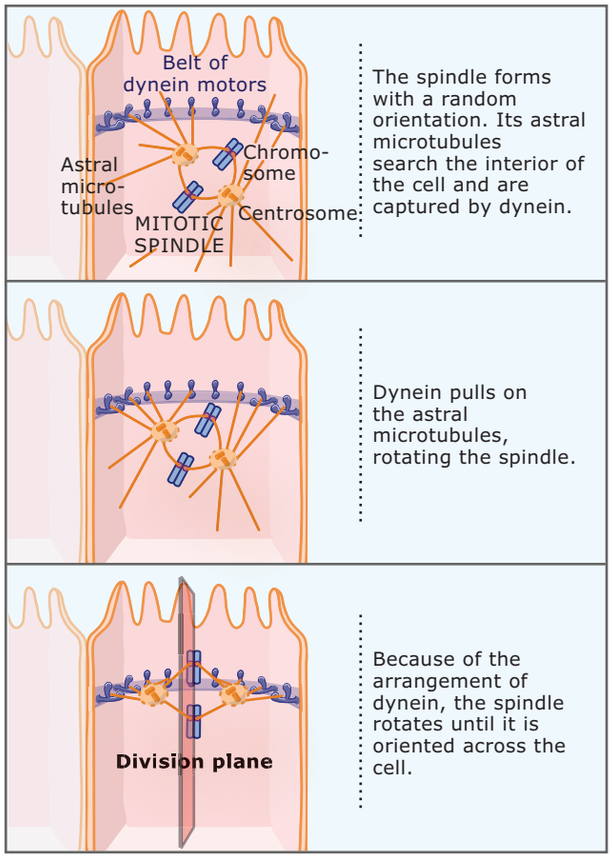


FIGURE 11.55 This cell is one of many within an epithelial sheet that extends to the left and right. The arrangement of dynein as a ring around the cell ensures that the spindle comes to rest oriented from side to side, regardless of how it was oriented when it formed. This orientation ensures that the cell will divide from top to bottom, creating two new cells for the sheet. The dynein is located at tight junctions, the points where adjacent cells in the sheet contact one another.

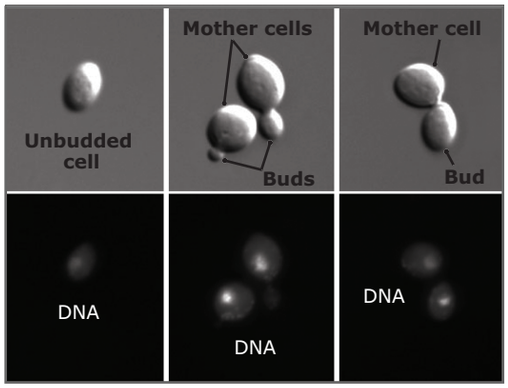


FIGURE 11.56 The three top panels show cells at successive stages of the cell cycle (from left to right). As the cell cycle progresses, the buds gradually increase in size until they are as large as the mother cells. The bottom panels show the DNA in the cells. The middle- and lower-right panels show that DNA appears in the bud only after it is as large as the mother cell. Photos courtesy of Robert Skibbens, Lehigh University.

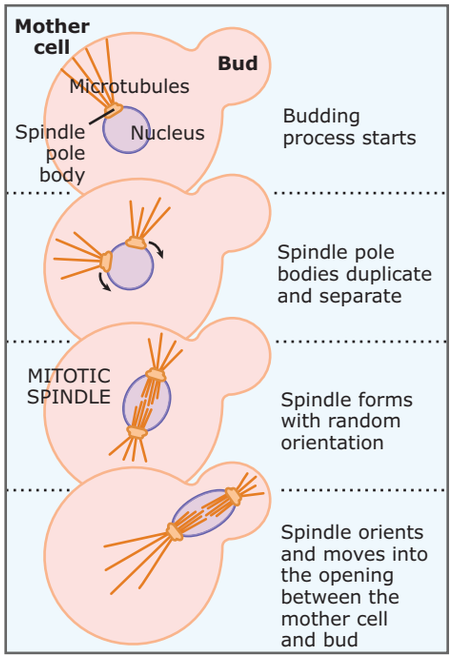


FIGURE 11.57 Budding yeast cells divide without breaking down their nucleus. The spindle pole body, a structure embedded in the nuclear envelope, nucleates microtubules both in the cytoplasm and inside the nucleus, where they form the mitotic spindle. The spindle forms in the mother cell and must be positioned in the opening between the mother cell and the bud. Properly positioning the spindle requires that it be both moved toward the bud and oriented along the axis between the bud and the mother cell.

The best understood example of how cell asymmetry is generated comes from studies of cell division in the yeast *Saccharomyces cerevisiae*, the yeast used to bake bread and brew beer. **FIGURE 11.56** shows that these yeast divide by a budding process in which a small area on the surface of a cell (the "mother" cell) grows outward, forming a bud that expands over the course of the cell cycle to eventually become the daughter cell. At about the same time the budding process starts, the lone microtubule-organizing center in the mother cell—a structure called the spindle pole body which is embedded in the nuclear membrane—duplicates, and the two spindle pole bodies that result separate and move to opposite ends of the nucleus as shown in **FIGURE 11.57**. Yeast

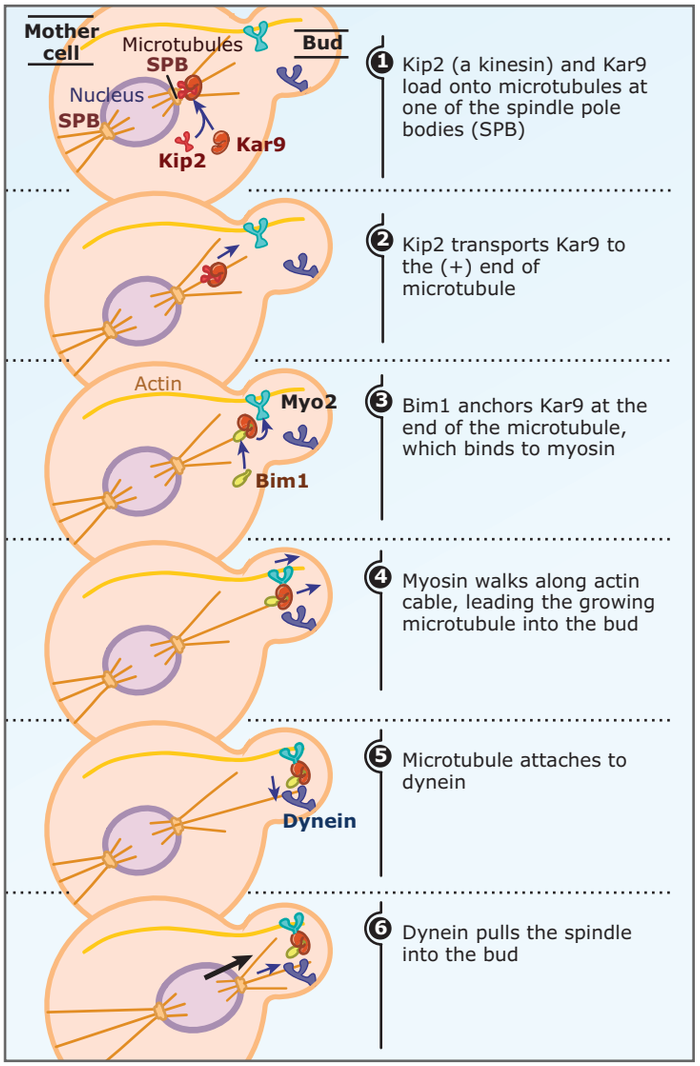


FIGURE 11.58 Getting the mitotic spindle into the bud is a collaborative effort between the actin and microtubule cytoskeletons. Polar actin cables run from the mother cell into the bud and are used to lead microtubules from one of the two spindle pole bodies in the right direction. Once within the bud, the microtubules can be captured by dynein. Because the dynein is attached to the membrane, its action pulls the spindle into the bud. This mechanism would not be possible if Kar9 and Bim1 could be loaded onto microtubules at both spindle pole bodies.

divide without breaking down their nuclear envelope, the spindle forming within the interior of the nucleus. The spindle is formed by microtubules nucleated by the two spindle pole bodies, which also nucleate microtubules on the outside of the nucleus. The spindle forms with a random orientation, uninfluenced by the location of the bud.

All of this occurs within the mother cell. In order for both the mother and daughter cells to inherit a set of chromosomes, the spindle must be aligned along the axis between the mother and the bud and moved so that it lies in the opening between them.

Both aligning and moving the spindle are performed by microtubules that extend from one of the spindle pole bodies out into the cytoplasm. How they do this is illustrated in **FIGURE 11.58**. At one of the two spindle pole bodies, a protein called Kar9 is loaded onto the microtubules. From the spindle pole body it is transported out along the microtubules to their plus ends by a member of the kinesin family (Kip2). Once it reaches an end, Kar9 remains there by binding to a +TIP (Bim1). At some point in the process, Kar9 also binds to a highly processive form of myosin (a member of the myosin V family capable of walking long distances along an actin filament without falling off), allowing the formation of a link between the tips of the cytoplasmic microtubules and actin filaments. Actin filaments exist in yeast as large cables that run through the cytoplasm of the mother cell into the bud, where they converge and are attached to the cortex at the bud's tip. All of the actin filaments in the cables have the same polarity, so the cables are polar structures that indicate the direction from the mother to the bud. Myosin moves the ends of the microtubules along the actin cables, leading the microtubules into the bud. Once there, the microtubules become attached to minus end-directed motors (dynein and a form of kinesin) anchored within the cortex. These then pull on the microtubules and hold onto their ends as they depolymerize. Thus, a combination of active pulling and force caused by controlled depolymerization of the microtubules moves the spindle through the cytoplasm of the mother cell and into the bud.

Microtubule dynamics plays a major role in orienting and positioning the spindle in both yeast cells and epithelial cells. The two situations have in common that microtubule ends must find a spot on the cortex of the cell, but the means by which dynamics are employed to do it are very different. The microtubules in yeast cells are much more stable and do not find their target by a search-and-capture mechanism that requires that the microtubules constantly grow and shrink. Rather, the cytoplasmic microtubules in yeast are led to their target. Microtubule dynamics comes into play as the tip of a microtubule is being towed along an actin filament by a myosin molecule. As the tip of the microtubule is being moved it must be able to add subunits without its connection

with the actin filament being broken. This may be possible because of the Bim1 protein, the mammalian version of which appears to allow cargoes to hang onto the end of a growing microtubule.

The mechanism that positions the yeast spindle would clearly not work if the cytoplasmic microtubules from both spindle pole bodies formed links with actin. If so, the spindle would end up stuck in the mother cell with its axis perpendicular to that between the mother and the bud. The means by which this is prevented is apparently based upon the mechanism of spindle pole body duplication. Like centrioles, an old and a new spindle pole body can be distinguished after each spindle pole body duplication. And like the centrosomes that form around the mother and daughter centrioles, the compositions of the old and new spindle pole bodies differ. In yeast, this is used to ensure the presence of proteins that inactivate Kar9 only on the new spindle pole body. As a result, Kar9 can only be loaded onto microtubules that extend out from the original, older spindle pole body. Thus, only microtubules from that spindle pole body can be led into the bud.

11.15 Interactions between microtubules and actin filaments

Key concepts

- Microtubules and actin filaments function together during cell locomotion and cell division.
- In general, microtubules direct where and when actin assembles or generates contractile forces. Microtubules influence the actin cytoskeleton through direct binding or indirect signaling.
- The two cytoskeletal systems can be bound together by linker proteins that attach microtubules to actin filaments.
- The dynamic growth and shortening of microtubules can activate a subset of G proteins; these activated G proteins control actin assembly and cell contraction.

Many dynamic cell functions require cooperation between the different cytoskeletal filaments. For example, microtubules work with actin filaments to move a cell over a substrate or to divide a cell into two (see *11.14 Microtubule dynamics and motors combine to generate the asymmetric organization of cells*). Intermediate filaments also interact with both microtubules and actin filaments to maintain cell and tissue integrity. In this section, we examine several aspects of microtubule-actin interactions during motility and division.

Several observations suggest that microtubules and actin filaments interact with each other within the cell. Researchers have known for about 30 years that when they depolymerize microtubules by adding a drug such as colchicine, the cell contracts. Contraction is driven by the actin cytoskeleton and the motor protein myosin, showing that microtubules normally resist or inhibit contraction. Cells whose microtubules are depolymerized also lose their polarized shape. For cells crawling over a surface, actin filaments are normally abundant at the front of the cell where their polymerization drives movement. When microtubules are depolymerized in these cells, the actin filaments are no longer properly localized to the front of the cell. These experimental observations suggest a general theme: microtubules function as directors, determining where actin should assemble and where it should contract. In this way, actin is used to provide force, whereas microtubules are used to organize or control where these forces act. By functioning together, actin and microtubules generate forces at the right place and time for specific cell functions.

How do the actin and microtubule cytoskeletons interact with each other at the molecular level? One way, shown in **FIGURE 11.59**, is through a linker, or a set of linkers, that binds an actin filament to a microtubule. A number of MAPs play this role by binding actin filaments as well as microtubules, forming static linkages

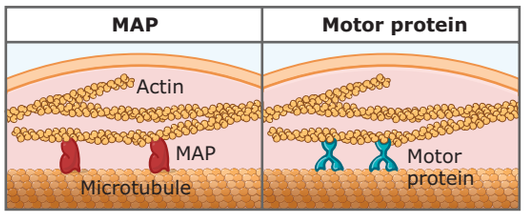


FIGURE 11.59 Several proteins or protein complexes bind to both microtubules and actin filaments and link them together. On the left is a protein that binds directly to both types of filaments and simply holds them together. Linkage can also occur through a motor, as on the right. In this case, the motor domains bind to one of the two types of filament while the tail—or other proteins bound to the tail—binds to the other. This type of interaction causes the microtubules and actin filaments to move relative to one another.

between the two. The neuronal MAP, MAP2c, is one such protein that can bind to both actin and microtubules. In growing neurons, binding of actin to microtubules is likely important as the neurons begin to form and send out long projections. Physical links between actin and microtubules may also occur through motor proteins. In this case, the linkage is dynamic, allowing one polymer to pull on the other. Such links can tether microtubules to the cell cortex, as we saw during spindle orientation in epithelial cells or spindle positioning in yeast (see *11.14 Microtubule dynamics and motors combine to generate the asymmetric organization of cells*). In both of those examples, a microtubule-based motor is anchored to the actin cytoskeleton and pulls on microtubules in order to move the spindle to the right place for cell division.

Linking microtubules to actin filaments may also guide growing microtubules to specific sites in the cell. In moving cells, some dynamic microtubules grow toward focal contacts, sites of cell adhesion to the extracellular matrix (see *12 Actin*). These dynamic microtubules are guided to the small focal contacts by the actin filament bundles attached to the contact sites. It is thought that +TIPs bound to microtubule ends may link microtubules to actin bundles, directing microtubule growth to the focal contact. Microtubules are targeted to adhesions at the rear of the cell and may deliver a signal that causes these adhesions to break down, selectively releasing the back of the cell from the substrate. Once the back of the cell is free it can contract, which moves the body of the cell forward. Many repetitions of this process coordinated with extension of the front of the cell allow the cell to move. Thus, specifically targeting adhesions at the rear of the cell for disassembly is one way that microtubules help direct a cell's movement.

Actin filaments and microtubules can also work together without being physically connected. It is now clear that the two types of polymer often relay signals to each other to regulate when and where the other polymer grows. The ability to signal and communicate with each other is critical; such communication allows microtubules and actin filaments to coordinate their activities and regulate when and where each polymer is built up, taken apart, or used to generate force. Although microtubules and actin filaments can relay signals to each other, they are also controlled by signaling pathways that respond to other inputs from inside or outside the cell. These signaling pathways act on many downstream targets in addition to the microtubule and actin cytoskeleton. We understand best the upstream signals that regulate actin filament assembly and organization. Most of the organization of actin filaments in cells is controlled by a small number of proteins called G proteins. When activated, these G proteins cause the formation of filopodia (spikelike actin projections that stick out at the front of a cell), lamellipodia (thin sheets of cytoplasm filled with actin filaments that also stick out at the front of the cell), or contractile actin bundles, such as the stress fibers that link up with focal adhesions (discussed above) and allow the cell to pull on the substrate. In general, an active G protein stimulates (often indirectly) an actin-binding protein, which then regulates the actin cytoskeleton. Remarkably, microtubule assembly or disassembly can control these G proteins by turning them on or off. In this way, dynamic microtubules direct actin assembly or contraction without physically binding to the actin filaments.

Signaling between microtubules and actin filaments is critical for cells to crawl over a substrate. Cell movement requires constant actin polymerization at the front of the moving cell to drive the cell forward, and contraction at the rear to move the body of the cell forward. At the front of the cell, actin polymerization pushes out a lamellipodium, and this polymerization is stimulated by the G protein Rac1. What activates Rac1 at the front of the cell, and why does the cell keep moving in the same direction? We now know that growing microtubules can activate Rac1, although we have no idea how they can do this. Microtubule activation of Rac1 is noteworthy because it demonstrates that the dynamic state of a microtubule can activate a signaling cascade in a specific region of the cell.

The communication between microtubules and Rac1 is not just one way; as shown in **FIGURE 11.60**, once activated, Rac1 may also relays a signal to microtubules to keep them in a growing state. Active Rac1 is thought to indirectly turns off a microtubule-destabilizing protein (oncoprotein 18), stimulating more microtubule growth. In this way, the communication between Rac1 and microtubules generates a local positive feedback loop where growing microtubules activate Rac1 and active Rac1 stimulates microtubule growth. This feedback loop keeps microtubules growing toward the front of the cell and stimulating actin polymerization there. As actin polymerization extends

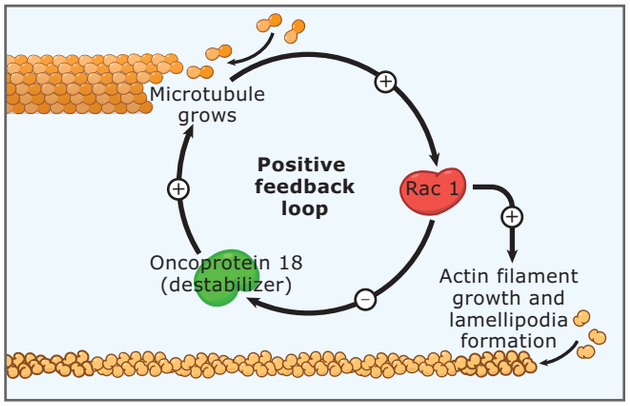


FIGURE 11.60 The polymerization state (growing or shrinking) of microtubules can indirectly change the dynamics and organization of actin filaments. A small G protein usually acts as an intermediate. In the example in this figure, growing microtubules activate Rac1, a small G protein that stimulates actin to polymerize with the organization needed to form a lamellipodium. Activated Rac1 also (indirectly) turns off the microtubule destabilizing protein Oncoprotein 18, thus creating a positive feedback loop that helps maintain microtubule growth and the formation of filopodia. Shrinking microtubules activate a different small G protein that promotes a different type of actin structure.

the front of the cell, Rac1 also stimulates microtubules to grow into the newly expanded region. Thus, as a consequence of the feedback between microtubules and Rac1, the cell can maintain its polarity and move continuously in the same direction.

Depolymerizing microtubules also start a signaling cascade. When microtubules depolymerize, they activate another G protein, RhoA. Active RhoA stimulates stress fiber and focal adhesion assembly, and indirectly activates the actin-based motor, myosin. These changes to the actin cytoskeleton cause the cell to contract. It is interesting that active RhoA can also initiate a signaling cascade that stabilizes a subset of microtubules and makes them nondynamic. Whether active RhoA limits its own activity by stabilizing a subset of microtubules is not yet known.

We still have much to learn about how the actin and microtubule cytoskeletons communicate and signal to each other. By studying these interactions, and the signaling proteins that function as intermediaries between the two cytoskeletal polymers, we will learn much about how cell locomotion and cell division are regulated and how to control these cell processes in disease states.

11.16 Cilia and flagella are motile structures

Key concepts

- Cilia and flagella contain a highly ordered core structure called an axoneme.
- The axoneme is composed of nine outer doublet microtubules surrounding a pair of central microtubules.
- Radial spokes, a complex of several polypeptides, link each outer doublet to the center of the axoneme.
- Dyneins are bound to each outer doublet and extend their motor domains toward the adjacent outer doublet.
- Dynein slides the outer doublets past each other; the structural links between outer doublets converts the sliding motion into a bending of the axoneme.
- Kinesins participate in flagellar assembly by transporting axonemal proteins to the distal tip of flagella.
- Nonmotile primary cilia participate in sensory processes.

In addition to moving cargos within cells, microtubules also play a role in moving cells relative to their environment. As can be seen in **FIGURE 11.61**, this is accomplished by cilia and

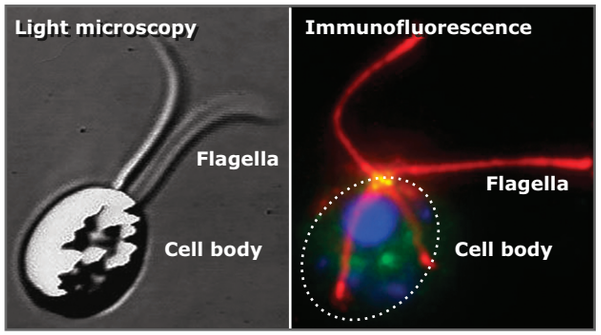


FIGURE 11.61 Light and fluorescence images of *Chlamydomonas reinhardtii*, a unicellular alga. Two very prominent flagella extend from the top of each cell. Microtubules are in red in the fluorescence image, showing that flagella are microtubule-based structures. *Chlamydomonas* cells swim by moving their flagella in a regular beating pattern. Left photo courtesy of Lynne Cassimeris, Lehigh University and right photo courtesy of Naomi Morrissette and Susan Dutcher, Washington University School of Medicine.

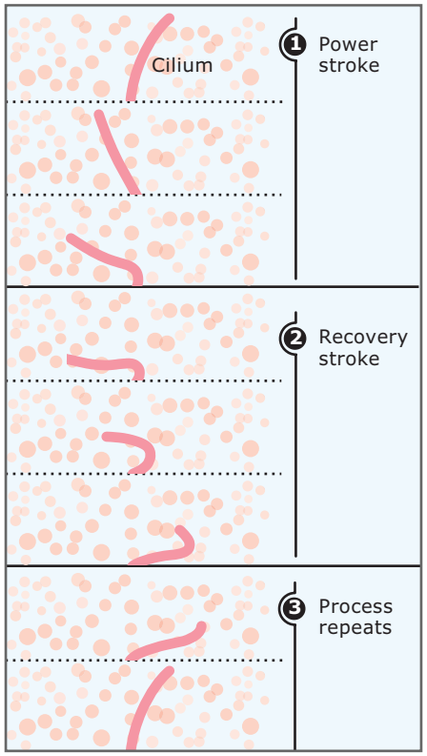


FIGURE 11.62 The beat of a cilium is divided into two parts. During the power stroke, the cilium is fully extended and moves liquid past the surface of the cell. During the recovery stroke that follows, the cilium bends from one end to the other, returning it to the starting position for another power stroke.

flagella, long, thin structures that project like hairs from the surface of many cells. Each of these organelles is composed of a long bundle of microtubules surrounded by an extension of the plasma membrane. Through interactions among the microtubules within the bundle the structures bend, allowing them to beat back and forth and move fluid past the surface of the cell as shown in **FIGURE 11.62**. For a stationary cell within a large group of cells, such as an epithelium, this allows fluid and objects to be moved over the surface of the tissue. For an individual, unattached cell, the cell itself is propelled through the fluid (i.e., it swims). Cilia and flagella are found on a number of unicellular organisms, such as *Paramecium* and *Chlamydomonas* (a green alga), as well as on the sperm cells of most eukaryotes. In mammals, cilia cover the apical domains of some types of epithelial cells and beat in synchrony, creating waves of ciliary motion that move across the surface of the tissue. Within the trachea this motion is used to clear mucus and debris from the respiratory tract; in the oviduct it transports eggs from the ovary to the uterus; and in the brain it circulates the cerebrospinal fluid.

Cilia and flagella share the same general structure and move by similar mechanisms but differ in several respects. Most significant are differences in length, the number of each per cell, and the beat pattern that they generate. Cilia are shorter (10–15 μm) and often number 100 or more per cell. Each cilium generates force by bending near its base (see Figure 11.62). The outer part of the cilium remains stiff and the bend at the base moves it in a motion resembling the powerstroke of an oar pulled through the water. This is followed by a recovery stroke, during which the cilium's bend is propagated from base to tip, readying the cilium for the next power stroke. **FIGURE 11.63** shows the movement of an actual cilium as it beats. (The online video has been slowed considerably in order to catch the stages of the motion. In reality, cilia beat so fast [many times per second] that they are little more than a blur.)

Flagella are usually longer (10–200 μm) than cilia and typically number only one or a few per cell. Flagella also generate force by bending; an S-shaped wave is propagated from the base to the tip of the flagellum, as illustrated in **FIGURE 11.64**. The beat patterns of both cilia and flagella share an underlying mechanism based on generation of the bend in the structure. Differences in the ways the bend is

propagated along the length of the organelle generate the different waveforms of cilia and flagella. Because the two types of organelle are variations on the same theme, we will focus on their common properties and we will use the term *flagella* to describe the structure and motility of either organelle unless specifically referring to the ciliary waveform.

Flagella will continue to beat if removed from the cell, demonstrating that the motion is generated solely within the organelle. Isolated flagella will also continue to beat after removal of the plasma membrane, provided ATP is present. The results of these experimental manipulations demonstrate that force is generated by the protein core of the flagellum in concert with ATP hydrolysis.

The core of a flagellum is a highly ordered structure composed of at least 250 different types of polypeptides. This structure is termed an **axoneme**. The structure of the axoneme is well conserved between the flagella of organisms as diverse as *Chlamydomonas*, a single-celled protozoan, and humans.

The major structural features of the axoneme are shown in **FIGURE 11.65**. Most prominent, particularly in the cross section, is a precisely organized bundle of microtubules that run continuously for the structure's entire length. Arranged in a circle are nine unusual "doublet microtubules," each composed of one conventional 13-protofilament microtubule (called the A tubule) with a second, incomplete microtubule of 10–11 protofilaments (called the B tubule) attached to its wall (see Figure 11.65). At the center of the ring of doublet microtubules are two conventional microtubules with 13 protofilaments (the "central pair"). This characteristic arrangement of microtubules within the axoneme is described as "9 + 2." All of the microtubules have the same polarity, oriented with their plus ends at the tip of the flagellum and their minus ends at its base. A variety of proteins binds and stabilizes the microtubules.

The microtubules within the axoneme are extensively interconnected by several types of links (see Figure 11.65). The proteins that form these connections are essential for organizing the microtubules into a single coherent unit, allowing it to move, and coordinating its movement to generate a waveform. Adjacent doublet microtubules are connected around the circumference of the axoneme by a protein called nexin. The doublet microtubules are also connected to the central pair of microtubules by

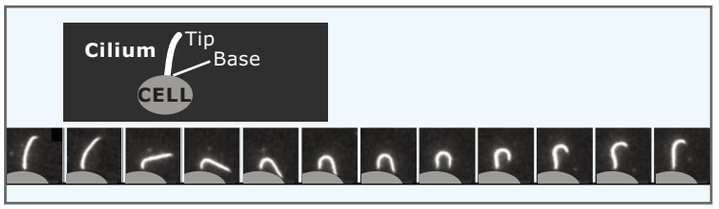


FIGURE 11.63 The beating of a cilium, shown using dark field microscopy. Note how sharply the cilium bends around its base during the power stroke and how smoothly it unfolds during the recovery stroke. Images of the cilium are taken from a video. Photos courtesy of D. R. Mitchell, SUNY Upstate Medical University.

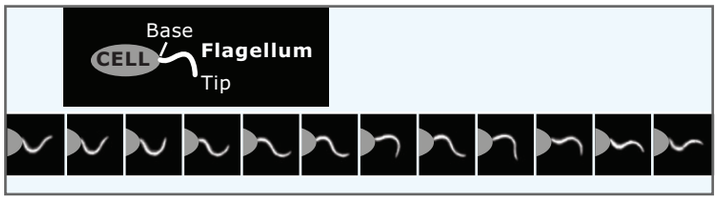


FIGURE 11.64 The beating of a flagellum, shown using dark field microscopy. Images of the flagellum are taken from a video. Photos courtesy of D. R. Mitchell, SUNY Upstate Medical University.

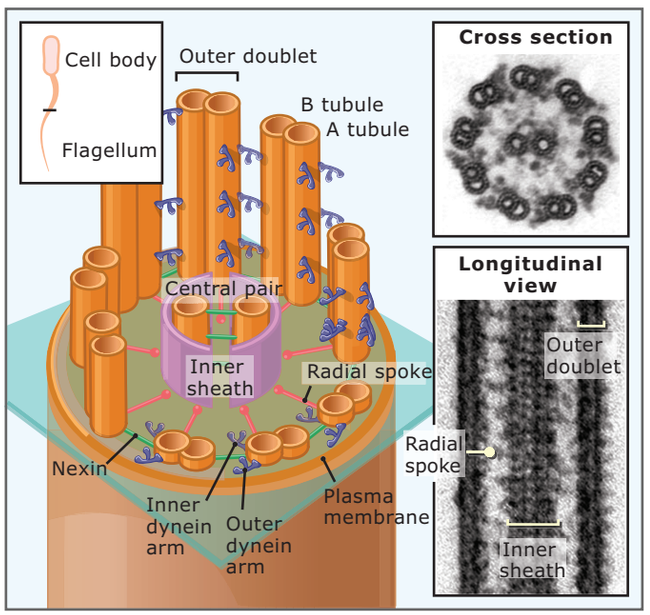


FIGURE 11.65 The structure of an axoneme, showing the highly ordered arrangement of microtubules within it. The microtubules are extensively interconnected by several different types of links. The different types of connections work together to create the beat pattern of a flagellum. On the right are electron micrographs. In the cross section the inner and outer dynein arms that link the outer doublets are visible. One very clear radial spoke and its head (on the lower left) is also visible. Photos courtesy of Dr. Gerald Rupp, Institute for Science and Health.

polypeptide complexes that form radial spokes with visible spokeheads. These two structures alone are remarkably complex: together the spokes and spokeheads contain 17 different polypeptides. The spokeheads are arranged around the inner sheath, a structure that surrounds the two central microtubules. Force is generated within the axoneme by axonemal (also called "ciliary" or "flagellar") dyneins that connect adjacent doublet microtubules; the tail domain binds to the A tubule of one doublet and the head domain to the B tubule of the next. The different connections formed by the nexins, the radial spokes, and the dyneins all occur at regular intervals along the length of the axoneme but have different periodicities. This makes it difficult to detect all three in electron micrographs that show cross sections through axonemes. When they are all visible, however, the structure resembles a wheel with thick spokes and a prominent hub.

Like the rest of the axoneme, the structure and arrangement of dyneins within it is complex. Axonemes contain more than one form of dynein, each larger and composed of more different polypeptides than cytoplasmic dynein. The different forms contain one, two, or three motor domains and are positioned at different places within the axoneme. Adjacent doublet microtubules are connected by two sets of dynein molecules, called the inner and outer arms (see Figure 11.65). The outer arms contain only dyneins with two or three heads, whereas dyneins in the inner arms have one or two.

How do all these connections allow flagella to move and generate a beat? The most basic question to be asked is how dyneins work within the structure, since they are the motors and motion must start with them. To understand the contribution of dynein in flagellar motility, flagella can be isolated from cells and the membrane removed from around the axoneme. Such demembranated axonemes can be treated briefly with a protease to break the nexin linkages between the outer doublet microtubules. **FIGURE 11.66** shows that if ATP is then added, those microtubules slide apart. Sliding is caused by the dyneins attached by their tails to one doublet microtubule generating force in a plus-to-minus end direction on the adjacent one. In an intact axoneme, dynein cannot slide the outer doublets apart because they are connected together by the nexin linkages. Instead, the force generated by dyneins is converted into a bending motion.

Cilia and flagella generate a beating motion by propagating a bend in the axoneme. A bend begins at the base of the cilium or flagellum and propagates toward the distal tip. The bend occurs because at any instant dyneins are active only within a small region of the axoneme. Dyneins are activated sequentially both along the length and around the circumference of the axoneme in order to propagate the bend. Dyneins are regulated by the central pair microtubules and the radial spokes; mutant flagella lacking these structures are paralyzed and unable to beat. In some organisms, the central pair microtubules rotate rapidly and as they spin may transmit signals to the radial spokes, which in turn activate dynein activity. Several kinases and phosphatases are localized to the central pair and radial spokes, and it is thought that rotation of the central pair activates a local signal transduction network to activate nearby dyneins. Through rapid, local activation and inactivation of specific dynein isoforms, axonemes generate either flagellar or ciliary beating and regulate the power and frequency of the beat.

At the base of a flagellum is a structure called a **basal body**. Basal bodies have the same structure as centrioles (see *11.7 Cells use*

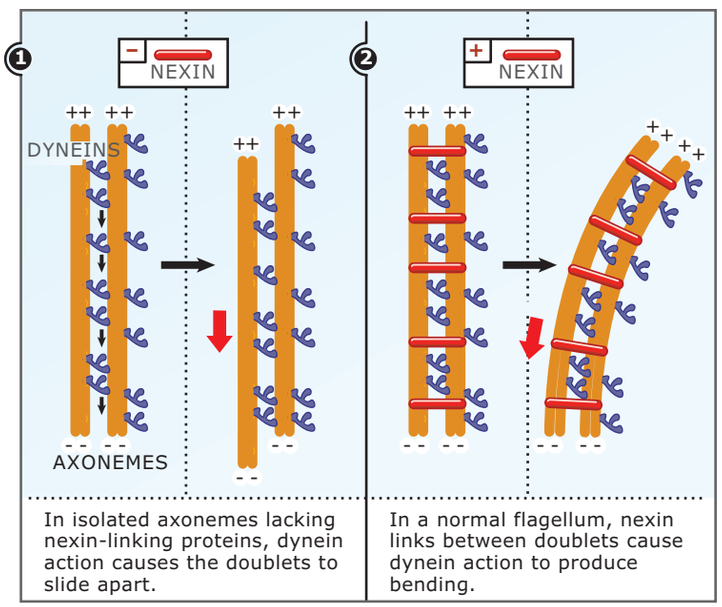


FIGURE 11.66 These two frames from an animation show two outer doublets (gold) connected by dynein. The first part of the animation illustrates what happens in an experiment in which the doublets have been purified out of a flagellum and the nexin links selectively removed. The second part shows what happens in an intact flagellum. The presence of nexin links between the doublets causes the flagellum to bend when dynein exerts force.

microtubule-organizing centers to nucleate microtubule assembly). Each basal body is a cylinder composed of 9 triplet microtubules, each with a complete 13-protofilament A tubule and 11 protofilament B and C tubules. The A and B tubules of the basal body serve as templates for assembly of the 9 outer doublet microtubules of the axoneme. The basal body remains associated with the base of the axoneme it generates and serves to anchor the axoneme to the body of the cell.

The assembly of flagella can be understood by severing the existing flagella off a cell and watching new flagella grow. Flagella regenerate in less than an hour and are functional (i.e., they beat) during the regeneration process. Growth of new flagella occurs at the plus ends of axonemal microtubules, located at the distal tip of each flagellum. Assembly of a flagellum requires that the necessary axonemal components be transported to the tip and assembled into the axoneme as it grows. Transport occurs in large protein complexes that have been observed to move toward the tip along the outer surface of the axoneme, just beneath the plasma membrane. This movement has been termed **intraflagellar transport (IFT)** and is powered by kinesin. Protein complexes are also moved from the tip of the flagellum to the base (toward the microtubule minus ends), but the function of transport in this direction is not known. IFT toward the cell body is powered by cytoplasmic dynein.

Although most cilia are motile structures, a related nonmotile form of cilia that plays a much different role in cells exists. Primary cilia are nonmotile organelles found on nearly all vertebrate cells, with the exception of blood cells. Unlike the case with motile cilia, cells typically have only a single primary cilium. A particularly striking example is shown in **FIGURE 11.67**. The axoneme of a primary cilium lacks a central pair of microtubules and, therefore, is often referred to as having a "9 + 0" structure. Most primary cilia look much like a regular cilium from the exterior, simply extending from the surface of the cell like a single short hair. In some highly differentiated cell types, however, the distal tip of the primary cilium is highly expanded and elaborated into a specialized domain that can be as large as the cell body. This is the case, for example, in the rod and cone cells that contain the photoreceptors where light is absorbed in our retinas. In these cells, the tip of the cilium is expanded into a large domain, called the outer segment, which contains stacks of membrane disks filled with the photoreceptor protein rhodopsin. An example of this is shown in **FIGURE 11.68**. The base of the primary cilium connects the outer segment to the rest of the cell, the axoneme extending only slightly beyond the point where the outer segment begins. IFT-type transport moves membrane vesicles

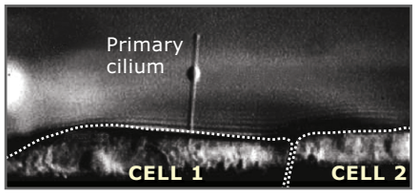


FIGURE 11.67 A very prominent primary cilium extending from the surface of a cell. The cell appears roughly in cross section, with its membrane and that of the neighboring cell indicated by dotted lines. The bulge in the primary cilium may be caused by cargo that is being moved between its axoneme and the surrounding membrane. Photo courtesy of Dr. Sam Bowser, Wadsworth Center.

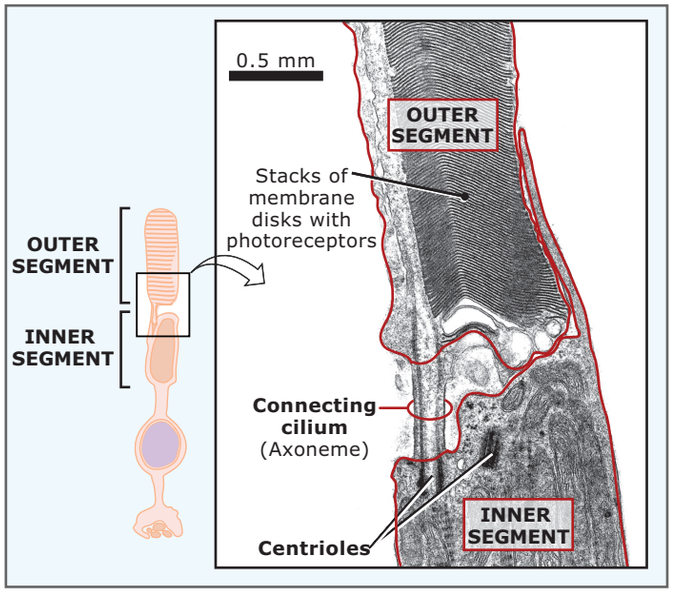


FIGURE 11.68 On the left is a drawing of an entire rod cell, showing its inner and outer segments and the thin connection between them. On the right is an electron micrograph of the region where the two are connected (indicated by the black box on the drawing). For a short distance near the point where it leaves the inner segment, the connecting cilium looks like a normal cilium. Its tip, however, is elaborated into the entire outer segment. Reprinted from *Histology of the Human Eye*, M. J. Hogan, J. A. Alvarado and J. E. Weddell, p. 425. Copyright 1971, with permission from Elsevier.

containing rhodopsin from the cell body to the outer segment and is likely to be necessary for its construction and maintenance.

Use of the outer segment of a rod cell as a light-sensing device may be an extreme example of a property that is widespread among primary cilia. A possibly general function for primary cilia as sensory devices is just beginning to be appreciated; other cell types that have more modest, unelaborated primary cilia than rod cells also specifically localize various types of receptors there. Localizing receptors to primary cilia may turn them into a kind of antenna that can detect changes in the extracellular environment and relay this information to the cell body.

A number of rare human diseases result from mutations that leave cilia and flagella nonmotile. Patients who inherit these mutations usually suffer repeated respiratory tract infections because their nonmotile cilia are unable to transport mucus and trapped pathogens and irritants out of the respiratory tract. Male patients are often sterile because their sperm are nonmotile. One of the best-known diseases resulting from nonmotile cilia and flagella is Kartagener's syndrome. In addition to respiratory tract infections and male sterility, half of all patients with Kartagener's syndrome have situs invertus, in which the normal left-right asymmetry of the internal organs is reversed. It is thought that early in normal development, before the organs are formed, flagellar beating drives a circular flow of fluid within the embryo and creates a gradient of secreted morphogens that defines left-right asymmetry. In the absence of a morphogen gradient, organ positioning is random along the left-right axis. Mice with mutations in flagellar dyneins or the motors responsible for IFT also have situs invertus, indicating that mutations effecting either flagellar motility or flagellar assembly can result in developmental defects.

Concepts and Reasoning Check

1. Describe the core structure of a flagellum and how force is generated by this organelle.

11.17 What's next?

The dynamic instability of microtubule assembly/disassembly was first described in 1984, the same year that the first kinesin was discovered. These two discoveries began an explosion in our understanding of how the microtubule cytoskeleton is put together and how motor proteins generate motility. The rapid pace of discovery shows no signs of slowing and several recent discoveries are opening up new frontiers for study, expanding our knowledge of how microtubules function in diverse organisms and in a wide range of cellular functions.

Experiments over the last decade have identified a large number of proteins that associate with microtubules, regulating assembly, producing force, or anchoring microtubules to other cell components. Despite the number of microtubule-interacting proteins that we are already aware of, there are likely more to be found. Recently, proteomic approaches have been applied to the centrosome and the yeast kinetochore, identifying all the protein components of these organelles. Many of these newly identified proteins are also likely to interact with the microtubule cytoskeleton. A logical next step is to identify functions for these proteins. Once we understand how individual proteins interact with microtubules, it will then be critical to figure out how all the protein "parts" work in concert during various cell functions. In particular, understanding the interplay between MAPs and motors will help us understand how cells divide chromosomes during mitosis, deliver vesicles to the correct location, or change the shape of a cell.

Many MAPs, and perhaps even some motors, are likely to function only within specific regions of the cell. Are they active in one place because they are only located there? If so, how did they get there? Or, are MAPs and motors regulated so that they are turned on and off in local areas of the cell? What localizes the on/off signal and sets the boundary that keeps an internal signal confined to a small area of the cell?

Cytoskeletal proteins were identified in eukaryotic cells and were thought to be unique to eukaryotes. We now know that prokaryotes also have their own cytoskeletal polymers: FtsZ (a tubulin homolog) and MreB (an actin homolog). FtsZ subunits look very much like a tubulin monomer of α- or β-tubulin. These FtsZ monomers assemble into filaments that look like a microtubule's protofilament, and they even bind and hydrolyze GTP and assemble and disassemble continuously within the bacterial cell. FtsZ polymers are associated with the bacterial membrane, where they help a dividing cell cleave into two by constricting the membrane at the center of the dividing cell. How

FtsZ functions (and how bacteria divide) is an open question. Future comparisons between FtsZ and tubulins will provide insight into how each protein functions and what features make tubulin polymerize into a microtubule and FtsZ into a single protofilament-like filament. These comparative studies may also help us understand when a cytoskeleton first appeared in an ancestral cell and how that cytoskeleton evolved in eukaryotes and prokaryotes.

Another area that has not yet been well explored is how physical forces influence microtubule assembly dynamics. Physical forces can be imposed by motors pulling on microtubules or by polymerization against barriers, such as the plasma membrane. When a microtubule reaches the plasma membrane it often bends and follows the contour of the membrane. Other times the microtubule buckles, rather than bends, and it can even break if buckled too far. Membranes or other physical barriers may also inhibit tubulin subunit addition, blocking further assembly and resulting in a catastrophe. What determines whether a polymerizing microtubule bends, buckles, breaks, or starts shortening when it hits a physical barrier such as the plasma membrane? Does a motor pulling on a microtubule alter its dynamics? How do MAPs and physical forces work together to regulate microtubule assembly and organization?

We still have much to learn about how the microtubule and actin cytoskeletons function together within the cell. Interactions between these two cytoskeletal polymers have important functions in cell locomotion and cell polarization. The microtubule and actin cytoskeletons can physically interact, being linked together by additional proteins. The two cytoskeletal polymers also communicate indirectly, relaying signals to each other through activation of kinases or other signaling molecules, and possibly also through physical forces. The indirect communication between actin filaments and microtubules can generate a positive feedback loop, where polymerization of one filament signals the other to polymerize as well. How such feedback loops are generated and how they function in processes such as directed cell locomotion are presently under study.

Our understanding of how motors work individually, in groups, or when different motors attach to the same cargo will continue to expand. Understanding how motors work in groups and how the activities of motors, which pull cargo in opposite directions, are coordinated will be critical to understanding how cargoes, including chromosomes in mitosis, are moved to correct destinations. Recent experiments have shown that dynein can walk backwards—moving toward the plus end of a microtubule. Understanding how and when dynein walks backwards will add significantly to how we view motor functions.

We are now learning that mutations in genes encoding microtubule-based motors and MAPs contribute to defects in neuronal morphogenesis and cell growth control. For example, a mutation in one member of the dynactin complex has been linked to a rare familial motor neuron disease. How mutated forms of MAPs and motors disrupt normal intracellular transport and cytoskeletal organization are important questions for the future. The identification of more human diseases linked to mutations in microtubule cytoskeletal proteins is likely to occur in the coming years and add to our understanding of how changes to the microtubule cytoskeleton influence the cell's physiology and general health.

While defects in the microtubule cytoskeleton contribute to disease, the microtubule cytoskeleton can also be a target for development of new drugs to combat other types of disease. Screens for small molecules able to disrupt mitosis identified monastrol, a small molecule that blocks the motor activity of a specific kinesin whose function is limited to the mitotic spindle. Small molecules such as monastrol may be useful drugs to treat cancers by blocking mitosis. Monastrol and other small molecules that bind proteins other than tubulin may have benefits of reduced toxicity and fewer side effects when used to treat disease, compared to the tubulin-binding drugs in use today (see *11.1 Introduction*). Other drugs are in development that bind only to tubulins from a limited number of organisms. Although tubulins are highly conserved among eukaryotes, it has been possible to isolate small molecules specifically targeted to fungal tubulins or to those in some disease-causing parasites, including the parasite responsible for malaria.

11.18 Summary

Microtubules are dynamic, polarized polymers that function in cell organization, polarity, and motility. Centrosomes—the MTOCs of animal

cells—nucleate microtubules and anchor them at their minus ends. In this way, the position of the centrosome dictates the overall pattern of microtubules present in a cell. Typically, the plus ends of microtubules are located near the plasma membrane and the minus ends are located near the cell center. The rapid assembly and disassembly of microtubules by dynamic instability makes them adaptable to new situations and allows them to easily reorganize into new patterns.

A major function of microtubules is to serve as a polarized track for the molecular motors kinesin and dynein. These motors attach to cargoes, including membrane vesicles, organelles, and chromosomes, and pull their cargoes toward the plus or minus ends of microtubules. The polarized organization of microtubules provides the navigational information necessary to direct cargo to the proper destination in the cell.

Specialized cells use microtubules as the major structural protein of cilia and flagella. Nine doublet microtubules and two center single microtubules form the core of the axoneme. Additional proteins link the doublet and center microtubules. Axonemal dyneins power ciliary or flagellar motility by forcing the outer doublets to slide relative to each other. In the highly crosslinked axoneme, sliding forces generate a bend in the axoneme. Rapid switching on and off of the axonemal dyneins allows propagation of the bend to the cilium or flagellum tip.

11.19 Supplement: What if tubulin did not hydrolyze GTP?

Key concepts

- If microtubules were equilibrium polymers they would depolymerize very slowly and would not easily reorganize.
- Tubulin dimers hydrolyze GTP when they assemble, making the microtubule a nonequilibrium polymer that can depolymerize rapidly.

Microtubules are not equilibrium polymers because tubulin subunits hydrolyze GTP after they assemble into polymers. GTP hydrolysis is not required for microtubules to assemble but instead functions to make it easy to take them apart.

Consider how hard it would be from a cell's perspective to take microtubules apart if they were equilibrium polymers. If so, gain and loss of subunits at the ends of the microtubules would be a simple equilibrium. Recall that Equation 11.1 described the rate of microtubule polymer formation:

$$dP/dt = k_{on}[\text{tubulin}] - k_{off}$$

At equilibrium, the rate of polymer formation is zero, and, therefore, as Equation 11.2 indicates

$$[\text{tubulin}] = C_c = k_{off}/k_{on}$$

A microtubule could experience a net gain or loss of subunits (i.e., an increase or decrease in length) only if the concentration of free subunits changed. Particularly revealing is to consider what would be required to make the microtubules depolymerize, since that is what is needed in order to allow the interior of a cell to reorganize. The maximum disassembly rate would occur when the tubulin subunit concentration is reduced to zero (see Equation 11.1 above). We can calculate how long it would take for a microtubule to depolymerize using an off rate of 15 dimers per second (the off rate of GTP-tubulin) and the estimate that 1624 tubulin dimers make up one micron of a microtubule. Using these values, a microtubule that is 100 µm long (as they are in some interphase cells) would take three hours to depolymerize. Yet, cells completely depolymerize their entire interphase microtubule array within minutes in preparation for mitosis. If the off rate were faster it would be possible to have faster disassembly, but it would also be harder to have any microtubules in the first place. This is because a faster off rate would also require a higher critical concentration (see Equation 11.2 above). To make a hypothetical equilibrium microtubule depolymerize at the rates observed in cells, the off rate in Equation 11.1 would have to be 540 dimers/second. At this high off rate, the critical concentration (Equation 11.2) would be significantly increased. Using the off rates given above, the critical concentration would increase approximately 36-fold, to 250 µM—ten times the actual concentration of tubulin within cells and approaching the intracellular concentration of ATP (~1 mM). The cell would have little in it other than tubulin!

So, if microtubules were equilibrium polymers, a cell could have microtubules but would have great difficulty disassembling them in order to rearrange them. Because disassembling them would require altering the equilibrium

between microtubules and free tubulin subunits, the only way the cell could disassemble its microtubules would be by destroying most of its tubulin! One consequence of this would be that cells would have great difficulty adapting their shape to changes in their environment. Cells would be sluggish and unable to adapt quickly.

These problems are avoided by having tubulin hydrolyze GTP after it has assembled, making microtubules nonequilibrium polymers. Unlike an equilibrium polymer, a nonequilibrium microtubule can both assemble and disassemble rapidly and can do both at the same tubulin concentration. This is because the gain and loss of subunits from the end of a microtubule are no longer one reaction and its exact reverse, as in a simple binding equilibrium. Instead, binding is by one species (GTP-tubulin) and loss is by another (GDP-tubulin). The release of energy by GTP hydrolysis between the two reactions means that the on and off rate constants can be independent of one another—in other words, each can be whatever is convenient for the cell. Evolution has selected the off rate constant to be very high so that a cell can take its microtubules apart quickly and without having to change the tubulin concentration, allowing fast rearrangements and a great deal of adaptability for the cell.

11.20 Supplement: Fluorescence recovery after photobleaching

Key concepts

- The fluorescent tag on proteins or lipids can be locally destroyed using very bright light from a laser.
- Recovery of fluorescence into the photobleached area occurs as unbleached proteins or lipids move into the bleached area, changing places with the photobleached protein or lipid.
- Recovery of photobleached regions on fluorescently tagged microtubules requires disassembly of the photobleached microtubule and new polymerization incorporating unbleached, fluorescent tubulin dimers.

FRAP is a method used to measure how fast a particular molecule or structure exchanges for others of its kind in a small region of a cell. With individual molecules (usually lipids or proteins that are not part of large structures), FRAP indicates how fast the molecules diffuse and what fraction of them are mobile. With proteins that are components of large, immobile structures (such as cytoskeletal filaments), it indicates how frequently the structures disassemble and reassemble.

To perform a FRAP experiment, a fluorescently tagged version of the protein or lipid of interest is first introduced into the cell. The cell is viewed by fluorescence microscopy, and the fluorescent tag is then destroyed in the region of interest by exposing the area to a laser beam. (Destroying the tag with the intense light of the laser is called bleaching, hence the term *photobleaching*.) Only the tag is destroyed; the attached protein or lipid is still functional (and whatever structure it may be part of is still intact) but is now invisible by fluorescence microscopy. If unbleached fluorescently tagged molecules are able to diffuse or assemble in the bleached zone, then fluorescence recovers within the bleached zone.

For microtubules, fluorescently tagged tubulins are introduced into a cell and enough time is allowed for them to incorporate evenly throughout all the cell's microtubules. The fluorescent tag is either a small fluorescent chemical covalently attached to purified tubulin or a fusion protein of γ-tubulin and green fluorescent protein. **FIGURE 11.69** shows that

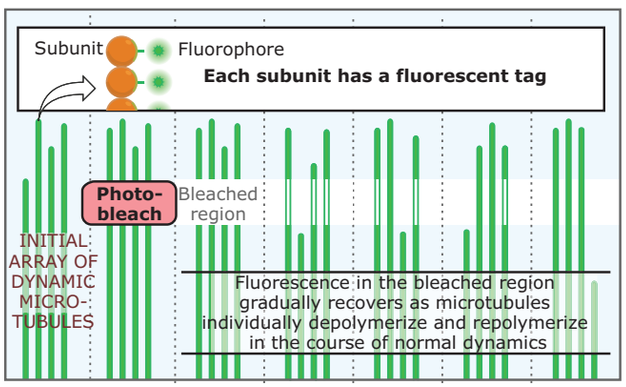


FIGURE 11.69 The green lines represent individual microtubules composed of subunits with fluorescent tags attached. To start an experiment, the tags in a small region are bleached by a very bright light. If the microtubules are not dynamic, the bleached region will remain indefinitely. If they are dynamic, as in the figure, fluorescence will gradually return to the region as bleached microtubules depolymerize and are replaced by new microtubules that polymerize and incorporate unbleached subunits. Because the number of bleached subunits in the cell is small compared to the total pool of fluorescently tagged subunits, the newly polymerized microtubules appear uniformly fluorescent along their lengths. The rate at which the fluorescence in a region recovers is thus a measure of how dynamic the microtubules there are.

after fluorescence is destroyed in a region, recovery of fluorescence to the photobleached area requires depolymerization of the photobleached microtubules and assembly of new microtubules that incorporate unbleached tubulins. The rate of fluorescence recovery is thus proportional to the rate of microtubule turnover.

11.21 Supplement: Tubulin synthesis and modification

Key concepts

- Synthesis of new tubulin is regulated by the concentration of dimers in the cytoplasm.
- α- and β-tubulins require cytosolic chaperonins and additional cofactors to fold properly and assemble into a heterodimer.
- Tubulins are subject to a number of posttranslational modifications.
- Some modifications only occur on tubulins in polymers. These modifications are associated with a stable subpopulation of microtubules.
- In some organisms, the presence of posttranslationally modified tubulins within a microtubule enhances the binding of motor proteins and provides an additional mechanism to regulate vesicle traffic in the cell.

Synthesis of α- and β-tubulins is regulated by a feedback mechanism that responds to the amount of tubulin available in a cell, as shown in **FIGURE 11.70**. During translation, a nascent tubulin polypeptide emerging from the ribosome can be bound by an already assembled tubulin dimer, activating an RNase that specifically degrades the mRNA. Thus, in a cell with an elevated concentration of tubulin dimers, tubulin mRNAs will be less stable and less new tubulin will be synthesized. Conversely, a cell whose tubulin concentration has somehow dropped below normal will have more stable tubulin mRNAs and will synthesize tubulins to replenish the tubulin pool. By having tubulin protein levels determine the stability of tubulin mRNAs, a cell is able to maintain its pool of tubulin dimers within a concentration range that allows its microtubules to function properly.

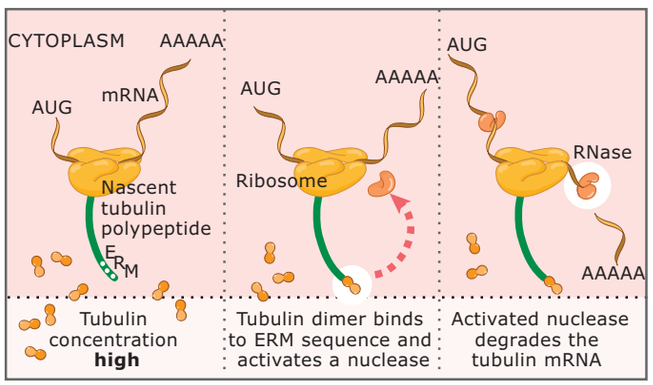


FIGURE 11.70 Interaction between a tubulin dimer and a short sequence at the end of tubulin polypeptides leads to the degradation of tubulin mRNA and decreased synthesis of tubulin. This feedback mechanism maintains the cytoplasmic tubulin concentration within narrow limits. In this figure, only tubulin and a nuclease are shown, but other components may participate as well.

α- and β-tubulin cannot fold or assemble properly by themselves. Several additional proteins are required, several of which are specific for tubulin. After translation, tubulin monomers are first bound and folded by CCT, a cytosolic chaperonin, in an ATP-dependent process. Several additional proteins that form a large complex (cofactors A-E) are then required to assemble the folded α and β subunits into heterodimers, as outlined in **FIGURE 11.71**. At some point in the assembly process each of the subunits binds a molecule of GTP. Release of the assembled dimer from the cofactor complex requires that the β subunit hydrolyze the GTP it has bound, but the GTP associated with the α subunit remains unhydrolyzed. Once the properly folded tubulin dimer is released, the β subunit rapidly exchanges GDP for GTP and the dimer is finally competent to assemble into microtubules.

Tubulin subunits are targets for a remarkable variety of posttranslational covalent modifications, including phosphorylation (on either α- or β-tubulin), detyrosination (removal of the terminal tyrosine residue from α-tubulin), acetylation (acetylation of a specific lysine residue within α-tubulin), and polyglutamylation and polyglycylation (covalent addition of chains of glutamate and glycine, respectively, to either α- or β-tubulin). It has long been recognized that certain modifications, such as acetylation and detyrosination, are always found on subsets of microtubules that are significantly more stable than most. High concentrations of modified tubulins are found in brain tissue and within the axonemal microtubules of cilia and flagella. Other cell types contain varying amounts of modified tubulins. In most cases, it is not yet clear how modification contributes to the function of a microtubule.

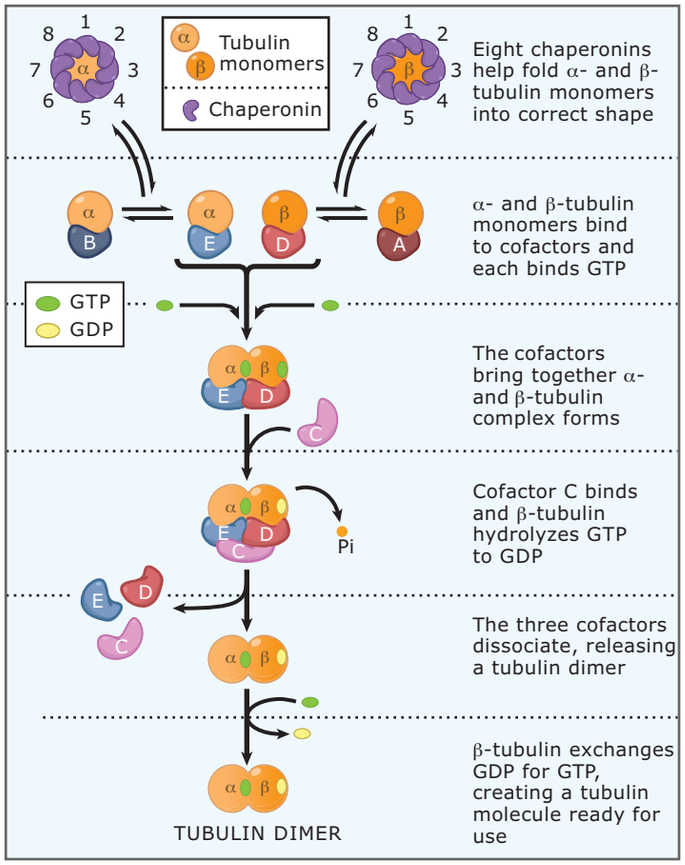


FIGURE 11.71 The steps required to assemble a tubulin heterodimer. The two tubulin subunits are folded separately and then brought together with the assistance of cofactors, several of which are specific to tubulin. The A, B, D, and E cofactors may be required because the folded conformations of the tubulin subunits are not stable until they are part of a tubulin dimer. Alternatively, the structures of the α and β subunits may need to be slightly contorted in order to get them to fit together. Cofactor C and energy input through hydrolysis of GTP by β-tubulin are necessary to dissociate the cofactors after assembly is complete.

The presence of modified tubulin subunits provides a convenient marker of microtubule stability, but the posttranslational modifications themselves are not responsible for making the microtubules more stable. At this time, it is not known how a subset of microtubules becomes much more stable, but it is likely that these microtubules are stabilized by the binding of specific MAPs to their walls or ends. Once the microtubule is stabilized, the tubulin subunits within it are modified in one or more of the ways previously described. In some cells, stable microtubules are preferentially bound by motor proteins, which enhances vesicle movement specifically on this subset of microtubules.

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