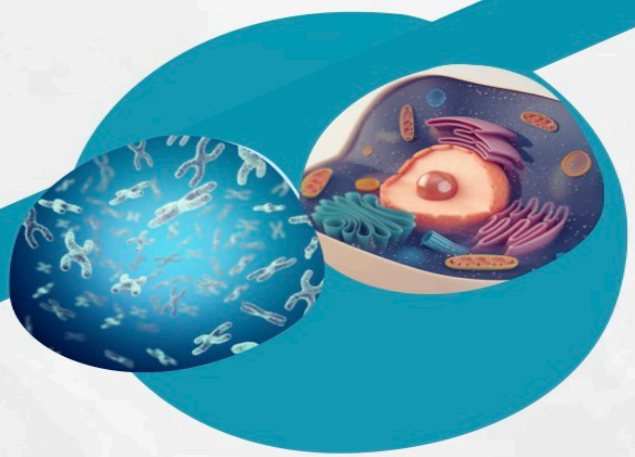
Sheets

Cell biology

Lecture 9,5~9,11



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9.5 Microtubule-Organizing Centers (MTOCs)

The function of a microtubule within a living cell depends on its location and orientation, which makes it important to understand why it assembles in one place as opposed to another. When studied in vitro, the assembly of microtubules from αβ-tubulin dimers occurs in two distinct phases: a slow phase of nucleation, in which a small portion of the microtubule is initially formed, and a much more rapid phase of elongation. Unlike the case in vitro, nucleation of microtubules takes place rapidly inside a cell, where it occurs in association with a variety of specialized structures called **microtubule-organizing centers** (or **MTOCs**). The best studied MTOC is the centrosome.

Centrosomes

In animal cells, the microtubules of the cytoskeleton are typically nucleated by the **centrosome**, a complex structure that contains two barrel-shaped **centrioles** surrounded by amorphous, electron-dense **pericentriolar material** (or **PCM**) (**Figure 9.14a,b**). Centrioles are cylindrical structures about 0.2 μm in diameter and typically about twice as long. Centrioles contain nine evenly-spaced blades, each of which contain three microtubules, designated A, B, and C. Only the A tubule is a complete microtubule (Figure 9.14*a,b*). The nine A tubules are connected to a central hub with nine spokes called the cartwheel. The ninefold symmetry of the centriole results from the structure of the SAS-6 protein, which self-assembles into a ninefold symmetric disc that forms the core of the cartwheel. New centrioles typically form adjacent to, and at right angles with, preexisting centrioles (Figure 9.14*a,c*). This process of centriole duplication means that most cells contain a pair of connected centrioles in which one termed the mother, is at least one cell cycle older than the other, which is termed the daughter. Daughter centrioles initially form at right angles to the mother centriole, but this precise spatial relation is subsequently lost. Further discussion of centriole duplication is found in Chapter 14. Centrioles recruit PCM to form a new centrosome with centrioles embedded in a cloud of PCM (Figure 9.14*d*). As discussed shortly, the centrosome is the major site of microtubule initiation in animal cells and typically remains at the center of the cell's microtubular network (Figure 9.14*e*).

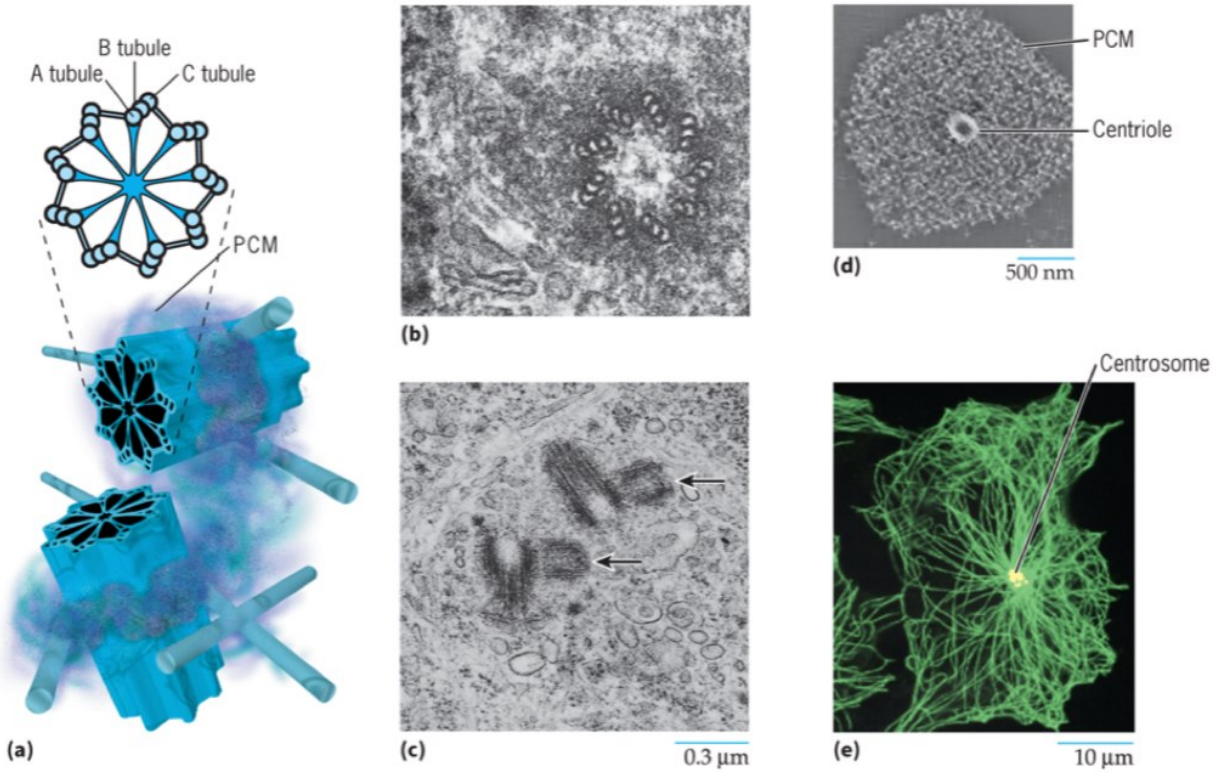

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FIGURE 9.14 The centrosome. (a) Schematic diagram of a centrosome showing the paired centrioles; the surrounding pericentriolar material (PCM); and microtubules emanating from the PCM, where nucleation occurs. (b) Electron micrograph of a cross section of a centriole showing the pinwheel arrangement of the nine peripheral fibrils, each of which consists of one complete microtubule and two incomplete microtubules. (c) Electron micrograph showing two pairs of centrioles. Each pair consists of a longer parental centriole and a smaller daughter centriole (arrow), which is undergoing elongation in this phase of the cell cycle (discussed in Section 14.1). (d) Electron micrographic reconstruction of a 1.0 M potassium iodide–extracted centrosome, showing the PCM to contain a loosely organized fibrous lattice. (e) Fluorescence micrograph of a cultured mammalian cell showing the centrosome (stained yellow by an antibody against a centrosomal protein) at the center of an extensive microtubular network.

Figure 9.15a shows an early experiment that demonstrates the role of the centrosome in the initiation and organization of the microtubular cytoskeleton. The microtubules of a cultured animal cell were first depolymerized by incubating the cells in nocodazole. Microtubule reassembly was then monitored by removing the chemical, fixing cells at various time intervals, and treating the fixed cells with fluorescent anti-tubulin antibodies. Within a few minutes after removal of the inhibiting conditions, one or two bright fluorescent spots are

seen in the cytoplasm of each cell. Within 15–30 minutes (Figure 9.15*a*), the number of labeled filaments radiating out of these foci increases dramatically. When these same cells are sectioned and examined in the electron microscope, the newly formed microtubules are found to radiate outward from a centrosome. Close examination shows that the microtubules do not actually penetrate into the centrosome and make contact with the centrioles but terminate in the dense PCM that resides at the centrosome periphery. It is this material that initiates the formation of microtubules (see Figure 9.16*c*).

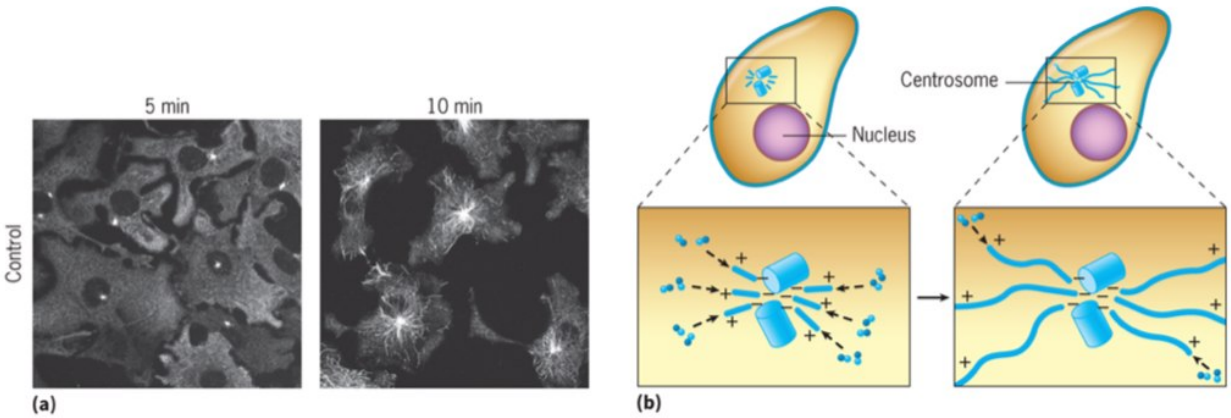

FIGURE 9.15 Microtubule nucleation at the centrosome. (a) Human MKN-1 cells were treated with nocodazole to depolymerize microtubules. The drug was then washed out to allow microtubules to regrow. Images show fluorescence microscopy of cells stained with antibodies recognizing microtubules at 5 and 10 minutes after nocodazole washout. At the five-minute time point, microtubules can be seen starting to all grow out from the same point in the cell—this is the centrosome. (b) Microtubules are nucleated at the centrosome and grow at their plus ends, while the minus ends remain anchored at the centrosome.

Because centrosomes are sites of microtubule nucleation, the microtubules of the cytoskeleton are all polarized the same way: The minus end is associated with the centrosome, and the plus (or growing) end is situated at the opposite tip (Figure 9.15*b*). The fraction of microtubules that remain associated with the centrosome is highly variable from one cell type to another. The centrosome of a nonpolarized cell (e.g., a fibroblast) is typically situated near the center of the cell and tends to remain associated with the minus ends of a large number of microtubules (as in Figure 9.14*e*). In contrast, many of the microtubules in a polarized epithelial cell are anchored by their minus ends at dispersed sites near the apical end of the cell as their plus ends extend toward the cell's basal surface (Figure 9.1). Similarly, the axon of a nerve cell contains large numbers of microtubules that have no association with the centrosome, which is located in the neuron's cell body. Many of these axonal microtubules are thought to form in conjunction with the centrosome but are then severed from the MTOC and transported into the axon by motor proteins. Certain animal cells, including mouse oocytes, lack centrosomes entirely, yet they are still capable of forming complex microtubular structures, such as the meiotic spindle (as discussed in Chapter 14). Human patients with genetic defects in centrosome-associated proteins display microcephaly, a reduction in brain size, presumably because the neuronal proliferation and migration are particularly sensitive to reductions in centrosome function.

Basal Bodies and Other MTOCs

Centrosomes are not the only MTOCs in cells. The outer microtubules in a cilium or flagellum are generated as outgrowths from the microtubules in a structure called a **basal body**, which resides at the base of the cilium or flagellum (see Figure 9.30). Basal bodies are identical in structure to centrioles, and in fact, they can turn into centrioles and vice versa. For example, the basal body that gives rise to the flagellum of a sperm cell is derived from a centriole that had been part of the meiotic spindle of the spermatocyte from which the sperm arose. Conversely, the sperm basal body typically becomes a centriole during the first mitotic division of the fertilized egg. Plant cells lack both centrosomes and centrioles, or any other type of obvious MTOCs. Instead, microtubules in a plant cell are nucleated around the surface of the nucleus and widely throughout the cortex (see Figure 9.17).

Microtubule Nucleation

Regardless of their diverse appearance, all MTOCs play similar roles in all cells: They control the number of microtubules, their polarity, the number of protofilaments that make up their walls, and the time and location of their assembly. In addition, all MTOCs share a common protein component—a type of tubulin called **γ-tubulin**, which was first discovered in the late 1980s by Berl Oakley and coworkers using genetic screens in the fungus *Aspergillus*. Unlike α- and β-tubulins, which make up about 2.5 percent of the protein of a nonneural cell, γ-tubulin constitutes only about 0.005 percent of the cell's total protein. Fluorescent antibodies to γ-tubulin stain all types of MTOCs, including the PCM of centrosomes (**Figure 9.16a**), suggesting that γ-tubulin is a critical component in microtubule nucleation. This conclusion is supported by other studies. For example, mutations in γ-tubulin lead to reduced microtubule nucleation at MTOCs.

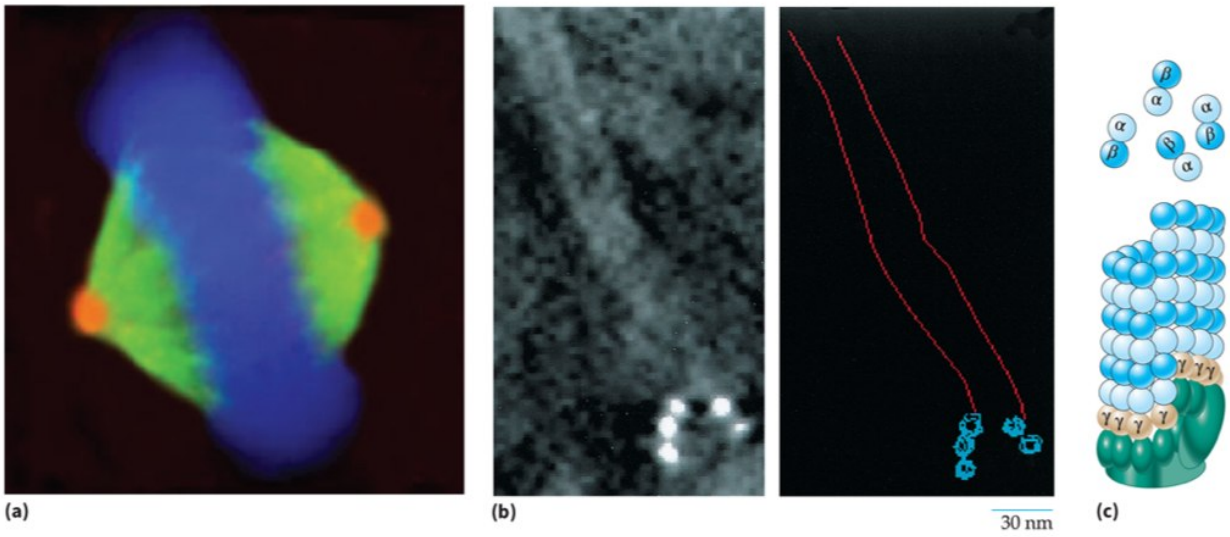

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FIGURE 9.16 The role of γ-tubulin in centrosome function. (a) Image shows a mitotic spindle in a human U2OS cell stained for γ-tubulin (orange), tubulin (green), and DNA (blue). (b) A reconstruction based on the electron micrographs of a portion of a centrosome that had been incubated with purified tubulin in vitro and then labeled with antibodies against γ-tubulin. The antibodies were linked to gold particles to make them visible (as white dots) in the reconstruction. During the incubation with tubulin, the centrosome served as an MTOC to nucleate microtubules with minus ends seen to be labeled with clusters of gold, which are often arranged in semicircles or rings. The accompanying drawing shows the outline of the microtubule seen in the reconstruction. (c) A model for γ-tubulin function during the assembly of microtubules. Nucleation begins as αβ-tubulin dimers bind to an open ring of γ-tubulin molecules (brown), which are held in place by a number of non-tubulin proteins (green) that make up the large γ-tubulin ring complex (γ-TuRC). Nucleation by the γ-TuRC defines microtubule polarity with a ring of α-tubulin monomers situated at the minus end of the structure.

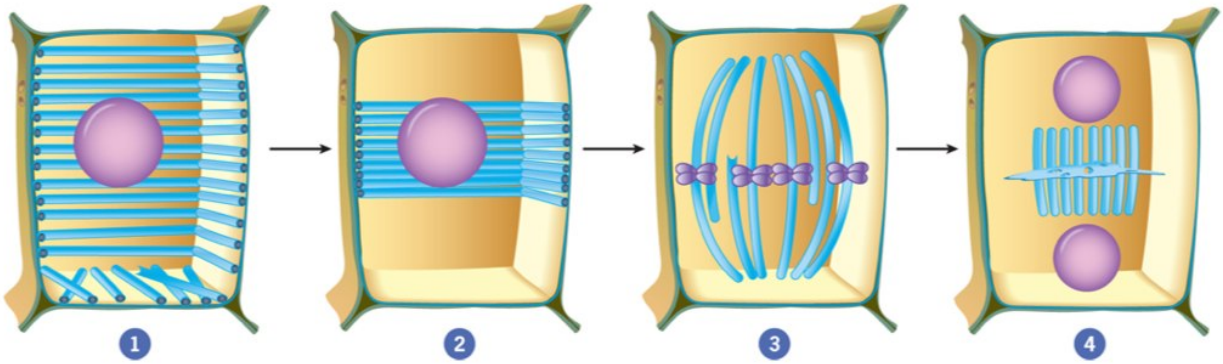
To understand the mechanism of microtubule nucleation, researchers have focused on the structure and composition of the PCM at the periphery of centrosomes. The insoluble fibers of the PCM (Figure 9.14*d*) are thought to serve as attachment sites for ring-shaped structures that have the same diameter as microtubules (25 nm) and contain γ-tubulin. These ring-shaped structures were discovered when centrosomes were purified and incubated with gold-labeled antibodies that bound to γ-tubulin. The gold particles were seen in the electron microscope to be clustered in semicircles or rings situated at the minus ends of microtubules (Figure 9.16*b*). These are the ends of the microtubules that are embedded in the PCM of the centrosome where nucleation occurs. Similar γ-tubulin ring complexes (or γ-TuRCs) have been isolated from cell extracts and shown to nucleate the microtubule assembly in vitro. Structural studies have shown that the γ-TuRC is a helical array of γ-tubulin (brown) subunits, each bound with a set of accessory proteins. The resulting open, lock-washer-like ring-shaped template has 13 γ-tubulins per turn on which the first row of αβ-tubulin dimers assemble (Figure 9.16*c*). The number of protofilaments in the microtubule is apparently

dictated by the number of γ-tubulins around the ring. In this model, only the α-tubulin of the heterodimer can bind to a ring of γ-tubulin subunits. Thus, the γ-TuRC determines the polarity of the entire microtubule and also forms a cap at its minus end, preventing the gain or loss of tubulin subunits.

The Dynamic Properties of Microtubules

The microtubules of the cytoskeleton are dynamic polymers that are subject to shortening, lengthening, disassembly, and reassembly. Although all microtubules appear quite similar morphologically, there are marked differences in their stability. Microtubules of the mitotic spindle or the cytoskeleton are extremely *labile*, that is, sensitive to disassembly. Microtubules of mature neurons are much less labile, and those of centrioles, cilia, and flagella are highly stable. These differences in microtubule stability are determined by microtubule-interacting proteins including MAPs (Figure 9.4), which stabilize microtubules; proteins known as +TIPs, which bind to the plus end of growing microtubules; and an enzyme called katanin, named after the samurai sword, which severs microtubules into shorter pieces. Microtubule stability is also regulated by posttranslational modifications to the tubulin subunits such as the covalent attachment of multiple glutamates onto the C-terminus of tubulin. Living cells can be subjected to a variety of artificial treatments that lead to the disassembly of labile cytoskeletal microtubules without disrupting other cellular structures. Disassembly can be induced by cold temperature; hydrostatic pressure; elevated Ca^{2+} concentration; and a variety of chemicals, including colchicine, vinblastine, vincristine, and nocodazole. The drug taxol stops the dynamic activities of microtubules by a very different mechanism. Taxol binds to the microtubule polymer, inhibiting its disassembly, thereby preventing the cell from assembling new microtubular structures as required. Many of these compounds, including taxol, are used in chemotherapy against cancer because they preferentially kill tumor cells.

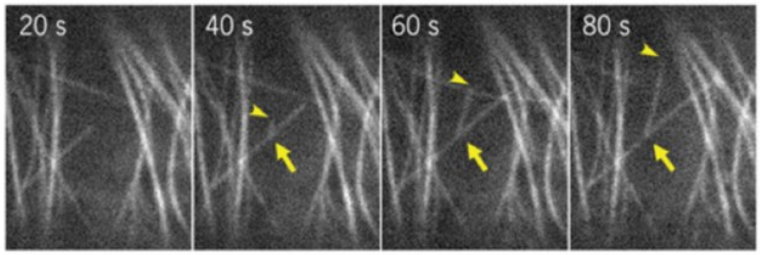
The lability of cytoskeletal microtubules reflects the fact that they are polymers formed by the noncovalent association of protein building blocks. The microtubules of the cytoskeleton are normally subject to depolymerization and repolymerization as the requirements of the cell change from one time to another. The dynamic character of the microtubular cytoskeleton is well illustrated by plant cells. If a typical plant cell is followed from one mitotic division to the next, four distinct arrays of microtubules appear, one after another (**Figure 9.17**).



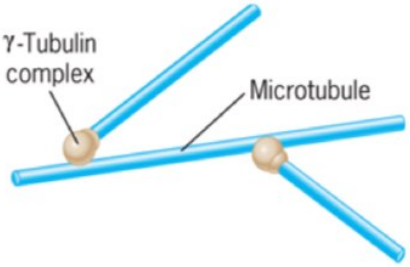
Source: Adapted from R.H. Goddard et al., *Plant Physiol.* 104:2, 1994. Plant Physiology by American Society of Plant Physiologists.

FIGURE 9.17 Four major arrays of microtubules present during the cell cycle of a plant cell. The organization of the microtubules at each stage is described in the text.

1. During most of interphase, the microtubules of a plant cell are distributed widely throughout the cortex, as depicted in Figure 9.17, stage 1. A search for γ-tubulin shows this nucleation factor to be localized along the lengths of the cortical microtubules, suggesting that new microtubules might form directly on the surface of existing microtubules. This idea is supported by studies of tubulin incorporation in living cells (**Figure 9.18a**) and by in vitro assays (Figure 9.18*b*) that show newly formed microtubules branching at an angle from the sides of preexisting microtubules. Once formed, the daughter microtubules are likely severed from the parent microtubule and incorporated into the parallel bundles that encircle the cell (Figures 9.6 and 9.17).
2. As the cell approaches mitosis, the microtubules disappear from most of the cortex, leaving only a single transverse band, called the preprophase band, that encircles the cell like a belt (Figure 9.17, stage 2). The preprophase band marks the site of the future division plane.
3. As the cell progresses into mitosis, the preprophase band is lost and microtubules reappear in the form of the mitotic spindle (Figure 9.17, stage 3).
4. After the chromosomes have been separated, the mitotic spindle disappears and is replaced by a bundle of microtubules called the phragmoplast (Figure 9.17, stage 4), which plays a role in the formation of the cell wall that separates the two daughter cells (see Figure 14.39).



(a)



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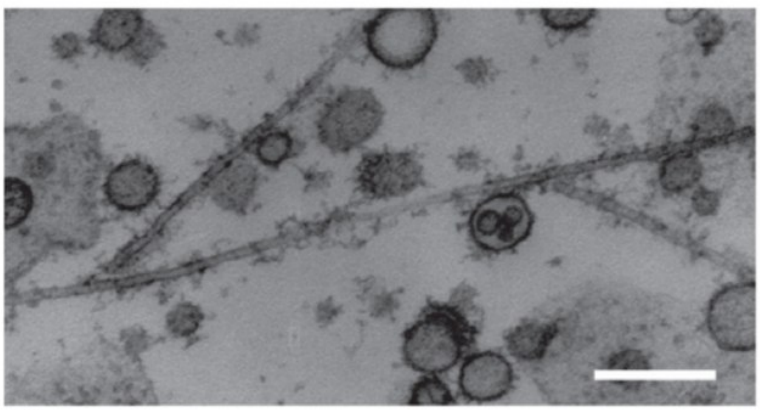

(b)

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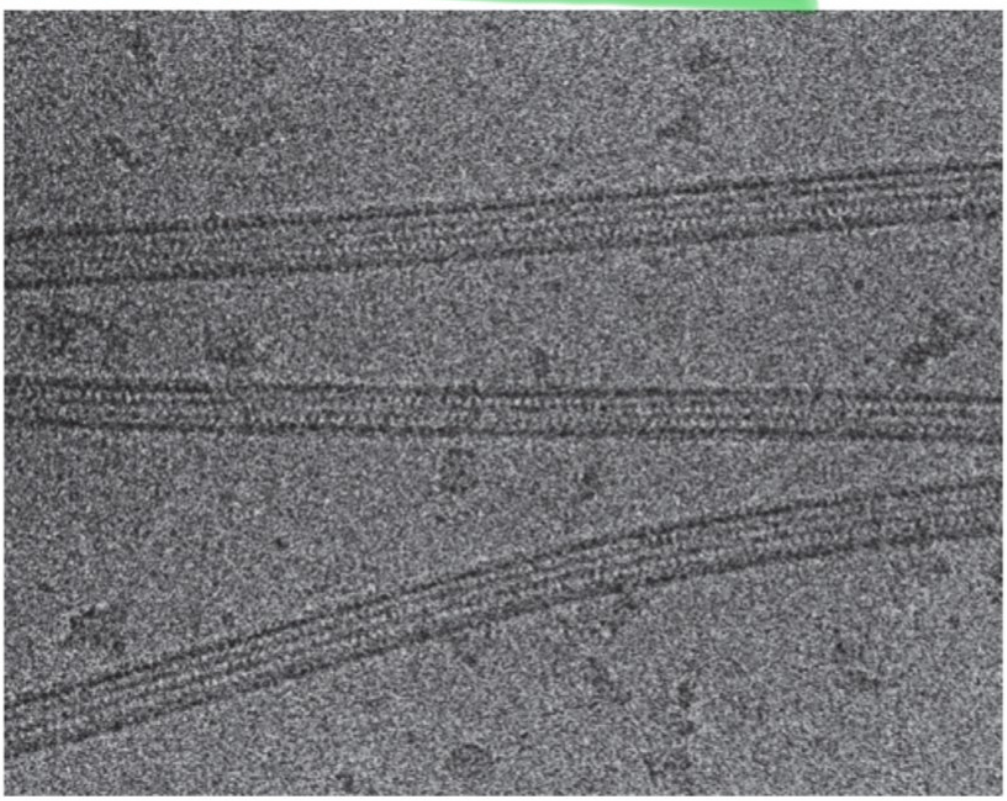
FIGURE 9.18 Nucleation of plant cortical microtubules. (a) The micrographs show a portion of a live cultured tobacco cell that is expressing fluorescently labeled GFP-tubulin. During the period of observation, an existing microtubule of the cortex nucleates the assembly of a daughter microtubule, which grows outward, forming a Y-shaped branch. The end of a newly formed microtubule is indicated by the arrowhead, the branchpoint by the arrow. (b) Electron micrograph of a microtubule with two daughter microtubules branching from its surface. The branched microtubules were assembled in a cell-free system containing tubulin subunits. Bar, 10 μm. (c) A schematic model showing how new microtubules are nucleated at the sites of γ-tubulin present on the surface of an existing microtubule.

These dramatic changes in the spatial organization of microtubules are thought to be accomplished by a combination of two separate mechanisms: (1) the rearrangement of existing microtubules and (2) the disassembly of existing microtubules and reassembly of new ones in different regions of the cell. In the latter case, the microtubules that make up the preprophase band are formed from the same subunits that a few minutes earlier were part of the cortical array or, before that, the phragmoplast.

The Underlying Basis of Microtubule Dynamics

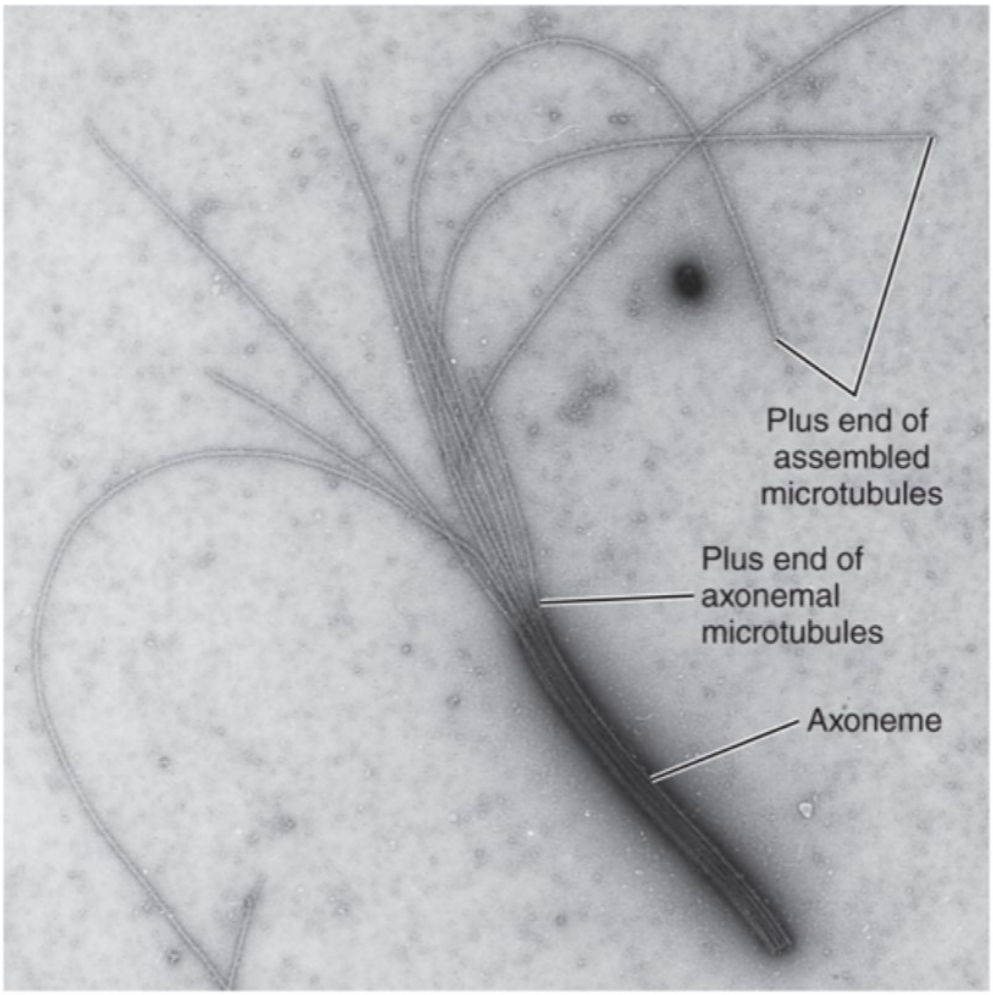
Insight into factors that influence the rates of microtubule assembly and disassembly came initially from studies carried out in vitro. The first successful approach to the in vitro assembly of microtubules was taken in 1972 by Richard Weisenberg of Temple University,

who found that microtubules could be disassembled and reassembled over and over simply by lowering and raising the temperature of the incubation mixture. **Figure 9.19** shows three microtubules that were assembled in the test tube from purified tubulin. One of the three microtubules in this figure contains only 11 protofilaments (as indicated by its thinner diameter). It is not unexpected that microtubules assembled in vitro might have abnormal protofilament numbers because they lack a proper template (Figure 9.16c) normally provided by the γ-TuRCs in vivo. In vitro assembly of microtubules is aided greatly by the addition of MAPs or by pieces of microtubules or structures that contain microtubules (**Figure 9.20**), which serve as templates for the addition of free subunits. In these in vitro studies, tubulin subunits are added primarily to the plus end of the existing polymer.



Source: Courtesy of R.H. Wade, Institut de Biologie Structurale, Grenoble, France.

FIGURE 9.19 Microtubules assembled in the test tube. Electron micrograph of frozen, unfixed microtubules that had polymerized in vitro. The individual protofilaments and their globular tubulin subunits are visible. Note that the middle of the three microtubules contains only 11 protofilaments.



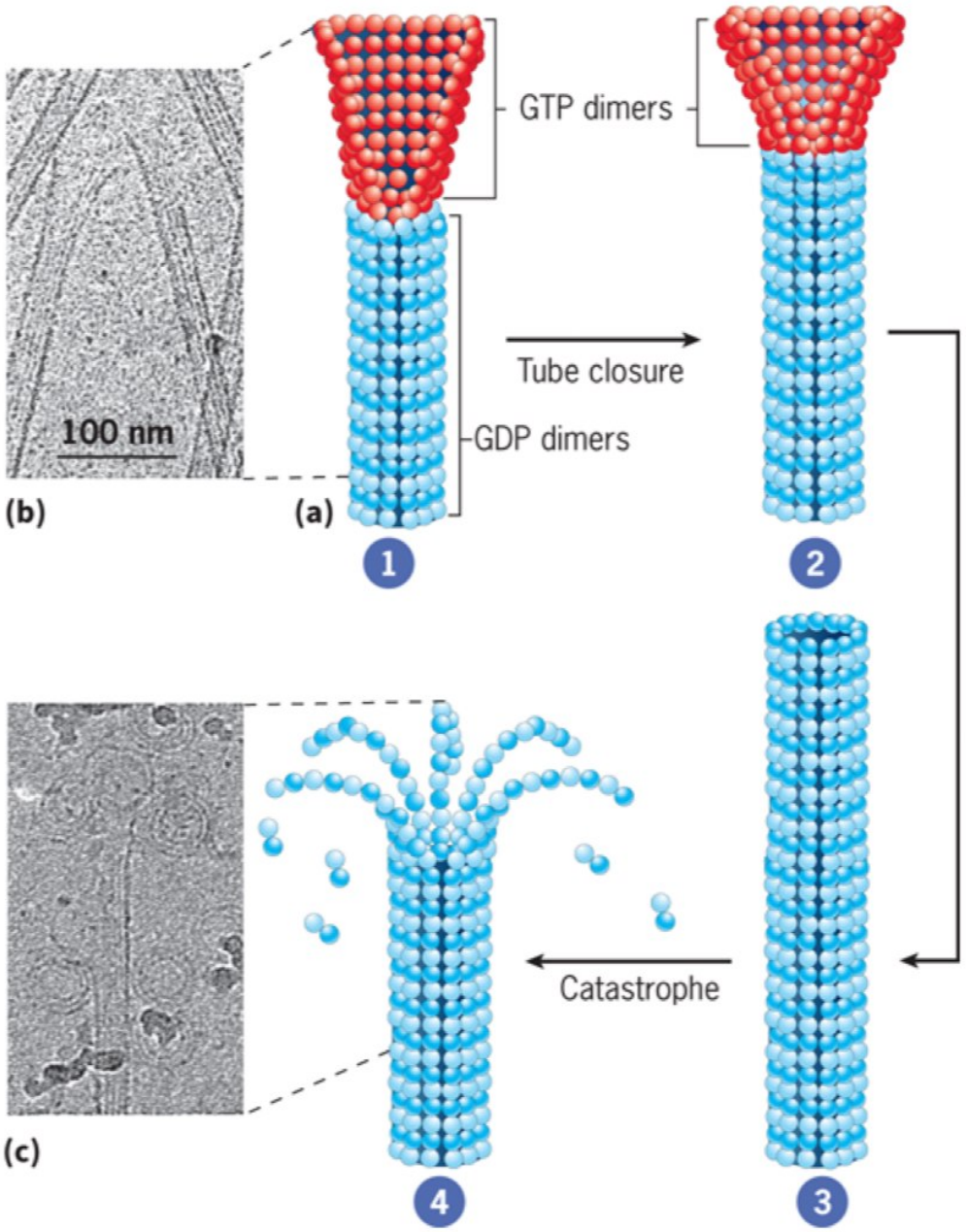
Source: Courtesy of L.I. Binder and Joel L. Rosenbaum.

FIGURE 9.20 The assembly of tubulin onto an existing microtubular structure. Electron micrograph showing the in vitro assembly of brain tubulin onto the plus ends of the microtubules of an axoneme from a *Chlamydomonas* flagellum.

Early in vitro studies established that GTP is required for microtubule assembly. Assembly of tubulin dimers requires that a GTP molecule be bound to the β-tubulin subunit.[2] As it turns out, β-tubulin is not only a structural protein, it is also an enzyme, a GTPase. GTP hydrolysis is not required for the actual incorporation of the dimer onto the end of a microtubule. Rather, the GTP is hydrolyzed to GDP shortly after the dimer is incorporated into a microtubule, and the resulting GDP remains bound to the assembled polymer. After a dimer is released from a microtubule during disassembly and enters the soluble pool, the GDP is replaced by a new GTP. This nucleotide exchange "recharges" the dimer, allowing it to serve once again as a building block for polymerization. Because it includes GTP hydrolysis, assembly of a microtubule is not an inexpensive cellular activity. Why has such a costly polymerization pathway evolved? To answer this question, it is useful to consider the effect of GTP hydrolysis on microtubule structure. When a microtubule is growing, the plus end is seen under the electron microscope as an open sheet to which GTP dimers are added (step 1, **Figure 9.21a, b**). During times of rapid microtubule growth, tubulin dimers are added more rapidly than their GTP can be hydrolyzed. The presence of a cap of tubulin-GTP dimers at the plus ends of the protofilaments is thought to favor the addition of more subunits and the

growth of the microtubule. However, microtubules with open ends, as in step 1, Figure 9.21*a*, are thought to undergo a spontaneous reaction that leads to closure of the tube (steps 2 and 3). In this model, tube closure is accompanied by the hydrolysis of the bound GTP, which generates subunits that contain GDP-bound tubulin. GDP-tubulin subunits have a different conformation from their GTP-tubulin precursors and are less suited to fit into the microtubule lattice. The resulting mechanical strain destabilizes the microtubule. The strain energy is released as the protofilaments curl outward from the plus end of the tubule and undergo catastrophic depolymerization (Figure 9.21*a*, step 4; 9.21*c*). Thus, it would appear that GTP hydrolysis is a fundamental component of the dynamic quality of microtubules. The strain energy stored in a microtubule as a result of GTP hydrolysis makes the microtubule inherently unstable and—in the absence of other stabilizing factors such as MAPs—capable of disassembling soon after its formation. Microtubules can shrink remarkably fast, especially in vivo, which allows a cell to disassemble its microtubular cytoskeleton very rapidly.

Recent cryo-electron microscopy (cryo-EM) data have suggested that even growing ends of the MT have curved protofilaments, implying that both GDP- and GTP-bound tubulins may tend to have a curved conformation, which is straightened out by lateral interactions in the microtubule lattice. If this result holds up, it will change the visual image of Figure 9.21 but will not alter the fundamental idea of lateral interactions stabilizing protofilaments in a straight conformation. The question becomes how GTP hydrolysis affects stability. Recent detailed cryo-EM structures suggest that the difference may lie in subtle conformation differences that affect how GTP- versus GDP-bound tubulins interact with neighboring tubulins in the lattice.


GTP dimers
Tube closure
GDP dimers
100 nm
(b)
(a)
1
2
Catastrophe
(c)
4
3

Source: (*a*) Adapted from A.A. Hyman and E. Karsenti, *Cell* 84:402, 1996, by Cell by Cell Press; (*b, c*) Courtesy of Thomas Mueller-Reichert, T.U. Dresden, Medical Theoretical Center (MTZ) and Anthony A. Hyman, Max Planck Institute of Molecular Cell Biology and Genetics.

FIGURE 9.21 The structural cap model of dynamic instability. According to the model, the growth or shrinkage of a microtubule depends on the state of the tubulin dimers at the plus end of the microtubule. Tubulin-GTP dimers are depicted in red. Tubulin-GDP dimers are depicted in blue. In a growing microtubule (step 1), the tip consists of an open sheet containing tubulin-GTP subunits. In step 2, the tube has begun to close, forcing the hydrolysis of the bound GTP. In step 3, the tube has closed to its end, leaving only tubulin-GDP subunits. GDP-tubulin subunits have a curved conformation compared to their GTP-bound counterparts, which makes them less able to fit into a the microtubule lattice. The strain resulting from the presence of GDP-tubulin subunits at the plus end of the microtubule is released as the protofilaments curl outward from the tubule and undergo catastrophic shrinkage (step 4). (b) Cryo-electron microscopy (Cryo-EM) image of the growing end of a microtubule showing a curved, open sheet. (c) Cryo-EM image of the shrinking end of a microtubule showing outwardly curved protofilaments.

The dynamic character of the microtubular cytoskeleton inside a cell can be revealed by expressing GFP-tagged tubulin in living cells or injecting tubulin with chemical tags that allow it to be visualized in fixed cells (**Figure 9.22**). If individual microtubules are observed under a fluorescence microscope, they appear to grow slowly for a period of time and then shrink rapidly and unexpectedly, as illustrated by the microtubule being followed in **Figure 9.23**. Because the switch from growth to shrinkage (an event termed *catastrophe*) occurs with high frequency in vivo, most microtubules disappear from the cell in a matter of minutes and are replaced by new microtubules that grow out from the centrosome. If particularly small amounts of fluorescently labeled protein are injected, the cytoskeletal filaments are no longer uniformly labeled as in Figure 9.5 but instead contain irregularly spaced fluorescent speckles as seen in Figure 9.23. These fluorescent speckles serve as fixed markers to follow dynamic changes in length or orientation of the filament. This technique, known as flourescence speckle microscopy, allows microtubule dynamics to be measured with high precision inside living cells.

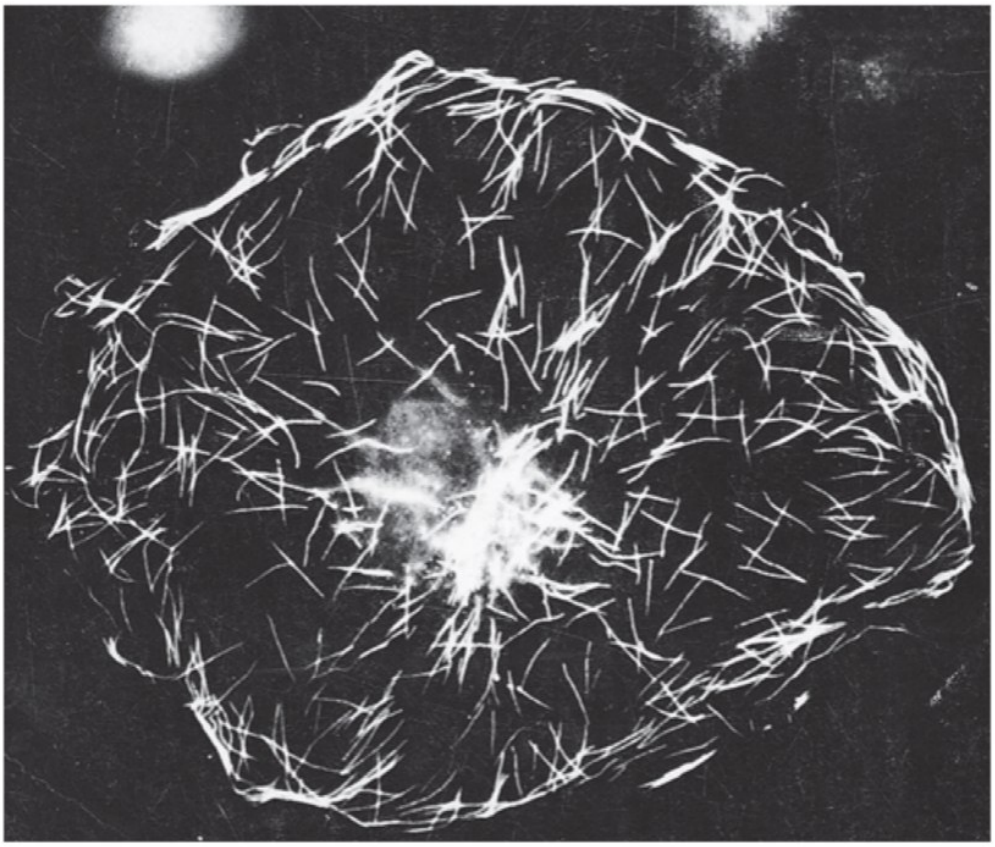


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FIGURE 9.22 Microtubule dynamics in living cells. This cultured fibroblast was injected with a small volume of tubulin that had been covalently linked to biotin; the location in the cell of this small molecule is readily determined using fluorescent anti-biotin antibodies. Approximately one minute following injection, the cells were fixed, and the location of biotinylated tubulin that had been incorporated into insoluble microtubules was determined. It is evident from this fluorescence micrograph that, even during periods as short as one minute, tubulin subunits are widely incorporated at the growing ends of cytoskeletal microtubules.

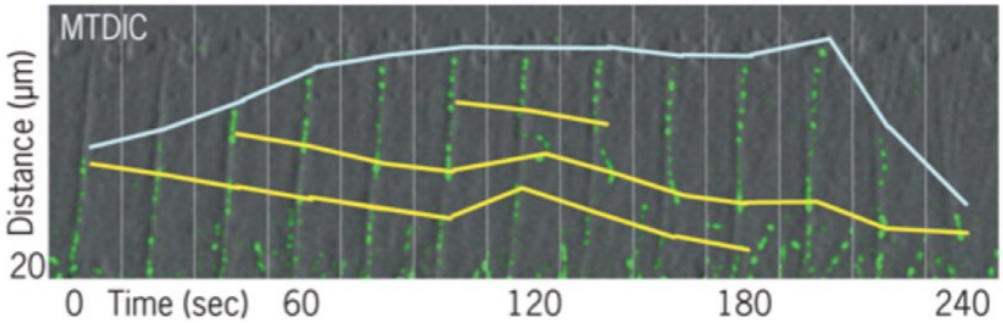

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FIGURE 9.23 Dynamic instability. This series of photographs shows the changes in length of a single microtubule in the growth cone of a neuron. The cell was microinjected with fluorescently labeled tubulin at sufficiently low concentration to produce green fluorescent speckles along the length of microtubules. Such speckles provide fixed reference points that can be followed over time. Each of the yellow horizontal lines connects one of these speckles from one time point to another. The blue line indicates the approximate plus end of the microtubule at various time points. From 0 to about 200 seconds, the microtubule undergoes a gradual addition of tubulin subunits at its plus end. Then, from about 200 to 240 seconds, the microtubule experiences rapid shrinkage.

In 1984, Timothy Mitchison and Marc Kirschner of the University of California, San Francisco, reported on the properties of individual microtubules and proposed that microtubule behavior in vivo could be explained by a phenomenon they termed **dynamic instability**. Dynamic instability explains the observation (1) that growing and shrinking microtubules can coexist in the same region of a cell and (2) that a given microtubule can switch back and forth unpredictably (*stochastically*) between growing and shortening phases, as in Figure 9.23. Dynamic instability is an inherent property of the microtuble itself and, more specifically, of the plus end of the microtubule. As indicated in Figure 9.20, it is the plus end where subunits are added during growth and lost during shrinkage. This does not mean that dynamic instability cannot be influenced by external factors. For example, cells contain a diverse array of proteins [called microtubule plus-end tracking proteins (+TIPs)] that bind to the dynamic plus ends of microtubules (**Figure 9.24**). Some of these +TIPs regulate the rate of the microtubule's growth or shrinkage or the frequency of interconversion between the two phases. Other +TIPs mediate the attachment of the plus end of the microtubule to a specific cellular structure, such as the kinetochore of a mitotic chromosome during cell division or the actin cytoskeleton of the cortex during vesicle transport. Once a subcellular structure is attached to the plus end of a microtubule, the dynamic properties of the microtubule can exert force on that structure. Polymerization of a microtubule can push on an attached object, whereas depolymerization of a microtubule can pull on an attached object. For instance, this type of pulling force plays a major role in the segregation of chromosomes during cell division (Section 14.1).

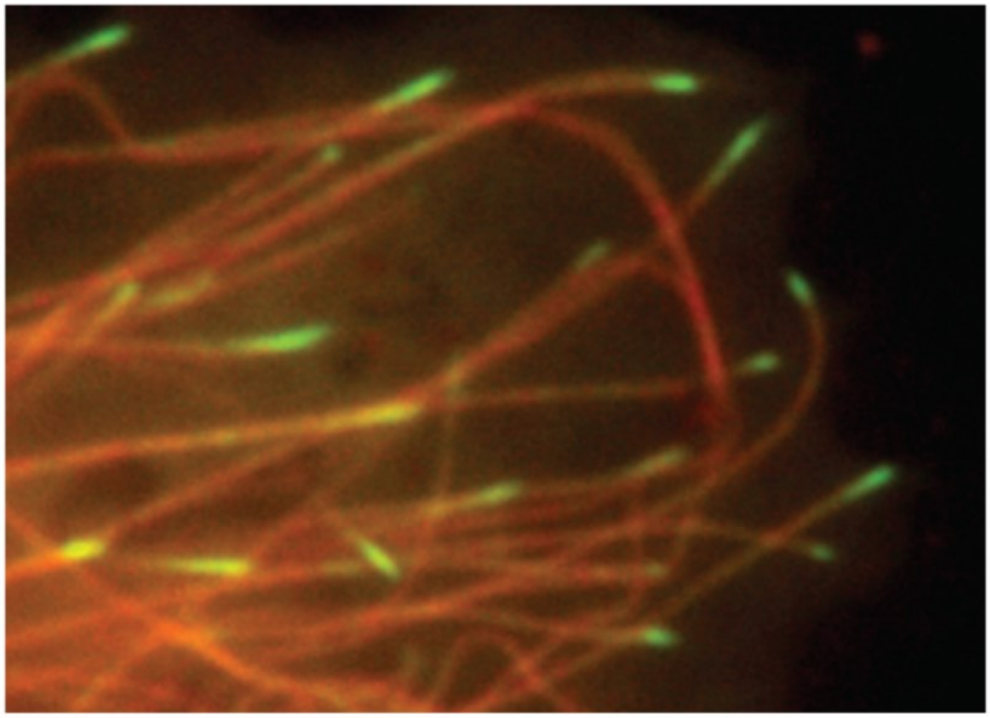

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FIGURE 9.24 Binding of a microtubule plus-end tracking protein (+TIP) The micrograph shows a live image of a human lung fibroblast expressing mCherry-labeled tubulin (generating red microtubules) and GFP-labeled EB3, a +TIP (appearing green). EB3 is seen to bind to the plus ends of the microtubules.

Dynamic instability provides a mechanism by which the plus ends of microtubules can rapidly explore the cytoplasm for appropriate sites of attachment. Attachment temporarily stabilizes microtubules and allows the cell to build the complex cytoskeletal networks discussed in this chapter. Dynamic instability also allows cells to respond rapidly to changing conditions that require remodeling of the microtubular cytoskeleton. One of the most dramatic examples of such remodeling occurs during mitosis, when the microtubules of the cytoskeleton are disassembled and remodeled into a bipolar mitotic spindle. This reorganization is associated with a marked change in microtubule stability; microtubules in interphase cells have half-lives that are 5–10 times longer than microtubules in mitotic cells. Unlike the microtubules of the mitotic spindle or cytoskeleton, the microtubules of the organelles to be discussed in the following sections lack dynamic activity and, instead, are highly stable.

Review

1. What is the centrosome? What component of the centrosome nucleates microtubules and how does it dictate the number of protofilaments?
2. What is the role of GTP in the assembly of microtubules? What is meant by the term *dynamic instability*? What role does it play in cellular activity?

9.7 Intermediate Filaments

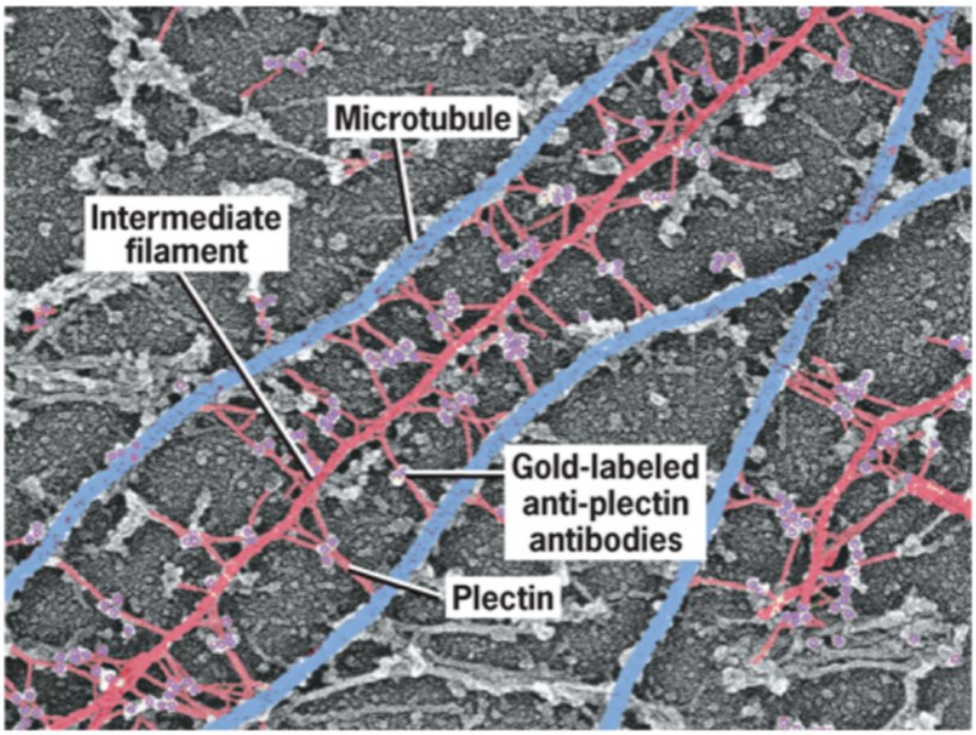
The second of the three major cytoskeletal elements to be discussed was seen in the electron microscope as solid, unbranched filaments with a diameter of 10–12 nm. They were named *intermediate filaments* (or *IFs*; see Section 9.1). To date, intermediate filaments have only been identified in animal cells, although similar insoluble filaments, based on completely different protein sequences, are seen in many other eukaryotes. Intermediate filaments are strong, flexible, ropelike fibers that provide mechanical strength to cells that are subjected to physical stress, including neurons, muscle cells, and the epithelial cells that line the body's cavities. Unlike actin filaments and microtubules, IFs are a chemically heterogeneous group of structures that, in humans, are encoded by approximately 70 different genes. The polypeptide subunits of IFs can be divided into five major classes based on the type of cell in which they are found (**Table 9.2**) as well as biochemical, genetic, and immunologic criteria. We restrict the present discussion to classes I–IV, which are found in the construction of cytoplasmic filaments, and consider type V IFs (the lamins), which are present as part of the inner lining of the nuclear envelope, in Section 12.3. IFs radiate through the cytoplasm of a wide variety of animal cells and are often interconnected to other cytoskeletal filaments by thin, wispy cross-bridges (**Figure 9.35**). In many cells, these cross-bridges consist of an elongated dimeric protein called plectin that can exist in numerous isoforms. Each plectin molecule has a binding site for an intermediate filament at one end and, depending on the isoform, a binding site for another intermediate filament, actin filament, or microtubule at the other end.

TABLE 9.2

Properties and Distribution of the Major Mammalian Intermediate Filament Proteins

More detailed tables can be found in *Trends Biochem Sci.* 31:384, 2006, *Genes and Development* 21:1582, 2007, and *Trends Cell Biol.* 18:29, 2008.

IF protein	Sequence type	Primary tissue distribution
Keratin (acidic) (28 different polypeptides)	I	Epithelia
Keratin (basic) (26 different polypeptides)	II	Epithelia
Vimentin	III	Mesenchymal cells
Desmin	III	Muscle
Glial fibrillary acidic protein (GFAP)	III	Astrocytes
Peripherin	III	Peripheral neurons
Neurofilament proteins		Neurons of central
NF-L	IV	and peripheral
NF-M	IV	nerves
NF-H	IV	
Nestin	IV	Neuroepithelia
Lamin proteins		All cell types
Lamin A	V	(nuclear envelopes)
Lamin B	V	
Lamin C	V	



Source: Courtesy of Tatyana Svitkina and Gary Borisy

FIGURE 9.35 Cytoskeletal elements are connected to one another by protein cross-bridges. Electron micrograph of a replica of a small portion of the cytoskeleton of a fibroblast after selective removal of actin filaments. Individual components have been digitally colorized to assist visualization. Intermediate filaments (red) are seen to be connected to microtubules (blue) by long wispy cross-bridges consisting of the fibrous protein plectin. Plectin is localized by antibodies linked to colloidal gold particles.

Although IF polypeptides have diverse amino acid sequences, all share a similar structural organization that allows them to form similar-looking filaments. Most notably, the polypeptides of IFs all contain a central, rod-shaped, α-helical domain of similar length and homologous amino acid sequence. This long fibrous domain makes the subunits of intermediate filaments very different from the globular tubulin and actin subunits of microtubules and actin filaments. The central fibrous domain is flanked on each side by globular domains of variable size and sequence (step 1, **Figure 9.36**). Two such polypeptides spontaneously interact as their α-helical rods wrap around each other to form a ropelike dimer approximately 45 nm in length (step 2). Because the two polypeptides are aligned parallel to one another in the same orientation, the dimer has polarity, with one end defined by the C-termini of the polypeptides and the opposite end by their N-termini.

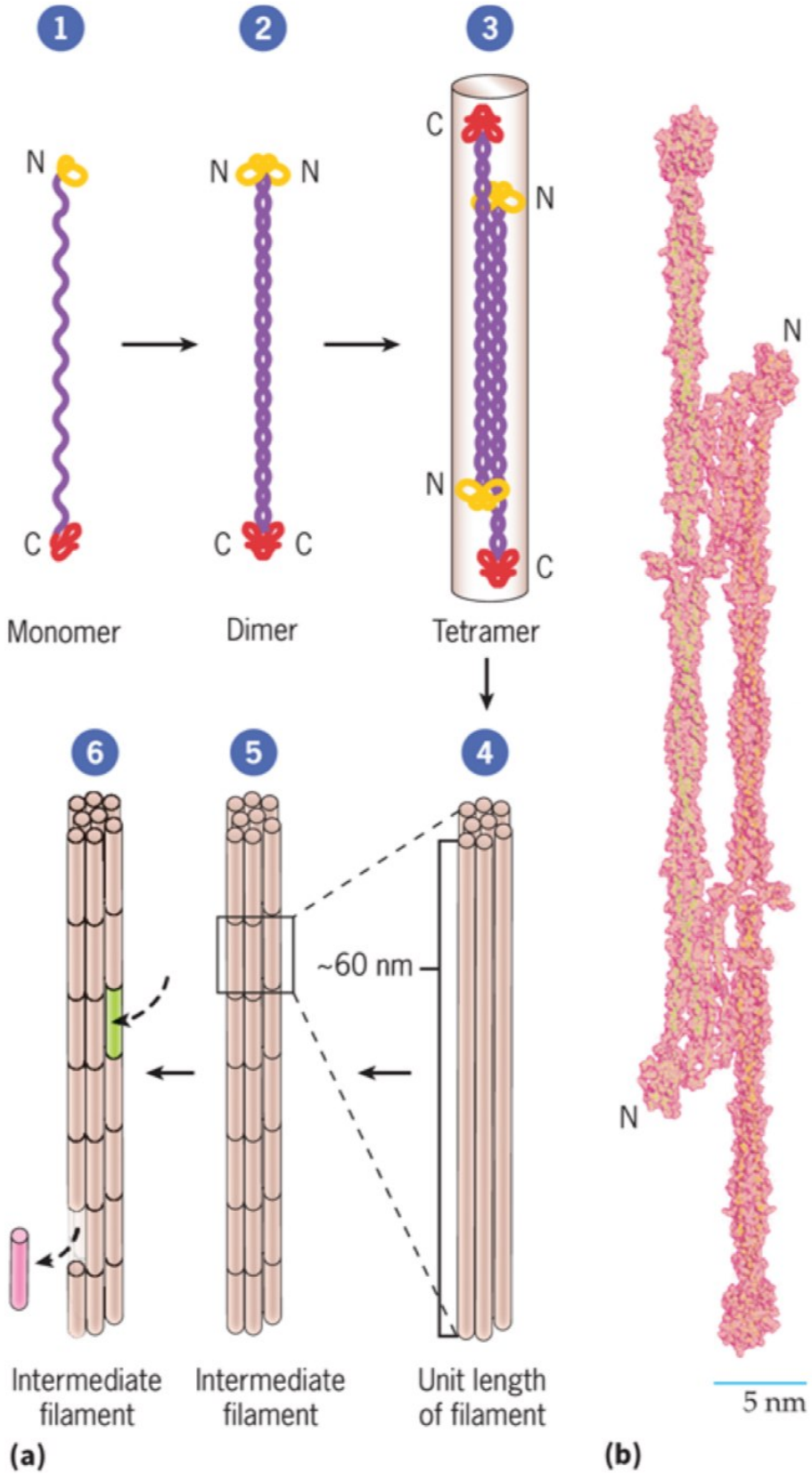
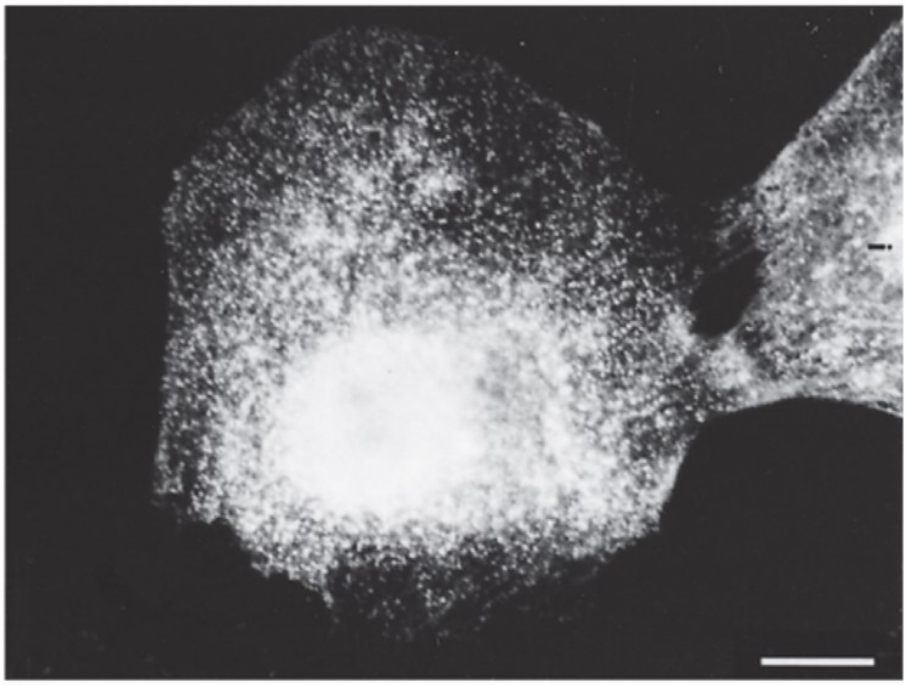

1
2
3
N
N
N
C
C
C
C
N
N
C
Monomer
Dimer
Tetramer
4
5
6
~60 nm
Unit length
of filament
Intermediate
filament
Intermediate
filament
(a)
N
N
5 nm
(b)

FIGURE 9.36 A model of intermediate filament assembly and architecture. (a) Each monomer has a pair of globular terminal domains (red or yellow) separated by a long α-helical region (step 1). Pairs of monomers associate in parallel orientation with their ends aligned to form dimers (step 2). Depending on the type of intermediate filament, the dimers may be composed of identical monomers (*homodimers*) or nonidentical monomers (*heterodimers*). Dimers in turn associate in an antiparallel, staggered fashion to form tetramers (step 3), which are thought to be the basic subunit in the assembly of intermediate filaments. In the model shown here, eight tetramers associate laterally to form a unit length of the intermediate filament (step 4). Highly elongated intermediate filaments are then formed from the end-to-end association of these unit lengths (step 5). Once formed, intermediate filaments undergo a process of dynamic remodeling that is thought to involve the intercalation of unit lengths of filament into the body of an existing filament (green structure is intercalating while pink structure dissociates, step 6). (b) A model of a tetramer of the IF protein vimentin.

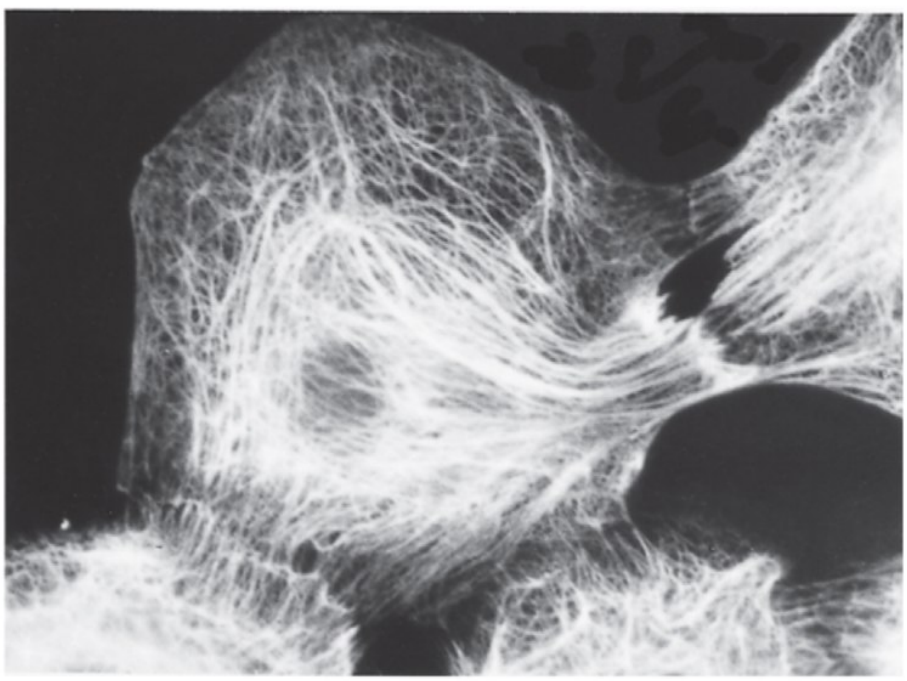
Intermediate Filament Assembly and Disassembly

The basic building block of IF assembly is thought to be a rodlike tetramer formed by two dimers that become aligned side by side in a staggered fashion with their N- and C-termini pointing in opposite (antiparallel) directions, as shown in Figure 9.36*a*, step 3, and in Figure 9.36*b*. Because the dimers point in opposite directions, the tetramer itself lacks polarity. Eight tetramers associate with one another in a side-by-side (lateral) arrangement to form a filament that is one unit in length (about 60 nm) (step 4). Subsequent growth of the polymer is accomplished as these unit lengths of filaments associate with one another in an end-to-end fashion to form the highly elongated intermediate filament (step 5). None of these assembly steps is thought to require the direct involvement of either ATP or GTP. Because the tetrameric building blocks lack polarity, so too does the assembled filament, which is another important feature that distinguishes IFs from other cytoskeletal elements.

Intermediate filaments tend to be less sensitive to chemical agents than other types of cytoskeletal elements and more difficult to solubilize. In fact, treatment of a cell with ionic detergents extracts just about everything inside the cell except the cell's intermediate filaments. Because of their insolubility, IFs were initially thought to be permanent, unchanging structures, so it came as a surprise to find that they behave dynamically in vivo. When labeled keratin subunits are injected into cultured skin cells, they are rapidly incorporated into existing IFs. Surprisingly, the subunits are not incorporated at the ends of the filament, as might have been expected by analogy with microtubule and actin filament assembly, but rather into the filament's interior (**Figure 9.37a**). The results depicted in Figure 9.37 might reflect the exchange of unit lengths of filament directly into an existing IF network (as shown in step 6, Figure 9.36*a*). Unlike the other two major cytoskeletal elements, assembly and disassembly of IFs are controlled primarily by phosphorylation and dephosphorylation of the subunits. For example, phosphorylation of vimentin filaments by protein kinase A leads to their disassembly.



(a)



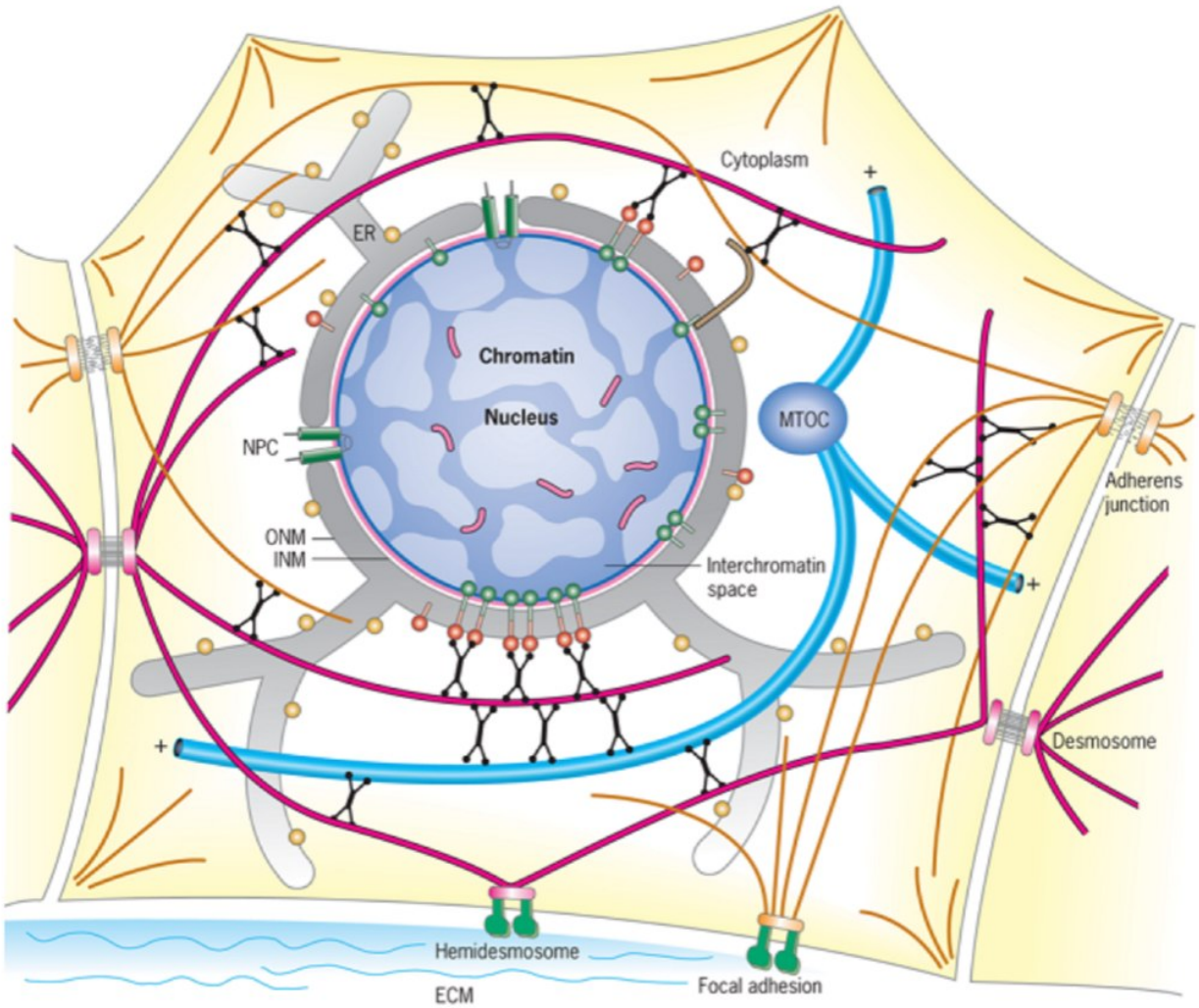
(b)

Source: ©1991 R.K.Miller et al. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.113.4.843

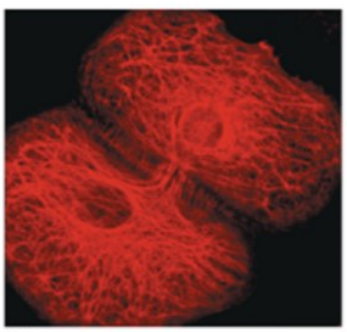
FIGURE 9.37 Experimental demonstration of the dynamic character of intermediate filaments. These photographs show the results of an experiment in which biotin-labeled type I keratin was microinjected into cultured epithelial cells and localized 20 minutes later using immunofluorescence. The photograph in *a* shows the localization of the injected biotinylated keratin (as revealed by fluorescent anti-biotin antibodies) that had become incorporated into filaments during the 20-minute period following injection. The photograph in *b* shows the distribution of intermediate filaments in the cell as revealed by anti-keratin antibodies. The dotlike pattern of fluorescence in *a* indicates that the injected subunits are incorporated into the existing filaments at sites throughout their length, rather than at their ends. (Compare with a similar experiment with labeled tubulin in Figure 9.22.) Bar, 10 μm.

Types and Functions of Intermediate Filaments

Keratin filaments constitute the primary structural proteins of epithelial cells (including epidermal cells, liver hepatocytes, and pancreatic acinar cells). **Figure 9.38a** shows a schematic view of the spatial arrangement of the keratin filaments of a generalized epithelial cell, and Figure 9.38*b* shows the actual arrangement within a pair of cultured epidermal cells. Keratin-containing IFs radiate through the cytoplasm, tethered to the nuclear envelope in the center of the cell and anchored at the outer edge of the cell by connections to the cytoplasmic plaques of desmosomes and hemidesmosomes (Section 7.4). Figure 9.38*a* also depicts the interconnections between IFs and the cell's microtubules and actin filaments, which transforms these otherwise separate elements into an integrated cytoskeleton. Because of these various physical connections, the IF network is able to serve as a scaffold for organizing and maintaining the cellular architecture and for absorbing the mechanical stresses applied by the extracellular environment.


Cytoplasm
ER
Chromatin
Nucleus
MTOC
NPC
ONM
INM
Interchromatin space
Adherens junction
Desmosome
Hemidesmosome
ECM
Focal adhesion
Protein of outer nuclear membrane (ONM)
Protein of inner nuclear membrane (INM)
Plakin-type cross-bridging molecule (e.g., plectin)
Nesprin
IF-anchoring plaques
Actin-anchoring plaques
Ribosome
AFs
IFs
MTs
Integrins
Lamins
ONM Outer nuclear membrane
INM Inner nuclear membrane
ER Endoplasmic reticulum
NPC Nuclear pore complex
ECM Extracellular matrix

(a)



(b)

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FIGURE 9.38 The organization of intermediate filaments (IFs) within an epithelial cell. (a) In this schematic drawing, IFs, shown in red, are seen to radiate throughout the cell, being anchored at both the outer surface of the nucleus and the inner surface of the plasma membrane. Connections to the nucleus are made via proteins that span both membranes of the nuclear envelope and to the plasma membrane via specialized sites of adhesion such as desmosomes and hemidesmosomes. IFs are also seen to be interconnected to both of the other types of cytoskeletal fibers. Connections to microtubules (MTs) and actin filaments (AFs) are made primarily by members of the plakin family of proteins, such as the dimeric plectin molecule shown in Figure 9.35. (b) Distribution of keratin-containing IFs in cultured skin cells (keratinocytes). The filaments are seen to form a cagelike network around the nucleus (which appears as a circular dark region towards the center of the cell) and also extend to the cell periphery.

The cytoplasm of neurons contains loosely packed bundles of intermediate filaments with long axes oriented parallel to that of the axon (see Figure 9.7*b*). These IFs, or **neurofilaments (NFs)**, as they are called, are composed of three distinct proteins: NF-L, NF-H, and NF-M, all of the type IV group of Table 9.2. Unlike the polypeptides of other IFs, NF-H and NF-M have sidearms that project outward from the neurofilament. These sidearms are thought to maintain the proper spacing between the parallel neurofilaments of the axon (see Figure 9.7*b*). In the early stages of differentiation when the axon is growing toward a target cell, it contains very few neurofilaments but large numbers of supporting microtubules. Once the nerve cell has become fully extended, it becomes filled with neurofilaments that provide support as the axon increases dramatically in diameter. Aggregation of NFs is seen in several human neurodegenerative disorders, including ALS and Parkinson's disease. These NF aggregates may block axonal transport, leading to the death of neurons.

Efforts to probe IF function have relied largely on genetically engineered mice that fail to produce a particular IF polypeptide (a gene knockout) or produce an altered IF polypeptide. These studies have revealed the importance of intermediate filaments in particular cell types. For example, mice carrying deletions in the gene encoding K14, a type I keratin polypeptide normally synthesized by cells of the basal epidermal layer, have serious health problems. These mice are so sensitive to mechanical pressure that even mild trauma, such as that occurring during passage through the birth canal or during nursing by the newborn, can cause severe blistering of the skin or tongue. This phenotype bears strong resemblance to a rare skin-blistering disease in humans, called epidermolysis bullosa simplex (EBS).[3] Subsequent analysis of EBS patients has shown that they carry mutations in the gene that encodes the homologous K14 polypeptide (or the K5 polypeptide, which forms dimers with K14). These studies confirm the role of IFs in imparting mechanical strength to cells situated in epithelial layers. Similarly, knockout mice that fail to produce the desmin polypeptide exhibit serious cardiac and skeletal muscle abnormalities. Desmin plays a key structural role in maintaining the alignment of the myofibrils of a muscle cell, and the absence of these IFs makes the cells extremely fragile. An inherited human disease, named desmin-related myopathy, is caused by mutations in the gene that encodes desmin. Persons with this disorder suffer from skeletal muscle weakness, cardiac arrhythmias, and eventual congestive heart failure. Not all IF polypeptides have such essential functions. For example, mice that lack the vimentin gene, which is expressed in fibroblasts, macrophages, and white blood cells, show relatively minor abnormalities, even though the affected cells lack cytoplasmic IFs. It is evident from these studies that IFs have tissue-specific functions, which are more important in some cells than in others.

Review

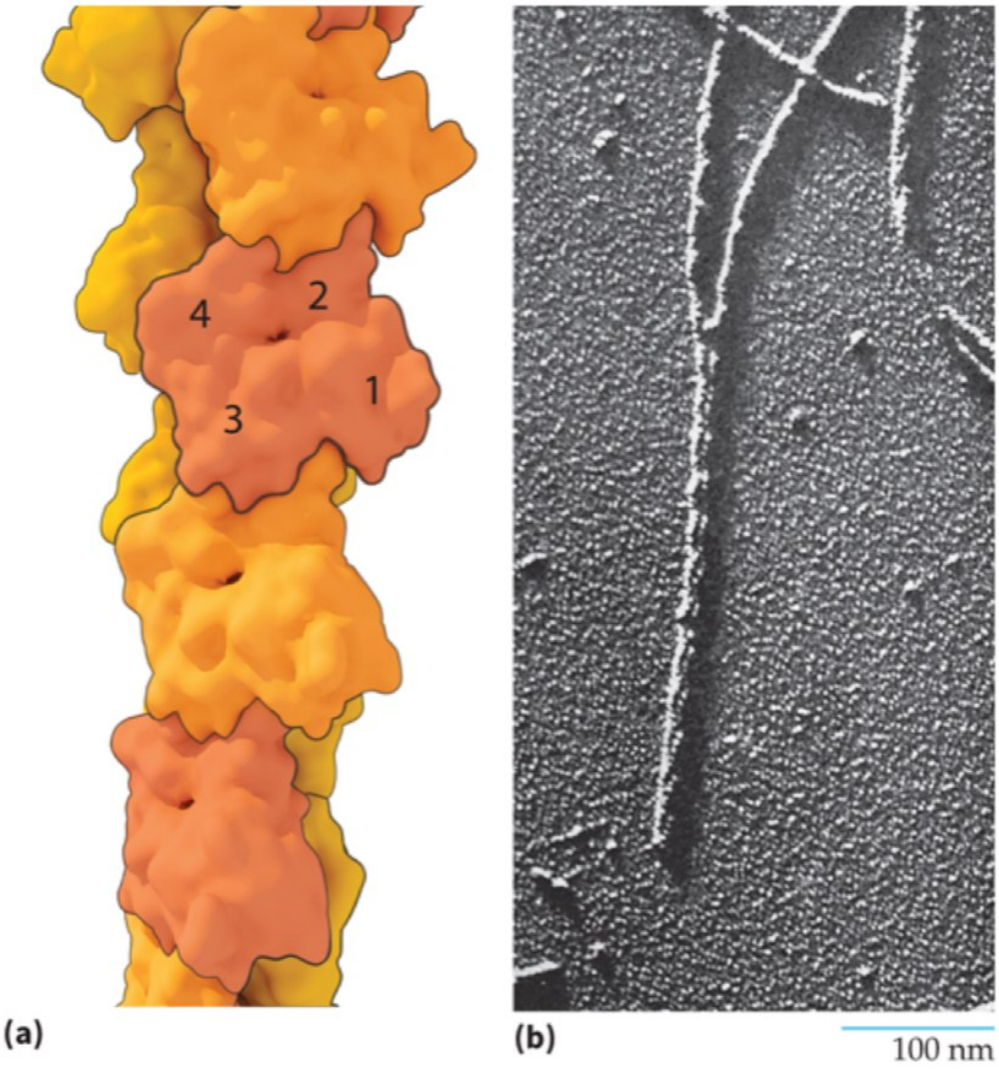
1. Give some examples that reinforce the suggestion that intermediate filaments are important primarily in tissue-specific functions rather than in basic activities that are common to all cells.
2. Compare and contrast microtubule assembly and intermediate filament assembly.

9.8 Actin and Myosin

Cells are capable of remarkable motility. The neural crest cells in a vertebrate embryo leave the developing nervous system and migrate across the entire width of the embryo, forming such diverse products as the pigment cells of the skin, teeth, and cartilage of the jaws (see Figure 7.11). Legions of white blood cells patrol the tissues of the body searching for debris and microorganisms. Certain parts of cells can also be motile; broad projections of epithelial cells at the edge of a wound act as motile devices that pull the sheet of cells over the damaged area, sealing the wound. Similarly, the leading edge of a growing axon sends out microscopic processes that survey the substratum and guide the cell toward a synaptic target. All of these various examples of motility share at least one component: They all depend on actin, the third major type of cytoskeletal element. **Actin** is also involved in intracellular motile processes, such as the movement of vesicles, phagocytosis, and cytokinesis. In fact, plant cells rely primarily on actin, rather than microtubules, to serve as tracks for the long-distance transport of cytoplasmic vesicles and organelles. This bias toward actin-based motility reflects the rather restricted distribution of microtubules in many plant cells (see Figure 9.6). Actin also plays an important role in determining the shapes of cells and can provide structural support for various types of cellular projections (as in Figure 9.69).

Actin Structure

Actin filaments are approximately 8 nm in diameter and composed of globular subunits of the protein actin, which is the most abundant protein in most cells. In the presence of ATP, actin monomers polymerize to form a flexible, helical filament. As a result of its subunit organization (**Figure 9.39a**), an actin filament is essentially a two-stranded structure with two helical grooves running along its length (Figure 9.39*b*). The terms *actin filament*, *F-actin*, and *microfilament* are basically synonyms for this type of filament. Depending on the type of cell and the activity in which it is engaged, actin filaments can be organized into ordered arrays, highly branched networks, or tightly anchored bundles.



Source: (*b*) From Robert H. Depue, Jr. and Robert V. Rice, *J. Mol. Biol.* 12:302, 1965. Reproduced with permission of Elsevier.

FIGURE 9.39 Actin filament structure. (a) Three-dimensional structure of an actin filament. The subdomains in one of the actin subunits are labeled 1, 2, 3, and 4, and the ATP-binding cleft in each subunit is evident. Actin filaments have polarity, with a plus and minus end. In this illustration, the filament's plus end is pointed towards the bottom of this page, and the minus end is pointed towards the top. (b) Electron micrograph of a replica of an actin filament showing its double-helical architecture.

All of the monomers within an actin filament are pointed in the same direction, resulting in a polar filament with so-called barbed and pointed ends. Note that the barbed end is also called the plus end, and the pointed end as the minus end of the actin filament. This naming convention originated from a technique used to identify and label actin filaments in preparation for electron microscopy. This method utilized the ability of a proteolytic fragment of myosin, called S1 (see Figure 9.43), to bind tightly and "decorate" the sides of actin filaments. When S1 fragments are bound, one end of the actin filament appears pointed like an arrowhead (the "pointed" end), while the other end looks barbed. An example of this arrowhead "decoration" is shown in the microvilli of intestinal epithelial cells of **Figure 9.40**. Using light microscopy, actin can be readily identified and localized using fluorescently

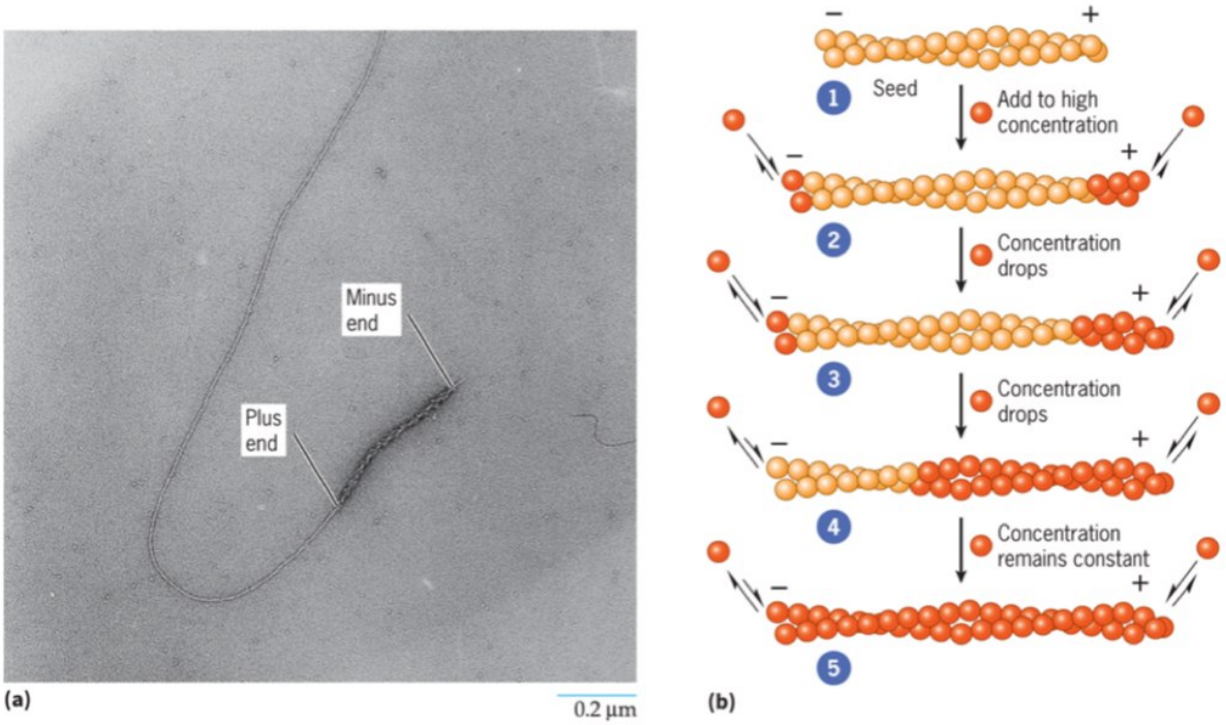
labeled phalloidin (Figure 9.68*a*), a chemical that binds to the sides of actin filaments; by expressing fluorescently labeled actin (such as GFP actin); or by labeling with anti-actin antibodies.

Actin was identified more than 50 years ago as one of the major contractile proteins of muscle cells. Since then, it has been identified as a major protein in virtually every eukaryotic cell that has been examined. Higher plant and animal species possess a number of actin-coding genes with products specialized for different types of processes and structures. Actins have been conserved remarkably during the evolution of eukaryotes. For example, the amino acid sequences of actin molecules from a yeast cell and from rabbit skeletal muscle are 88 percent identical. In fact, actin molecules from diverse sources can copolymerize to form hybrid filaments. Although actin filaments can generate forces on their own (see Experimental Pathway: Studying Actin-Based Motility Without Cells in Section 9.12), most processes involving actin require the activity of motor proteins, specifically those of the myosin superfamily.

Actin Filament Assembly and Disassembly

Before it is incorporated into a filament, an actin monomer binds a molecule of ATP. Actin is an ATPase, just as tubulin is a GTPase, and the role of ATP in actin assembly is similar to that of GTP in microtubule assembly (Section 9.5). The ATP associated with the actin monomer is hydrolyzed to ADP at some time after it is incorporated into the end of a growing actin filament. As a consequence, the bulk of an actin filament consists of ADP–actin subunits.

Actin polymerization is readily demonstrated in vitro in solutions containing ATP-actin monomers. As in the case of microtubules, the initial nucleation event in filament formation occurs slowly in vitro, whereas the subsequent stage of filament elongation occurs much more rapidly. The slow nucleation stage of filament formation can be bypassed by including preformed actin filaments in the reaction mixture. When preformed actin filaments are incubated with a high concentration of labeled ATP-actin monomers, both ends of the filament become labeled, but the fast-growing barbed end incorporates the monomers at a rate approximately 10 times that of the pointed end (**Figure 9.41a**).



Source: Courtesy of M.S. Runge and T. D. Pollard

FIGURE 9.41 Actin assembly in vitro. (a) Electron micrograph of a short actin filament that was labeled with S1 myosin and then used to nucleate actin polymerization. The addition of actin subunits occurs much more rapidly at the barbed (plus) end than at the pointed (minus) end of the existing filament. (b) Schematic diagram of the kinetics of actin filament assembly in vitro. All of the orange subunits are part of the original seed; red subunits were present in solution at the beginning of the incubation. The steps are described in the text. Once a steady-state concentration of monomers is reached, subunits are added to the plus end at the same rate they are released from the minus end. As a result, subunits treadmill through the filament in vitro. Note: No attempt is made to distinguish between subunits with a bound ATP versus ADP.

Figure 9.41*b* illustrates how the events that occur during actin assembly/disassembly in vitro depend on the concentration of actin monomers and on the elongation dynamics of the filament ends. The barbed and pointed ends require different minimal concentrations of ATP-actin monomers in order to elongate, a measure known as the critical concentration. The critical concentration of the barbed end is much lower than that of the pointed end, meaning that the barbed end can continue to elongate at lower ATP-actin concentrations than the pointed end can. Suppose we were to begin by adding preformed actin filaments (seeds) to a solution of actin in the presence of ATP (step 1). As long as the concentration of ATP-actin monomers remains high, subunits will continue to be added at both ends of the filament (step 2, Figure 9.41*b*). As the monomers in the reaction mixture are consumed by addition to the ends of the filaments, the concentration of free ATP-actin continues to drop until a point is reached where net addition of monomers continues at the barbed end, which has a lower critical concentration of ATP-actin, but stops at the pointed end, which has a higher critical concentration for ATP-actin (step 3). As filament elongation continues, the free monomer concentration drops further. At this point, monomers continue to be added to the barbed ends of the filaments, but a net loss of subunits occurs at their pointed end. As the

free monomer concentration falls, a point is reached where the two reactions at opposite ends of the filaments are balanced so that both the lengths of the filaments and the concentration of free monomers remain constant (step 4). This type of balance between two opposing activities is an example of steady state (Section 3.1) and occurs when the ATP-actin concentration is approximately 0.3 μM. Because subunits are being added to the barbed ends and removed from the pointed ends of each filament at steady state, the relative position of individual subunits within each filament is continually moving—a process known as "treadmilling" (steps 4 and 5). Studies on living cells containing fluorescently labeled actin subunits have demonstrated the occurrence of treadmilling in vivo (see Figure 9.48*c*).

As discussed in Section 9.11, the rate of assembly and disassembly of actin filaments in the cell can be influenced by a number of different accessory proteins. Changes in the local conditions in a particular part of the cell can push events there toward either the assembly or the disassembly of microfilaments. By controlling this dynamic behavior, the cell can reorganize its actin cytoskeleton. Such reorganization is required for dynamic processes such as cell locomotion, changes in cell shape, phagocytosis, and cytokinesis.

As noted previously, actin filaments play a role in nearly all of a cell's motile processes. The involvement of these filaments is most readily demonstrated by treating cells with one of the following drugs that disrupt dynamic actin-based activities: cytochalasin, derived from a mold, which blocks the barbed ends of actin filaments and allows depolymerization at the pointed end; phalloidin, obtained from a poisonous mushroom, which binds to intact actin filaments and prevents their turnover; and latrunculin, obtained from a sponge, which binds to free monomers and blocks their incorporation into the polymer. Actin-mediated processes rapidly grind to a halt when cells contain one of these compounds.

Myosin: The Molecular Motor of Actin

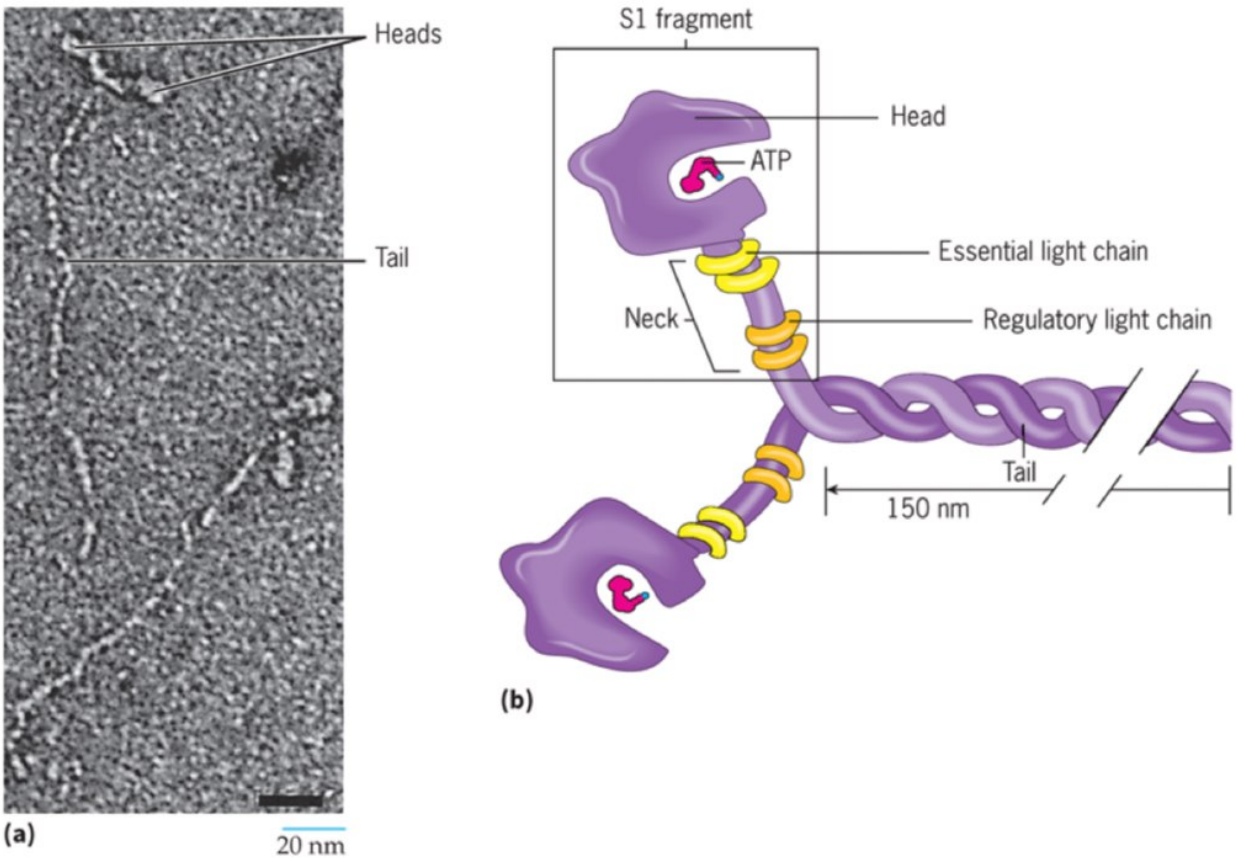
We previously examined the structure and actions of two molecular motors—kinesin and dynein—that operate in opposite directions over tracks of microtubules. To date, all of the motors known to operate in conjunction with actin filaments are members of the myosin superfamily. Myosins—with the major exception of myosin VI, which is discussed below—move toward the barbed end of an actin filament.

Myosin was first isolated from mammalian skeletal muscle tissue and has subsequently been found in virtually all eukaryotic cells. All myosins share a characteristic motor (head) domain. The head contains a site that binds an actin filament and a site that binds and hydrolyzes ATP to drive the myosin motor. Whereas the head domains of various myosins are similar, the tail domains are highly divergent. Myosins also contain a variety of low-molecular-weight (light) chains. They are generally divided into two broad groups: the **conventional (or type II) myosins**, which were first identified in muscle tissue, and the **unconventional myosins**. The unconventional myosins are subdivided on the basis of amino acid sequence into at least 17 different classes (type I and types III–XVIII). Some of these classes are expressed widely among eukaryotes, whereas others are restricted. Myosin X, for example, is found only in vertebrates, and myosins VIII and XI are present only in plants. Humans contain about 40 different myosins from at least 12 classes, each presumed to have its own specialized function(s). Of the various myosins, those in the conventional (type II) class are best understood.

Conventional (Type II) Myosins

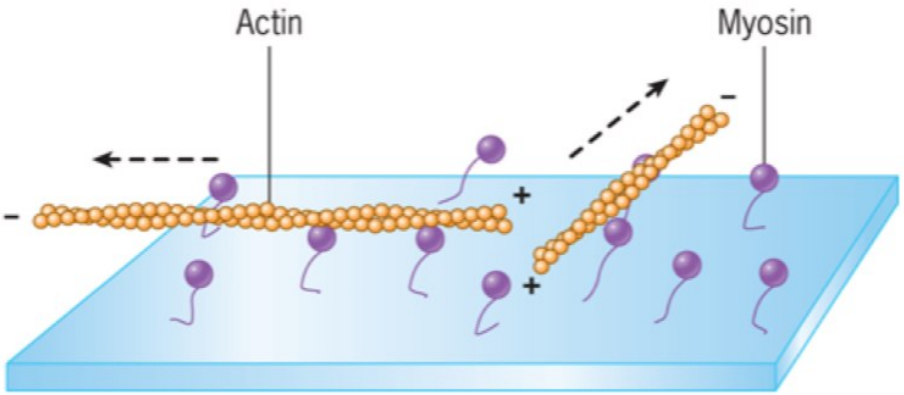
Proteins of the myosin II class are the primary motors for muscle contraction but are also found in a variety of non-muscle cells. The human genome encodes 16 different myosin II heavy chains, 3 of which function in non-muscle cells. All type II myosins move toward the barbed end of an actin filament. Among their non-muscle activities, type II myosins are required for splitting a cell into two during cell division, generating tension at focal adhesions, cell migration, and the turning behavior of growth cones (see Figure 9.70). The effect of inhibiting myosin II activity in an advancing growth cone is demonstrated in **Figure 9.42**.

An electron micrograph of a pair of myosin II molecules is shown in **Figure 9.43a**. Each myosin II molecule is composed of six polypeptide chains—one pair of heavy chains and two pairs of light chains—organized in such a way as to produce a highly asymmetric protein (Figure 9.43*a*). Examination of the molecule in Figure 9.43*b* shows that it consists of (1) a pair of globular heads that contain the catalytic site of the molecule; (2) a pair of necks, each consisting of a single, uninterrupted α-helix and two associated light chains; and (3) a single, long, rod-shaped tail formed by the intertwining of long α-helical sections of the two heavy chains. Isolated myosin heads (S1 fragments of Figure 9.43*b*) that have been immobilized on the surface of a glass coverslip are capable of sliding attached actin filaments in an in vitro assay such as that shown in **Figure 9.44**. Thus, all of the machinery required for motor activity is contained in a single head. The mechanism of action of the myosin head and the key role played by the neck are discussed in Figure 9.54. The fibrous tail portion of a myosin II molecule plays a structural role, allowing the protein to form filaments. Myosin II molecules assemble so that the ends of the tails point toward the center of the filament and the globular heads point away from the center (**Figures 9.45** and 9.51). As a result, the filament is described as bipolar, indicating a reversal of polarity at the filament's center. Because they are bipolar, the myosin heads at the opposite ends of a myosin filament have the ability to pull actin filaments toward one another, as occurs in a muscle cell. As described in the following section, some myosin II filaments assemble in transient construction, assembling when and where they are needed and then disassembling after they have acted.



Source: (*a*) ©1997 S.A. Burgess et al. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.139.3.675

FIGURE 9.43 Structure of a myosin II molecule. (a) Electron micrograph of negatively stained myosin molecules. The two heads and tail of each molecule are clearly visible. (b) A highly schematic drawing of a myosin II molecule (molecular mass of 520,000 Da). The molecule consists of one pair of heavy chains (purple) and two pairs of light chains, which are named as indicated. The paired heavy chains consist of a rod-shaped tail in which portions of the two polypeptide chains wrap around one another to form a coiled coil and a pair of globular heads. When treated with a protease under mild conditions, the molecule is cleaved at the junction between the neck and the tail, which generates the S1 fragment.

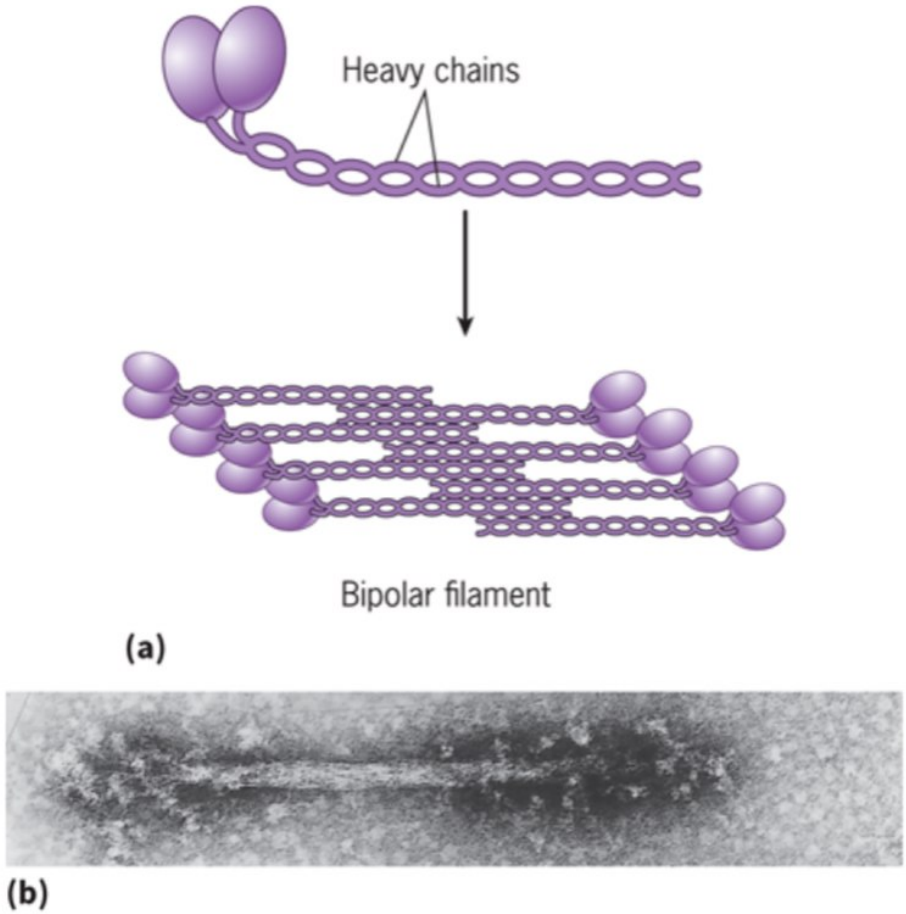


(a)

(b)

Source: (*b*) Based on work by T. Yanagida.

FIGURE 9.44 In vitro motility assay for myosin. (a) Schematic drawing in which myosin heads are bound to a silicone-coated coverslip, which is then incubated with a preparation of actin filaments. (b) Results of the experiment depicted in *a*. Two images were taken 1.5 seconds apart and photographed as a double exposure on the same frame of film. The dashed lines with arrowheads show the sliding movement of the actin filaments over the myosin heads during the brief period between exposures.



Source: (*b*) Courtesy Hugh Huxley

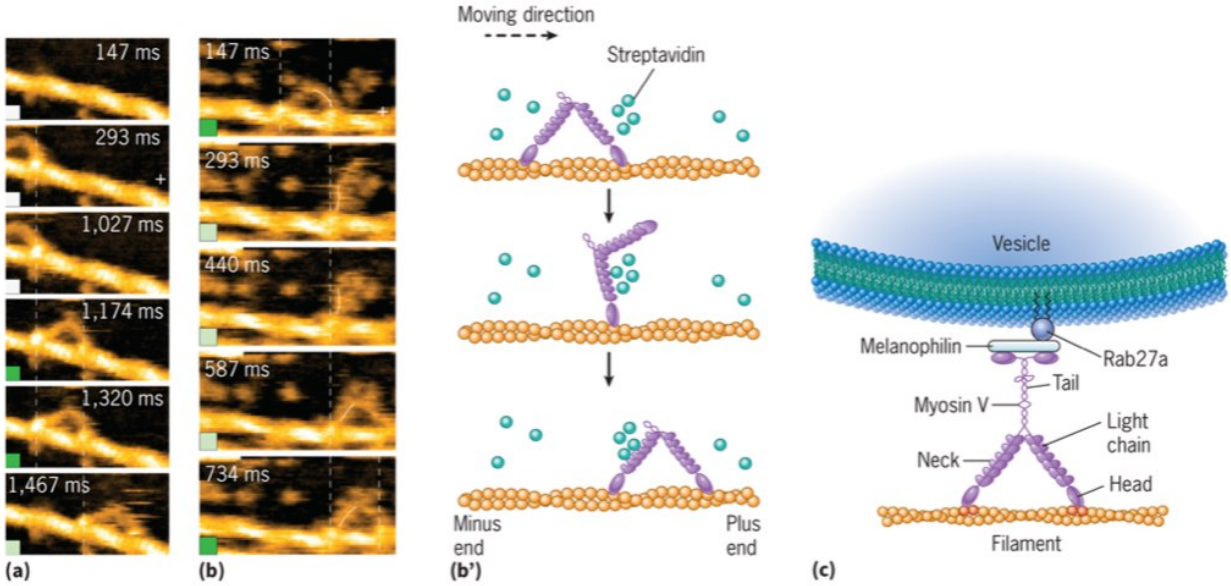
FIGURE 9.45 Structure of a bipolar myosin II filament. (a) Schematic diagram of the staggered arrangement of the individual myosin molecules in a myosin II filament. (b) Electron micrograph of a bipolar myosin filament formed in vitro. The heads of the filament are seen at both ends, leaving a smooth section in the center of the filament.

Unconventional Myosins

In 1973, Thomas Pollard and Edward Korn of the National Institutes of Health described a unique myosin-like protein that was extracted from the protist *Acanthamoeba*. Unlike muscle myosin, this smaller unconventional myosin had only a single head and was unable to assemble into filaments in vitro; the protein became known as myosin I. As reflected in the drawing of a microvillus in Figure 9.61, myosin I often serves as a cross-link between actin filaments of the cytoskeleton and the lipid bilayer of the plasma membrane. It has been suggested that myosin I can exert tension on the plasma membrane, which could play a role in processes that require movement or deformation of the membrane.

None of the unconventional myosins are capable of filament formation and instead appear to operate primarily as individual protein molecules. The best-studied unconventional myosins are capable of moving processively along actin filaments in a manner analogous to the way that kinesins and cytoplasmic dynein move along microtubules. The steps taken by one of these myosins—myosin V—have been revealed in a remarkable series of atomic force micrographs that capture a single molecule as it moves rapidly along an actin filament in vitro (**Figure 9.46a**). In order to visualize the various steps in the protein's mechanical

cycle, these researchers placed molecular obstacles in the path of the moving motor protein, which slowed the rate at which the protein was able to travel. Figure 9.46*b* shows a series of images that reveal the movements of the molecule during a single mechanical cycle. (Keep in mind that myosin V normally has a sizable tail segment, as depicted in Figure 9.46*c*, that has been removed for these particular experiments.)



Source: (*a, b*) Reprinted by permission from Springer Nature: Noriyuki Kodera et al. *Nature* 468, pages 72–76, 2010

FIGURE 9.46 Myosin V—a two-headed unconventional myosin involved in organelle transport. (a) Direct visualization of a single myosin V molecule (one that is lacking its normal tail domain) as it moves along an actin filament in vitro in the presence of ATP. Using high-speed atomic force microscopy (HS-AFM), this series of images shows the movement of the molecule over a period of about one second. (b) Succesive HS-AFM images showing the hand-over-hand movement of a single myosin V molecule as it passes through a cluster of obstacles (consisting of streptavidin protein molecules). The swinging neck (or lever arm) is highlighted with a thin white line. Interpretive drawings (*b'*) depict the motor protein as seen in several of the corresponding HS-AFM images. (c) Schematic drawing of a complete dimeric myosin V molecule, including its numerous light chains, with both heads bound to an actin filament and its tail domain bound to a vesicle. Rab27a and melanophilin serve as adaptors that link the globular ends of the tail to the vesicle membrane. The long neck of myosin V binds six light chains.

In the first frame of Figure 9.46*b*, both heads of the dimeric protein are seen to be attached to the underlying filament, and a cluster of obstacles are seen in the path of the motor. In the second frame, the trailing head of the myosin V molecule has detached from the filament and is in the process of moving forward through the roadblocks in its path. By the fourth frame, this swinging head has made contact with the filament at a forward position along the filament and thus has become the new leading head. These images provide visual confirmation that myosin V moves in a hand-over-hand fashion similar to that of the kinesin molecule in Figure 9.11*b*. To accomplish this type of movement, at least one of the two heads must be bound to its polarized track at all times. Figure 9.46*c* shows an illustration of an

intact myosin V molecule. As seen in this illustration, myosin V is noteworthy for the length of each neck, which at 23 nm is about three times that of myosin II.

Because of its long necks, which act as a swinging arm (or lever) during movement, myosin V can take very large steps. This is very important for a motor protein that moves processively along an actin filament made up of helical strands of subunits. The actin helix repeats itself about every 13 subunits (36 nm), which is just about the step size of a myosin V molecule (Figure 9.46*b*). To accomplish this type of movement, each myosin head must move a distance of 72 nm, twice the distance between two successive binding sites on the actin filament (Figure 9.46*b*). As a result of its giant strides, myosin V can walk along the filament in a straight path even though the underlying "roadway" spirals 360 degrees between its "feet." Several unconventional myosins (including myosins I, V, and VI) are associated with various types of cytoplasmic vesicles and organelles. In some cases, these myosins may act primarily as organelle tethers and in other cases as organelle transporters. Some vesicles have been shown to contain microtubule-based motors (kinesins and/or cytoplasmic dynein) and microfilament-based motors (unconventional myosins). In fact, the two types of motors may be physically linked to one another. The movement of vesicles and other membranous carriers over long distances within animal cells occurs on microtubules, as previously described. However, once they approach the end of the microtubule, these membranous vesicles are often thought to switch over to the microfilament tracks for local movement through the actin-rich periphery of the cell, which is mediated by myosins (**Figure 9.47**).

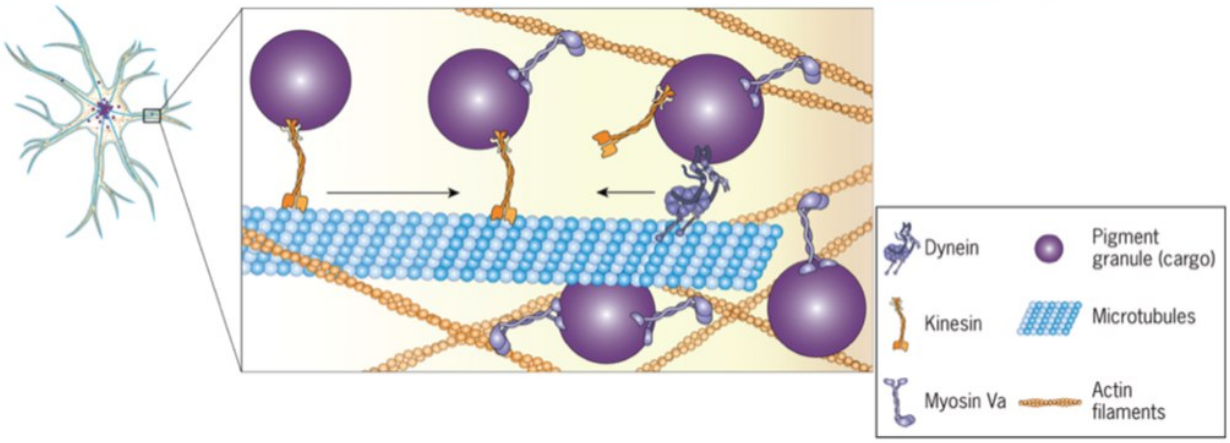
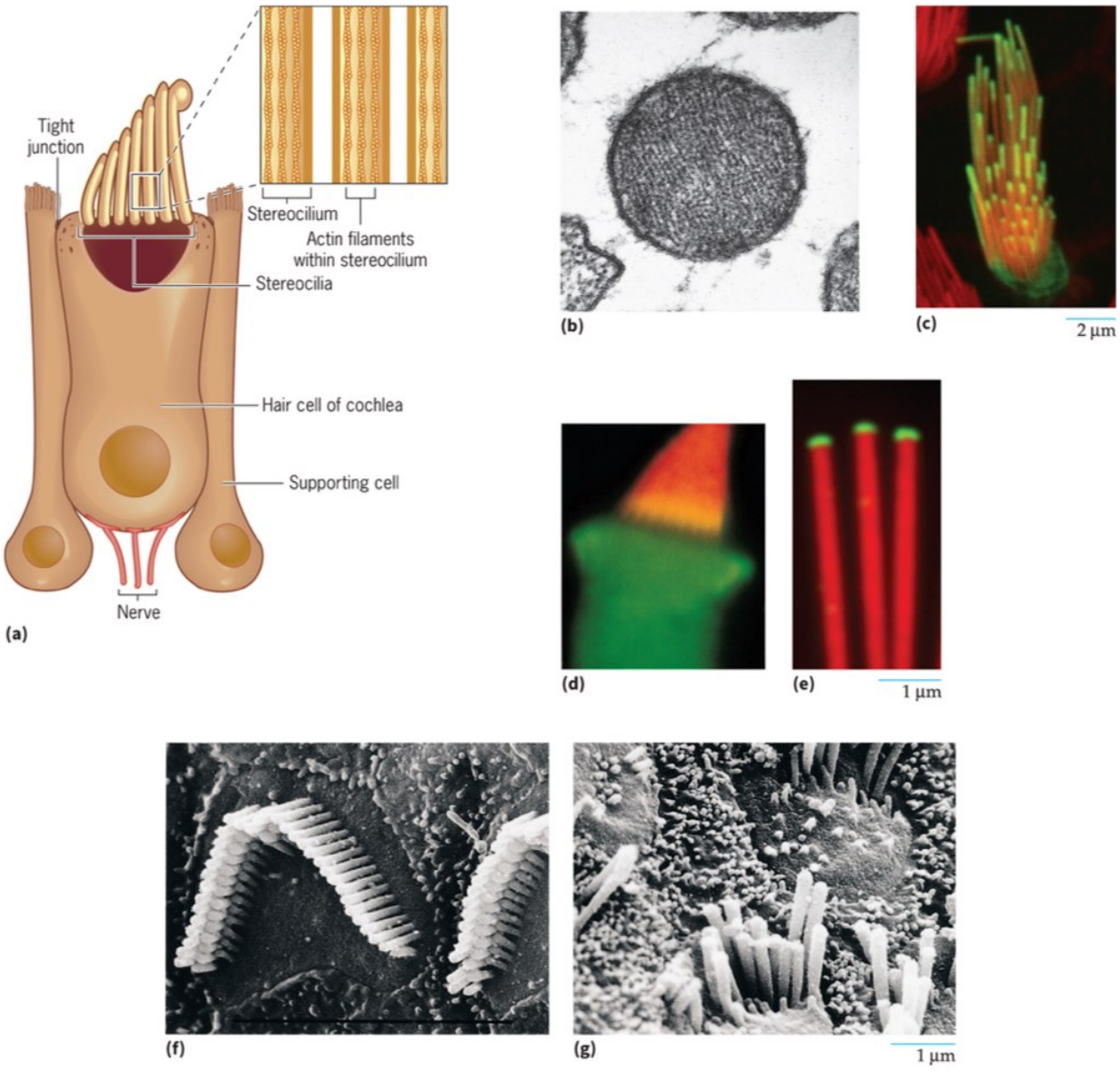


FIGURE 9.47 The contrasting roles of microtubule- and microfilament-based motors in intracellular transport. Most vesicle transport is thought to be mediated by members of the kinesin and dynein families, which carry their cargo over relatively long distances. It is thought that some vesicles also carry myosin motors, such as myosin Va, which transport their cargo over microfilaments, including those present in the peripheral (cortical) regions of the cell. The two types of motors may act in a cooperative manner, as illustrated here in the case of a frog pigment cell in which pigment granules move back and forth within extended cellular processes.

Cooperation between microtubules and microfilaments has been best studied in pigment cells (Figure 9.47). In mammals, pigment granules (melanosomes) are transported to fine peripheral processes of the pigment cell by one of the myosin V isoforms called Va. Melanosomes are then transferred to hair follicles where they become incorporated into a developing hair. Mice lacking myosin Va activity are unable to transfer melanosomes into

hair follicles, causing their coat to have a much lighter color. Humans lacking a normal gene encoding myosin Va suffer from a rare disorder called Griscelli syndrome; these individuals exhibit partial albinism (lack of skin coloration), exhibit silver-gray hair in infancy, and suffer other symptoms thought to be related to defects in vesicle transport. In 2000, it was discovered that a subset of Griscelli patients had a normal myosin Va gene, but lacked a functional gene encoding a peripheral membrane protein called Rab27a. The Rab family of proteins was discussed in Section 8.5 as molecules that regulate vesicle trafficking. Rabs are also involved in the attachment of myosin (and kinesin) motors to membrane surfaces (Figure 9.46*c*).

Inner ear hair cells, with the structure shown in **Figure 9.48a**, have been a particularly good system for studying the functions of unconventional myosins. Hair cells are named for the bundle of stiff, hairlike stereocilia that project from the apical surface of the cell into the fluid-filled cavity of the inner ear. Displacement of the stereocilia by mechanical stimuli leads to the opening of Ca^{2+} channels in the plasma membrane and the subsequent generation of nerve impulses that we perceive as sound. Stereocilia have no relation to the true cilia discussed earlier. Instead of containing microtubules, each stereocilium is supported by a bundle of parallel actin filaments (Figure 9.48*b*) with barbed ends located at the outer tip of the projection and pointed ends at the base. Stereocilia have provided some of the most striking images of the dynamic nature of the actin cytoskeleton. While the stereocilia themselves are permanent structures, the actin bundles are in constant flux. Actin monomers continually bind to the barbed end of each filament, treadmill through the body of the filament, and dissociate from the pointed end. This process is captured in the fluorescence micrograph of Figure 9.48*c*, which shows the incorporation of GFP-labeled actin subunits at the barbed end of each filament. Several unconventional myosins (I, III, V, VI, VII, and XV) are localized at various sites within the hair cells of the inner ear; two of these are shown in Figure 9.48*d, e*. Mutations in myosin VIIa are the cause of Usher 1B syndrome, which is characterized by both deafness and blindness. The morphologic effects of mutations in the myosin VIIa gene on the hair cells of the inner ear of mice are shown in Figure 9.48*f, g*. As in humans, mice that are homozygous for the mutant allele encoding this motor protein are deaf.


Tight junction
Stereocilium
Actin filaments within stereocilium
Stereocilia
Hair cell of cochlea
Supporting cell
Nerve
(a)
(b)
(c)
2 µm
(d)
(e)
1 µm
(f)
(g)
1 µm

Source: (*a*) Adapted from T. Hasson, Curr Biology 9:22, 1999; from Elsevier Science; (*b*) Courtesy of A.J. Hudspeth, R.A. Jacobs and P.G. Gillespie; (*c*) ©2004 Agnieszka K. Rzadzinska et al. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.200310055; (*d*) ©1997 Tama Hasson et al. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.137.6.1287; (*e*) ©2004 Agnieszka K. Rzadzinska et al. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.200310055; (*f*, *g*) Republished with permission of The Company of Biologists Ltd, from Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells, Development, Tim Self et al., 1998; permission conveyed through Copyright Clearance Center, Inc.

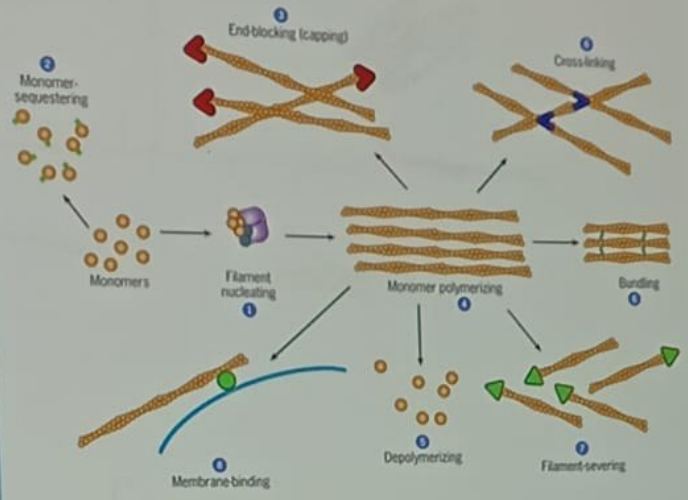
FIGURE 9.48 Hair cells, actin bundles, and unconventional myosins. (a) Drawing of a hair cell of the cochlea. The inset shows a portion of several of the stereocilia, which are composed of a tightly grouped bundle of actin filaments. (b) Transmission electron micrograph of a cross section of a stereocilium showing that it is composed of a dense bundle of actin filaments. (c) Fluorescence micrograph of a hair cell from the vestibule of a rat inner ear. The tips of the stereocilia are labeled green due to the incorporation of GFP-actin monomers at their barbed ends. Taller stereocilia contain a longer column of GFP-labeled subunits, which reflects a more rapid incorporation of actin monomers. The stereocilia appear red due to labeling by rhodamine-labeled phalloidin, which binds to actin filaments. (d) A hair cell from the bullfrog inner ear. The localization of myosin VIIa is indicated in green. The orange bands near the bases of the stereocilia (due to red and green overlap) indicate a concentration of myosin VIIa. (e) Myosin XVa (green) is localized at the tips of the stereocilia of a rat auditory hair cell. (f) Scanning electron micrograph of the hair cells of the cochlea of a control mouse. The stereocilia are arranged in V-shaped rows. (g) A corresponding micrograph of the hair cells of a mouse with mutations in the gene encoding myosin VI. The stereocilia of the hair cells exhibit a disorganized arrangement. Both myosin VI and myosin VIIa are required for proper hair cell development and maintenance.

Myosin VI, a processive organelle transporter in the cytoplasm of many cells, is distinguished by its movement in a "reverse direction," that is, toward the pointed end of an actin filament. Myosin VI has been found to function in diverse membrane trafficking processes, including clathrin-mediated endocytosis, the movement of uncoated vesicles to early endosomes, the maintenance of Golgi morphology, and autophagy. In stereocilia and microvilli, myosin VI functions as an actin tether that is required for the maintenance of normal cellular morphology. Mutations in myosin VI are the cause of several inherited diseases.

Now that we have described the basics of actin and myosin structure and function, you can see how these two proteins interact to mediate a complex cellular activity.

13.11 | Nonmuscle motility

1) **Nucleating proteins** – provide a template for adding actin monomers. Examples: *Arp2/3 complex*; *formin* branched versus unbranched filaments
2) **Monomer-sequestering proteins** – bind to actin-ATP monomers and prevent them from polymerizing. Example: *thymosin* b_4
3) **End-blocking (capping) proteins** – regulate the length of actin filaments Examples: *capZ*; *tropomodulin*).
4) **Monomer-polymerizing proteins** –promote the growth of actin filaments. Example: *profilin*



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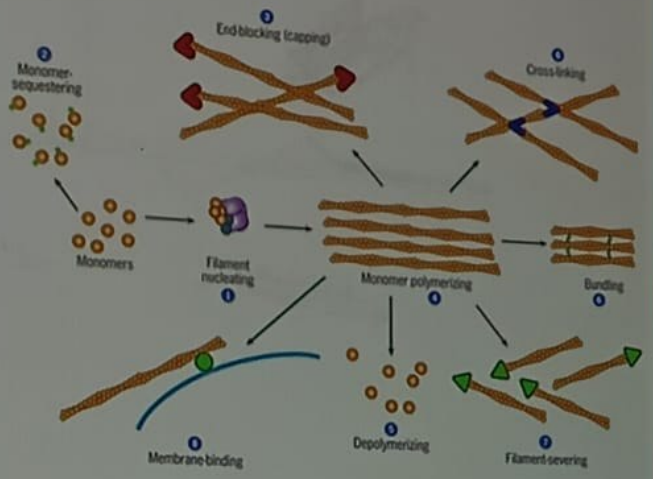
13.11 | Nonmuscle motility

5) **Actin filament depolymerizing proteins** – bind actin-ADP subunits for rapid turnover of actin filaments. Example: *cofilin*
6) **Cross-linking proteins** – alter the three-dimensional organization of actin filaments. Examples: *filamin (X-link), villin, fimbrin (bundling)*
7) **Filament-severing proteins** – shorten filaments and decrease cytoplasmic viscosity. Example: *gelsolin*
8) **Membrane-binding proteins** – link contractile proteins to plasma membrane. Examples: *vinculin, spectrin (dystrophin)*



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