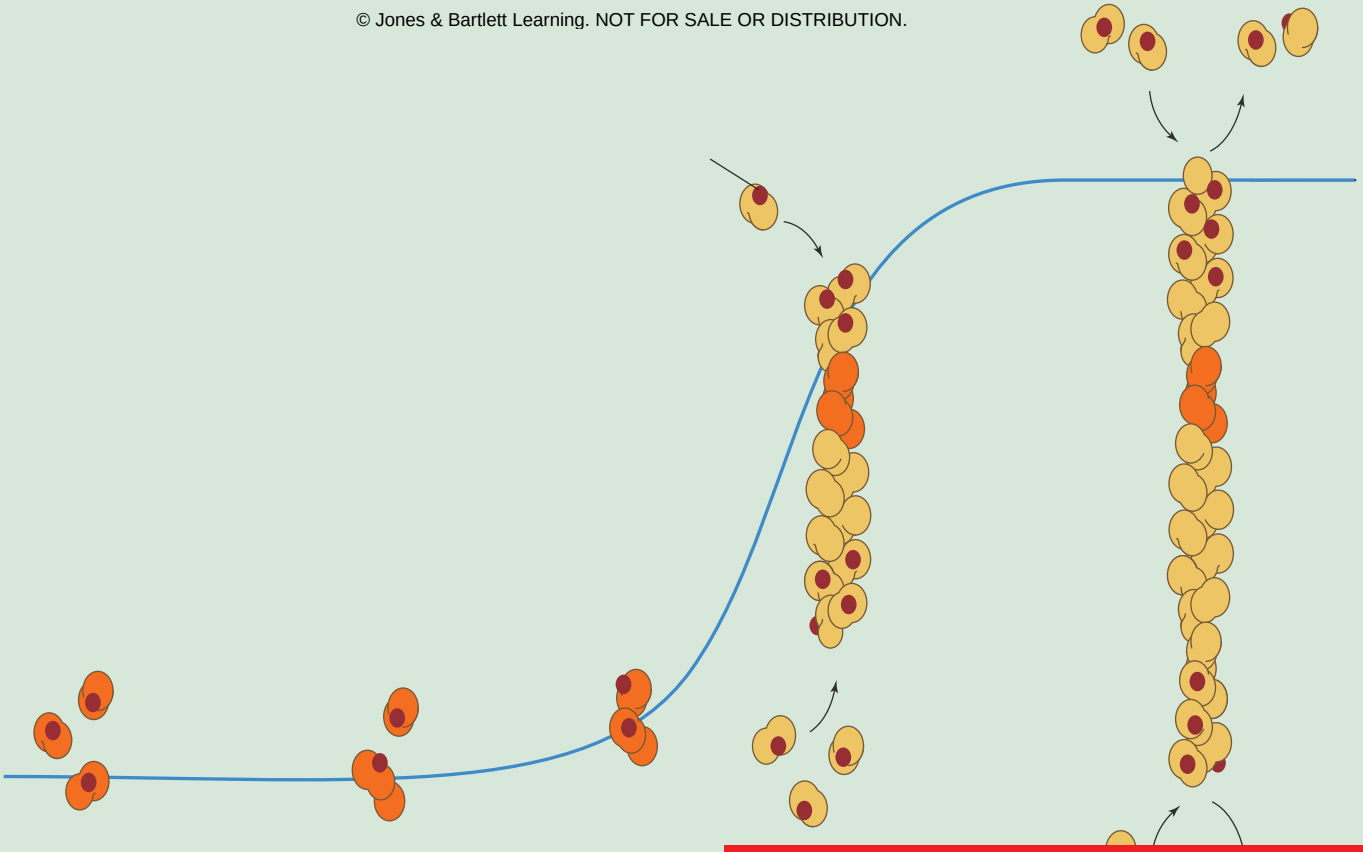


Chapter 5

The Cytoskeleton and Cellular Architecture

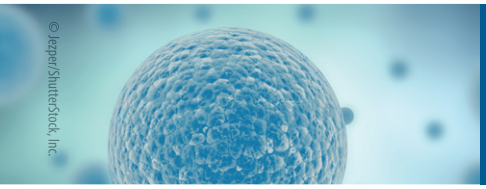
CHAPTER OUTLINE

5.1 The Big Picture

The first four chapters of this text have focused on describing the molecular constituents found in all cells. So far, the emphasis has been on the general traits of the four classes of essential cellular molecules (sugars, nucleic acids, proteins, and lipids) that allow them to perform their functions in cells. Now it's time to put this information to work, by applying the concepts in the first four chapters to specific molecules in cells. We will start by examining proteins known as cytoskeletal proteins, which collectively constitute the cytoskeleton of a cell.

We are embarking on a detailed examination of cellular structure and function, so let's have a look at what we expect to find. Using the cytoskeleton as an example, we are going to

start amassing enough information to build **cell biology principle #1: Cells are always in motion**.



CELL BIOLOGY PRINCIPLE #1

Cells are always in motion.

At first, this principle might be surprising, because we rarely think of ourselves as being in constant motion. Most cellular motion is not the standard type we experience in our everyday lives; instead, it means the cells in our bodies (which we can't see), as well as every other living cell, are undergoing some kind of movement *to stay alive*. We're referring to types of motion that may be quite unfamiliar at this point, which is why it takes a whole chapter to explain this principle (**BOX 5-1**).

There are five major ideas in this chapter:

- All cytoskeletal proteins share two important features. One is that they form polymers inside cells; this means that, just as many amino acids polymerize to form a functioning polypeptide, cytoskeletal proteins polymerize to form a functioning *skeleton* inside cells. Unlike polypeptides, however, the individual units in the skeleton are not always held together by covalent bonds. Instead, many bind via noncovalent bonds, and this means these parts of the skeleton can be disassembled and reassembled relatively easily. The second important feature of cytoskeletal proteins is that they function in much the same way as our own bony skeleton, in that they help provide the mechanical strength necessary to resist physical forces, hold cells in place, and determine the shape and motion of individual cells. Whereas our bony skeleton is primarily composed of one material (bone), the cytoskeleton is made up of three very different classes of proteins, each specializing in a subset of these functions.
- The class of cytoskeletal proteins called the *intermediate filaments* is primarily associated with the mechanical strength function in cells. What does this have to do with cellular motion? Any moving object has to have enough strength to both generate the motion *and* resist the resulting forces without tearing apart, and intermediate filaments help cells resist external forces. Relative to the other two cytoskeletal protein types covered in this chapter, intermediate filaments are specialized to perform a small number of tasks, based on their simple yet distinctive shapes. Pay particular attention to how the method of assembly helps to make these proteins especially tough and to costs/benefits of this strategy. These features will be handy when comparing the three types of cytoskeletal proteins.
- The class of cytoskeletal proteins called *microtubules* is primarily associated with the trafficking function in cells. Trafficking is a word that is rarely used in everyday conversation, but cell biologists use it because it describes the kind of

BOX 5-1 TIP

My goal in each chapter of this section of the book is for students to understand one principle of cell biology at a level that is suitable for their background and interests. This means that, depending on the degree of detail they are seeking, it may be unnecessary to read an entire chapter, or they may have to seek more advanced texts. As in Chapters 1, 2, 3, and 4, it is important to ask the course instructor what level of sophistication is appropriate for their course.

remodeling of a cell's contents that takes place continuously throughout a cell's lifetime. Given the structure–function relationship that forms the foundation of cell biology, we can reasonably expect that because these proteins perform a different *function* from intermediate filaments, they will also have a significantly different *structure.* As we discuss the polymerization of microtubules, pay attention to the differences between this strategy and that used for intermediate filament polymerization.

- Another class of cytoskeletal proteins, called *actin*, is associated with large-scale movement of cells (keep in mind that because cells are so small, moving a few millionths of a meter is considered large scale for them). Actin is by far the most complex of the three types of cytoskeletal proteins because it forms so many different shapes. Therefore, comparing its polymerization mechanism with intermediate filaments and microtubules will not be sufficient to explain how it functions; instead, we will address some of the basic functions of actin in this chapter, and examine more specific examples in later chapters.
- Eukaryotic cytoskeletal proteins have a long evolutionary history, evolving from similar proteins that first appeared in prokaryotic cells. If all modern cells move all the time, the earliest prokaryotes likely did, too. A brief look at these prokaryotic cytoskeletal proteins suggests a great deal about the roles they played in the earliest forms of life.

5.2 The Cytoskeleton Is Represented by Three Functional Classes of Proteins

KEY CONCEPTS

- The cytoskeleton is a complex mixture of three different types of proteins that are responsible for providing mechanical strength to cells and supporting movement of cellular contents.
- The most visible form of cytoskeletal proteins are long filaments found in the cytosol, but these proteins also form smaller shapes that are equally important for cellular function.
- The structural differences between the three cytoskeletal protein types underscores their four different functions in cells.

The term *cytoskeleton* is used to describe a network of filamentous proteins that occupy a large portion of the cytosol in most cells, where they appear to link most of the organelles to each other and to the plasma membrane, as shown in **FIGURE 5-1**. This network plays a significant role in determining the distribution of cellular contents, and even the overall shape of the cell. If we consider the function of our own bony skeleton, the analogy makes sense. Our skeleton defines most of our body shape (our height, the length of our limbs, the shape of our hands and feet, etc.), and permits movement by providing the attachment sites for muscles. As we will see, the cytoskeleton contributes greatly to the motion of cells as well.

If we continue the skeleton analogy, we can see additional similarities: Just as our skeleton is built from a relatively small number of materials (mostly bones, ligaments, and cartilage), the cytoskeleton is composed largely of three different types of proteins. Bones can vary widely in size and shape despite their similar molecular makeup, and this same idea applies to cytoskeletal proteins. Damage to our bones and ligaments can have devastating effects on our ability to function, while damage of the cytoskeleton can likewise be fatal to a cell.

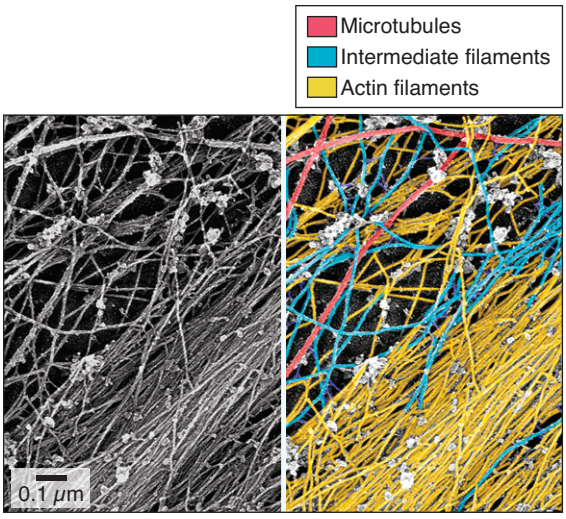


FIGURE 5-1 The cytoskeleton forms an interconnected network of filaments in the cytosol of animal cells.

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.

If we apply the three traits of proteins from Chapter 3 to the cytoskeletal proteins, we see even more similarities. By necessity, all of them bind to a target; in many cases, the target is simply another copy of the same type of protein. When they bind, they change shape and form polymers that are typically the largest protein-based structures in cells. Intermediate filament proteins bind to other intermediate filament proteins to form long, filamentous polymers named intermediate filaments. Likewise, tubulin proteins bind to other tubulin proteins to form long, tube-shaped polymers called microtubules, and actin proteins polymerize to form actin filaments, sometimes known as microfilaments. Note that they do not form mixed polymers: each protein type will only polymerize with others of the same type. As we will see below, these cytoskeletal proteins can bind to many different proteins as well; these proteins can connect the polymers to organelles or the plasma membrane, for example. Finally, different filament types can be interconnected by bridging proteins to form a diverse network.

Only when we consider the third trait of proteins do we see obvious differences between these filaments. Each filament performs a distinct set of functions in cells. When one considers how many different proteins each cytoskeletal polymer can bind to, the number of combined functions increases tremendously. How do we keep track of all of these functions? By clustering similar functions together into a small, manageable set. Each of the cytoskeletal protein types will be classified into a different functional category, and our mission is to find the information that will justify classifying them in this way.

5.3 Intermediate Filaments Are the Strongest, Most Stable Elements of the Cytoskeleton

KEY CONCEPTS

- Intermediate filaments are highly stable polymers that have great mechanical strength.
- Intermediate filament polymers are composed of tetramers of individual intermediate filament proteins.
- Several different genes encode intermediate filament proteins, and their expression is often cell and tissue specific.
- Intermediate filament assembly and disassembly are controlled by posttranslational modification of individual intermediate filament proteins.
- Specialized intermediate-filament-containing structures protect the nucleus, support strong adhesion by epithelial cells, and provide muscle cells with great mechanical strength.

One striking similarity between our bony skeleton and the cytoskeleton is that each is strong and durable. Without bones, we would not be able to resist the force of gravity enough to allow us to stand, walk, or even sit upright. Likewise, without a cytoskeleton, our cells cannot resist the mechanical stresses that we mostly ignore in everyday life, such as the frictional forces created when our clothing rubs against us. One of the major functions of the cytoskeleton, therefore, is to provide the mechanical strength necessary for cells to resist these forces. The intermediate filament portion of the cytoskeleton, shown in **FIGURE 5-2**, is primarily responsible for supplying this strength to cells, as illustrated in **FIGURE 5-3**. Whenever we discuss intermediate filaments, we will map them to this function (**BOX 5-2**).

Intermediate Filaments Are Formed from a Family of Related Proteins

Different cells in a multicellular organism are exposed to different types and degrees of mechanical stress (defined as the amount of force applied to a given surface area). For example, the cells lining our blood vessels are exposed to varying degrees of fluid sheer stress caused by the constant flow of blood over their surface, while our skeletal muscle cells experience stress when they contract. Cells have the ability to construct different intermediate filaments suited to resist the types of force they are exposed to. Pathologists often look for the specific makeup of these "custom" intermediate filaments as an easy way to distinguish one cell type in a tissue from another.

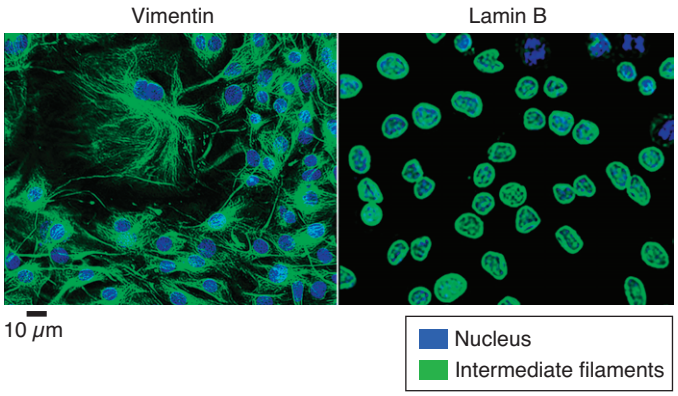


FIGURE 5-2 Two types of intermediate filaments. Fluorescence microscopy was used to label a type III intermediate filament protein (vimentin, left panel) and a type V intermediate filament protein (lamin, right panel) in cultured fibroblast cells.
Photos courtesy of John Common and Birgit Lane, Institute of Medical Biology, Singapore.

The Intermediate Filament Genes in Humans Are Classified into Six Groups

Each type of intermediate filament protein is encoded by a separate gene. Humans contain 70 intermediate filament genes that form approximately 75 different filament proteins (the number of proteins exceeds the number of genes due to alternative splicing of some of these genes; see Chapter 8, *In Eukaryotes, Messenger RNAs Undergo Processing Prior to Leaving the Nucleus*). These 70 genes are classified into 6 groups, named type I, type II, and so on, as shown in **TABLE 5-1**. Clusters of closely related genes (those that share the most sequence homology) are given a single name (e.g., type I and type II keratins, type IV neurofilaments, type V lamins). In humans, the type I and type II keratins are the most numerous intermediate filament proteins.

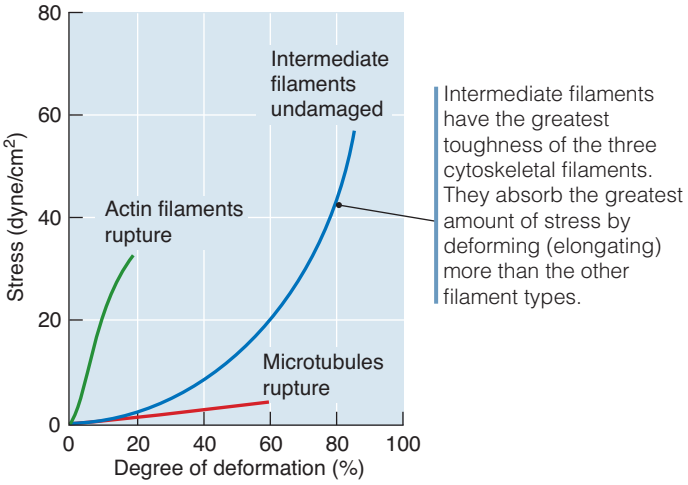


FIGURE 5-3 Intermediate filaments have the most mechanical strength of the cytoskeletal proteins, as measured by the strain (deformation) of the filaments in response to application of shear stress (force/area). Note that each of three cytoskeletal filaments has a different response to the application of stress.
Adapted from P. A. Janmey, et al., *J. Cell Biol.* 113 (1991): 155–160.

Intermediate Filament Expression Is Largely Cell and Tissue Specific

The expression pattern for each of the six groups of intermediate filament proteins is usually restricted to a specific cellular location, cell, or tissue type, as shown in Table 5-1. This reflects the customization property of intermediate filaments, in that each different filament serves a slightly different purpose. For example, the type V intermediate filaments (known as lamins) are found exclusively in the nucleus

BOX 5-2

The steel cable / highrise building analogy. To get a good idea of how intermediate filaments function in cells, try visualizing how one might use steel cables (intermediate filaments) to reinforce a tall building (a cell). Perhaps steel beams would be better for that job, but cells are much more dynamic than buildings, and steel beams are not flexible enough to adapt to the shape changes that cells experience. If you were required to use cables, you'd have to also develop a means of connecting the cables to the walls of the building, as leaving cables lying around would essentially be useless. This means that intermediate filaments alone are not enough; consider some possible ways cells could attach them to other structures in cells, too.

TABLE 5-1 The major classes of intermediate filaments in mammals.

Class	Protein	Distribution	Proposed Function
I	Acidic keratins	Epithelial cells	Tissue strength and integrity
II	Basic keratins	Epithelial cells	Tissue strength and integrity
III	Desmin, GFAP, vimentin, peripherin	Muscle, glial cells, mesenchymal cells, peripherin neurons	Sarcomere organization, integrity
IV	Neurofilaments (NFL, NFM, and NFH)	Neurons	Axon organization
V	Lamins	Nucleus	Nuclear structure and organization
VI	Nestin	Neurons	Axon growth

(see Chapter 2 and Chapter 8), neurofilaments are expressed only in nerve cells, and the expression of type VI intermediate proteins (phakinin and filensin) is restricted to the eye lens.

The Primary Building Block of Intermediate Filaments Is a Filamentous Subunit

To better understand how intermediate filaments provide cells with mechanical strength, we need to examine their molecular structure. The first notable feature is that *all intermediate filament proteins are only effective when they form polymers.* While there is some turnover of intermediate filaments in cells (e.g., during cell division), these polymers are the most stable of the three types of cytoskeletal filaments.

Let's use the fact that intermediate filaments must be mechanically strong to understand how they are made. What property of proteins can we exploit to make them especially strong? Let's start at the simplest level, the 1° structure. Recall from Chapter 3 that all proteins are linear polymers of amino acids linked together by the same covalent (peptide) bonds. This means that the backbone of all proteins has essentially the same strength, and suggests that changes in only the 1° structure will not be sufficient to provide the extra strength needed in an intermediate filament.

A Central Alpha-Helical Domain Confers Tremendous Tensile Strength to Intermediate Filament Subunits

If we look at the 2° structure of proteins, we see some clues. Recall that there are three 2° structures: α-helices, β-sheets, and random coils. Are any of these stronger than the others? Both α-helices and β-sheets are stabilized by hydrogen bonds, but random coils may not be; this suggests that either of the first two may be especially abundant in intermediate filament proteins. When we look at their general structure, as shown in **FIGURE 5-4**, we do indeed see an abundance of α-helices in the central rod domain (in fact, this is where α-helices were first discovered), flanked on either side by small, globular head and tail domains at the amino and carboxy ends, respectively. This makes sense, because α-helices are long and thin: remember that we are trying to make a long, thin polymer from these protein subunits. It is easy to imagine that if one tugs on the head and tail ends of this structure, the abundance of hydrogen bonds stabilizing the α-helices will prevent

the protein from stretching out and/or collapsing. This simple property contributes a great deal to the function of these proteins.

Intermediate Filament Subunits Form Coiled-Coil Dimers

Now let's look at the 3° structure of intermediate filaments. **FIGURE 5-5** illustrates how complete intermediate filaments are assembled. Notice that two intermediate filament proteins (also called *monomers* or *subunits* in this context) wrap around one another in parallel to form a dimer (**BOX 5-3**). Some intermediate filament dimers only form when the two subunit types are different (e.g., keratins and neurofilaments make heterodimers), some can assemble from identical or

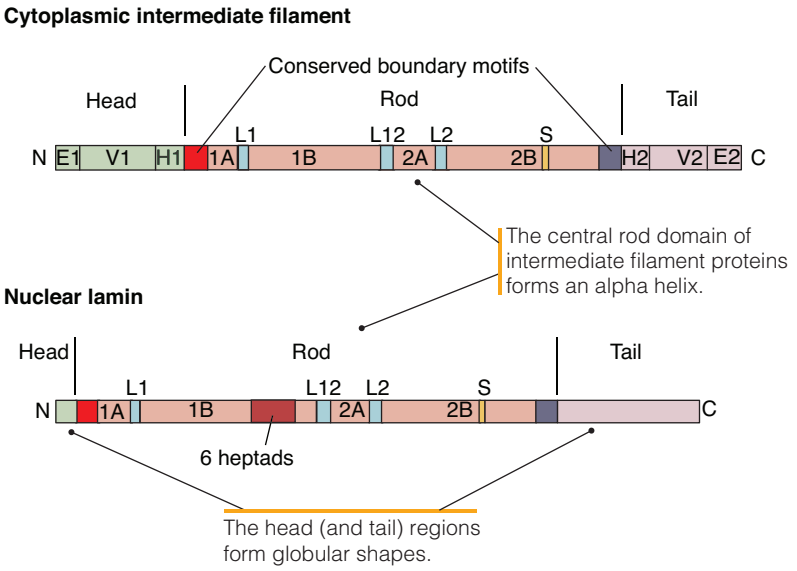


FIGURE 5-4 The general structure of two different intermediate filament proteins.

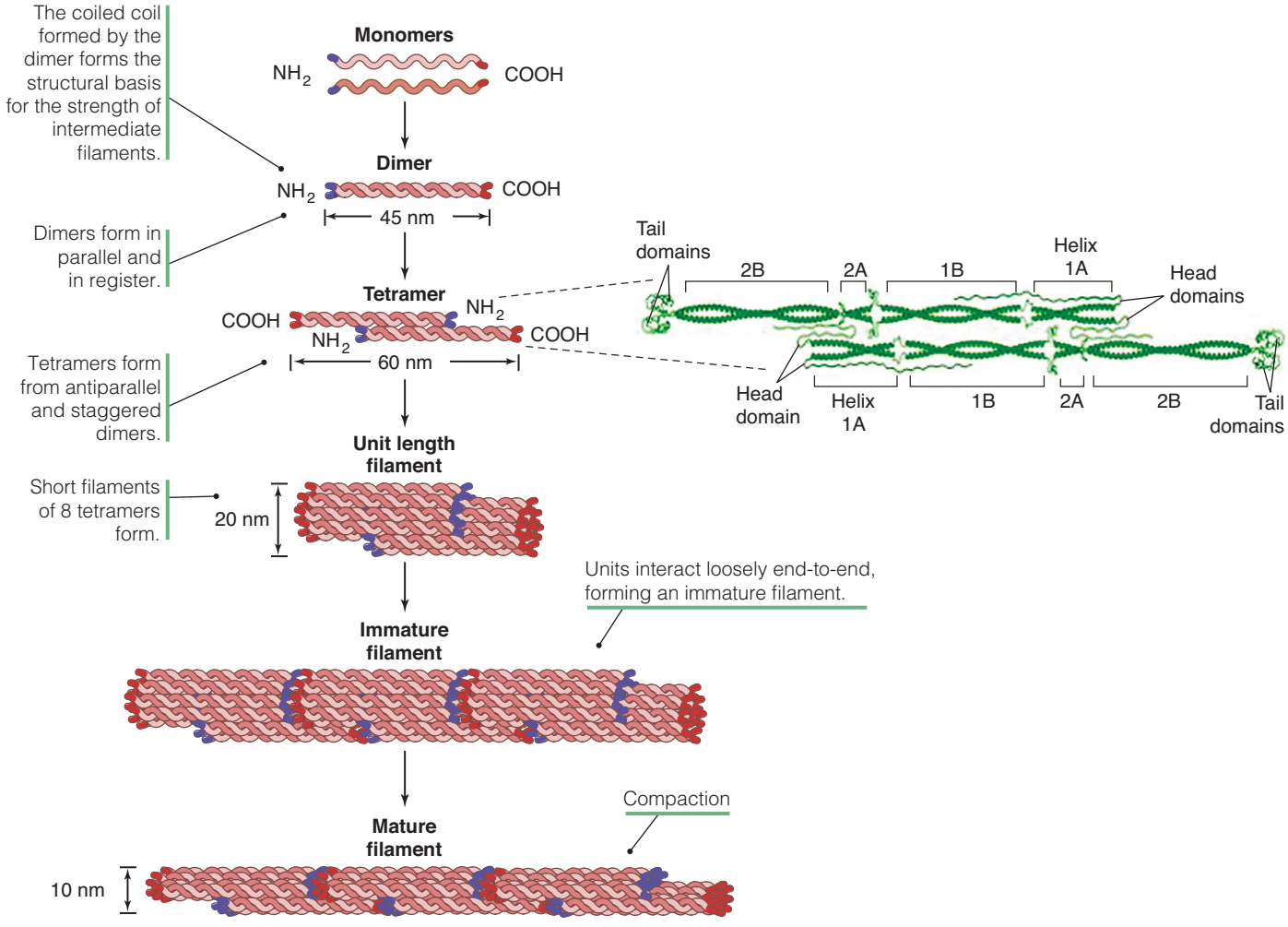


FIGURE 5-5 A model for intermediate filament assembly.

BOX 5-3 TIP

Revisiting the protein/subunit issue. In Chapter 3, we discussed the use of different words to describe the functions of polypeptides. In most cases, the word "subunit" cannot be used interchangeably with "protein." But, be careful: when we talk about the cytoskeleton, the rules change. A cytoskeletal protein is very often called a subunit, because these proteins are frequently considered functional only when they form polymers. This applies to all three cytoskeletal filament types. Because they can form functional polymers of different sizes, there is no rigorous standard to define when a "subunit" becomes part of a functional polymer. As frustrating as this may be for new cell biology students, it's simply the way things are, and no real effort has been made to fix it. We all just have to get used to this exception.

different subunits (e.g., vimentin and nestin make homo- and heterodimers), and the rest make only homodimers. Because it is a coil formed by two α-helical polypeptides, this structure is called a **coiled coil** (the redundancy is intentional). Coiled coils are some of the strongest structures found in proteins, because they maximize the amount of surface contact between the two polypeptide chains; the close proximity of the amino acid side chains permits hydrophobic bonds to form between the two chains. Because many of these bonds form between leucine amino acids, this structure is sometimes referred to as a leucine-zipper motif.

Heterodimers Overlap to Form Filamentous Tetramers

The next level of intermediate filament assembly is an antiparallel staggered tetramer, as seen in Figure 5-5. Notice that at this stage, even the coiled coils line up and interact with one another, further increasing the number of bonds stabilizing the structure. These tetramers are the smallest stable form of intermediate filaments in cells, and usually represent the "replacement pool" on hand to replace damaged tetramers in the polymers. It is important to note that these tetramers have no structural polarity; the amino termini (tails) of each dimer project outward at both ends, and the carboxy termini (heads) interact with the rod domains on the adjacent subunit. This means that the two ends of the tetramer are identical; there is no front or back to this structure. This feature is important because we will see later that the other two types of cytoskeletal filaments *do* have structural polarity, and this difference helps explain why these filaments play different functional roles in cells (**BOX 5-4**).

Assembly of a Mature Intermediate Filament from Tetramers Occurs in Three Stages

Intermediate filament assembly has been studied mostly *in vitro,* using purified proteins. Under conditions where the assembly can be slowed down enough to watch it progress, it occurs in three stages, illustrated in Figure 5-5. First, eight tetramers associate into a

BOX 5-4

The Tetris analogy. If the nonpolarized tetramer is the basic building block of intermediate filaments, let's use a popular videogame to illustrate it. In the game Tetris®, the goal is to arrange different geometric shapes into a continuous horizontal line. One of the shapes, called the S-unit, resembles the outline shape of an intermediate filament tetramer. In this analogy, a fully formed intermediate filament is like an enormous Tetris game containing only S-units, and all of them fit together perfectly to make many complete horizontal lines. Considering how difficult that would be in the game, it is amazing that cells do this routinely.

loosely packed unit-length filament approximately 17 nm in diameter. Next, these unit-length filaments align end-to-end to form an immature filament, still 20 nm in diameter. Finally, the filament compacts to form a dense, mature, 10-nm-thick filament. Note that no additional proteins are required to form intermediate filaments this way, and assembly is spontaneous. It has been very difficult to verify that the filament assembly occurs in the exact same steps in cells, but the evidence accumulated thus far suggests that the assembly process is at least very similar.

Posttranslational Modifications Control the Shape of Intermediate Filaments

Recall from Chapter 3 (see *Cells Chemically Modify Proteins to Control Their Shape and Function*) that proteins form both covalent and noncovalent bonds with other molecules. Intermediate filaments undergo many posttranslational modifications, including phosphorylation, glycosylation, farnesylation, and transglutamination of the head and tail domains on the individual intermediate filament subunits. While the function of most of these modifications is only now being truly appreciated, it is clear that the phosphorylation/dephosphorylation of domains in intermediate filament proteins helps control the assembly of the subunits into mature filaments. This is especially evident when intermediate filaments disassemble during cell division: the nuclear envelope dissolves (requiring dissolution of the lamin intermediate filaments) and the contents of the cell (including the cytosolic intermediate filament proteins) are partitioned into roughly equal halves during cytokinesis (see Chapter 7, *Cytokinesis Completes Mitosis by Partitioning the Cytoplasm to Form Two New Daughter Cells*). In general, phosphorylation of the globular domains dissolves intermediate filaments; upon removal of the phosphates by protein phosphatases (see Chapter 3, *Covalent Modifications are Relatively Long Lasting*), the subunits spontaneously reassemble into filaments.

Intermediate Filaments Form Specialized Structures

The restricted cell and tissue distribution of intermediate filament proteins further emphasizes the importance of cell specialization in multicellular animals, and allows us to examine how the mechanical strength function is applied to different cell and tissue types in greater detail.

Lamins Form a Strong Cage inside the Nucleus

Lamins (not to be confused with *laminins,* see Chapter 6, *Laminins Provide an Adhesive Substrate for Cells*) are found exclusively in the nucleus of cells, and are structurally quite different from all of the other intermediate filament proteins. Three different lamin genes in mammals undergo alternative splicing (see Chapter 8, *The Spliceosome Controls RNA Splicing*) to yield six different lamin subunits, called lamins A, B1, B2, B3, C1, and C2. Each cell contains at least one version of all three (A, B, C) types, resulting in three different homodimers. All of these proteins contain a longer helical domain than cytoplasmic intermediate filaments; this difference helps ensure that lamins do not copolymerize with the shorter cytoplasmic intermediate filament subunits.

Lamins form strong filaments that line the inner surface of the nucleus, protecting chromatin from potential damage caused by mechanical trauma (see Figure 2-26). More recent evidence suggests that the lamins are linked to the chromatin by other structural proteins and may play a role in controlling gene expression by influencing the degree of chromatin compaction. When the nuclear envelope dissolves during prometaphase in

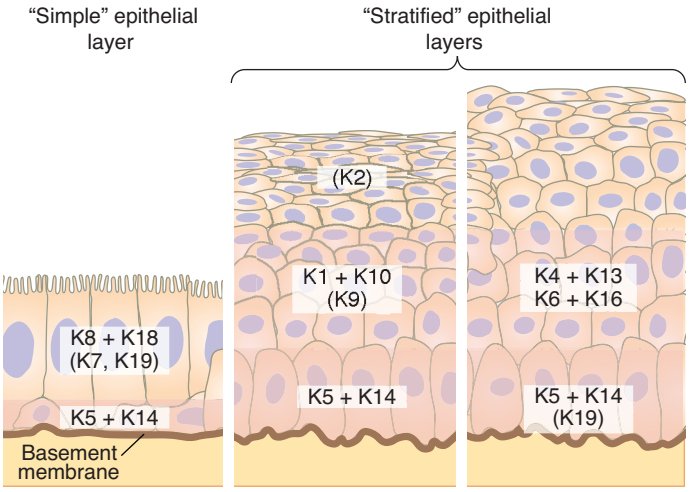


FIGURE 5-6 Keratin expression patterns vary in different epithelial tissues. Note that K5/K14 is expressed in all three types of epithelia shown, but K8/K18 is restricted to simple epithelia.

cell division, lamins are phosphorylated in the head and tail globular domains, and the resulting shape change of the proteins causes them to dissolve into individual tetramers. It is not yet known whether this phosphorylation causes nuclear envelope disassembly, or merely coincides with it.

Epithelial Intermediate Filaments Form Strong Attachment Sites at the Cell Surface

The largest and most diverse group of intermediate filament proteins is the keratins expressed in epithelial cells (see Chapter 14, *Epithelial Tissues Form Protective, Semipermeable Barriers between Compartments*). Humans have 54 different keratin genes (28 type I and 26 type II) that encode proteins, and these are named according to the convention "K#," where # is a number between 1 and 86 (the details of these numbers are not important for us here). Keratins are considered **obligate heterodimers**, in that the dimers forming their filaments must contain one type I and one type II subunit. Different combinations of keratin pairs are expressed in different stages of development, and in different kinds of epithelial tissues, as shown in **FIGURE 5-6**.

Keratins are always located in the cytosol of epithelial cells, and in some cases they are the most abundant proteins these cells express (keratins can represent up to 80% of proteins in the cytosol). Keratins are essential components of two important cell adhesion complexes in epithelial cells: desmosomes and hemidesmosomes (see Chapter 6, *The Hemidesmosome Is a Specialized Junction Formed between Cells and the Basal Lamina* and *Desmosomes Are Intermediate Filament-Based Cell Adhesion Complexes*). The primary function of these complexes is to ensure strong adhesion between neighboring epithelial cells and between epithelial cells and their underlying extracellular matrix.

The Protein Desmin Is Essential for Muscle Function

Desmin is a type III intermediate filament protein that forms homodimers and is found in all three types of muscle tissue (skeletal, cardiac, smooth; see Chapter 14 for a more detailed description of these tissues). The distribution of desmin differs in each type of muscle cell, but desmin is always part of the contractile apparatus. In smooth muscle cells, desmin is found in the dense bodies that connect to the actin microfilament network (see *Actin-Binding Motor Proteins Exert Force on Actin Filaments to Induce Cell Movement* later in this chapter), while in skeletal and cardiac muscle cells, desmin is concentrated in the Z-lines that border the sarcomeres and intercalated discs. Desmin is somewhat unusual in that it does not form the long filamentous structures that we typically see formed by most intermediate filament proteins; instead, it appears to function as part of molecular clusters that link disparate parts of muscle cells together. One good example is a structure called a **costamere**, illustrated in **FIGURE 5-7**. Costameres link the contractile apparatus of muscle cells with receptor protein complexes in the plasma membrane, which in turn anchor the cells to the extracellular matrix. Mutations in costamere proteins, including desmin, lead to a variety of muscular diseases, including muscular dystrophies. Experiments in three different animal model systems (developing chicken embryos, mice, and zebrafish) confirm that desmin is essential for proper muscular function. When desmin is mutated or deleted in these animals, muscle cells will continue to form, but their ability to contract is severely compromised.

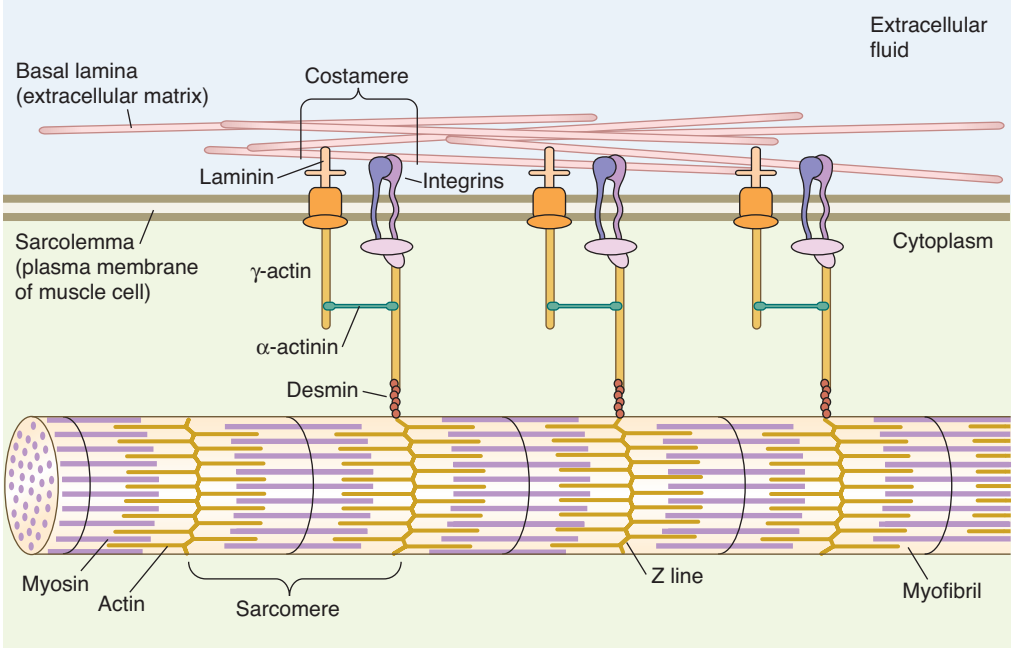


FIGURE 5-7 Costameres link the contractile apparatus of muscle cells to the plasma membrane and extracellular matrix.

CONCEPT CHECK 1

Compare the properties of intermediate filaments with six other materials that have great mechanical strength: rope, chain, steel cable, steel rebar, silk, and Kevlar. In what ways are intermediate filaments similar to/dissimilar from these structures? Using evolution by natural selection as a driving force for optimizing intermediate filament structure and function, suggest an argument for why the current properties of intermediate filaments are the best means for providing mechanical strength inside cells (for example, why don't cells build a structure that more closely resembles steel chains?).

5.4 Microtubules Organize Movement inside a Cell

KEY CONCEPTS

- Microtubules are hollow, tube-shaped polymers composed of proteins called tubulins.
- Microtubules serve as "roads" or tracks that guide the intracellular movement of cellular contents.
- Microtubule formation is initiated at specific sites in the cytosol called microtubule-organizing centers. The basic building block of a microtubule is a dimer of two different tubulin proteins.
- Microtubules have structural polarity, which determines the direction of the molecular transport they support. This polarity is caused by the binding orientation of the proteins in the tubulin dimer.
- The stability of microtubules is determined, at least in part, by the type of guanine nucleotides bound by the tubulin dimers within it.
- Dynamic instability is caused by the rapid growth and shrinkage of microtubules at one end, which permits cells to rapidly reorganize their microtubules.
- Microtubule-binding proteins play numerous roles in controlling the location, stability, and function of microtubules.
- Dyneins and kinesins are the motor proteins that use ATP energy to transport molecular "cargo" along microtubules.
- Cilia and flagella are specialized microtubule-based structures responsible for motility in some cells.

Microtubules are cytoskeletal proteins that guide the trafficking of proteins, vesicles, and even organelles inside a cell. Before we pursue this idea any further, let's have a closer look at the concept of trafficking. The Oxford English Dictionary defines trafficking as "To pass to and fro upon; to frequent (a road, etc.); to traverse." When we discuss trafficking in the context of cell biology, we are referring to the mechanisms cells use to move their contents around. We have already encountered an excellent example of how this occurs during the assembly of cellular membranes in Chapter 4 (see *Most Membrane Assembly Begins in the SER and Is Completed in the Target Organelle*). This illustrates an important theme in cell biology: the function of a molecule in a cell is often determined by its location. This means that one good way of regulating cellular functions is to permit movement of molecules in cells, then regulate this movement very closely.

Why don't we just call this molecular movement? The answer lies in a single word in the above definition of trafficking: road. The difference between simple movement and trafficking is that trafficking takes place on defined routes, and is therefore easier to regulate. In cells, the microtubule cytoskeleton serves as a network of "roads" upon which molecules "pass to and fro" (**BOX 5-5**). This has four important implications:

- First, it demonstrates that cells are far more than bags of molecules. Even stationary cells constantly move and sort their contents, and the distribution of material in cells is far from random.
- Second, microtubule-based movement of molecules limits where some of these molecules can go; if a region of a cell (e.g., the interior of the nucleus or the matrix of a mitochondrion) lacks microtubules, molecules that require microtubules for movement will not be transported there. Conversely, if a region of a cell contains many microtubules (e.g., the mitotic spindle), molecular traffic in that region can be tightly controlled.
- Third, the transport of microtubule-dependent cargo depends upon the structural stability of the microtubules it rides on. Molecular traffic in cells can easily be redirected by simply dissolving some microtubules in one region of the cell and building new microtubules in another.
- Fourth, transport along microtubule "roads" requires energy. Just like the roads in our everyday lives, these roads don't move by themselves; rather, we have to generate some sort of force to push or pull our cargo. The same holds true for cells. We will encounter proteins specialized to convert metabolic energy into forces that move cargo along microtubule roads later in this chapter.

The distribution of this microtubule network varies greatly from one cell type to another (e.g., in some yeast cells, the microtubules actually penetrate the nucleus), but a good example of such a network is shown in **FIGURE 5-8**. Notice that it is most dense in the center of the cell, and appears to radiate outward toward the cell periphery. These are

BOX 5-5

The highway network analogy. Let's expand the notion of trafficking on a road a bit further to highlight a few other features of microtubules. Roads come in many sizes and forms, from one-lane dirt roads to multilane superhighways. Microtubules resemble the highways more than the dirt roads because they permit two-way trafficking and several different "vehicles" can travel on them at once. Also, sometimes very large structures are transported on microtubules, analogous to the "wide load" convoys that are used to move large structures on highways. Finally, microtubules are, in proportion to the cargo that travels on them, extremely long. Cargo can be carried tremendous distances (up to hundreds of millimeters in very long cells) on a single microtubule. One important difference between microtubules and highways is that cargo can attach to and detach from a microtubule anywhere along its length.

the "roads" upon which many molecules travel as they make their way through the cytosol. When we discuss microtubules, we'll map them to the *intracellular transport* function.

Microtubule Assembly Begins at a Microtubule-Organizing Center

Because of their different functions in cells, it makes sense that the assembly and structure of microtubules are different than that for intermediate filaments. One striking difference between the two is that microtubule assembly typically requires a large complex of proteins, whereas all intermediate filaments spontaneously assemble. An important similarity between them is that both are made up of two protein subunits; the subunits that form microtubules belong to a protein family called the **tubulins**.

There is an advantage to investing so many resources on controlling microtubule assembly. In Chapter 4 we discussed how microtubule-based transport of membrane vesicles is crucial for building membranes. Recall from Chapter 4, *Concept Check #3* and *Concept Check Answers #3* that at least three different strategies exist for organizing the steps required to complete a complex process, such as the synthesis and sorting of membrane components. Microtubules are an excellent example of two of these strategies: the **centralized network** and the **distributed network**.

The centralized network appears in cells that contain **microtubule-organizing centers** (**MTOCs**) called **centrosomes**. While many animal cells contain centrosomes, this feature is not found in all eukaryotes (e.g., plant cells) (**BOX 5-6**). This MTOC was so-named because it can initiate the assembly of microtubules. During interphase, it typically lies close to the nucleus, as shown in Figure 5-8. During the S phase of the cell replication cycle (see *The Cell Cycle Is Divided into Five Phases* in Chapter 13), it is copied, and the two new MTOCs form the poles of the mitotic spindle (see *Mitosis and Cytokinesis Occur in M Phase* in Chapter 13). At the completion of the cell cycle, each pole of the spindle becomes the centrosome for the daughter cells.

The distributed network is represented by cells that do not always contain an MTOC. During the interphase stage of the cell cycle, microtubules in these cells arise instead from clusters of proteins distributed throughout the cytosol. Some of these proteins are also found in MTOCs, as we will discuss below.

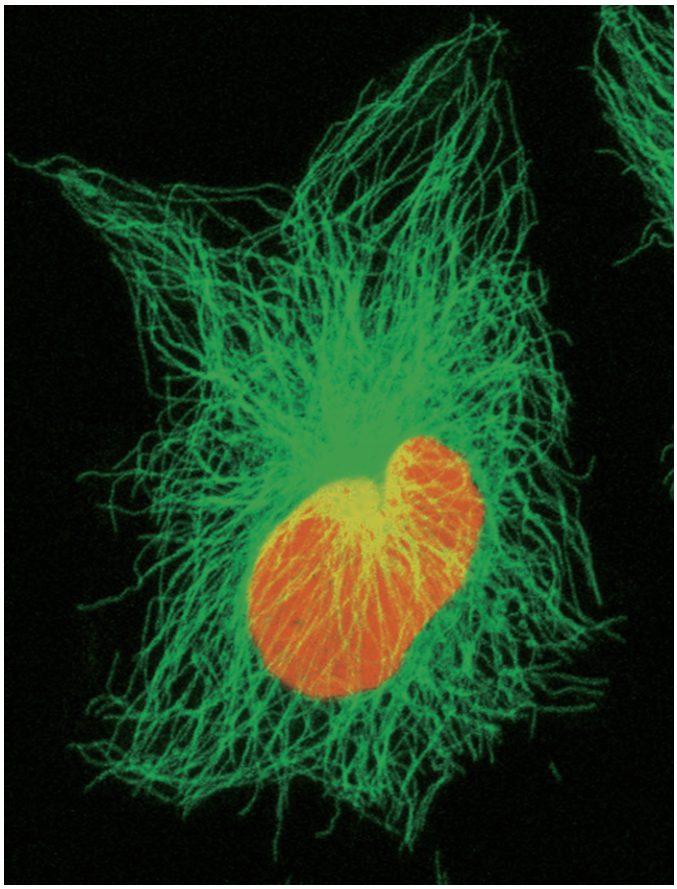


FIGURE 5-8 The distribution of microtubules in a human epithelial cell. The microtubules are stained green in this fluorescence micrograph, and the DNA is stained red to show the location of the nucleus.

Photo courtesy of Holger Lorenz, Zentrum für Molekulare Biologie der Universität Heidelberg, Germany.

BOX 5-6 TIP

Centrosomes vs. MTOCs. The history of these terms is somewhat complicated, so let's clarify what each refers to. The word centrosome was coined first, by microscopists who literally saw a "body" (in Greek, *soma*) in the "center" of a cell. They didn't know what its function was, so they decided to leave the name at that. But, at the time, use of Latin and Greek words to derive biological terms was still very popular—recall the history of naming proteins in Chapter 1—so "central body" was converted to *centrosome*. Much later, when it became clear that the function of the centrosome is to nucleate microtubules, it was also called an MTOC to reflect this fact. However, considering that other cellular structures also nucleate microtubules, the term MTOC is now used as a blanket term for them all (like "organelle"), and each structure has its own name. So we come full circle, and the centrosome is still the name of the MTOC near the nucleus, even though we now know what it does.

The MTOC Contains the Gamma Tubulin Ring Complex (γTuRC) That Nucleates Microtubule Formation

FIGURE 5-9 shows two electron micrographs of the centrosome. Notice that two structures are clearly visible in this MTOC; these are called the centrioles. Each centrosome contains two centrioles, arranged perpendicular to one another. Intact centrioles are very difficult to isolate from most cells. So far, we know that these structures are composed primarily of tubulins, plus at least 100 other protein types. How they achieve their perpendicular orientation, and what significance this orientation may have, are still not well understood, and the function of these centrioles is not known.

Figure 5-9 also shows the cluster of centrosome proteins that surrounds the centrioles and appears as a dense cloud when viewed with an electron microscope. This cloud is called the **pericentriolar material**, and like the centrioles it is composed of many different proteins. Among these is a set of proteins that forms a scaffold, or lattice, around the centrioles. One protein in the lattice, called **gamma-** (γ) **tubulin**, is organized into a helical or ring shape, somewhat like a single coil of a spring. Two additional proteins help form the coil, and many more attach to one face of this coil, forming a structure called the **gamma-tubulin ring complex** (**γTuRC**). Because microtubules grow outward from the γTuRCs, most cytosolic microtubules appear to originate from a single place in these cells. Cells lacking interphase MTOCs also contain γ-tubulin in many of the protein clusters that promote microtubule growth.

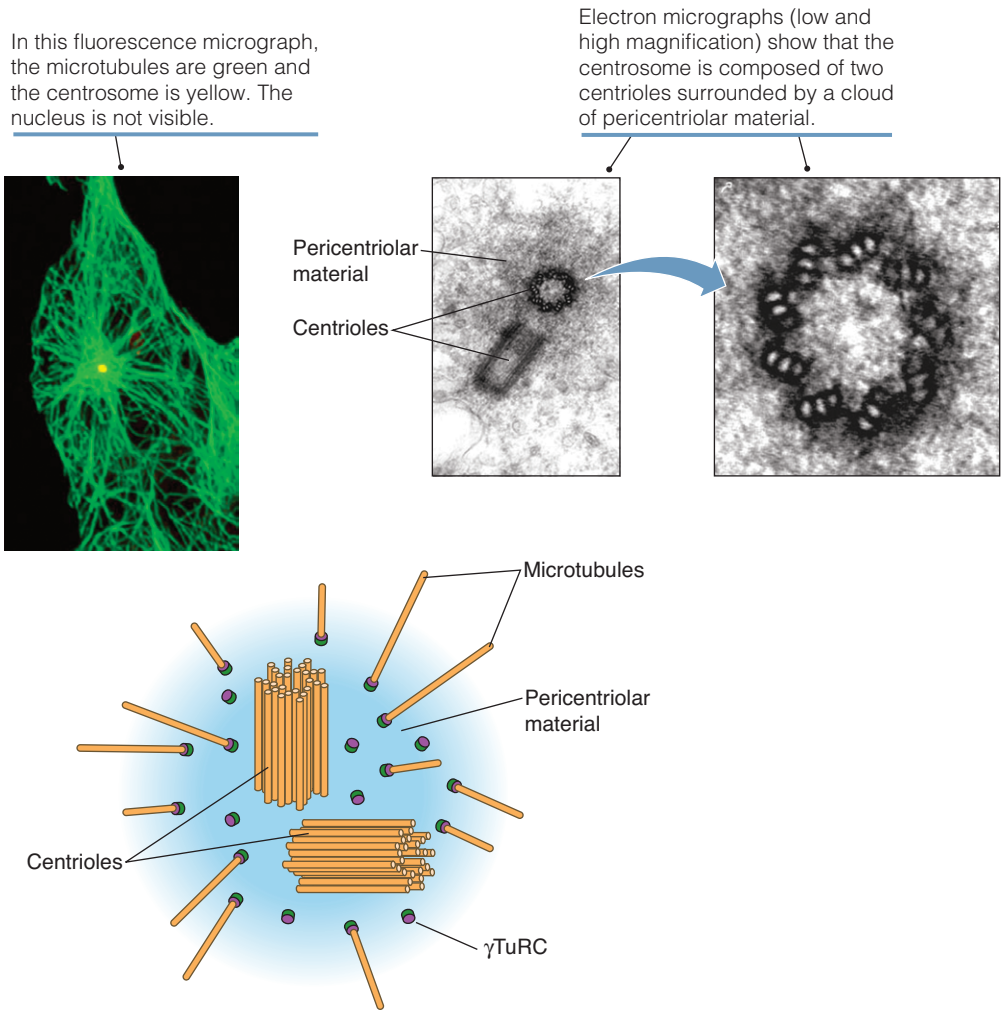


FIGURE 5-9 The structure and location of the centrosome.
Photos courtesy of Lynne Cassimeris, Lehigh University.

Most microtubules that grow from the centrosome remain attached to it, but microtubule formation and anchoring are not performed by the same proteins. The function of γ-tubulin is to initiate the formation of microtubules in cells, but it does not hold the resulting microtubules in place. At least some of the other proteins in the γTuRC play this role, though the exact mechanism is still not known. Gamma-tubulin rings have been found in many different locations in cells, strongly suggesting that these other sites initiate microtubule formation as well. Many of these sites contain additional proteins that could anchor the microtubules.

The Primary Building Block of Microtubules Is an Alpha-Beta Tubulin Dimer

Regardless of where they form, microtubules always assemble the same way. It is easiest to observe this assembly in a test tube (*in vitro*) with purified proteins, and that is how we gained most of our understanding of this process. Microtubules are composed of only two proteins, called α- and β-tubulin. These two proteins bind to one another so strongly that they form a very stable dimer, shown in **FIGURE 5-10**. Both are similarly shaped, globular proteins, and they align front-to-back to form a distinctive shape that looks roughly like a peanut or a figure eight.

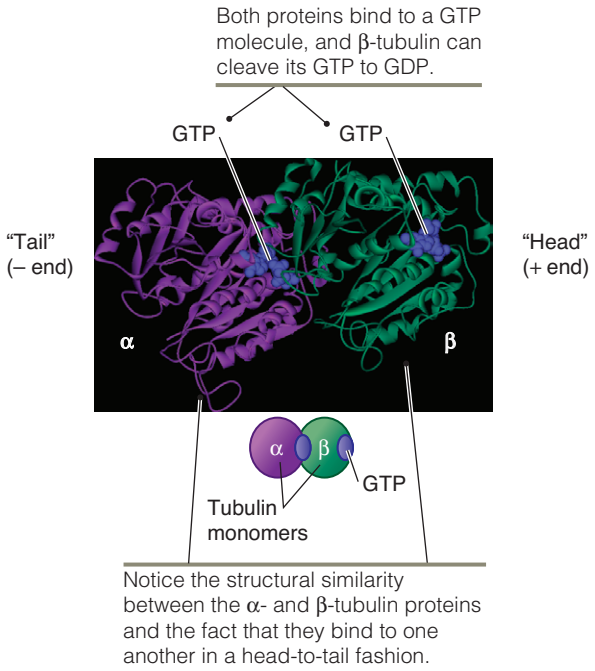


FIGURE 5-10 A three-dimensional model of the dimer formed by α- and β-tubulin.
Structure from Protein Data Bank 1TUB. E. Nogales, S. G. Wolf, and K. H. Downing, Nature 391 (1998): 199–203.

A second important observation is that each α- and β-tubulin protein binds to a GTP molecule. The α-tubulin never releases its GTP, but the β-tubulin is capable of cleaving off the terminal phosphate to create GDP (a process called **GTP hydrolysis**), and has the ability to release this GDP and bind a new GTP to take its place. This swapping of GDP for GTP is crucial for controlling the assembly of α-β tubulin dimers into microtubules, as we will discuss below.

The difference in the way α-tubulin and β-tubulin bind GTP is an excellent illustration of how the positioning of the nucleotide-binding pocket affects the relationship between secondary and tertiary structures of these proteins. The GTP that is bound to α-tubulin is buried within the dimer (resulting in stabilization), whereas the GTP bound to β-tubulin is exposed. If the exposed α-β dimer on the plus end of the microtubule undergoes a conformational change, it is hypothesized that the nucleotide in the β-tubulin is hydrolyzed and this alters the shape of the tubulin dimer—from straight to curved—which then promotes disassembly, at least in part, by disrupting lateral interactions between adjacent dimers. We'll examine the impact of this conformational change more closely when we discuss dynamic instability later.

A third property of microtubule assembly that we can easily observe in a test tube is that it is spontaneous, rapid, and reversible. If purified α-β tubulin dimers bound to GTP are concentrated enough (i.e., they reach what is called the **critical concentration**), they will spontaneously form microtubules, as shown in **FIGURE 5-11**. This is easy to measure: as the microtubules elongate, they deflect more light passing through the test tube (making it appear cloudy, a property called *turbidity*), so we can simply shine light through the tube and watch what happens over time. Later, if no additional GTP is added and the initial GTP is cleaved to GDP by β-tubulin, the microtubules will spontaneously disassemble, and the turbidity will drop. This switch from growth to shrinkage can take place in seconds.

Microtubules Are Hollow "Tubes" Composed of 13 Protofilaments

Let's have a look at the molecular mechanisms behind this rapid assembly and disassembly. Based on studies of purified solutions of α-β tubulin dimers, we know that the first step

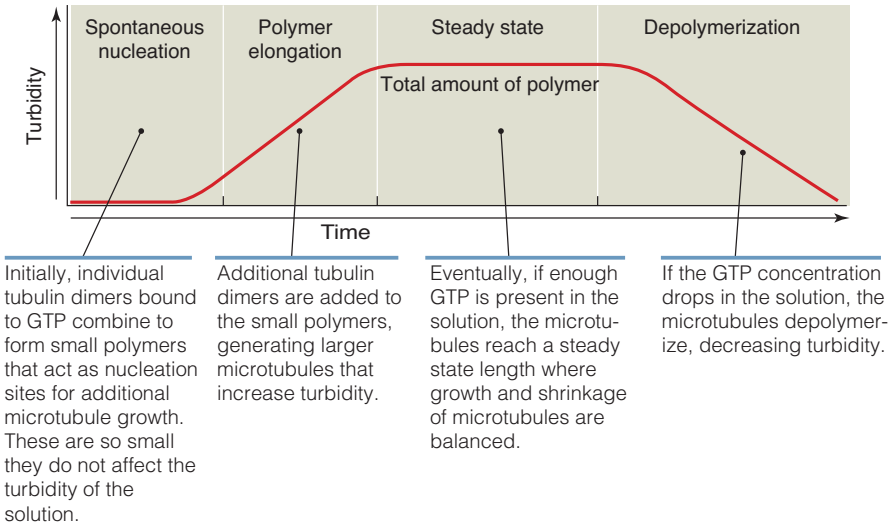


FIGURE 5-11 *In vitro* assembly of microtubules is spontaneous and GTP dependent. The graph represents the turbidity of a solution of α-β tubulin dimers over time.

in building a microtubule is the assembly of small polymers of these dimers, shown in **FIGURE 5-12**. Most of these polymers are unstable, and quickly dissociate, but some reach a critical size (between 6 and 12 dimers), and these begin to grow. Additional dimers are added to the tips and sides of the polymer, until a sheet composed of 13 **protofilaments**

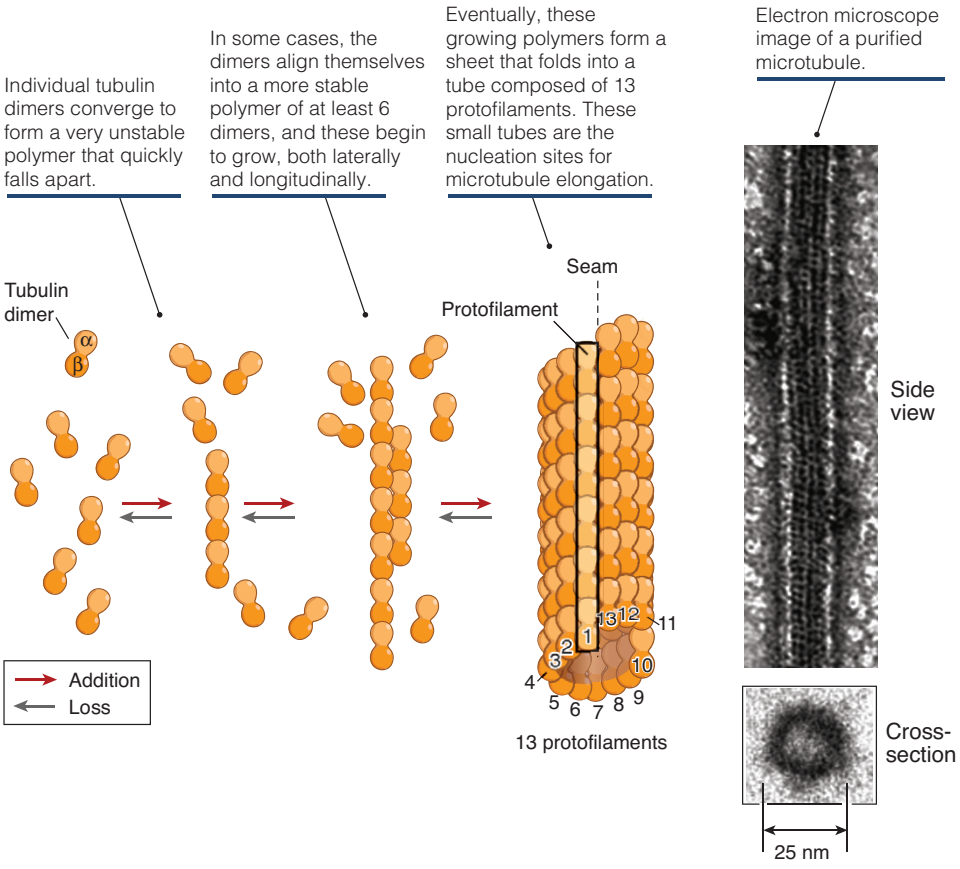


FIGURE 5-12 A simple model of microtubule assembly.
Micrograph provided by Harold Erickson, Duke University School of Medicine. Top photo courtesy of Lynne Cassimeris, Lehigh University.

is formed. This sheet then folds into a tube shape, giving rise to the final microtubule structure, approximately 25 nm in diameter. These tubes, also called nuclei (not to be confused with the organelles of the same name), are rather short, but can quickly increase in length by addition of more dimers to both ends. This association is not permanent, however; eventually, the dimers fall off the ends of the microtubule. At steady state, the rate of dimer addition will equal that of dimer dissociation, keeping the average length of the microtubules constant.

GTP Binding and Hydrolysis Regulate Microtubule Polymerization and Disassembly

To help explain how microtubules can grow and shrink at the same time, let's use two of the three traits of proteins (see Chapter 3, *Proteins Are Polymers of Amino Acids That Possess Three Important Traits*). First, tubulin dimers adopt at least two different stable shapes (trait #1). One appears when both the α- and β-tubulin proteins are bound to GTP. Dimers in this condition favor polymerization, and therefore readily attach themselves to the ends of microtubules (trait #2). The second shape appears when the β-tubulin in the dimer cleaves its GTP to GDP (recall that the α-tubulin never cleaves its GTP, so we will ignore it). This cleavage is a spontaneous process. Because the β-tubulin is now bound to a different molecule (GDP has a different shape than GTP), it changes shape. This change causes dimers to adopt a shape that promotes depolymerization. Thus, at steady state, some dimers are in the GTP-bound state, while others are in the GDP-bound state, and the resulting shapes of these dimers causes some dimers to add to a microtubule, while other dimers will not.

Because the α- and β-tubulin proteins are aligned front-to-back in the dimer, one end of the dimer is structurally different from the other. This means that the dimer, as well as the microtubule it forms, has structural polarity. By convention, one end is called the "plus end" and the other the "minus end." These names were chosen because microtubules do not grow at the same rate from both ends; *in vitro,* α-β dimers in the GTP-bound state preferentially attach to the plus end, so the plus end grows faster than the minus end. Note that this is quite different from intermediate filaments, which do not possess structural polarity when they are polymers.

The Growth and Shrinkage of Microtubules Is Called Dynamic Instability

Studying microtubule assembly *in vitro* is a great way to grasp the fundamentals of the process, but it is clearly not as realistic as studying microtubule dynamics *in vivo* (i.e., in living cells). *In vivo* research is typically much more difficult than working with purified molecules, but it provides an opportunity to tackle questions that cannot be otherwise addressed. An excellent example concerns the complex mechanisms cells use to control the location, stability, and growth rate of microtubules. To help us get started, let's mention three important differences between *in vitro* and *in vivo* studies of microtubules.

- First, microtubules are seldom "naked" in cells and therefore do not all behave the same way. Microtubules are known to bind to many different molecules in cells—so many that one cannot easily keep track of them all at once. Thus, we can only estimate the combined effect that all of these molecules have on any given microtubule.
- Second, every microtubule in a cell lies in its own unique environment. *In vitro,* one can suspend all of the microtubules in a single solution and observe their collective behavior in that one environment, but the interior of a cell is so heterogeneous that no two microtubules are ever in the exact same state.

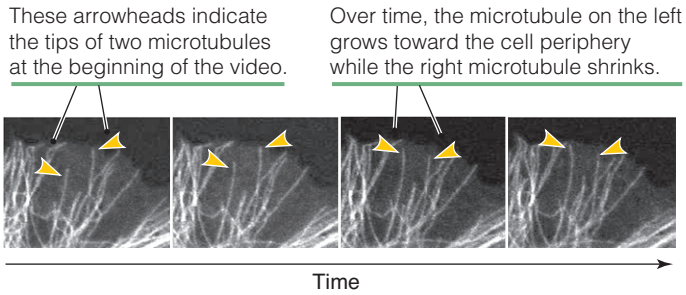


FIGURE 5-13 Growth and shrinkage of microtubules in a living cell. The microtubules have been tagged with a fluorescent molecule, and recorded by video over time.
Photos courtesy of Lynne Cassimeris, Lehigh University.

- Third, living cells don't "hold still." Regardless of what our wishes are, cells have their own agenda: to maintain their disequilibrium with the surrounding environment, and thereby stay alive. This means that the internal state of a cell changes over time, so the microtubules we see at the end of an experiment may have been influenced by factors we cannot (or did not) control during the experiment.

Despite these limitations, researchers have made considerable progress toward understating how cells control their microtubules. One of the most important advances is our understanding of how microtubule assembly, elongation, and stabilization occur. The centrosome and other MTOCs in cells play a central role in this.

Some Microtubules Rapidly Grow and Shrink in Cells

Using specially prepared light microscopes, one can see that the length and position of microtubules changes rapidly in most cells, as shown in **FIGURE 5-13**. This growing/shrinking behavior is collectively called **dynamic instability**. The growth of microtubules begins when γ-tubulin rings nucleate polymerization of the α-β tubulin dimers, such as in MTOCs like the centrosome. Because the centrosome is the most heavily studied MTOC, we use it to illustrate how dynamic instability takes place.

Microtubule growth begins when enough GTP-bound α-β tubulin dimers bind to the γ-tubulin ring (located on the surface of the centrosome) to form a microtubule nucleus, as we discussed above. These nuclei always have the same orientation: their minus ends are nearest the γ-tubulin, and their plus ends face away from the γ-tubulin, into the cytosol. At some point, the minus end of a growing microtubule releases from the γ-tubulin, but remains attached to the centrosome by additional proteins in the pericentriolar material (most of these have yet to be identified). If additional GTP-bound dimers are nearby, they will add to the nucleus, but mostly at the plus end, as shown in **FIGURE 5-14**. This causes

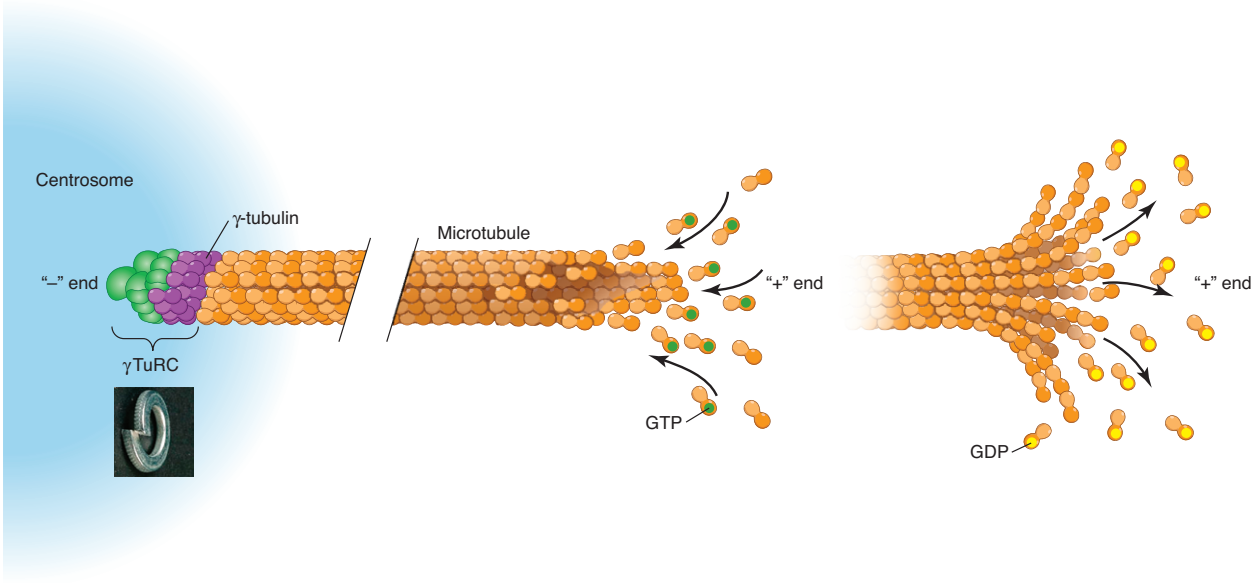


FIGURE 5-14 The growth of microtubules begins at the gamma tubulin ring and continues as long as the plus end contains GTP-bound tubulin dimers.
Photo courtesy of Lynne Cassimeris, Lehigh University

the microtubule to elongate, and it will continue as long as enough GTP-bound tubulin dimers are present at the plus end (this is often referred to as a GTP cap). We see this in the microscope as the emergence of a microtubule from the centrosome and its trajectory through the cytosol. Where a microtubule ends up is determined by a host of factors, including several proteins in the cytosol that attach to it as it elongates.

Eventually, the supply of GTP-bound tubulin dimers runs out, and the microtubule stops growing. At this point, two things can happen. The most common outcome, illustrated in **FIGURE 5-15**, is that the microtubule begins to depolymerize at the plus end (and perhaps also at the minus end), and the microtubule literally falls apart, somewhat like a rope uncoiling. Cell biologists who study this process gave the action the dramatic name **catastrophe** to emphasize the rapidity of this collapse. What causes catastrophe? Recall that all α-β tubulin dimers bind to GTP molecules, and the β-tubulin cleaves its GTP to GDP, switching it from a pro-polymerization shape to a shape that does not favor polymerization. When the last of the GTP-bound tubulin dimers is added to the end of a microtubule, they eventually convert the GTP bound to the β-tubulin into GDP, and this drives them to drop off the end of the microtubule. Once they fall off, the dimers that were previously added to the microtubule now face the same choice: to remain with the microtubule, or fall off. Because they have been part of the microtubule longer than those at the very tip, there is a greater probability that they are now in the GDP-bound form, so they too fall off.

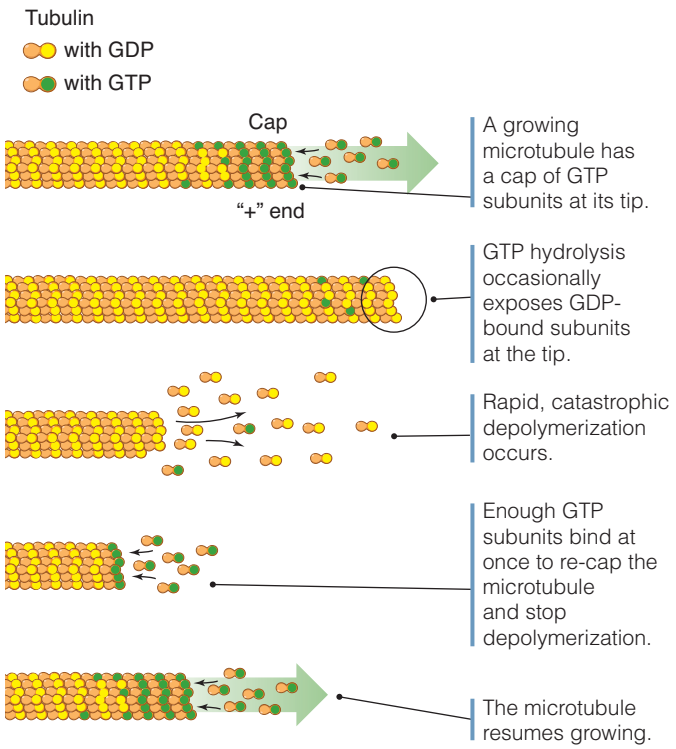


FIGURE 5-15 Two fates of the plus ends of microtubules.

The second outcome for a microtubule that has stopped growing, is that its plus end is bound by a set of **microtubule capping proteins** that stabilize the plus end, such that even if all of the dimers in the microtubule are in the GDP-bound form, they still do not depolymerize. As long as the capping proteins remain attached to the plus end, the microtubule will remain intact. Attachment of the pericentriolar proteins to the minus end stabilizes it, too.

Cells Can Regulate the Rate of Microtubule Growth and Shrinkage

Despite its name, catastrophe does not necessarily lead to complete dissolution of a microtubule. Often, the depolymerization is reversed by a process given the equally dramatic name of **rescue**. Microtubules undergoing rescue regain their GTP cap, usually through one of two mechanisms. First, if the shrinking microtubule happens to have its plus end in a region of the cell where enough GTP-bound tubulin dimers are present, they simply attach themselves to the shrinking plus end, thereby stopping further dissolution. The second mechanism is a bit more complicated, because it requires additional proteins to rescue the microtubule; these proteins attach themselves to the shrinking plus end and promote the attachment of new GTP-bound dimers. When viewed under a microscope, these proteins appear to "track" with the plus end of the regrowing microtubules, though researchers do not yet fully understand the mechanisms underlying these behaviors.

Some Microtubules Exhibit Treadmilling

In cases where neither end of a microtubule is stabilized, tubulin dimers are added to the plus end and lost from the minus end. The overall length of these microtubules remains

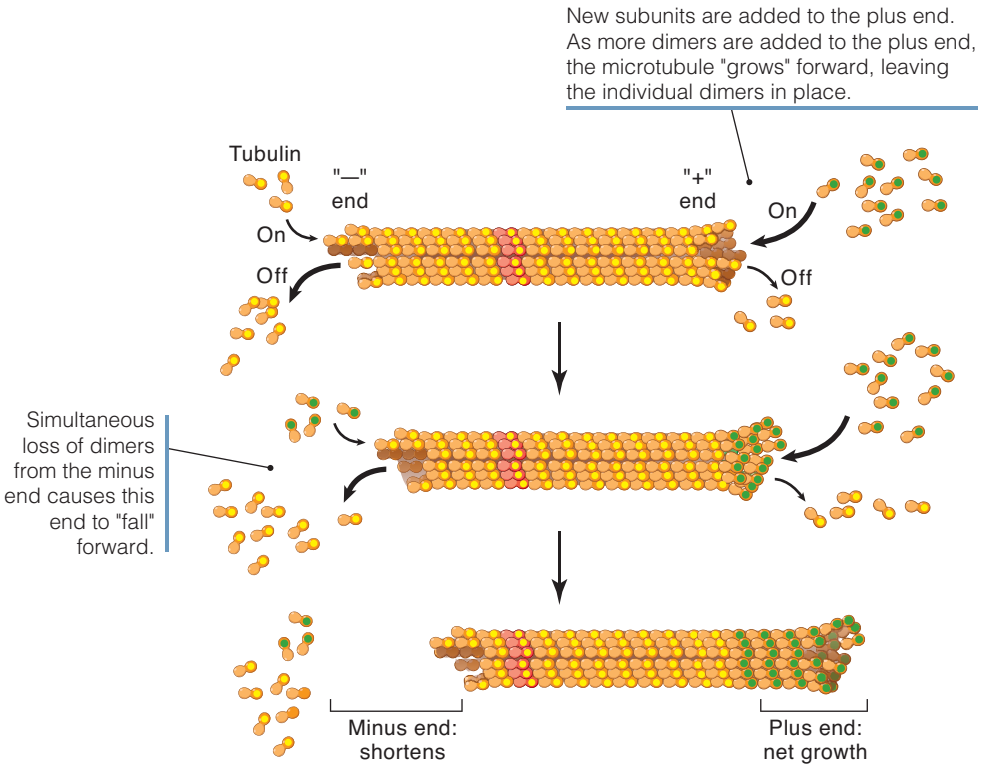


FIGURE 5-16 Treadmilling in microtubules.

fairly constant, but the dimers are always in flux, as shown in **FIGURE 5-16**. This process is called **treadmilling** because it somewhat resembles the motion of an exercise treadmill. But be careful about over interpreting this: an exercise treadmill *actually moves* (rotates), but a treadmilling microtubule doesn't move in the same way. To understand this concept, it is important to keep in mind that the *relative* position of a given dimer in a microtubule changes over time, even if the *absolute* position does not change; that is, when it is first added to the microtubule, a dimer lies at the extreme plus end, but over time, as more dimers are added on top of it, the dimer is now somewhere in the middle of the microtubule. Eventually, after enough other dimers have fallen off the minus end, the same dimer is now found at the minus end. Assuming that enough time has passed between its incorporation into the plus end and its appearance at the minus end, it switches from a GTP-bound state to a GDP-bound state, and eventually falls off. Though the name treadmilling has stuck, it might be more useful to consider the motion of a tread on a tractor as it moves: once a given part of the tread contacts the ground, it remains there, stationary, until it is pulled back up by the passing wheels, as shown in Figure 5-16.

Dynamic Instability Allows Cells to Explore Their Cytosol

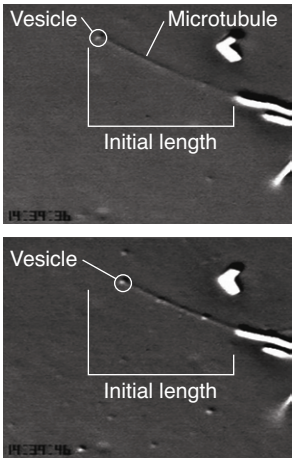
How does a microtubule undergoing dynamic instability benefit cells? If it won't hold still, how can it serve as any kind of useful "road" for molecular traffic? The answer, simply put, is that a moving microtubule isn't supposed to support trafficking. Instead, dynamic instability is a clever way to allow cells to do something we can't do with a conventional road: move it from location to location, according to where it is needed most; once it is stabilized in its new location, trafficking resumes. In some special cases, the stabilized road can be pushed or pulled, even as cargo is moving on it. This is very useful for cells that are undergoing major shape changes, or for cells attempting to convert their interphase

BOX 5-7

The fishing analogy. One way of understanding the value of repeatedly building and disassembling microtubules at the centrosome is to consider why someone using a fishing pole does something similar. Imagine fishing from a boat, by casting a hook at the end of a fishing line into the water. You might wait for a few seconds or minutes, and if no fish bites the hook, you reel the hook in and try again, perhaps in a different part of the water. This is similar to the behavior of the centrosome, in that microtubules are extended and then left to drift for a few seconds or minutes, and if a capping protein does not capture the plus end (i.e., bite the hook), the microtubule is disassembled (rather than "reeled in") and recycled so a new microtubule can emerge from the centrosome, perhaps in a different region of the cytosol.

microtubules into a mitotic spindle at the beginning of mitosis (BOX 5-7). In effect, it allows a cell to explore its cytosol as it changes position, or to redirect molecular traffic to a different region of the cell as necessary.

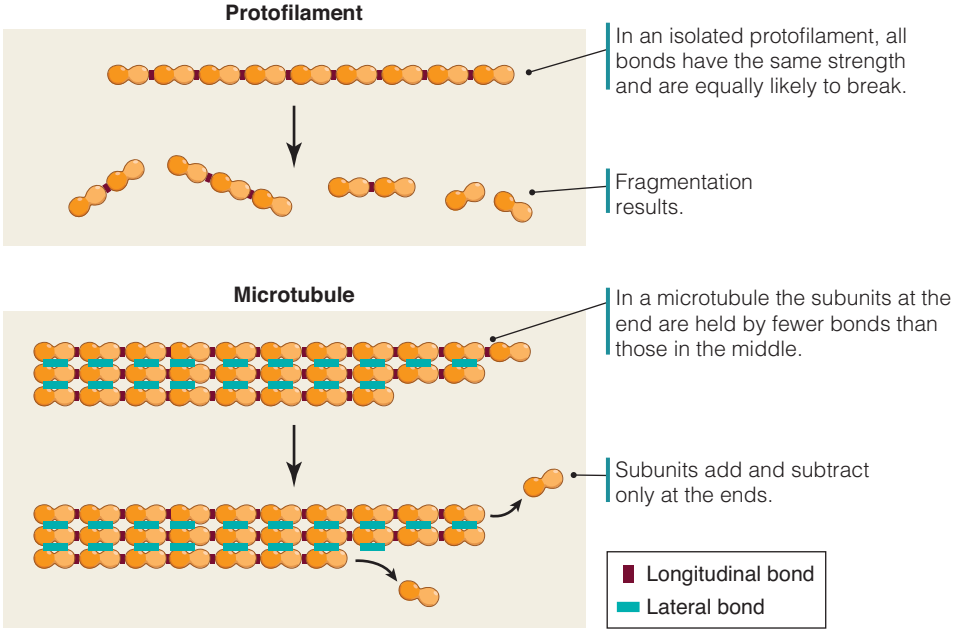
Another benefit of dynamic instability is that it allows cells to exert force. If a molecule can remain attached to the plus end of a moving microtubule, it can be transported through the cell as the microtubule shrinks or grows, as shown in FIGURE 5-17. The structural basis for this force is provided by extensive, noncovalent, intramolecular bonding in microtubules illustrated in FIGURE 5-18. Because the 13 protofilaments in a microtubule are stabilized by both longitudinal bonds (between successive dimers in the same protofilament) and lateral bonds (between dimers in adjacent protofilaments), microtubules are strong enough to transport even relatively large items, such as the vesicle shown in Figure 5-17.



In this figure, a shortening microtubule has a small vesicle attached at its end. As the microtubule shortens, the vesicle remains attached and is moved toward the nucleation site.

This was an *in vitro* experiment using purified tubulin added to diluted cytoplasm. *In vivo*, microtubule depolymerization may help move kinetochores during mitosis.

FIGURE 5-17 Microtubules exert enough force to move cargo by dynamic instability.
Photos courtesy of Lynne Cassimeris, Lehigh University.



In an isolated protofilament, all bonds have the same strength and are equally likely to break.

Fragmentation results.

In a microtubule the subunits at the end are held by fewer bonds than those in the middle.

Subunits add and subtract only at the ends.

FIGURE 5-18 Longitudinal and lateral bonds make microtubules strong.

Microtubule-Associated Proteins Regulate the Stability and Function of Microtubules

The capping proteins, rescue-associated proteins, and proteins that govern the motion in Figure 5-17 are examples of a large group of proteins called **microtubule-associated proteins (MAPs)**. Other examples of MAPs include proteins that crosslink, bundle, or sever microtubules; proteins that control microtubule stability; and proteins that link microtubules to other elements of the cytoskeleton. Some of these proteins bind only to the tips of microtubules, while others can bind along the entire length of a microtubule.

Microtubule-Based Motor Proteins Transport Organelles and Vesicles

Some of the most important MAPs are those that push/drag cargo along the length of a microtubule; called motor proteins, these are the proteins that perform the actual trafficking of molecules in a cell. In our analogy of microtubules serving as "roads," motor proteins often play the role of flatbed trucks. Actual cargo is attached to one side of these proteins, while the other side is responsible for determining the speed and direction of the movement. In some cases, this cargo may be a vesicle, such as those we discussed in Chapter 4, or, entire organelles such as the endoplasmic reticulum, Golgi apparatus, and mitochondria. During mitosis, condensed chromosomes are transported along the length of microtubules in the mitotic spindle to ensure their proper segregation to the resulting daughter cells; in other cases, the microtubule motor proteins remain stationary and move the microtubule. There is no easy example of this in our road analogy—it would be the equivalent of the trucks being held in place, spinning their wheels and moving the road beneath them.

The two families of microtubule motor proteins are called dyneins and kinesins, shown in **FIGURE 5-19**. Most dyneins move in the same direction, toward the minus end of a microtubule; most kinesins move toward the plus end of a microtubule, but a few move in the opposite direction. To accomplish this movement, the proteins use ATP energy, but more specifically, they adopt at least four different shapes, depending on their ATP/ADP and target-binding status, as shown for kinesins in **FIGURE 5-20**. The portion of each protein that binds to microtubules is called a head domain, and that is where ATP is used to generate the motion. Notice that the two heads swing past one another, very similar to the way a person walks. This is one of the rare instances where a cellular event (motor protein movement) very closely resembles an equivalent activity in our own lives (walking along a road). Also, notice that all movement is relative: if a microtubule is anchored (e.g., to a centrosome), the motor protein will move along it, but if the motor protein is anchored (we will see examples of this in later chapters), then the microtubule will move.

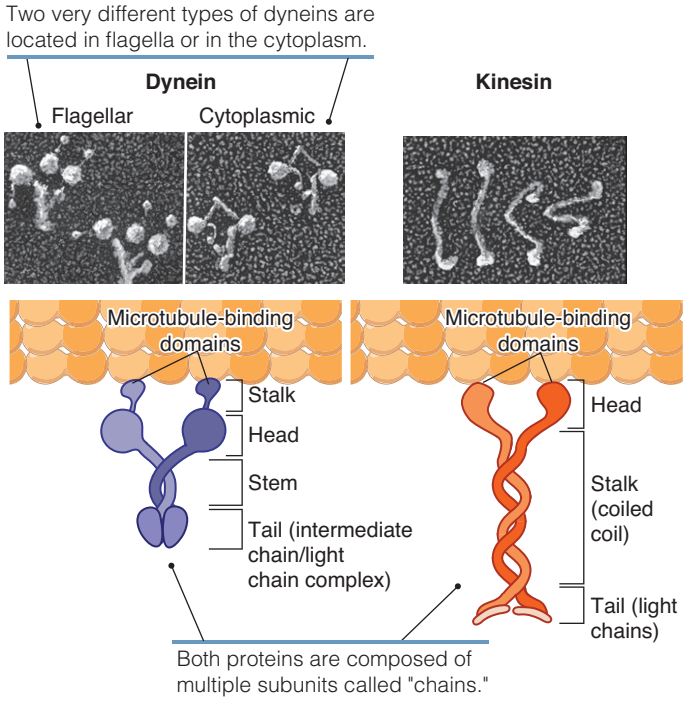


FIGURE 5-19 The structure of dynein and kinesin, the two most common motor proteins that bind to microtubules.
Photos courtesy of John Heuser, Washington University School of Medicine.

Microtubule-based transport in cultured cells is illustrated in **FIGURE 5-21**. In this figure, pigment granules are concentrated in the center of a pigment cell in response to a chemical stimulant. Notice that all of the granules move in the same direction with respect to the polarized organization of the microtubule network: they move toward the

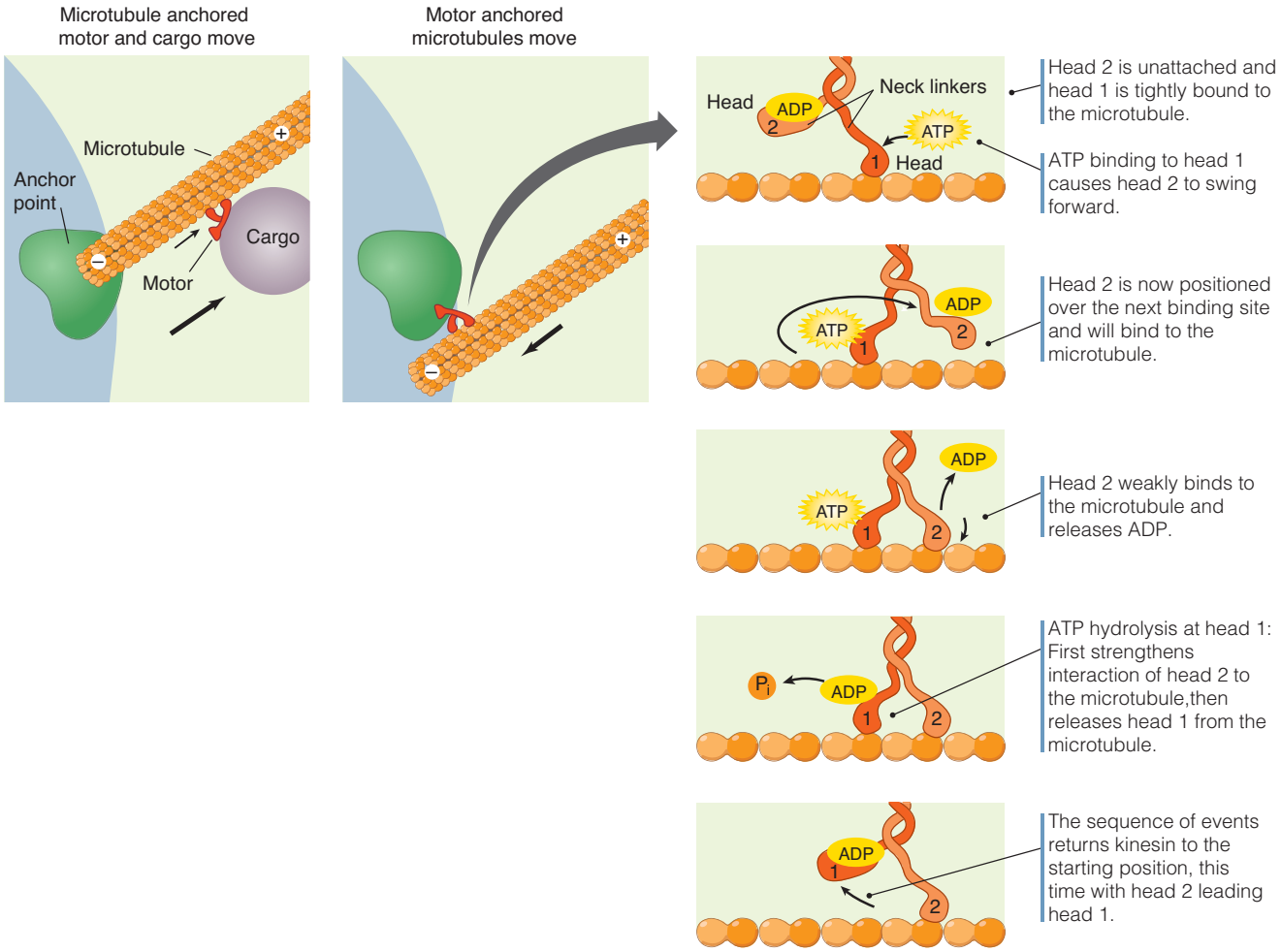


FIGURE 5-20 How a microtubule motor protein moves along a microtubule.

minus ends of the microtubules, which are attached to the centrosome. This illustrates another important trait of microtubule trafficking: individual motor proteins "walk" along microtubules in only one direction. When the stimulant was added to the pigment cell in Figure 5-19, only those motor proteins that walk toward the minus end were activated. If any plus-end motor proteins were attached to the pigment granules, they were inactive, and were therefore simply dragged along as excess baggage, as shown in **FIGURE 5-22**. Once the stimulant is removed (not shown in Figure 5-19), the plus-end motors are active again, and the pigment granules are dragged in the opposite direction, dispersing them throughout the cytosol. This is how some animals can rapidly change their coloring: when they condense pigment granules in their pigment cells, the cells adopt the color of the pigment, and dispersal of the granules changes the color of the cells back to their original state. We'll have a much closer look at the microtubule motor proteins in Chapters 7 and 9.

This microtubule trafficking network generates and maintains structural polarity in cells. No cell is perfectly symmetrical: an unbalanced distribution of cellular contents is often essential to cell function

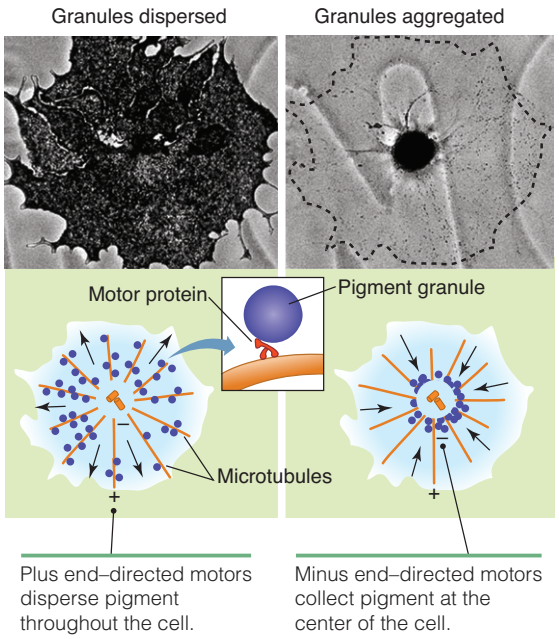
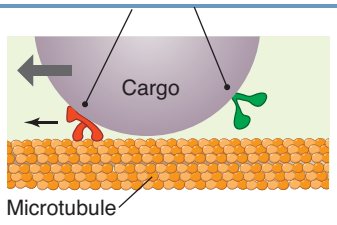
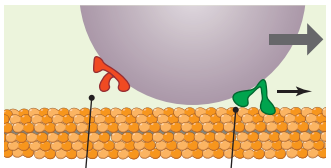


FIGURE 5-21 Microtubule motor proteins transport pigment granules in a pigment cell.
Photos courtesy of Vladimir Rodionov, University of Connecticut Health Center.

One motor protein binds both the cargo and the microtubule and actively transports in one direction, while the other does not bind the microtubule.





Switching between different motor proteins determines the direction that the cargo moves.

FIGURE 5-22 A model for biodirectional movement on a microtube.

BOX 5-8 TIP

Different definitions of the word "polarity." So far in this text, we have used the word polarity in several different contexts, and each has a different meaning. With reference to chemical bonds, the term polarity refers to an unequal distribution of electrons, such as are found between oxygen and hydrogen in water. This is *electrical polarity. Structural polarity* has several different meanings: it can mean an unequal distribution of atoms (the 5′ end of a nucleic acid has a different chemical structure than the 3′ end, for example), or it can be applied to larger structures, such as the arrangement of polypeptides in a protein, or the organization of proteins in a polymer. In this chapter, the structural polarity we are referring to concerns the three-dimensional arrangement of proteins in a polymer, such as intermediate filaments. A handy way to determine if a structure has this kind of polarity is to simply ask: Is one end of the structure the same as the other? For example, a single intermediate filament protein is polarized because it has an amino and a carboxy end; the dimer formed by these proteins is also polar, because the dimer has different ends. When the dimer forms a tetramer, however, the polarity disappears because now both ends of the tetramer have the same structure. Finally, the word *polarity* can be associated with entire cells, such as epithelial cells or neurons. These cells have very different regions on their plasma membranes, so one can always distinguish one side from the other. Skeletal muscle cells are less polarized, because they pull (contract) from both ends with the same structures. In fact, just about every cell is polarized at least to some degree.

(consider how useful a skeletal muscle cell would be if it spread its contractile proteins randomly throughout the cytosol). This distribution does not happen by accident. Rather, it represents a complex and carefully coordinated program of trafficking. This is especially evident in highly polarized cells such as epithelial cells and neurons (see Chapter 14 for more discussion of these cell types). It is speculated that the centrioles, which lie in the middle of the centrosome and are always oriented perpendicular to each other, may also contribute to establishing this polarized organization (**BOX 5-8**).

Cilia and Flagella Are Specialized Microtubule-Based Structures Responsible for Motility in Some Cells

In addition to coordinating molecular trafficking, some cells use microtubules to generate force *outside* the cell. The microtubules are clustered into special structures called cilia and flagella (singular: cilium, flagellum) (**BOX 5-9**). In single-celled organisms or specialized motile cells in multicellular organisms (e.g., sperm cells), these structures propel the cell through the aqueous environment, a motion often referred to as swimming; in stationary cells, the force is used to move fluid over the surface of the cell. Cilia and flagella are fairly similar in structure, and both share the same structure, called an **axoneme**, that organizes their microtubules (**FIGURE 5-23**). Axonemes are composed of a pair of central microtubules that run the length of the cilium, surrounded by nine pairs of microtubules that form a "cage" around the central pair. This is commonly referred to as a 9+2 arrangement. Both cilia and flagella move in a whip-like fashion in liquid, shown in **FIGURE 5-24**, and this is caused by the dynein-driven sliding of the peripheral microtubules past one another in a coordinated fashion. The extensive crosslinking of microtubules within the axoneme provides the strength necessary to resist the bending forces induced by the dynein motor proteins. The minus ends of the microtubules are anchored in an MTOC called the **basal body** that lies at the cytoplasmic end of these structures.

BOX 5-9 FAQ

Are prokaryotic cilia and flagella the same as those in eukaryotes? No. First, bacteria only form structures called flagella, not cilia. Also, despite the fact that prokaryotic and eukaryotic flagella appear to perform the same functions, they are completely different structures: prokaryotic flagella are composed of a protein called flagellin and contain no microtubules at all.

In mammals, a modified nonmotile cilium called the **primary cilium** forms on almost every cell. While it cannot "whip" like normal cilia (it lacks the central pair of

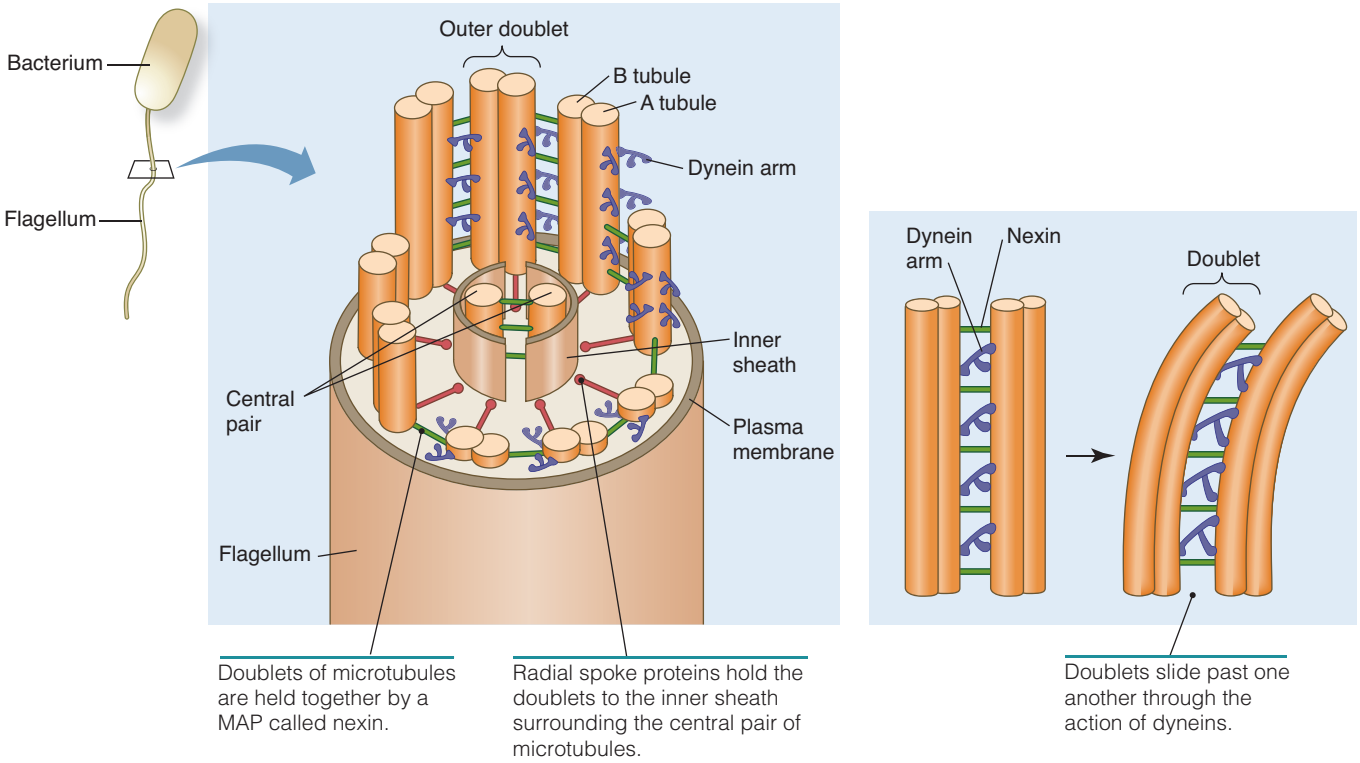


FIGURE 5-23 The structure of an axoneme.

microtubules), it is thought to play a central role in sensing extracellular stimuli, including photons of light, chemical "odorants," and shear forces in the extracellular space caused by fluid flowing past a cell.

CONCEPT CHECK 2

When microtubules are grown *in vitro* from purified tubulin proteins and poured onto a glass slide that has been coated with dynein or kinesin proteins, the microtubules appear to "surf" across the slide if ATP and GTP are also present. How can you explain this behavior? What would happen if you then added a large amount of ADP or GDP instead of ATP or GTP?

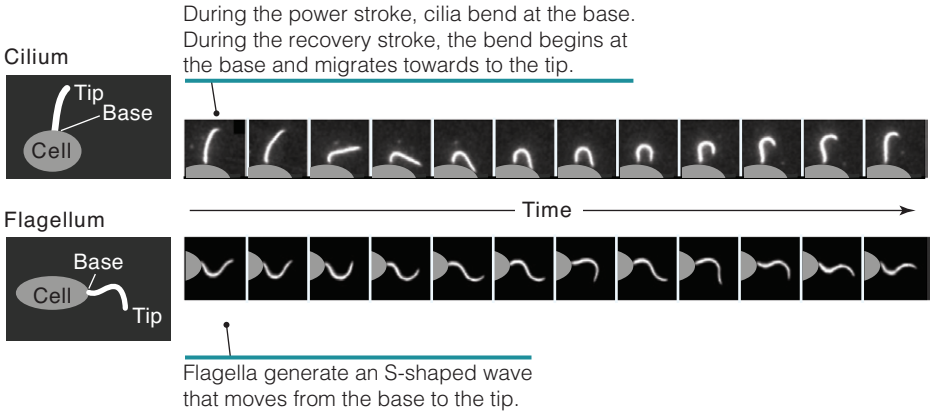


FIGURE 5-24 The coordinated motion of a cilium and a flagellum.
Photos courtesy of D. R. Mitchell, SUNY Upstate Medical University.

5.5 Actin Filaments Control the Movement of Cells

KEY CONCEPTS

- Actin filaments are thin polymers of actin proteins.
- Actin filaments are responsible for large-scale changes in cell shape, including most cell movement.
- Actin filament polymerization is initiated at numerous sites in the cytosol by actin-nucleating proteins.
- Actin filaments have structural polarity, which determines the direction that force is exerted on them by myosin motor proteins.
- The stability of actin filaments is determined by the type of adenine nucleotides bound by the actin proteins within them.
- Actin-binding proteins play numerous roles in controlling the location, stability, and function of actin filaments.
- Cell migration is a complex process, requiring assembly and disassembly of different types of actin filament networks.

The actin cytoskeleton is, in many ways, similar to the microtubule cytoskeleton. For example, both are composed of filaments made from globular protein subunits, and both are acted upon by motor proteins to induce cellular movement, but the kind of movement induced by the actin cytoskeleton is dramatically different from that associated with microtubules. Actin and its associated proteins have a much greater impact on the overall shape of a cell than microtubule-based movement, so we see them dominate in cells where *large-scale movement* is especially important, such as in muscle cells. In this section, we will follow the same format as that used for describing the other two cytoskeletal filaments, to help compare and contrast them.

The Building Block of Actin Filaments Is the Actin Monomer

The simplest building block of actin filaments is a monomeric actin protein. There are as many as six different isoforms of the actin protein (named α, β, γ, δ, etc.) expressed in a given cell, each encoded by a different (but highly homologous) gene. Actin monomers bind together to form an actin filament, also called a microfilament because it has the smallest diameter—7 nm—of the three cytoskeletal filament types. A microfilament is actually composed of two strands that are intertwined, shown in **FIGURE 5-25**, somewhat like the coiled coils formed by intermediate filament subunits. Both lateral and longitudinal bonds hold the actin monomers together; this suggests that these filaments share an important functional feature with intermediate filaments: they have great tensile strength, and they can resist pulling forces much better than microtubules can. But like microtubules, these filaments have structural polarity: the plus end is often called the barbed end, and the minus end is known as the pointed end. These names were coined by microscopists who used an electron microscope to examine actin filaments bound to another protein called myosin—the complex these two proteins form looks somewhat like an arrowhead, as shown in **FIGURE 5-26**.

As a rule, actin filaments are found wherever large-scale movement and/or tensile strength are required in a cell. Because these are common requirements, actin filaments are found in a wide variety of locations and in a myriad of configurations; some of these are shown in **FIGURE 5-27**. We will have a closer look at many of these structures in later chapters, when we can focus on how they help solve specific problems cells encounter.

ATP Binding and Hydrolysis Regulate Actin Filament Polymerization and Disassembly

Like tubulin proteins, actin monomers adopt at least two different shapes, and each is determined by the nucleotide phosphate bound to the monomer, plus a single divalent

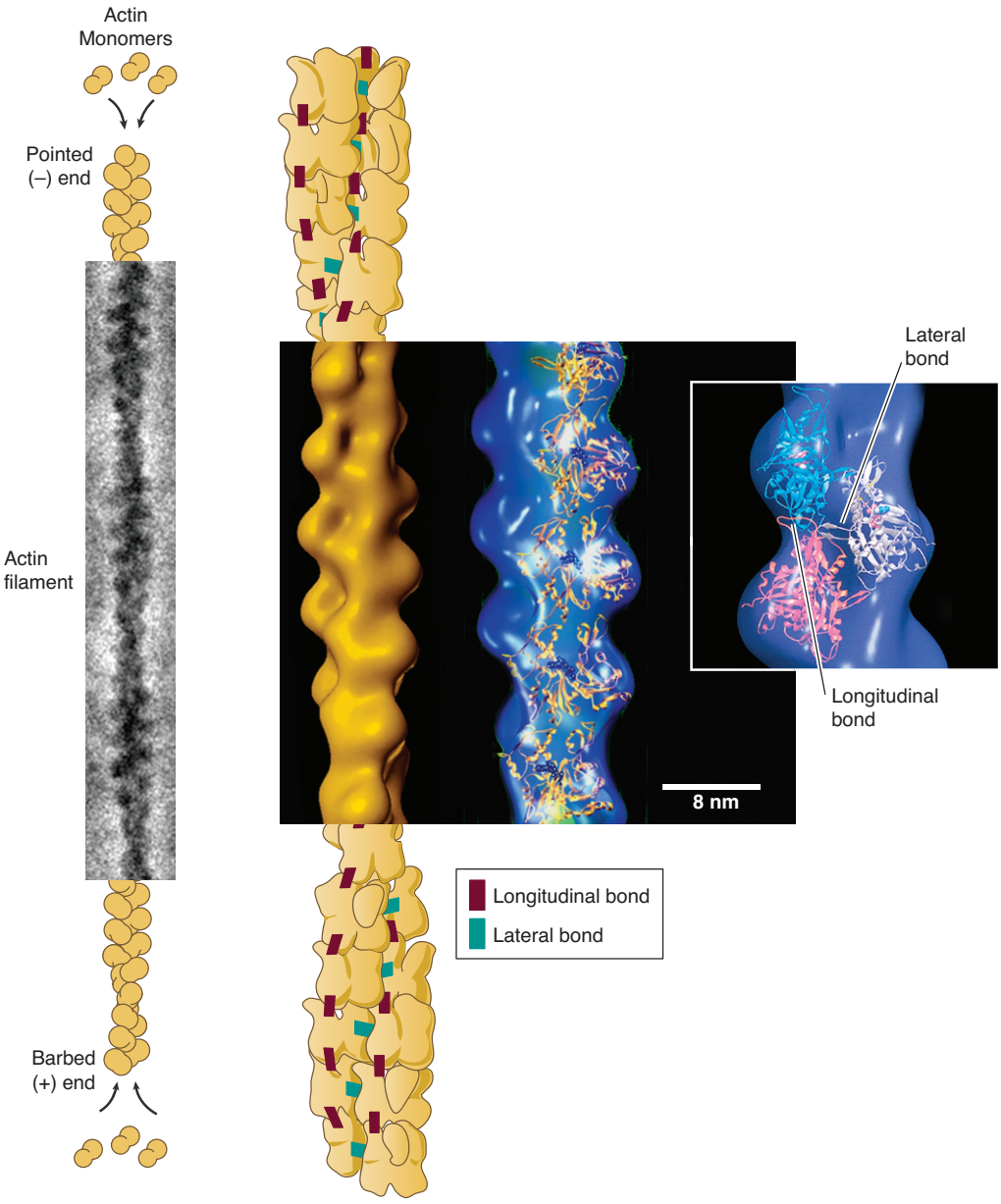


FIGURE 5-25 The general structure of an actin filament. The lateral and longitudinal bonds holding actin monomers together are indicated on the right.
Photos courtesy of Ueli Aebi, University of Basel.

cation (e.g., Ca^{+2}, Mg^{+2}). Actin proteins bind to adenosine nucleotides: when they bind to ATP, they adopt a configuration that *promotes* polymerization, and when they cleave the terminal phosphate to convert ATP to ADP, the protein changes to a shape that *discourages* polymerization. When the ADP-bound protein encounters a fresh ATP molecule, it drops the ADP and picks up the ATP. In short, actin monomers behave very similar to α-β tubulin dimers, except they bind to ATP/ADP instead of GTP/GDP. This ATP/ADP is held in a cleft in the middle of the monomer, as shown in **FIGURE 5-28** and discussed in **BOX 5-10**.

Actin Polymerization Occurs in Three Stages

Like microtubules, much has been learned about actin polymerization by using purified proteins *in vitro*. The polymerization mechanisms of these two cytoskeletal proteins are

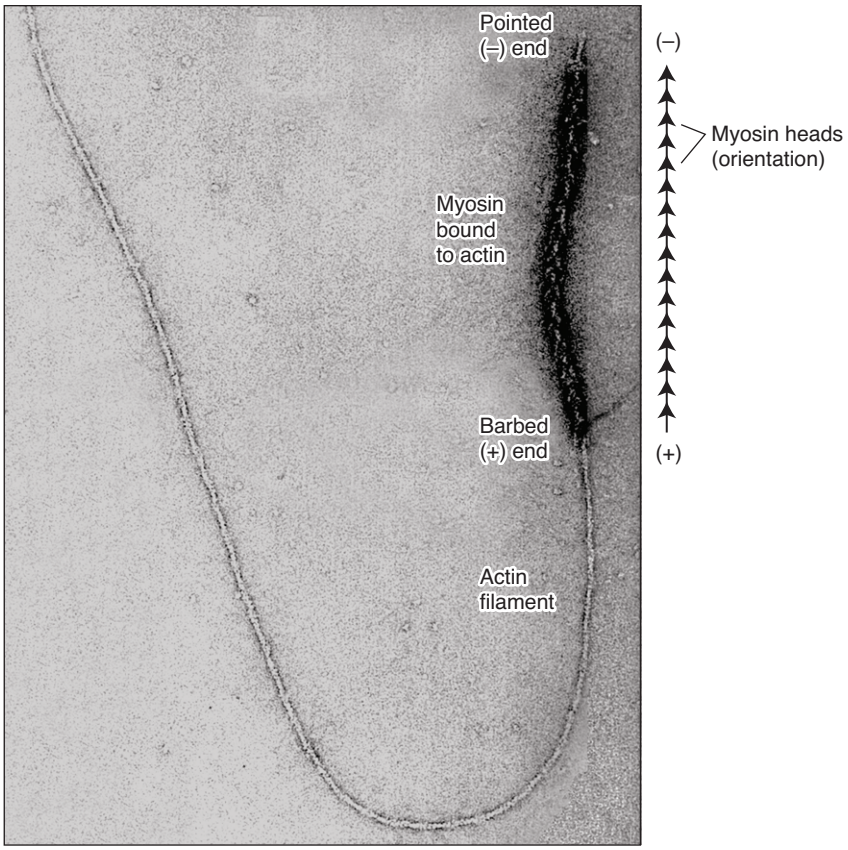


FIGURE 5-26 An electron micrograph of an actin filament partially coated with myosin proteins.
Photo courtesy of Marschall Runge, John Hopkins School of Medicine and Thomas Pollard, Yale University.

strikingly similar, so we can capitalize on our earlier discussion of microtubule assembly to help us learn how actin behaves. For example, actin filaments form spontaneously once a critical concentration is reached, and filament assembly occurs in the same three stages we saw for microtubules, as demonstrated in **FIGURE 5-29**. First, nuclei form from the binding of three actin proteins, and then additional monomers are added to the nuclei (called the elongation stage) until a steady state is reached, where the rate of monomer addition is equal to the rate of disassembly.

Actin Filaments Have Structural Polarity

In a growing actin filament, the ATP-bound monomers are preferentially added to the barbed (plus) end. They align in a front-to-back orientation with the monomers at the end of the filament, and this gives the entire filament the same kind of structural polarity we see in microtubules. This polarity confers the same advantage to microfilaments that we saw in microtubules: motor proteins that bind to actin filaments can easily determine which direction to pull on them. We will examine the motor proteins in greater detail below.

Actin Filaments Undergo Treadmilling

Yet another similarity between actin filaments and microtubules is that both undergo treadmilling. Notice how closely the treadmilling shown in **FIGURE 5-30** resembles that shown in Figure 5-16. As you might expect, the ATP-bound monomers added to the plus end

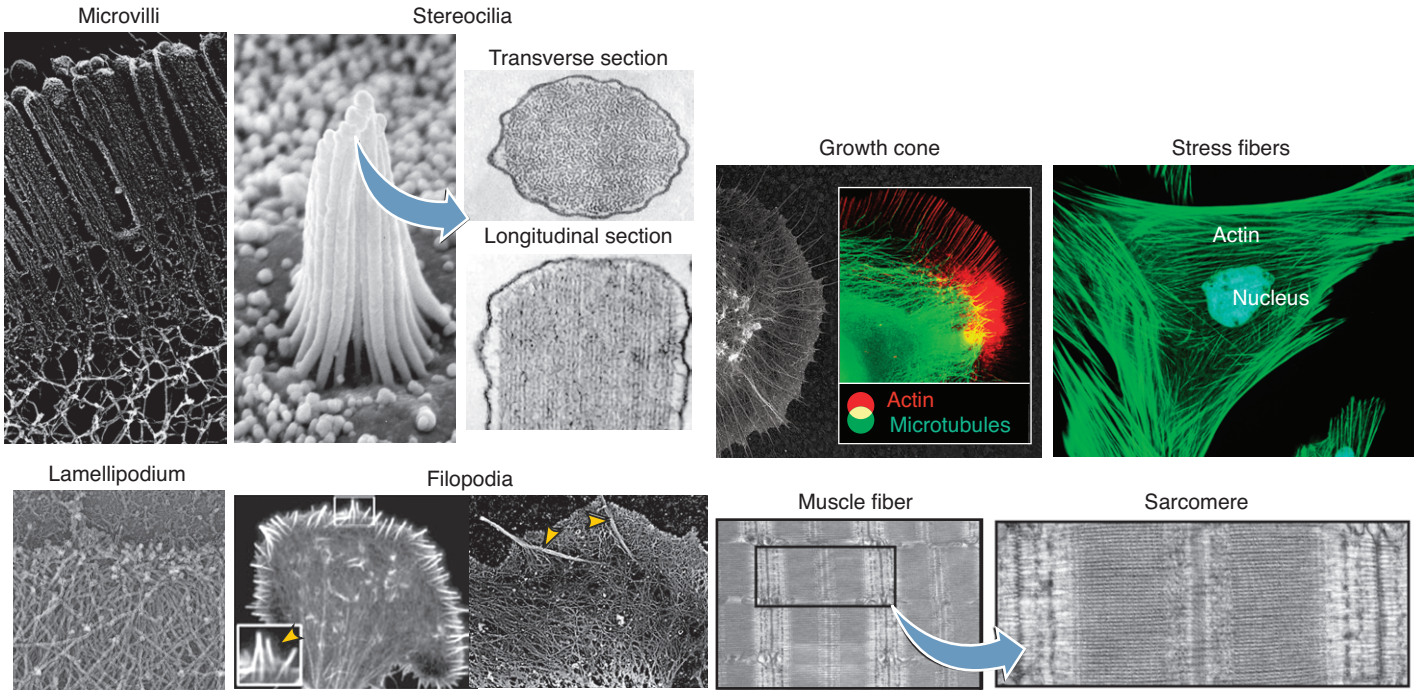


FIGURE 5-27 A number of different actin filament–based structures in cells.

Neuronal growth cone photos © Schaefer, Kabir, and Forscher, 2002. Originally published in *The Journal of Cell Biology*, 158: 139–152. Used with permission of Rockefeller University Press. Photos courtesy of Paul Forscher, Yale University. Neuronal growth cone photos © Schaefer, Kabir, and Forscher, 2002. Originally published in *The Journal of Cell Biology*, 158: 139–152. Used with permission of Rockefeller University Press. Photos courtesy of Paul Forscher, Yale University. Photo of cell with stress fibers courtesy of Michael W. Davidson and Florida State University Research Foundation. Lamellipodium photo courtesy of Tatyana M. Svitkina, University of Pennsylvania. Filopodia photos reprinted from *Cell*, vol. 118, M. R. Mejiliano, et al., Lamellipodial versus filopodial mode of the actin . . . , pp. 363–373, Copyright (2004) with permission from Elsevier [http://www.sciencedirect.com/science/journal/00928674]. Photo courtesy of Tatyana M. Svitkina, University of Pennsylvania. Filopodia photos reprinted from *Cell*, vol. 118, M. R. Mejiliano, et al., Lamellipodial versus filopodial mode of the actin . . . , pp. 363–373, Copyright (2004) with permission from Elsevier [http://www.sciencedirect.com/science/journal/00928674]. Photo courtesy of Tatyana M. Svitkina, University of Pennsylvania./science/journal/00928674]. Photo courtesy of Tatyana M. Svitkina, University of Pennsylvania. Electron micrograph of microvilli © Hirokawa, et al., 1982. Originally published in *The Journal of Cell Biology*, 94: 425–443. Used with permission of Rockefeller University Press. Photos courtesy of John E. Heuser, Washington University in St. Louis. Scanning micrograph of stereocilia reproduced from A. J. Hudspeth and R. Jacobs, *Proc. Natl. Acad. Sci. USA* 76 (1979): 1506–1509. Photo courtesy of A. J. Hudspeth and R. A. Jacobs. Electron micrographs of stereocilia © Tilney, DeRosier, and Mulroy, 1980. Originally published in *The Journal of Cell Biology*, 86: 244–259. Used with permission of Rockefeller University Press. Photos courtesy of Michael J. Mulroy, Medical College of Georgia.

eventually hydrolyze the ATP to form ADP, and once these monomers reach the minus end, they tend to fall off. One important difference between actin filaments and microtubules is that actin filaments are not generally nucleated by a single structure in cells, and so there is no equivalent to an MTOC for actin. As a result, many more actin filaments have exposed minus ends, and treadmilling is therefore more likely for actin filaments than for microtubules.

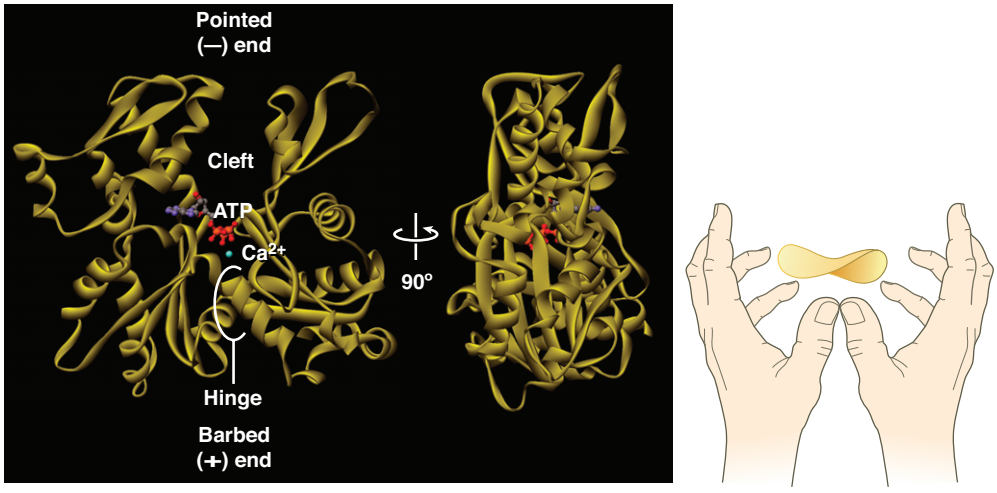


FIGURE 5-28 The structure of an actin monomer. Left, a ribbon model, derived from a crystallized form of the protein. Right is an analogy of an actin monomer as a pair of hands.

Structures from Protein Data Bank 1ATN. W. Kabsch, et al., *Nature* 347 (1990): 21–22.

BOX 5-10

The clasping hands analogy. One simple way to visualize the structure of actin is to press your hands together at the base of your thumbs so that they form a clam-shaped structure that can open and close by pivoting at the base (see Figure 5-28). Next, pick up a small potato chip with your two hands and have it settle into your palm: this is the equivalent of an actin protein bound to ATP. Holding the chip in your hands keeps your palm slightly open and keeps your hand in a shape that somewhat resembles two parentheses: (). This is the shape of actin when it prefers polymerization, which would be roughly equivalent to having your two hands grasp the base of someone else's hand held the same way.

Now, squeeze your palms together to break the chip (no need to crush it, just break it). This is the equivalent of cleaving the ATP to ADP. Notice that your hands are now flatter, more like two parallel lines: ||. Your hands are less inclined to grab someone else's, because there is less space between them. To switch back to the ATP-bound form, you simply drop the broken chip and pick up a new one. Actin never tries to glue the broken chip (ADP) back together (to make ATP); that's the job of the metabolic enzymes, which we will discuss in Chapter 7.

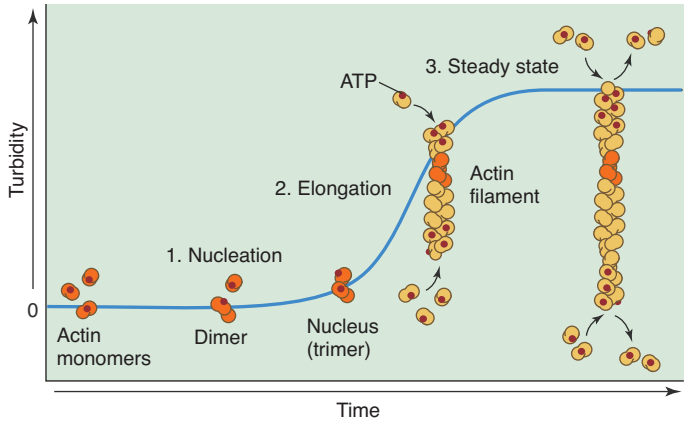


FIGURE 5-29 The three stages of actin filament assembly *in vitro*.

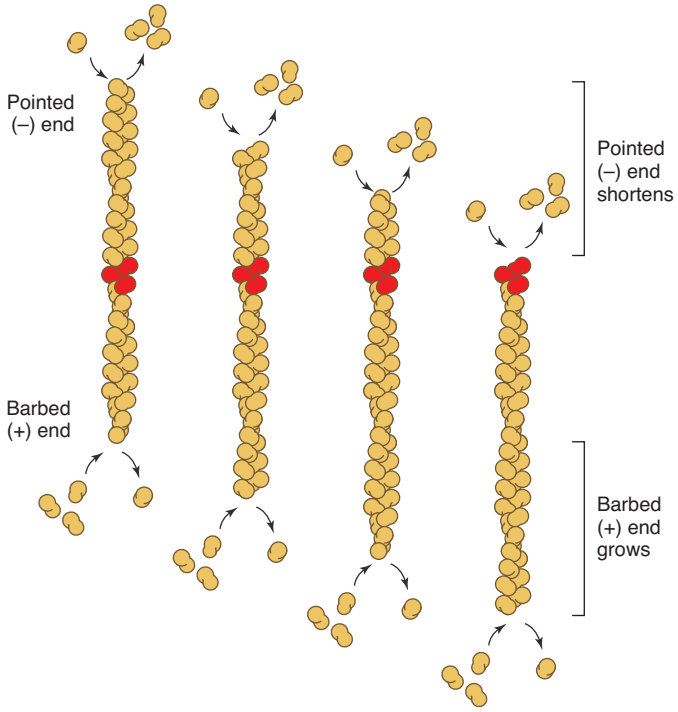


FIGURE 5-30 Treadmilling in actin filaments. Note the similarity of this treadmilling with that shown for microtubules in Figure 5-16.

The abundance of free minus ends in the actin cytoskeleton requires us to look more closely at how they behave in cells. Technically speaking, each end of an actin filament has its own critical concentration. Because ATP–actin monomers prefer to add to the plus end, we can conclude that the critical concentration for the plus end is lower than that for the minus end—it takes a lower concentration of ATP–actin to reach the polymerization point at the plus end. If we increase the ATP–actin concentration enough, we will reach the critical concentration for the minus end as well, and actin monomers will begin adding to both ends. Notice that treadmilling of actin therefore only happens when the concentration of ATP–actin monomers falls between the two critical concentrations.

This matters because it offers cells a tool to help control the organization of their cytoskeleton. If they require rapid assembly of a strong cytoskeletal filament in a new region of the cytosol, they can trigger the disassembly of existing actin filaments by simply reducing the amount of free ATP–actin monomers. This can be done in several ways, including binding of free monomers by actin-binding proteins. Recall that the disassembly of the other strong filaments, the intermediate filaments, requires phosphorylation of the subunits. The strategy for removing actin filaments is much simpler and faster. The corollary to this is that assembly of new actin filaments is likewise much faster, because they can initially grow from both ends when the pool of ATP-actin is highest, then level off once steady state is reached.

The main point here is simply that treadmilling is an especially important trait for actin filaments, and we will see examples of it being put to use in many different instances. When we discuss actin filaments in other chapters, keep in mind that treadmilling will often be invoked to help explain how these filaments function.

Six Classes of Proteins Bind to Actin to Control Its Polymerization and Organization

Given how dynamic actin filaments can be, for them to be of any use, cells must be able to control when they form, where they form, how fast they form, their orientation with respect to one another (and other cellular structures), and so on. That's a tall order for a filament made out of only one protein. Recall from Chapter 3 that posttranslational modification is a common method for controlling protein shape and function. Actin proteins are modified by phosphorylation, alkylation, and formation of intrachain disulfide bonds, but because actin filaments are used in so many different cellular structures (recall Figure 5-27), even these modifications are not enough to fully control actin filaments. A tremendous number (close to 300, by some estimates) of **actin-binding proteins** are expressed by cells to assist. We cannot possibly discuss them all, but we can have a look at some representatives. The actin-binding proteins are grouped into six classes, according to the effects they have on actin filaments.

Monomer-Binding Proteins Regulate Actin Polymerization

Perhaps the simplest way to regulate actin filament behavior is to simply control how many actin monomers are available for polymerization; this is the role played by **monomer-binding proteins**. In metazoan cells, the two most abundant proteins in this class are thymosin β4 and profilin. Thymosin β4 binds to actin monomers (in a 1:1 ratio) and prevents the monomers from polymerizing, even if the monomer is also bound to ATP. This is an excellent example of how the function of one protein is to bind to and control the function of another. Profilin plays a slightly different role. It too binds 1:1 with actin monomers, but only prevents the monomer from binding to the minus end of an actin filament. Because it still has a free minus end, a monomer bound to profilin can still add to the plus end, as shown in FIGURE 5-31. This is a good example of how a cell can drive actin filaments to grow in a preferred direction.

Nucleating Proteins Regulate Actin Polymerization

For all three types of cytoskeletal filaments, the rate-limiting step is always the initial nucleation process. Small clusters of subunits are quite unstable, and easily fall apart. The role of actin **nucleating proteins** is to stabilize these clusters to facilitate the formation of actin nuclei. Some of the best-known nucleating proteins belong to a multi-subunit cluster called the ARP2/3 complex, so named because it contains *a*ctin *r*elated *p*roteins 2 and 3, plus five other proteins. The ARP2/3 complex, in conjunction with several other regulatory proteins, initiates the formation of actin nuclei. Interestingly, it does this by binding to the side of an existing actin filament, and therefore promotes the formation of branched actin filaments networks, as shown in FIGURE 5-32. Other nucleating proteins can initiate the formation of new linear filaments as well.

Capping Proteins Affect the Length and Stability of Actin Filaments

Capping proteins inhibit actin filament elongation by binding to either the plus or minus ends of the filaments. They can also prevent the shortening of filaments by inhibiting loss

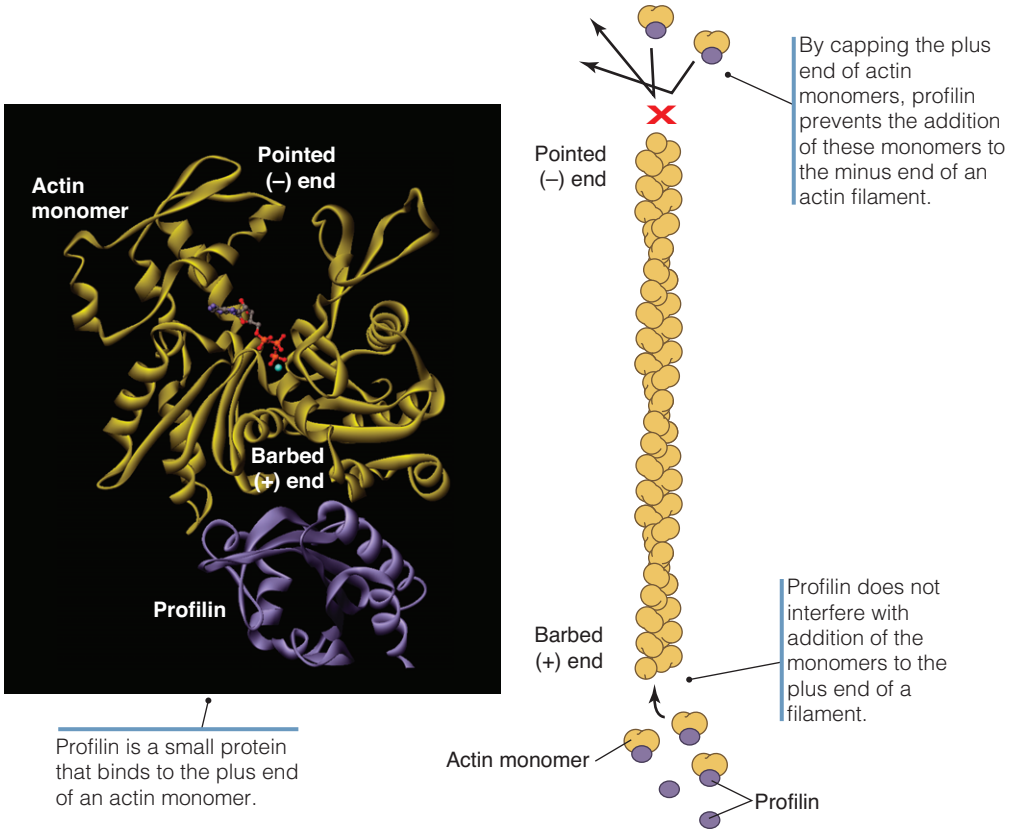


FIGURE 5-31 The structure and function of profilin, an actin monomer-binding protein.
Structure from Protein Data Bank 2ITF. J. C. Grigg, et al., *Mol. Microbiol*. 63 (2007): 139–149.

of monomers from the ends. This is important because it prevents the runaway polymerization/depolymerization of actin, and permits cells to build up a high concentration of monomers without risking their random attachment to existing filaments. In short, it uncouples actin filament growth from the concentration of monomers in the cytosol. When used with the monomer-binding proteins, these proteins give cells considerable latitude for deciding when and where to build new actin filaments. Examples of capping proteins include CapZ and gelsolin, and we will see how muscle cells use CapZ to control the length of actin filaments in striated muscle cells in Chapter 14.

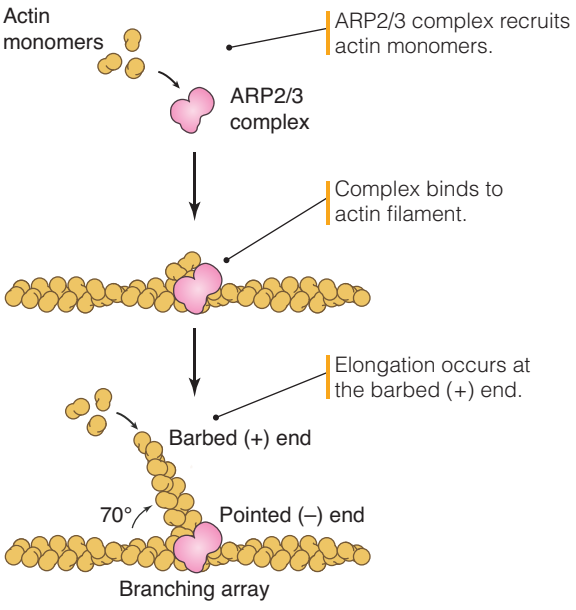


FIGURE 5-32 ARP2/3 nucleates the formation of a new actin filament off the side of an existing filament.

Severing and Depolymerizing Proteins Control Actin Filament Disassembly

As important as actin filaments are, there are times when their presence is a problem for a cell. For example, when a cell divides, it must eventually split into two, and the presence of long actin filaments in the cytosol of the dividing cell can interfere if these filaments are longer than the diameter of the resulting daughter cells. Likewise, when a cell is actively migrating, it literally has to pick itself up and crawl forward; any long actin filaments that remain in place once the cell begins to move must be disassembled or the cell runs the risk of ripping itself apart. For these reasons, cells usually keep a small pool

of **depolymerizing proteins** on hand to facilitate the rapid breakdown of the filaments. Cofilin and other members of the actin depolymerizing factor (ADF) family are a widely used group of depolymerizing proteins. **Severing proteins** are aptly named—they break actin filaments all along the length of the filaments (rather than at the ends), and when combined with the depolymerizing proteins, can dissolve a network of actin filaments in seconds.

Cross-Linking Proteins Organize Actin Filaments into Bundles and Networks

Thus far, we've focused our attention on actin-binding proteins that target one filament at a time. The last class of actin-binding proteins contains those that work with *groups* of actin filaments. These proteins are called **cross-linking proteins**, because their function is to form linkages between actin filaments. It is important to point out that these links are *not* the covalent bonds that sometimes cross link proteins. In this case, the cross links are the actin-binding proteins themselves, and the bonds holding the filaments to the cross-linking proteins are noncovalent. Actin cross-linking proteins are classified into the three groups shown in **FIGURE 5-33**. The cross-linked filaments can take the form of tight parallel bundles, loose bundles, or branched networks, as **FIGURE 5-34** shows.

Group	Protein	Molecular Weight (kDa)	Location
I	Fascin	55	• Acrosomal process • Filopodia • Lamellipodia • Microvilli • Stress fibers
	Scruin	102	• Acrosomal process
II	Villin	92	• Intestinal and kidney brush border microvilli
III Calponin homology-domain superfamily	Fimbrin	68	• Adhesion plaques • Microvilli • Stereocilia • Yeast actin cables
	Dystrophin	427	• Muscle cortical networks
	ABP120 (dimer)	92	• Pseudopodia
	α-actinin (dimer)	102	• Adhesion plaques • Filopodia • Lamellipodia • Stress fibers
	Filamin (dimer)	280	• Filopodia • Pseudopodia
	Spectrin (tetramer)	α 280 β 246–275	• Cortical networks

FIGURE 5-33 Actin cross-linking proteins are organized into three groups, based on the way they bind actin filaments.

Actin-Binding Motor Proteins Exert Force on Actin Filaments to Induce Cell Movement

To help distinguish actin from the other two cytoskeletal networks in cells, we'll again emphasize that actin and its associated proteins are responsible for the large-scale movement of cells. To move an entire cell is an enormous undertaking, especially when one considers how carefully organized it is. The exact mechanisms cells use to control their movements are likely as varied as the cells themselves, but we can nonetheless discuss some common features shared by almost all moving cells.

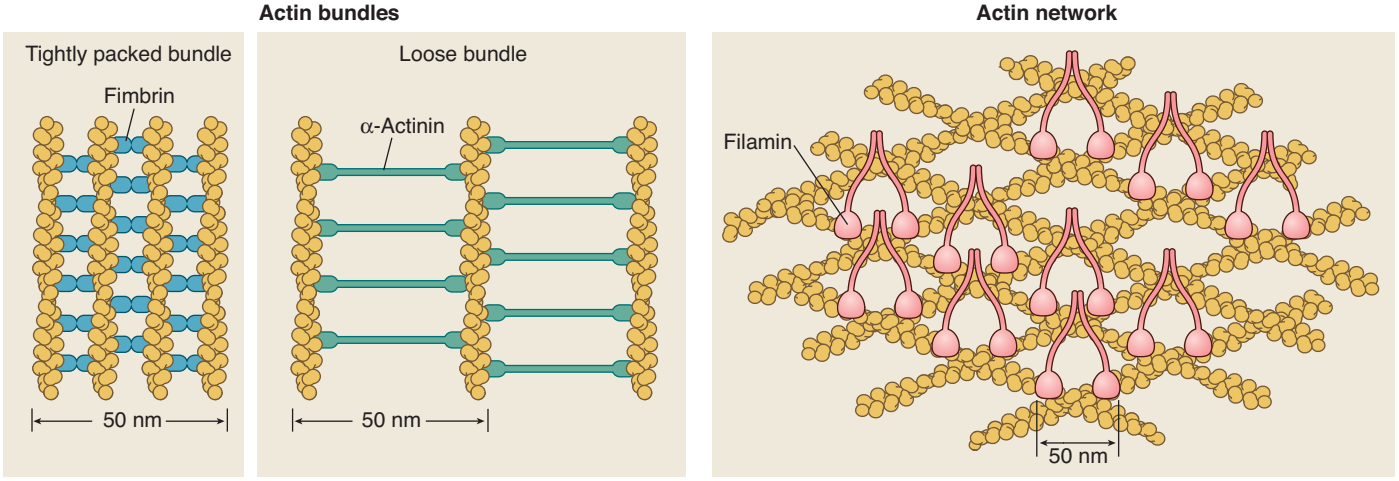


FIGURE 5-34 Three forms of cross-linked actin filaments created by different cross-linking proteins.

Central to all directed movement is the need for some form of propulsive force. Actin-binding motor proteins provide this force by converting the metabolic (potential) energy stored in ATP into kinetic energy (actual motion). These proteins are so special that they are not generally grouped with the six classes of actin-binding proteins we've just discussed. This special status is based on a simple fact: without these motor proteins, most actin networks (regardless of how they were organized) would be practically useless. The one feature all of these motor proteins possess is the ability to reversibly bind to actin filaments and to exert force on them during the period when they are bound. This results in a bind-move-release-shift cycle for these proteins, not unlike the way a person tugs on a rope. Also, these motors can cooperate, like tug-of-war teams. The more motor proteins that are used, the stronger the force a cell can exert on its actin network.

Motor-based motion serves a tremendous number of purposes for cells. During development, when a multicellular organism is still only a disorganized cluster of cells, actin-based movement is responsible for reorganizing the cluster into a primitive body plan. Organisms deprived of this early cell-migratory ability die well before maturity; in mammals, this is known as an embryonic lethal phenotype. Later in development, cells must be able to move away from the central mass to create distinct organs, limbs, and the networks (nervous system, circulatory system, lymphatic system) that connect them. Even in adulthood, cell migration is absolutely essential—it permits some cells to heal a wound, and others to maintain an active immune system. Even perfectly normal tissues require cell movement to remain healthy; for example, the cells lining our gastrointestinal (GI) tract are constantly being replaced by cells migrating from deeper layers in the GI lining. Some highly specialized motor proteins even control molecular trafficking by moving cargo along actin filaments, analogous to the function of microtubule motor proteins.

Cell Migration Is a Complex, Dynamic Reorganization of an Entire Cell

Rather than attempt to describe how every cell moves, let's envision a generic, eukaryotic animal cell and use it to integrate some well-known aspects of cell migration into a model of how most real cells move. Keep in mind that the goal here is not to comprehensively understand how any given cell moves, but simply to put what we've learned about the cytoskeleton to work and see how simple concepts can be combined to reveal a portion of a cell's elegant inner workings.

We can start by deciding what kind of cell we want to move. Let's make it the simplest cell possible, by throwing out any specialized structures that aren't necessary, such as cilia or flagella, special sensors for environmental stimuli (e.g., light-sensing structures in the visual system), or highly specialized cell shapes (e.g., the elongated shape of many neurons, the cell–cell junctions that hold epithelial cells together). What we're left with is a slightly oval-shaped cell that can move in any direction it pleases, with only the most basic structural organization.

Now, consider what it will take to move this cell. Keep in mind that this cannot be a rash decision; we already know from previous chapters that almost every part of a cell is connected to some other part, so randomly moving one part of the cell forward could have drastic consequences for the rest. Cell migration is therefore carefully orchestrated, ensuring that every part of a cell remains as functional as possible. Let's use an analogy: if each of us was roughly the size of an average protein in our generic cell, that cell would be at least as big as any major sports stadium in the United States. Imagine attending a game at such a stadium, then being told that the entire stadium would have to be moved two miles (or kilometers) in one direction while the game continued. Seems nearly impossible, doesn't it?

In one sense, it really is impossible, as stadiums aren't designed to be moved. Let's imagine how we would construct a building of that size that could be moved at will. Most of our building materials should possess at least one of these traits:

- **Modular Construction**. The structure should be almost as easy to take apart as it is to assemble. Let's make almost nothing permanent, so we would stay away from concrete as a building material, for example.
- **Lightweight and Strong**. Concrete is strong, but it is used primarily to resist compressive forces (pushing). We want our materials to resist shear forces, tensile strain (pulling), *and* compression. Not only is concrete heavy, but pulling forces can easily crack it.
- **Elasticity.** Elastic materials can absorb kinetic energy by deforming without breaking, as well as spontaneously return to their original shape once the kinetic energy is removed. Elasticity would give us some latitude when we start moving large sections of our stadium/cell.

Recall that almost all of the cellular molecules we have described so far in this book possess at least one of these three traits. We can briefly describe how our imaginary stadium could be moved: we would first partially disassemble the largest stadium structures, and then apply some motive force to all of the pieces to move them forward. Remember that we can't push or pull the structure from the outside, because cells can't do that, either; all of the motion has to be generated *inside* the plasma membrane. And the plasma membrane has to remain intact throughout the entire process, of course, or the cell would rupture and die. If this sounds a bit daunting, it should, because it's a tougher problem than most building contractors ever have to face.

Migrating Cells Produce Three Characteristic Forms of Actin Filaments: Filopodia, Lamellipodia, and Contractile Filaments

Let's leave the analogy for a while, and return to the cellular solution to this problem. How does a cell generate enough force to move it a long distance without help from an outside (pushing/pulling) force? Fortunately, the answer is elegantly simple: cells use actin filaments and their associated motor proteins to both push and pull themselves forward. To explain this, we will need actin-binding proteins and actin-based motor proteins to help control the location, shape, and size of actin filament networks.

Cell migration begins when a cell pushes a portion of its plasma membrane outward. This pushing force comes from the polymerization of actin filaments near the plasma membrane, and it requires the coordinated action of several different actin-binding proteins: nucleating proteins initiate filament formation, capping proteins cap the minus ends to prevent filament breakdown, severing and disassembly proteins dissolve existing actin filaments to provide new monomers, monomer-binding proteins release their monomers to add to the plus ends, and cross-linking proteins organize the filaments into networks. As the filaments grow, they distort the plasma membrane; the elasticity provided by the hydrophobic interactions between the membrane phospholipids helps ensure that the membrane does not rip when the actin filaments push against it.

One type of actin cluster that pushes the plasma membrane is called a **filopodium** (plural, **filopodia**), which means "thread-like foot" (BOX 5-11). These structures are thin, parallel bundles of filaments that all have the same polarity, with the minus ends pointing toward the cytosol, and the plus ends pointing toward the plasma membrane, as shown in FIGURE 5-35. A second type of actin cluster is called a **lamellipodium** (plural, **lamellipodia**), which means "sheet-like foot." Lamellipodia contain branched networks of filaments, held together by different cross-linking proteins than those found in filopodia. Lamellipodia cause broad, relatively shallow distortions of the plasma membrane.

BOX 5-11

Clarifying the "foot" vs. "hand" comparisons. Part of the difficulty in understanding actin dynamics comes from the way actin structures are described. The names of most actin structures were developed by microscopists, who were the first biologists to see them. Because some of the structures cause the plasma membrane to protrude, they looked a bit like the "feet" that protrude from the surfaces of some furniture, appliances, electronics, etc., that support or stabilize them. This was unfortunate, however, in that the word "foot" conjures up more than this image for most of us. For example, our feet can be used for walking, of course, but the "feet" that actin structures form *do not* function like that at all. Humans *push* off of their feet to move forward and backward, but cells do not; the only "pushing" equivalent for actin is the initial process of building the feet, by polymerizing the actin. However, once these feet have been built, they never push again. Cells always *pull* with these "feet," in the sense that the feet serve as anchors for tugging the rest of the cell behind them, and the natural analogy for pulling is the hand/arm. So, be careful to distinguish between the appearance-based names for these structures, and the analogous functions they perform. Actin protractions may look like a foot at the end of a leg, but they function like a hand at the end of an arm.

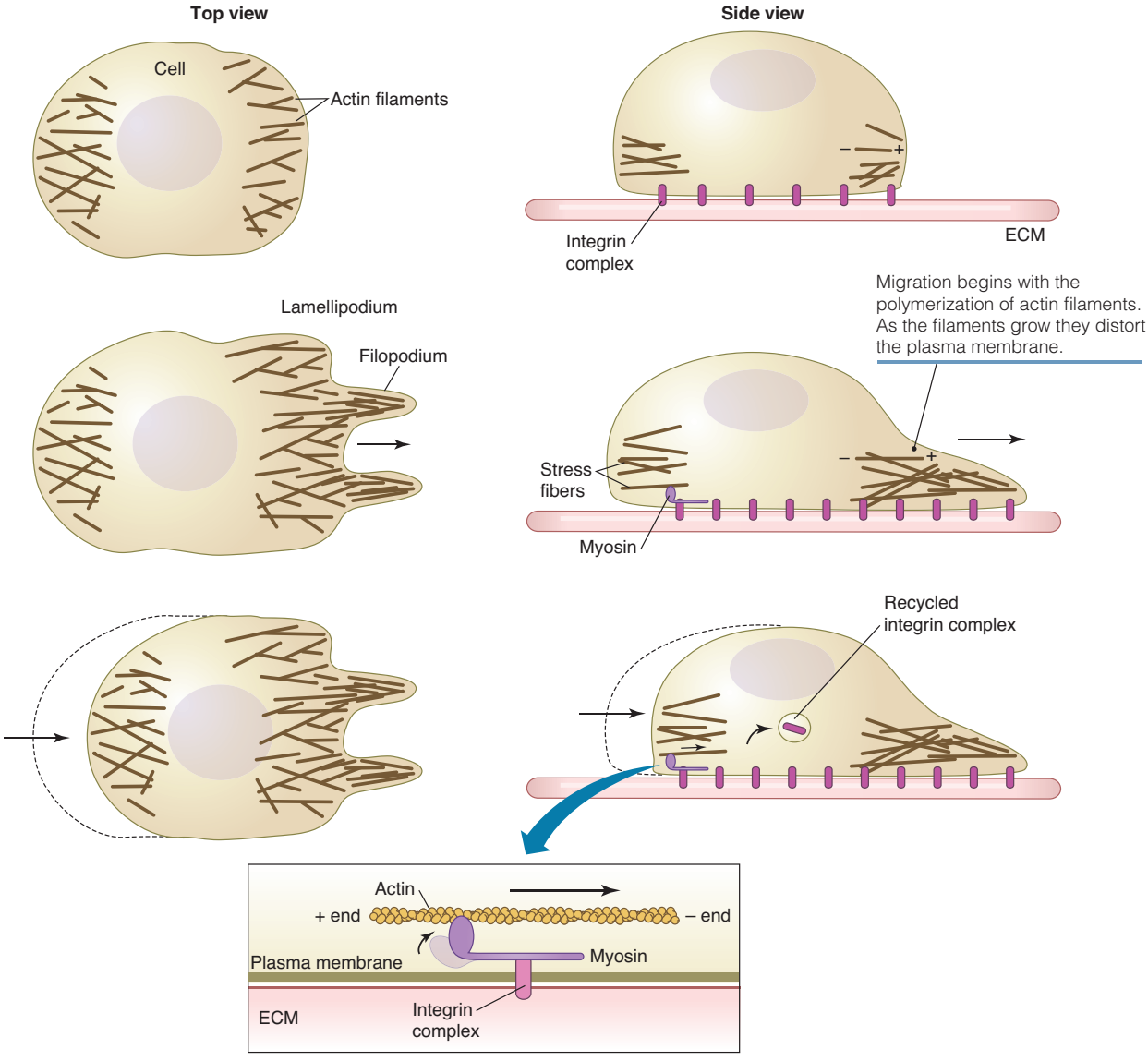


FIGURE 5-35 Cells crawl forward when actin at the leading edge elongates, pushing the plasma membrane outward. These actin filaments are anchored to the plasma membrane by binding to integrin complexes and are organized into larger networks called filopodia and lamellipodia. At the rear (trailing) edge of the cell, myosin motor proteins pull on actin filaments connected to the plasma membrane, dragging the rear of the cell forward.

Once the plasma membrane has been distended, the next step is to secure it in its new location. This requires a different set of membrane-associated proteins that we will discuss in detail in Chapter 6, but for now, we will simply call them **integrin complexes**. Filopodia and lamellipodia are called "feet" because they are analogous to the feet of a walking animal: they are the first structures to make contact with the new surface the cell is walking on, and they must be able to generate some traction if the cell is going to move forward. The adhesive proteins help keep the plasma membrane in place, like the treads on the sole of a shoe.

One important feature of these integrin complexes is that they can bind to actin filaments. The first filaments they bind are usually those making up the filopodia and lamellipodia, but later, once several adhesive proteins have been positioned in these "feet," a new type of actin bundle attaches to them. These bundles resemble filopodia in some ways in that they are parallel bundles with identical polarity, but rather than growing outward toward the periphery of the cell, the plus ends of these actin filaments grow inward, deeper into the cytosol. Whether the integrin complexes actually nucleate actin filament growth, or simply capture neighboring clusters, is still not clear. The thickest of these bundles are called **stress fibers**. Stress fibers also contain motor proteins that cause individual actin proteins in the fibers to slide past one another, shortening the fibers.

Keep in mind that the modularity of actin filaments makes their assembly and disassembly easy to control, and a host of actin-binding proteins are responsible for keeping this cycle properly balanced. Actin is also the smallest of the cytoskeletal filaments, and the lateral and longitudinal bonds made between monomers make actin filaments very strong. This helps explain why actin is the protein of choice for moving entire cells.

Myosins Are a Family of Actin-Binding Motor Proteins

Having assembled a network of actin filaments in our generic cell, we now have to explain how a cell pulls itself forward. To help us, let's briefly introduce a new analogy. Imagine we are watching a rock climber scale a cliff face. As she climbs up, we see a repeating pattern: she extends her arms, clutches a stable portion of the cliff with her hands, and then pulls herself upward to a new location, where the process repeats itself (we must ignore her legs pushing upward, because cells cannot do this). Like the rock climber, a cell must *contract* to actually crawl forward.

The contractile force in a cell is provided by the motor proteins we mentioned previously. The most prominent actin-associated motors are proteins called **myosins**. There are a multitude of different myosin proteins, and they are classified into 18 different families (named myosin I, myosin II, and so on, up to myosin XVIII). The most widely expressed myosins in humans are different forms of myosin II, and these are classified into muscle and nonmuscle types. Many cell biologists call these conventional myosins, and they are by far the best understood.

Despite the great variability between myosins, they all share some common properties that explain how they make cells move. First, myosins are multi-subunit proteins organized into three structural domains, each of which performs a distinct job (**FIGURE 5-36**). The different subunits are called **light and heavy chains**, based on their relative size. The motor domain, which is formed by the heavy chain, binds to both the actin filament and to ATP. It is also responsible for cleaving the ATP to ADP to generate the contractile force. The **regulatory domain**, composed of both a heavy chain and two light chains, serves as the "lever arm" that swings back and forth as the myosin slides along an actin filament. The tail domain determines which other proteins each myosin protein binds to; some bind to other myosins, enabling cells to form large clusters of myosins that can generate tremendous force.

To move our generic cell forward, we simply introduce myosin II molecules into the integrin complexes and actin stress fibers. The tail domains of some myosin II proteins can

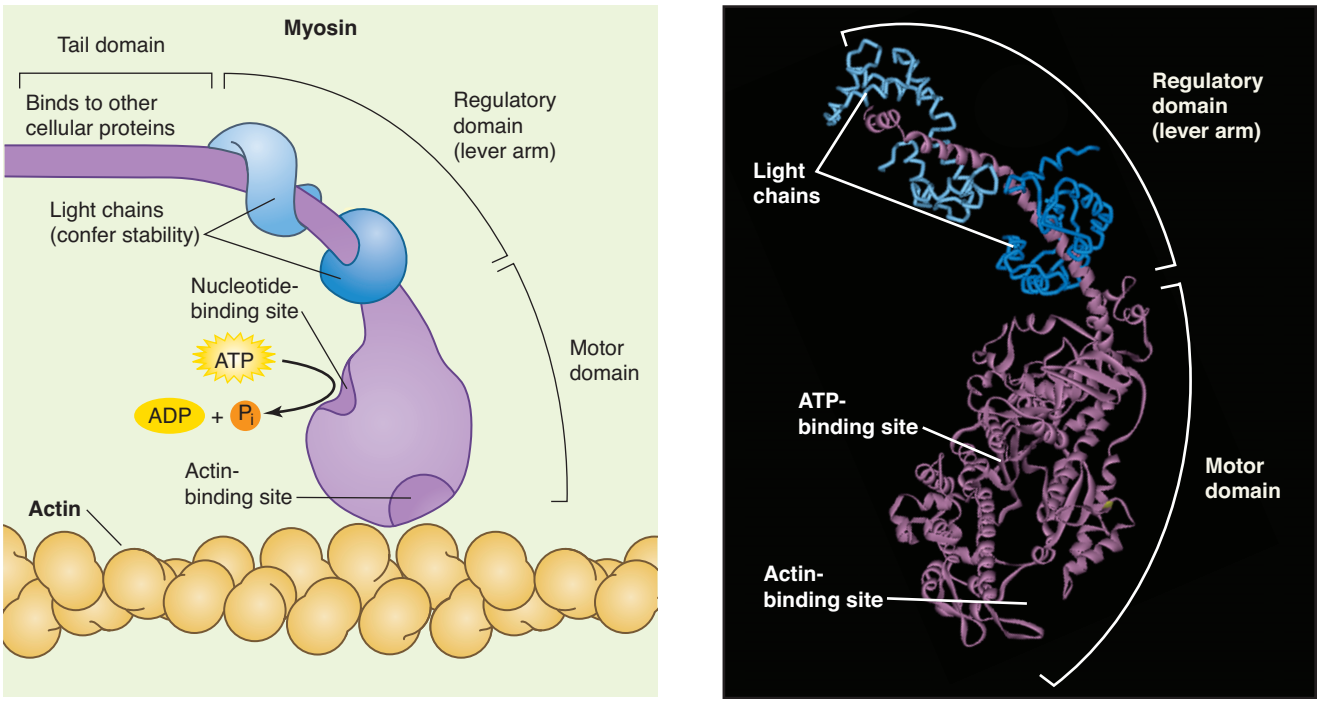


FIGURE 5-36 Myosin proteins contain three functional domains.
Structure from Protein Data Bank 2MYS. I. Rayment, et al., *Science* 261 (1993): 50–58.

bind directly to proteins in integrin complexes, and the head domains bind to the actin stress fibers. Once activated, the myosin begins a **contractile cycle** that causes it to move toward one end of the actin filaments (myosin V crawls toward the minus end, all other myosins crawl toward the plus end). The contractile cycle for myosin requires ATP binding and hydrolysis by the head domain.

Now we need to have a close look at a very subtle feature that will help put the whole picture together. Notice that when the myosins attached to the integrin complexes at the front of migrating cells attempt to crawl toward the plus end of the actin filaments, they can't move; they are stuck in place by the integrin complexes. This is extremely important. If the myosins can't move, what happens to the force they exert on the actin? Inevitably, the actin filaments must be pulled forward instead, or the filaments will rip apart (this is why actin filaments must be so strong). Notice that this is what we mean when we say that a cell pulls itself forward: whatever is attached to the actin filament gets pulled along with it. For example, if other actin-binding proteins are attached to the plasma membrane at the rear of the cell, the force exerted by the myosins is sufficient to drag the attached membrane forward too. If a lot of myosins in stress fibers work together, they can easily tug the rest of the cell, including the organelles, along with the membrane. To make things even easier, some actin filaments form close to and parallel with the plasma membrane at the rear of the cell, creating a **cortical-actin network** (the word *cortical* in this context refers to the outer edge of the cytosol). When myosins pull on these filaments, the cell essentially squeezes itself forward, somewhat like the pastry bags used to decorate cakes. Combined with successive waves of filopodia and lamellipodia, followed by integrin complexes that can pull actin filaments, the cell both "rolls" and "squeezes" itself forward.

This leaves one final question: what happens when the cell is pulled so far forward that the first integrin complexes are now at the rear of the cell? The answer, fortunately, is simple: cells capitalize on the modular nature of the contractile apparatus, and simply disassemble the integrin complex, releasing the rear of the cell from the surface it was attached to. Any remaining actin filaments attached to the integrin complexes are also disassembled and recycled.

Note that this entire operation is under the control of many types of signaling molecules that orchestrate the timing and location of each molecular event. The molecular mechanisms that cells use to pass signals from one location to another in a single cell, such as those that take place here, are discussed in Chapter 11.

Not All Cell Movement Is the Same

The above scenario is just one example of one type of cellular movement, and cells move in innumerable ways. For example:

- Cortical actin–myosin complexes can pinch one region of a cell to compensate for expansion in another region.
- Cortical actin–myosin complexes can squeeze a dividing cell in the middle so tightly that the two halves break apart to create daughter cells.
- The cells lining the blood vessels can change shape to regulate the amount of fluid they allow to flow between them.

Remember, cells are always in motion, even seemingly stationary cells.

Striated Muscle Contraction Is a Well-Studied Example of Cell Movement

Perhaps the easiest cells to observe contracting are the muscle cells that move our bones and cause our heart to beat. These cells have a very distinctive and highly specialized actin cytoskeleton; in fact, nearly 20% of the total protein in these cells is actin. Skeletal muscle cells are specialized to contract in only one direction, and so their actin and myosin filaments are all arranged in parallel, forming what appears as stripes under a microscope. Cardiac muscle cells have a very similar arrangement (though they form branched rather than entirely linear patterns). For this reason, both cell types are called striated muscle (striated is another word for striped) cells. **FIGURE 5-37** shows the overall structure of a typical skeletal muscle. Notice that the stripes are actually distinct regions of a highly organized structure called a sarcomere. A large skeletal muscle, such as a triceps muscle in the arm, contains millions of sarcomeres, all capable of pulling in the same direction. Note that smooth muscle cells, the third type of muscle cells, lack sarcomeres because they are capable of contracting in multiple directions at once. We will have a much closer look at the structure and function of all three muscle cells and contractile tissues in Chapter 14.

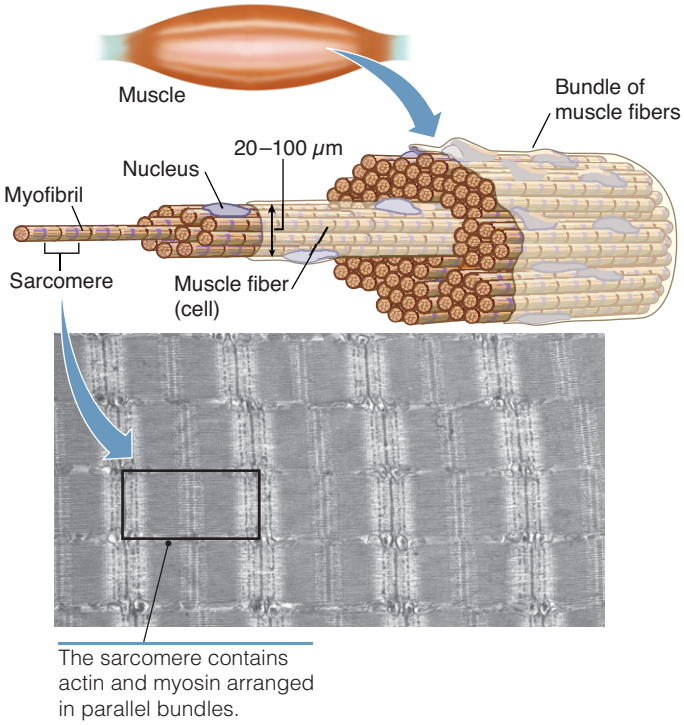


FIGURE 5-37 Striated muscle cells contain parallel bundles of actin and myosin that appear as stripes when viewed with microscopes.
Photo courtesy of Clara Franzini-Armstrong, University of Pennsylvania, School of Medicine.

CONCEPT CHECK 3

Actin and myosin work so closely together that they are sometimes called by a single name, *actomyosin*. These proteins provide the kinetic energy necessary to move cells, including muscle cells, and sometimes the actomyosin cytoskeleton is compared to real muscles, like our pectoral muscles. Actomyosin is sometimes called "the '*muscle element*' that allows *muscle cells* to form *entire muscles*."[3] Is this a fair comparison? Develop an argument that supports, and another that rejects, this comparison.

[3] Patel TJ & RL Lieber (1997) Force transmission in skeletal muscle: from actomyosin to external tendons, *Exec Sports Sci Rev*, 25:321–363.

5.6 Eukaryotic Cytoskeletal Proteins Arose from Prokaryotic Ancestors

The great benefits provided by cytoskeletal proteins are not reserved for eukaryotic cells only; these proteins evolved from ancestral cytoskeletal proteins that first appeared in prokaryotic cells. Modern prokaryotic cells express a number of cytoskeletal proteins that are homologous to eukaryotic cytoskeletal proteins and play very similar roles in these cells. For example, despite the fact that prokaryotes do not possess true intermediate filaments, the intermediate filament protein vimentin contains binding regions for single-stranded DNA, and is thought to be a descendent of a DNA-binding protein that first appeared in primitive prokaryotes.

Likewise, a protein called FtsZ is a member of a prokaryotic family of proteins closely related to eukaryotic tubulin proteins. It is widely expressed in bacteria, where it binds and cleaves GTP and adopts two different shapes accordingly. FtsZ also forms dimers that generate protofilament polymers, and organizes into a ring around the middle of bacterial cells that contracts during cell division. One interesting observation is that this protein is also found in mitochondria and chloroplasts, suggesting eukaryotic cells may have inherited this protein directly from its own prokaryote-derived organelles. At least four other bacterial proteins are known to bind to and alter the structure and function of FtsZ polymers, and these are likely related to an ancestral protein that gave rise to MAPs in eukaryotes.

At least two bacterial homologs for actin proteins have also been identified, though their gene sequence similarity to eukaryotic actin genes is quite low, suggesting their common ancestor was encoded by one of the earliest genes in cells. The protein MreB binds ATP and forms helical filaments in bacteria that promote cell movement. Despite the weak sequence homology, the three-dimensional structure of this protein is remarkably similar to that of actin. ParM is another filament-forming protein that resembles actin filaments, and is known to participate in the segregation of plasmids in bacteria, again suggesting a role in controlling movement in these cells.

Together, these proteins conjure up an image of a primitive, single-celled organism that gained the ability to specialize by producing proteins that performed a subset of the behaviors it needed to survive. Some of these proteins were the ancestors of modern cytoskeletal proteins. It underscores that protecting DNA, compartmentalizing the cell's contents, and moving through the environment are some of the most important properties of all living organisms.

5.7 Chapter Summary

All cells adopt different shapes, reflecting the wide range of functions they perform; however, these shapes do not come for free. Left by itself, a phospholipid bilayer will always form a sphere, the lowest energy state in an aqueous environment. This means that without some active intervention by proteins, all cells would be spherical in shape. The name "cytoskeleton" is given to the proteins discussed in this chapter because they play a role analogous to our own skeleton: they distort the plasma membrane to create different shapes, sort the cell's contents into specific regions of these shapes, and stabilize these shapes by providing resistance to mechanical forces that would otherwise distort (or even destroy) the cell.

Intermediate filaments are the most stable type of cytoskeleton proteins, and they are especially effective at resisting mechanical forces. This arises from the way they are built: they are highly coiled filaments, which permits maximum contact between the individual proteins and increases the number of bonds holding these proteins together. Intermediate filaments

are found primarily in the cytosol, but one type forms a cage-like structure in the nucleus that protects the DNA. Different cell types express different intermediate filament proteins, permitting them to customize this part of their cytoskeleton to suit their specific needs.

Microtubules behave quite differently from intermediate filaments. These filaments can be very dynamic in cells; in fact, the term *dynamic instability* was coined to describe their rapid assembly and disassembly in cells. Cells capitalize on this dynamic instability by using microtubule-associated proteins (MAPs) to control when and where microtubules form. Once a microtubule has been stabilized, its primary role is to serve as a track for directing the trafficking of molecules in the cytosol. Examples of this transport include vesicle traffic between the ER and the Golgi apparatus, movement of mitochondria from one region of the cell to another, and segregation of chromosomes during mitosis. The transport of most molecules on microtubules is driven by specialized MAPs called motor proteins. The structure of microtubules makes them quite strong; tubulin proteins form both lateral and longitudinal bonds with a microtubule, and this gives them enough strength to literally push and pull molecules attached to their tips as they undergo dynamic instability. Tubulin proteins are a good example of a common theme in cell biology: they bind to GTP and GDP, and use the difference in shape between these two molecules to influence their own shape and function.

Actin filaments are the most complex of the three cytoskeletal elements. This complexity arises from two features of these structures: they can easily form both linear and branched networks, and they undergo a process called treadmilling that keeps them constantly in flux, even if their overall shape remains constant. Both properties are controlled by a wide variety of actin-binding proteins that are analogous to the MAPs that bind microtubules. Actin filaments are also smaller and simpler than intermediate filaments or microtubules, making them the preferred choice for rapid filament assembly and disassembly in subregions of the cytosol. Motor proteins called myosins pull on actin filaments; when these actin filaments are immobilized on the cell surface, the resulting force causes the plasma membrane to move. Coordinated teams of actin and myosin can cause an entire cell to change its shape or even move to a new location.

Every cell has a cytoskeleton. The importance of cytoskeletal proteins to cellular function is evident when one considers how long they have been present in cells. Evidence from modern prokaryotes suggest that the ancestral proteins that gave rise to modern cytoskeletal networks were some of the earliest proteins to appear in cells. These proteins also illustrate how important cytoskeletal functions are for all organisms.

? CHAPTER STUDY QUESTIONS

1. Which of the three major classes of cytoskeletal proteins do you expect to contain the highest proportion of hydrophobic amino acids, and why?

2. Though IFs do not bind to motor proteins, the IF protein called desmin is essential for proper muscle cell contraction. Review Figure 5-7 and explain how desmin contributes to cell contraction.

3. In 1992, scientists conducted an experiment to test the effect of GTP hydrolysis on microtubule assembly and disassembly *in vitro*. In the test sample, they added an analog of GTP that tubulin hydrolyzes much more slowly than GTP. What effect do you expect they observed, relative to the control sample (tubulin incubated with real GTP), and why?

4. Why does the "GTP cap" typically only form on one end of a microtubule?

5. Briefly explain how a microtubule motor protein can transport a chromosome from the midpoint (metaphase plate) to the tip of the mitotic spindle. Assume the poles of the spindle are microtubule organizing centers.

6. Though it is most commonly used for microtubules, the term "cap" to refer to the plus end of a growing filament is also applicable to an actin filament. Explain what this actin cap is and how it impacts actin filament dynamics.

7. Explain the different roles played by actin bundling proteins, myosins, and actin severing proteins during cell migration.

8. Why don't intermediate filaments (IFs) exhibit dynamic instability?

9. Some of the most commonly used anticancer drugs target microtubules. Explain the rationale for disrupting the function of the microtubules cytoskeleton as an effective anti-drug therapy. Do you think the other cytoskeletal proteins are attractive targets for anticancer research? What side effects would you expect in patients receiving drugs that target the cytoskeleton?

10. The stiffening of muscles known as "rigor mortis" occurs approximately 2 to 6 hours after death, and then persists for approximately 48 hours until the muscles return to a "relaxed" (flexible) state. Using Figure 5-37 as a guide, explain why rigor mortis lasts much longer than it takes to occur. (As a hint, also review Section 3.5 in Chapter 3.)

? MULTIPLE CHOICE QUESTIONS

1. What is the function of actin capping proteins?
   - **A.** They control the length and stability of actin filaments.
   - **B.** They control actin filament severing.
   - **C.** They organize actin filaments into bundles.
   - **D.** They organize actin filaments into networks.
   - **E.** They regulate actin ubiquitination.

2. Why are intermediate filaments considered strong?
   - **A.** Because they have a high proportion of beta sheet secondary structure, which is very stable.
   - **B.** Because they protect the nucleus, which is capable of resisting a great deal of physical force.
   - **C.** Because they have quaternary structure, which is the strongest form of protein.
   - **D.** Because they absorb a large amount of stress without breaking.
   - **E.** Because they require covalent modification (phosphorylation) to depolymerize.

3. Turbidity is used to measure the polymerization of actin filaments *in vitro* because:
   - **A.** Turbidity increases when the number of actin filaments increases.
   - **B.** Turbidity is directly proportional to actin monomer concentration.
   - **C.** Turbidity increases as a function of actin treadmilling.
   - **D.** Turbidity measures the rate of ATP hydrolysis by actin.
   - **E.** Turbidity increases when actin filaments are severed by actin binding proteins.

4. The function of the γTuRC is...

A. to promote binding of GTP to αβ tubulin dimers.
B. to stimulate increased microtubule turnover during dynamic instability.
C. to prevent treadmilling of actin filaments.
D. to stimulate treadmilling of actin filaments.
E. to nucleate the formation of αβ tubulin dimers into polymers.

5. Why is it significant that two of the three classes of cytoskeletal protein polymers have "plus" and "minus" ends?

A. These polymers can only grow at one end, so it is important to know which end is facing outward from the MTOC.
B. The plus and minus ends on these two polymers prevent them from entering the nucleus.
C. The amino acids comprising these polymers are primarily ionic, and they create an electrical charge across the polymers.
D. These polymers bind to motor proteins that move toward the plus or minus ends, specifically.
E. The monomers fold up from the minus end to the plus end as they are synthesized.