CHAPTER

14

# The Cytoskeleton and Cell Movement

The membrane-enclosed organelles discussed in the preceding chapters constitute one level of the organizational substructure of eukaryotic cells. A further level of organization is provided by the cytoskeleton, which consists of a network of protein filaments extending throughout the cytoplasm. The cytoskeleton provides a structural framework for the cell, serving as a scaffold that determines cell shape and the positions of organelles. In addition to this structural role, the cytoskeleton is responsible for cell movement and the transport of organelles and other structures (such as mitotic chromosomes) through the cytoplasm. Importantly, the cytoskeleton is much less rigid and permanent than its name implies. Rather, it is a dynamic structure that is continually reorganized as cells move and change shape—for example, during mitosis and cell division.

The cytoskeleton is composed of three principal types of protein filaments: actin filaments, microtubules, and intermediate filaments (**Figure 14.1**), which are held together and linked to subcellular organelles and the plasma membrane by a variety of accessory proteins. This chapter discusses the structure and organization of each of these three major components of the cytoskeleton as well as the roles of actin filaments and microtubules in cell motility, organelle transport, and cell division.



## 14.1 Structure and Organization of Actin Filaments

### Learning Objectives

**You should be able to:**

- Summarize the dynamics of actin filaments and the roles of actin-binding proteins.
- Illustrate the organization of actin filaments underlying the plasma membrane.
- Describe the structure and function of microvilli.
- Explain how remodeling of actin filaments is responsible for cell motility.

The most abundant cytoskeletal protein of most cells is **actin**, which polymerizes to form actin filaments—thin, flexible fibers approximately 7 nm in diameter and up to several $\mu$m in length (**Figure 14.2**). Within the cell, actin

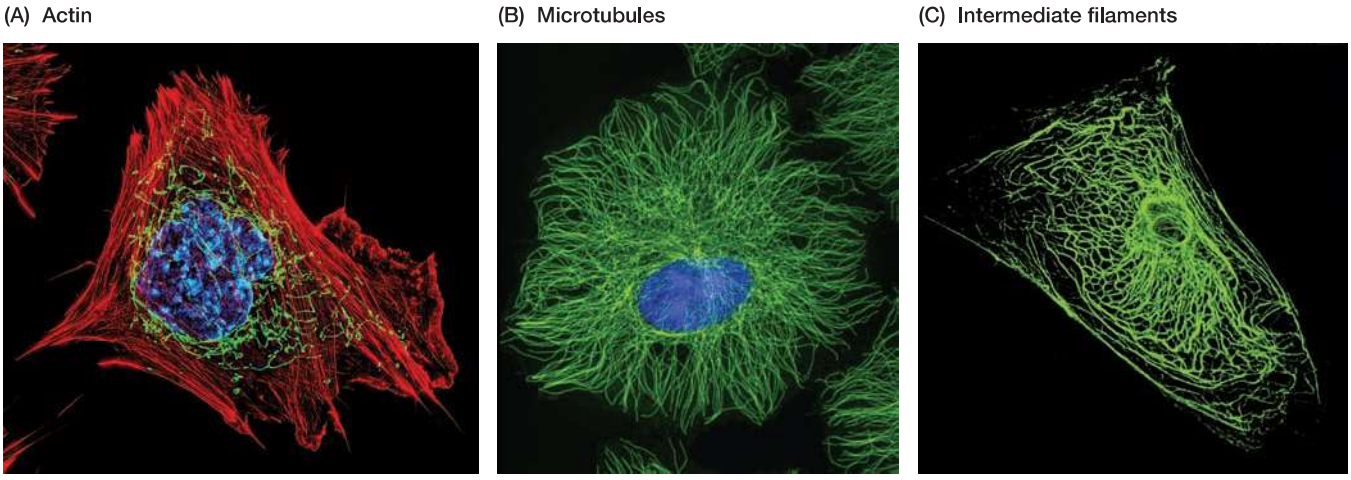


**Figure 14.1 Cytoskeletal filaments** Fluorescent micrographs of actin filaments (A), microtubules (B), and intermediate filaments (C) in cultured cells. (A) actin is stained red, mitochondria green, and DNA blue. (B) Tubulin is stained green and DNA blue. (C) Keratin, an intermediate filament protein, is stained green. (A, © NIH/NICHD/ Dylan Burnette and Jennifer Lippincott-Schwartz/Science Source; B, © Thomas Deerinck, NCMIR/Science Source; © Alvin Telser/Science Source.)

filaments (also called **microfilaments**) are organized into higher-order structures, forming bundles or three-dimensional networks with the properties of semisolid gels. The assembly and disassembly of actin filaments, their cross-linking into bundles and networks, and their association with other cell structures (such as the plasma membrane) are regulated by a variety of **actin-binding proteins**, which are critical components of the actin cytoskeleton. Actin filaments are particularly abundant beneath the plasma membrane where they form a network that provides mechanical support, determines cell shape, and allows movement of the cell surface, thereby enabling cells to migrate, engulf particles, and divide.

### *Assembly and organization of actin filaments*

Actin was first isolated from muscle cells, in which it constitutes approximately 20% of total cell protein, in 1942. Although actin was initially thought to be uniquely involved in muscle contraction, it is now known to be an extremely abundant protein (typically 5–10% of total protein) in all types of eukaryotic cells. Yeasts have only a single actin gene, but higher eukaryotes have several distinct types of actin, which are encoded by different members of the actin gene family. Mammals, for example, have six distinct actin genes: Four are expressed in different types of muscle (skeletal, cardiac, and smooth muscle) and two are expressed in nonmuscle cells. All of the actins, however, are very similar in amino acid sequence and have been highly conserved throughout the evolution of eukaryotes. Yeast actin, for example, is 90% identical in amino acid sequence to the actins of mammalian cells.

The three-dimensional structures of both individual actin molecules and actin filaments were determined in 1990 by Kenneth Holmes, Wolfgang

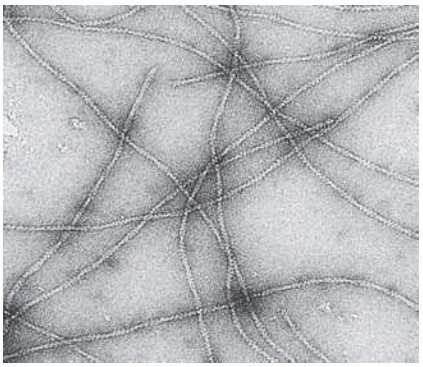


**Figure 14.2 Actin filaments** Electron micrograph of actin filaments. (Courtesy of Roger Craig, University of Massachusetts Medical Center.)

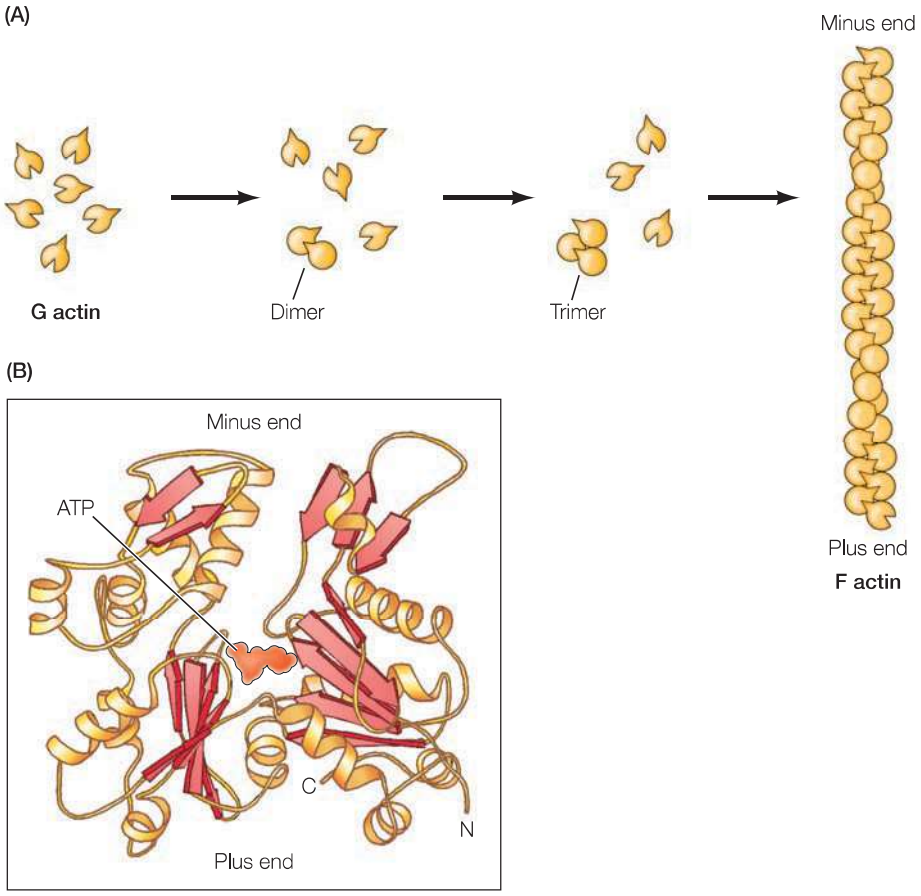


**Figure 14.3 Assembly and structure of actin filaments** (A) Actin monomers (G actin) polymerize to form actin filaments (F actin). The first step is the formation of dimers and trimers, which then grow by the addition of monomers to both ends. (B) Structure of an actin monomer with ATP bound.

**Animation 14.1**
Assembly of an Actin Filament

Kabsch, and their colleagues. Individual actin molecules are globular proteins of 375 amino acids (43 kd). Each actin monomer (**globular [G] actin**) has tight binding sites that mediate head-to-tail interactions with two other actin monomers, so actin monomers polymerize to form filaments (**filamentous [F] actin**) (Figure 14.3). Each monomer is rotated by 166 degrees in the filaments, which therefore have the appearance of a double-stranded helix. Because all the actin monomers are oriented in the same direction, actin filaments have a distinct polarity and their ends (called plus ends and minus ends) are distinguishable from one another. This polarity of actin filaments is important both in their assembly and in establishing a specific direction of myosin movement relative to actin, as discussed later in the chapter.

**Actin monomers polymerize to form filaments with distinct plus and minus ends.**

The behavior of actin monomers and filaments is regulated within cells to take advantage of the intrinsic properties of actin. Under physiological conditions, actin monomers polymerize to form filaments. The first step in actin polymerization (called nucleation) is the formation of a small aggregate consisting of three actin monomers. Actin filaments are then able to grow by the reversible addition of monomers to both ends, but the plus end elongates five to ten times faster than the minus end. The actin monomers bind ATP

**The plus end of actin filaments elongates by the addition of ATP-actin monomers.**

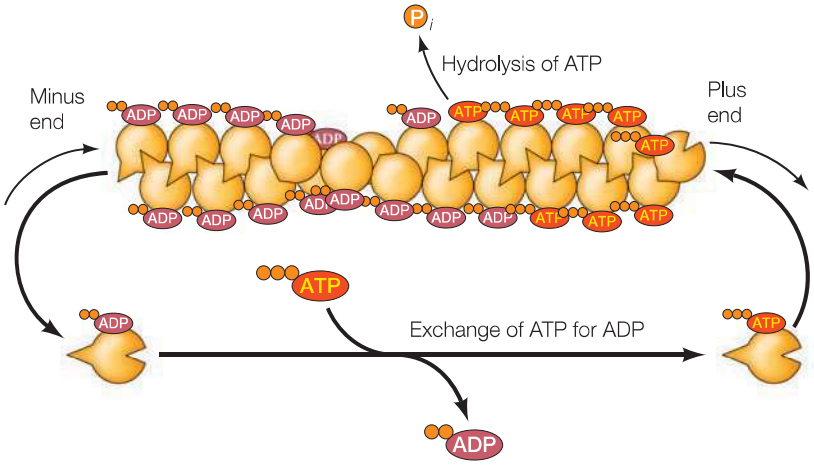


**Figure 14.4 Treadmilling and the role of ATP in actin filament polymerization** Actin bound to ATP associates with the rapidly growing plus ends, and the ATP is then hydrolyzed to ADP within the filament. Because ADP-actin dissociates from filaments more readily than ATP-actin, actin monomers bound to ADP dissociate from the minus end while monomers bound to ATP are added to the plus end.

(see Figure 14.3), which is hydrolyzed to ADP following filament assembly. Although ATP is not required to provide energy for polymerization, actin monomers to which ATP is bound polymerize more readily than those to which ADP is bound. As a result, ATP-actin monomers are added rapidly to the plus ends of filaments, and the ATP is then hydrolyzed to ADP after polymerization. The ADP-actin is less tightly bound and can dissociate from the minus end. This can result in the phenomenon known as **treadmilling**, in which ATP-actin is added to the plus end while ADP-actin dissociates from the minus end of a filament (**Figure 14.4**). Treadmilling illustrates the dynamic behavior of actin filaments, which, as discussed below, is critical in regulating the structure and function of actin filaments.

Within the cell, the assembly and disassembly of actin filaments is regulated by actin-binding proteins. Some of these proteins initiate the formation of actin filaments or stimulate their growth. Others stabilize actin filaments by binding along their length or by capping their ends and preventing the dissociation of actin monomers. Conversely, some proteins act to disassemble actin filaments by severing them and stimulating their depolymerization.

**The growth of filaments is initiated by actin-binding proteins that can form either linear or branched filaments.**

The principal proteins that stimulate the initiation and elongation of actin filaments are **formin** and the **Arp2/3 complex** (*a*ctin-*r*elated *p*rotein) (**Figure 14.5**). The activities of these proteins are regulated in response to a variety of signals to determine where filaments are formed within the cell. Formins are a family of proteins that bind ATP-actin and nucleate the initial polymerization of actin monomers (**Figure 14.5A**). They then move along the growing filament, adding new monomers to the plus end. Formins nucleate long unbranched actin filaments that make up stress fibers, the contractile ring, filopodia, and the thin filaments of muscle cells (discussed later in this chapter). Formins are associated with another actin-binding protein called **profilin**, which binds actin monomers and stimulates the exchange of bound ADP for ATP. This promotes actin polymerization by increasing the local concentration of ATP-actin.

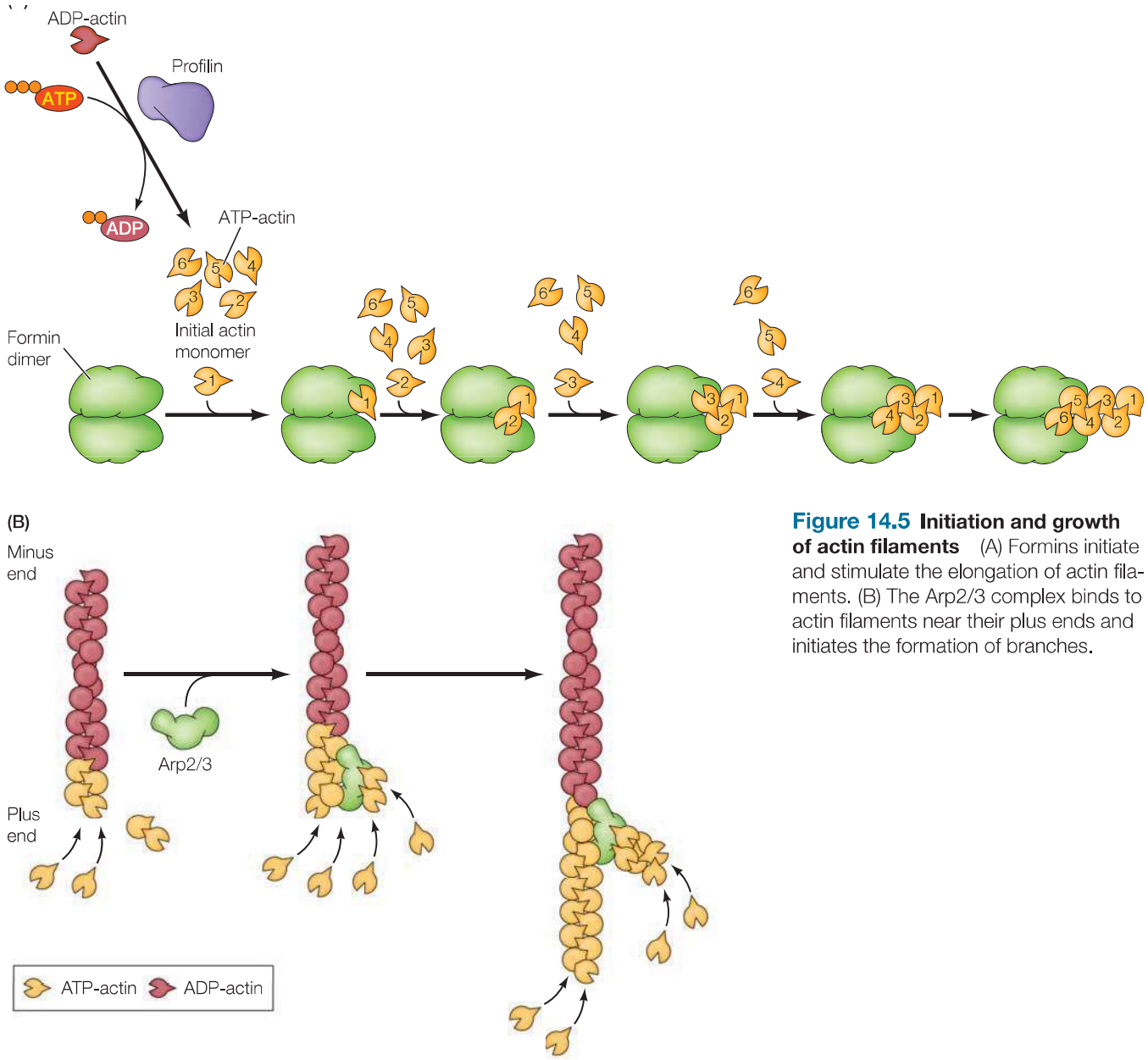


**Figure 14.5 Initiation and growth of actin filaments** (A) Formins initiate and stimulate the elongation of actin filaments. (B) The Arp2/3 complex binds to actin filaments near their plus ends and initiates the formation of branches.

The Arp2/3 proteins initiate growth of branched actin filaments, which play a key role in driving cell movement at the plasma membrane (see **Figure 14.5B**). The Arp2/3 proteins bind ATP-actin near the plus ends of filaments and initiate the formation of a new branch. The activity of the Arp2/3 proteins is controlled by several other cellular proteins, so that the formation of branched actin filaments is regulated by the physiological needs of the cell.

Many actin filaments are relatively stable within cells due to capping proteins that bind to their ends and to filament-stabilizing proteins, such as members of the **tropomyosin** family, which bind lengthwise along the groove of actin filaments (**Figure 14.6**). Other proteins that bind along the length of actin filaments serve to cross-link actin filaments into two

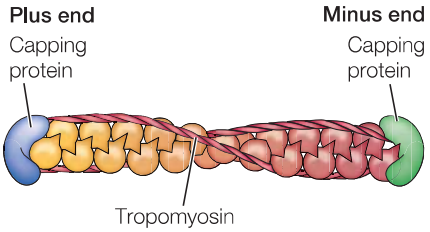


**Figure 14.6 Stabilization of actin filaments** Actin filaments can be stabilized by capping proteins that bind to their plus or minus ends and by filament-stabilizing proteins (e.g., tropomyosin) that bind along their length.

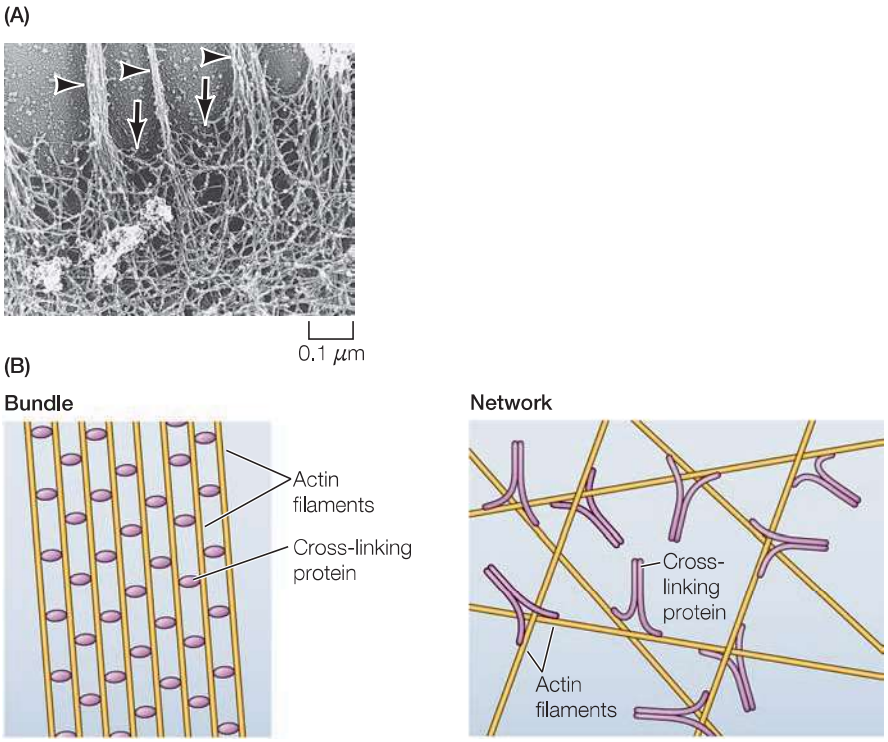


**Figure 14.7 Actin bundles and networks** (A) Electron micrograph of actin bundles (arrowheads) projecting from the actin network (arrows) underlying the plasma membrane of a macrophage. The bundles support cell surface projections called filopodia (see Figure 14.15). (B) Schematic organization of a bundle and a network. Actin filaments in bundles are cross-linked into parallel arrays by small proteins that align the filaments closely with one another. In contrast, networks are formed by large flexible proteins that cross-link orthogonal filaments. (A, courtesy of John H. Hartwig, Brigham & Women's Hospital.)

**Actin-binding proteins can stabilize and organize filaments into bundles or networks.**

general types of structures, called **actin bundles** and **actin networks** (**Figure 14.7**). In bundles, the actin filaments are cross-linked into closely packed parallel arrays. In networks, the actin filaments are cross-linked in orthogonal arrays that form three-dimensional meshworks with the properties of semisolid gels. The nature of the association between these filaments is determined by the size and shape of the cross-linking proteins. The proteins that cross-link actin filaments into bundles are small rigid proteins that force the filaments to align closely with one another. In contrast, the proteins that organize actin filaments into networks tend to be large flexible proteins that can cross-link perpendicular filaments. The roles of these assemblies in a variety of cell structures and processes are discussed in the following sections.

Other actin-binding proteins remodel or modify, rather than stabilize, existing filaments. An example is the actin-binding protein **cofilin**, which severs actin filaments (**Figure 14.8**). This generates new ends of the filaments, which are then accessible either for depolymerization from minus ends or growth by addition of new actin monomers to their plus ends. This regulated turnover of actin filaments plays a key role in dynamic processes, such as cell motility and cell division following mitosis.

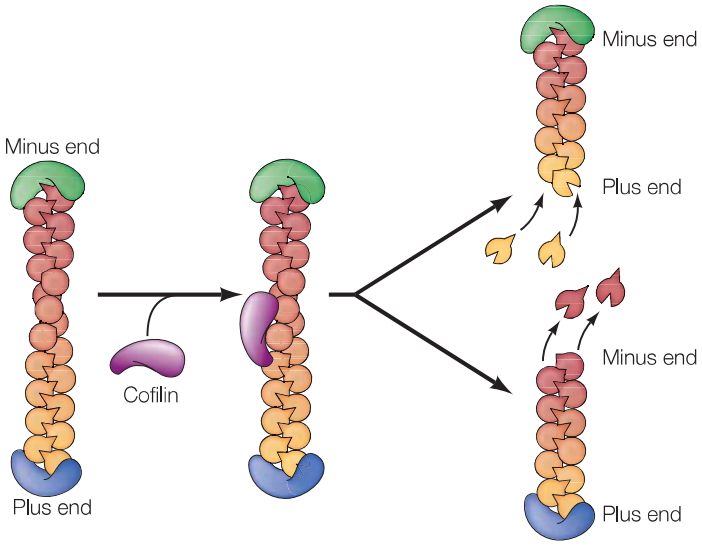


**Figure 14.8 Filament severing by cofilin** Cofilin binds to and severs actin filaments. The newly formed filament ends are then available for the polymerization or depolymerization of actin monomers.

### *Association of actin filaments with the plasma membrane*

Actin filaments are highly concentrated at the periphery of the cell, where they form a three-dimensional network beneath the plasma membrane (see Figure 14.7). This network of actin filaments and associated