Microtubules

Microtubules are nonbranching and rigid hollow tubes of protein that can rapidly disassemble in one location and reassemble in another. In general, they grow from the ***microtubule-organizing center (MTOC)*** located near the nucleus (page 53) and extend toward the cell periphery. Microtubules create a system of connections within the cell, frequently compared to railroad tracks, along which vesicular movement occurs.

Microtubules are elongated polymeric structures composed of equal parts of α-tubulin and β-tubulin

Microtubules measure 20 to 25 nm in diameter (Fig. 2.37). The wall of the microtubule is approximately 5 nm thick and consists of 13 circularly arrayed globular ***dimeric tubulin molecules.*** The tubulin dimer has a molecular weight of 110 kDa and is formed from an α-tubulin and a β-tubulin molecule, each with a molecular weight of 55 kDa. (Fig. 2.38). The dimers polymerize in an end-to-end fashion, head to tail, with the α molecule of one dimer bound to the β molecule of the next dimer in a repeating pattern. The polymer thus formed is called a protofilament. Axial periodicity seen along the 5-nm-diameter dimers corresponds to the length of the protein molecules. A small, 1-μm segment of microtubule contains approximately 16,000 tubulin dimers.

Microtubules grow from γ-tubulin rings within the MTOC that serve as nucleation sites for each microtubule

Microtubule formation can be traced to hundreds of γ-tubulin rings that form an integral part of the MTOC (Fig. 2.39). The α- and β-tubulin dimers are added to a γ-tubulin ring in an end-to-end fashion (see Fig. 2.38). Polymerization of tubulin dimers requires the presence of guanosine triphosphate (GTP) and Mg^{2+}. Each tubulin molecule binds GTP before it is incorporated into the forming microtubule. The GTP–tubulin complex is then polymerized, and at some point GTP is hydrolyzed to guanosine diphosphate (GDP). As a result of this polymerization pattern, each microtubule possesses a ***minus*** (nongrowing) ***end*** embedded in the MTOC and a ***plus*** (growing) ***end*** elongating toward the cell periphery. Tubulin dimers dissociate from microtubules in the steady state, which adds a pool of free tubulin dimers to the cytoplasm. This pool is in equilibrium with the polymerized tubulin in the microtubules; therefore, polymerization and depolymerization are in equilibrium. The equilibrium can be shifted in the direction of depolymerization by exposing the cell or isolated microtubules to low temperatures or high pressure. Repeated exposure to alternating low and high temperature is the basis of the purification technique for tubulin and microtubules. The speed of polymerization or depolymerization can also be modified by interaction with specific ***microtubule-associated proteins (MAPs).*** These proteins, such as MAP-1, 2, 3, and 4, MAP-t, and TOGp, regulate microtubule assembly and anchor the microtubules to specific organelles. MAPs are also responsible for the existence of stable populations of nondepolymerizing microtubes in the cell, such as those found in cilia and flagella.

The length of microtubules changes dynamically as tubulin dimers are added or removed in a process of dynamic instability

Microtubules observed in cultured cells with real-time video microscopy appear to be constantly growing toward the cell periphery (by addition of tubulin dimers) and then suddenly shrinking in the direction of the MTOC (by removal of tubulin dimers). This constant remodeling process, known as ***dynamic instability,*** is linked to a pattern of GTP hydrolysis during the microtubule assembly and disassembly process. The MTOC can be compared to a feeding chameleon, which fires its long, projectile tongue to make contact with potential food. The chameleon then retracts its tongue back into its mouth and repeats this process until it is successful in obtaining food. The same strategy of "firing" microtubules from the MTOC toward the cell periphery and subsequently retracting them enables the cell to establish an organized system of microtubules linking peripheral structures and organelles with the MTOC. As mentioned above, association of a microtubule with MAPs, such as occurs within the axoneme of a cilium or flagellum, effectively blocks this dynamic instability and stabilizes the microtubules.

The structure and function of microtubules in mitosis and in cilia and flagella are discussed later in this chapter and in Chapter 4.

Microtubules can be visualized in the light microscope and are involved in intracellular transport and cell motility

Microtubules may be seen in the light microscope by using special stains, polarization, or phase contrast optics.

Because of the limited resolution of the light microscope, in the past microtubules were erroneously called fibers, such as the "fibers" of the mitotic spindle. Microtubules may now be distinguished from filamentous and fibrillar cytoplasmic components even at the light microscopic level by using antibodies to tubulin, the primary protein component of microtubules, conjugated with fluorescent dyes (Fig. 2.39).

In general, microtubules are found in the cytoplasm, where they originate from the MTOC; in cilia and flagella, where they form the axoneme and its anchoring basal body; in centrioles and the mitotic spindle; and in elongating processes of the cell, such as those in growing axons.

Microtubules are involved in numerous essential cellular functions:

- Intracellular vesicular transport (e.g., movement of secretory vesicles, endosomes, lysosomes)
- Movement of cilia and flagella
- Attachment of chromosomes to the mitotic spindle and their movement during mitosis and meiosis
- Cell elongation and movement (migration)
- Maintenance of cell shape, particularly its asymmetry

Movement of intracellular organelles is generated by molecular motor proteins associated with microtubules

In cellular activities that involve movement of organelles and other cytoplasmic structures, such as transport vesicles, mitochondria, and lysosomes, microtubules serve as guides to the appropriate destinations. ***Molecular motor proteins*** attach to these organelles or structures and ratchet along the microtubule track (Fig. 2.40). The energy required for the ratcheting movement is derived from ATP hydrolysis. Two families of molecular motors have been identified that allow for unidirectional movement:

- ***Dyneins*** constitute one family of molecular motors. They move along the microtubules toward the minus end of the tubule. Therefore, ***cytoplasmic dyneins*** are capable of transporting organelles from the cell periphery toward the MTOC. One member of the dynein family, ***axonemal dynein,*** is present in cilia and flagella. It is responsible for the sliding of one microtubule against an adjacent microtubule of the axoneme that effects their movement.
- ***Kinesins,*** members of the other family, move along the microtubules toward the plus end; therefore, they are capable of moving organelles from the cell center toward the cell periphery.

Both dyneins and kinesins are involved in mitosis and meiosis. In these activities, dyneins move the chromosomes along the kinetochore microtubules toward the spindle pole. Kinesins are simultaneously involved in movement of polar microtubules. These microtubules extend from one spindle pole past the metaphase plate and overlap with microtubules extending from the opposite spindle pole. Kinesins located between these microtubules generate a sliding movement that reduces the overlap, thereby pushing the two spindle poles apart to each daughter cell (Fig. 2.41).

Actin Filaments

Actin filaments are present in virtually all cell types

Actin molecules (42 kDa) are abundant and may constitute up to 20% of the total protein of some nonmuscle cells (Fig. 2.42). Similar to the tubulin in microtubules, actin molecules also assemble spontaneously by polymerization into a linear helical array to form filaments 6 to 8 nm in diameter. They are thinner, shorter, and more flexible than microtubules. Free actin molecules in the cytoplasm are referred to as ***G-actin (globular actin)*** in contrast to the polymerized actin of the filament, called ***F-actin (filamentous actin).*** Actin filaments are polarized structures; their fast-growing end is referred to as the ***plus*** or ***barbed end,*** and their slow-growing end is referred to as the ***minus*** or ***pointed end.*** The dynamic process of actin polymerization requires the presence of K^+, Mg^{2+}, and ATP, which is hydrolyzed to ADP after each G-actin molecule is incorporated into the filament (Fig. 2.43). The control and regulation of the polymerization process depends on the local concentration of G-actin and the interaction of ***actin-binding proteins (ABPs),*** which can prevent or enhance polymerization.

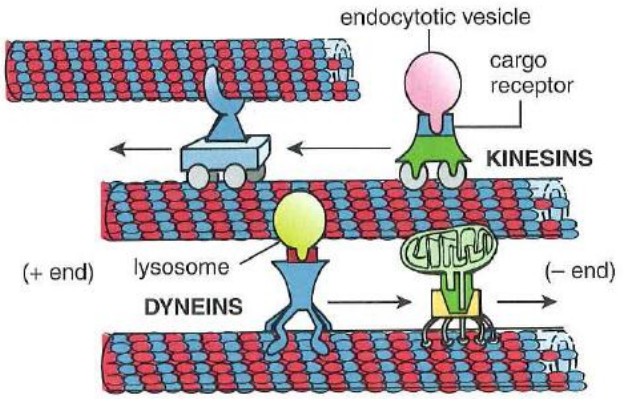

FIGURE 2.40
The molecular motor proteins associated with microtubules. Microtubules serve as guides for molecular motor proteins. These ATP-driven microtubule-associated motor proteins are attached to moving structures (such as organelles) and ratchet them along a tubular track. Two types of molecular motors have been identified: dyneins that move along microtubules toward their minus (–) end (i.e., toward the center of the cell) and kinesins that move toward their plus (+) end (i.e., toward the cell periphery).

In addition to controlling the rate of polymerization of actin filaments, ABPs are responsible for their organization. For example, a number of proteins can modify or act on actin filaments to give them various specific characteristics:

- ***Actin-bundling proteins*** cross-link actin filaments into parallel arrays, creating actin filament bundles. An example of this modification occurs inside the microvillus, where actin filaments are cross-linked by the actin-bundling proteins ***fascin*** and ***fimbrin*** (see Fig. 4.3, page 92). This cross-linkage provides support and imparts rigidity to the microvilli.
- ***Actin filament–severing proteins*** sever long actin filaments into short fragments. An example of such proteins is ***gelsolin***, a 90-kDa ABP that normally initiates actin polymerization but at high Ca^{2+} concentrations causes severing of the actin filaments, converting an actin gel into a fluid state.

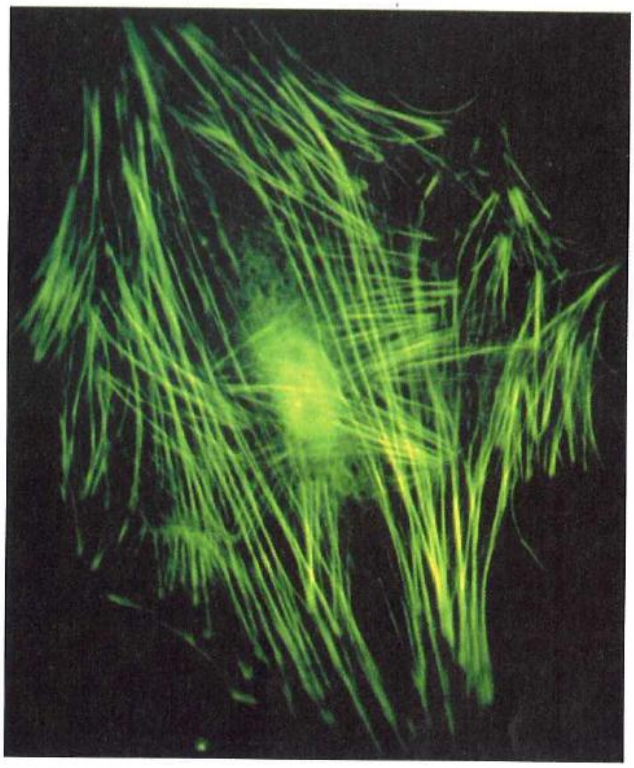

FIGURE 2.42
Distribution of actin filaments in a human fibroblast in culture. The cell was stained with an actin-specific antibody conjugated with the dye fluorescein. The antigen–antibody reaction, performed directly in the culture, results in localization of the actin. The actin filaments organized in linear bundles fluoresce and thus show their distribution in this nonmigrating cell. (Courtesy of Dr. Elias Lazarides.)

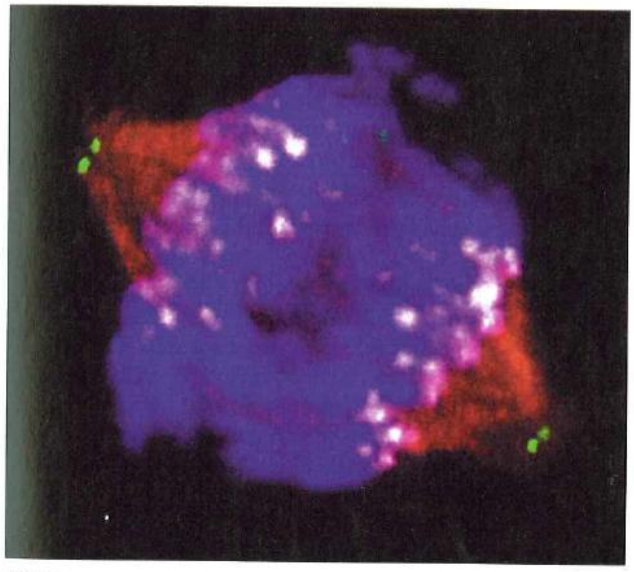

FIGURE 2.41
Distribution of kinesin-like motor protein within the mitotic spindle. This confocal immunofluorescent image shows a mammary gland epithelial cell in anaphase of mitosis, Each mitotic spindle pole contains two centrioles *(green)*. A mitosis-specific kinesin-like molecule called Eg5 *(red)* is associated with the subset of the mitotic spindle microtubules that connect the kinetochores *(white)* to the spindle poles. The motor action of Eg5 is required to separate the sister chromatids *(blue)* into the daughter cells. This cell was first immunostained with three primary antibodies against Eg5 *(red)*, centrin *(green)*, and kinetochores *(white)* and then incubated in three different fluorescently tagged secondary antibodies that recognize the primary antibodies. Chromosomes were stained with a fluorescent molecule that intercalates into the DNA double helix. ×3,500. (Courtesy of Dr. Wilma L. Lingle and Ms. Vivian A. Negron.)

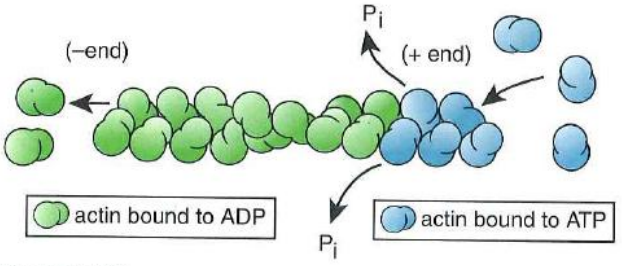

FIGURE 2.43
Polymerization of actin filaments. Actin filaments are polarized structures. Their fast-growing end is referred to as the plus (+) or barbed end; the slow-growing end is referred to as the minus (–) or pointed end. The dynamic process of actin polymerization requires energy in the form of an ATP molecule that is hydrolyzed to ADP after a G-actin molecule is incorporated into the filament.