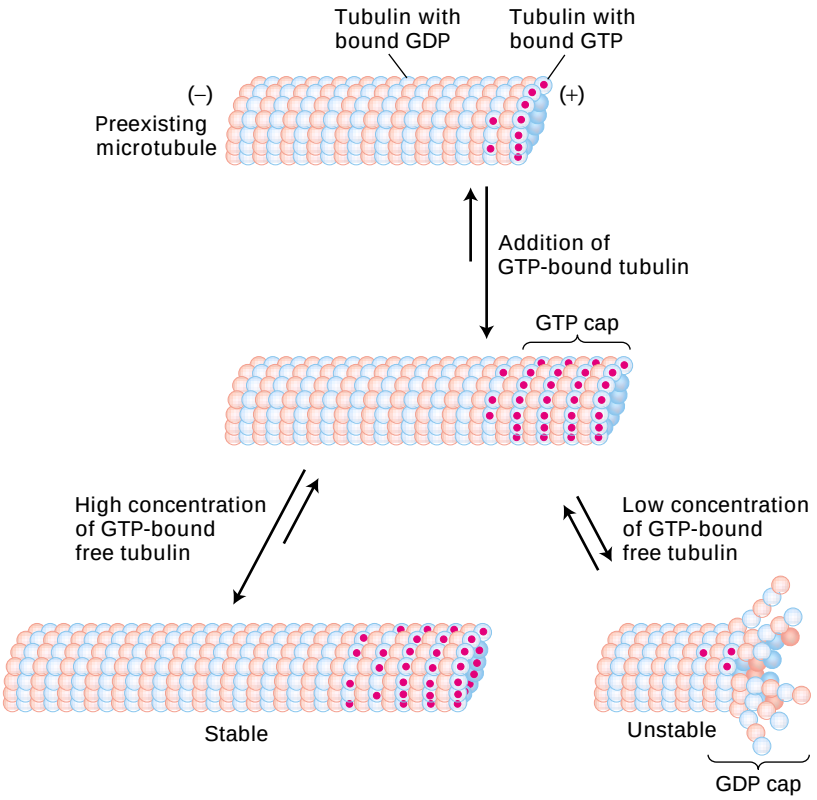
The results of subsequent in vivo studies showed that individual cytosolic and mitotic microtubules display dynamic instability. In one set of experiments, fluorescent αβ–tubulin subunits were microinjected into live cultured cells. The cells were chilled to depolymerize preexisting microtubules into tubulin dimers and then incubated at 37 °C to allow repolymerization, thus incorporating the fluorescent tubulin into all the cellular microtubules. Video recordings of a small region in labeled cells showed that some microtubules became longer, others became shorter, and some appeared alternately to grow and to shrink over a period of several minutes (Figure 20-10). Because most microtubules in a cell associate by their (−) ends with MTOCs, their instability is largely limited to the (+) ends.

Two conditions influence the stability of microtubules. First, the oscillations between growth and shrinkage in vitro occur at tubulin concentrations near the C_c. As already stated, at tubulin concentrations above the C_c, the entire population of microtubules grows and, at concentrations below the C_c, all microtubules shrink. At concentrations near the C_c, however, some microtubules grow, whereas others shrink. The second condition affecting microtubule stability is whether GTP or GDP occupies the exchangeable nucleotide-binding site on β-tubulin at the (+) end of a microtubule (Figure 20-11). Because dissociation ("off" rate) of a GDP-tubulin dimer is four orders of magnitude as fast as that of a GTP-tubulin dimer, a microtubule is destabilized and depolymerizes rapidly if the (+) end becomes capped with subunits containing GDP–β-tubulin rather than GTP–β-tubulin. This situation can arise when a microtubule shrinks rapidly, exposing GDP–β-tubulin in the walls of the microtubule, or when a microtubule grows so slowly that the hydrolysis of β-tubulin–bound GTP converts it into GDP before additional subunits can be added to the (+) end of the microtubule. Before a shortening microtubule vanishes entirely, it can be "rescued" and start to grow if tubulin subunits with bound GTP add to the (+) end before the bound GTP hydrolyzes. Thus the parameters that determine the stability of a microtubule are the growth rate, the shrinkage rate, the catastrophe frequency, and the rescue frequency.

Numerous Proteins Regulate Microtubule Dynamics and Cross-Linkage to Other Structures

A large number of proteins influence the assembly and stability of microtubules and their association with other cell structures (Table 20-1). These proteins are collectively called **microtubule-associated proteins (MAPs)** because most copurify with microtubules isolated from cells. The results of immunofluorescence localization studies also have shown a parallel distribution of MAPs and microtubules in cells—strong evidence for their interaction in vivo.

MAPs are classified into two groups on the basis of their function. One group stabilizes microtubules. The stucture of a stabilizing MAP consists of two domains—a basic microtubule-binding domain and an acidic projection domain. In the electron microscope, the projection domain appears as a filamentous arm that extends from the wall of the microtubule. This arm can bind to membranes, intermediate



◀ FIGURE 20-11 **Dynamic instability model of microtubule growth and shrinkage.** GTP-bound αβ–tubulin subunits (red) add preferentially to the (+) end of a preexisting microtubule. After incorporation of a subunit, the GTP (red dot) bound to the β-tubulin monomer is hydrolyzed to GDP. Only microtubules whose (+) ends are associated with GTP-tubulin (those with a GTP cap) are stable and can serve as primers for the polymerization of additional tubulin. Microtubules with GDP-tubulin (blue) at the (+) end (those with a GDP cap) are rapidly depolymerized and may disappear within 1 minute. At high concentrations of unpolymerized GTP-tubulin, the rate of addition of tubulin is faster than the rate of hydrolysis of the GTP bound in the microtubule or the rate of dissociation of GTP-tubulin from microtubule ends; thus the microtubule grows. At low concentrations of unpolymerized GTP-tubulin, the rate of addition of tubulin is decreased; consequently, the rate of GTP hydrolysis exceeds the rate of addition of tubulin subunits and a GDP cap forms. Because the GDP cap is unstable, the microtubule end peels apart to release tubulin subunits. [See T. Mitchison and M. Kirschner, 1984, *Nature* **312:**237; M. Kirschner and T. Mitchison, 1986, *Cell* **45:**329; and R. A. Walker et al., 1988, *J. Cell Biol.* **107:**1437.]

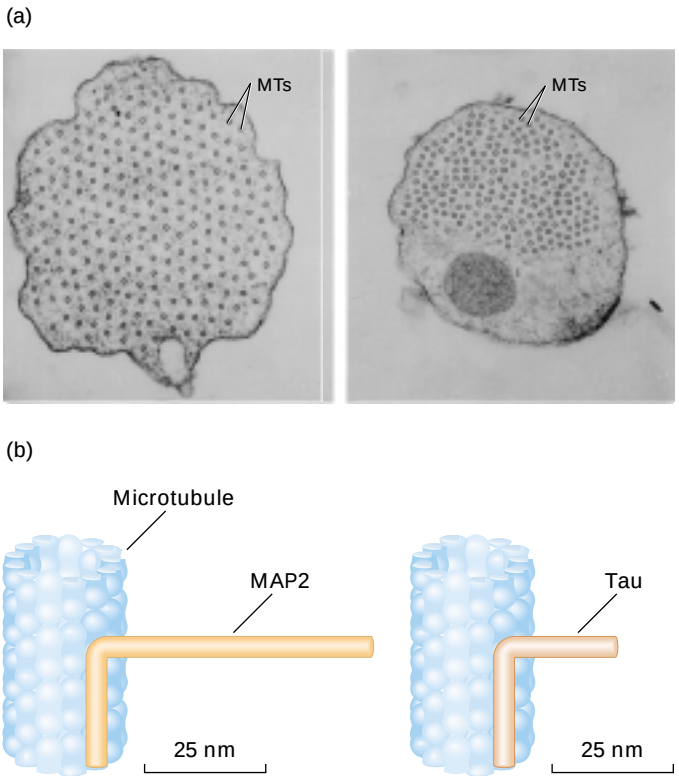
TABLE 20-1 Proteins That Modulate Microtubule (MT) Dynamics

Protein	MW	Location	Function
MICROTUBULE-STABILIZING PROTEINS			
MAP1	250,000–300,000 (heavy chain)	Dendrites and axons; non-neuronal cells	Assembles and stabilizes MTs
MAP2	42,000 and 200,000	Dendrites	Assembles and cross-links MTs to one another and to intermediate filaments
MAP4	210,000	Most cell types	Stabilizes MTs
Tau	55,000–62,000	Dendrites and axons	Assembles, stabilizes, and cross-links MTs
CLIP170	170,000	Most cell types	Cross-links MTs to endosomes and chromosomes
MICROTUBULE-DESTABILIZING PROTEINS			
Katanin	84,000	Most cell types	Microtubule severing
Op18 (stathmin)	18,000	Most cell types	Binds tubulin dimers

filaments, or other microtubules, and its length controls how far apart microtubules are spaced (Figure 20-12).

The microtubule-binding domain contains several repeats of a conserved, positively charged four-residue amino acid sequence that binds the negatively charged C-terminal part of tubulin. This binding is postulated to neutralize the charge repulsion between tubulin subunits within a microtubule, thereby stabilizing the polymer. MAP1A and MAP1B are large, filamentous molecules found in axons and dendrites of neurons as well as in non-neuronal cells. Each of these MAPs is derived from a single precursor polypeptide, which is proteolytically processed in a cell to generate one light chain and one heavy chain.

Other stabilizing MAPs include MAP2, MAP4, Tau, and CLIP170. MAP4, the most widespread of all the MAPs, is found in neuronal and non-neuronal cells. In mitosis, MAP4 regulates microtubule stability, and CLIP170 cross-links microtubules to chromosomes. MAP2 is found only in dendrites, where it forms fibrous cross-bridges between microtubules and links microtubules to intermediate filaments. Tau, which is much smaller than most other MAPs, is present in both axons and dendrites. This protein exists in several iso-



◀ EXPERIMENTAL FIGURE 20-12 **Spacing of microtubules depends on length of projection domain in bound microtubule-associated proteins.** Insect cells transfected with DNA expressing either long-armed MAP2 protein or short-armed Tau protein grow long axonlike processes. (a) Electron micrographs of cross sections through the processes induced by the expression of MAP2 (*left*) or Tau (*right*) in transfected cells. Note that the spacing between microtubules (MTs) in MAP2-containing cells is larger than in Tau-containing cells. Both cell types contain approximately the same number of microtubules, but the effect of MAP2 is to enlarge the caliber of the axonlike process. (b) Diagrams of association between microtubules and MAPs. Note the difference in the lengths of the projection arms in MAP2 and Tau. [Part (a) from J. Chen et al., 1992, *Nature* **360:**674.]

forms derived from alternative splicing of a *tau* mRNA. The ability of Tau to cross-link microtubules into thick bundles may contribute to the stability of axonal microtubules. Findings from gene transfection experiments implicate Tau in axonal elongation. Deletion of the genes encoding Tau and MAPIB leads to more severe phenotypes of axonal dysgenesis and lethality. Furthermore, aberrant polymerization of Tau into filaments is linked to neurodegenerative diseases such as human dementia in Alzheimer's patients.

When stabilizing MAPs coat the outer wall of a microtubule, tubulin subunits are unable to dissociate from the ends of that microtubule. Although bound MAPs generally dampen the rate of microtubule disassembly, the assembly of microtubules is affected to varying degrees: some MAPs, such as Tau and MAP4, stabilize microtubules, whereas other MAPs do not. Because of the effect of assembly MAPs on microtubule dynamics, modulating the binding of MAPs can control the length of microtubules. In most cases, this control is accomplished by the reversible phosphorylation of the MAP projection domain. Phosphorylated MAPs are unable to bind to microtubules; thus they promote microtubule disassembly. **MAP kinase,** a key enzyme for phosphorylating MAPs, is a participant in many signal-transduction pathways (Chapter 14), indicating that MAPs are targets of many extracellular signals. MAPs, especially MAP4, are also phosphorylated by a **cyclin-dependent kinase (CDK)** that plays a major role in controlling the activities of various proteins in the course of the cell cycle (Chapter 21).

A second group of MAPs directly destabilizes microtubules in many cell types. One of this group, called katanin, severs intact cytosolic microtubules by an ATP-dependent process. Internal bonds between tubulin subunits in the microtubule wall are broken, causing microtubules to fragment. This activity may release microtubules at the MTOC. Another protein, called Op18 or stathmin, increases the frequency of rapid disassembly of microtubules in the mitotic spindle. This protein may act by binding tubulin dimers, thereby reducing the pool of dimers available for polymerization. Phosphorylation inactivates Op18 and inhibits its destabilizing effect.

Colchicine and Other Drugs Disrupt Microtubule Dynamics

Some of the earliest studies of microtubules employed several drugs that inhibit mitosis, a cell process that depends on microtubule assembly and disassembly. Two such drugs isolated from plants, *colchicine* and *taxol,* have proved to be very powerful tools for probing microtubule function, partly because they bind only to αβ-tubulin or microtubules and not to other proteins and because their concentrations in cells can be easily controlled.

Colchicine and a synthetic relative, colcemid, have long been used as mitotic inhibitors. In cells exposed to high concentrations of colcemid, cytosolic microtubules depolymerize, leaving an MTOC. However, when plant or animal cells are exposed to low concentrations of colcemid, the microtubules remain and the cells become "blocked" at **metaphase,** the mitotic stage at which the duplicated chromosomes are fully condensed (see Figure 9-3). When the treated cells are washed with a colcemid-free solution, colcemid diffuses from the cell and mitosis resumes normally. Thus experimenters commonly use colcemid to accumulate metaphase cells for cytogenetic studies; removal of the colcemid leaves a population of cells whose cell cycle is in synchrony. Such synchronous populations are advantageous for studies of the cell cycle (Chapter 21).

The interface between α-tubulin and β-tubulin monomers in dimeric tubulin contains a high-affinity but reversible binding site for colchicine. Colchicine-bearing tubulin dimers, at concentrations much less than the concentration of free tubulin subunits, can add to the end of a growing microtubule. However, the presence of one or two colchicine-bearing tubulins at the end of a microtubule prevents the subsequent addition or loss of other tubulin subunits. Thus colchicine "poisons" the end of a microtubule and alters the steady-state balance between assembly and disassembly. As a result of this disruption of microtubule dynamics, mitosis is inhibited in cells treated with low concentrations of colchicine.

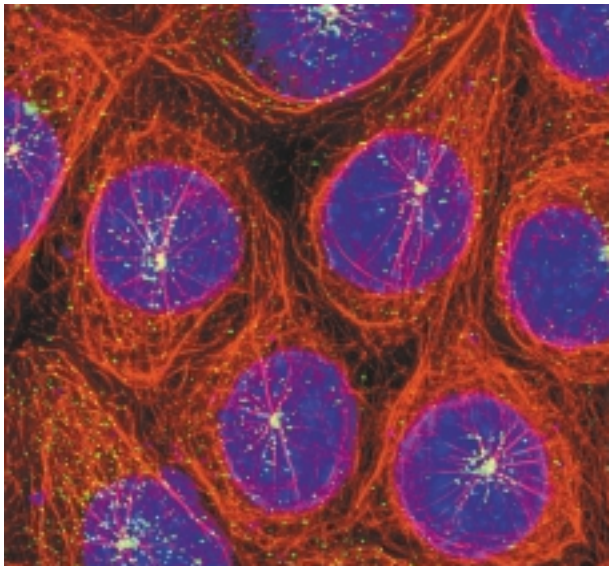
Other drugs bind to different sites on tubulin dimers or to microtubules and therefore affect microtubule stability through different mechanisms. For example, at low concentrations, taxol binds to microtubules and stabilizes them by inhibiting their shortening.

Drugs that disturb the assembly and disassembly of microtubules have been widely used to treat various diseases. Indeed, more than 2500 years ago, the ancient Egyptians treated heart problems with colchicine. Nowadays, this drug is used primarily in the treatment of gout and certain other diseases affecting the joints and skin. Other inhibitors of microtubule dynamics, including taxol, are effective anticancer agents and are used in the treatment of ovarian cancer. ▮

MTOCs Orient Most Microtubules and Determine Cell Polarity

In an interphase fibroblast cell, cytosolic microtubules are arranged in a distinctive hub-and-spoke array that lies at the center of a cell (Figure 20-13a). The microtubule spokes radiate from a central site occupied by the **centrosome,** which is the primary microtubule-organizing center in many interphase cells. We will use the term *MTOC* to refer to any of the structures used by cells to nucleate and organize microtubules. In animal cells, the MTOC is usually a centrosome, a collection of microtubule-associated proteins that sometimes but not always contains a pair of **centrioles** (Figure 20-13b). The centrioles, each a pinwheel array of triplet microtubules, lie in the center of the MTOC but do not make direct contact with the (−) ends of the cytosolic microtubules. Centrioles are not present in the MTOCs of plants and fungi; moreover, some epithelial cells and newly fertilized eggs from animals also lack centrioles. Thus, it is the associated

(a)



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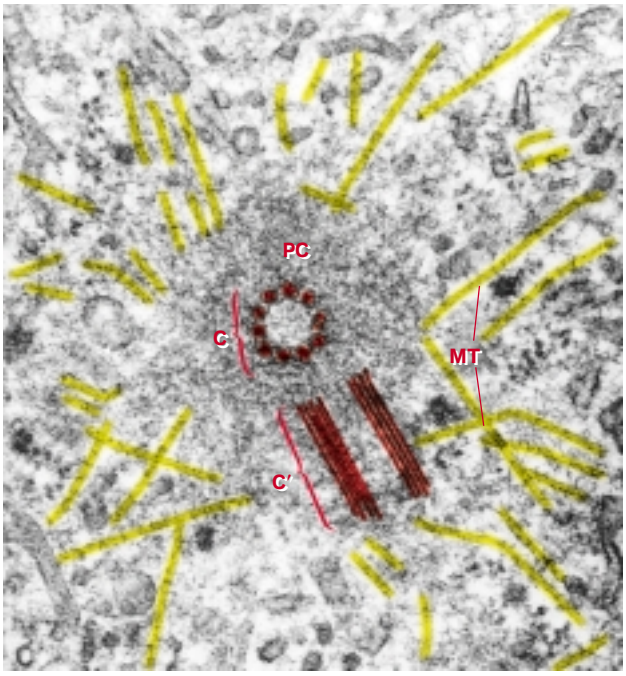
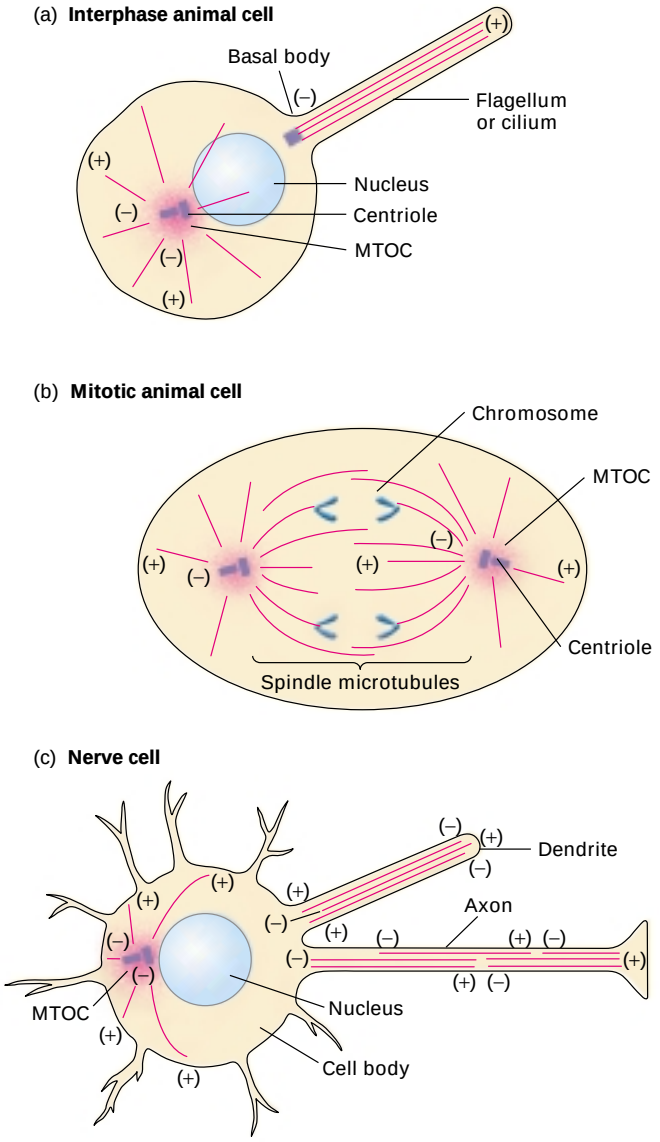


▲ EXPERIMENTAL FIGURE 20-13 **The centrosome, which functions as a microtubule-organizing center, contains a pair of orthogonal centrioles in most animal cells.** (a) Micrograph showing several cells each with an MTOC identified by fluorescently labeled antibodies against PCM1, a centrosomal protein. (b) Electron micrograph of the MTOC in an animal cell. The pair of centrioles (red), C and C′, in the center are oriented at right angles; thus one centriole is seen in cross section, and the other longitudinally. Surrounding the centrioles is a cloud of material, the pericentriolar (PC) matrix, which contains γ-tubulin and pericentrin. Embedded within the MTOC, but not contacting the centrioles, are the (−) ends of microtubules (MT; yellow). [Part (a) from A. Kubo and S. Tsukita, 2003, *J. Cell Sci.* **116**:919. Part (b) from B. R. Brinkley, 1987, in *Encyclopedia of Neuroscience,* vol. 2, Birkhauser Press, p. 665; courtesy of B. R. Brinkley.]



▲ FIGURE 20-14 **Orientation of cellular microtubules.** (a) In interphase animal cells, the (−) ends of most microtubules are proximal to the MTOC. Similarly, the microtubules in flagella and cilia have their (−) ends continuous with the basal body, which acts as the MTOC for these structures. (b) As cells enter mitosis, the microtubule network rearranges, forming a mitotic spindle. The (−) ends of all spindle microtubules point toward one of the two MTOCs, or poles, as they are called in mitotic cells. (c) In nerve cells, the (−) ends of all axonal microtubules are oriented toward the base of the axon, but dendritic microtubules have mixed polarities.

proteins in an MTOC that have the capacity to organize cytosolic microtubules.

Because microtubules assemble from the MTOC, microtubule polarity becomes fixed in a characteristic orientation (Figure 20-14). In most interphase animal cells, for instance, the (−) ends of microtubules are closest to the MTOC. In mitosis, the centrosome duplicates and migrates to new positions flanking the nucleus. There the centrosome becomes

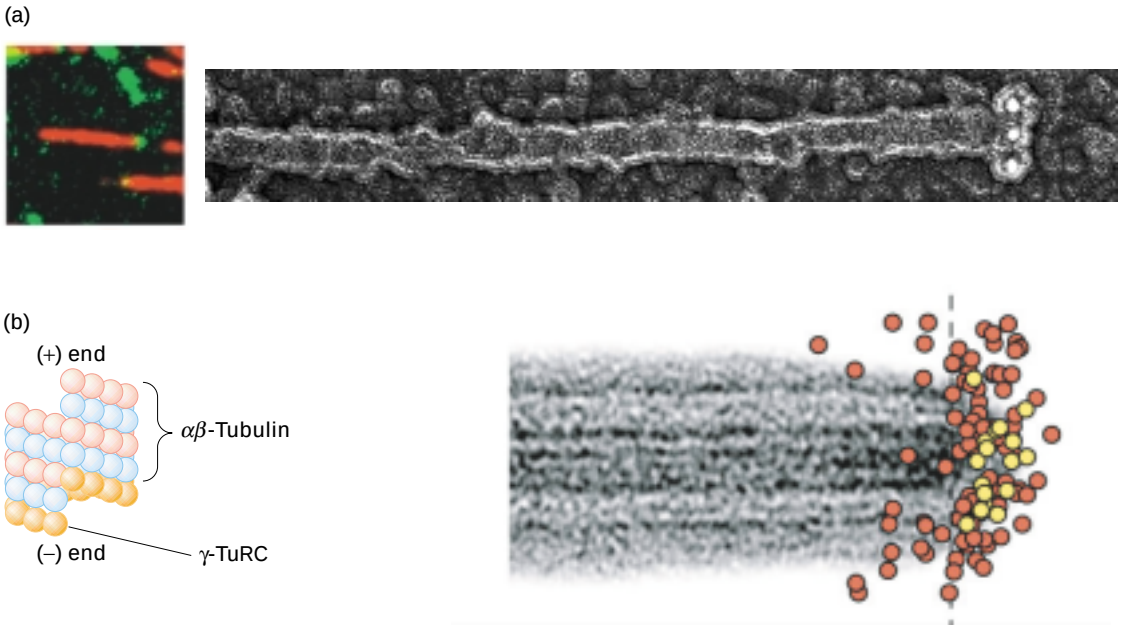
the organizing center for microtubules forming the mitotic apparatus, which will separate the chromosomes into the daughter cells in mitosis. The microtubules in the axon of a nerve cell, which help stabilize the long process, are all oriented in the same direction.

In contrast with the single perinuclear MTOC present in most interphase animal cells, plant cells, polarized epithelial cells, and embryonic cells contain hundreds of MTOCs, which are distributed throughout the cell, often near the cell cortex. In plant cells and polarized epithelial cells, a cortical array of microtubules aligns with the cell axis. In both cell types, the polarity of the cell is linked to the orientation of the microtubules.

The γ-Tubulin Ring Complex Nucleates Polymerization of Tubulin Subunits

The MTOC organizes cytosolic microtubules by first nucleating microtubule assembly and then anchoring and releasing microtubules. Despite its amorphous appearance, the pericentriolar material of an MTOC is an ordered lattice that contains many proteins that are necessary for initiating the assembly of microtubules (see Figure 20-13). One of these proteins, γ-tubulin, was first identified in genetic studies designed to discover proteins that interact with β-tubulin. The results of subsequent studies demonstrated that γ-tubulin and the lattice protein pericentrin are part of the pericentriolar material of centrosomes; these proteins have also been detected in MTOCs that lack a centriole. The finding that the introduction of antibodies against γ-tubulin into cells blocks microtubule assembly implicates γ-tubulin as a necessary factor in nucleating the polymerization of tubulin subunits.

Approximately 80 percent of the γ-tubulin in cells is part of a 25S complex, which has been isolated from extracts of frog oocytes and fly embryos. Named the *γ-tubulin ring complex* (*γ-TuRC*) for its ringlike appearance in the electron microscope, the complex comprises eight polypeptides and measures 25 nm in diameter. Findings from in vitro experiments show that the γ-TuRC can directly nucleate microtubule assembly at subcritical tubulin concentrations—that is, at concentrations below which polymerization would not take place in the absence of the γ-TuRC. To investigate how γ-TuRC associates with microtubules, scientists performed immunolabeling experiments with the use of gold-conjugated antibodies specific for γ-TuRC components, either γ-tubulin or XGRIP. The results of these studies reveal that complexes are localized to one end of a microtubule and are not present along the sides (Figure 20-15a). This location is consistent with a role for



▲ EXPERIMENTAL FIGURE 20-15 **The γ-tubulin ring complex (γ-TuRC) is localized to one end of the microtubule.** (a) A fluorescence micrograph (*left*) and an electron micrograph (*right*) of microtubules stained with gold-labeled antibodies to γ-tubulin or XGRIP, a microtubule-binding protein. Both proteins are components of the γ-TuRC. The labeled proteins are localized to one end of the microtubules. (b) A model of the γ-TuRC. This complex is thought to nucleate microtubule assembly by presenting a row of γ-tubulin subunits, which can directly bind αβ-tubulin subunits. This model is supported by plotting the positions of gold-labeled antibodies to either γ-tubulin (red) or XGRIP109 (yellow) from several experiments onto a microtubule end. [Parts (a) and (b, right) from T. J. Keating and G. G. Borisy, 2000, *Nature Cell Biol.* **2:**352; courtesy of T. J. Keating and G. G. Borisy. Part (b, left) modified from C. Wiese and Y. Zheng, 1999, *Curr. Opin. Struc. Biol.* **9:**250.]

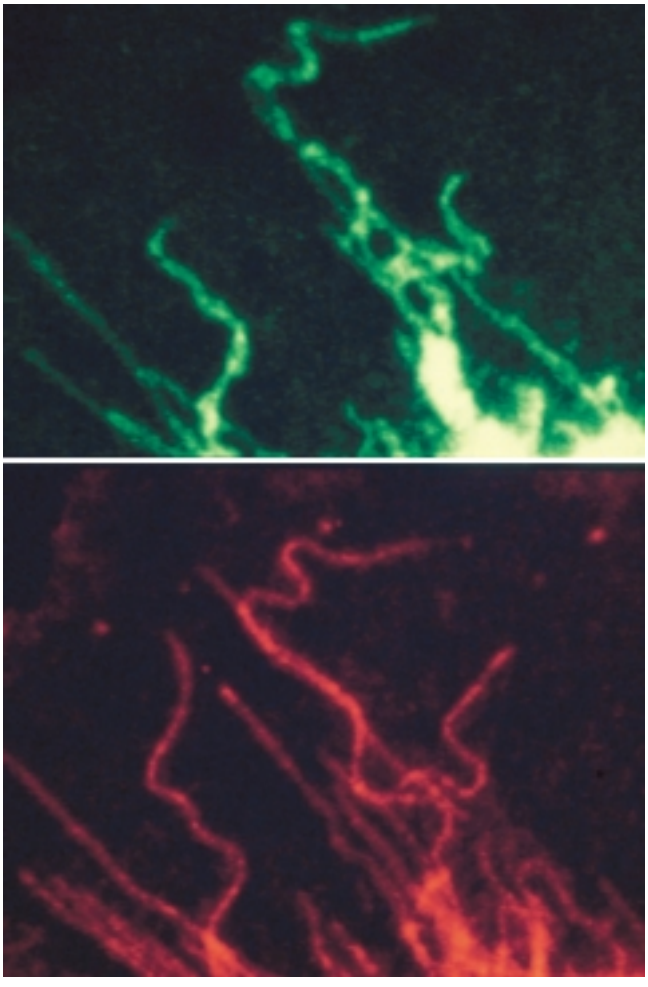
γ-TuRC in nucleating microtubule assembly. A model of γ-TuRC based on electron microscopy shows γ-tubulin in contact with the (−) end of a microtubule (Figure 20-15b).

Cytoplasmic Organelles and Vesicles Are Organized by Microtubules

Fluorescence microscopy reveals that membrane-limited organelles such as the endoplasmic reticulum (ER), Golgi, endosomes, and mitochondria are associated with microtubules. For instance, in cultured fibroblasts stained with anti-tubulin antibodies and $DiOC_6$, a fluorescent dye specific for the ER, the anastomosing ER network in the cytosol is seen to colocalize with microtubules (Figure 20-16). If cells are treated with a microtubule-depolymerizing drug, the ER loses its networklike organization. After the drug has been washed from the cells, tubular fingers of ER grow as new microtubules assemble. In cell-free systems, the ER can be reconstituted with microtubules and an ER-rich cell extract. Even under this cell-free regime, ER membranes elongate along microtubules. This close association between the ER and intact microtubules suggests that proteins bind ER membranes to microtubules.

The role of microtubules in organizing the Golgi complex also has been studied extensively. In interphase fibroblasts, the Golgi complex is concentrated near the MTOC. In mitosis (or after the depolymerization of microtubules by colcemid), the Golgi complex breaks into small vesicles that are dispersed throughout the cytosol. When the cytosolic microtubules re-form during interphase (or after removal of the colcemid), the Golgi vesicles move along these microtubule tracks toward the MTOC, where they reaggregate to form large membrane complexes.

These observations were among the first to suggest that microtubules play a role in the intracellular transport of membrane-limited organelles and vesicles. Other examples of such transport and the motor proteins that power them are described in Section 20.2.



▲ EXPERIMENTAL FIGURE 20-16 **Fluorescence microscopy reveals colocalization of endoplasmic reticulum membranes and cytosolic microtubules.** $DiOC_6$, an ER-binding fluorescent dye (green), and fluorescently-labeled anti-tubulin antibodies (red) were used to stain a cultured frog fibroblast. The alignment of the ER network and microtubules in many but not all regions of the cytoplasm is evident because the cell has sparse microtubules. [Courtesy of M. Terasaki.]

KEY CONCEPTS OF SECTION 20.1

Microtubule Organization and Dynamics

- Tubulins belong to an ancient family of GTPases that polymerize to form microtubules, hollow cylindrical structures 25 nm in diameter.
- Microtubules, like actin microfilaments, exhibit both structural and functional polarity.
- Dimeric αβ–tubulin subunits interact end-to-end to form protofilaments, which associate laterally into microtubules (see Figure 20-7).
- Microtubules exhibit structural polarity. Subunits are added and lost preferentially at one end, the (+) end.
- Assembly and disassembly of microtubules depends on the critical concentration, C_c, of αβ–tubulin subunits. Above the C_c, microtubules assemble; below the C_c, microtubules disassemble.
- Microtubules exhibit two dynamic phenomena that are pronounced at tubulin concentrations near the C_c: (1) treadmilling, the addition of subunits at one end and their loss at the other end, and (2) dynamic instability, the oscillation between lengthening and shortening (see Figure 20-9).
- The balance between growth and shrinkage of unstable microtubules depends on whether the exchangeable GTP bound to β-tubulin is present on the (+) end or whether it has been hydrolyzed to GDP (see Figure 20-11).
- Microtubule-associated proteins (MAPs) organize microtubules and affect their stability. Some MAPs prevent