Molecular biology of the cell
BIO 207

Prof Wouter R. Karthaus PhD
EPFL-SV-ISREC

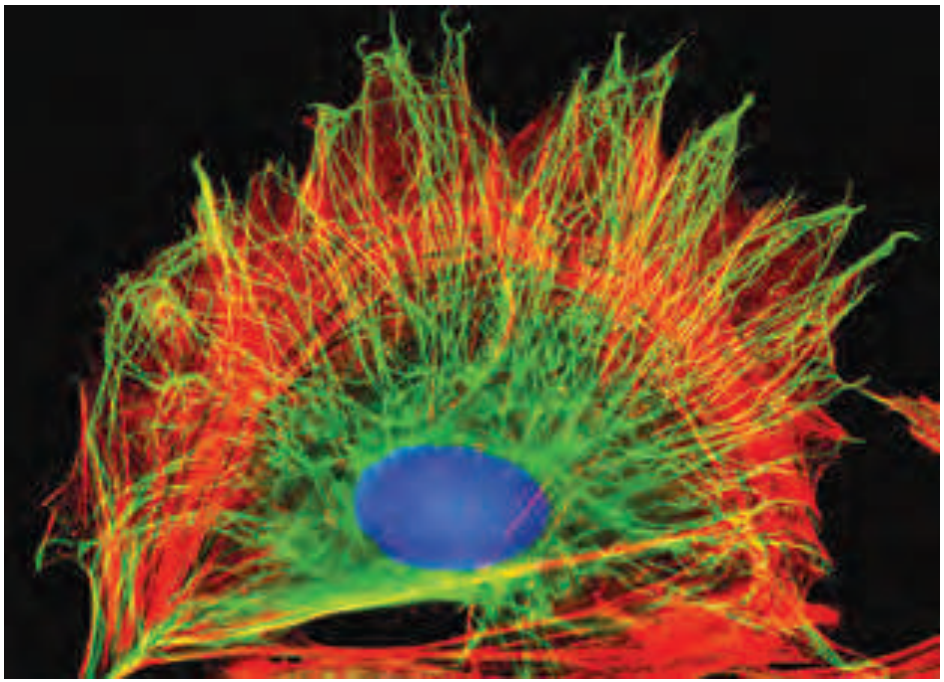
BIO207@EPFL.CH

The Cytoskeleton

CHAPTER
16

IN THIS CHAPTER

The cytoskeleton



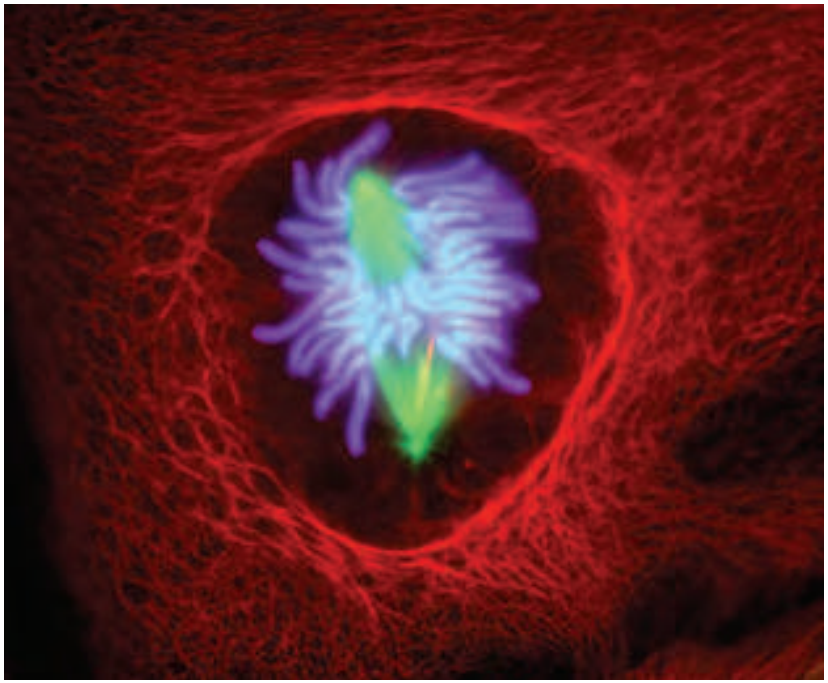


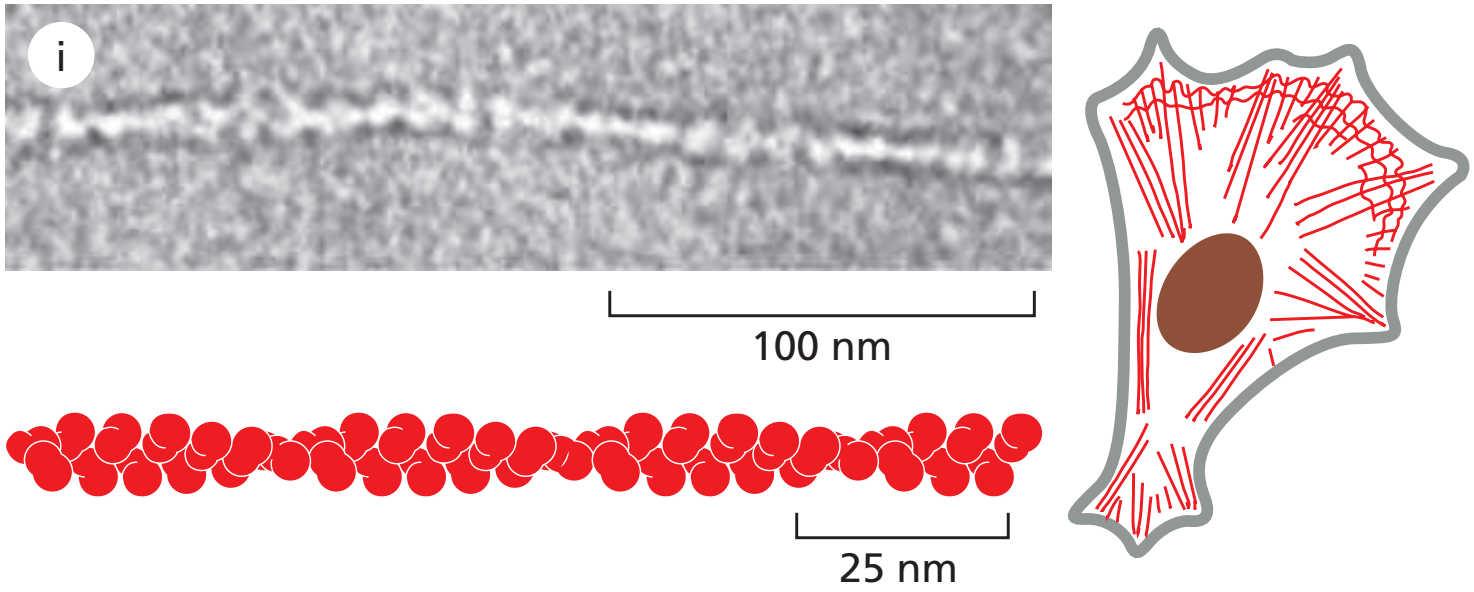
Figure 16–1 The cytoskeleton. (A) A cell in culture has been fixed and labeled to show its cytoplasmic arrays of microtubules (green) and actin filaments (red). (B) This dividing cell has been labeled to show its spindle microtubules (green) and surrounding cage of intermediate filaments (red).

Cytoskeleton functions

- Regulation of spatial and mechanical function of the cell
- Cell shape
  - Membrane shape etc.
- Movement
- Drives division of cells
- Rigidity (Strength)

There are 3 main players to know!

ACTIN FILAMENTS

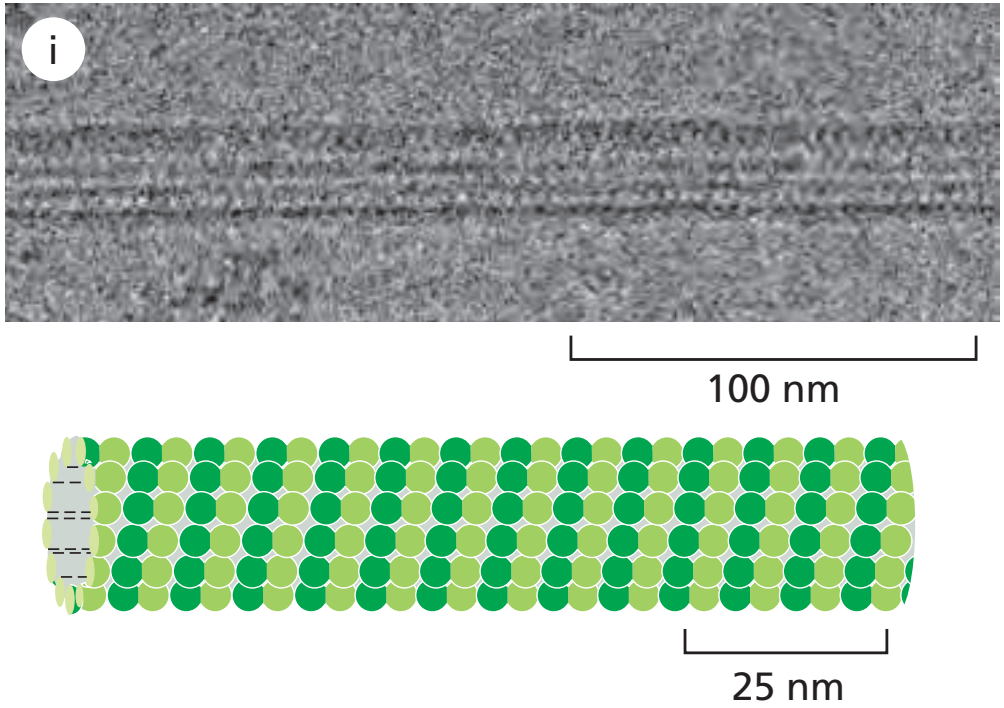


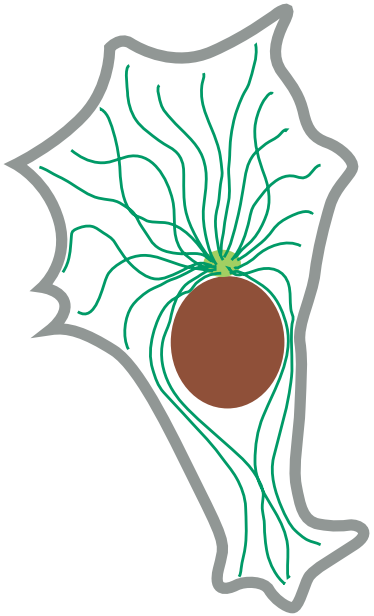
Involved in plasma membrane shape & whole cell locomotion

Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures with a diameter of 8 nm that organize into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane. (i) Single actin filament; (ii) microvilli; (iii) stress fibers (*red*) terminating in focal adhesions (*green*); (iv) striated muscle.

Micrc

MICROTUBULES





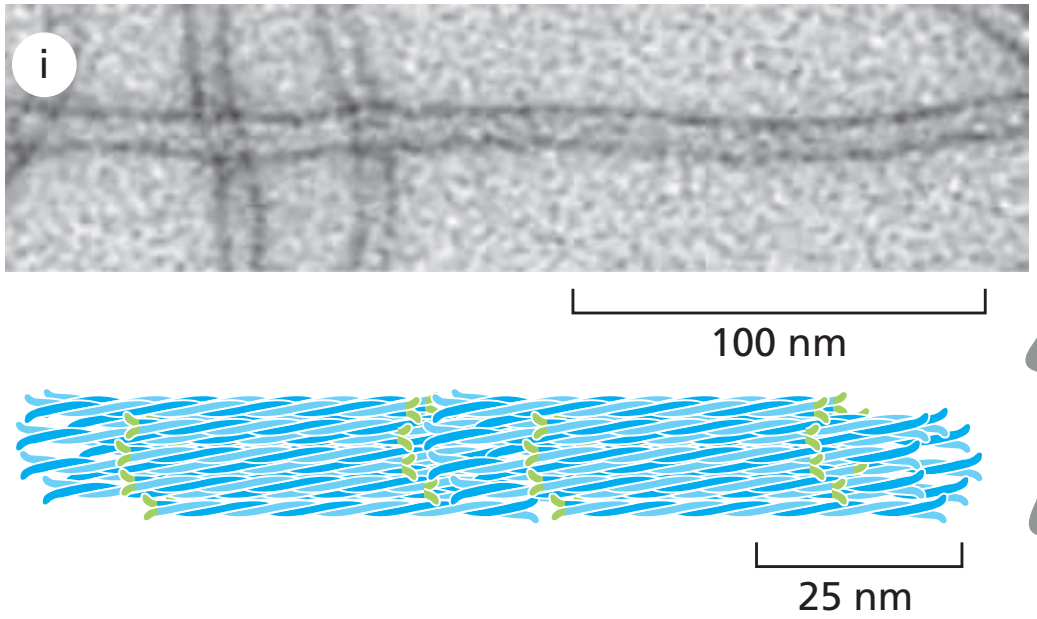
Involved in

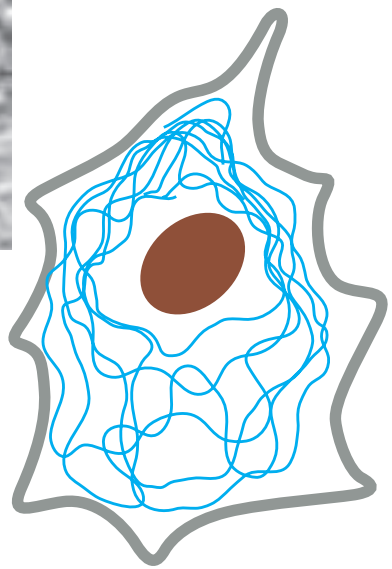
- positioning of organelles
- Transport
- Forming mitotic spindle during division and chromosome segregation

Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and frequently have one end attached to a microtubule-organizing center (MTOC) called a *centrosome*. (i) Single microtubule; (ii) cross section at the base of three cilia showing triplet microtubules; (iii) interphase microtubule array (*green*) and organelles (*red*); (iv) ciliated protozoan.

Micrograp

INTERMEDIATE FILAMENTS



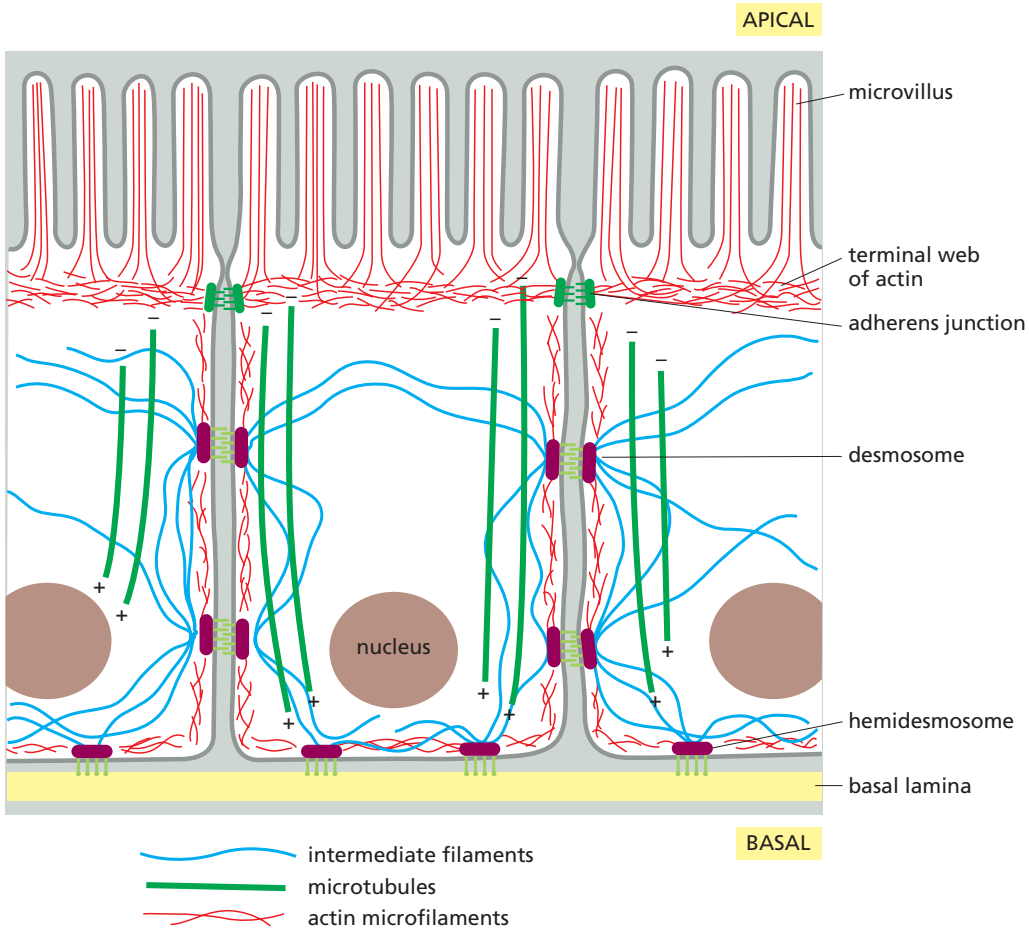


Cell strength and rigidity

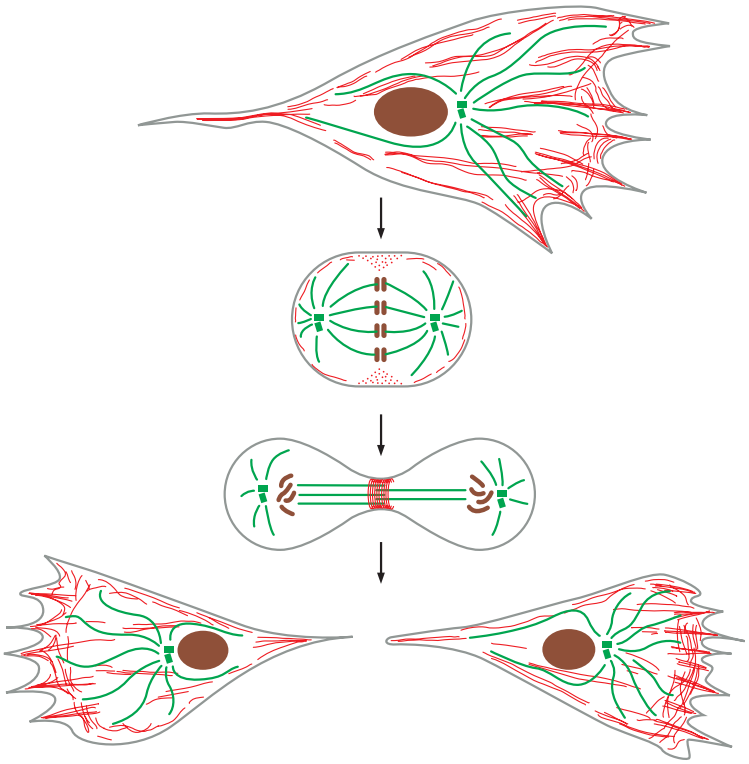
Many different kinds exist and these are cell type specific

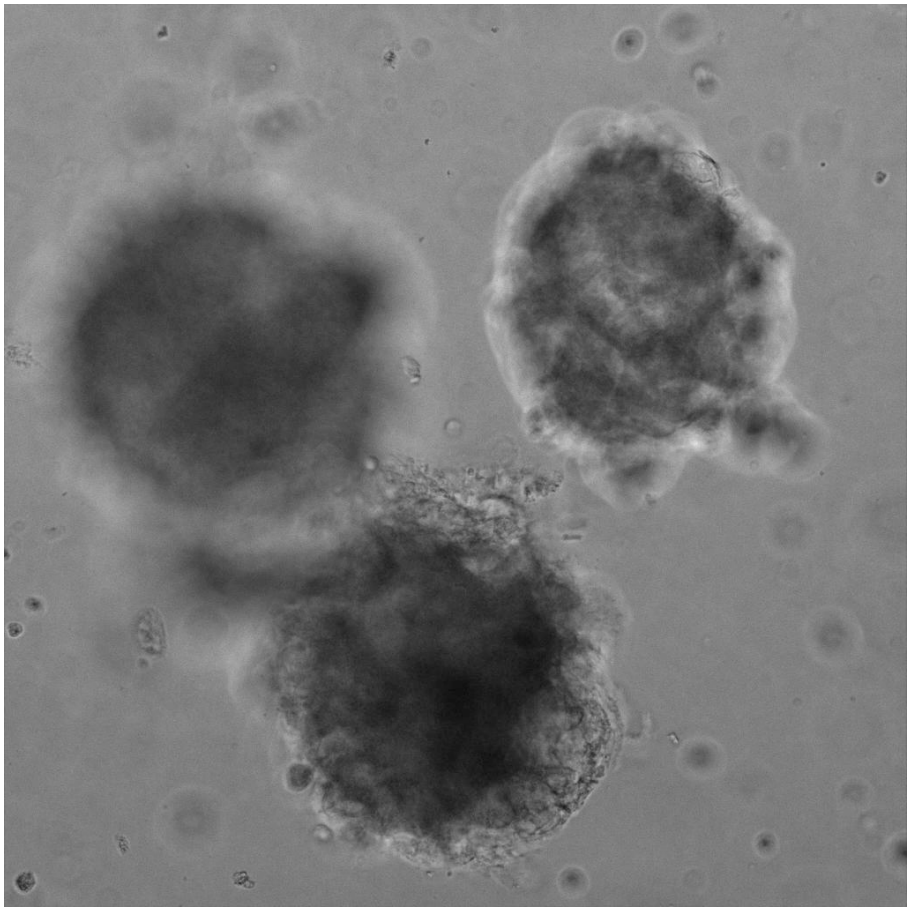
Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell–cell junction to another, thereby strengthening the entire epithelium. (i) Individual intermediate filaments; (ii) Intermediate filaments (*blue*) in neurons and (iii) epithelial cell; (iv) nuclear lamina.

Organization of the cytoskeleton in polarized epithelial cells.



The cytoskeleton is dynamic

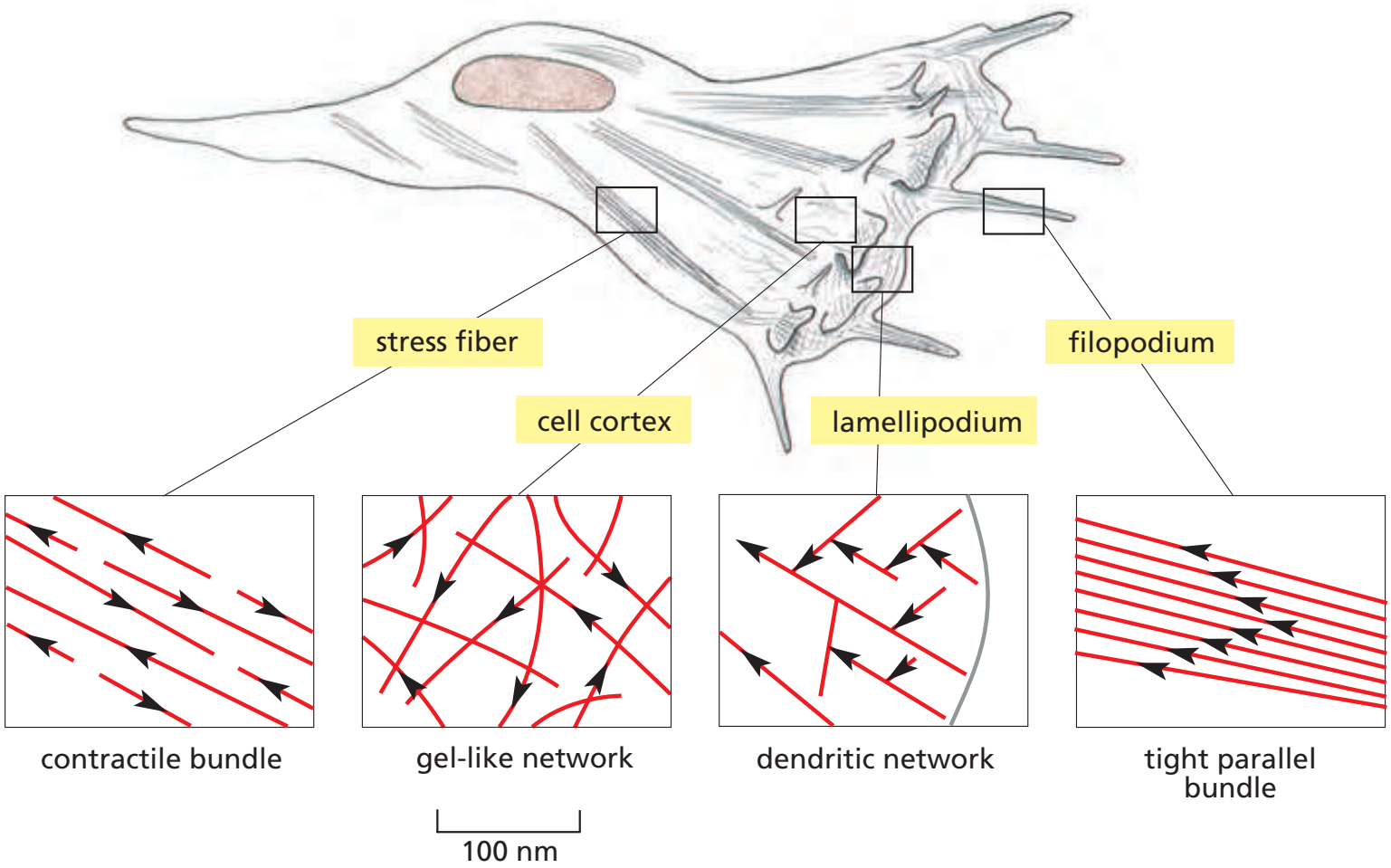




This dynamic nature is possible due to
the modular buildup of the cytoskeletion

Actin filaments

Actin filaments comes in many forms



structure of an actin monomer and actin filament

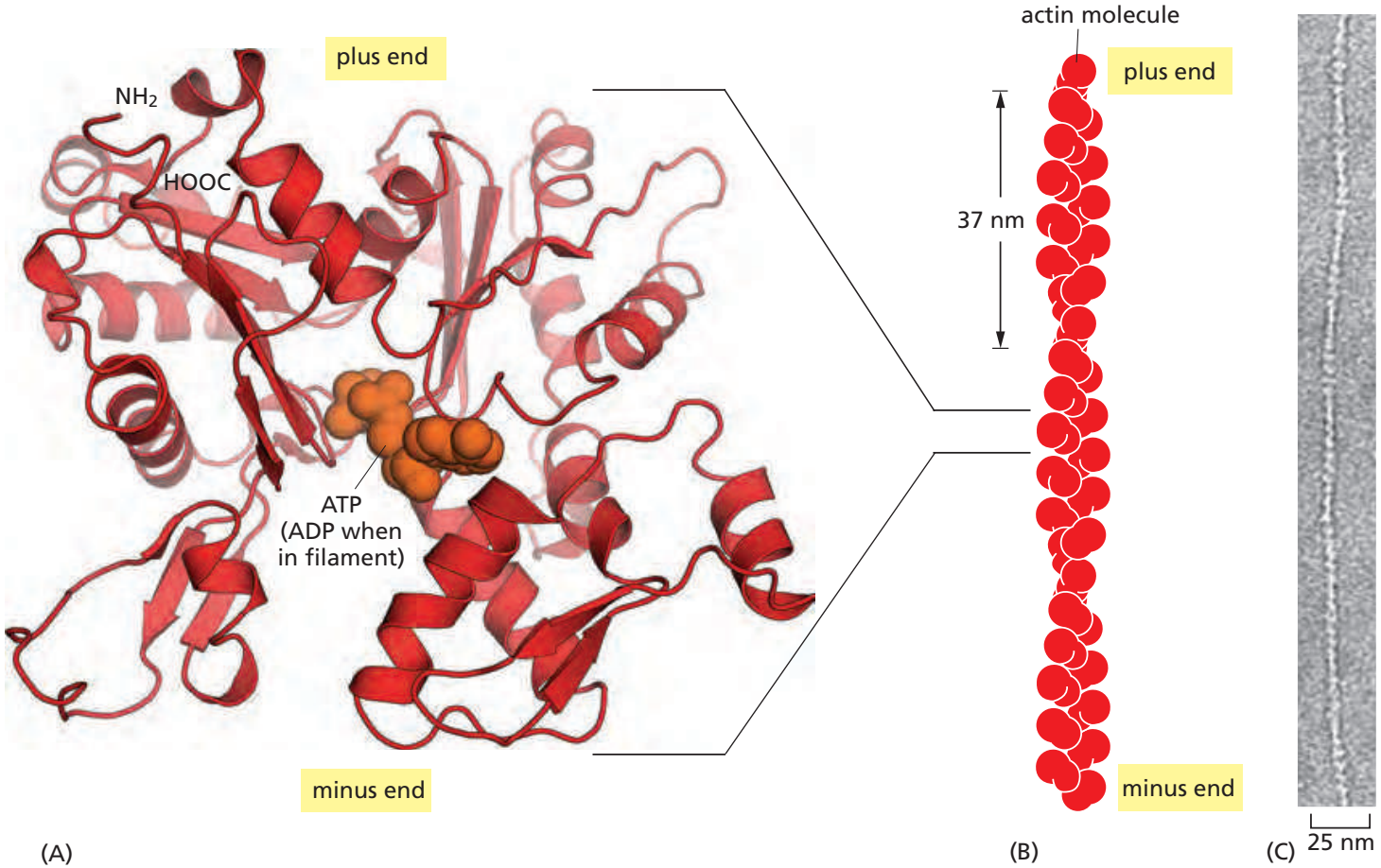
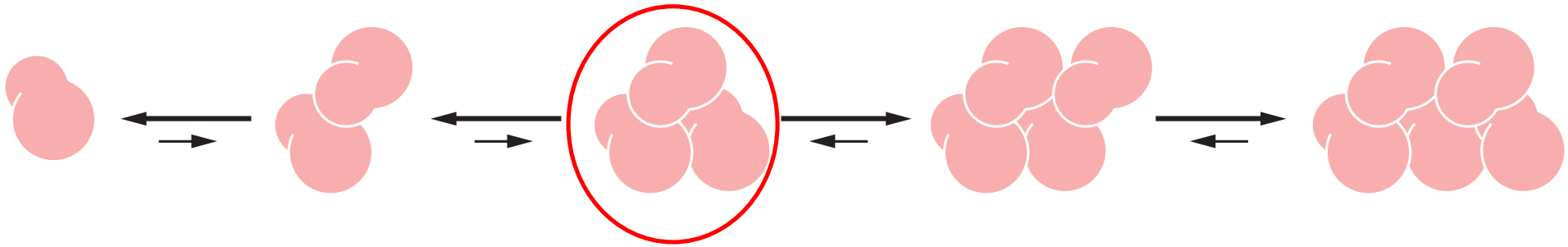


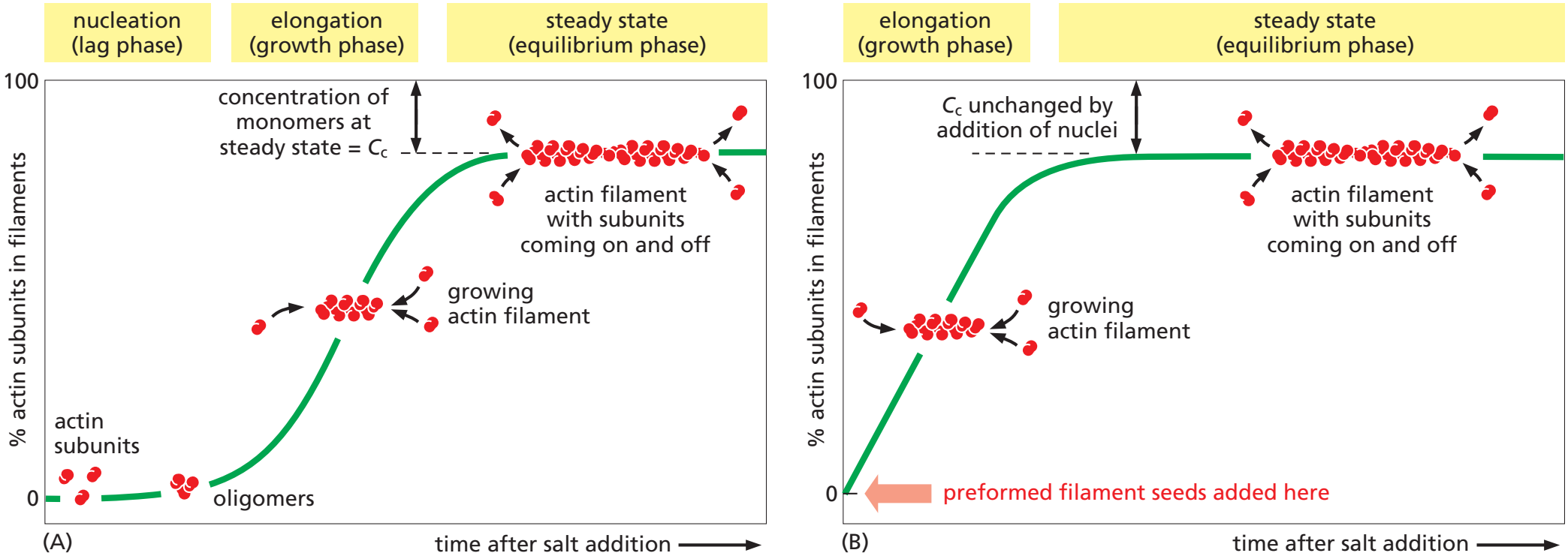
Figure 16–11 The structures of an actin monomer and actin filament. (A) The actin monomer has a nucleotide (either ATP or ADP) bound in a deep cleft in the center of the molecule. (B) Arrangement of monomers in a filament consisting of two protofilaments, held together by lateral contacts, which wind around each other as two parallel strands of a helix, with a twist repeating every 37 nm. All the subunits within the filament have the same orientation. (C) Electron micrograph of negatively stained actin filament. (C, courtesy of Roger Craig.)

Formation of actin filaments

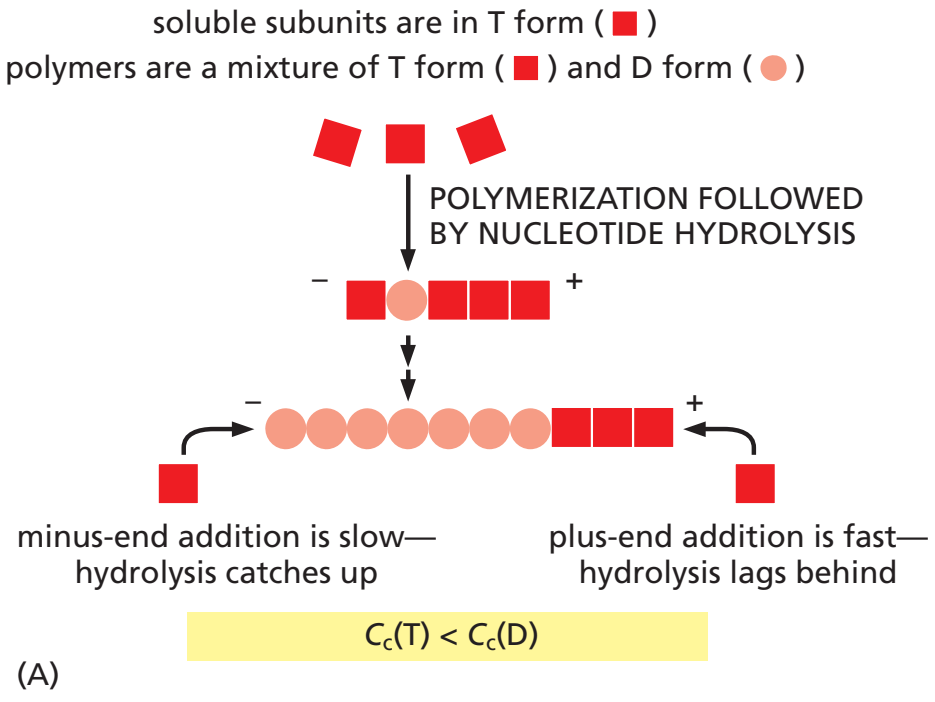
NUCLEATION A helical polymer is stabilized by multiple contacts between adjacent subunits. In the case of actin, two actin molecules bind relatively weakly to each other, but addition of a third actin monomer to form a trimer makes the entire group more stable.



In a test tube actin filaments a formed must faster if a premade >3 subunit is added



Treadmilling of an actin filament, made possible by the ATP hydrolysis that follows subunit addition



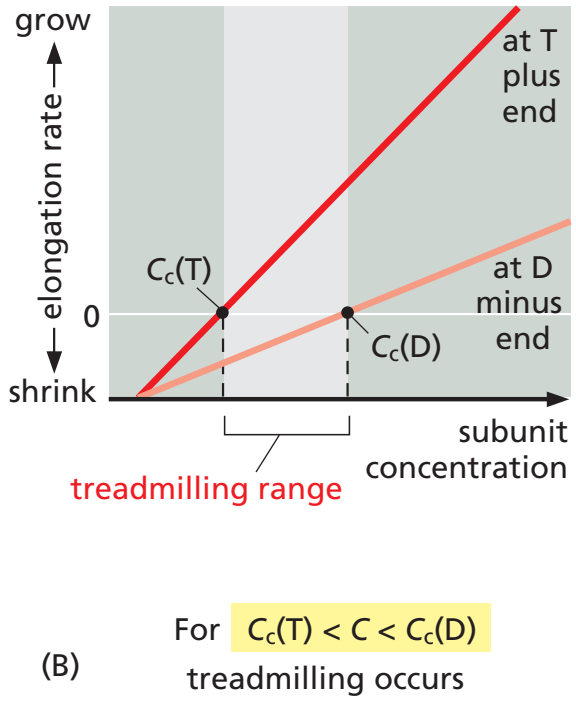


Figure 16–14 Treadmilling of an actin filament, made possible by the ATP hydrolysis that follows subunit addition. (A) Explanation for the different critical concentrations (Cc) at the plus and minus ends. Subunits with bound ATP (T-form subunits) polymerize at both ends of a growing filament, and then undergo nucleotide hydrolysis within the filament. As the filament grows, elongation is faster than hydrolysis at the plus end in this example, and the terminal subunits at this end are therefore always in the T form. However, hydrolysis is faster than elongation at the minus end, and so terminal subunits at this end are in the D form. (B) Treadmilling occurs at intermediate concentrations of free subunits. The critical concentration for polymerization on a filament end in the T form is lower than for a filament end in the D form. If the actual subunit concentration is somewhere between these two values, the plus end grows while the minus end shrinks, resulting in treadmilling.

Effects of thymosin and profilin on actin polymerization

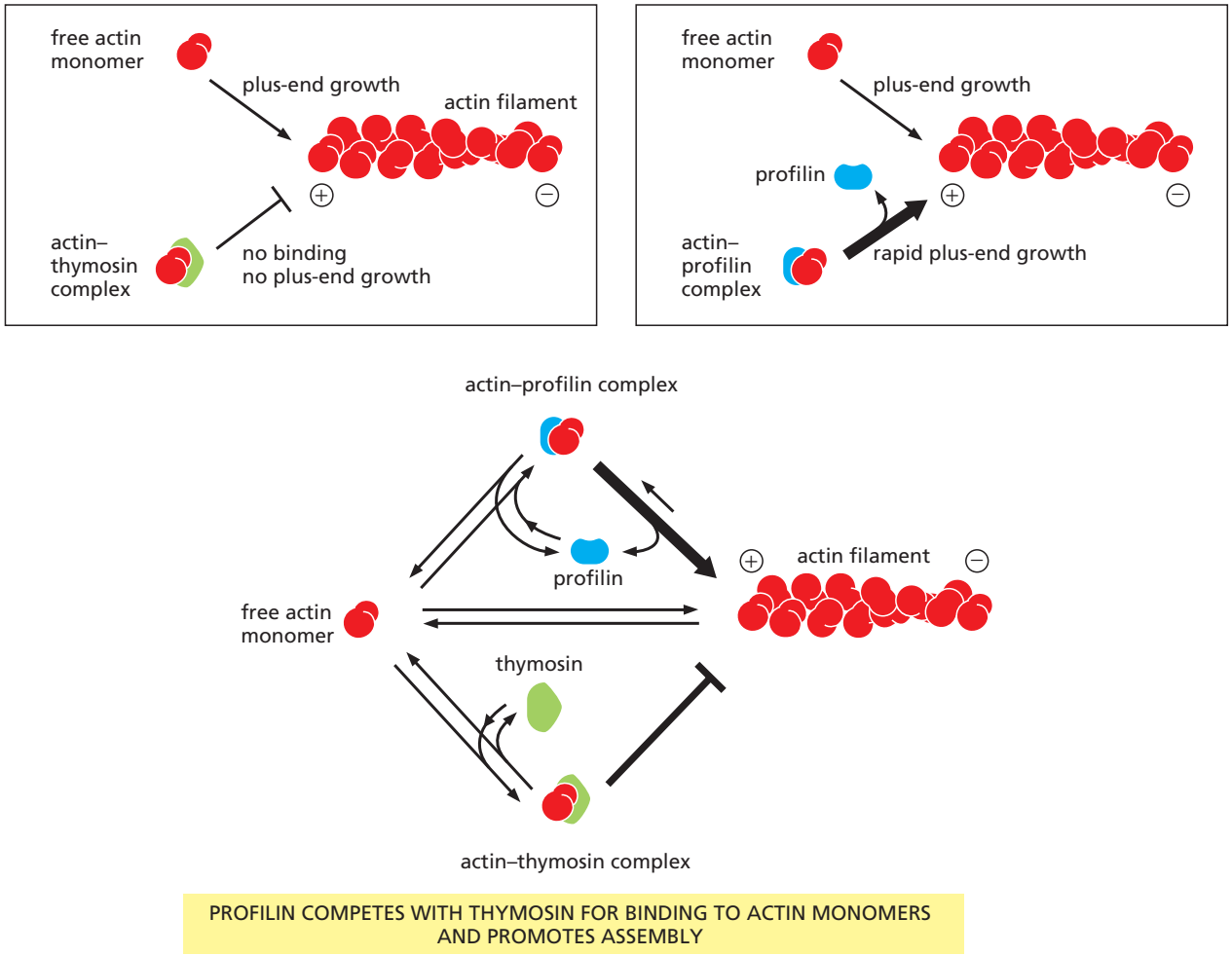


Figure 16–15 Effects of thymosin and profilin on actin polymerization. An actin monomer bound to thymosin is sterically prevented from binding to and elongating the plus end of an actin filament (left). An actin monomer bound to profilin, on the other hand, is capable of elongating a filament (right). Thymosin and profilin cannot both bind to a single actin monomer at the same time. In a cell in which most of the actin monomer is bound to thymosin, the activation of a small amount of profilin can produce rapid filament assembly. As indicated (bottom), profilin binds to actin monomers that are transiently released from the thymosin-bound monomer pool, shuttles them onto the plus ends of actin filaments, and is then released and recycled for further rounds of filament elongation.

Actin elongation mediated by formins

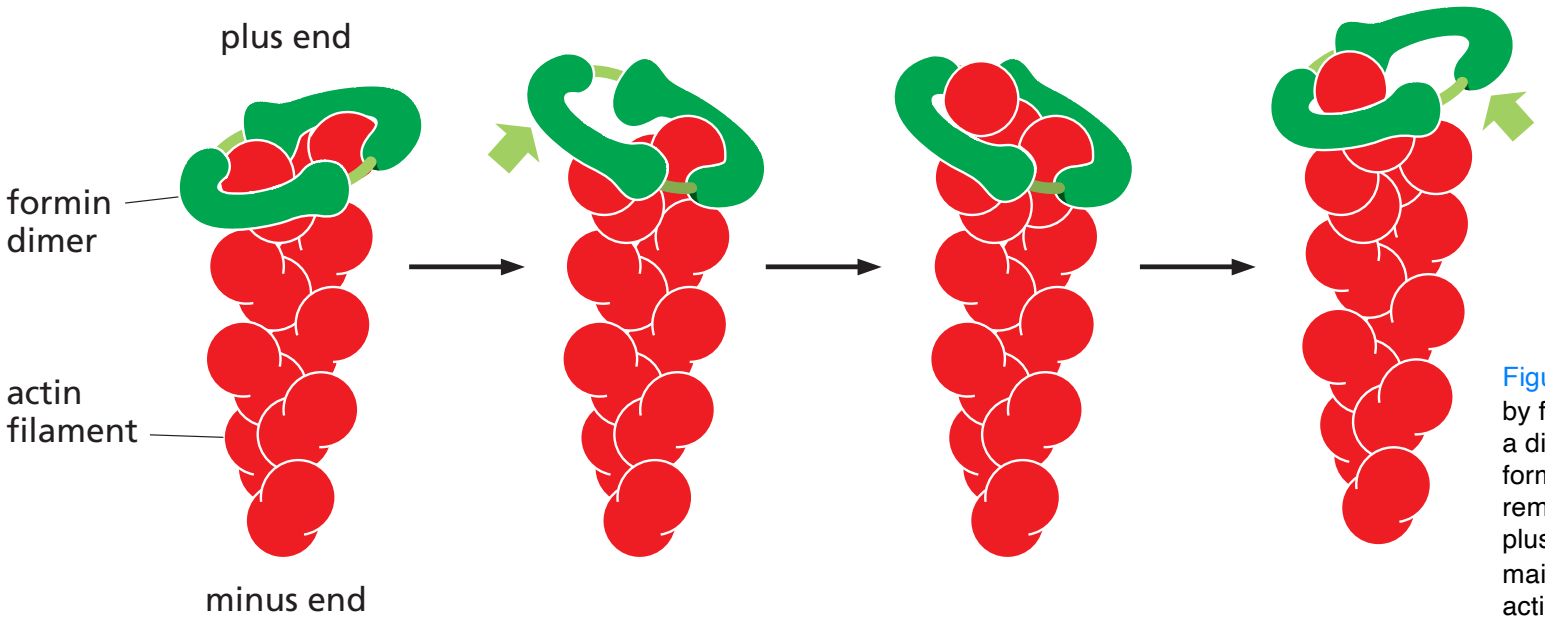


Figure 16–17 Actin elongation mediated by formins. Formin proteins (green) form a dimeric complex that can nucleate the formation of a new actin filament (red) and remain associated with the rapidly growing plus end as it elongates. The formin protein maintains its binding to one of the two actin subunits exposed at the plus end as it allows each new subunit to assemble. Only part of the large dimeric formin molecule is shown here. Other regions regulate its activity and link it to particular structures in the cell. Many formins are indirectly connected to the cell plasma membrane and aid the insertional polymerization of the actin filament directly beneath the membrane surface.

Nucleation and actin web formation by the Arp 2/3 complex

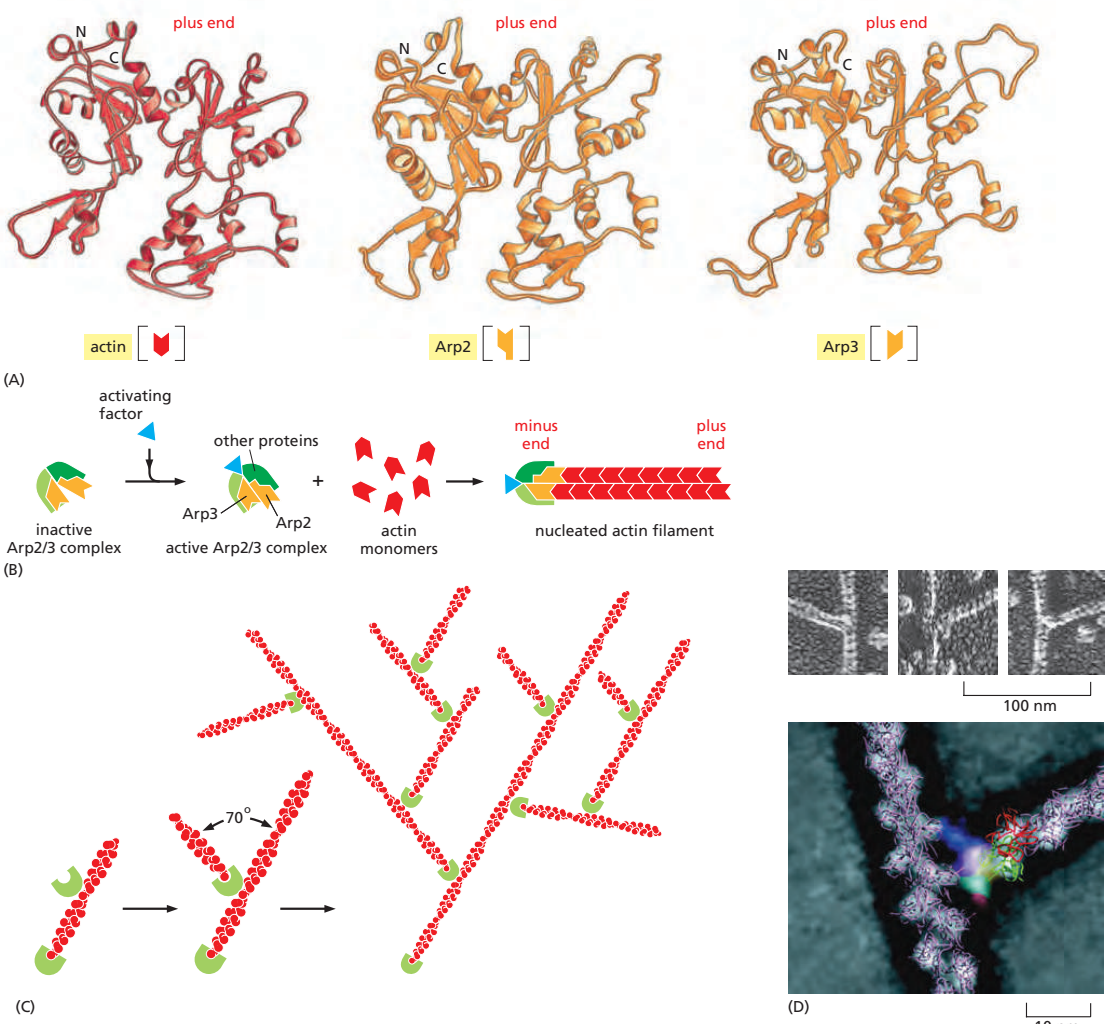


Figure 16–16 Nucleation and actin web formation by the Arp 2/3 complex. (A) The structures of Arp2 and Arp3 compared to the structure of actin. Although the face of the molecule equivalent to the plus end (top) in both Arp2 and Arp3 is very similar to the plus end of actin itself, differences on the sides and minus end prevent these actin-related proteins from forming filaments on their own or coassembling into filaments with actin. (B) A model for actin filament nucleation by the Arp 2/3 complex. In the absence of an activating factor, Arp2 and Arp3 are held by their accessory proteins in an orientation that prevents them from nucleating a new actin filament. When an activating factor (indicated by the blue triangle) binds the complex, Arp2 and Arp3 are brought together into a new configuration that resembles the plus end of an actin filament. Actin subunits can then assemble onto this structure, bypassing the rate-limiting step of filament nucleation. (C) The Arp 2/3 complex nucleates filaments most efficiently when it is bound to the side of a preexisting actin filament. The result is a filament branch that grows at a 70° angle relative to the original filament. Repeated rounds of branching nucleation result in a treelike web of actin filaments. (D) Top, electron micrographs of branched actin filaments formed by mixing purified actin subunits with purified Arp 2/3 complexes. Bottom, reconstructed image of a branch where the crystal structures of actin (pink) and the Arp 2/3 complex have been fitted to the electron density. The mother filament runs from top to bottom, and the daughter filament branches off to the right where the Arp 2/3 complex binds to three actin subunits in the mother filament. (D, top, from R.D. Mullins et al., Proc. Natl Acad. Sci. USA 95:6181–6186, 1998, with permission from National Academy of Sciences; botttom, from N. Volkmann et al., Science 293:2456–2459, 2001, with permission from AAAS.)

Filament capping and its effects on filament dynamics

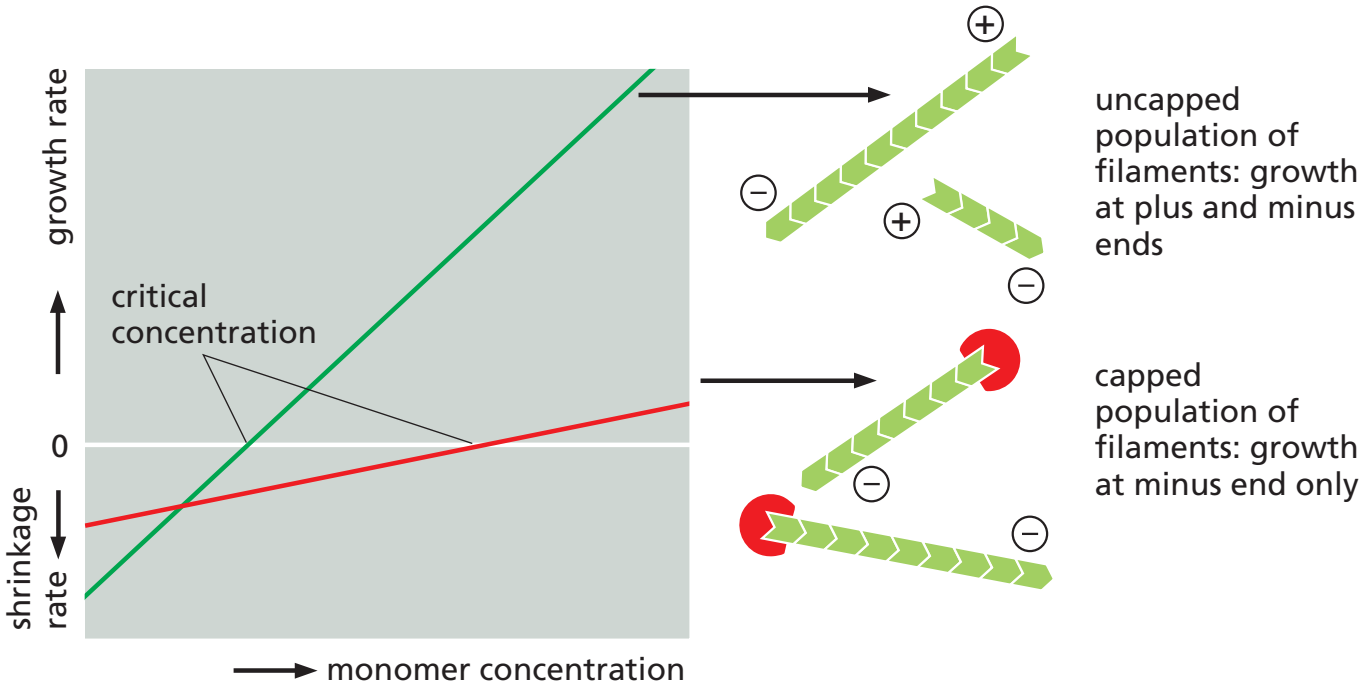


Figure 16–19 Filament capping and its effects on filament dynamics. A population of uncapped filaments adds and loses subunits at both the plus and minus ends, resulting in rapid growth or shrinkage, depending on the concentration of available free monomers (green line). In the presence of a protein that caps the plus end (red line), only the minus end is able to add or lose subunits; consequently, filament growth will be slower at all monomer concentrations above the critical concentration, and filament shrinkage will be slower at all monomer concentrations below the critical concentration. In addition, the critical concentration for the population shifts to that of the filament minus end.

Twisting of an actin filament induced by cofilin

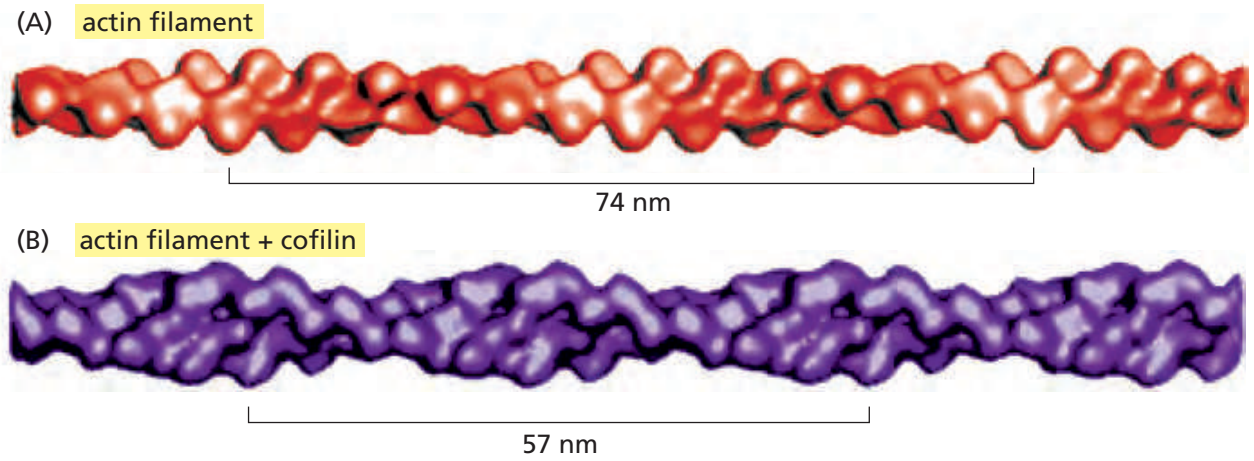


Figure 16–20 Twisting of an actin filament induced by cofilin. (A) Three-dimensional reconstruction from cryoelectron micrographs of filaments made of pure actin. The bracket shows the span of two twists of the actin helix. (B) Reconstruction of an actin filament coated with cofilin, which binds in a 1:1 stoichiometry to actin subunits all along the filament. Cofilin is a small protein (14 kD) compared to actin (43 kD), and so the filament appears only slightly thicker. The energy of cofilin binding serves to deform the actin filament, twisting it more tightly and reducing the distance spanned by each twist of the helix. (From A. McGough et al., *J. Cell Biol.* 138:771–781, 1997. With permission from the authors.)

The formation of two types of actin filament bundles

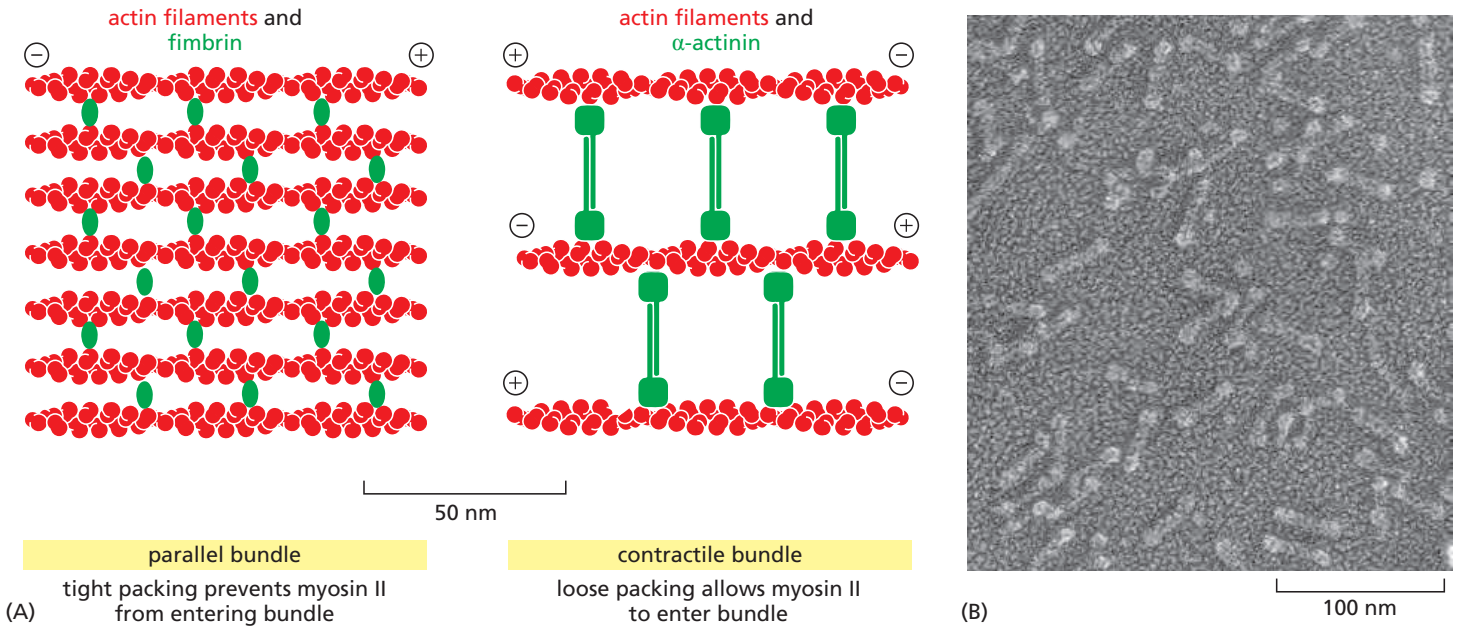
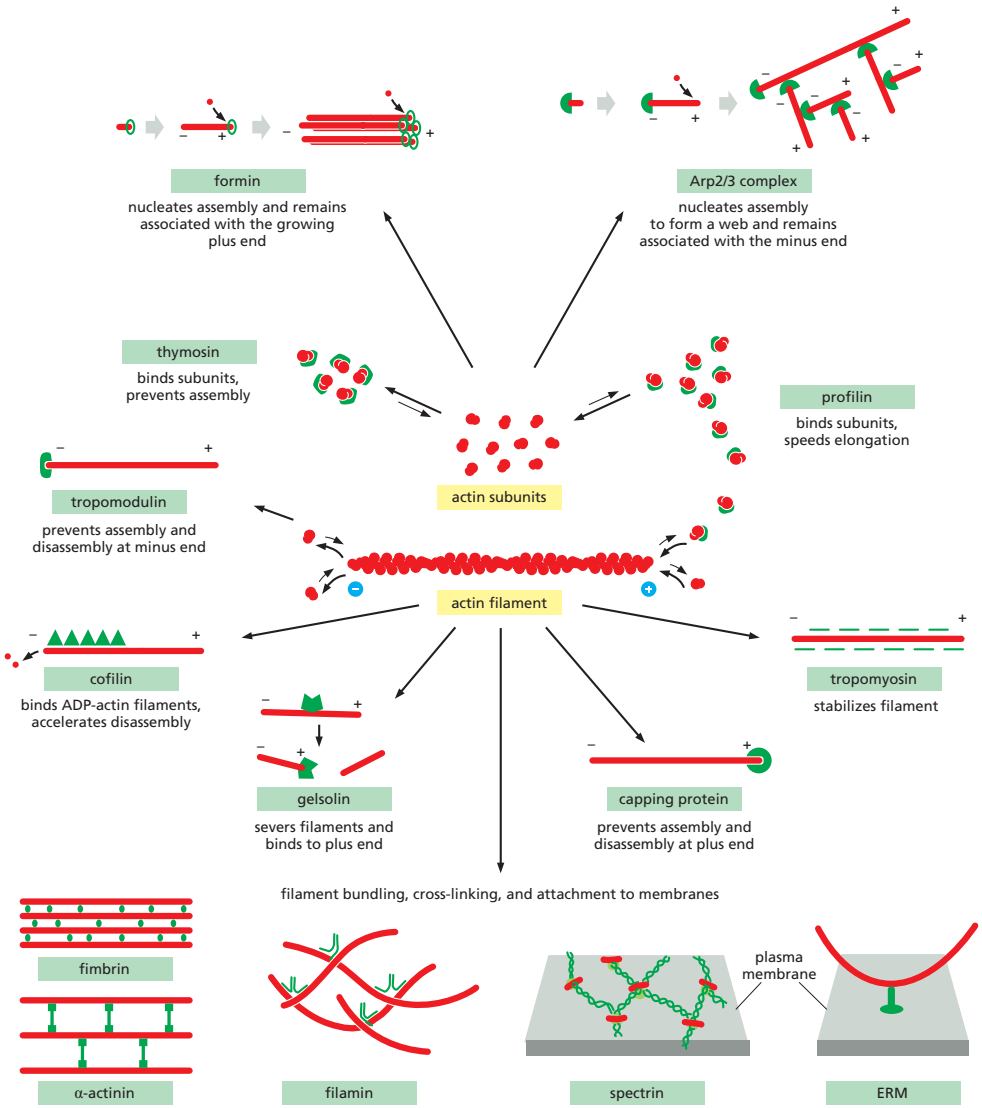


Figure 16–23 The formation of two types of actin filament bundles. (A) Fimbrin cross-links actin filaments into tight bundles, which exclude the motor protein myosin II from participating in the assembly. In contrast, α-actinin, which is a homodimer, cross-links actin filaments into loose bundles, which allow myosin (not shown) to incorporate into the bundle. Fimbrin and α-actinin tend to exclude one another because of the very different spacing of the actin filament bundles that they form. (B) Electron micrograph of purified α-actinin molecules. (B, courtesy of John Heuser.)

ACTIN FILAMENTS



Myosin motor proteins and the actin cytoskeleton

Myosin superfamily members

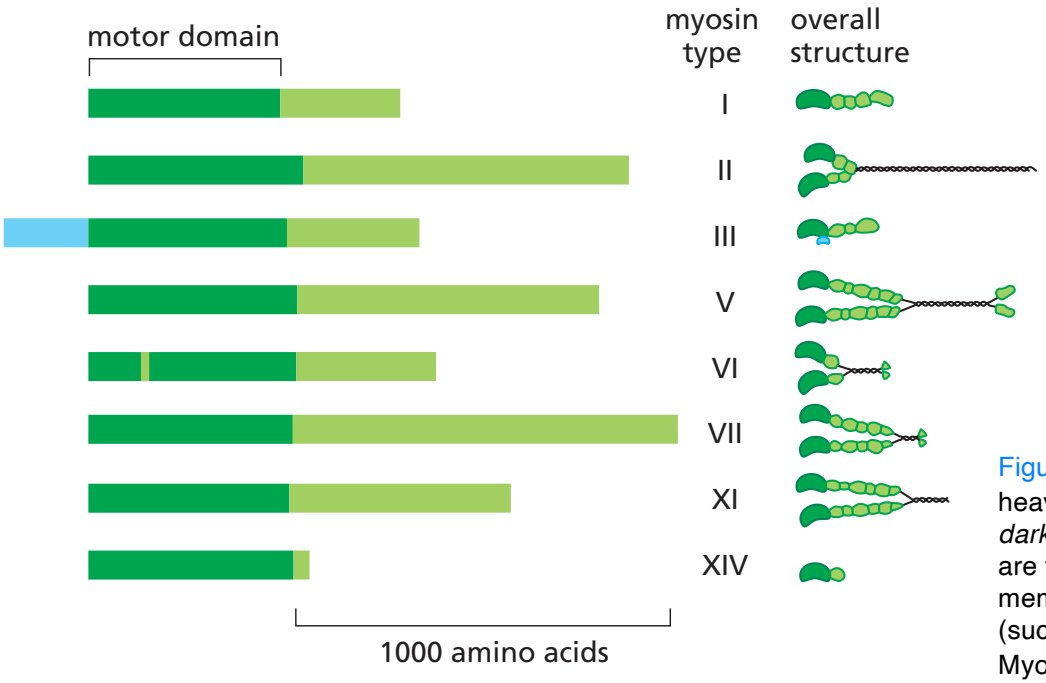


Figure 16–40 Myosin superfamily members. Comparison of the domain structure of the heavy chains of some myosin types. All myosins share similar motor domains (shown in *dark green*), but their C-terminal tails *(light green)* and N-terminal extensions *(light blue)* are very diverse. On the right are depictions of the molecular structure for these family members. Many myosins form dimers, with two motor domains per molecule, but a few (such as I, III, and XIV) seem to function as monomers, with just one motor domain. Myosin VI, despite its overall structural similarity to other family members, is unique in moving toward the minus end (instead of the plus end) of an actin filament. The small insertion within its motor head domain, not found in other myosins, is probably responsible for this change in direction.

Myosin V carries cargo along actin filaments

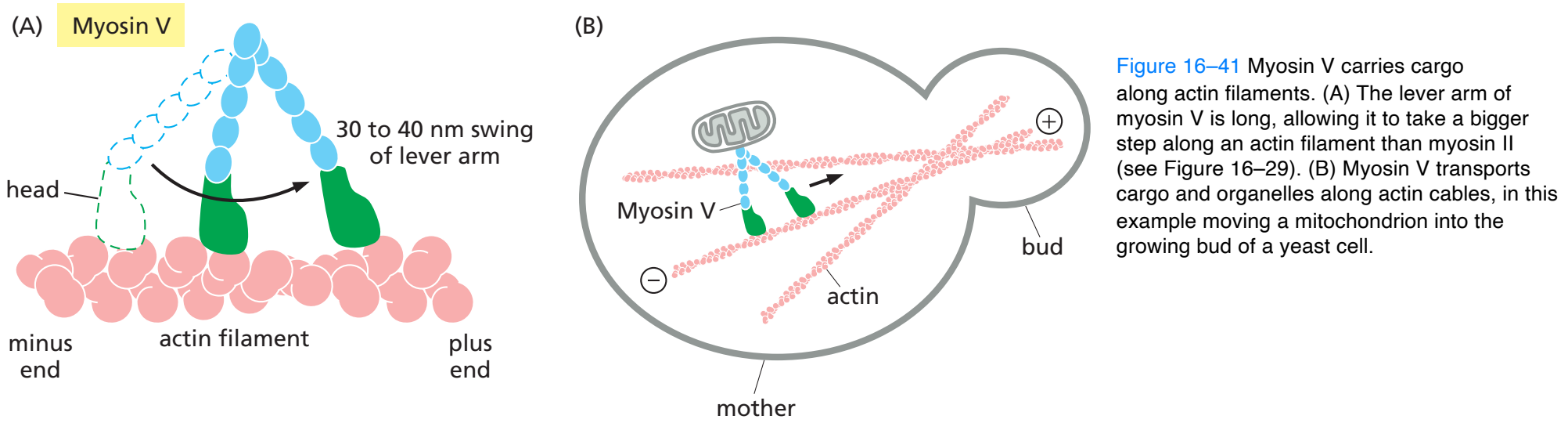
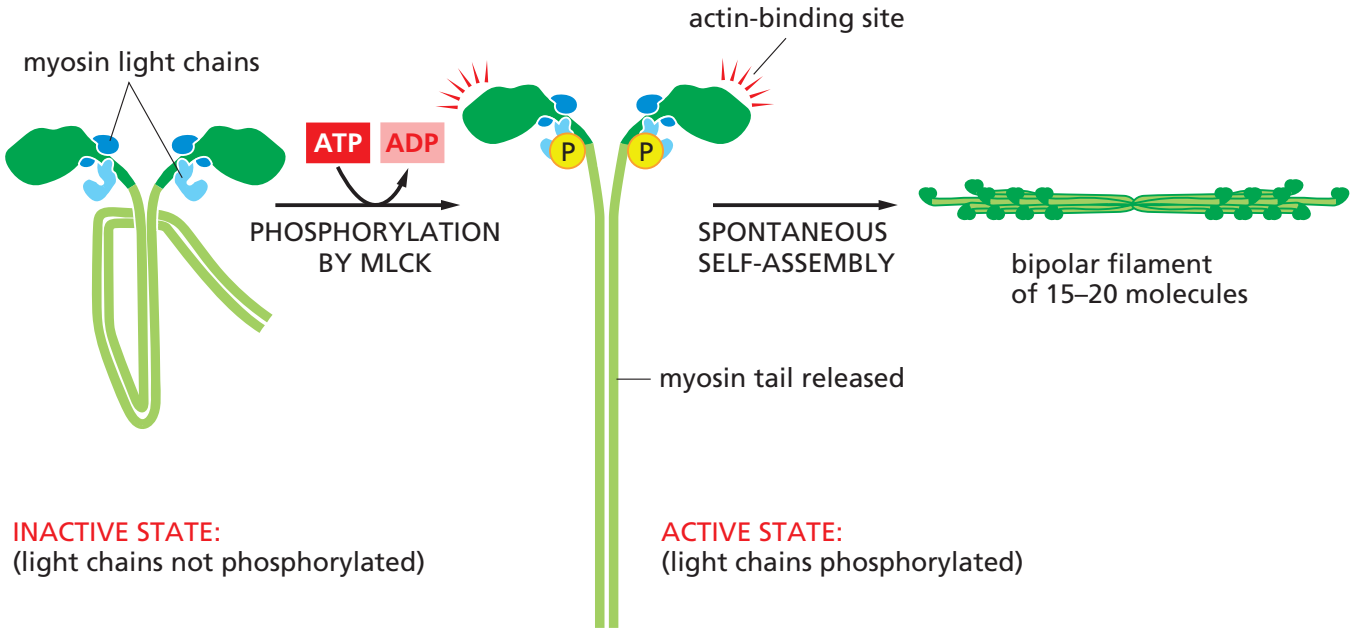
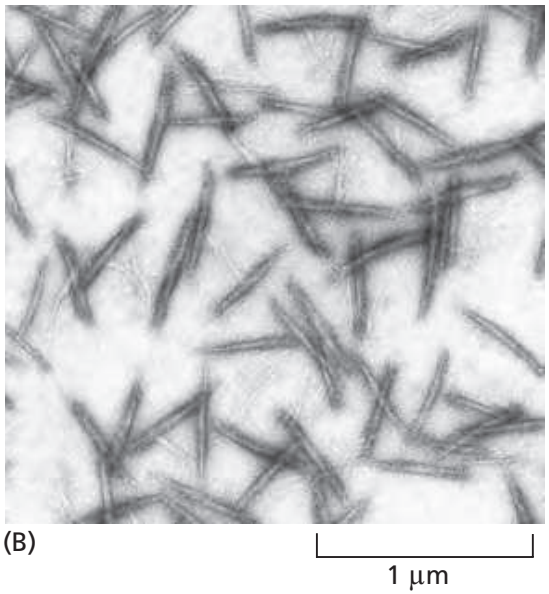


Figure 16–41 Myosin V carries cargo along actin filaments. (A) The lever arm of myosin V is long, allowing it to take a bigger step along an actin filament than myosin II (see Figure 16–29). (B) Myosin V transports cargo and organelles along actin cables, in this example moving a mitochondrion into the growing bud of a yeast cell.

Light-chain phosphorylation and the regulation of the assembly of myosin II into thick filaments





A model of how forces generated in the actin-rich cortex move a cell forward

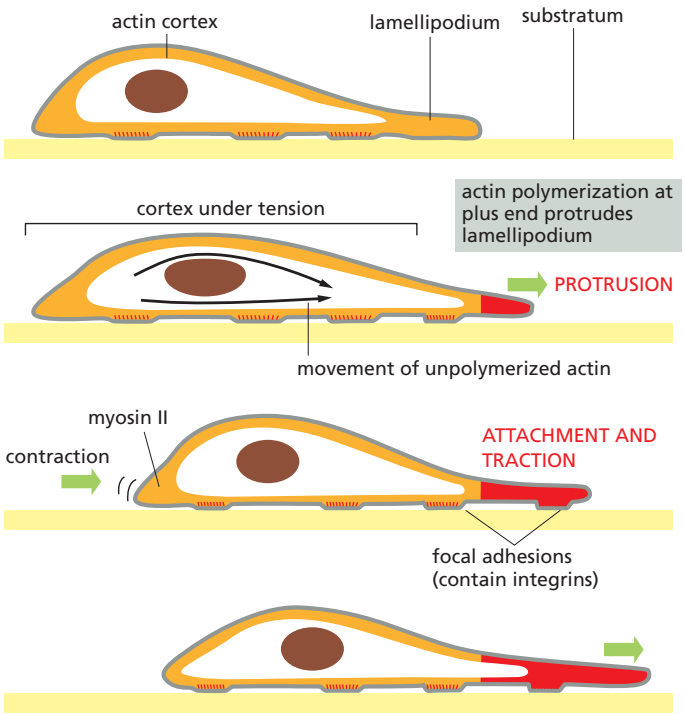


Figure 16–75 A model of how forces generated in the actin-rich cortex move a cell forward. The actin-polymerization- dependent protrusion and firm attachment of a lamellipodium at the leading edge of the cell move the edge forward (*green* arrows at front) and stretch the actin cortex. Contraction at the rear of the cell propels the body of the cell forward (*green* arrow at back) to relax some ofthe tension (traction). New focal contacts are made at the front, and old ones are disassembled at the back as the cell crawls forward. The same cycle can be repeated, moving the cell forward in a stepwise fashion. Alternatively, all steps can be tightly coordinated, moving the cell forward smoothly. The newly polymerized cortical actin is shown in *red*.

Control of cell– substratum adhesion at the leading edge of a migrating cell

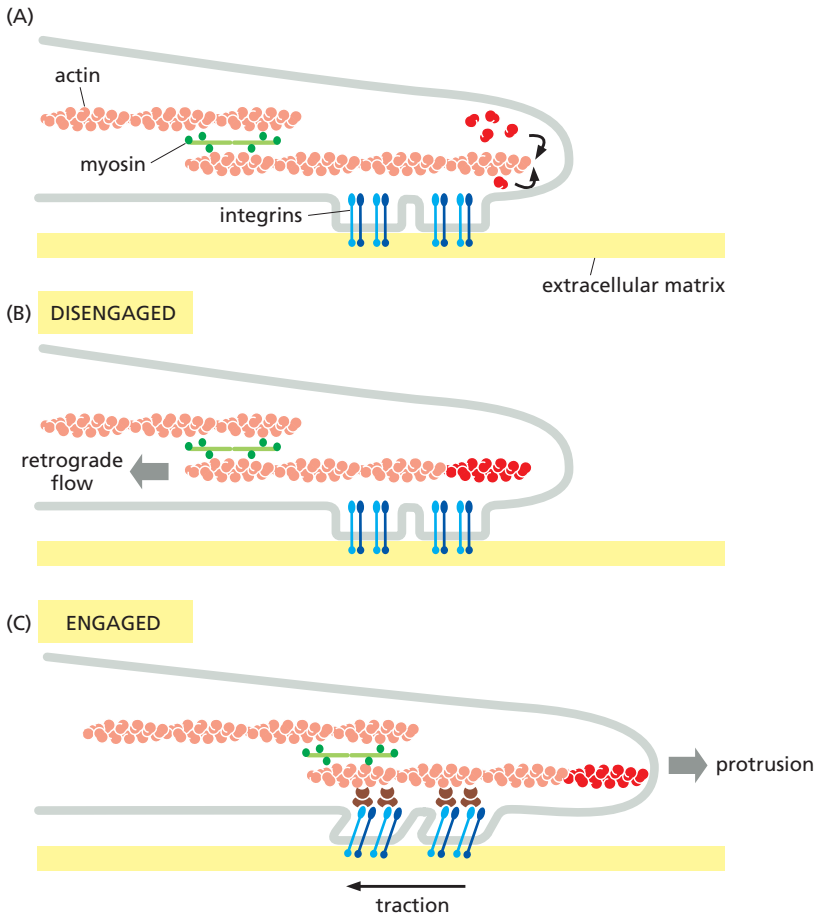


Figure 16–82 Control of cell– substratum adhesion at the leading edge of a migrating cell. (A) Actin monomers assemble on the barbed end of actin filaments at the leading edge. Transmembrane integrin proteins *(blue)* help form focal adhesions that link the cell membrane to the substrate. (B) If there is no interaction between the actin filaments and focal adhesions, the actin filament is driven rearward by newly assembled actin. Myosin motors *(green)* also contribute to filament movement. (C) Interactions between actin-binding adaptor proteins *(brown)* and integrins link the actin cytoskeleton to the substratum. Myosin-mediated contractile forces are then transmitted through the focal adhesion to generate traction on the extracellular matrix, and new actin polymerization drives the leading edge forward in a protrusion.

Membrane bleb induced by disruption of the actin cortex

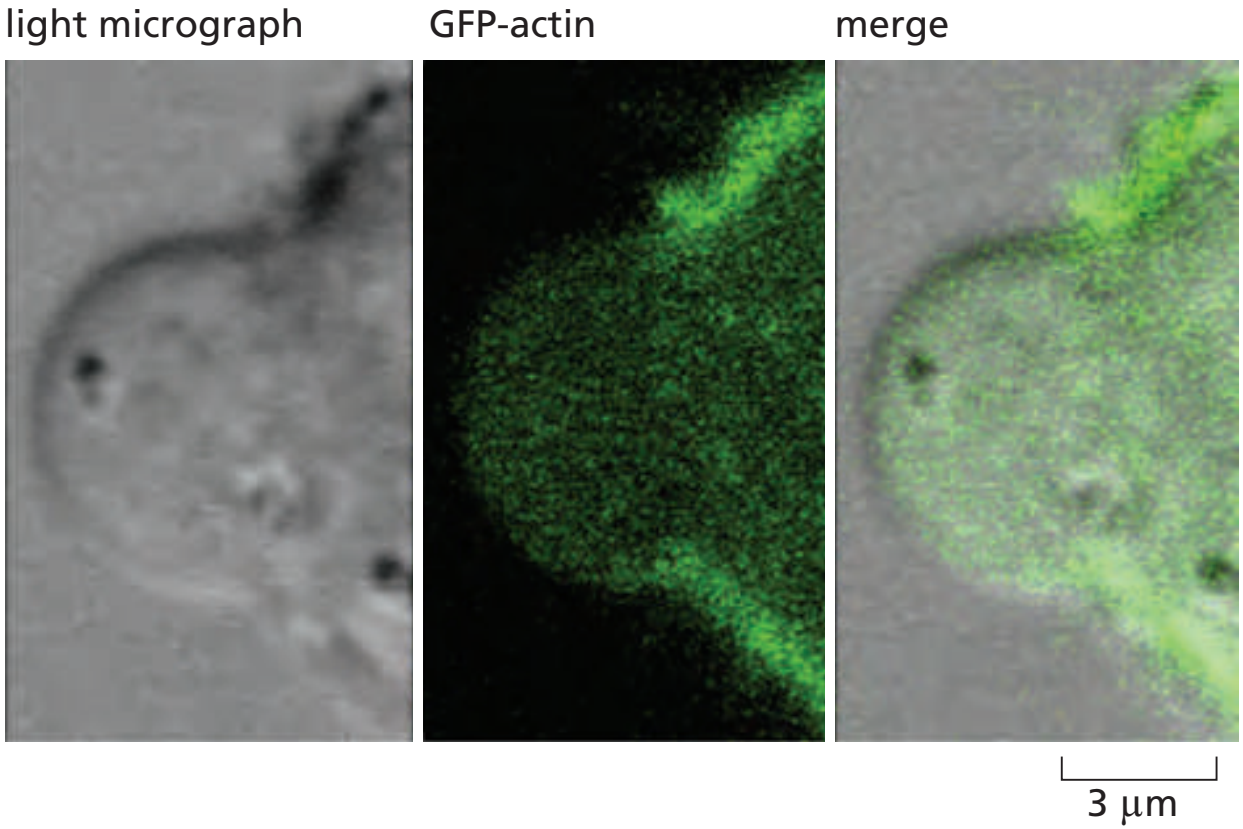


Figure 16–76 Membrane bleb induced by disruption of the actin cortex. On the left is a light micrograph showing a spherical membrane protrusion or bleb induced by laser ablation of a small region of the actin cortex. The cortex is labeled *green* in the *middle* image by expression of GFP-actin. (Courtesy of Ewa Paluch.)

The dramatic effects of Cdc42, Rac, and Rho on actin organization in fibroblasts

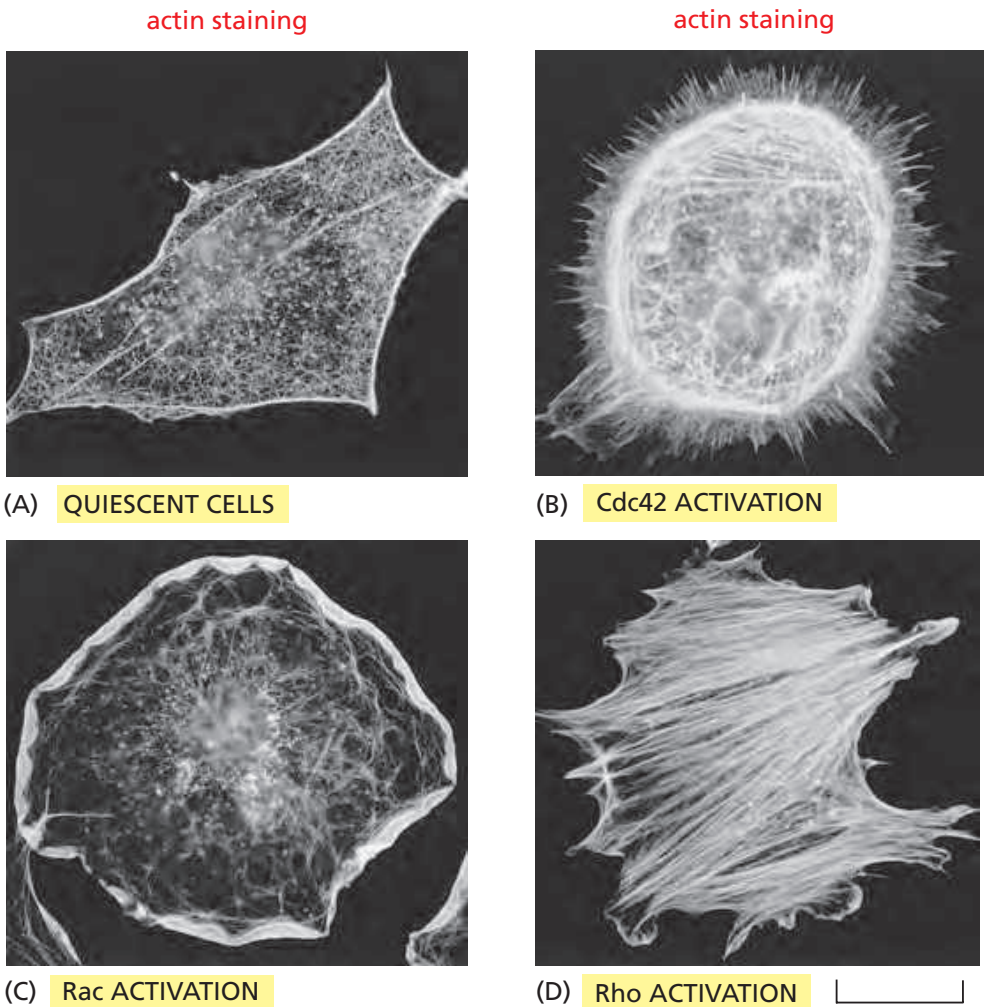


Figure 16–84 The dramatic effects of Cdc42, Rac, and Rho on actin organization in fibroblasts. In each case, the actin filaments have been labeled with fluorescent phalloidin. (A) Serum-starved fibroblasts have actin filaments primarily in the cortex, and relatively few stress fibers. (B) Microinjection of a constitutively activated form of Cdc42 causes the protrusion of many long filopodia at the cell periphery. (C) Microinjection of a constitutively activated form of Rac, a closely related monomeric GTPase, causes the formation of an enormous lamellipodium that extends from the entire circumference of the cell. (D) Microinjection of a constitutively activated form of Rho causes the rapid assembly of many prominent stress fibers. (From A. Hall, *Science* 279:509–514, 1998. With permission from AAAS.)

The contrasting effects of Rac and Rho activation on actin organization

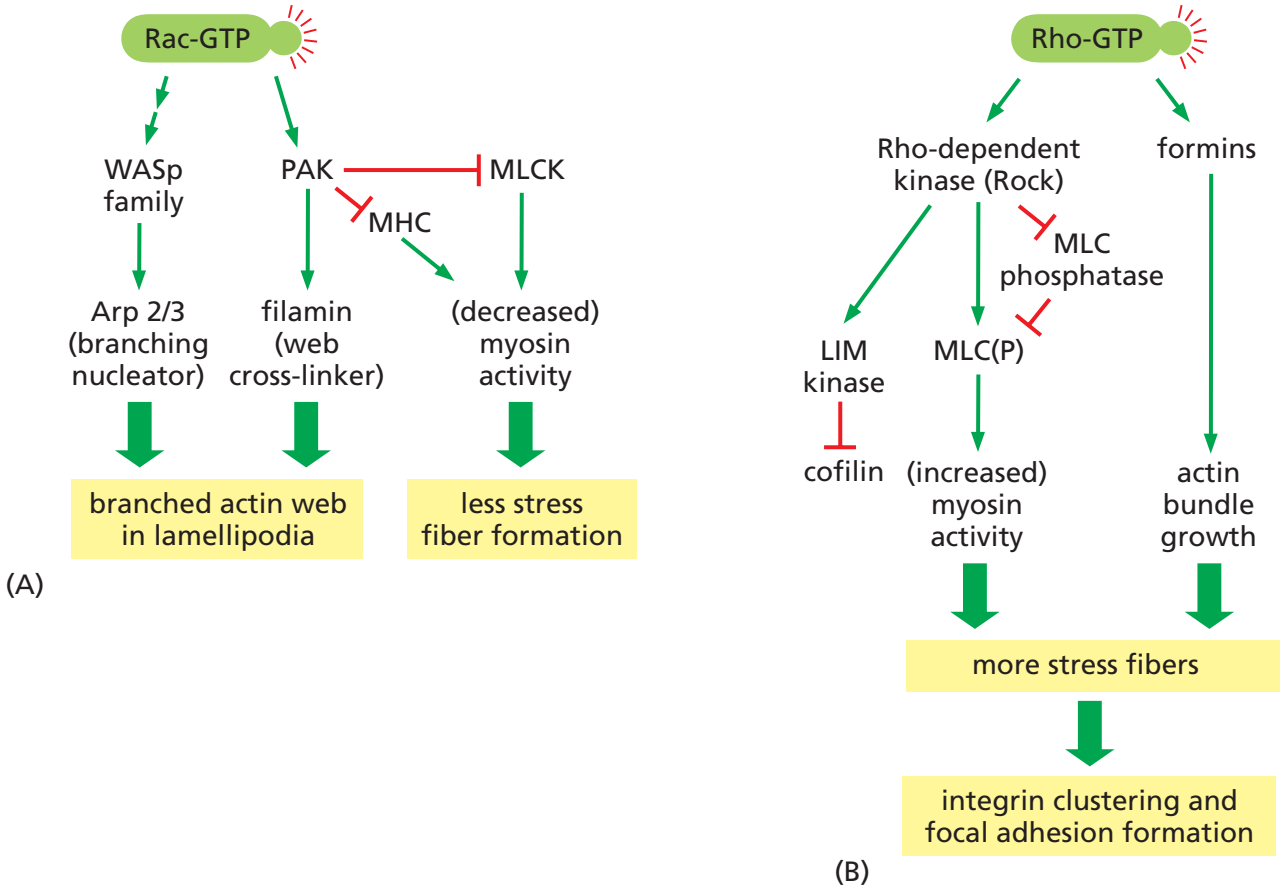


Figure 16–85 The contrasting effects of Rac and Rho activation on actin organization. (A) Activation of the small GTPase Rac leads to alterations in actin accessory proteins that tend to favor the formation of actin networks, as in lamellipodia. Several different pathways contribute independently. Rac-GTP activates members of the WASp protein family, which in turn activate actin nucleation and branched web formation by the Arp 2/3 complex. In a parallel pathway, Rac-GTP activates a protein kinase, PAK, which has several targets including the web-forming cross-linker filamin, which is activated by phosphorylation, and the myosin light chain kinase (MLCK), which is inhibited by phosphorylation. Inhibition of MLCK results in decreased phosphorylation of the myosin regulatory light chain and leads to myosin II filament disassembly and a decrease in contractile activity. In some cells, PAK also directly inhibits myosin II activity by phosphorylation of the myosin heavy chain (MHC). (B) Activation of the related GTPase Rho leads to nucleation of actin filaments by formins and increases contraction by myosin II, promoting the formation of contractile actin bundles such as stress fibers. Activation of myosin II by Rho requires a Rho-dependent protein kinase called Rock. This kinase inhibits the phosphatase that removes the activating phosphate groups from myosin II light chains (MLC); it may also directly phosphorylate the myosin light chains in some cell types. Rock also activates other protein kinases, such as LIM kinase, which in turn contributes to the formation of stable contractile actin filament bundles by inhibiting the actin depolymerizing factor cofilin. A similar signaling pathway is important for forming the contractile ring necessary for cytokinesis (see Figure 17–44).

Actin homologs in bacteria determine cell shape

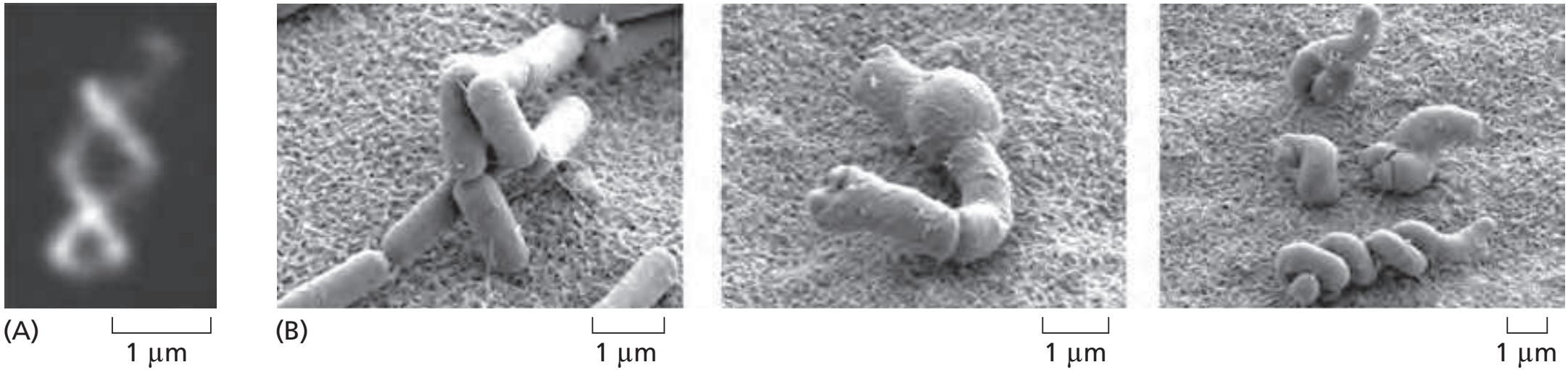


Figure 16–8 Actin homologs in bacteria determine cell shape. (A) The MreB protein forms abundant patches made up of many short, interwoven linear or helical filaments that are seen to move circumferentially along the length of the bacterium and are associated with sites of cell wall synthesis. (B) The common soil bacterium Bacillus subtilis normally forms cells with a regular rodlike shape when viewed by scanning electron microscopy (left). In contrast, B. subtilis cells lacking the actin homolog MreB or Mbl grow in distorted or twisted shapes and eventually die (center and right). (A, from P. Vats and L. Rothfield, Proc. Natl Acad. Sci. USA 104:17795–17800, 2007. With permission from National Academy of Sciences; B, from A. Chastanet and R. Carballido-Lopez, Front. Biosci. 4S:1582–1606, 2012. With permission Frontiers in Bioscience.)

Microtubuli

Tubulin & Microtubule structure

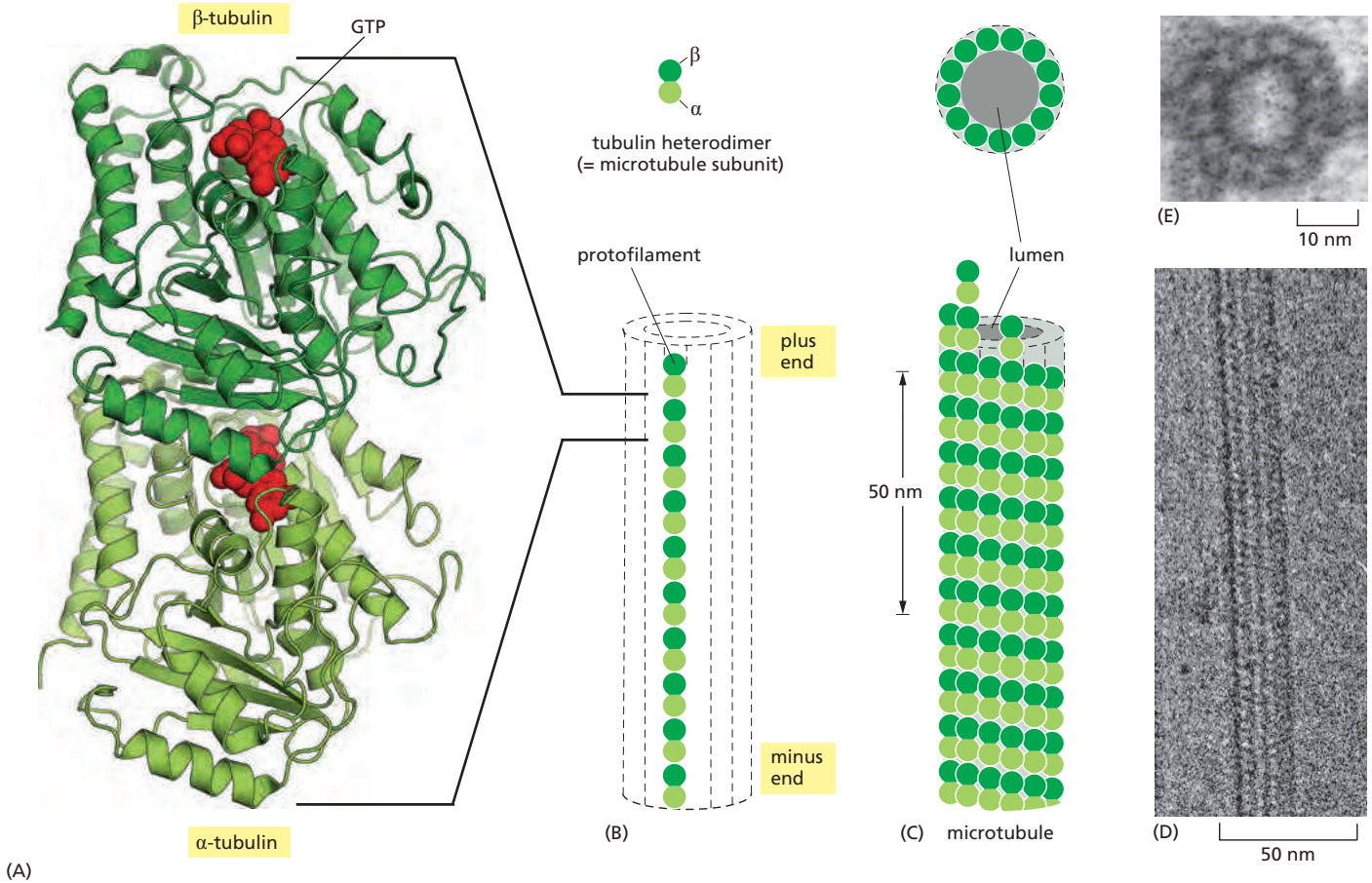


Figure 16–42 The structure of a microtubule and its subunit. (A) The subunit of each protofilament is a tubulin heterodimer, formed from a tightly linked pair of α- and β-tubulin monomers. The GTP molecule in the α-tubulin monomer is so tightly bound that it can be considered an integral part of the protein. The GTP molecule in the β-tubulin monomer, however, is less tightly bound and has an important role in filament dynamics. Both nucleotides are shown in *red*. (B) One tubulin subunit (αβ-heterodimer) and one protofilament are shown schematically. Each protofilament consists of many adjacent subunits with the same orientation. (C) The microtubule is a stiff hollow tube formed from 13 protofilaments aligned in parallel. (D) A short segment of a microtubule viewed in an electron microscope. (E) Electron micrograph of a cross section of a microtubule showing a ring of 13 distinct protofilaments. (D, courtesy of Richard Wade; E, courtesy of Richard Linck.)

The preferential growth of microtubules at the plus end

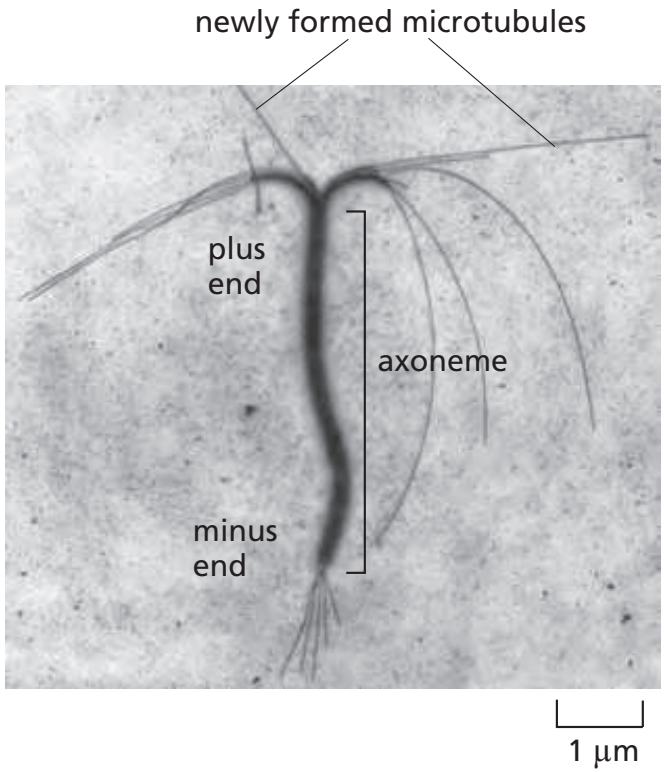
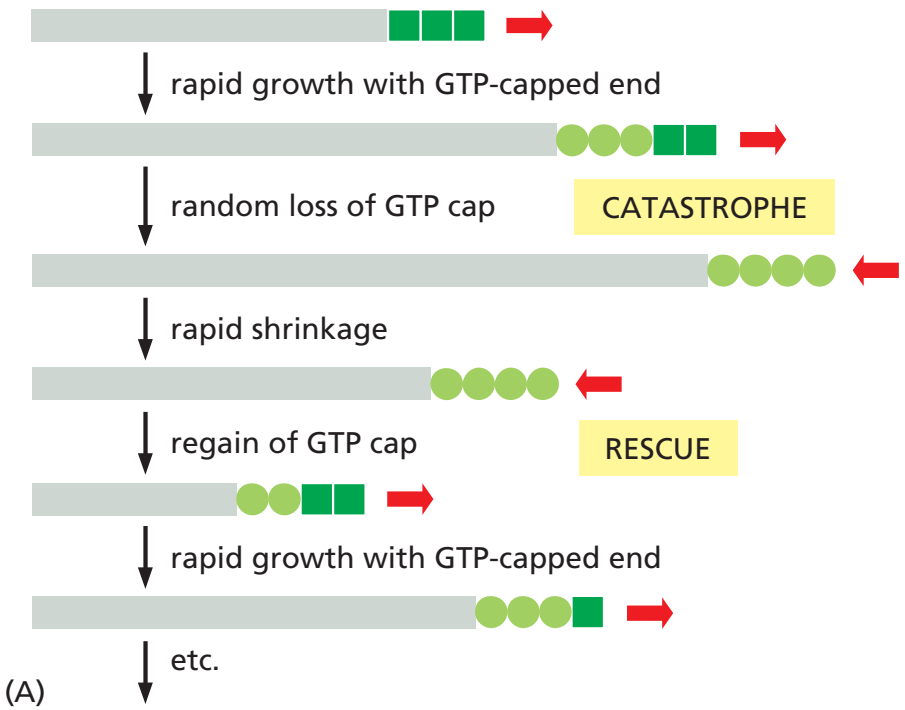
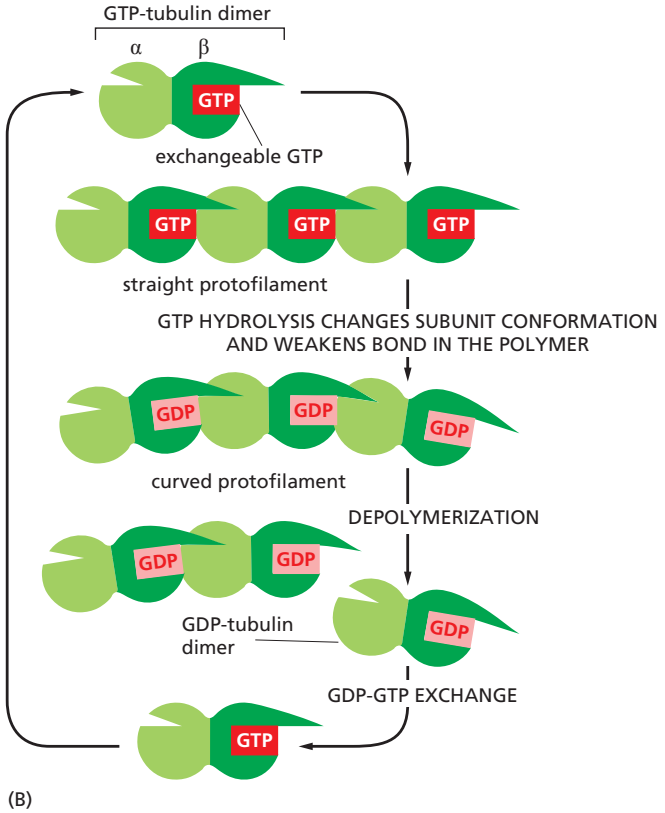


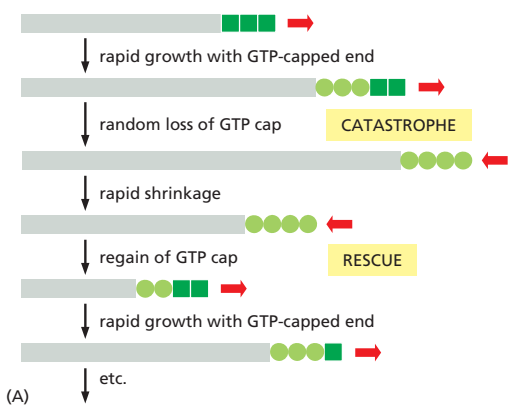
Figure 16–43 The preferential growth of microtubules at the plus end. Microtubules grow faster at one end than at the other. A stable bundle of microtubules obtained from the core of a cilium (called an axoneme) was incubated for a short time with tubulin subunits under polymerizing conditions. Microtubules grew fastest from the plus end of the microtubule bundle, the end at the *top* in this micrograph. (Courtesy of Gary Borisy.)

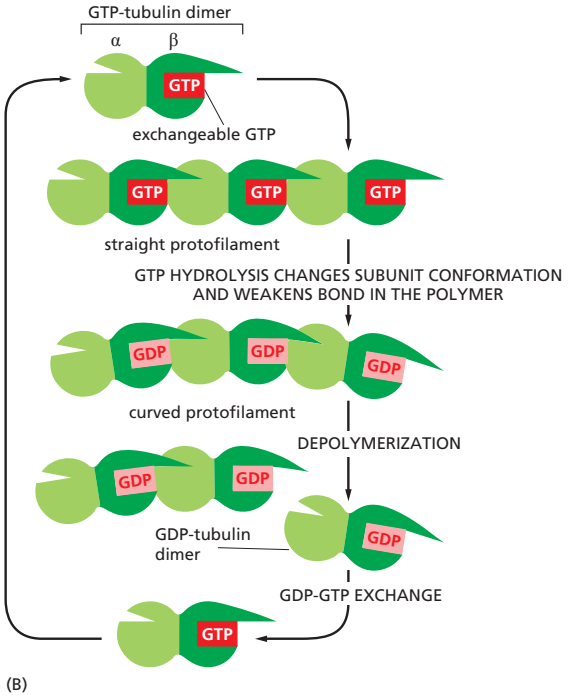
Dynamic instability due to the structural differences between a growing and a shrinking microtubule end

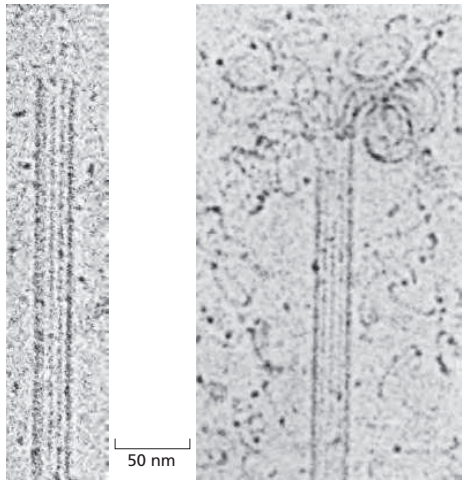


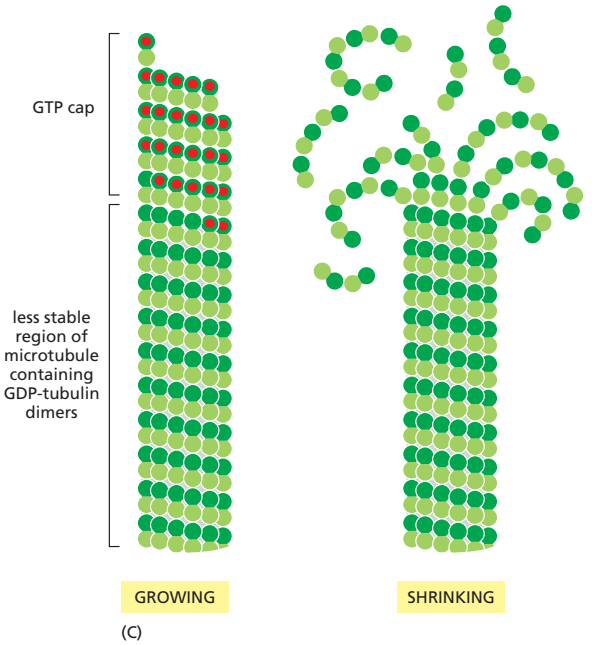


https://www.youtube.com/watch?v=YGU0uN_RAIo









The effects of proteins that bind to microtubule ends

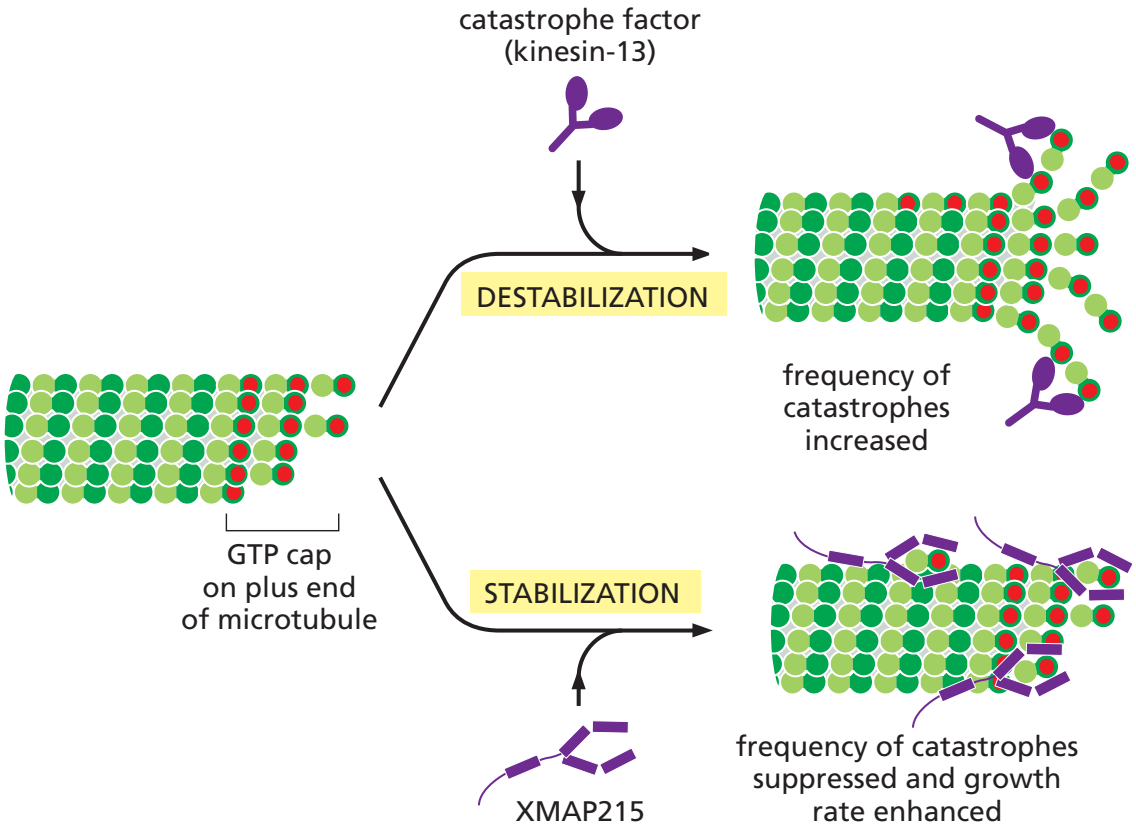


Figure 16–52 The effects of proteins that bind to microtubule ends. The transition between microtubule growth and shrinkage is controlled in cells by a variety of proteins. Catastrophe factors such as kinesin-13, a member of the kinesin motor protein superfamily, bind to microtubule ends and pry them apart, thereby promoting depolymerization. On the other hand, a MAP such as XMAP215 stabilizes the end of a growing microtubule (XMAP stands for *Xenopus* microtubule- associated protein, and the number refers to its molecular mass in kilodaltons). XMAP215 binds tubulin dimers and delivers them to the microtubule plus end, thereby increasing the microtubule growth rate and suppressing catastrophes.

Blocking of tubulin assembly by sequestration of tubulin by stathmin

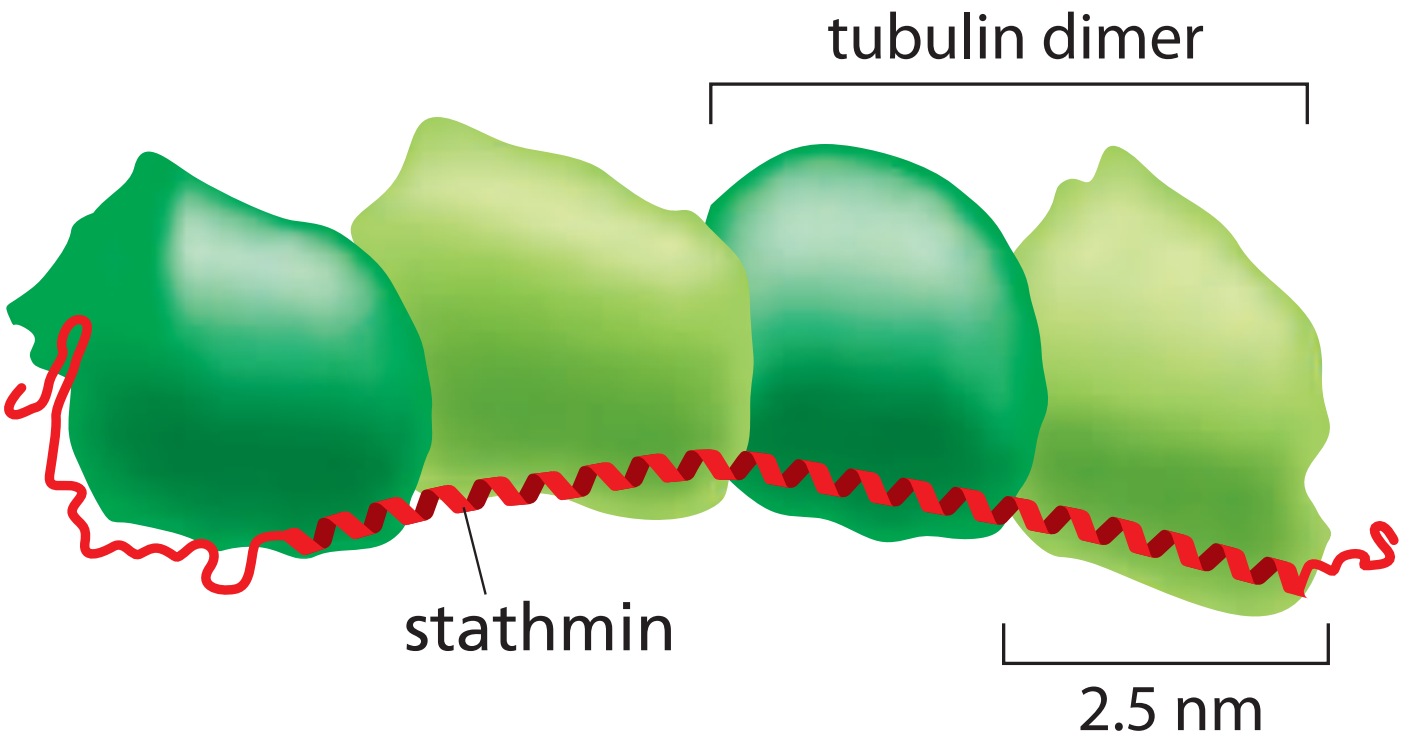


Figure 16–54 Sequestration of tubulin by stathmin. Structural studies with electron microscopy and crystallography suggest that the elongated stathmin protein binds along the side of two tubulin heterodimers. (Adapted from M.O. Steinmetz et al., *EMBO J.* 19:572–580, 2000. With permissiMoBnofCro6mn1J6o.h2n0W4/il1e6y.a5n4d Sons.)

Microtubule nucleation by the γ-tubulin ring complex

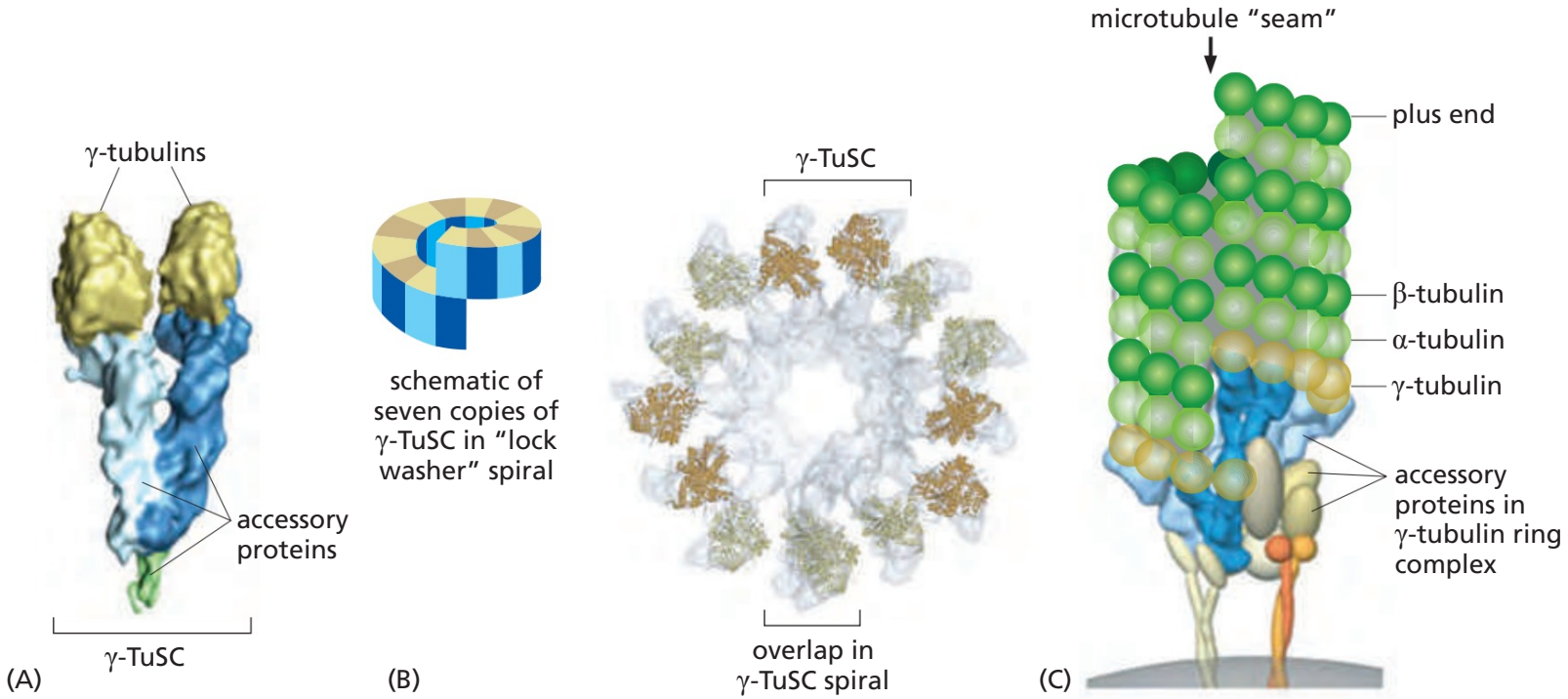
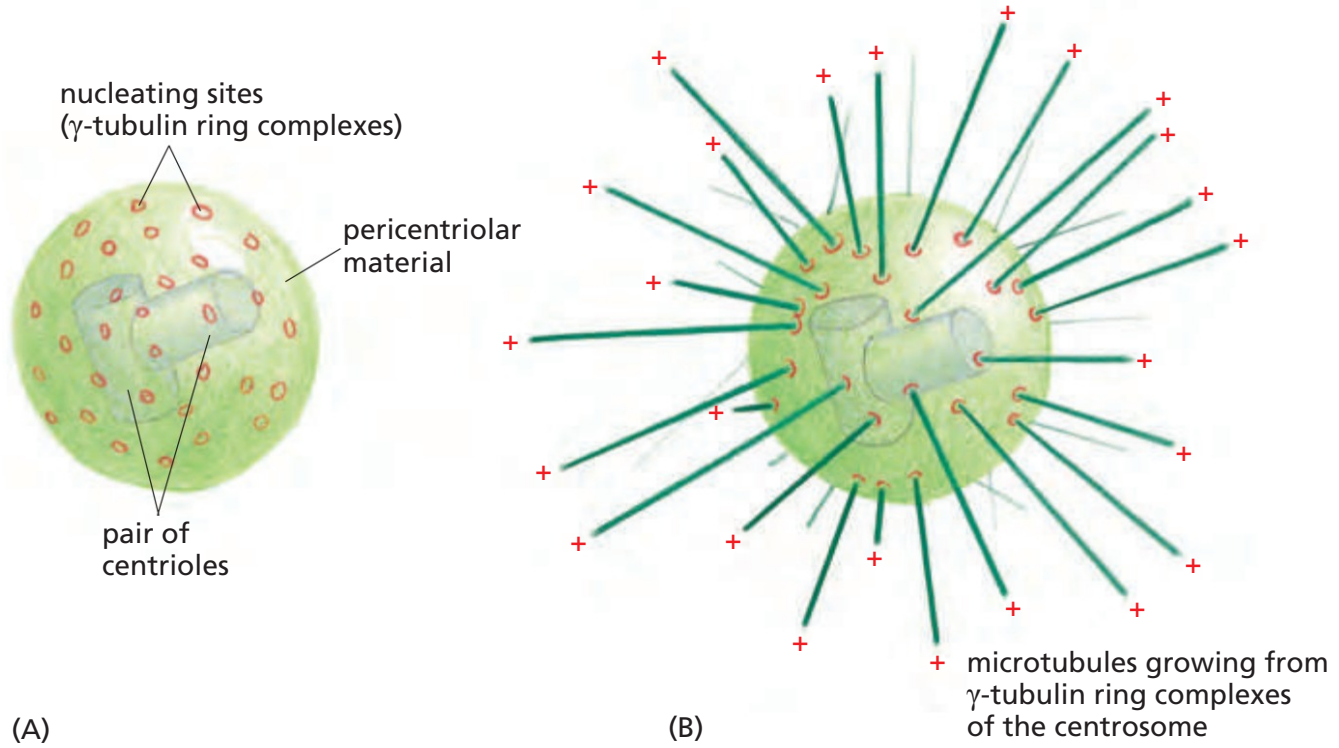
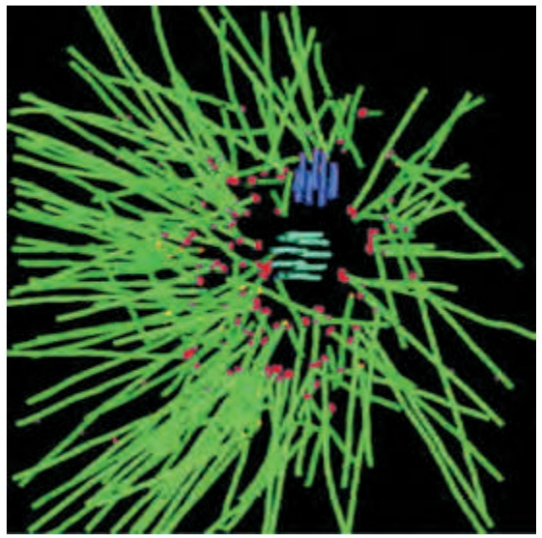


Figure 16–46 Microtubule nucleation by the γ-tubulin ring complex. (A) Two copies of γ-tubulin associate with a pair of accessory proteins to form the γ-tubulin small complex (γ-TuSC). This image was generated by high-resolution electron microscopy of individual purified complexes. (B) Seven copies of the γ-TuSC associate to form a spiral structure in which the last γ-tubulin lies beneath the first, resulting in 13 exposed γ-tubulin subunits in a circular orientation that matches the orientation of the 13 protofilaments in a microtubule. (C) In many cell types, the γ-TuSC spiral associates with additional accessory proteins to form the γ-tubulin ring complex (γ-TuRC), which is likely to nucleate the minus end of a microtubule as shown here. Note the longitudinal discontinuity between two protofilaments, which results from the spiral orientation of the γ-tubulin subunits. Microtubules often have one such "seam" breaking the otherwise uniform helical packing of the protofilaments. (A and B, from J.M. Kollman et al., *Nature* 466:879–883, 2010. With permission from Macmillan Publishers Ltd.)

The centrosome is a microtubule organizing center



(A) (B)



(C)

Figure 16–47 The centrosome. (A) The centrosome is the major MTOC of animal cells. Located in the cytoplasm next to the nucleus, it consists of an amorphous matrix of fibrous proteins to which the γ-tubulin ring complexes that nucleate microtubule growth are attached. This matrix is organized by a pair of centrioles, as described in the text. (B) A centrosome with attached microtubules. The minus end of each microtubule is embedded in the centrosome, having grown from a γ-tubulin ring complex, whereas the plus end of each microtubule is free in the cytoplasm. (C) In a reconstructed image of the MTOC from a *C. elegans* cell, a dense thicket of microtubules can be seen emanating from the centrosome. (C, from E.T. O'Toole et al., *J. Cell Biol.* 163:451–456, 2003. With permission from the authors.)

A pair of centrioles in the centrosome

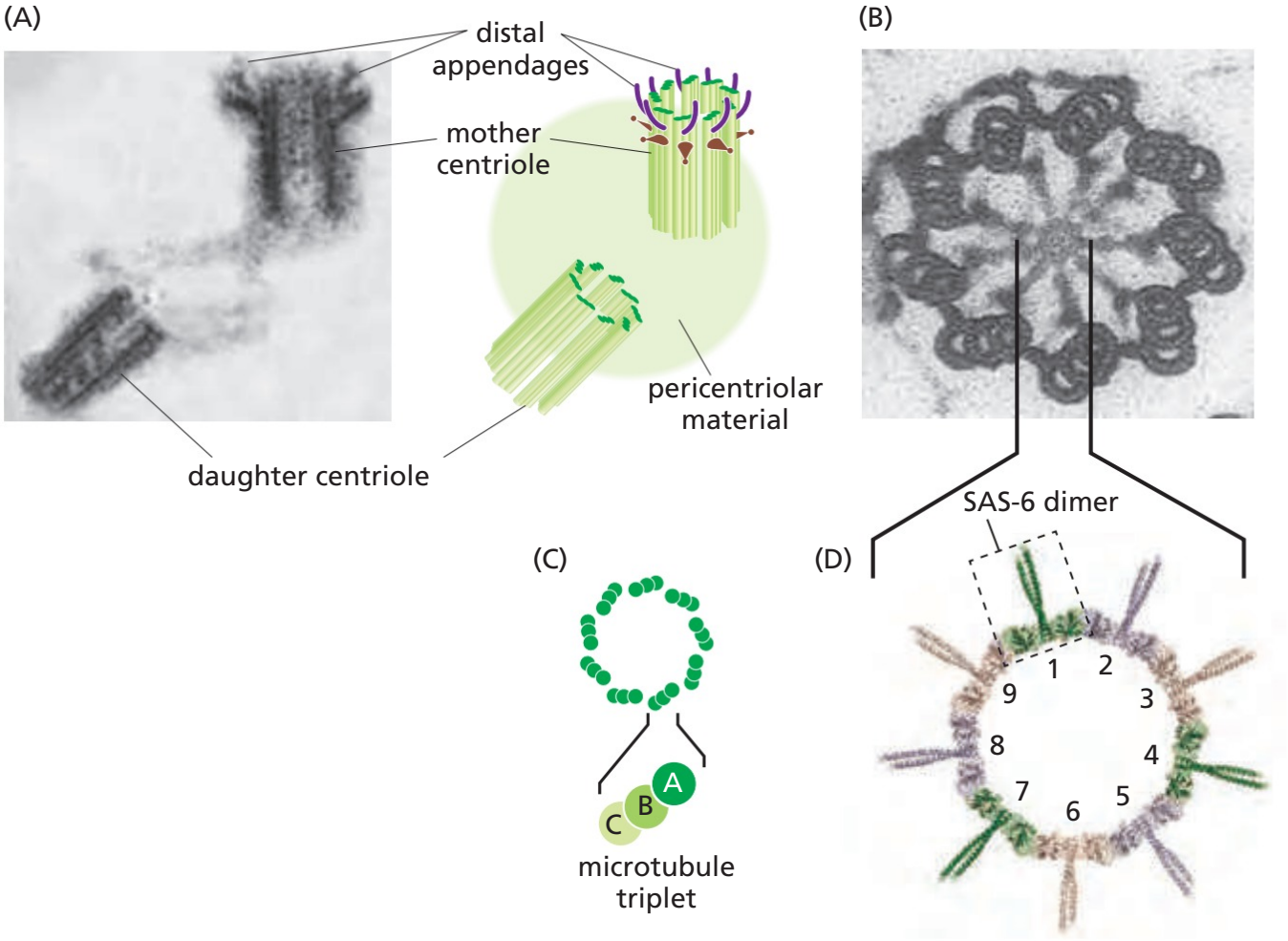


Figure 16–48 A pair of centrioles in the centrosome. (A) An electron micrograph of a thin section of an isolated centrosome showing the mother centriole with its distal appendages and the adjacent daughter centriole, which formed through a duplication event during S phase (see Figure 17–26). In the centrosome, the centriole pair is surrounded by a dense matrix of pericentriolar material from which microtubules nucleate. Centrioles also function as basal bodies to nucleate the formation of ciliary axonemes (see Figure 16–68). (B) Electron micrograph of a cross section through a centriole in the cortex of a protozoan. Each centriole is composed of nine sets of triplet microtubules arranged to form a cylinder. (C) Each triplet contains one complete microtubule (the A microtubule) fused to two incomplete microtubules (the B and C microtubules). (D) The centriolar protein SAS-6 forms a coiled-coil dimer. Nine SAS-6 dimers can self-associate to form a ring. Located at the hub of the centriole cartwheel structure, the SAS-6 ring is thought to generate the ninefold symmetry of the centriole. (A, from from M. Paintrand, et al. *J. Struct. Biol.* 108:107, 1992. With permission from Elsevier; B, courtesy of Richard Linck; D, courtesy of Michel Steinmetz.)

Self assembly of microtubule in the center

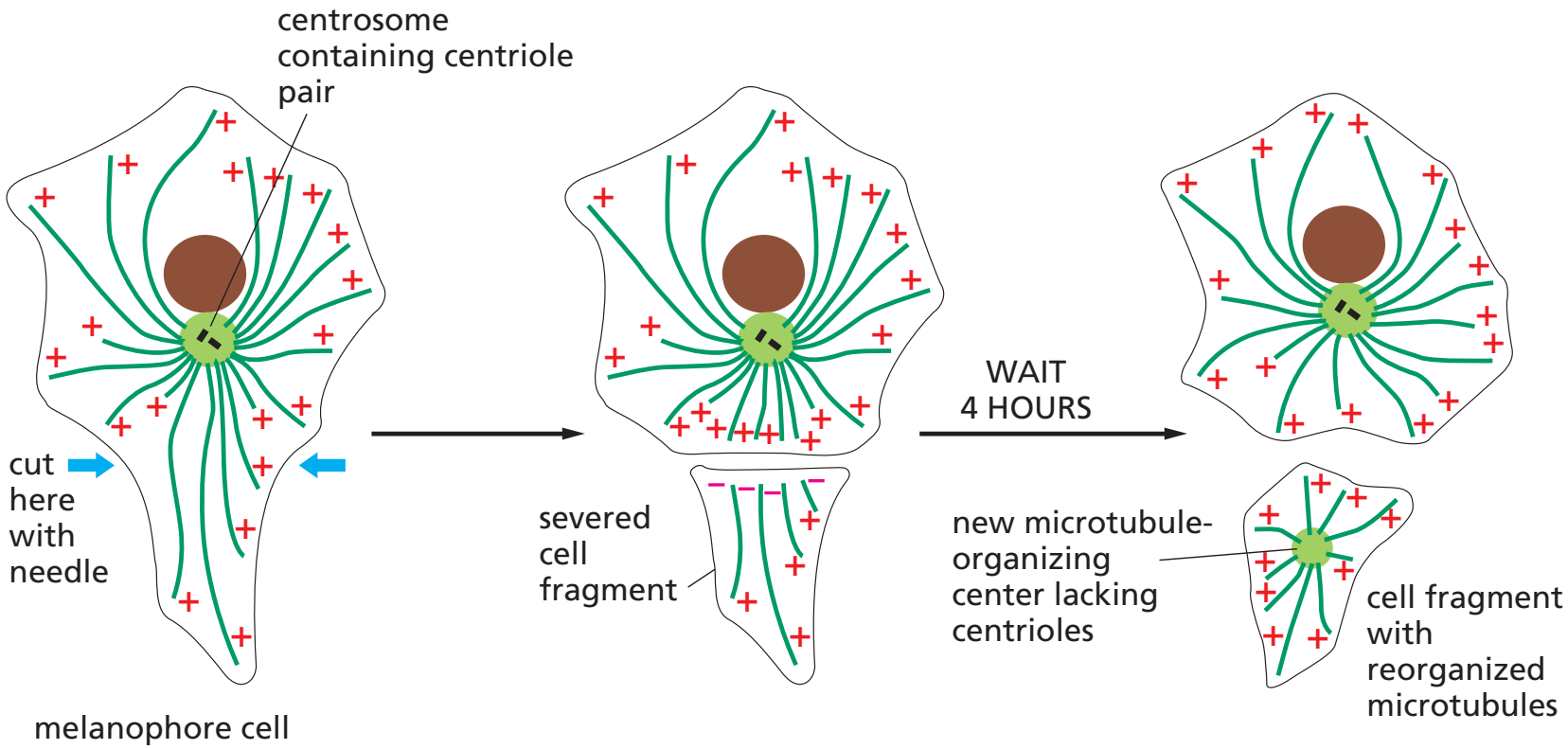
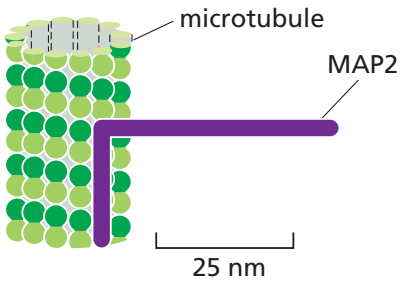
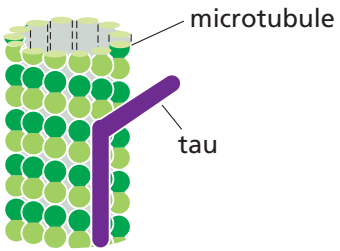


Figure 16–49 A microtubule array can find the center of a cell. After the arm of a fish pigment cell is cut off with a needle, the microtubules in the detached cell fragment reorganize so that their minus ends end up near the center of the fragment, buried in a new microtubule-organizing center.

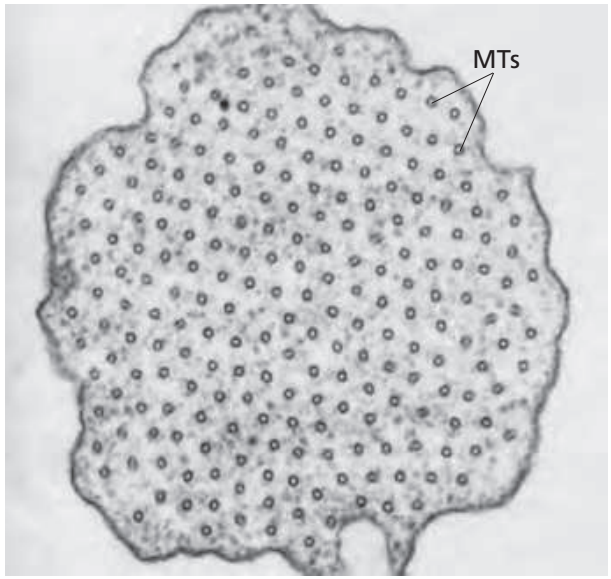
Organization of microtubule bundles by MAPs



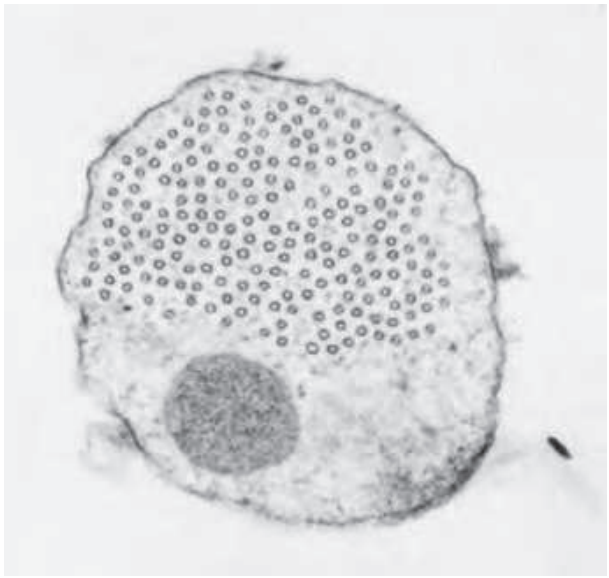
(A)



(B)



(C)



(D)

300 nm

Figure 16–51 Organization of microtubule bundles by MAPs. (A) MAP2 binds along the microtubule lattice at one of its ends and extends a long projecting arm with a second microtubule-binding domain at the other end. (B) Tau possesses a shorter microtubule cross-linking domain. (C) Electron micrograph showing a cross section through a microtubule bundle in a cell overexpressing MAP2. The regular spacing of the microtubules (MTs) in this bundle results from the constant length of the projecting arms of the MAP2.
(D) Similar cross section through a microtubule bundle in a cell overexpressing tau. Here the microtubules are spaced more closely together than they are in (C) because of tau's relatively short projecting arm. (C and D, courtesy of J. Chen et al., *Nature* 360:674–677, 1992. With permission from Macmillan Publishers Ltd.)

Microtubule organization in a neuron

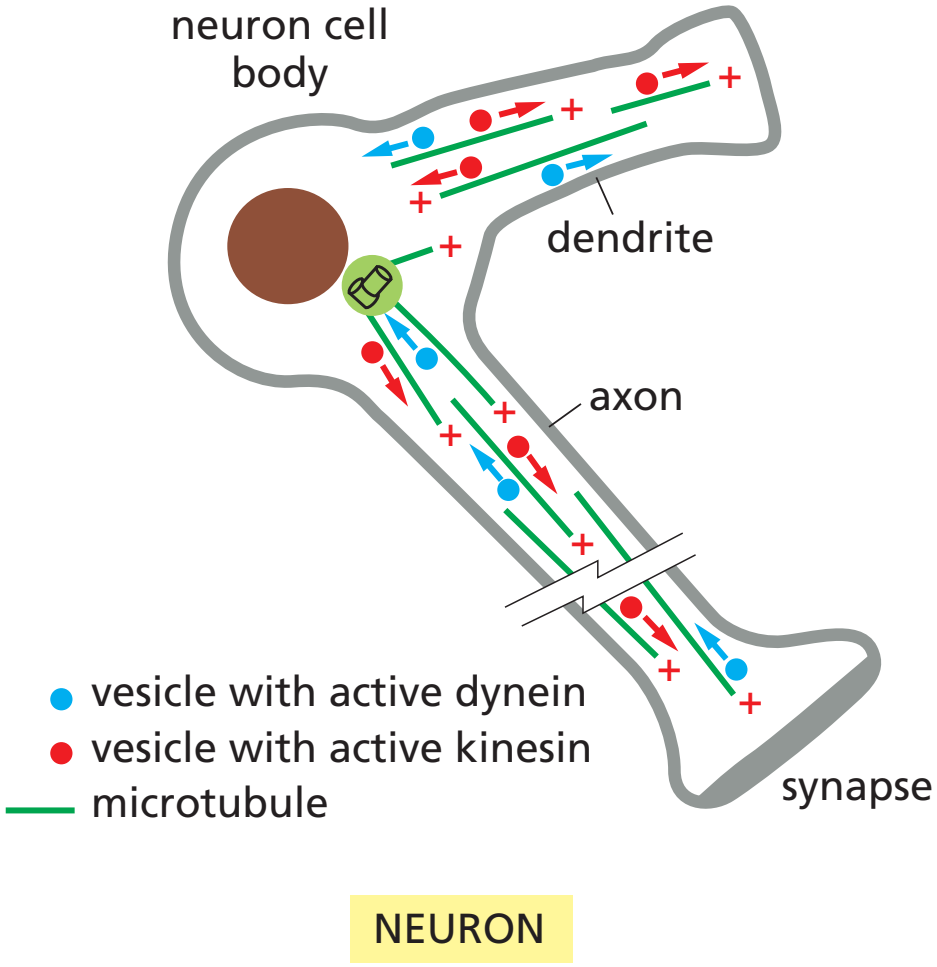
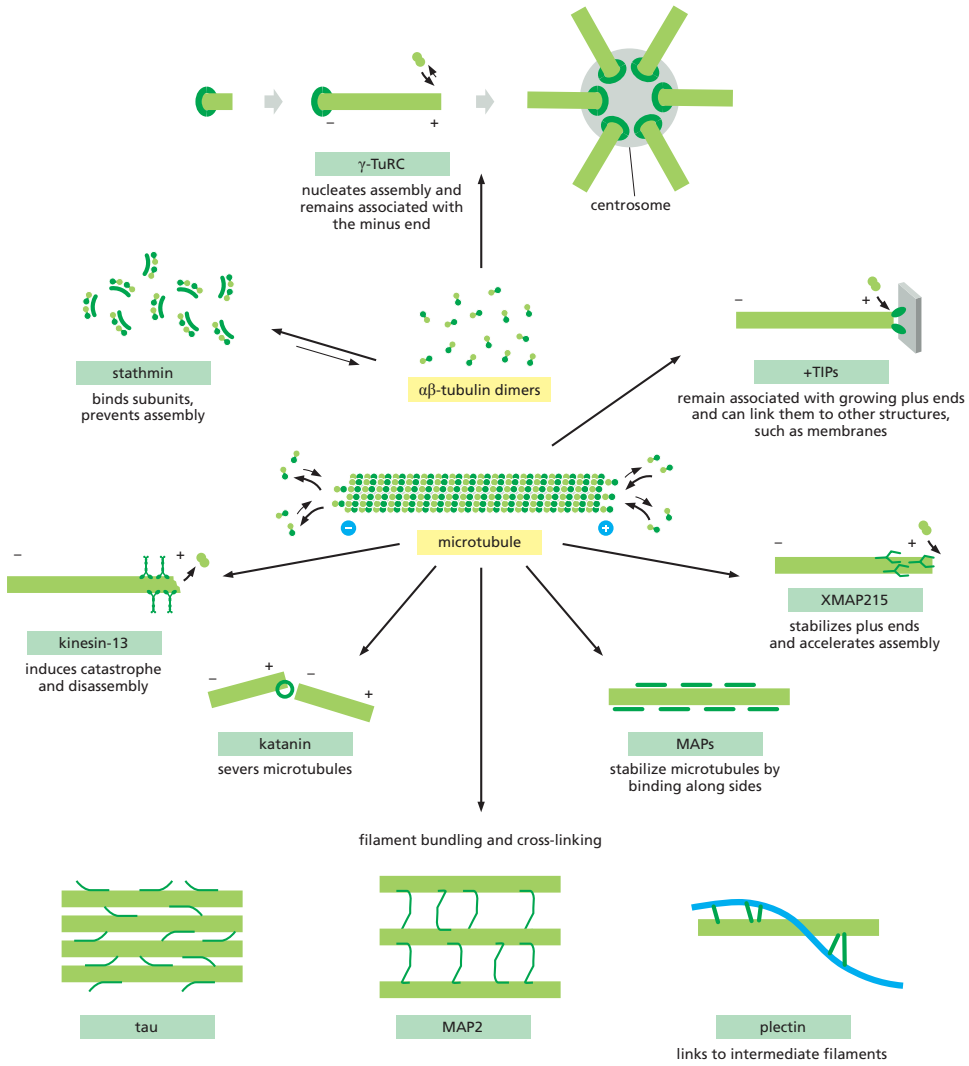


Figure 16–62 Microtubule organization in a neuron. In a neuron, microtubule organization is complex. In the axon, all microtubules share the same polarity, with the plus ends pointing outward toward the axon terminus. No single microtubule stretches the entire length of the axon; instead, short overlapping segments of parallel microtubules make the tracks for fast axonal transport. In dendrites, the microtubules are of mixed polarity, with some plus ends pointing outward and some pointing inward. Vesicles can associate with both kinesin and dynein and move in either direction along the microtubules in axons and dendrites, depending on which motor is active.

MICROTUBULES



Motor proteins associated with tubulin

Kinesin and kinesin- related proteins

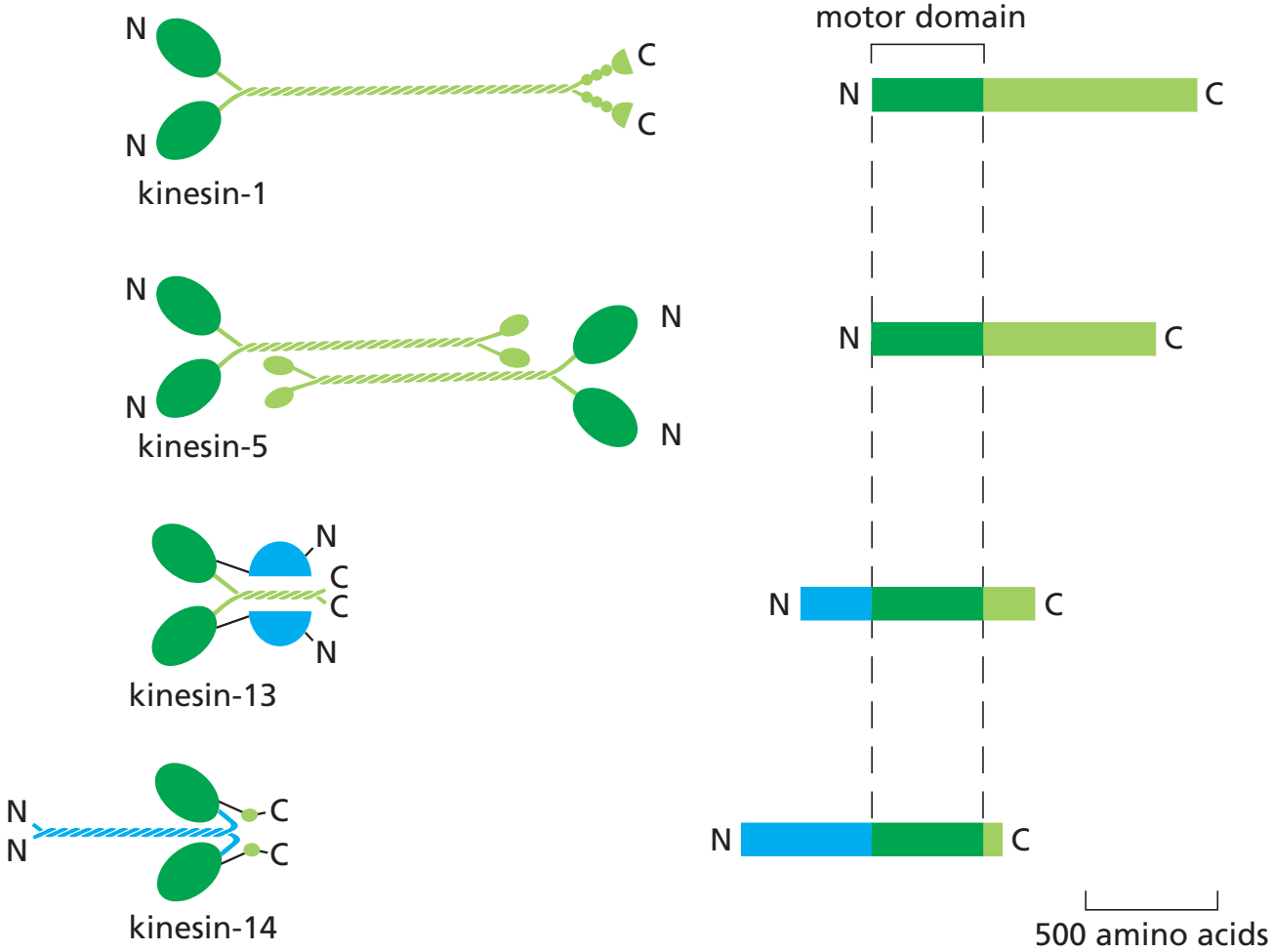


Figure 16–56 Kinesin and kinesin- related proteins. Structures of four kinesin superfamily members. As in the myosin superfamily, only the motor domains are conserved. Kinesin-1 has the motor domain at the N-terminus of the heavy chain. The middle domain forms a long coiled-coil, mediating dimerization. The C-terminal domain forms a tail that attaches to cargo, such as a membrane-enclosed organelle. Kinesin-5 forms tetramers where two dimers associate by their tails. The bipolar kinesin-5 tetramer is able to slide two microtubules past each other, analogous
to the activity of the bipolar thick filaments formed by myosin II. Kinesin-13 has its motor domain located in the middle of the heavy chain. It is a member of a family of kinesins that have lost typical motor activity and instead bind to microtubule ends to promote depolymerization (see Figure 16–52). Kinesin-14 is a C-terminal kinesin that includes the *Drosophila* protein Ncd and the yeast protein Kar3. These kinesins generally travel in the opposite direction from the majority of kinesins, toward the minus end instead of the plus end of a microtubule.

The mechanochemical cycle of kinesin

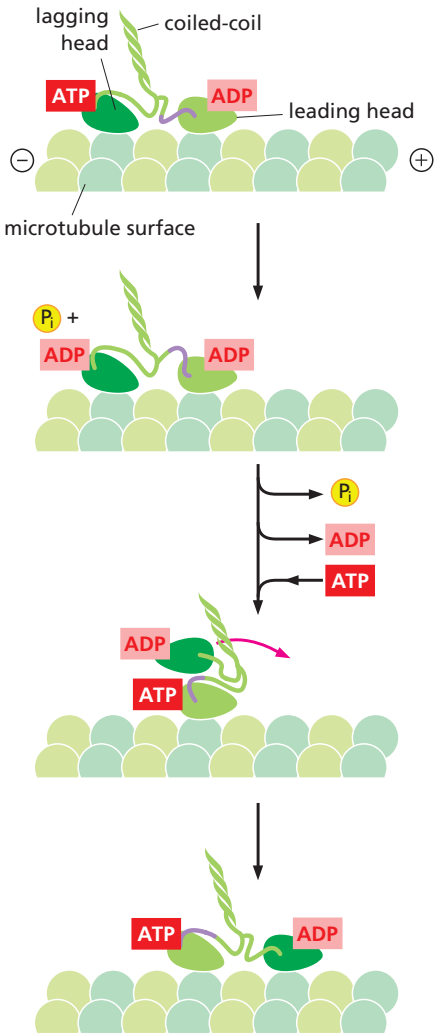
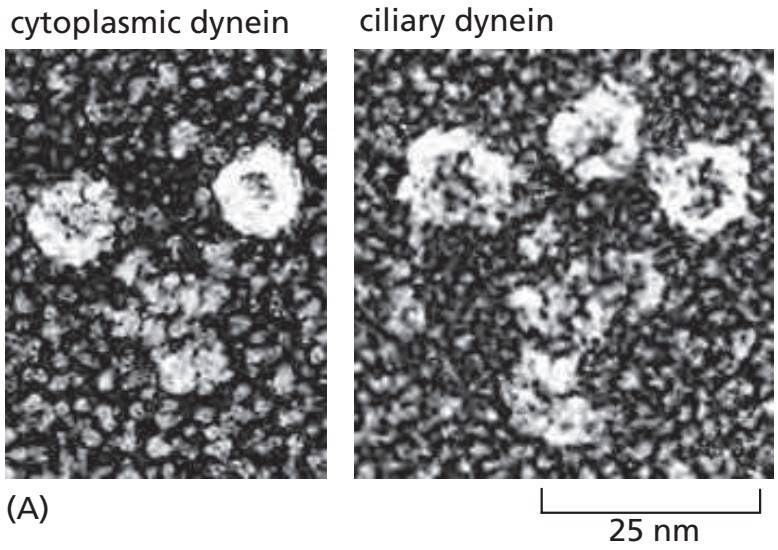


Figure 16–57 The mechanochemical cycle of kinesin. Kinesin-1 is a dimer of two nucleotide-binding motor domains (heads) that are connected through a long coiled-coil tail (see Figure 16–56). The two kinesin motor domains work in a coordinated manner; during a kinesin "step," the rear head detaches from its tubulin binding site, passes the partner motor domain, and then rebinds to the next available tubulin binding site. Using this "hand- over-hand" motion, the kinesin dimer can move for long distances on the microtubule without completely letting go of its track. At the start of each step, one of the two kinesin motor domain heads, the rear or lagging head *(dark green)*, is tightly bound to the microtubule and to ATP, while the front or leading head is loosely bound to the microtubule with ADP in its binding site. The forward displacement of the rear motor domain is driven by the dissociation of ADP and binding of ATP in the leading head (between panels 2 and 3 in this drawing). The binding of ATP to this motor domain causes a small peptide called the "neck linker" to shift from a rearward-pointing to a forward-pointing conformation (the neck linker is drawn here as a *purple* connecting line between the leading motor domain and the intertwined coiled-coil). This shift pulls the rear head forward, once it has detached from the microtubule with ADP bound [detachment requires ATP hydrolysis and phosphate (Pi) release]. The kinesin molecule is now poised for the next step, which proceeds by an exact repeat of the process shown (Movie 16.9).

Dyneins



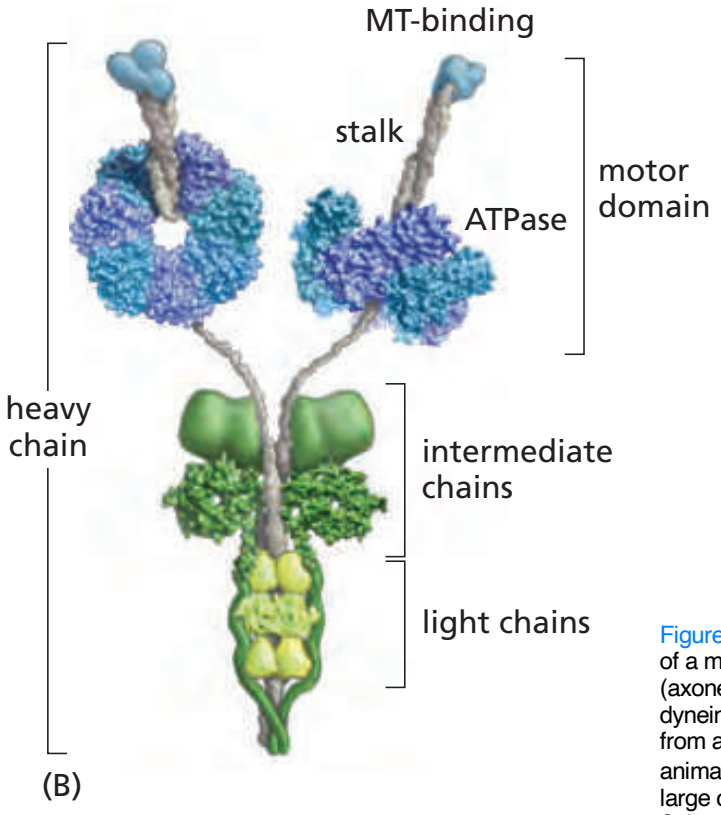


Figure 16–58 Dyneins. (A) Freeze-etch electron micrographs of a molecule of cytoplasmic dynein and a molecule of ciliary (axonemal) dynein. Like myosin II and kinesin-1, cytoplasmic dynein is a two-headed molecule. The ciliary dynein shown is from a protozoan and has three heads; ciliary dynein from animals has two heads. Note that the dynein head is very large compared with the head of either myosin or kinesin. (B) Schematic depiction of cytoplasmic dynein showing the two heavy chains *(blue and gray)* that contain domains for microtubule (MT) binding and ATP hydrolysis, connected by a long stalk. Bound to the heavy chain are multiple intermediate chains *(dark green)* and light chains *(light green)* that help to mediate many of dynein's functions. (A, courtesy of John Heuser; B, adapted from R. Vale, *Cell* 112:467–480, 2003. With permission from Cell Press.)

Dynein in action

Dynein is the only motor protein moving towards the minus end!

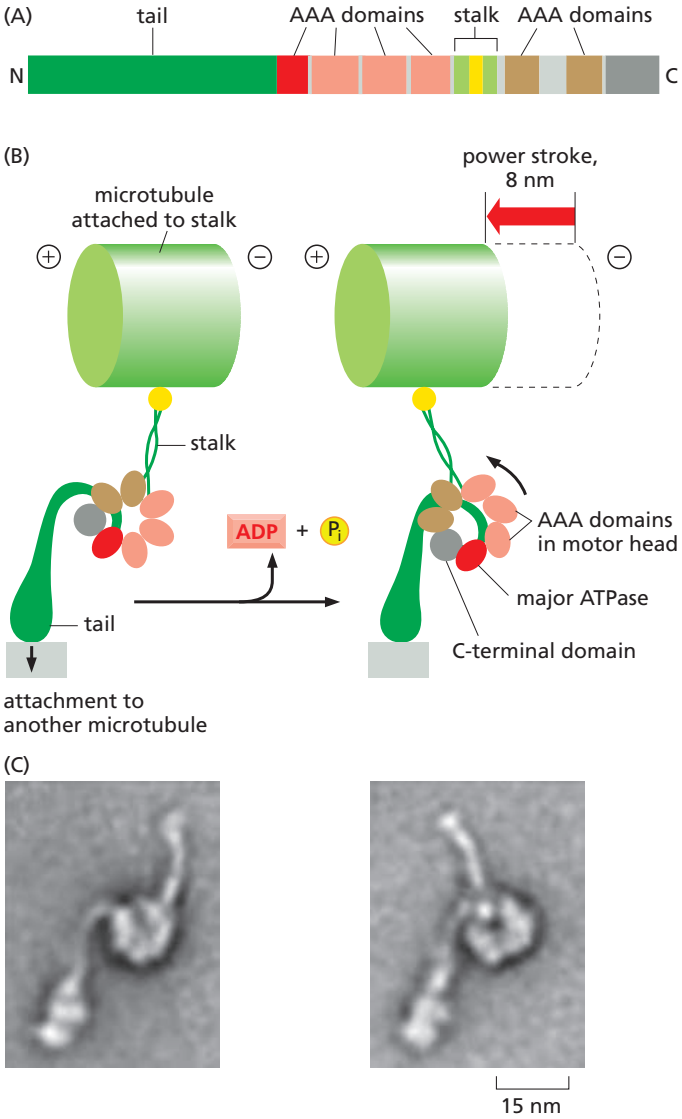


Figure 16–59 The power stroke of dynein. (A) The organization of the domains in each dynein heavy chain. This is a huge polypeptide, containing nearly 4000 amino acids. The number of heavy chains in a dynein is equal to its number of motor heads. (B) Illustration of dynein c, a monomeric axonemal dynein found in the unicellular green alga *Chlamydomonas reinhardtii.* The large dynein motor head is a planar ring containing a C-terminal domain *(gray)* and six AAA domains, four of which retain ATP-binding sequences, but only one of which *(dark red)* has the major ATPase activity. Extending from the head are a long, coiled-coil stalk with the microtubule-binding site at the tip, and a tail that attaches to an adjacent microtubule in the axoneme. In the ATP- bound state, the stalk is detached from the microtubule, but ATP hydrolysis causes stalk–microtubule attachment *(left).* Subsequent release of ADP and phosphate (Pi) then leads to a large conformational "power stroke" involving rotation of the head and stalk relative to the tail *(right).* Each cycle generates a step of about 8 nm, thereby contributing to flagellar beating (see Figure 16–65). In the case of cytoplasmic dynein, the tail is attached to a cargo such as a vesicle, and a single power stroke transports the cargo about 8-nm along the microtubule toward its minus end (see Figure 16–60). (C) Electron micrographs of purified monomeric dyneins in two different conformations representing different steps in the mechanochemical cycle. (C, from S.A. Burgess et al., *Nature* 421:715–718, 2003. With permission from Macmillan Publishers Ltd.)

Dynactin mediates the attachment of dynein to a membrane-enclosed organelle

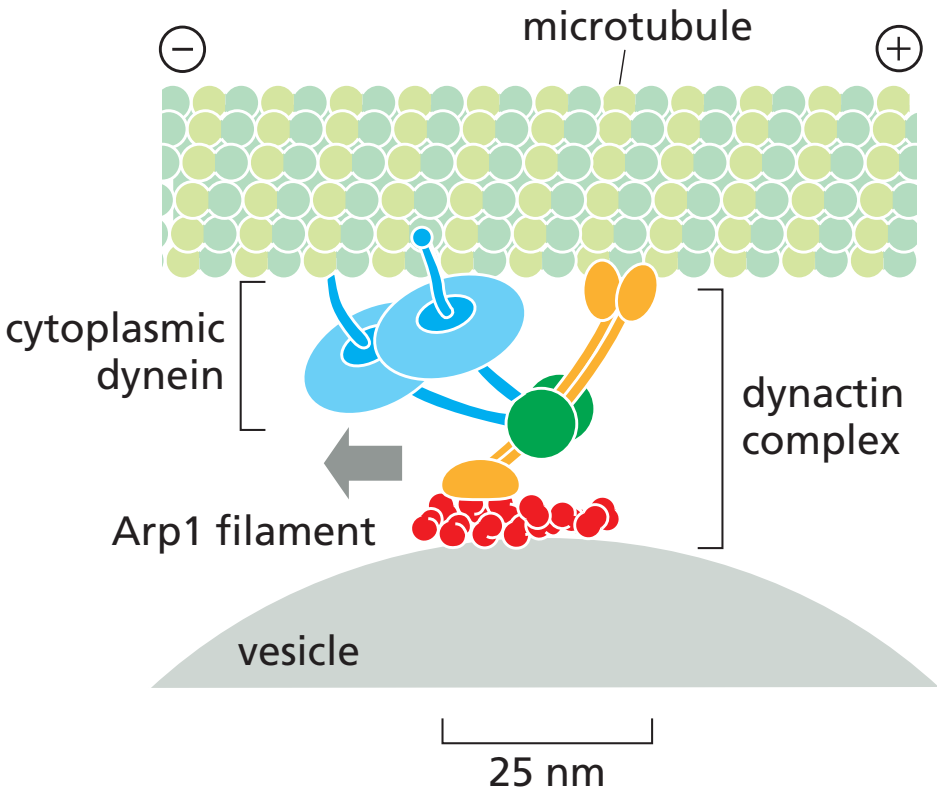
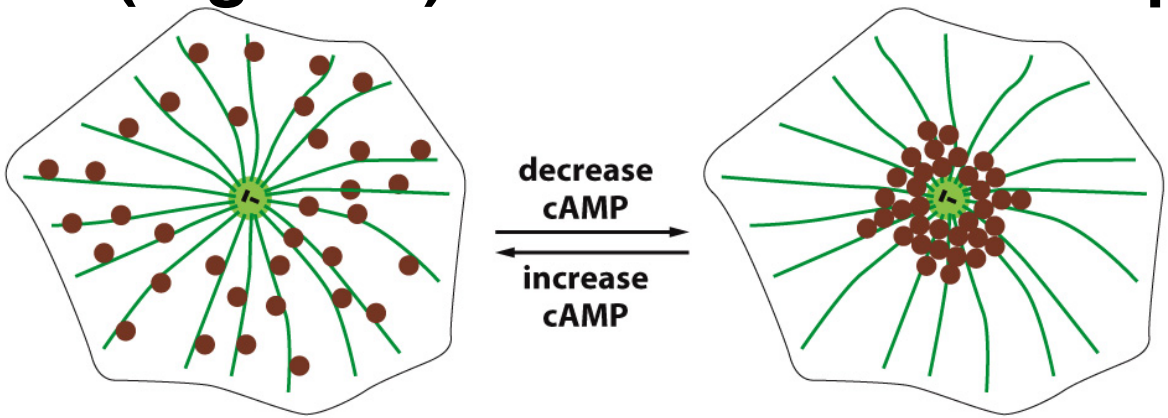
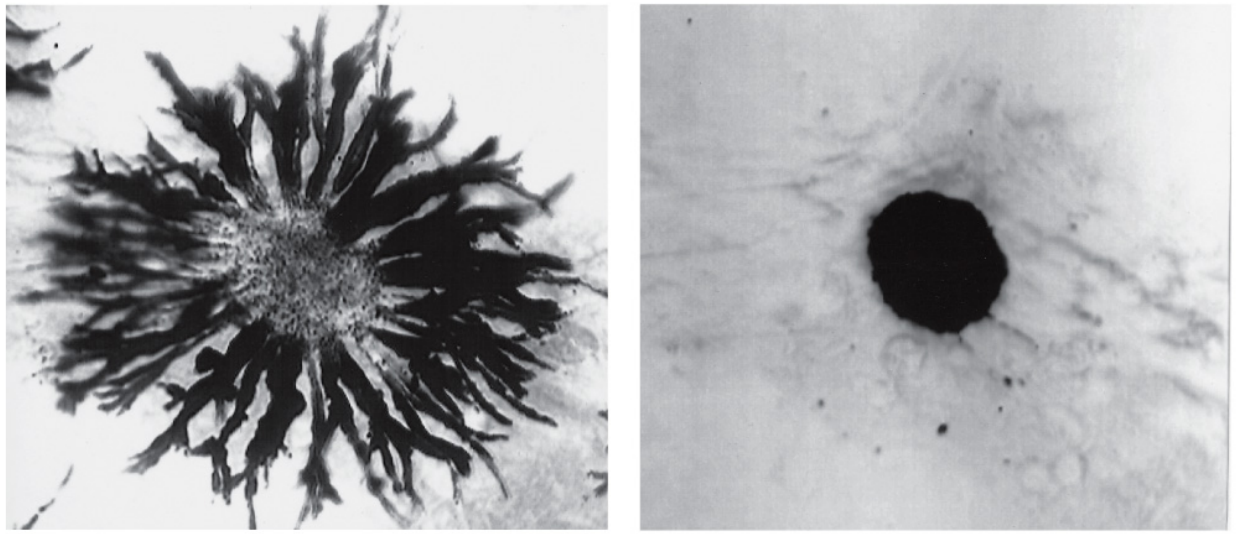


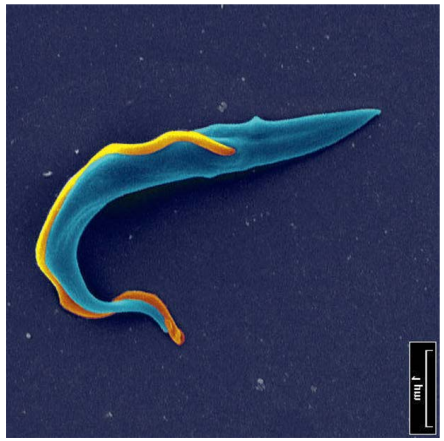
Figure 16–60 Dynactin mediates the attachment of dynein to a membrane- enclosed organelle. Dynein requires the presence of a large number of accessory proteins to associate with membrane- enclosed organelles. Dynactin is a large complex that includes components that bind weakly to microtubules, components that bind to dynein itself, and components that form a small, actin-like filament made of the actin-related protein Arp1.

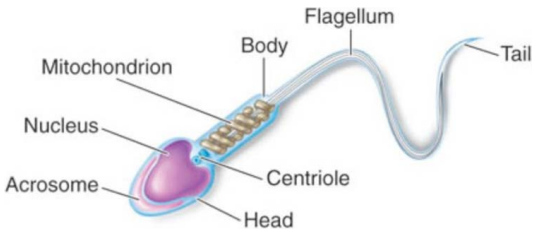
Melanosome (Pigment) movement in a fish pigment cell

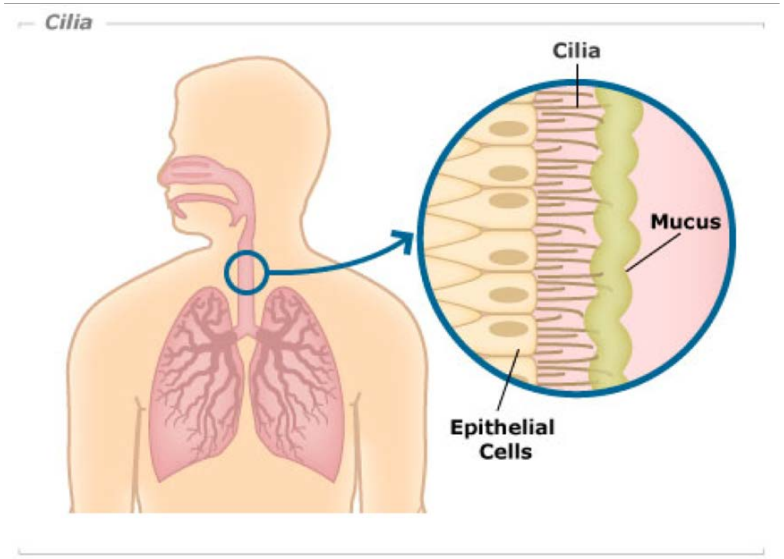


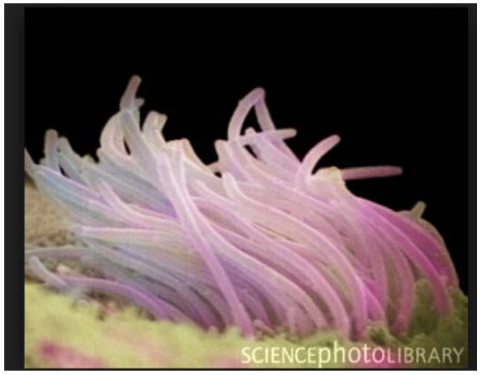


Cilia and Flagella









Cilia and Flagella

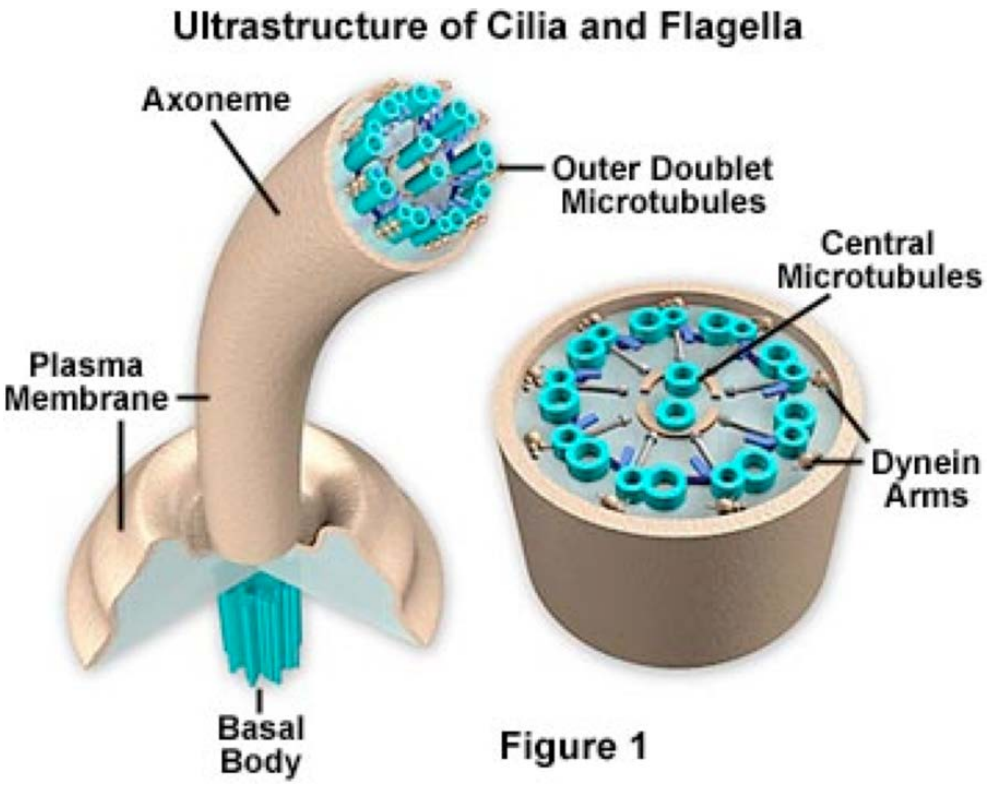


Figure 1

It has a similar ringlike structure
But it is assembled differently!

The arrangement of microtubules in a flagellum or cilium

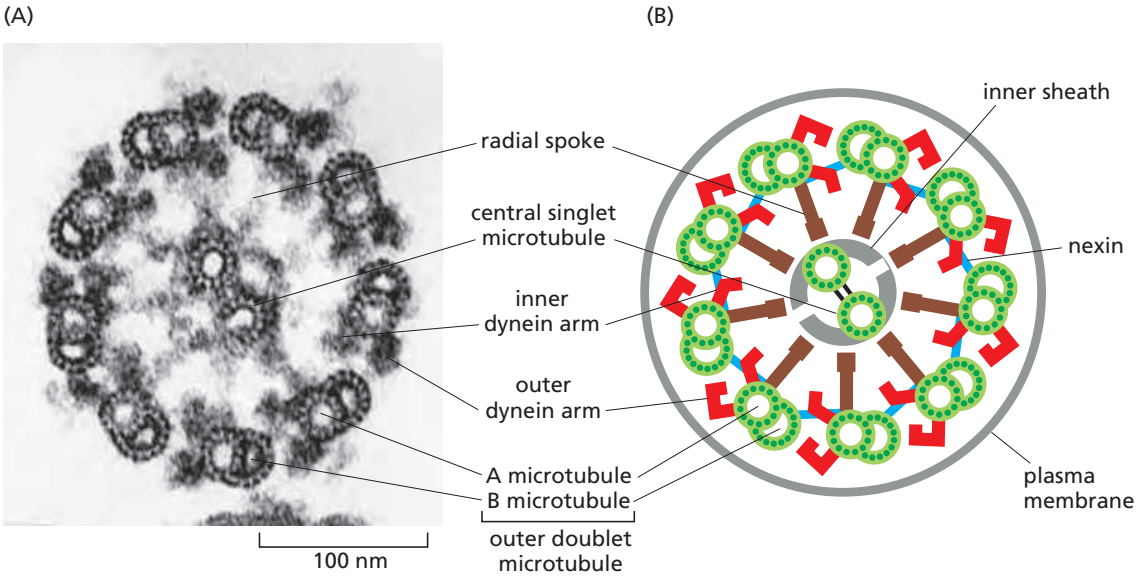


Figure 16–63 The arrangement of microtubules in a flagellum or cilium. (A) Electron micrograph of the flagellum of a green-alga cell (*Chlamydomonas*) shown in cross section, illustrating the distinctive "9 + 2" arrangement of microtubules. (B) Diagram of the parts of a flagellum or cilium. The various projections from the microtubules link the microtubules together and occur at regular intervals along the length of the axoneme. (C) High-resolution electron tomography image of an outer doublet microtubule showing structural details and features inside the microtubules called microtubule inner proteins (MIPs). (A, courtesy of Lewis Tilney; C, courtesy of Daniela Nicastro.)

The bending of an axoneme

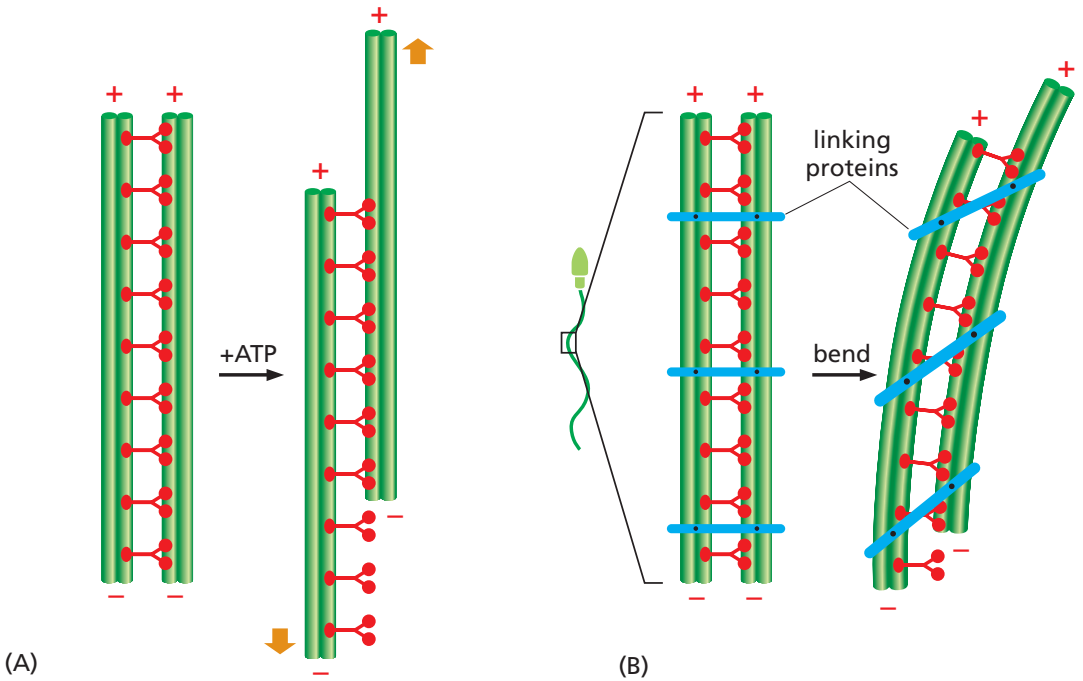
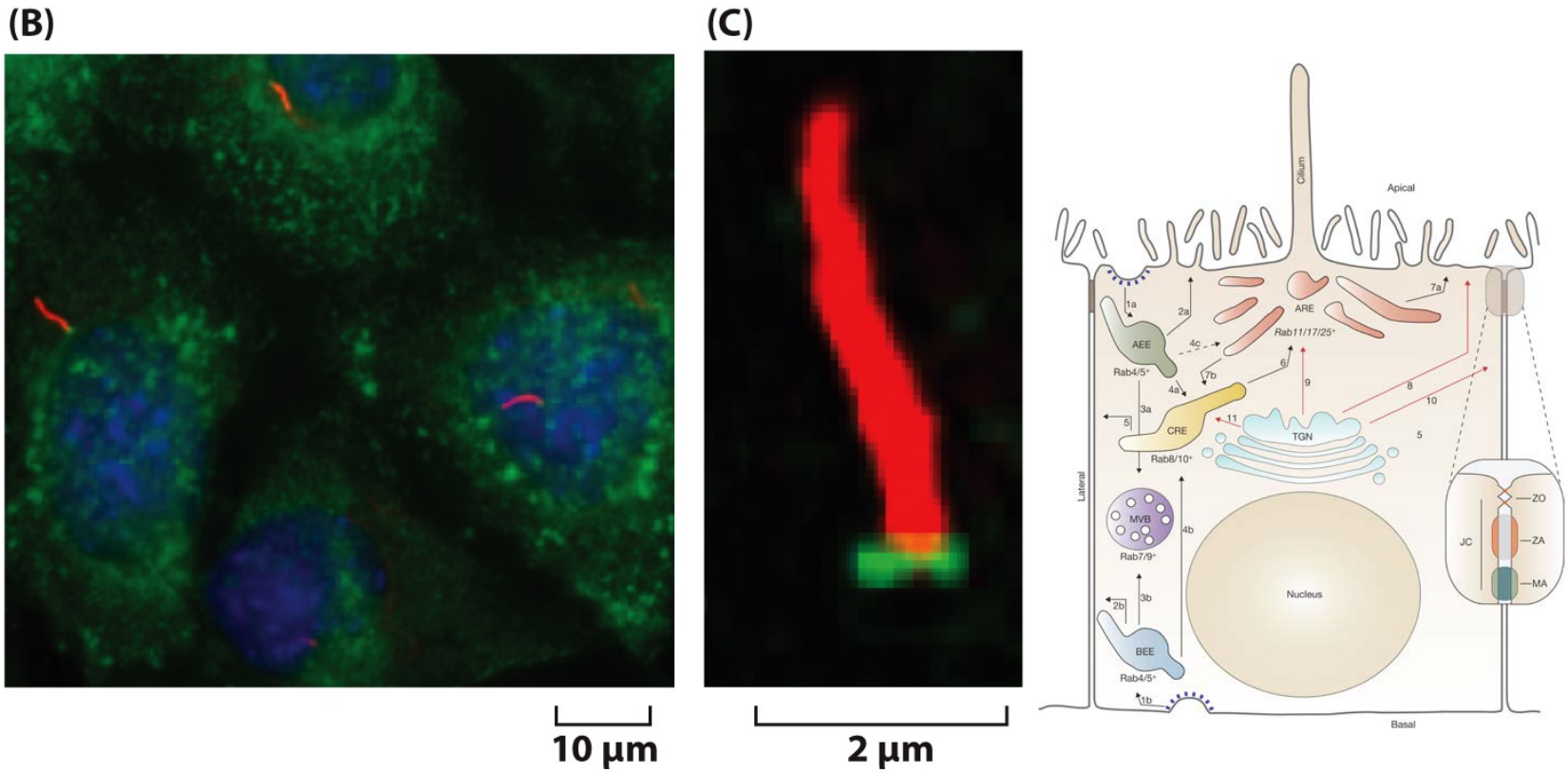


Figure 16–65 The bending of an axoneme. (A) When axonemes are exposed to the proteolytic enzyme trypsin, the linkages holding neighboring doublet microtubules together are broken. In this case, the addition of ATP allows the motor action of the dynein heads to slide one pair of doublet microtubules against the other pair. (B) In an intact axoneme (such as in a sperm), flexible protein links prevent the sliding of the doublet. The motor action therefore causes a bending motion, creating waves or beating motions.

If dynein hooks onto one filament and moves onto the other, the two filaments will slide relative to each other

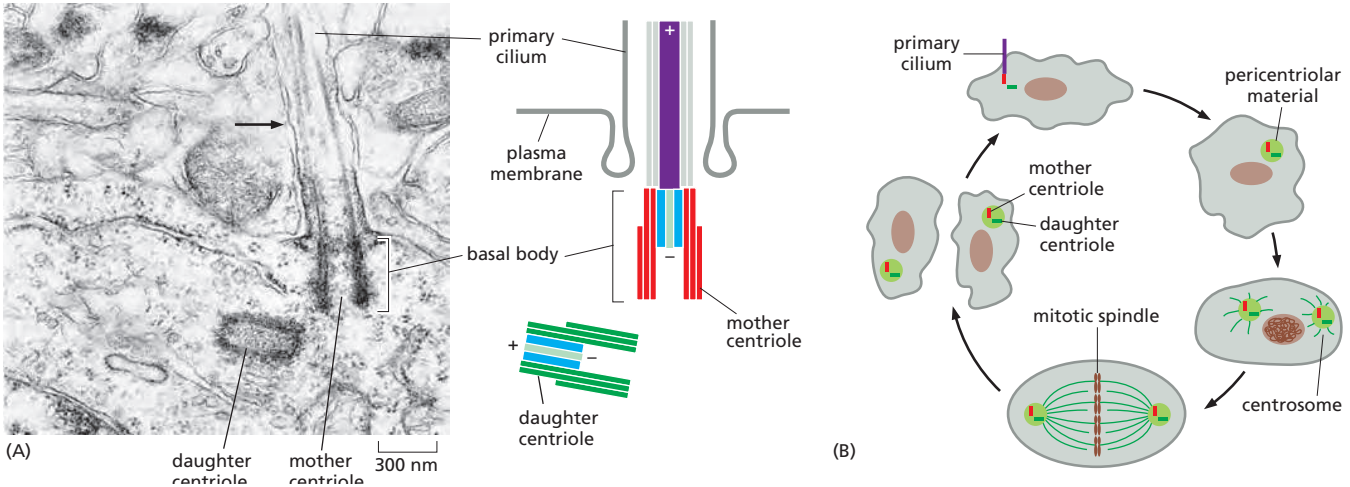
But if the sliding is blocked by a protein bridge, the whole thing will bend. If then the dyneins let go, a whipping movement will take place

Primary cilium



Most cells have a primary cilium, involved in sensing the environment.
Very active transport of lipids and proteins to and from the cilium
A structure very rich in signaling molecules (like hedgehog signaling components)

Primary cilium



During interphase, structures with a centriole in the center form under the plasma membrane to initiate the formation of cilia

Figure 16–66 Primary cilia. (A) Electron micrograph and diagram of the basal body of a mouse neuron primary cilium. The axoneme of the primary cilium (*black arrow*) is nucleated by the mother centriole at the basal body, which localizes at the plasma membrane near the cell surface.
(B) Centrioles function alternately as basal bodies and as the core of centrosomes. Before a cell enters the cell division cycle, the primary cilium is shed or resorbed. The centrioles recruit pericentriolar material and duplicate during S phase, generating two centrosomes, each of which contains a pair of centrioles. The centrosomes nucleate microtubules and localize to the poles of the mitotic spindle. Upon exit from mitosis, a primary cilium again grows from the mother centriole. (A, courtesy of Josef Spacek.)

Intermediate filaments

Intermediate filament construction

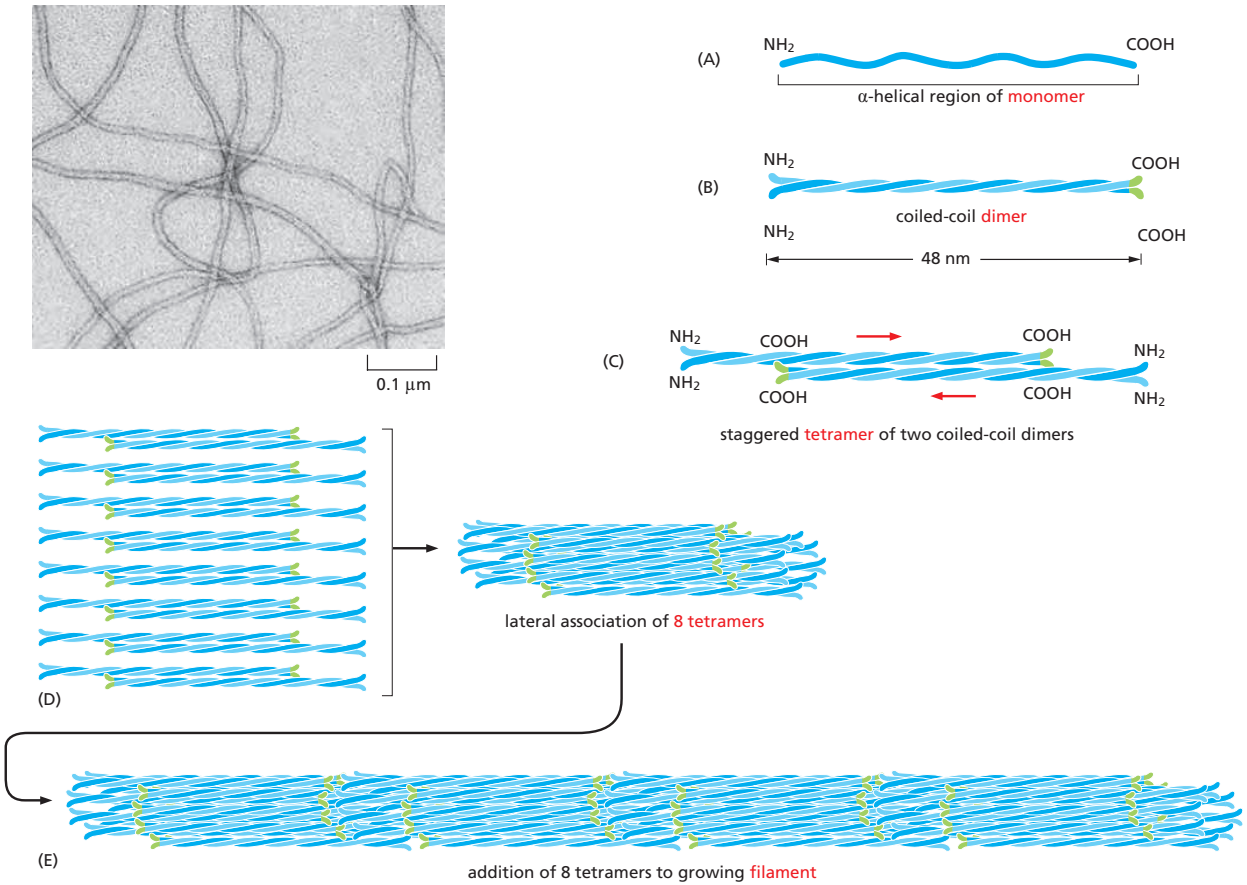


Figure 16–67 A model of intermediate filament construction. The monomer shown in (A) pairs with another monomer to form a dimer (B), in which the conserved central rod domains are aligned in parallel and wound together into a coiled-coil. (C) Two dimers then line up side by side to form an antiparallel tetramer of four polypeptide chains. Dimers and tetramers are the soluble subunits of intermediate filaments. (D) Within each tetramer, the two dimers are offset with respect to one another, thereby allowing it to associate with another tetramer. (E) In the final 10-nm ropelike filament, tetramers are packed together in a helical array, which has 16 dimers (32 coiled-coils) in cross section. Half of these dimers are pointing in each direction. An electron micrograph of intermediate filaments is shown on the upper left (Movie 16.12). (Electron micrograph courtesy of Roy Quinlan.)

Keratin intermediatefilaments in epithelial cells

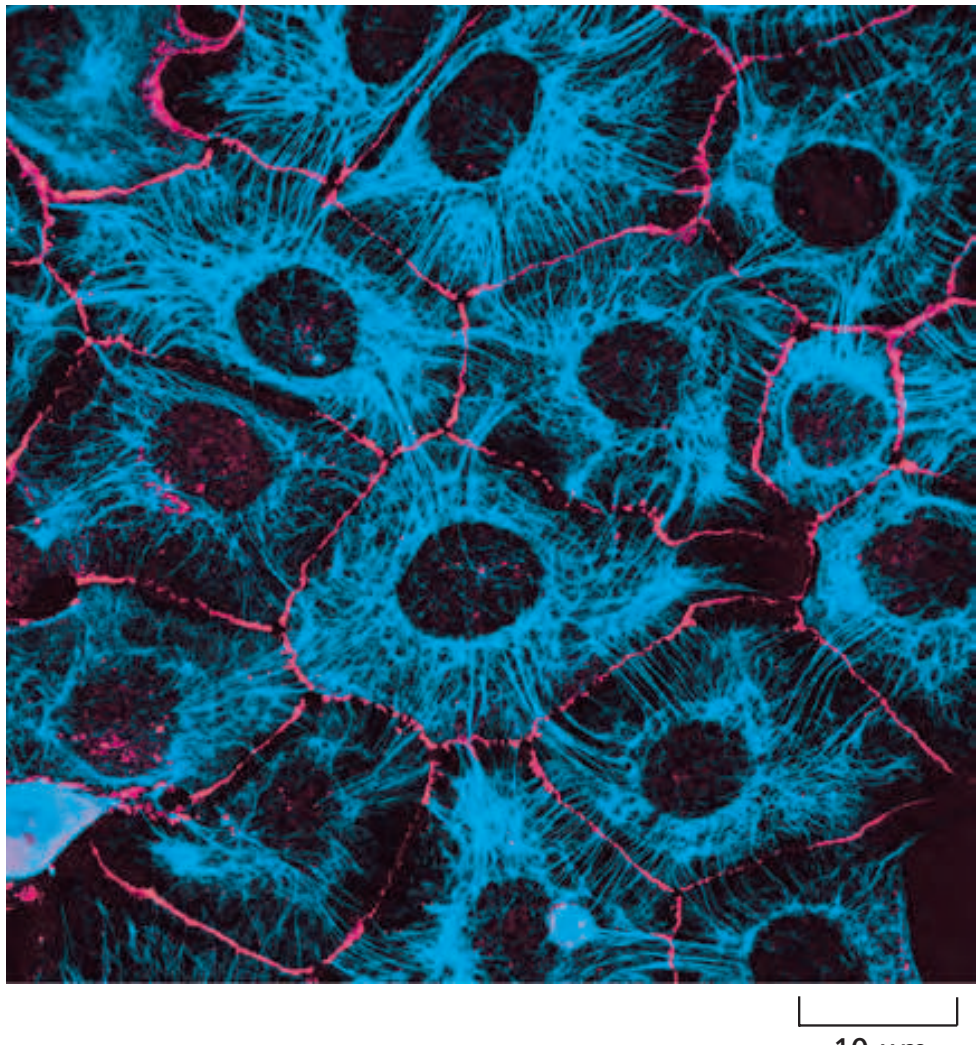


Figure 16–68 Keratin filaments in epithelial cells. Immunofluorescence micrograph of the network of keratin filaments *(blue)* in a sheet of epithelial cells in culture. The filaments in each cell are indirectly connected to those of its neighbors by desmosomes (discussed in Chapter 19). A second protein *(red)* has been stained to reveal the location of the cell boundaries. (Courtesy of Kathleen Green and Evangeline Amargo.)

Blistering of the skin caused by a mutant keratin gene

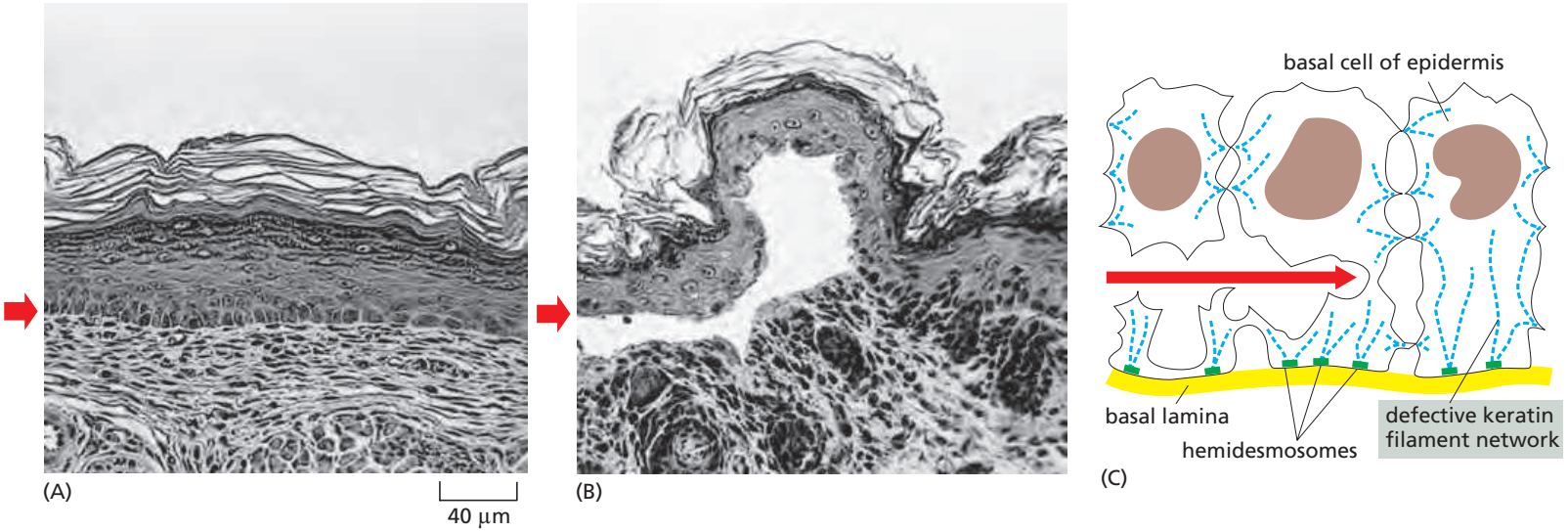


Figure 16–69 blistering of the skin caused by a mutant keratin gene. A mutant gene encoding a truncated keratin protein (lacking both the N- and C-terminal domains) was expressed in a transgenic mouse. The defective protein assembles with the normal keratins and thereby disrupts the keratin filament network in the basal cells of the skin. Light micrographs of cross sections of (A) normal and (B) mutant skin show that the blistering results from the rupturing of cells in the basal layer of the mutant epidermis (short *red* arrows). (C) A sketch of three cells in the basal layer of the mutant epidermis, as observed by electron microscopy. As indicated by the *red* arrow, the cells rupture between the nucleus and the hemidesmosomes (discussed in Chapter 19), which connect the keratin filaments to the underlying basal lamina.

Comparison to Actin and Tubulin

- Actin and tubulin are small, compact, globular proteins. The proteins of the intermediate filaments are very elongated
- Actin and tubulin form polarized filaments. Due to the head-to-tail assembly of dimers, intermediate filaments have no polarity.
- Actin and tubulin form enzymes (ATPases and GTPases respectively)
- Big differences in stability and mechanical properties