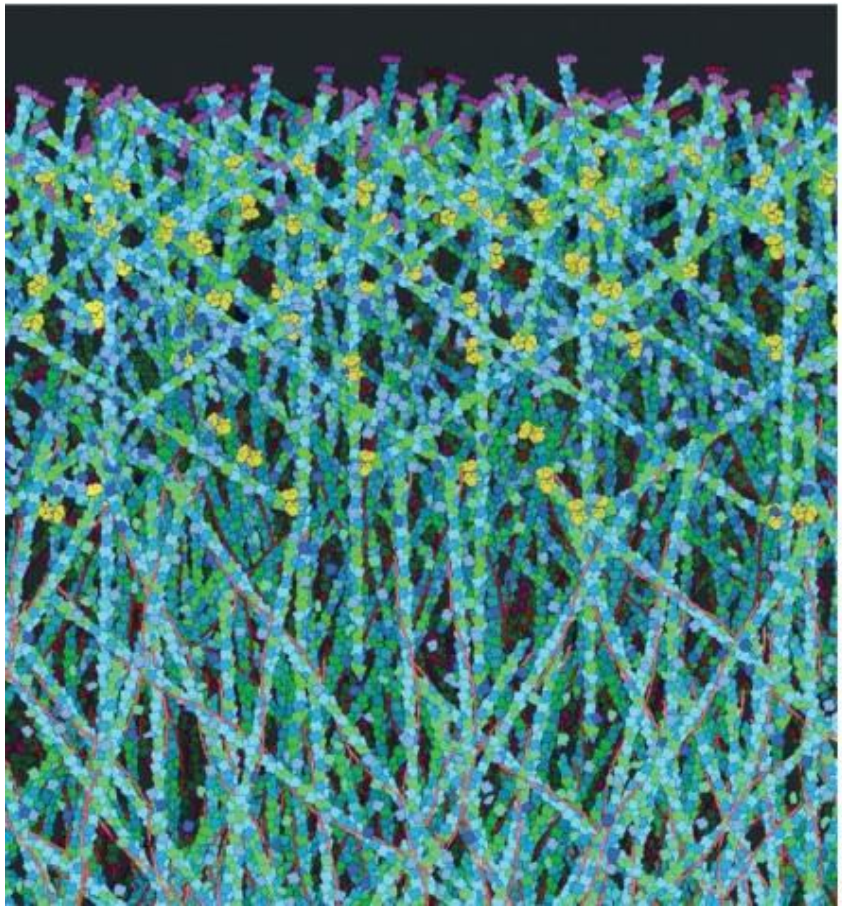
CHAPTER 13

The Cytoskeleton



Actin networks of the leading edge of a motile cell.

13.0 | Introduction

Poisons, Drugs and the Cytoskeleton

Toxins have been isolated from various plants that affect the cytoskeleton.

Two major groups of multicyclic peptide toxins are produced by the death cap:

1) **Amatoxins**, which are potent inhibitors of RNA polymerase II,
2) **Phallotoxins**, like phalloidin, which bind tightly and specifically to actin filaments of the cytoskeleton.

13.0 | Introduction

Poisons, Drugs and the Cytoskeleton

Stems of the autumn crocus (*Colchicum autumnale*) have been used to treat joint pain and gout since ancient times.

In the 1800s, chemists isolated the activity to a small molecule which they called colchicine.

Colchicine has a high binding affinity to tubulin, which prevents the assembly of microtubules.

13.0 | Introduction

Poisons, Drugs and the Cytoskeleton

Taxol, the most famous cytoskeletal drug, was found to be synthesized by an endosymbiont fungus living in the bark of the Pacific yew.

Taxol binds specifically and tightly to tubulin to stabilize microtubules and keep them from depolymerizing.

Taxol has been used since the 1980s as a key chemotherapeutic agent for a number of cancers, including those of the breast, lung and ovary.

13.1 | Overview of the Major Functions of the Cytoskeleton

Properties of cytoskeletal components

The vertebrate skeleton consists of hardened elements that support the soft tissues of the body and play a key role in mediating bodily movements.

Eukaryotic cells also possess a “skeletal system,” the cytoskeleton, with analogous functions.

The cytoskeleton is composed of three well defined filamentous structures (microtubules, actin filaments, and intermediate filaments) that together form an elaborate interactive and dynamic network.

Each of the three types of cytoskeletal filaments is a polymer of protein subunits held together by weak, noncovalent bonds.

13.1 | Overview of the Major Functions of the Cytoskeleton

Properties of cytoskeletal components

TABLE 9.1 Properties of Microtubules, Intermediate Filaments, and Actin Filaments

	Microtubules	Intermediate filaments	Actin filaments
Subunits incorporated into polymer	GTP-αβ-tubulin heterodimer	~70 different proteins, likely incorporated as tetramers	ATP-actin monomers
Preferential site of incorporation	+ End (β-tubulin)	Internal	+ End (barbed)
Polarity	Yes	No	Yes
Enzymatic activity	GTPase	None	ATPase
Motor proteins	Kinesins, dyneins	None	Myosins
Major group of associated proteins	MAPs	Plakins	Actin-binding proteins
Structure	Stiff, hollow, inextensible tube	Tough, flexible, extensible filament	Flexible, inextensible helical filament
Dimensions	25 nm outer diam.	10–12 nm diam.	8 nm diam.
Distribution	All eukaroytes	Animals	All eukaryotes
Primary functions	Support, intracellular transport, cell organization	Structural support, mechanical strength	Motility, contractility, intracellular transport
Subcellular distribution	Cytoplasm	Cytoplasm + nucleus	Cytoplasm

13.1 | Overview of the Major Functions of the Cytoskeleton

Roles of cytoskeletal components

The cytoskeleton:

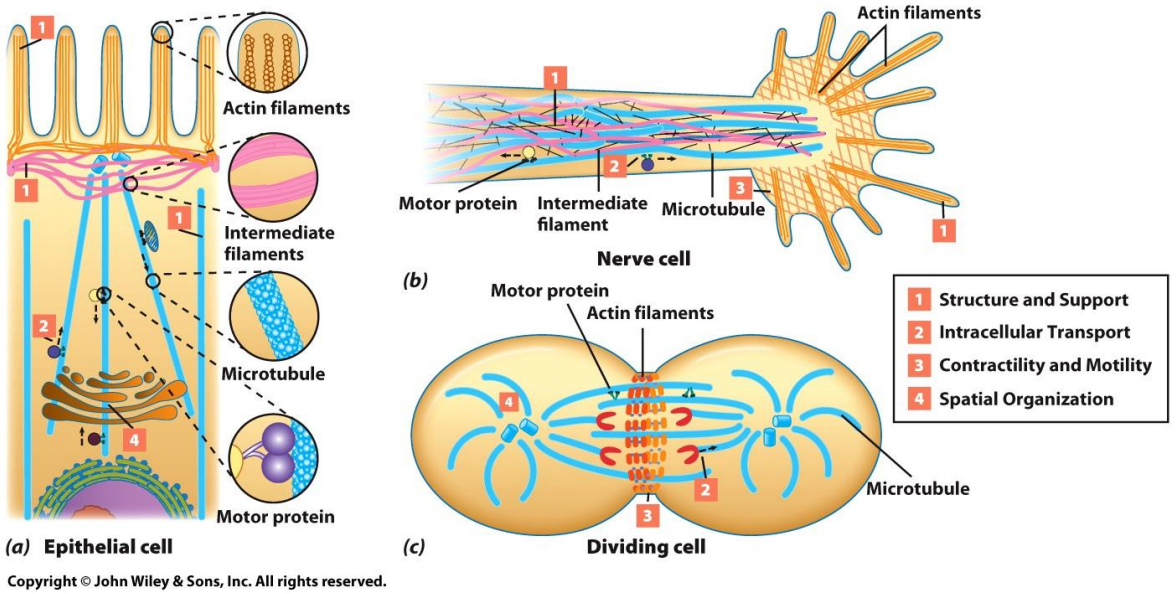
Serves as a scaffold to provide structural support and maintain cell shape.

Serves as an internal framework to position organelles.

Provides a network of tracks that direct the movement of materials and organelles.

Generates forces needed for cellular locomotion.

Makes up an essential part of the cell division machinery.



Structure and functions of the cytoskeleton

13.2 | Structure and Function of Microtubules

Structure and Composition of Microtubules

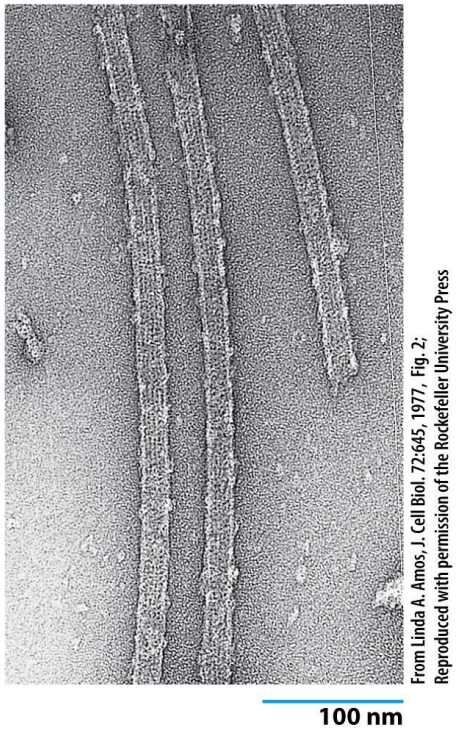
Microtubules are hollow, rigid, cylindrical structures.

The microtubule is a set of globular proteins arranged in longitudinal rows called **protofilaments**.

These rows are aligned parallel to the long axis of the tubule.

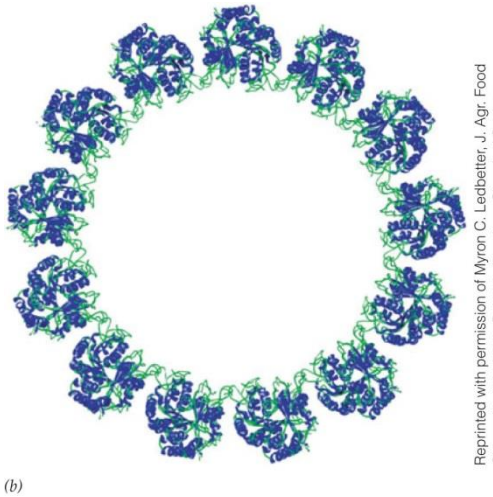
Microtubules have 13 protofilaments aligned side by side in a circular pattern within the wall.

Noncovalent interactions between adjacent protofilaments may help maintain microtubule structure.



From Linda A. Amos, J. Cell Biol. 72:645, 1977, Fig. 2; Reproduced with permission of the Rockefeller University Press

Electron micrograph of negatively stained microtubules



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Electron micrograph cross section through a microtubule revealing the 13 subunits

13.2 | Structure and Function of Microtubules

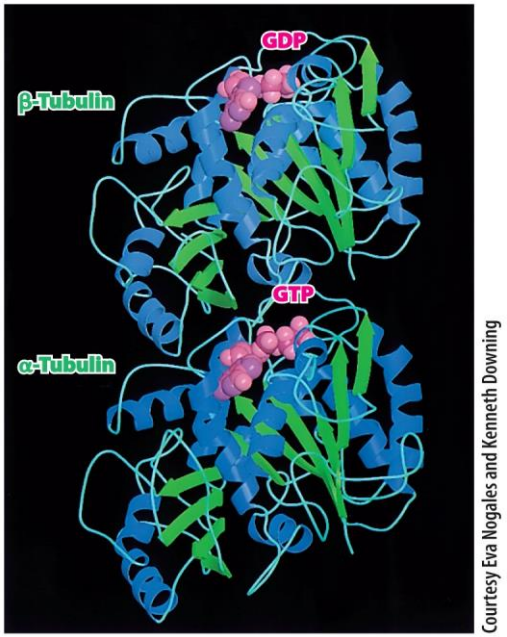
Structure and Composition of Microtubules

Protofilaments are assembled from dimeric building blocks consisting of one α-tubulin and one β-tubulin subunit.

The globular subunits have a similar 3D structure and fit tightly together.

The tubulin dimers are organized in a linear array along the length of each protofilament, which is asymmetric and polar.

The plus end is terminated by a row of β-tubulin subunits and the minus end and is terminated by a row of α-tubulin subunits.



Ribbon model: 3D structure of the αβ-tubulin heterodimer

13.2 | Structure and Function of Microtubules

Microtubule-Associated Proteins

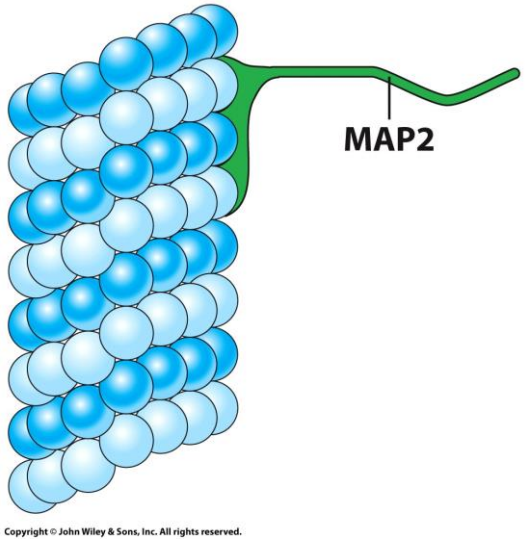
Microtubules typically contain additional proteins, called microtubule-associated proteins (or MAPs).

MAPs comprise a heterogeneous collection of proteins with one domain that attaches to the side of a microtubule and another domain that projects outward as a tail.

MAPs generally increase the stability of microtubules and promote their assembly.

MAP activity is controlled by the addition and removal of phosphate groups from amino acid residues.

An abnormally high level of phosphorylation of one particular MAP, called tau, has been implicated in Alzheimer's disease.



Schematic diagram of a brain MAP2 molecule bound to the surface of a microtubule.

13.2 | Structure and Function of Microtubules

Microtubules as Structural Supports and Organizers

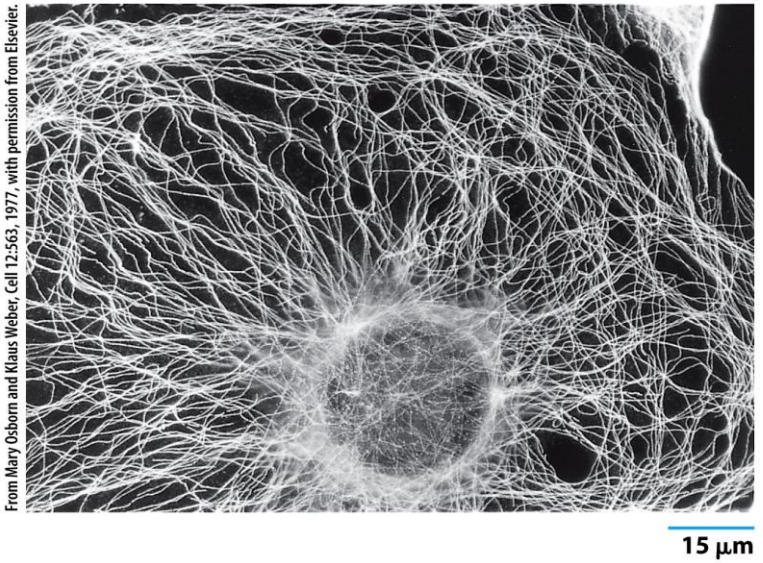
Microtubules resist forces that might compress or bend the fiber for mechanical support.

Distribution of microtubules helps determine the shape of that cell.

In cultured animal cells, microtubules extend in a radial array outward from near the nucleus, giving cells a round, flattened shape.

Microtubules of columnar epithelial cells are oriented with their long axis parallel to the long axis to support the cell's elongated shape.

Role of microtubules as skeletal elements is evident in highly elongated cellular processes like cilia and flagella and nerve cell axons.



Localization of microtubules in a cultured mouse cell shown by fluorescent anti-tubulin antibodies. Microtubules extend from the perinuclear region of the cell in a radial array and curve gradually as they conform to the shape of the cell.

13.2 | Structure and Function of Microtubules

Microtubules as Structural Supports and Organizers

Microtubules also play a key role in maintaining the internal organization of cells.

The Golgi complex is typically organized as a single ribbon located near the center of a mammalian cell, just outside the nucleus.

Treatment of cells with nocodazole or colchicine, which promote microtubule disassembly, can disperse the Golgi elements into separate Golgi stacks scattered throughout the cytoplasm.

When the drug is removed and microtubules reassemble, the Golgi membranes return to their normal position in the cell interior.

13.2 | Structure and Function of Microtubules

Microtubules as Agents of Intracellular Motility

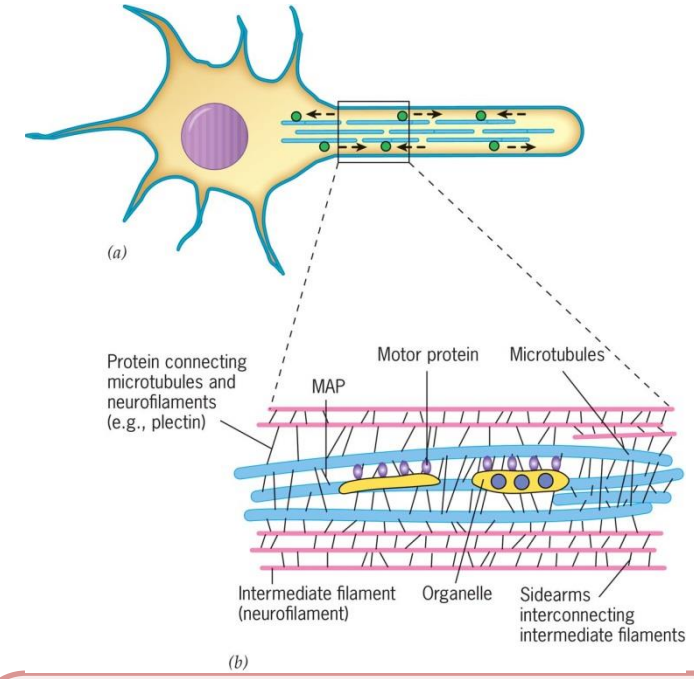
Transport of materials from one membrane compartment to another depends on microtubules.

Neurotransmitters are packaged in membranous vesicles in the ER and Golgi complex of the cell body and then transported down the axon.

Structures traveling from the cell body toward the neuron terminal move in an anterograde direction.

Structures that move in the opposite, or retrograde, direction go toward the cell body.

Defects in both anterograde and retrograde transport have been linked to neurological diseases like Amyotrophic Lateral Sclerosis (ALS).



Vesicles movement down the length of an axon along tracks of microtubules. Microtubule and intermediate filament organization in an axon

13.3 | Motor Proteins: Kinesins and Dyneins

- Motor Proteins that Traverse the Microtubular Cytoskeleton
  - Molecular motors convert energy from ATP into mechanical energy.
  - Molecular motors move unidirectionally along their cytoskeletal track in a stepwise manner.
  - Three categories of molecular motors:
    - Kinesin and dynein move along microtubule tracks.
    - Myosin moves along microfilament tracks.

13.3 | Motor Proteins: Kinesins and Dyneins

Motor Proteins Traverse the Microtubular Cytoskeleton

Motor proteins move unidirectionally along their cytoskeletal track in a stepwise manner and undergo a series of conformational changes that constitute a mechanical cycle.

The steps of the mechanical cycle are coupled to the steps of a chemical cycle, which provide the energy necessary to fuel the motor's activity and to move along the track.

The chemical cycle steps include the binding of ATP to the motor, the hydrolysis of ATP, the release of the products (ADP and Pi), and the binding of a new molecule of ATP.

13.3 | Motor Proteins: Kinesins and Dyneins

Kinesins

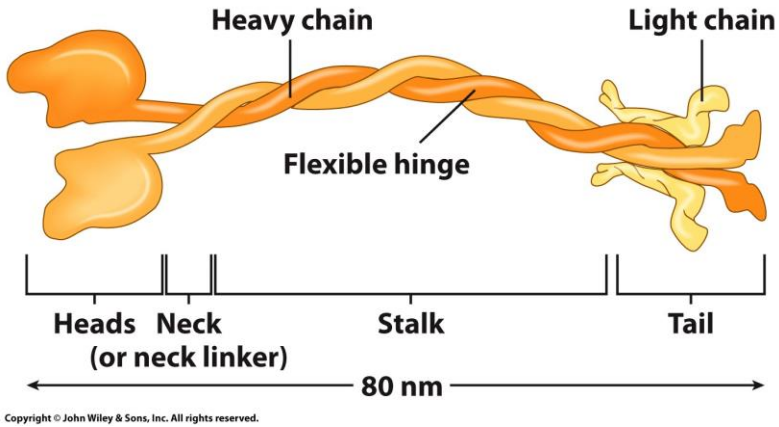
The kinesin molecule is in a superfamily of 14 KRPs (kinesin-related proteins).

A kinesin molecule is a tetramer of two identical heavy and two identical light chains.

The globular heads bind microtubules and act as ATP-hydrolyzing, force-generating engines.

Each head is connected to a neck, a rodlike stalk, and a fan-shaped tail that binds cargo.

A number of different proteins have been identified as potential adaptors that link specific KRPs and their cargoes.



Force-generating heads bind to the microtubule; tail binds to the cargo being transported

13.3 | Motor Proteins: Kinesins and Dyneins

Cytoplasmic Dynein

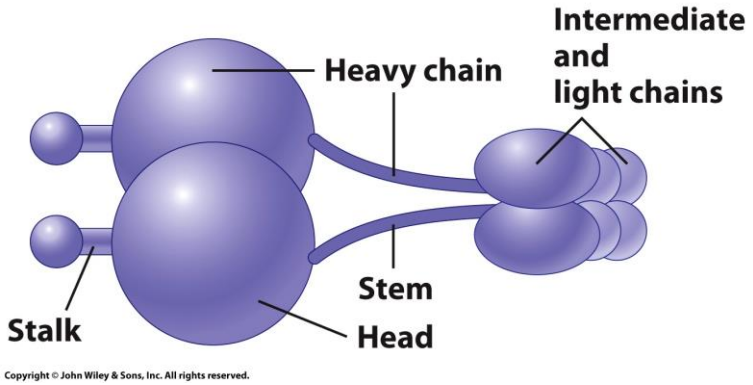
Cytoplasmic dynein is a huge protein composed of two identical heavy chains and a variety of intermediate and light chains.

The heavy chain consists of a large globular force-generating head and a microtubule-binding stalk.

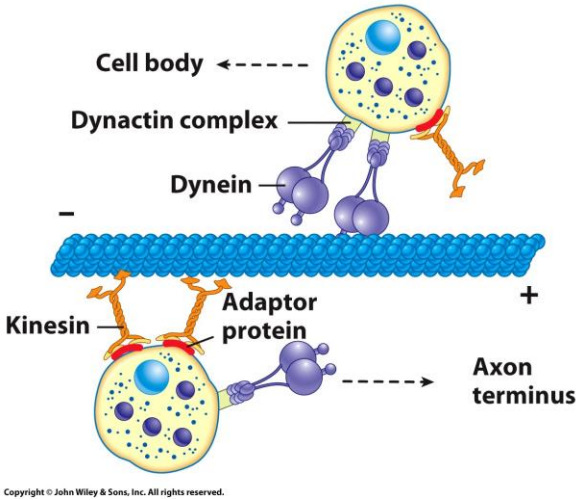
Cytoplasmic dynein moves processively along a microtubule toward the polymer's minus end—opposite that of most kinesins.

Role 1: Position the spindle and move chromosomes during mitosis.

Role 2: Position the centrosome and Golgi complex and moving organelles, vesicles, and particles through the cytoplasm.



Structure of a cytoplasmic dynein molecule



Schematic diagram of two vesicles moving in opposite directions along the same microtubule

13.3 | Motor Proteins: Kinesins and Dyneins

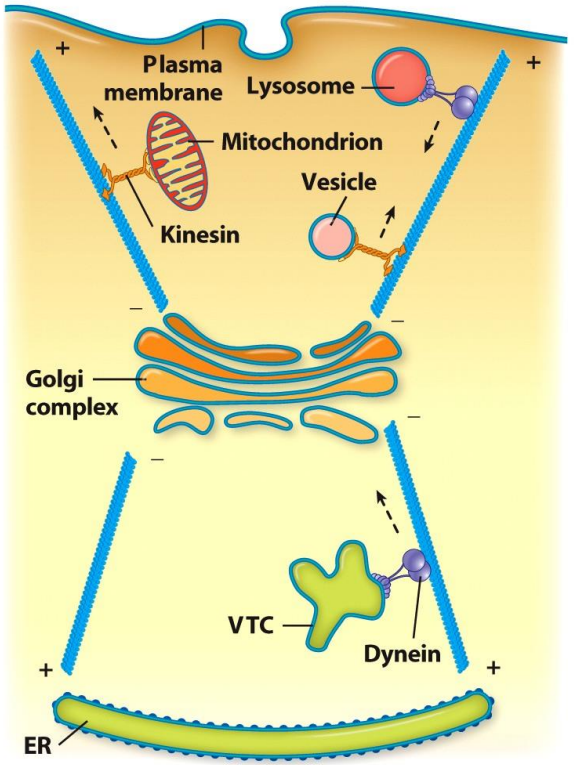
Cytoplasmic Dynein

Dynein-driven cargo includes endosomes and lysosomes, ER-derived vesicles heading toward the Golgi complex, RNA molecules, and the HIV virus which is transported to the nucleus of an infected cell.

Cytoplasmic dynein does not interact directly with membrane-bounded cargo but requires the intervening adaptor dynactin, a protein that also increases the processivity of dynein.

Kinesins and cytoplasmic dynein move similar materials in opposite directions over the same railway network.

Organelles may bind kinesin and dynein simultaneously.



Schematic illustration of kinesin-mediated and dynein-mediated transport of vesicles

13.4 | Microtubule Organizing Centers (MTOCs)

The function of a microtubule within a living cell depends on its location and orientation, which makes it important to understand why a microtubule assembles in one place as opposed to another.

When studied in vitro, the assembly of microtubules from αβ-tubulin dimers occurs in two distinct phases: a slow phase of nucleation, in which a small portion of the microtubule is initially formed, and a much more rapid phase of elongation.

Unlike the case in vitro, nucleation of microtubules takes place rapidly inside a cell, where it occurs in association with a variety of specialized structures called microtubule-organizing centers (or MTOCs).

All MTOCs play similar roles in all cells: they control the number of microtubules, their polarity, the number of protofilaments that make up their walls, and the time and location of their assembly.

The best studied MTOC is the centrosome.

13.4 | Microtubule Organizing Centers (MTOCs)

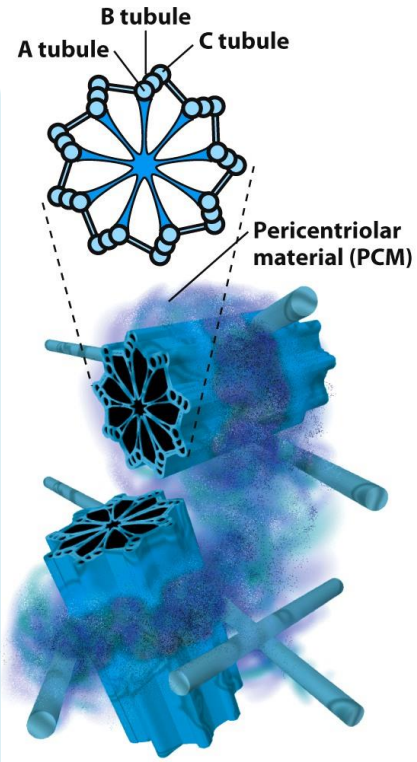
Centrosomes

In animal cells, microtubules are typically nucleated by the centrosome, a complex structure that contains two barrel-shaped centrioles surrounded by amorphous, pericentriolar material (PCM).

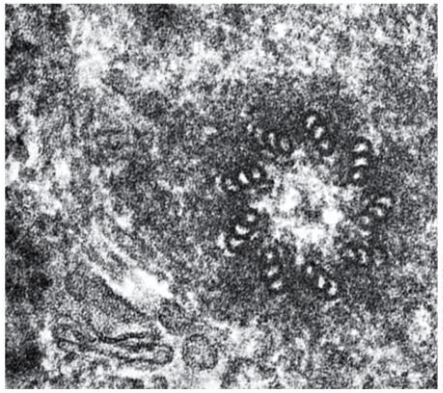
Centrioles are cylindrical structures about 0.2 μm in diameter and typically about twice as long.

Centrioles contain nine evenly spaced blades, each of which contain three microtubules, designated the A, B, and C tubules. Only the A tubule is a complete microtubule.

The nine A tubules are connected to a central hub with nine spokes called the cartwheel.



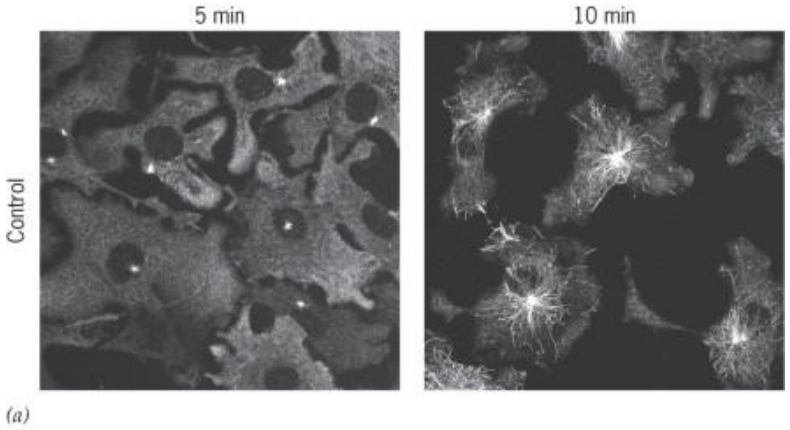
Schematic diagram of a centrosome shows the paired centrioles, surrounding PCM, and microtubules forming from the PCM



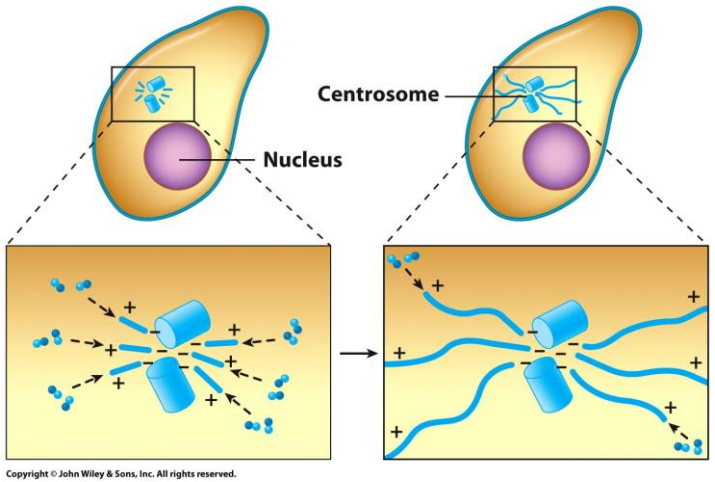
EM cross section of a centriole showing its pinwheel arrangement

13.4 | Microtubule Organizing Centers (MTOCs)

Centrosomes



Fumoto K, Kadono M, Izumi N, Kikuchi A. 2009. Axin localizes to the centrosome and is involved in microtubule nucleation. EMBO Rep.10, 606-13.



Microtubule nucleation at the centrosome.

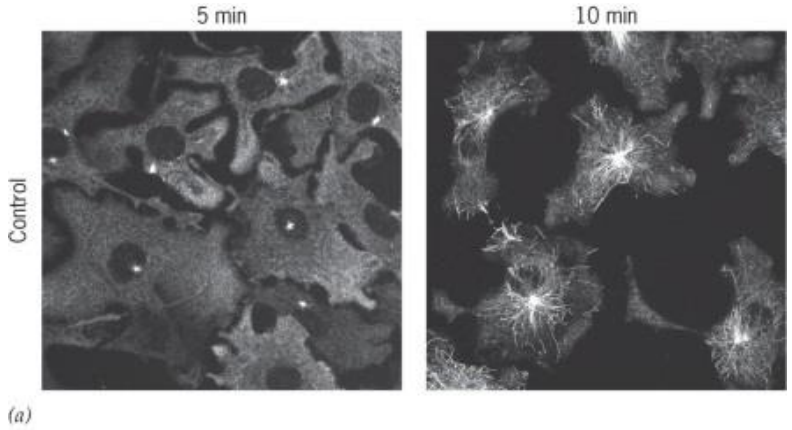
The growth of microtubules occurs by addition of subunits at the plus end of the polymer away from the centrosome

the centrosome play a crucial role in the initiation and organization of the microtubular cytoskeleton.

Because centrosomes are sites of microtubule nucleation, the microtubules of the cytoskeleton are all polarized the same way: the minus end is associated with the centrosome, and the plus (or growing) end is situated at the opposite tip.

13.4 | Microtubule Organizing Centers (MTOCs)

Centrosomes



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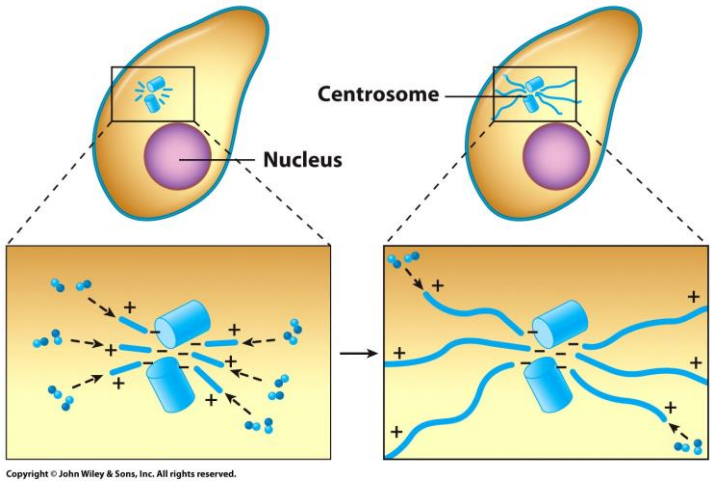
Microtubule nucleation at the centrosome.

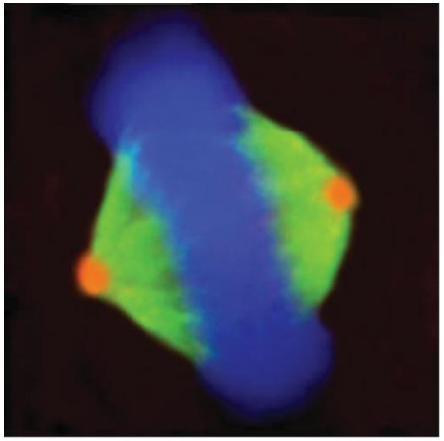


The growth of microtubules occurs by addition of subunits at the plus end of the polymer away from the centrosome

Genetic defects in centrosome-associated proteins cause microcephaly, because neuron proliferation and migration is sensitive to loss of centrosome function.

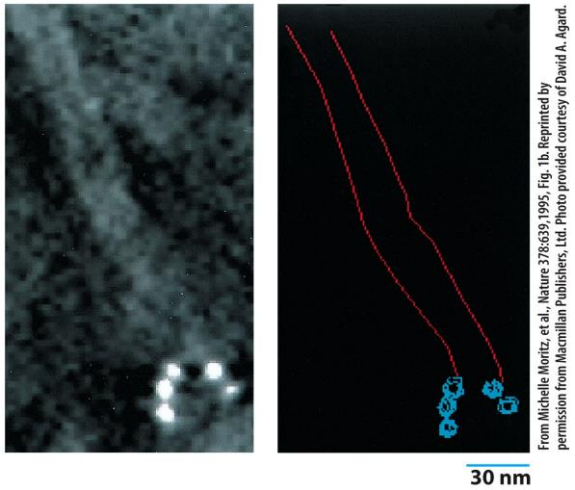
13.4 | Microtubule Organizing Centers (MTOCs)

Microtubule Nucleation



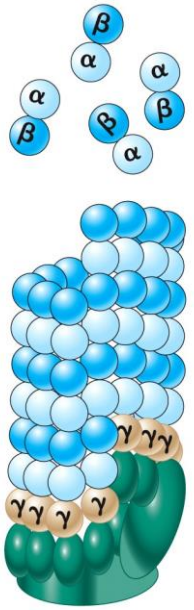
(a)

Fibroblast stained with γ-tubulin (red) and β-tubulin (green) Abs



From Michelle Moritz, et al., Nature 378:639, 1995, Fig. 1b. Reprinted by permission from Macmillan Publishers, Ltd. Photo provided courtesy of David A. Agard.

EM and drawing of a centrosome incubated with purified tubulin then labeled with γ-tubulin Abs



γ-tubulin ring complex model

MTOCs share a common factor, γ-tubulin, a critical protein in microtubule nucleation.

The PCM serves as attachment sites for ring-shaped structures that contain γ-tubulin

13.5 | Microtubule Dynamics

The Dynamic Properties of Microtubules

The microtubules of the cytoskeleton are dynamic polymers that are subject to shortening, lengthening, disassembly, and reassembly.

Cell differences in microtubule stability are determined by microtubule interacting proteins including MAPs which stabilize microtubules, proteins known as +TIPs which bind to the plus-end of growing microtubules, and the enzyme katanin, that severs microtubules into shorter pieces.

Disassembly can be induced by cold temperature; hydrostatic pressure; elevated Ca^{2+} concentration; and a variety of chemicals, including colchicine, vinblastine, vincristine, taxol, and nocodazole.

The lability of cytoskeletal microtubules reflects the fact that they are polymers formed by the noncovalent association of protein building blocks.

The microtubules of the cytoskeleton are normally subject to depolymerization and repolymerization as the requirements of the cell change from one time to another.

13.5 | Microtubule Dynamics

The Underlying Basis of Microtubule Dynamics

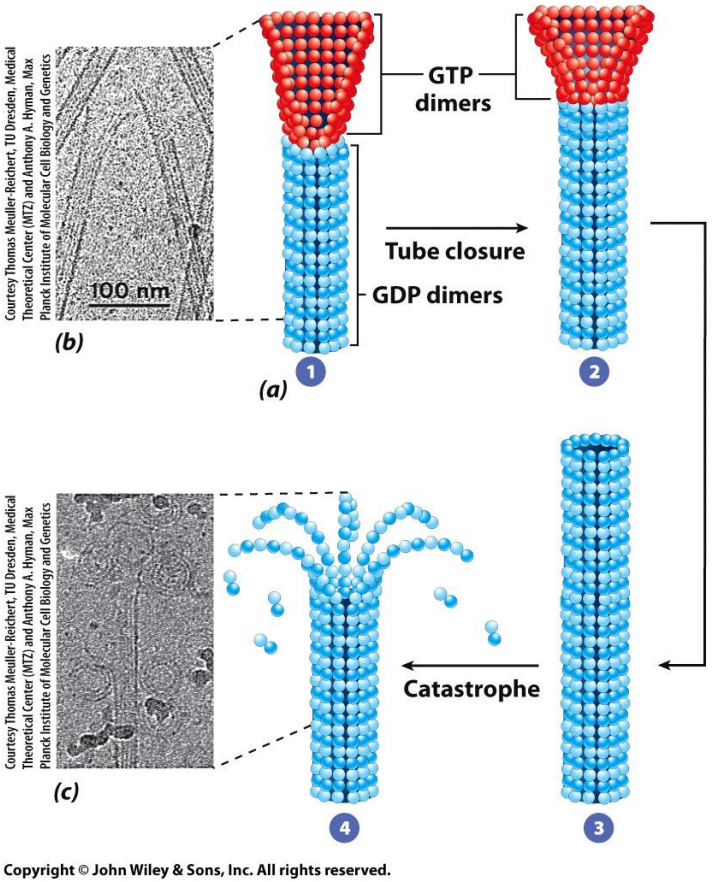
Assembly of tubulin dimers requires that a GTP molecule be bound to the β-tubulin subunit.

β-tubulin is a structural protein and a GTPase.

GTP hydrolysis is not required for tubulin dimer incorporation; GTP is hydrolyzed to GDP after the dimer is incorporated, and the resulting GDP remains bound to the assembled polymer.

After a dimer is released from a microtubule during disassembly and enters the soluble pool, the GDP is replaced by a new GTP.

This nucleotide exchange "recharges" the dimer, allowing it to serve once again as a building block for polymerization.



Structural cap model of dynamic instability

13.5 | Microtubule Dynamics

The Underlying Basis of Microtubule Dynamics

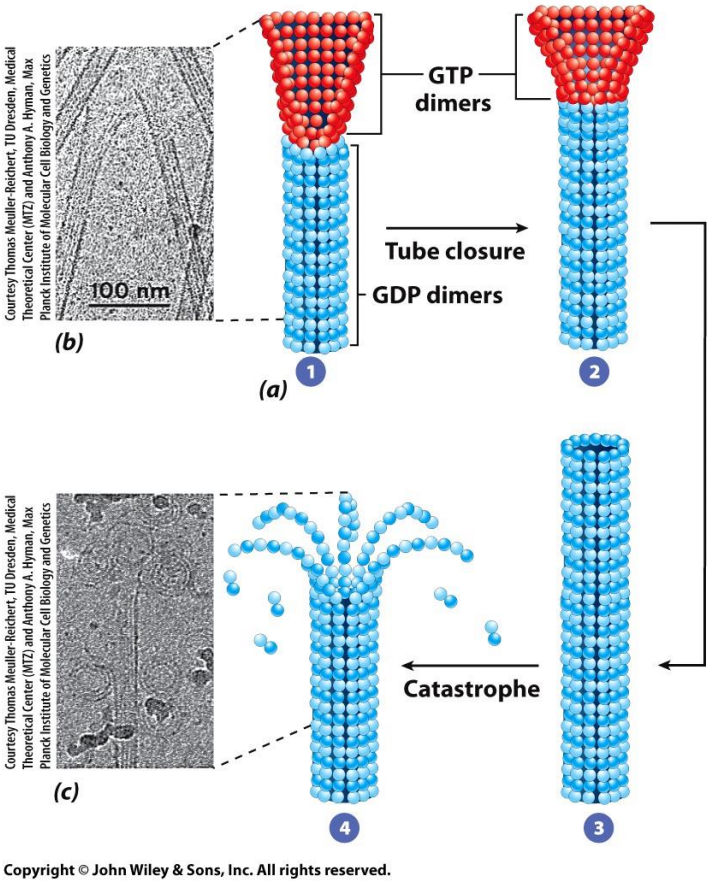
When a microtubule is growing, the plus end is an open sheet to which GTP-dimers are added.

During rapid growth, tubulin dimers are added more rapidly than their GTP can be hydrolyzed.

Microtubules with open ends undergo a spontaneous reaction that leads to closure of the tube, accompanied by the hydrolysis of the bound GTP to generate GDP-bound tubulin.

GDP-tubulin subunits have a different conformation than GTP-tubulin subunits and are less suited to fit into a straight protofilament.

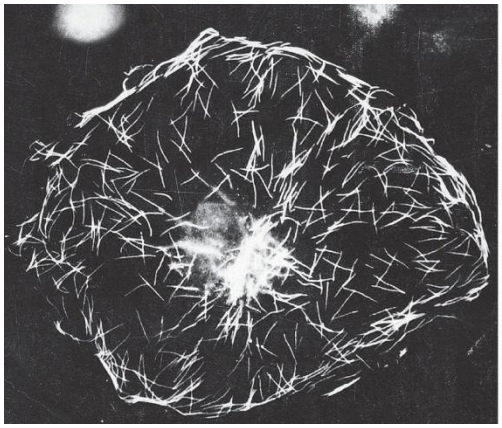
The strain energy is released as the protofilaments curl outward from the plus end of the tubule and undergo depolymerization.



Structural cap model of dynamic instability

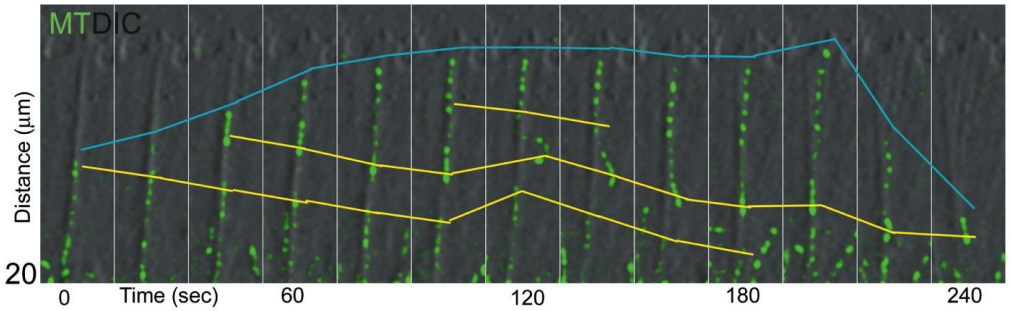
13.5 | Microtubule Dynamics

The Underlying Basis of Microtubule Dynamics



From Marc Kirschner, J. Cell Biol. 102, cover of No. 3, 1986; Reproduced with permission of the Rockefeller University Press.

Injection of tubulin (biotin) into fibroblast for 1 minute



From Andrew W. Schaefer, Nurul Kabir, and Paul Forscher, Yale University. J.Cell Biol. 158:145, 2002, Fig. 5, reproduced with permission of The Rockefeller University Press.

Dynamic instability: Length of a single MT in growth cone of a neuron over time. Reference tubulin-GFP (yellow) and plus end (blue) are shown.

Dynamic instability explains the observation (1) that growing and shrinking microtubules can coexist in the same region of a cell, and (2) that a given microtubule can switch back and forth unpredictably between growing and shortening phases.

13.7 | Intermediate Filaments

Intermediate filaments have only been identified in animal cells.

Intermediate filaments are strong, flexible, ropelike fibers that provide mechanical strength to cells

IFs are a chemically heterogeneous group of structures that are encoded by approximately 70 different genes.

IFs can be divided into five major classes based on the type of cell in which they are found as well as biochemical, genetic, and immunologic criteria.

TABLE 9.2 Properties and Distribution of the Major Mammalian Intermediate Filament Proteins

IF protein	Sequence type	Primary tissue distribution
Keratin (acidic) (28 different polypeptides)	I	Epithelia
Keratin (basic) (26 different polypeptides)	II	Epithelia
Vimentin	III	Mesenchymal cells
Desmin	III	Muscle
Glial fibrillary acidic protein (GFAP)	III	Astrocytes
Peripherin	III	Peripheral neurons
Neurofilament proteins		Neurons of central and peripheral nerves
NF-L	IV	
NF-M	IV	
NF-H	IV	
Nestin	IV	Neuroepithelia
Lamin proteins		All cell types (Nuclear envelopes)
Lamin A	V	
Lamin B	V	
Lamin C	V	

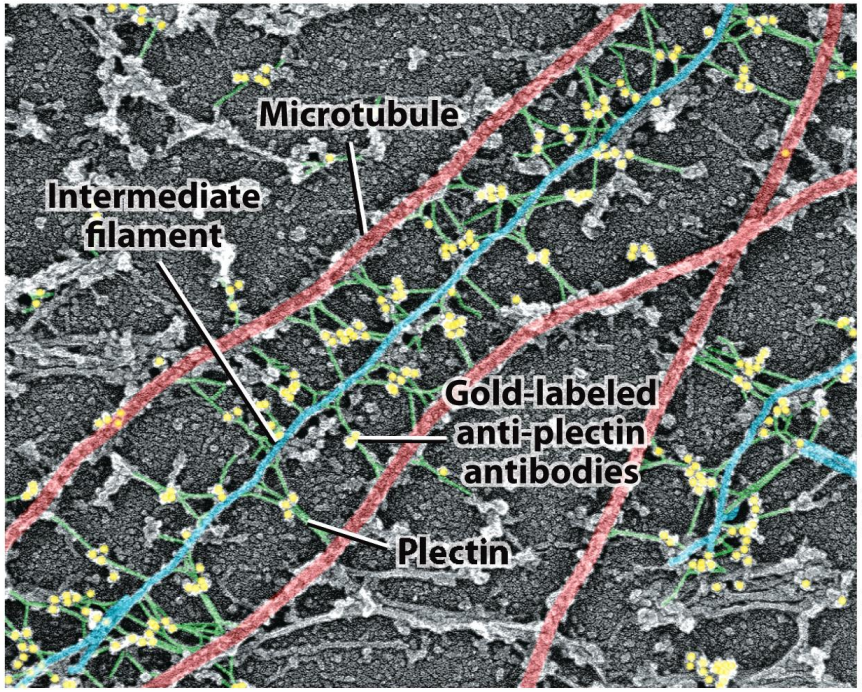
More detailed tables can be found in *Trends Biochem Sci.* 31:384, 2006, *Genes and Development* 21:1582, 2007, and *Trends Cell Biol.* 18:29, 2008.

13.7 | Intermediate Filaments

IFs radiate through the cytoplasm of a wide variety of animal cells and are often interconnected to other cytoskeletal filaments by thin cross-bridges.

In many cells, these cross-bridges consist of an elongated dimeric protein called **plectin**.

Each plectin molecule has a binding site for an intermediate filament at one end and, depending on the isoform, a binding site for another intermediate filament, microfilament, or microtubule at the other end.



Cytoskeletal elements are connected to one another by protein cross-bridges (plectin).

13.7 | Intermediate Filaments

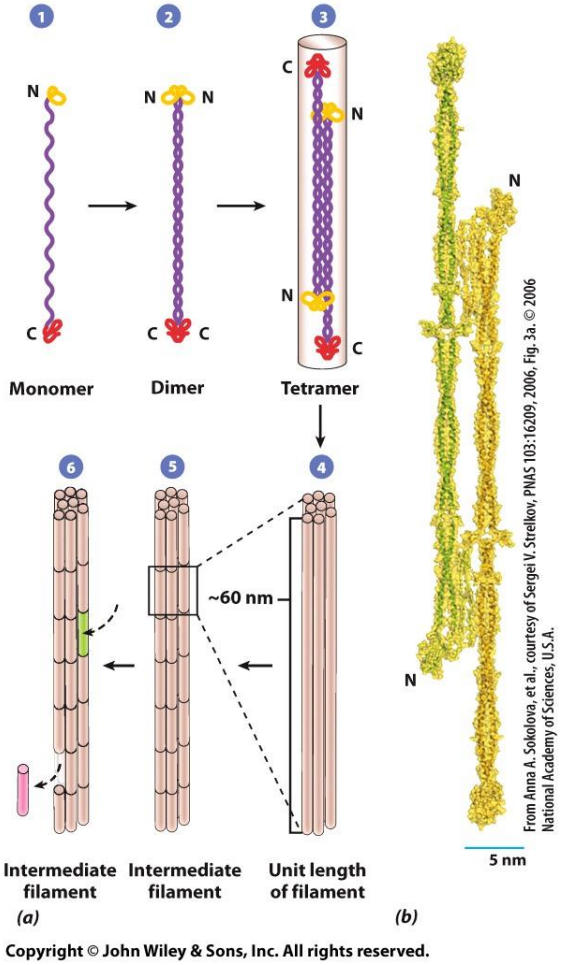
IF polypeptides have diverse amino acid sequences, yet all share a similar structural organization and form similar-looking filaments.

Polypeptides of IFs all contain a central, rod-shaped, α-helical domain of similar length.

The central fibrous domain is flanked on each side by globular domains of variable size.

Two polypeptides interact as their α-helical rods wrap around each other to form a ropelike dimer.

The two polypeptides are aligned parallel to one another so the dimer has polarity, with one end defined by the C-termini of the polypeptides and the opposite end by their N-termini.



Models of IF assembly and architecture

13.7 | Intermediate Filaments

Intermediate Assembly and Disassembly

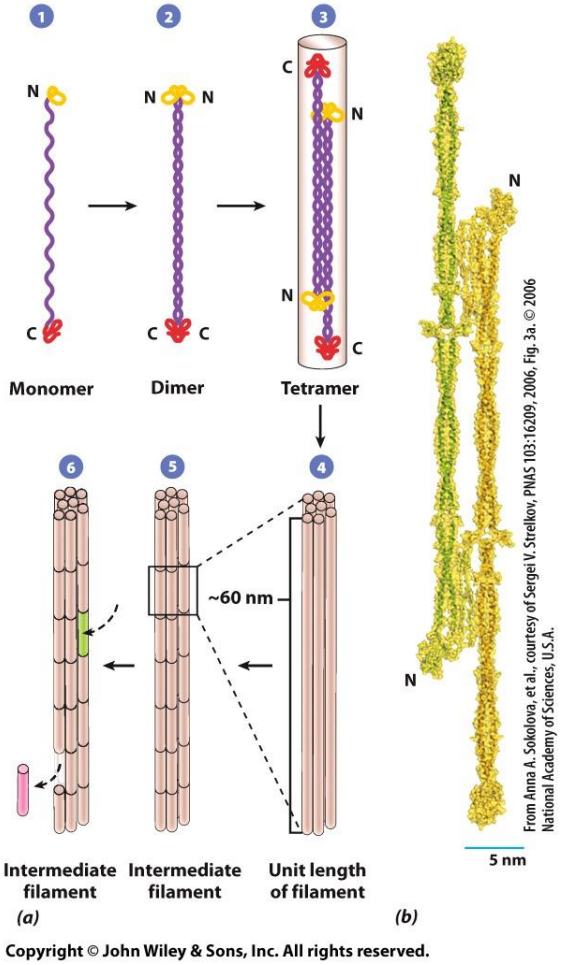
The basic building block of IF assembly is thought to be a rodlike tetramer.

Eight tetramers associate with one another in a lateral arrangement to form a filament that is one unit in length (about 60 nm).

Unit lengths of filaments associate with one another in an end-to-end fashion to form the highly elongated intermediate filament.

None of these assembly steps require the direct involvement of either ATP or GTP.

The tetrameric building blocks lack polarity as does the assembled filament, which distinguishes IFs from other cytoskeletal elements.



Models of IF assembly and architecture

13.8 | Actin

Cells can be motile: Neural crest cells leave the developing nervous system and migrate across the embryo, to form pigmented skin cells, teeth, and the cartilage of the jaws. White blood cells patrol the body searching for debris and microorganisms.

Cell parts can be motile: Projections of epithelial cells at the edge of a wound act as motile devices that pull the sheet of cells over the damaged area, sealing the wound. The leading edge of a growing axon sends out microscopic processes that survey the substratum and guide the cell toward a synaptic target.

All of these various examples of motility share at least one component: they all depend on actin, the third major type of cytoskeletal element.

Actin is also involved in intracellular motile processes, such as the movement of vesicles, phagocytosis, and cytokinesis.

Actin also plays an important role in determining the shapes of cells and can provide structural support for various types of cellular projections.

13.8 | Actin

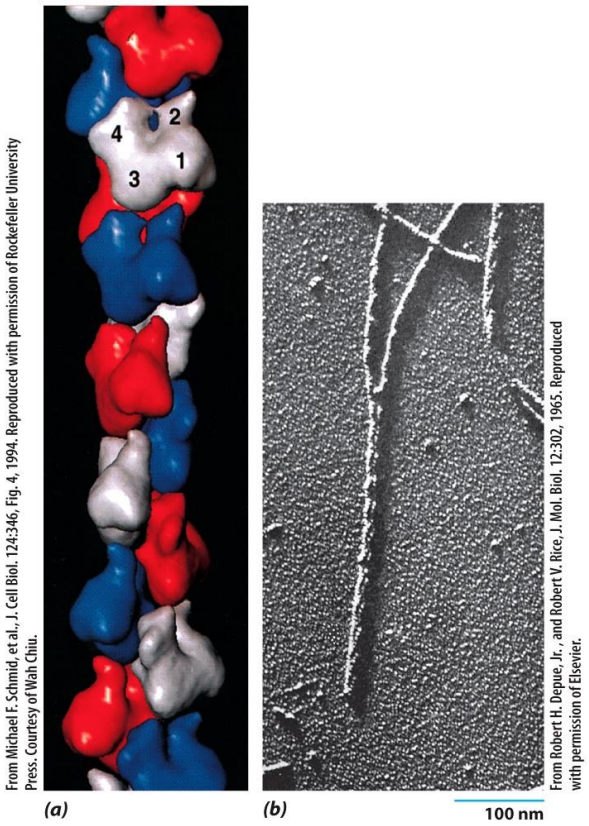
Actin Structure

Actin filaments are 8 nm in diameter and composed of globular subunits of actin, which is the most abundant protein in most cells.

In the presence of ATP, actin monomers polymerize to form a flexible, helical filament.

An actin filament (F-actin, microfilament) is a two-stranded structure with two helical grooves running along its length.

Depending on the type of cell and the activity in which it is engaged, actin filaments can be organized into ordered arrays, highly branched networks, or tightly anchored bundles.



Actin filament structure: model and EM showing its double-helical architecture

13.8 | Actin

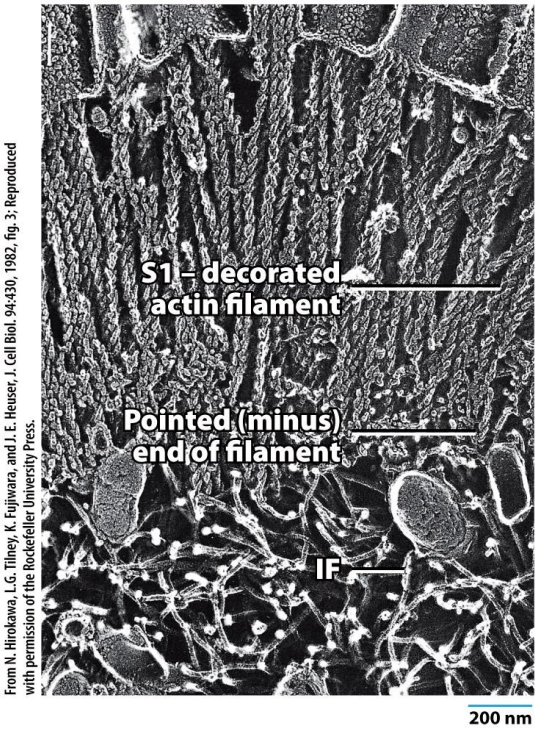
Actin Structure

All of the monomers within an actin filament are pointed in the same direction, resulting in a polar filament with so-called "barbed" and "pointed" ends.

Naming originated from a technique used to identify and label actin filaments in preparation for electron microscopy.

This method utilized the ability of a proteolytic fragment of myosin, called S1, to bind tightly and "decorate" the sides of actin filaments.

When S1 fragments are bound, one end of the actin filament appears pointed like an arrowhead (the "pointed" end), while the other end looks barbed.



From N. Hirokawa, L.G. Tilney, K. Fujiwara, and J. E. Heuser, J. Cell Biol. 94:430, 1982, fig. 3; Reproduced with permission of the Rockefeller University Press.

EM: Determining the location and polarity of actin filaments with the S1 subunit of myosin.

13.8 | Actin

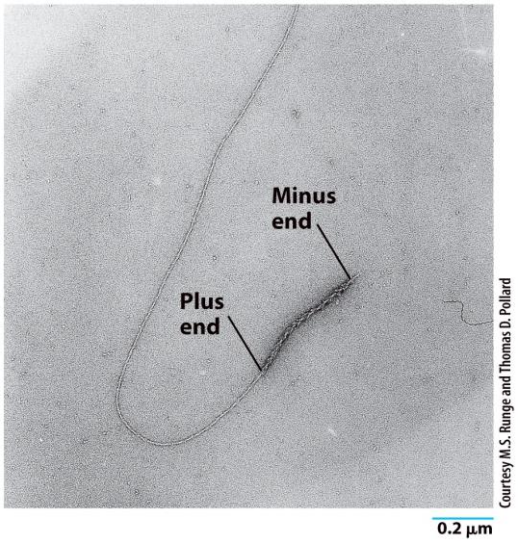
Actin Filament Assembly and Disassembly

Before it is incorporated into a filament, an actin monomer binds a molecule of ATP.

Actin is an ATPase, just as tubulin is a GTPase.

The ATP associated with the actin monomer is hydrolyzed to ADP at some time after it is incorporated into the end of a growing actin filament.

Both ends of a filament become labeled, but the fast-growing barbed end incorporates the monomers at a rate about 10 times that of the pointed end.



EM: short actin filament labeled with S1 myosin then used to nucleate actin polymerization. Addition occurs much more rapidly at the barbed (plus) end than at the pointed (minus)

13.8 | Actin

Actin Filament Assembly and Disassembly

Actin assembly/disassembly in vitro depend on the concentration of actin monomers and on the elongation dynamics of the filament ends.

The barbed and pointed ends require different minimal concentrations of ATP-actin monomers in order to elongate, a measure known as the critical concentration.

The critical concentration of the barbed end is much lower than the pointed end, meaning that the barbed end can continue to elongate at lower ATP-actin concentrations than the pointed end can.

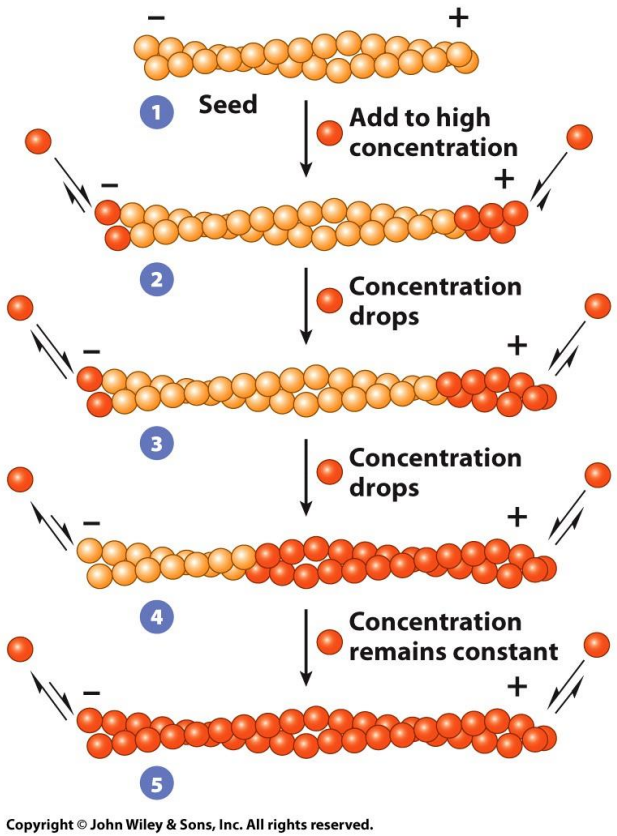


Diagram of the kinetics of actin-filament assembly in vitro to achieve treadmilling

13.8 | Actin

Actin Filament Assembly and Disassembly

As long as the concentration of ATP-actin monomers remains high, subunits will continue to be added at both ends of the filament.

As the concentration of free ATP-actin drops, a point is reached where net addition of monomers continues at the barbed end but stops at the pointed end.

As filament elongation continues, the free monomer concentration drops so that monomers continue to be added to the barbed ends of the filaments, but a net loss of subunits occurs at their pointed end.

A point is reached where the two reactions at opposite ends of the filaments are balanced so that both the lengths of the filaments and the concentration of free monomers remain constant, known as "**treadmilling**."

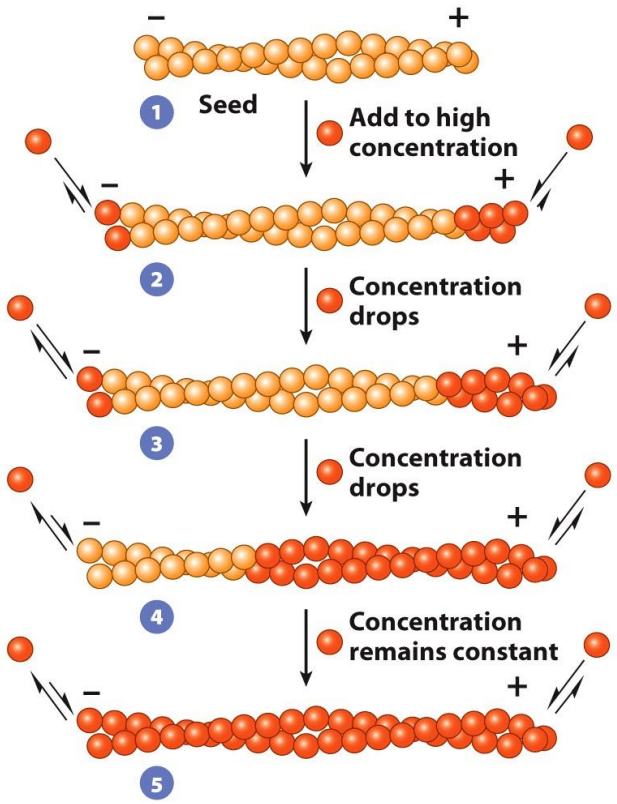


Diagram of the kinetics of actin-filament assembly in vitro to achieve treadmilling

13.8 | Actin

Actin Filament Assembly and Disassembly

The rate of assembly and disassembly of actin filaments in the cell can be influenced by a number of different accessory proteins.

By controlling this dynamic behavior, the cell can reorganize its actin cytoskeleton, required for dynamic processes such as cell locomotion, changes in cell shape, phagocytosis, and cytokinesis.

The involvement of these filaments is most readily demonstrated by treating the cells with one of the following drugs that disrupt dynamic actin-based activities:

Cytochalasin, derived from a mold, which blocks the barbed (+) ends of actin filaments and allows depolymerization at the pointed end;

Phalloidin, obtained from a poisonous mushroom, which binds to intact actin filaments and prevents their turnover;

Latrunculin, obtained from a sponge, which binds to free monomers and blocks their incorporation into the polymer.

13.9 | Myosin: The Molecular Motor of Actin

All of the motors known to operate in conjunction with actin filaments are members of the myosin superfamily.

Myosins, with the major exception of myosin VI, move toward the barbed end of an actin filament.

Myosin was first isolated from mammalian skeletal muscle tissue and has subsequently been found in virtually all eukaryotic cells.

All myosins share a characteristic motor (head) domain that contains a site that binds an actin filament and a site that binds and hydrolyzes ATP to drive the myosin motor.

Whereas the head domains of various myosins are similar, the tail domains are highly divergent.

Myosins are generally divided into two broad groups: the conventional (or type II) myosins and the unconventional myosins.

13.9 | Myosin: The Molecular Motor of Actin

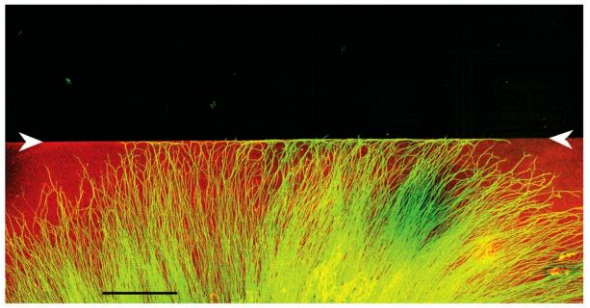
Conventional (Type II) Myosins

Proteins of the myosin II class are the primary motors for muscle contraction but are also found in a variety of nonmuscle cells.

The human genome encodes 16 different myosin II heavy chains, 3 of which function in nonmuscle cells.

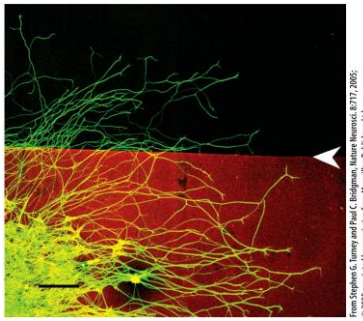
All myosin IIs move toward the barbed end of an actin filament.

Among their nonmuscle activities, type II myosins are required for splitting a cell in two during cell division, generating tension at focal adhesions and cell migration



From Stephen G. Turney and Paul C. Bridgman, Nature Neurosci. 8:717, 2005; © 2005, reprinted by permission from Macmillan Publishers, Ltd.

Fluorescence micrograph: neurites (green) growing out from mouse embryonic nervous tissue along a coverslip laminin-coated (red).



Tissue from a mouse embryo lacking myosin IIB.

13.9 | Myosin: The Molecular Motor of Actin

Conventional (Type II) Myosins

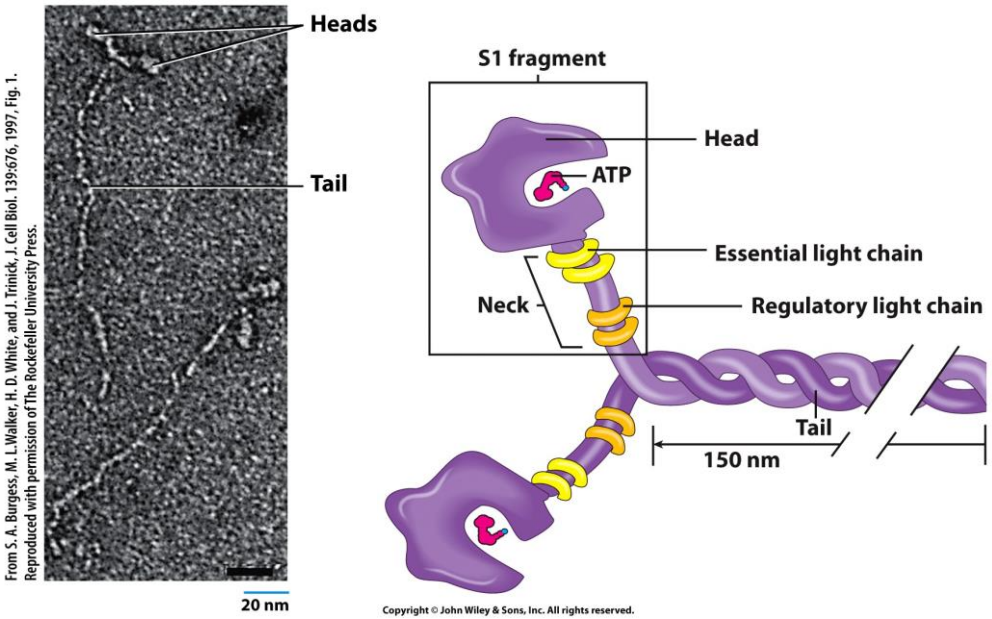
Each myosin II molecule is composed of six polypeptide chains, one pair of heavy chains and two pairs of light chains, organized in such a way as to produce a highly asymmetric protein.

Myosin II consists of

(1) a pair of globular heads that contain the catalytic site of the molecule;

(2) a pair of necks, each consisting of a single, uninterrupted α helix and two associated light chains;

(3) a single, long, rod-shaped tail formed by the intertwining of long α-helical sections of the two heavy chains.



Electron micrograph and schematic drawing of a myosin II molecule with one pair of heavy chains (blue) and two pairs of light chains

13.10 | Muscle Organization and Contraction

The Composition and Organization of Thick and Thin Filaments

Each thick filament is composed of several hundred myosin II molecules together with small amounts of other proteins.

The polarity of the thick filaments of muscle cells is reversed at the center of the sarcomere.

The center of the filament is composed of the opposing tail regions of the myosin molecules and is devoid of heads.

Myosin heads project from each thick filament along the remainder of its length due to the staggered position of the myosin proteins that make up the body of the filament.

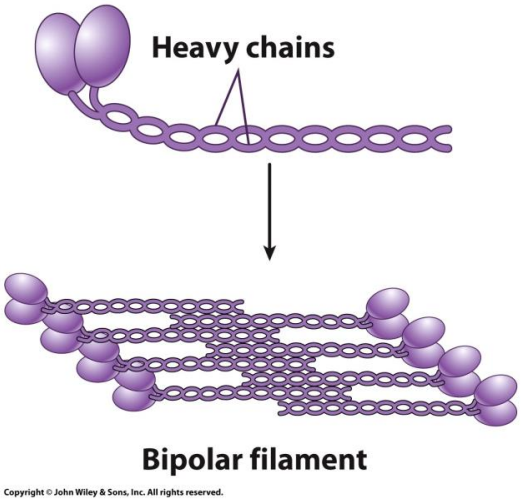


Diagram of the staggered arrangement of the individual myosin molecules in a myosin II filament and EM of a bipolar myosin filament formed in vitro

13.9 | Myosin: The Molecular Motor of Actin

Conventional (Type II) Myosins

The fibrous tail portion of myosin II plays a structural role so it can form filaments.

Myosin II molecules assemble so that the ends of the tails point toward the center of the filament and the globular heads point away from the center.

As a result, the filament is bipolar, indicating a reversal of polarity at the filament's center.

Because they are bipolar, the myosin heads at the opposite ends of a myosin filament have the ability to pull actin filaments toward one another.

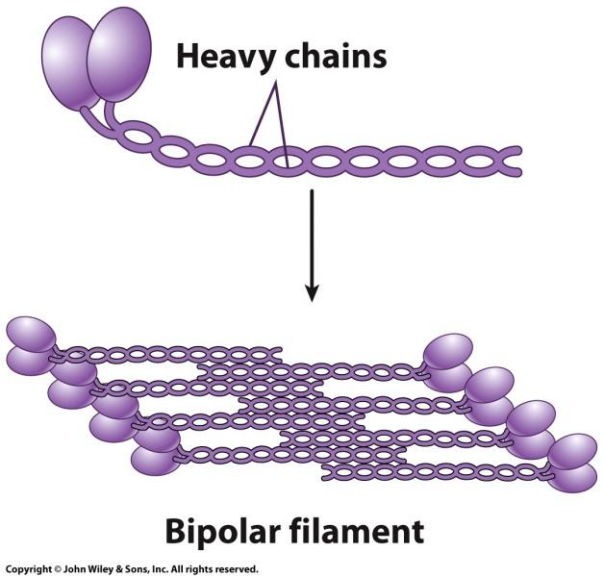
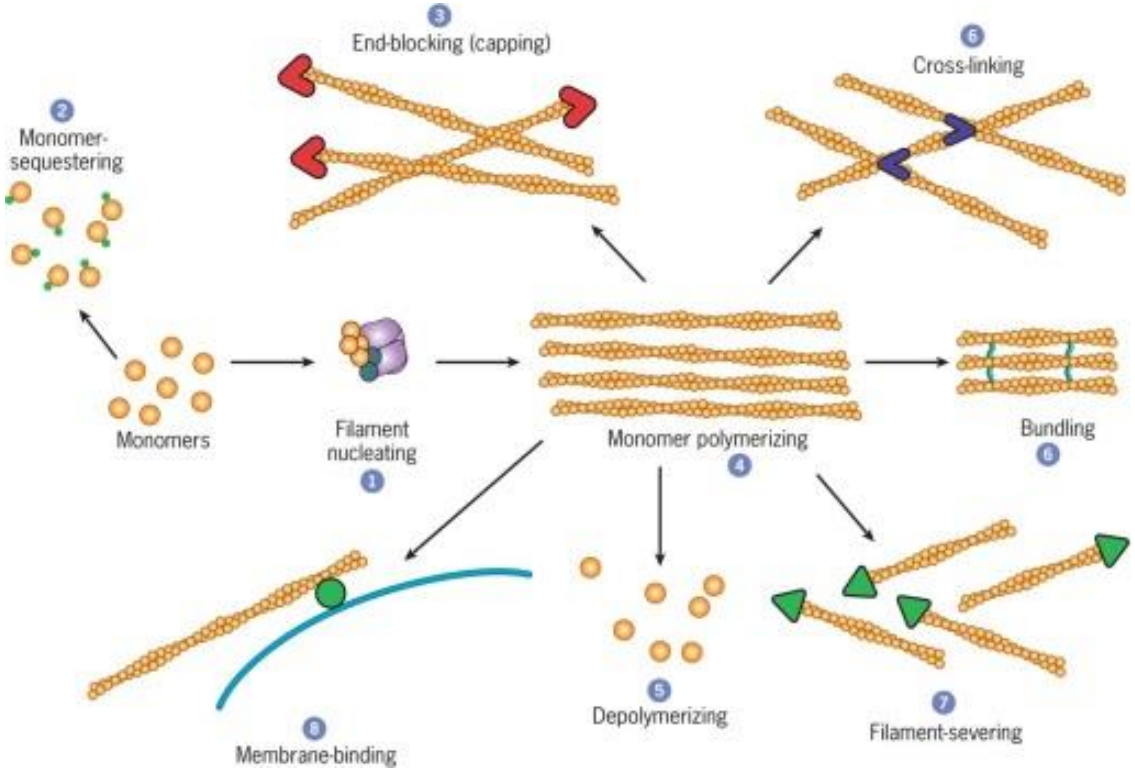


Diagram of the staggered arrangement of the individual myosin molecules in a myosin II filament and EM of a bipolar myosin filament formed in vitro

13.11 | Nonmuscle motility

1) **Nucleating proteins** – provide a template for adding actin monomers. Examples: *Arp2/3 complex; formin* branched versus unbranched filaments
2) **Monomer-sequestering proteins** – bind to actin-ATP monomers and prevent them from polymerizing. Example: *thymosin* b_4
3) **End-blocking (capping) proteins** – regulate the length of actin filaments Examples: *capZ; tropomodulin*).
4) **Monomer-polymerizing proteins** –promote the growth of actin filaments. Example: *profilin*



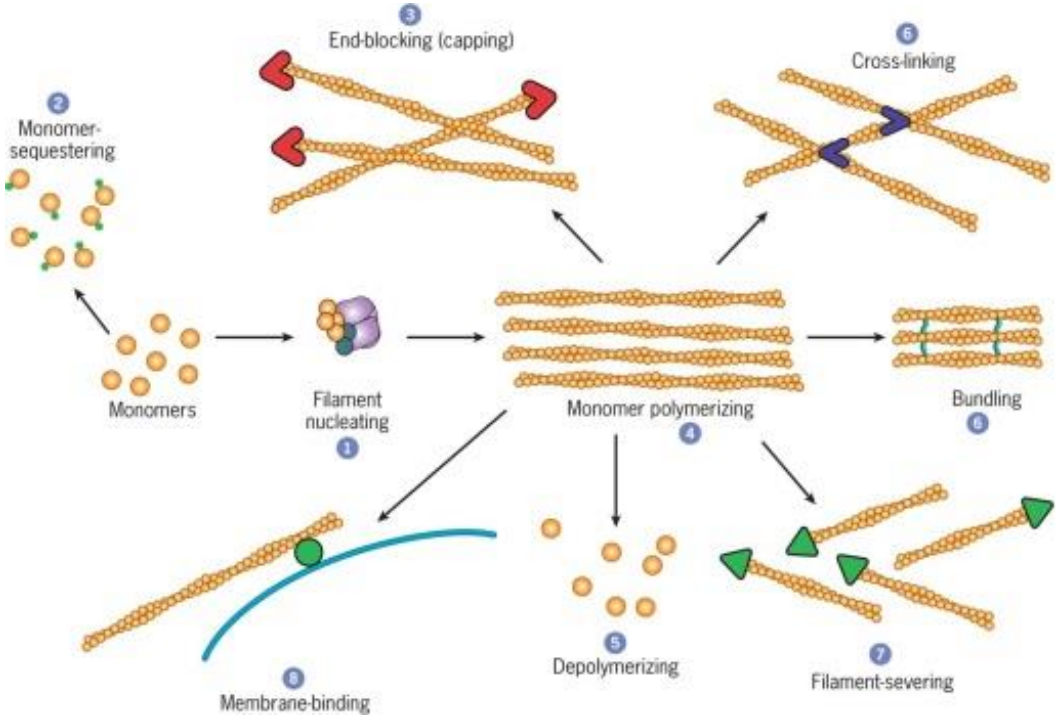
13.11 | Nonmuscle motility

5) Actin filament depolymerizing proteins – bind actin-ADP subunits for rapid turnover of actin filaments. Example: *cofilin*

6) Cross-linking proteins – alter the three-dimensional organization of actin filaments. Examples: *filamin (X-link), villin, fimbrin (bundling)*

7) Filament-severing proteins – shorten filaments and decrease cytoplasmic viscosity. Example: *gelsolin*

8) Membrane-binding proteins – link contractile proteins to plasma membrane. Examples: *vinculin, spectrin (dystrophin)*



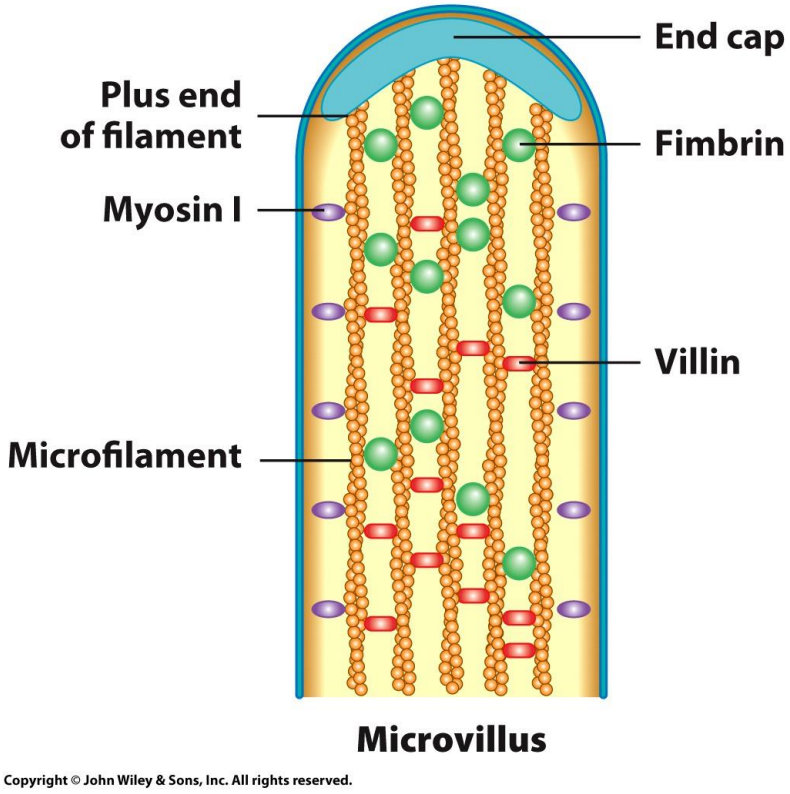
13.11 | Nonmuscle motility

Unconventional Myosins

Microvilli are present on the apical surface of epithelia that function in absorption of solutes, such as the lining of the intestine and wall of the kidney tubule.

Each microvillus contains about 25 actin filaments that are maintained in a highly ordered arrangement by the bundling proteins villin and fimbrin.

The role of myosin I, which is present between the plasma membrane of the microvillus and the peripheral actin filaments, remains unclear.



Actin filaments and actin-binding proteins in a microvillus.