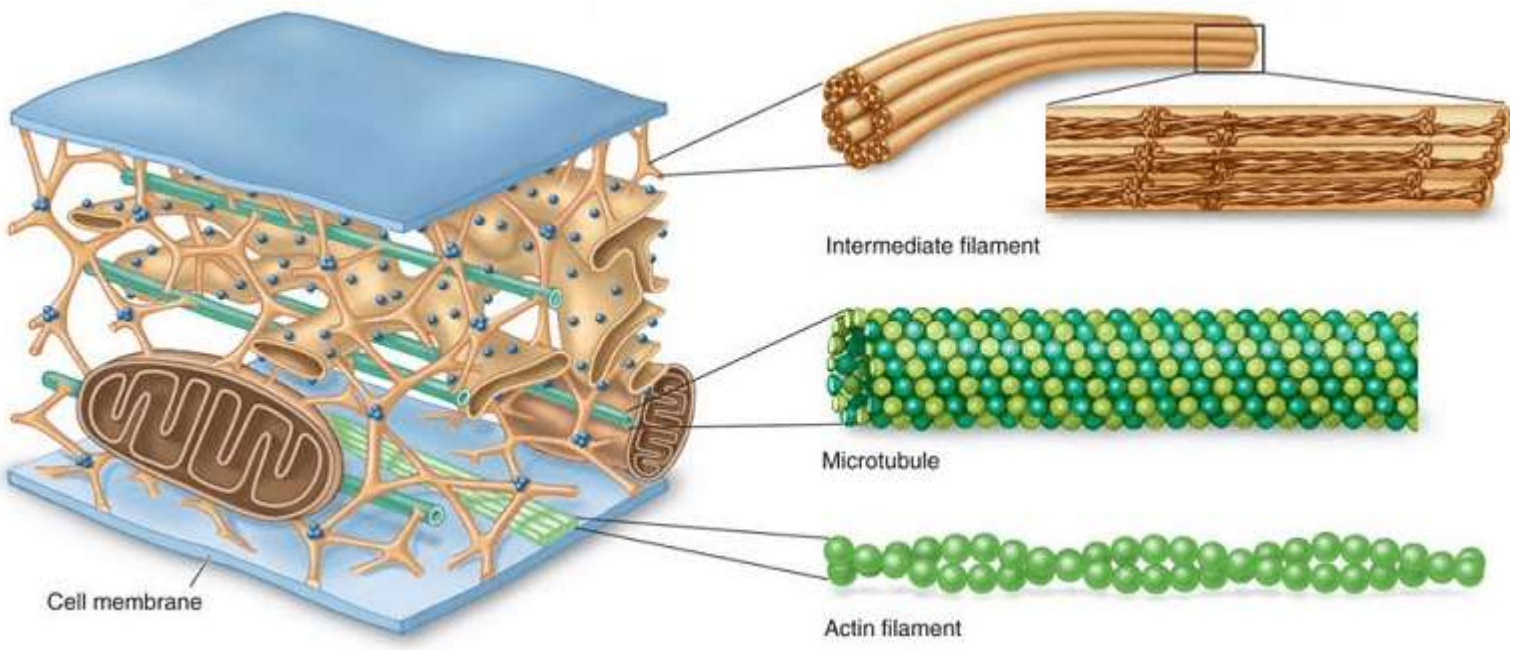


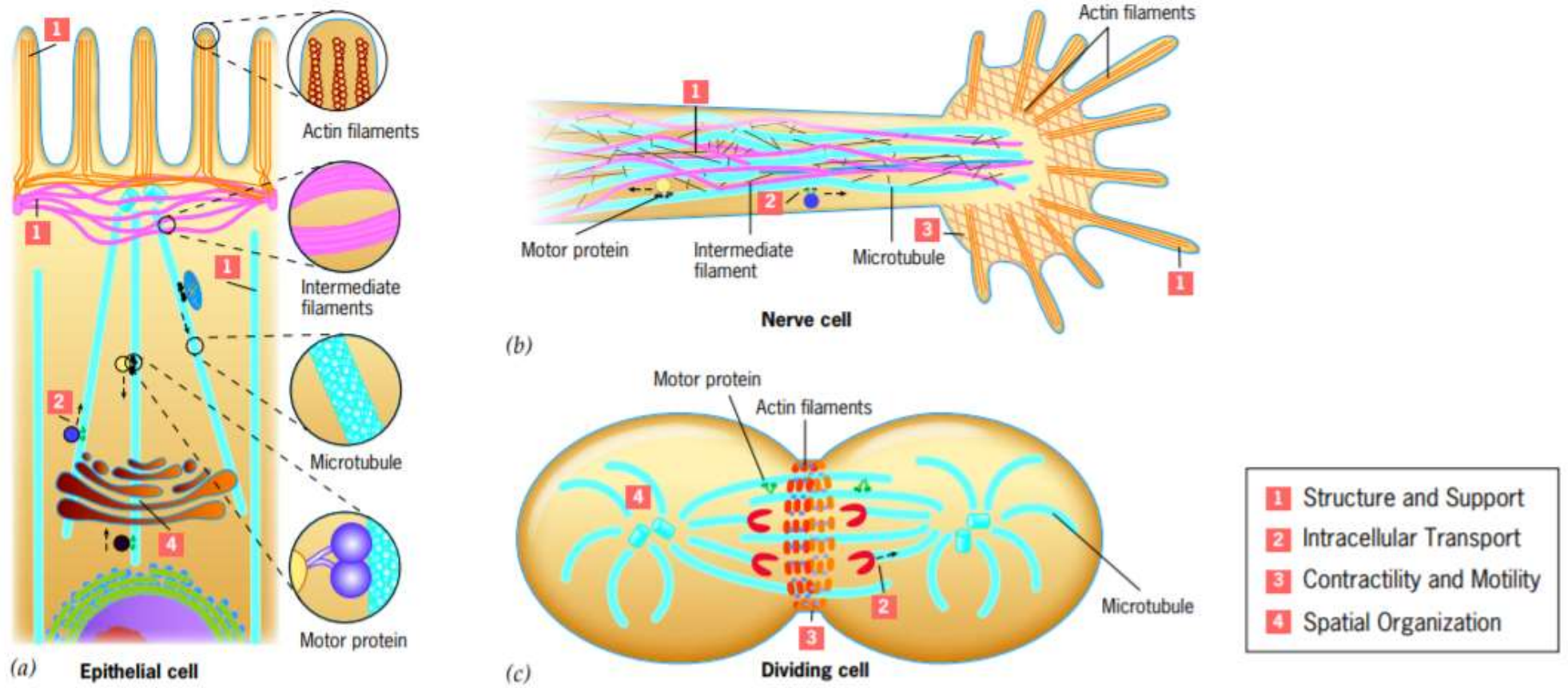

# Cytoskeleton

# Cytoskeleton

- Eukaryotic cells possess a “skeletal system”—a cytoskeleton
- The cytoskeleton is composed of three well defined filamentous structures—microtubules, microfilaments, and intermediate filaments

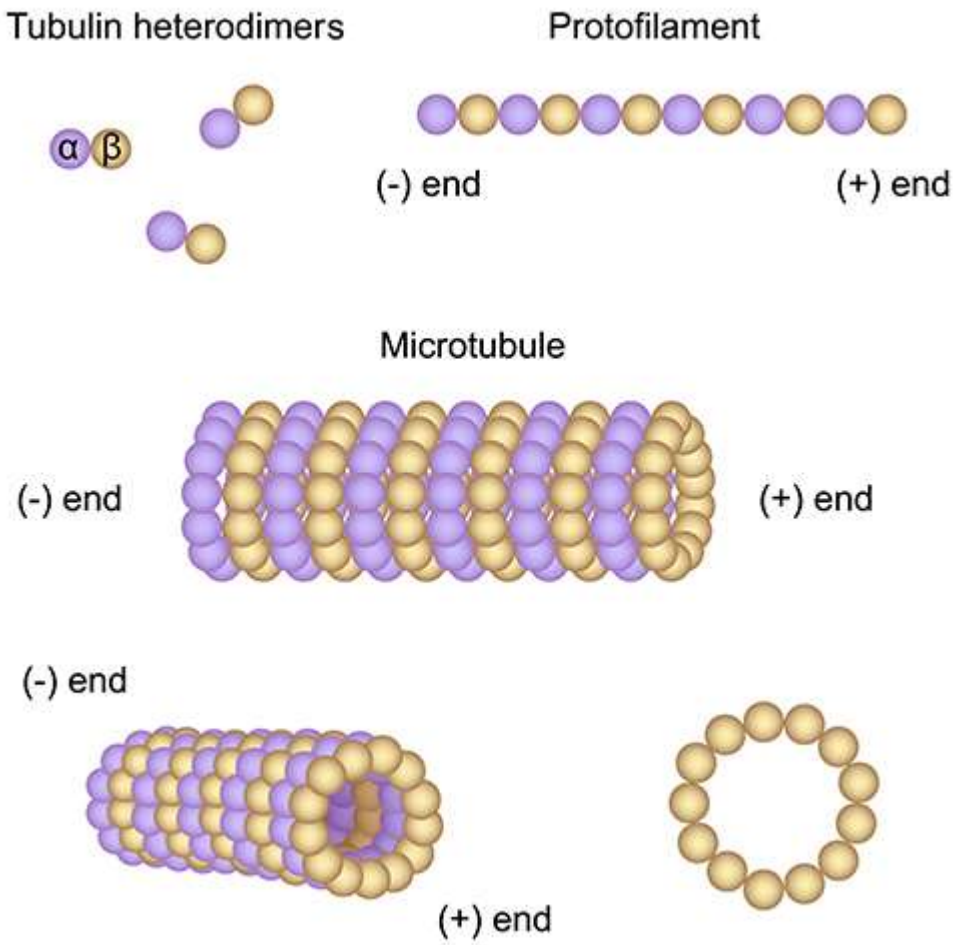
- **Microtubules** are long, hollow, unbranched tubes composed of subunits of the protein tubulin
- **Microfilaments** are solid, thinner structures, often organized into a branching network and composed of the protein actin
- **Intermediate filaments** are tough, ropelike fibers composed of a variety of related proteins.

# Overview of the Major Functions of the Cytoskeleton



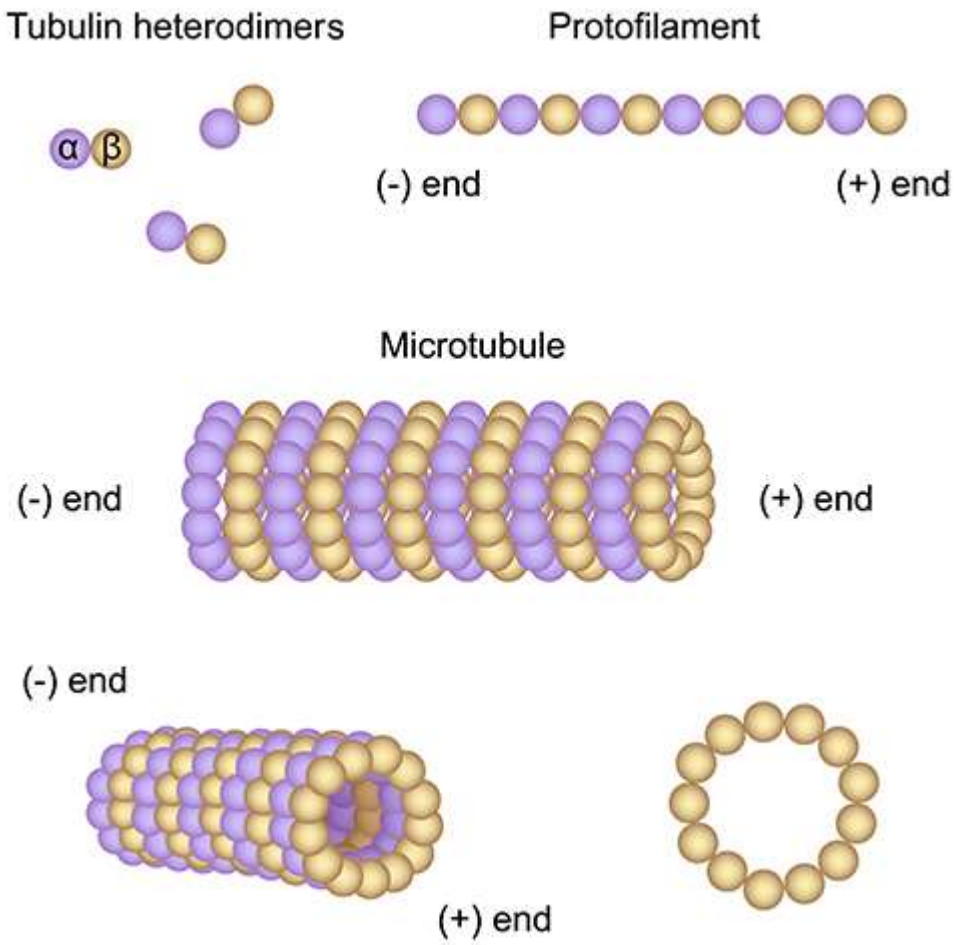
# Microtubules: Structure & Organization

- Microtubules are hollow, relatively rigid, tubular structures
- MTs have an outer diameter of 25 nm and a wall thickness of approximately 4 nm
- The wall of a MT is composed of globular proteins arranged in longitudinal rows, termed protofilaments
- Protofilaments are aligned parallel to the long axis of the tubule



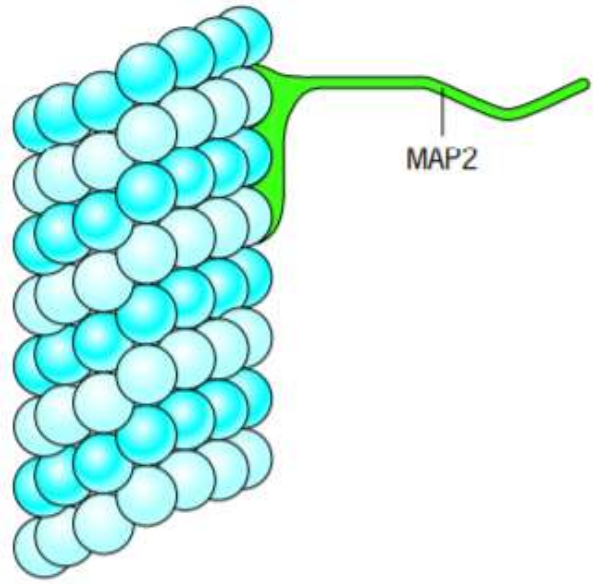
# Microtubules: Structure & Organization

- Each protofilament is assembled from dimeric building blocks consisting of one **α-tubulin** and one **β-tubulin** subunit
- Protofilament is asymmetric, with an α-tubulin at one end and a β-tubulin at the other end
- All of the protofilaments of a microtubule have the same polarity. Consequently, the entire polymer has polarity
- One end of a microtubule is known as the **plus** end and is terminated by a row of **β-tubulin** subunits
- The opposite end is the **minus** end and is terminated by a row of **α-tubulin** subunits



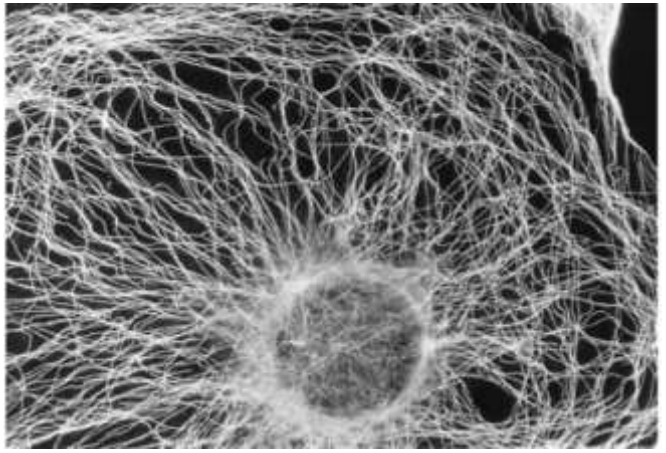
# Microtubule-associated proteins (MAPs)

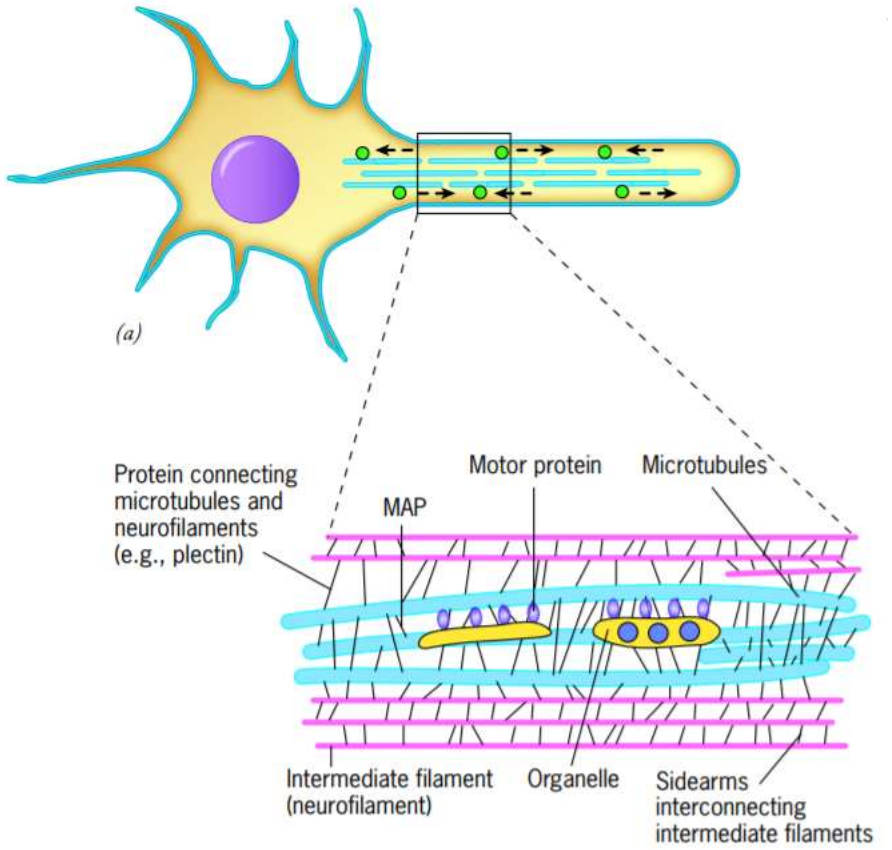
- MAPs comprise a heterogeneous collection of proteins
- The first MAPs to be identified are referred to as “classical MAPs”
- MAPs have one domain that attaches to the side of a MT and another domain that projects outward as a tail from the MT’s surface
- MAPs cross-bridges connecting microtubules to each other, thus maintaining their parallel alignment
- MAPs increase the stability of microtubules and promote their assembly
- The microtubule-binding activity of the various MAPs is controlled primarily by the addition and removal of phosphate groups from particular amino acid residues.

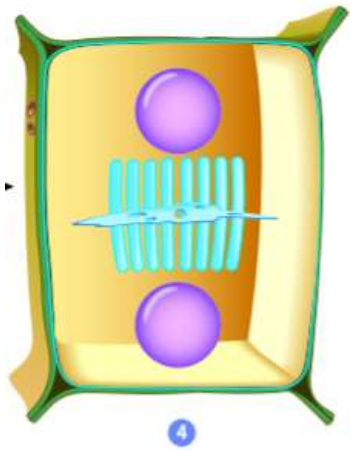


# Microtubules roles

- The distribution of cytoplasmic microtubules in a cell helps determine the shape of that cell
- Microtubules also play a key role in maintaining the internal organization of cells (localization of membranous organelles, including the ER and Golgi complex)
- Vesicles move along the microtubules of an axon, either toward or away from the cell body
- The formation of the cell wall that separates the two daughter plant cells

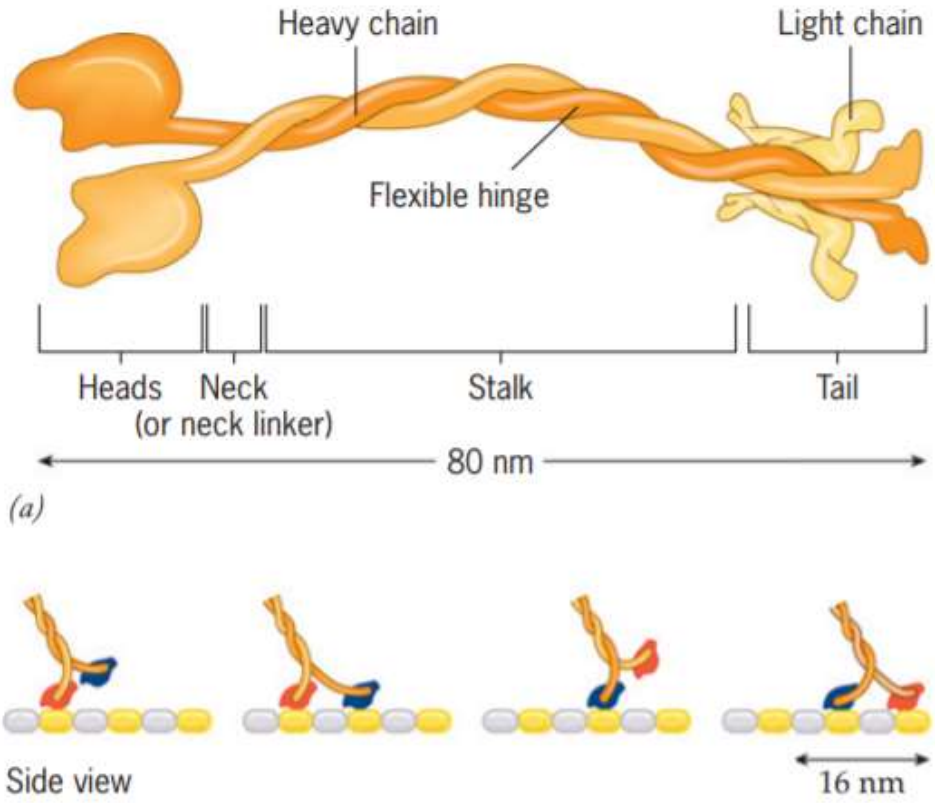




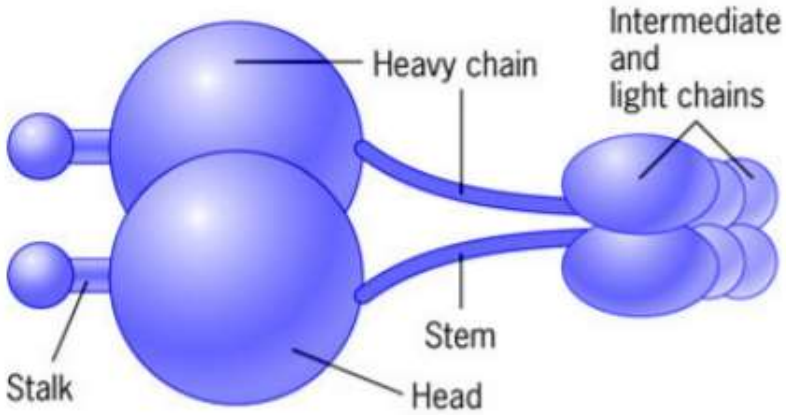
# Microtubule Motor protein: Kinesin

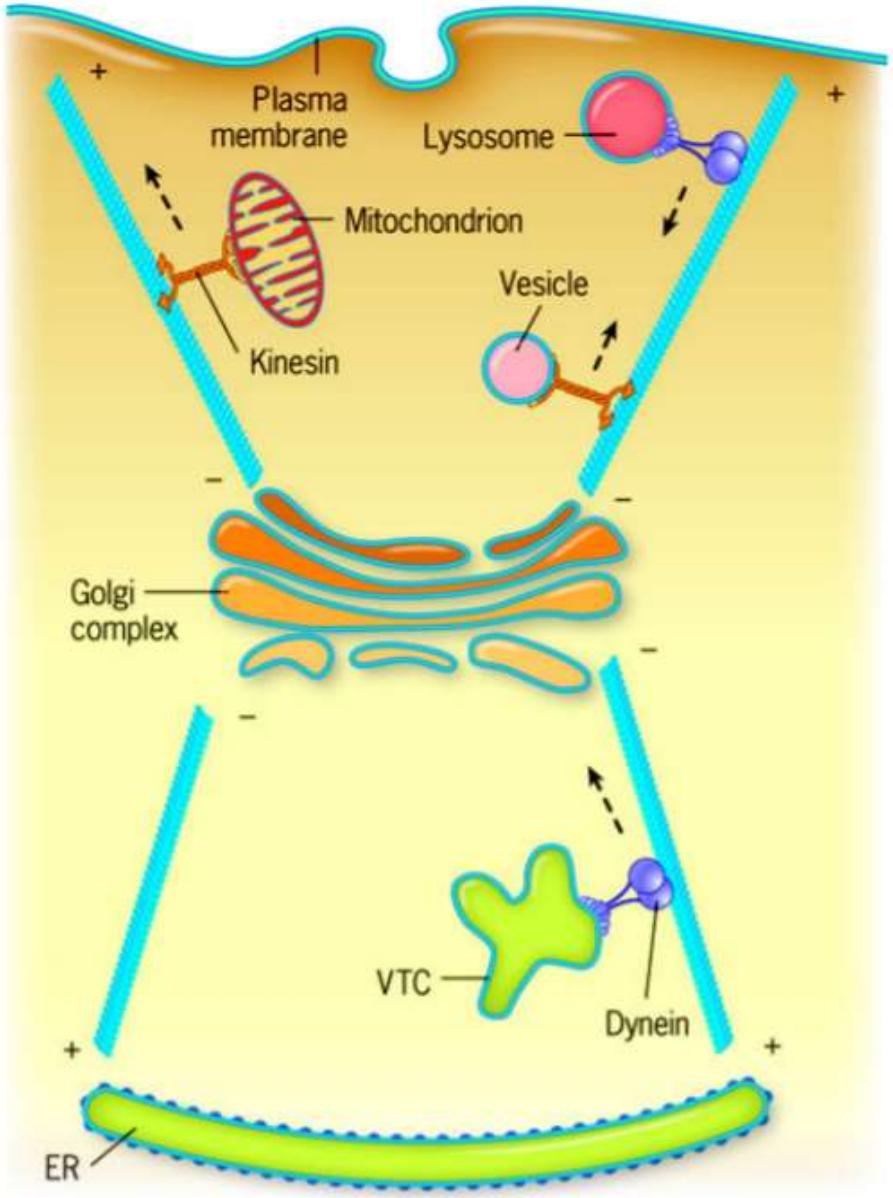
- Kinesin molecule is a tetramer constructed from two identical heavy chains and two identical light chains
- Kinesin molecule has several parts, including a pair of globular heads that bind a microtubule and act as ATP-hydrolyzing, force-generating “engines”
- Each head (or motor domain) is connected to a neck, a rodlike stalk, and a fan-shaped tail that binds cargo to be hauled
- Kinesin is a plus end-directed microtubular motor.

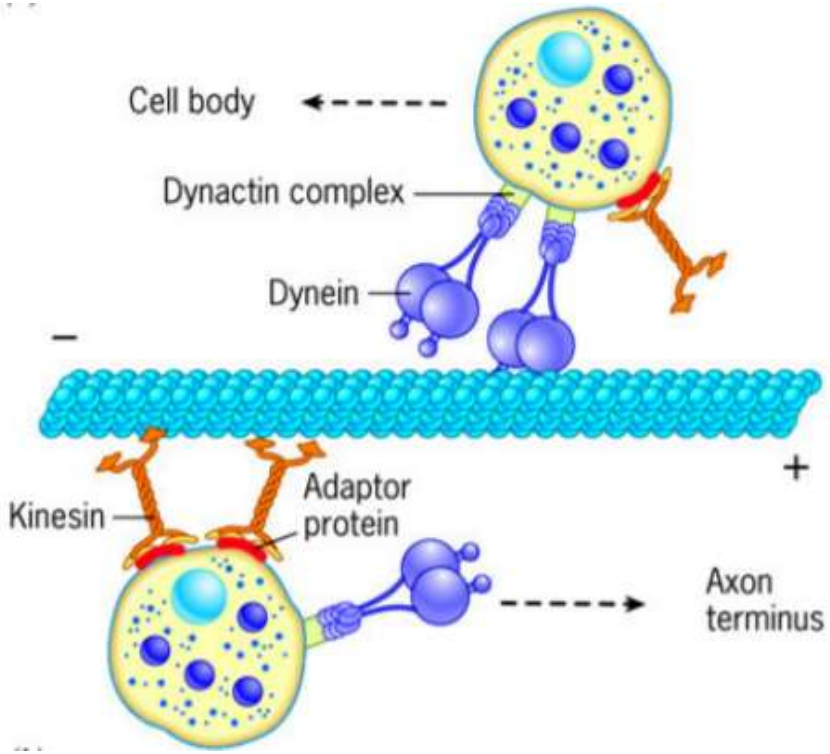


# Microtubule Motor protein: Dynein

- Dynein is a huge protein (molecular mass of approximately 1.5 million daltons) composed of two identical heavy chains and a variety of intermediate and light chains
- Each dynein heavy chain consists of a large globular head with an elongated projection (stalk)
- Dynein head, acts as a force-generating engine
- Each stalk contains the microtubule-binding site situated at its tip
- The longer projection, known as the stem (or tail), binds the intermediate and light chains
- Dynein is a minus end-directed microtubular motor.





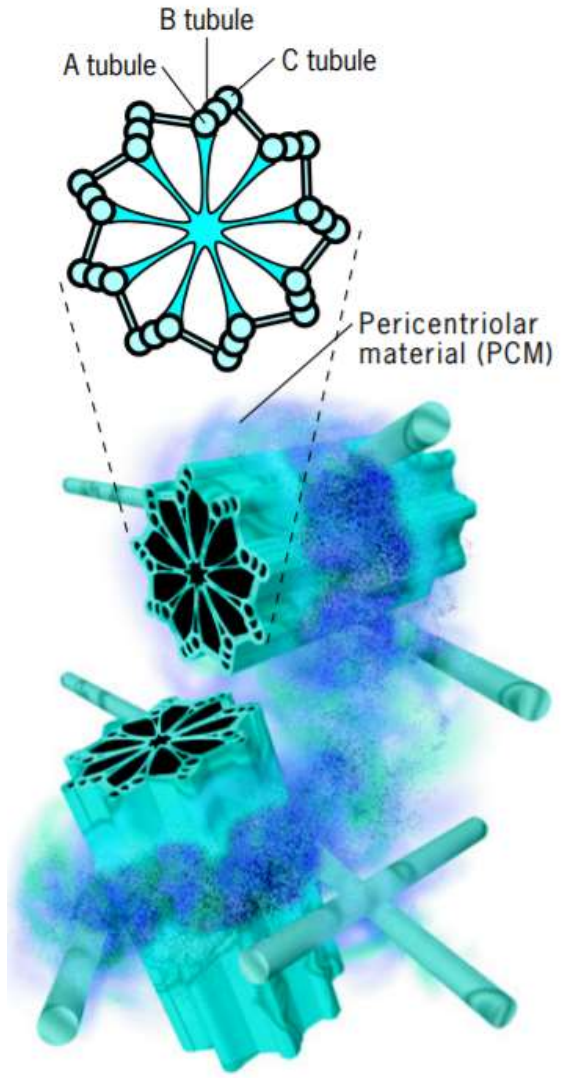




- **Cytoplasmic dynein** does not interact directly with membrane bounded cargo but requires an intervening adaptor—multisubunit protein **dynactin**

- **Kinesin** can be attached to vesicles by a variety of integral and peripheral membrane proteins

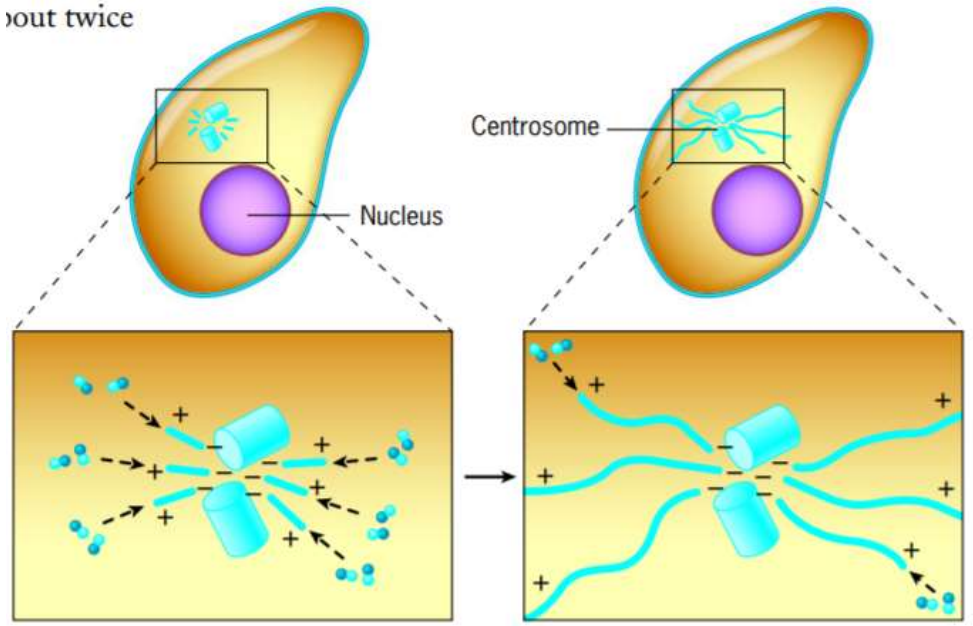
# Microtubule-organizing centers (MTOCs): Centrosome

- In animal cells, the microtubules of the cytoskeleton are typically nucleated by the centrosome, a complex structure that contains two barrel-shaped centrioles surrounded by amorphous, electron-dense pericentriolar material (or PCM)
- Centrioles contain nine evenly spaced fibrils, each of which contains three microtubules, designated the A, B, and C tubules
- Only the A tubule is a complete microtubule and it is connected to the center of the centriole by a radial spoke



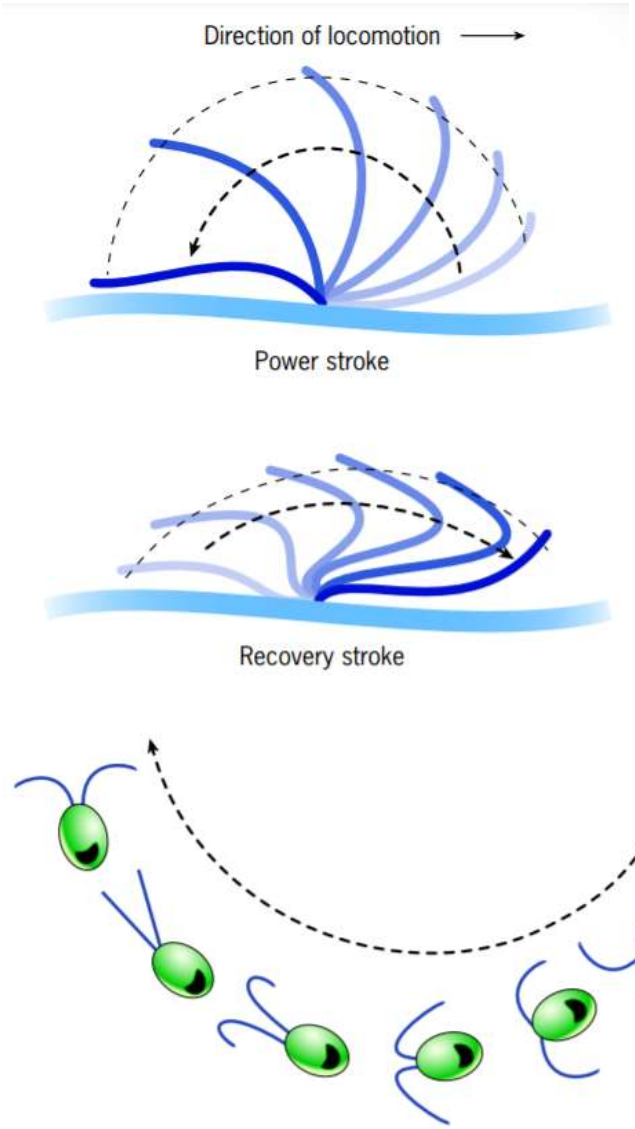
# Microtubule-organizing centers (MTOCs): Centrosome

- PCM initiates the formation of MTs
- Centrioles are not directly involved in microtubule nucleation
- The polarity of these microtubules is always the same: the minus end is associated with the centrosome, and the plus (or growing) end is situated at the opposite tip
- Thus, even though microtubules are nucleated at the MTOC, they are elongated at the opposite end of the polymer.

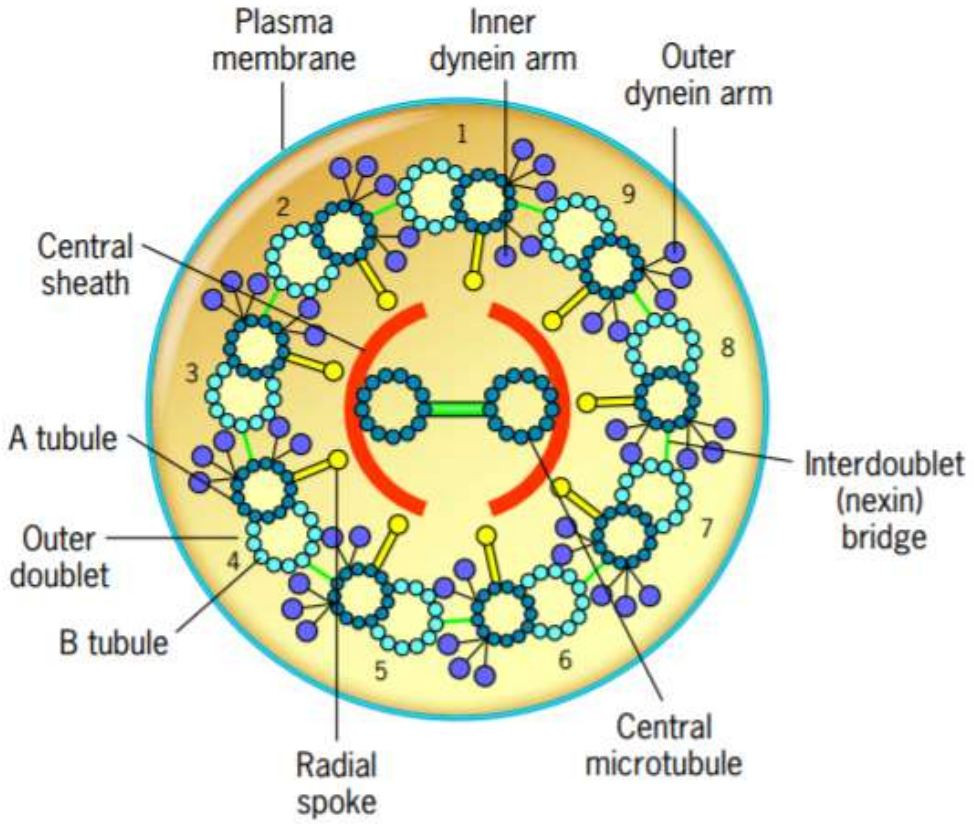


# Cilia and Flagella: Structure and Function

- Cilia tend to occur in large numbers on a cell's surface, and their beating activity is usually coordinated
- Flagella typically occur singly or in pairs and exhibit a variety of different beating patterns (waveforms), depending on the cell type
- In multicellular organisms, cilia move fluid and particulate material through various tracts
- In its power stroke, the cilium is maintained in a rigid state as it pushes against the surrounding medium
- In its recovery stroke, the cilium becomes flexible, offering little resistance to the medium

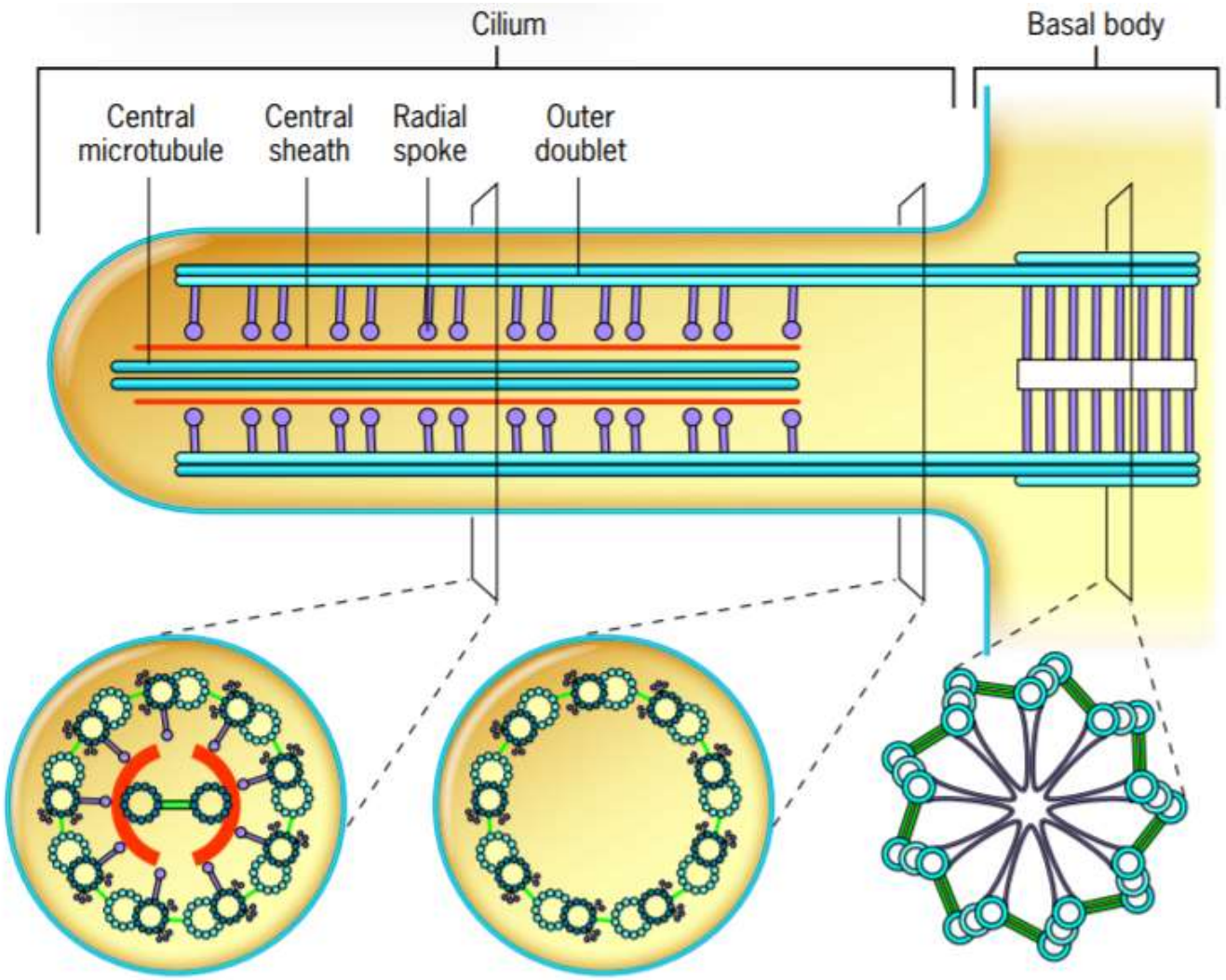


# Axoneme

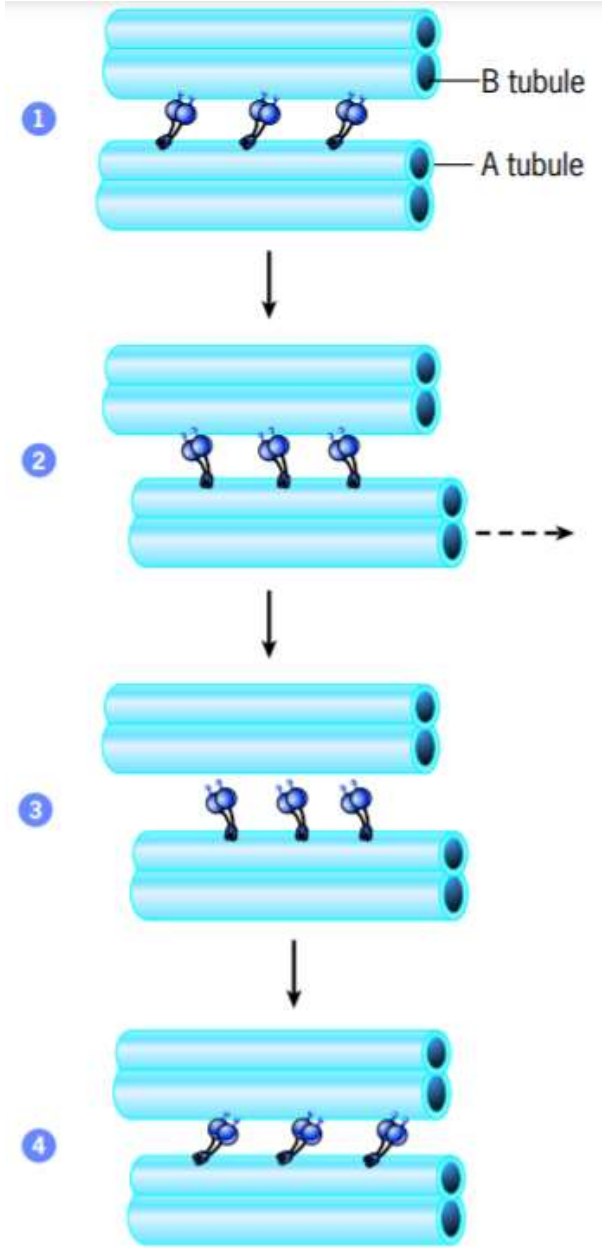



- Nine peripheral doublet microtubules surrounding a central pair of single microtubules (9+2)
- Peripheral doublets: A microtubule (complete) and B microtubule (incomplete)
- Central tubules are enclosed by the central sheath, which is connected to the A tubules of the peripheral doublets by a set of radial spokes
- The doublets are connected to one another by an inter-doublet bridge composed of an elastic protein, nexin
- Inner dynein arm and an outer dynein arm project from the A tubule
- All MTs of the axoneme have the same polarity: their plus ends are at the tip of the projection and their minus ends at the base

# Microtubule-organizing centers (MTOCs): Basal body




- Cilium or flagellum emerges from a **basal body**, similar in structure to the centriole
- The A and B tubules of the basal body elongate to form the doublets of the cilium or flagellum
- Dynein arms act as swinging cross-bridges that generate the forces required for ciliary or flagellar movement.
- The globular heads and stalks of dynein projecting from A tubule towards the B tubule of the neighboring doublet



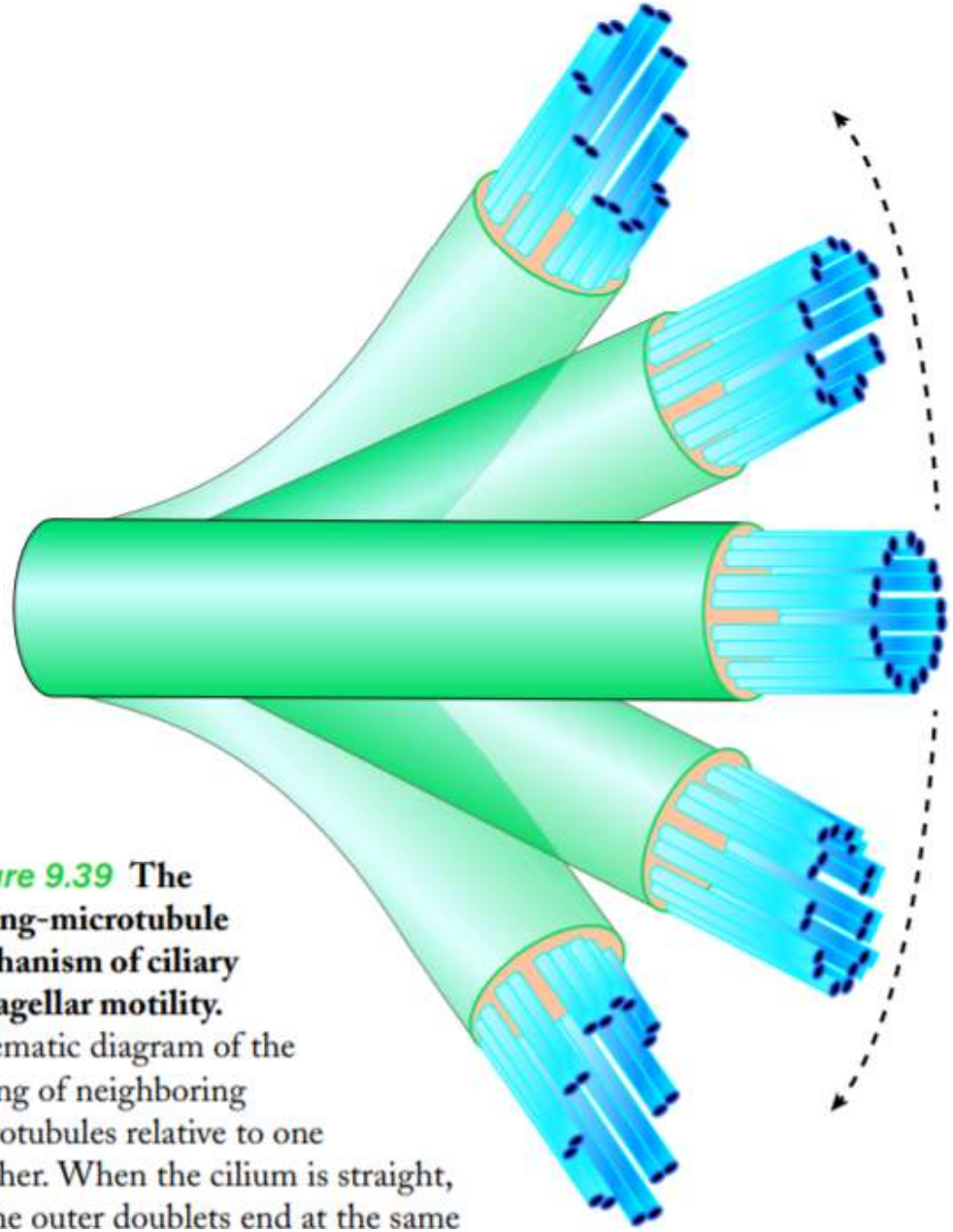
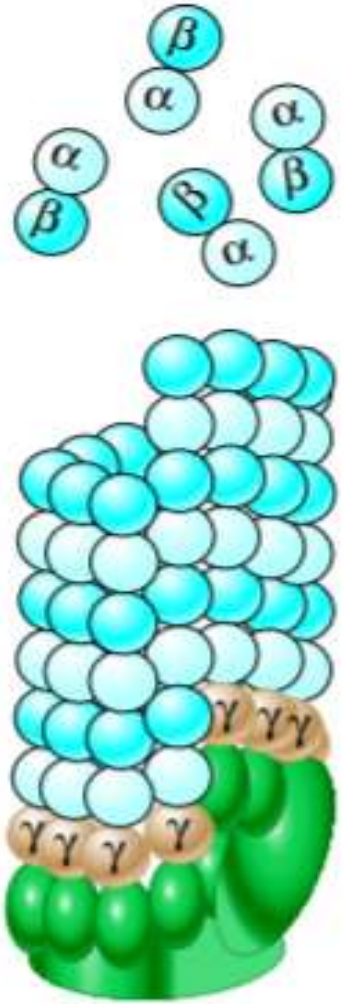



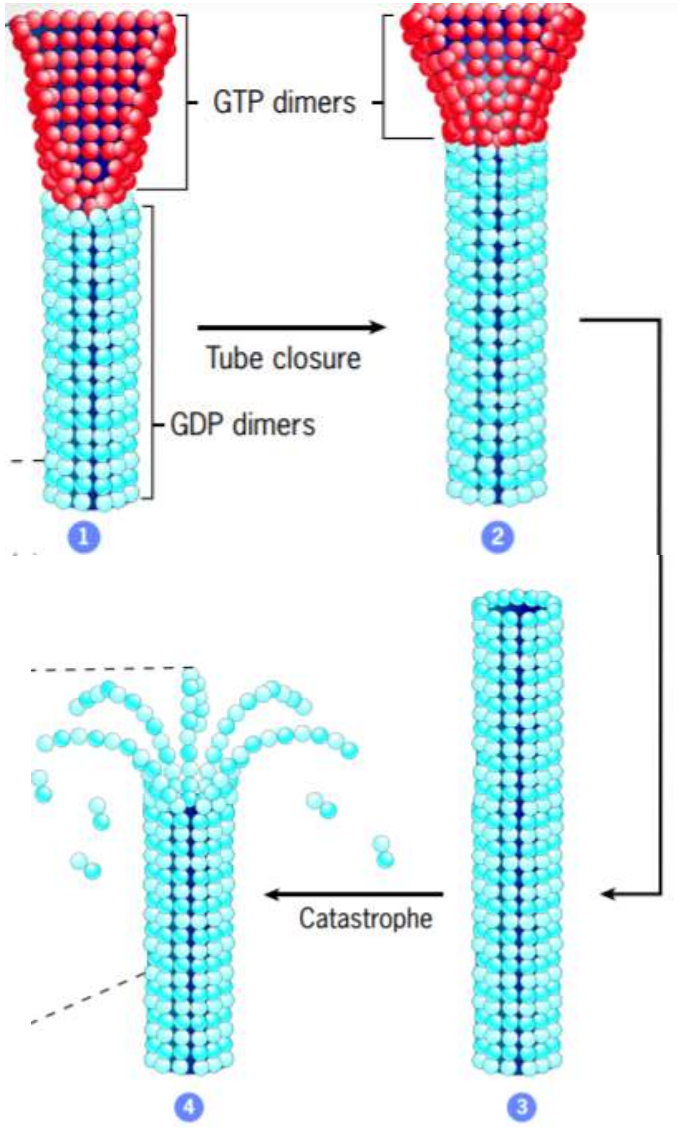
*Figure 9.39* **The sliding-microtubule mechanism of ciliary or flagellar motility.** Schematic diagram of the sliding of neighboring microtubules relative to one another. When the cilium is straight, all the outer doublets end at the same

# Microtubule Nucleation

- 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
- In addition, all MTOCs share a common protein component—a type of tubulin, called γ-tubulin
- γ -tubulin ring complexes have shown to nucleate microtubule assembly
- Only the α-tubulin of the heterodimer can bind to a ring of γ-subunits.
- γ -tubulin ring complexes determines the polarity of the entire microtubule and also forms a cap at its minus end, preventing the gain or loss of tubulin subunits.



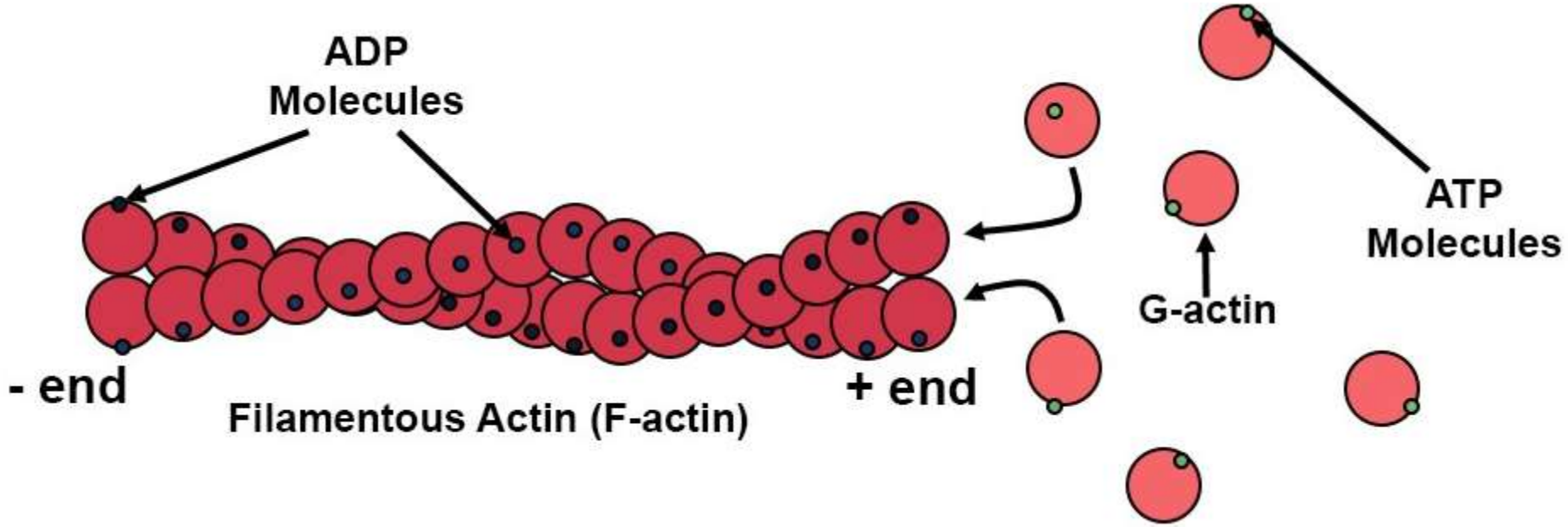
# Underlying Basis of Microtubule Dynamics




- **Step 1**: tip consists of an open sheet containing tubulin-GTP subunits
- **Step 2**: tube has begun to close, forcing the hydrolysis of the bound GTP
- **Step 3**: tube has closed to its end, leaving only tubulin-GDP subunits
- GDP-tubulin subunits have a curved conformation compared to their GTP-bound counterparts, which makes them less able to fit into a straight protofilament
- The strain resulting from the presence of GDP-tubulin subunits at the plus end of the microtubule is released as the protofilaments curl outward from the tubule and undergo catastrophic shrinkage (**Step 4**).

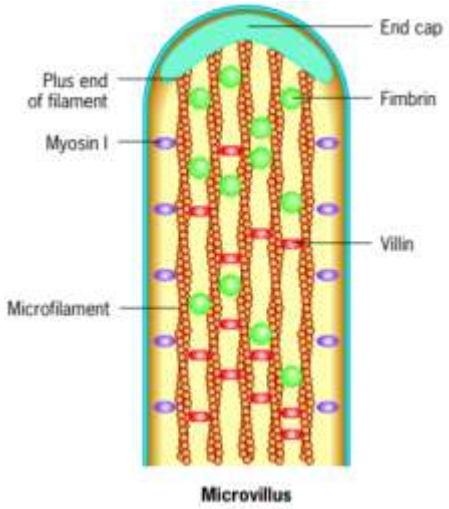
# Microfilaments

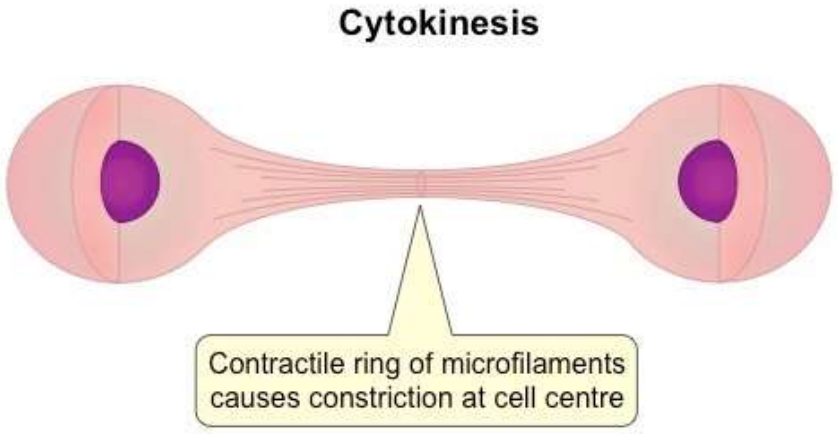
- Microfilaments are approximately 8 nm in diameter and composed of globular subunits of the protein actin
- In the presence of ATP, actin monomers polymerize to form a flexible, helical filament
- Actin filament is essentially a two-stranded structure with two helical grooves running along its length



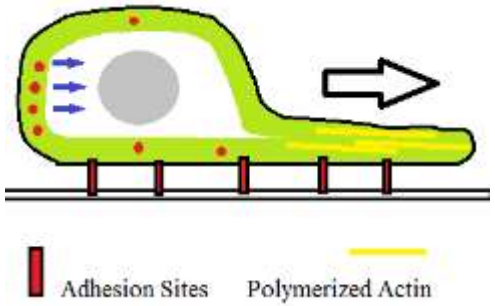
# Microfilament roles

- Bundles of microfilaments make up the core of microvilli of intestinal cells.
- Microfilaments are also involved in intracellular motile processes, such as the movement of vesicles, phagocytosis, and cytokinesis.
- Plant cells rely primarily on microfilaments, rather than microtubules, for the long-distance transport of cytoplasmic vesicles and organelles

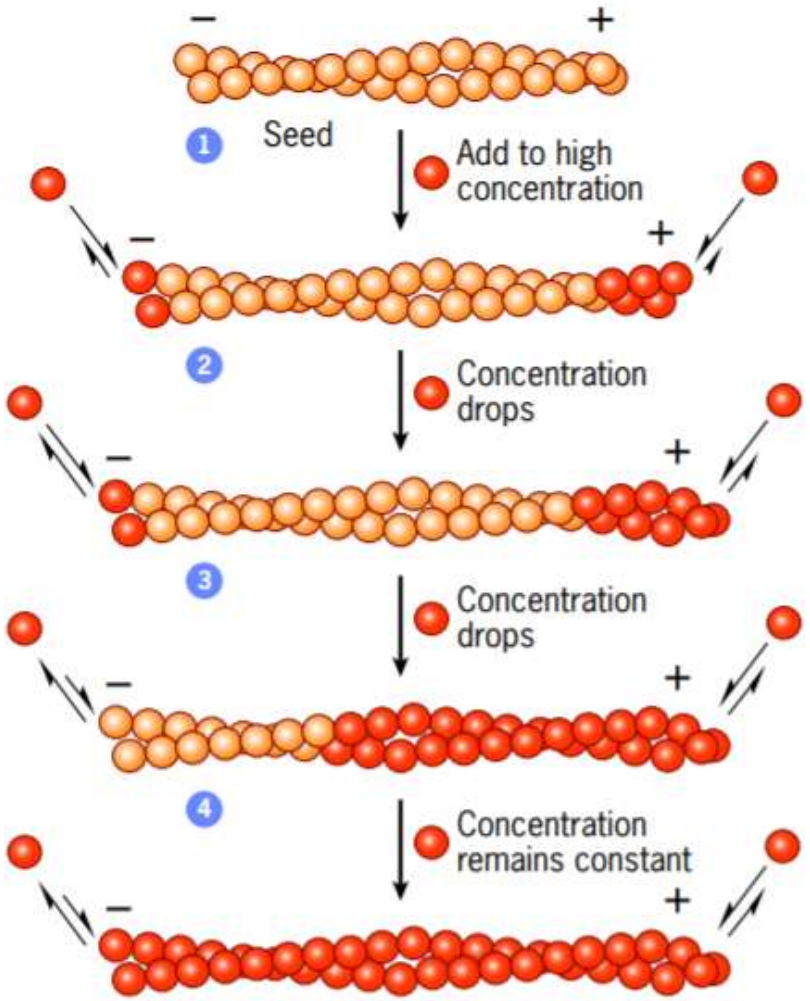



- to provide mechanical strength to the cell by forming a band under the plasma membrane
- link transmembrane proteins to cytoplasmic proteins
- form contractile ring during cytokinesis in animal cells
- cytoplasmic streaming
- generate locomotion in cells such as white blood cells and amoeba
- Interact with myosin to provide force of muscular contraction



# Microfilament Assembly and Disassembly




- **Step 1**: Preformed actin filaments (seeds) in the presence of ATP-actin monomers
- **Step 2**: Subunits continue to be added at both ends of the filament
- **Step 3**: Net addition of monomers continues at the plus (barbed) end, but stops at the minus (pointed) end
- **Step 4**: Two reactions at opposite ends of the filaments are balanced

# Myosin: Molecular Motor of Actin Filaments

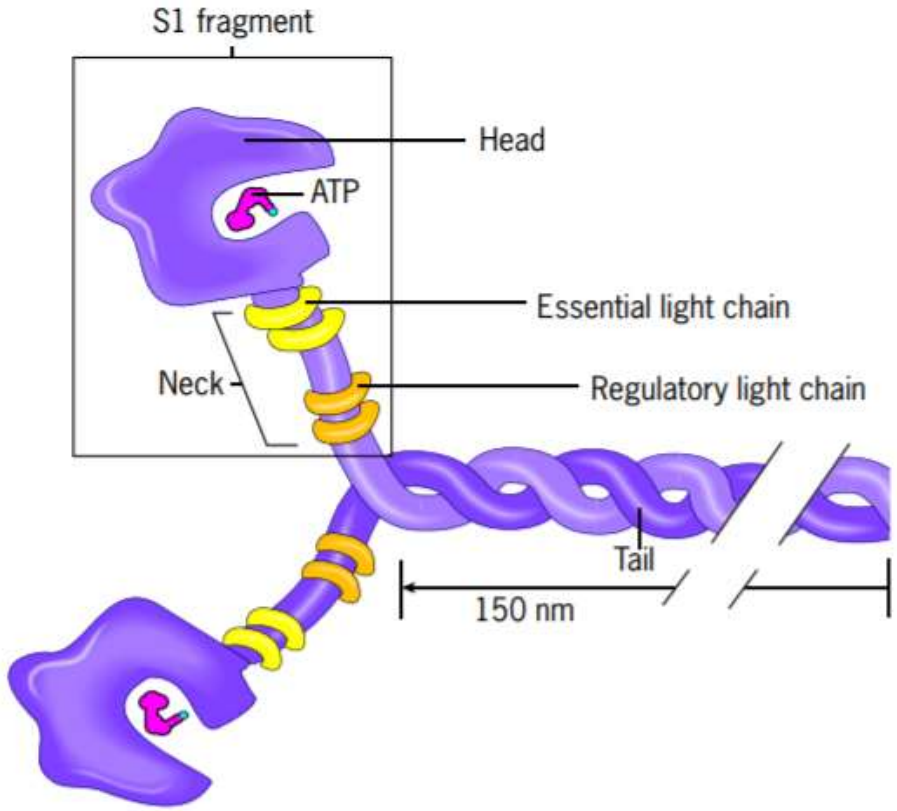
- Myosins are generally divided into two broad groups: the conventional (or type II) myosins and unconventional myosins
- The unconventional myosins are subdivided on the basis of amino acid sequence into at least 17 different classes (type I and types III–XVIII)
- Some of these classes are expressed widely among eukaryotes, whereas others are restricted
- Myosin X, for example, is found only in vertebrates
- Myosins VIII and XI are present only in plants
- Humans contain about 40 different myosins from at least 12 classes, each presumed to have its own specialized function(s)
- Myosins— with the major exception of myosin VI, move toward the plus end of an actin filament.

# 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 non-muscle cells
- The human genome encodes 16 different myosin II heavy chains, 3 of which function in non-muscle cells
- All myosin II's move toward the plus (barbed) end of an actin filament
- Among their non-muscle activities, type II myosins are required for splitting a cell in two during cell division, generating tension at focal adhesions, cell migration

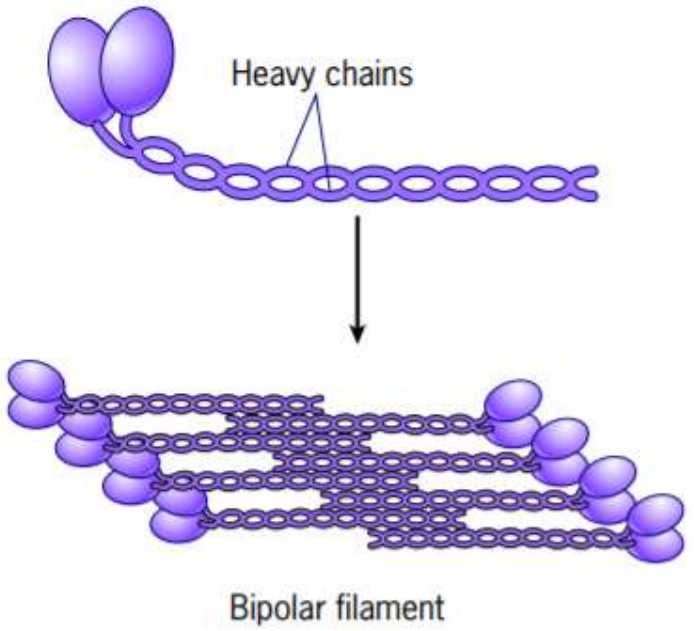
# Conventional (Type II) Myosins

- 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
- a pair of globular heads that contain the catalytic site of the molecule;
- a pair of necks, each consisting of a single, uninterrupted helix and two associated light chains
- a single, long, rod-shaped tail formed by the intertwining of long -helical sections of the two heavy chains



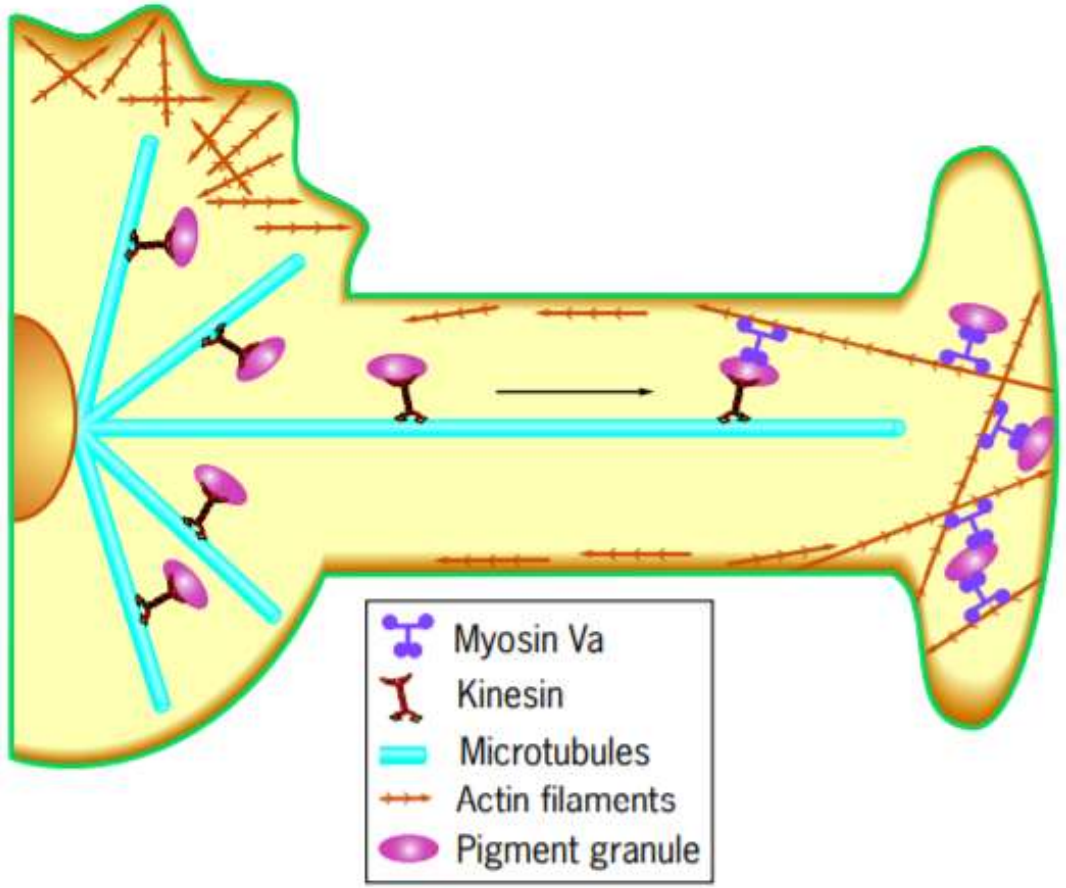
# Unconventional Myosins

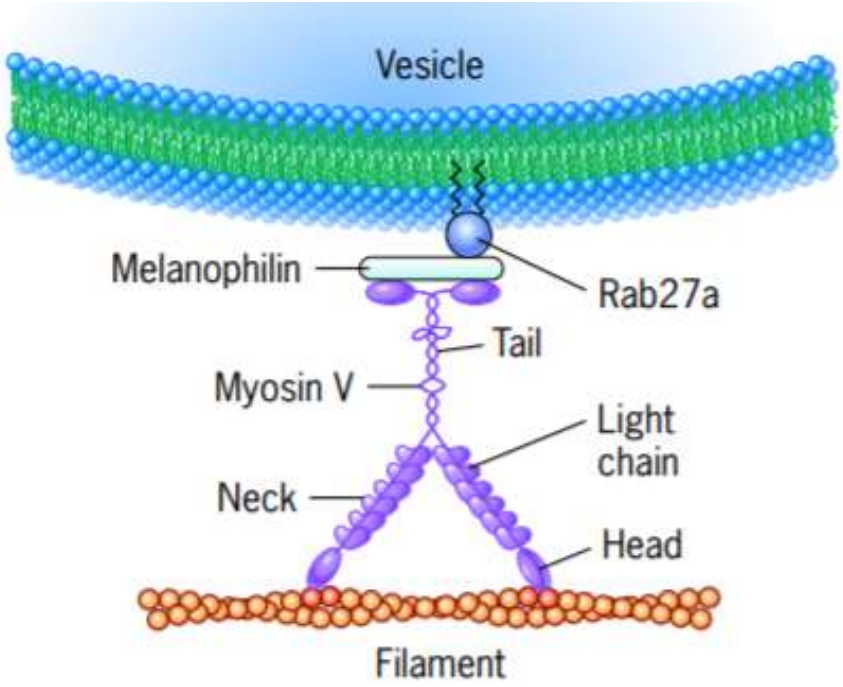
- Unconventional myosin have only a single head, thereby showing no assembly into filaments
- Myosin I often serves as a cross-link between actin filaments of the cytoskeleton and the lipid bilayer of the plasma membrane
- Several unconventional myosins (including myosin I, V, and VI) are associated with various types of cytoplasmic vesicles and organelles
- Mysoin VI, organelle transporter in the cytoplasm of many cells, shows movement towards the pointed (minus) end of an actin filament
- Myosin VI is located at the base of the stereocilia where it might connect the cytoskeleton to the overlying plasma membrane




**Myosin II (Conventional myosin)**

- In other types of cells, myosin VI is thought to be involved in the formation of clathrin-coated vesicles at the plasma membrane, the movement of uncoated vesicles to early endosomes, and the maintenance of Golgi morphology.

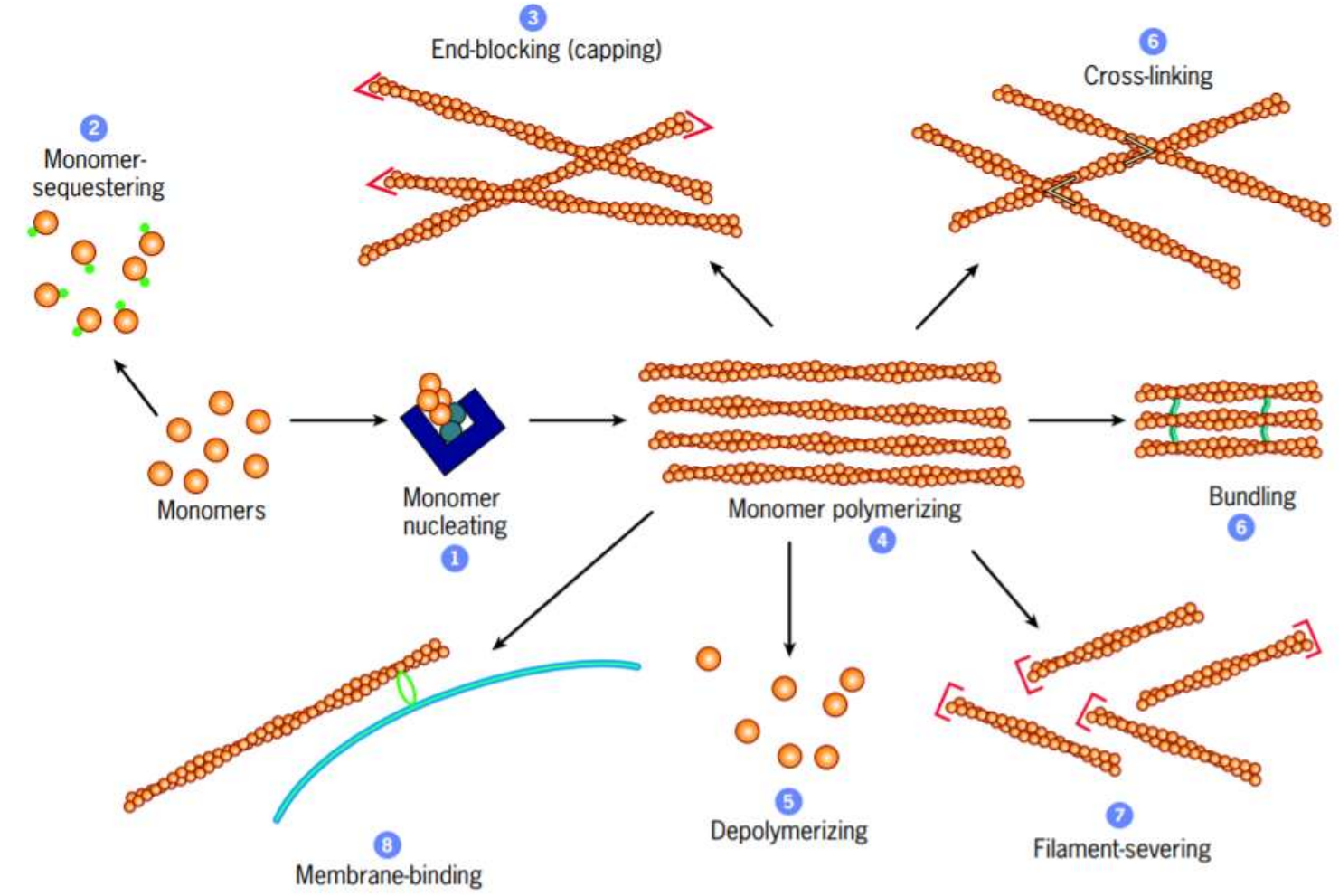



**Myosin V**

- Cooperation between microtubules and microfilaments has been best studied in pigment cells
- In mammals, pigment granules (melanosomes) are transported into fine peripheral processes of the pigment cell by one of the myosin V isoforms called Va.

# Actin-binding proteins




The roles of actin-binding proteins.

1. Arp2/3 complex
2. Thymosins (e.g., thymosin 4)
3. Tropomodulin
4. Profilin
5. Cofilin, ADF, and Depactin
6. Filamin, Villin and Fimbrin
7. Gelsolin
8. Vinculin, members of the ERM family (ezrin, radixin, and moesin), and members of the spectrin family (including dystrophin).

# Intermediate Filaments

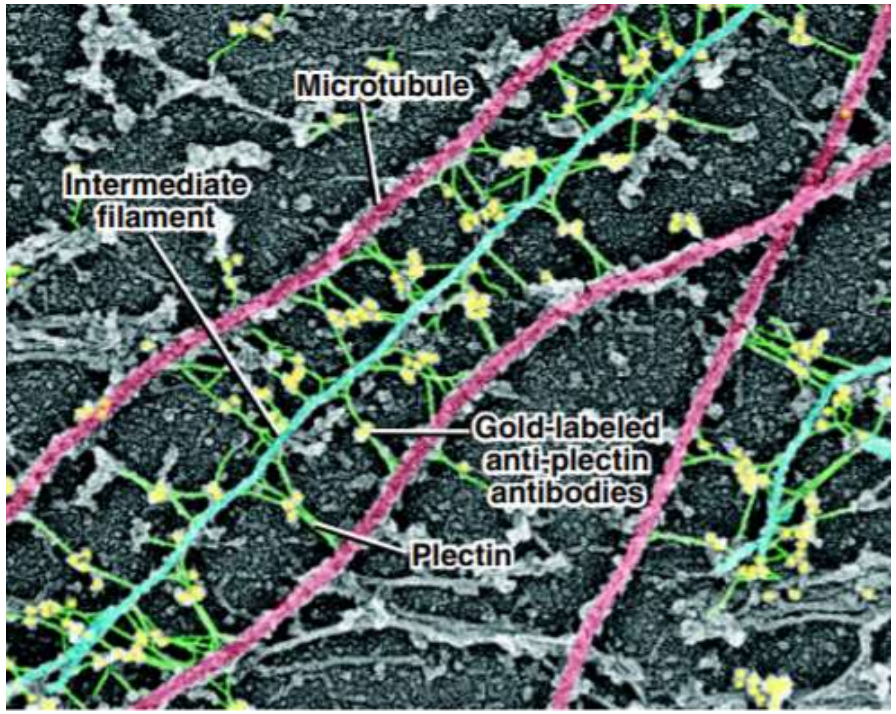
- Solid, unbranched filaments with a diameter of 10–12 nm
- Intermediate filaments are strong, flexible, ropelike fibers that provide mechanical strength
- Unlike MFs and MTs, IFs are a chemically heterogeneous group of structures that, in humans, are encoded by approximately 70 different genes
- The polypeptide subunits of IFs can be divided into five major classes based on the type of cell in which they are found.

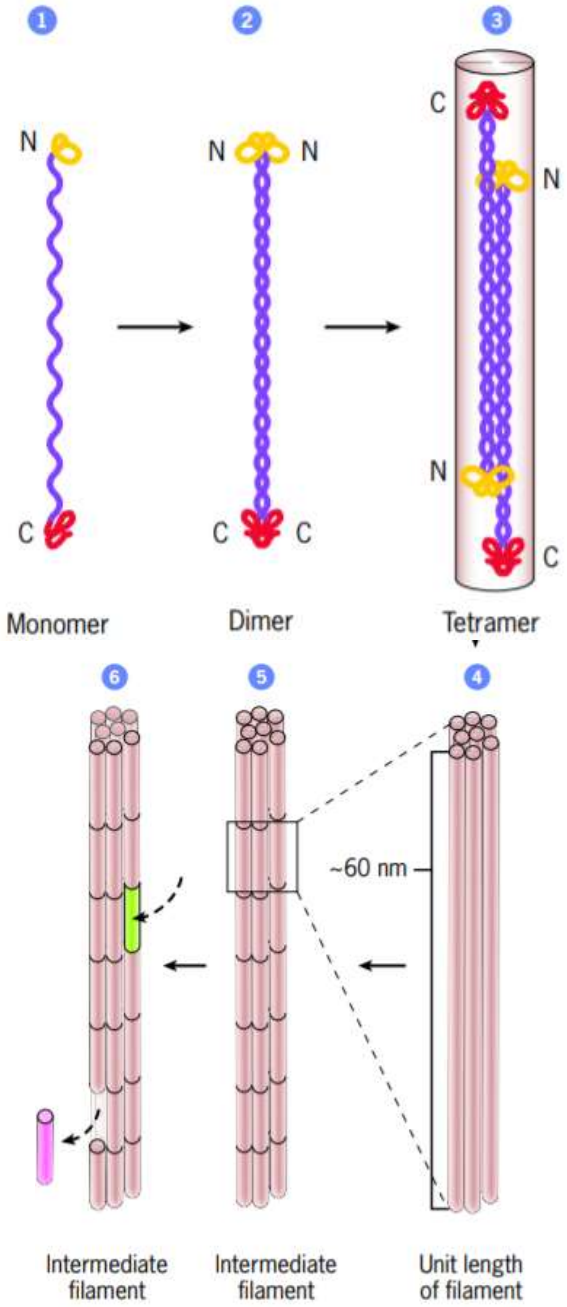
*Table 9.2* ***Properties and Distribution of the Major Mammalian Intermediate Filament Proteins***

| 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 | |

# Plectins

- IFs radiate through the cytoplasm of a wide variety of animal cells and are often interconnected to other cytoskeletal filaments by cross-bridges
- Cross-bridges consist of an elongated dimeric protein called plectin that can exist in numerous isoforms
- 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.






# IF Assembly and Disassembly

- The central fibrous domain is flanked on each side by globular domains of variable size and sequence **(step 1)**
- Two such polypeptides spontaneously interact as their α-helical rods wrap around each other to form a ropelike dimer approximately 45 nm in length **(step 2)**
- Two dimers that become aligned side by side in a staggered fashion with their N- and C-termini pointing in opposite (antiparallel) directions- Tetramer **(step 3)**
- 8 tetramers associate with one another in a side-by-side (lateral) arrangement to form a filament that is one unit in length (about 60 nm) **(step 4)**
- Unit lengths of filaments associate with one another in an end-to-end fashion to form the highly elongated intermediate filament **(step 5).**

# Types and Functions of Intermediate Filaments

*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 | |

## 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 |

## Table 16–2 Drugs That Affect Actin Filaments and Microtubules

| ACTIN-SPECIFIC DRUGS | |
|---|---|
| Phalloidin | binds and stabilizes filaments |
| Cytochalasin | caps filament plus ends |
| Swinholide | severs filaments |
| Latrunculin | binds subunits and prevents their polymerization |
| **MICROTUBULE-SPECIFIC DRUGS** | |
| Taxol | binds and stabilizes microtubules |
| Colchicine, colcemid | binds subunits and prevents their polymerization |
| Vinblastine, vincristine | binds subunits and prevents their polymerization |
| Nocodazole | binds subunits and prevents their polymerization |

# Question 1

Which of the following is a *false* statement?

a. Like microtubules and actin filaments, intermediate filaments form polarized structures and their function depends on this polarity.

b. A single epithelial cell can make a variety of keratins, all of which copolymerize into a single keratin filament system.

c. Keratins and vimentin-related proteins do not co-polymerize with each other: when they are expressed in the same cell, they form separate filament systems.

d. The most dramatic example of the importance of phosphorylation in the disassembly of intermediate filaments is provided by the nuclear lamins, which are phosphorylated and disassembled each time the cell enters mitosis.

# Question 2

All of the following cellular events involve actin filaments *except*

a. amoeboid movement.

b. cytokinesis in animal cells.

c. flagellar movement in bacteria.

d. contraction of smooth muscle.

# Question 3

Which of the following statements is *true* about intermediate filaments?

a. Rather than consisting of a single type of protein, they can be made up of a number of different proteins.

b. They are involved in cell movement.

c. The basic structure of an intermediate filament protein is a globular head and a long α-helical tail.

d. Like microfilaments, they exhibit treadmilling.

# Question 4

Treadmilling of actin filaments refers to

a. net assembly at both plus- and minus-ends.

b. net assembly at plus-end and net disassembly at minus-end.

c. net disassembly at plus-end and net assembly at minus-end.

d. net disassembly at both plus- and minus-end.

# Question 5

95.A cargo has to be delivered from the center of the cell to the cell periphery using the microtubule network. To which protein does it need to be associated with? 2018

- 1. Dynein
- 2. Kinesin
- 3. Microtubule associated protein 4
- 4. Myosin

# Question 6

76. An investigator expresses a GFP-fused protein that localizes to the outer membrane of Golgi apparatus. Upon visualising GFP-signal in the fluorescence microscope, it was noted that GFP is pericentrosomal in its localization (Fig A). Treatment of such GFP expressing cells with a newly identified drug disrupted the Golgi into small vesicles (Fig B).

Figure A

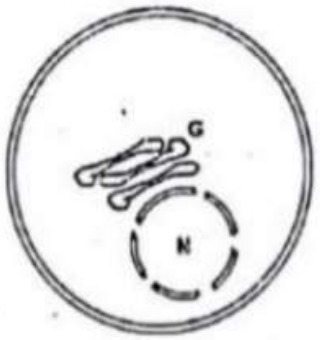
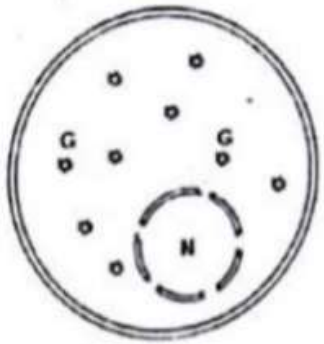



Figure B




Following is a list of potential targets of the drug:

A. Dyncin complex
B. Myosin
C. Microtubules
D. Dicer

Choose the combination with all correct targets:

1. A, B and D only
2. B and D only
3. A and C only
4. A only