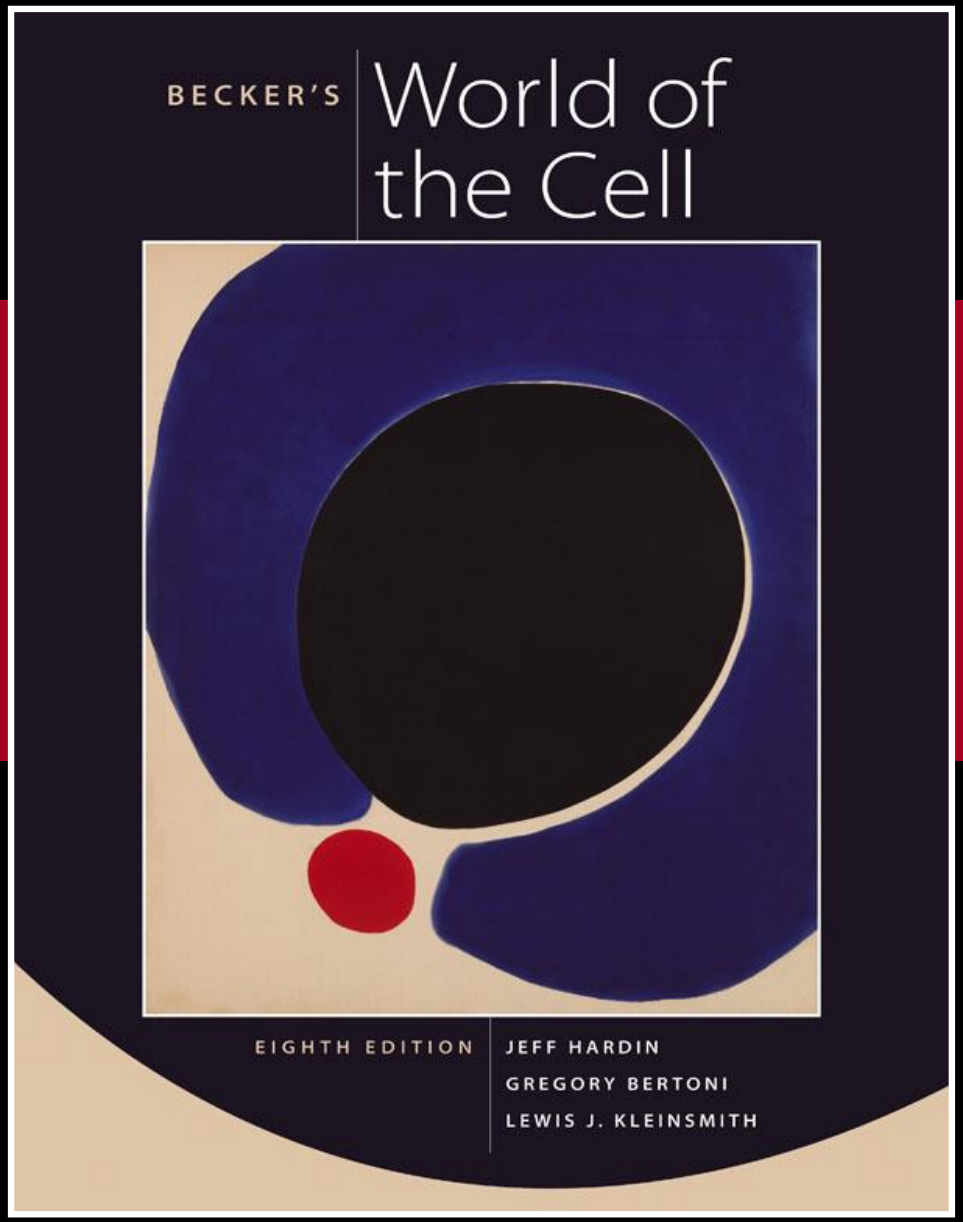


Chapter 14

Cellular Movement: Motility and Contractility

Lectures by
Kathleen Fitzpatrick
Simon Fraser University

Motile Systems

- Motility occurs at the tissue, cellular, and subcellular levels
- *Intracellular* components move, e.g., microtubules of the mitotic spindle play a role in the separation of chromosomes during cell division
- To generate movement, MTs and MFs provide a scaffold for **motor proteins** or **mechanoenzymes** that produce motion at the molecular level

Two eukaryotic motility systems

- 1. Interactions between motor proteins and microtubules
  - E.g., *fast axonal transport* in neurons, or the sliding of MTs in *cilia* and *flagella*
- 2. Interactions between actin and members of the *myosin* motor proteins
  - E.g., muscle contraction

Microtubule-Based Movement Inside cells: Kinesin and Dynein

- MTs provide a rigid set of tracks for transport of a variety of organelles and vesicles
- Traffic toward the minus ends of MTs is considered "inbound"; toward the plus end is "outbound"
- *Microtubule-associated motor proteins* walk along the MTs and provide the force needed for movement

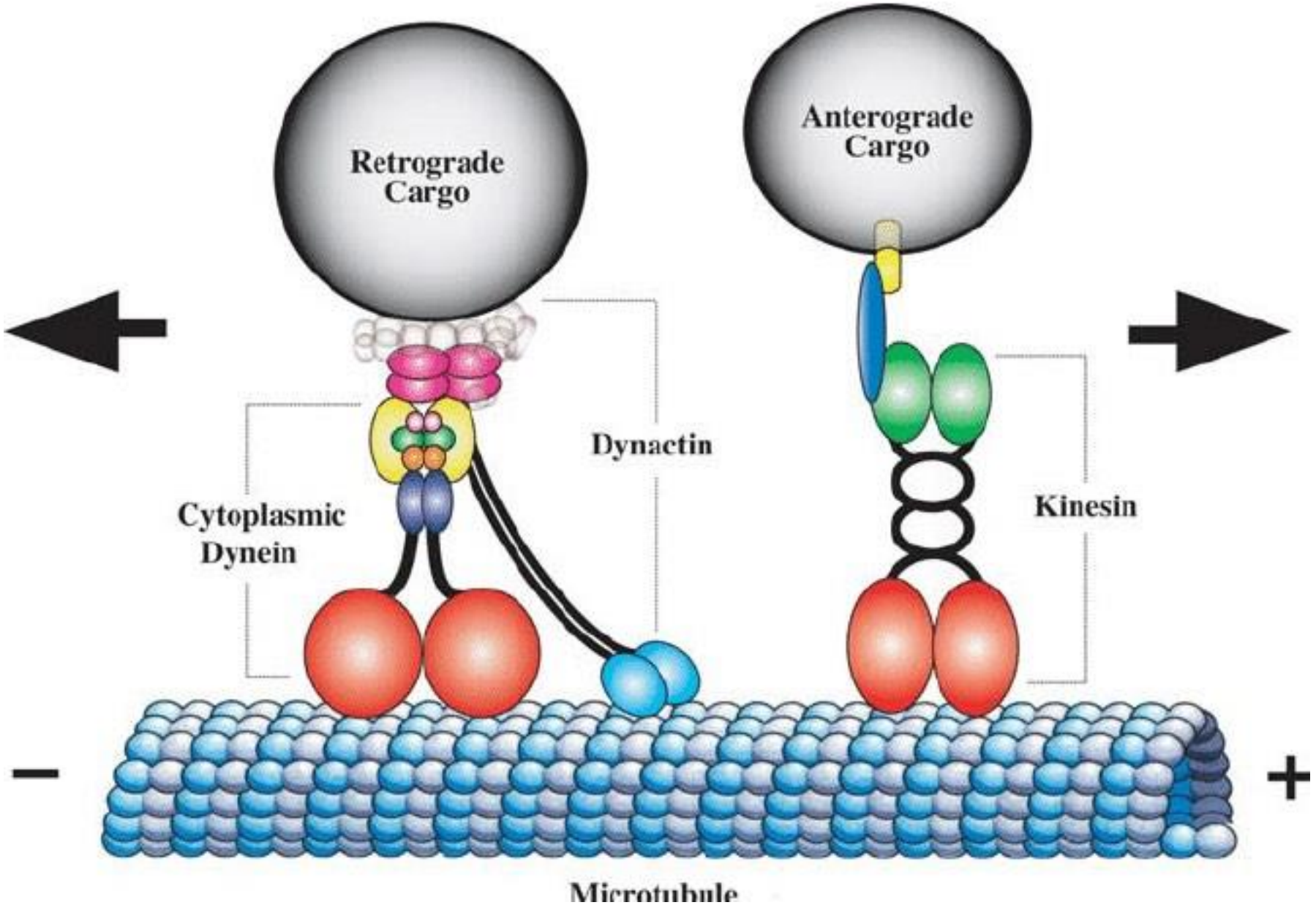


Table 14-1

Table 16-1 Selected Motor Proteins of Eukaryotic Cells

Motor Protein	Typical Function
Microtubule (MT)-Associated Motors	
Dyneins	
Cytoplasmic dynein	Moves cargo toward minus ends of MTs
Axonemal dynein	Activates sliding in flagellar MTs
Kinesins*	
Kinesin 1 (classic kinesin)	Dimer; moves cargo toward plus ends of MTs
Kinesin 3	Monomer; movement of synaptic vesicles in neurons
Kinesin 5	Bipolar, tetrameric; bidirectional sliding of MTs during anaphase of mitosis
Kinesin 6	Completion of cytokinesis
Kinesin 13 ("catastrophins")	Dimer; destabilization of plus ends of MTs
Kinesin 14	Spindle dynamics in meiosis and mitosis; moves toward minus end of MTs

Motor Proteins Move Cargoes Along MTs During Axonal Transport

- Proteins and neurotransmitters produced in the cell body must be transported to the nerve ending
- This process, **fast axonal transport**, involves movement of vesicles and organelles along MTs
- Organelles can be observed moving along filaments through cytoplasm of axons at rates of about 2 μm/sec

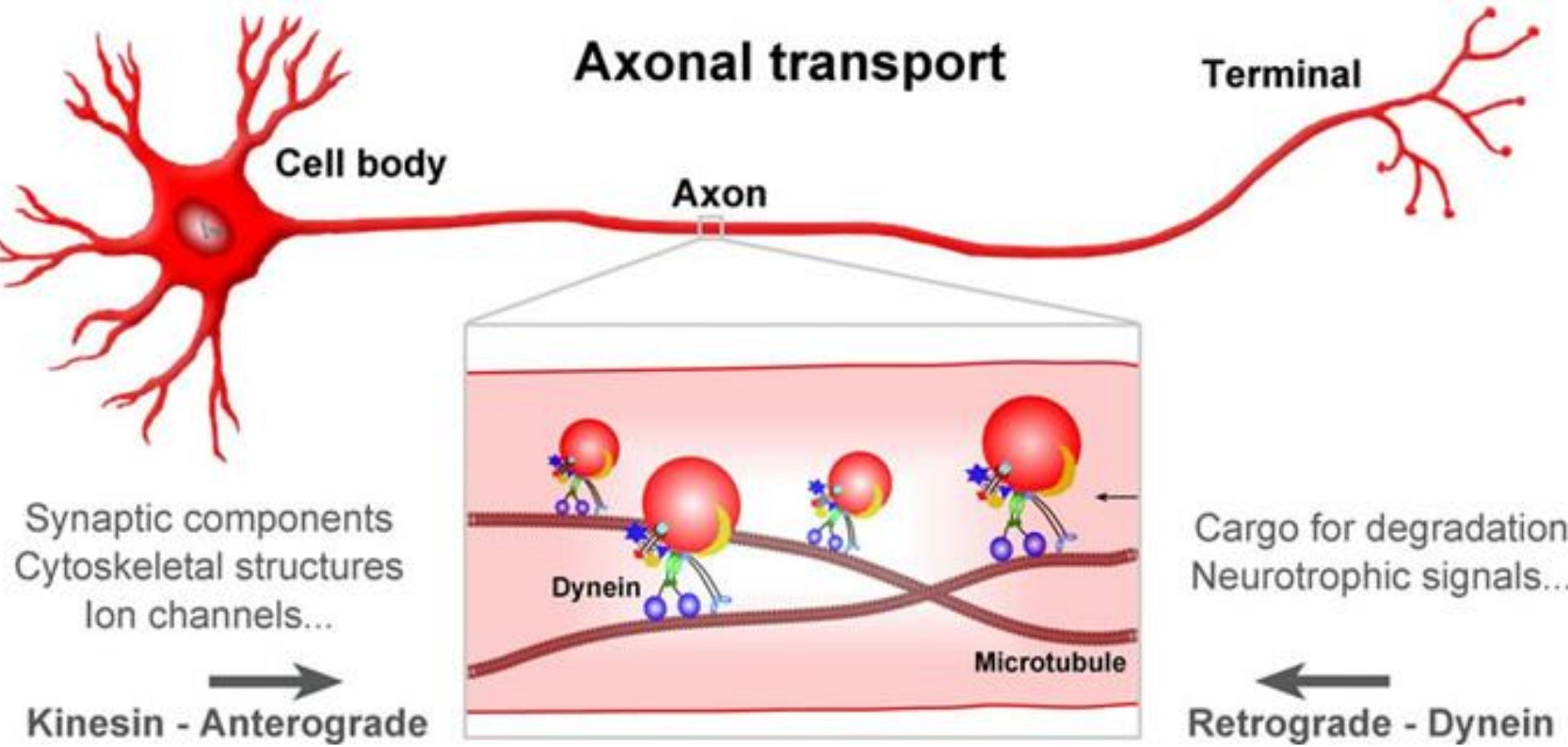
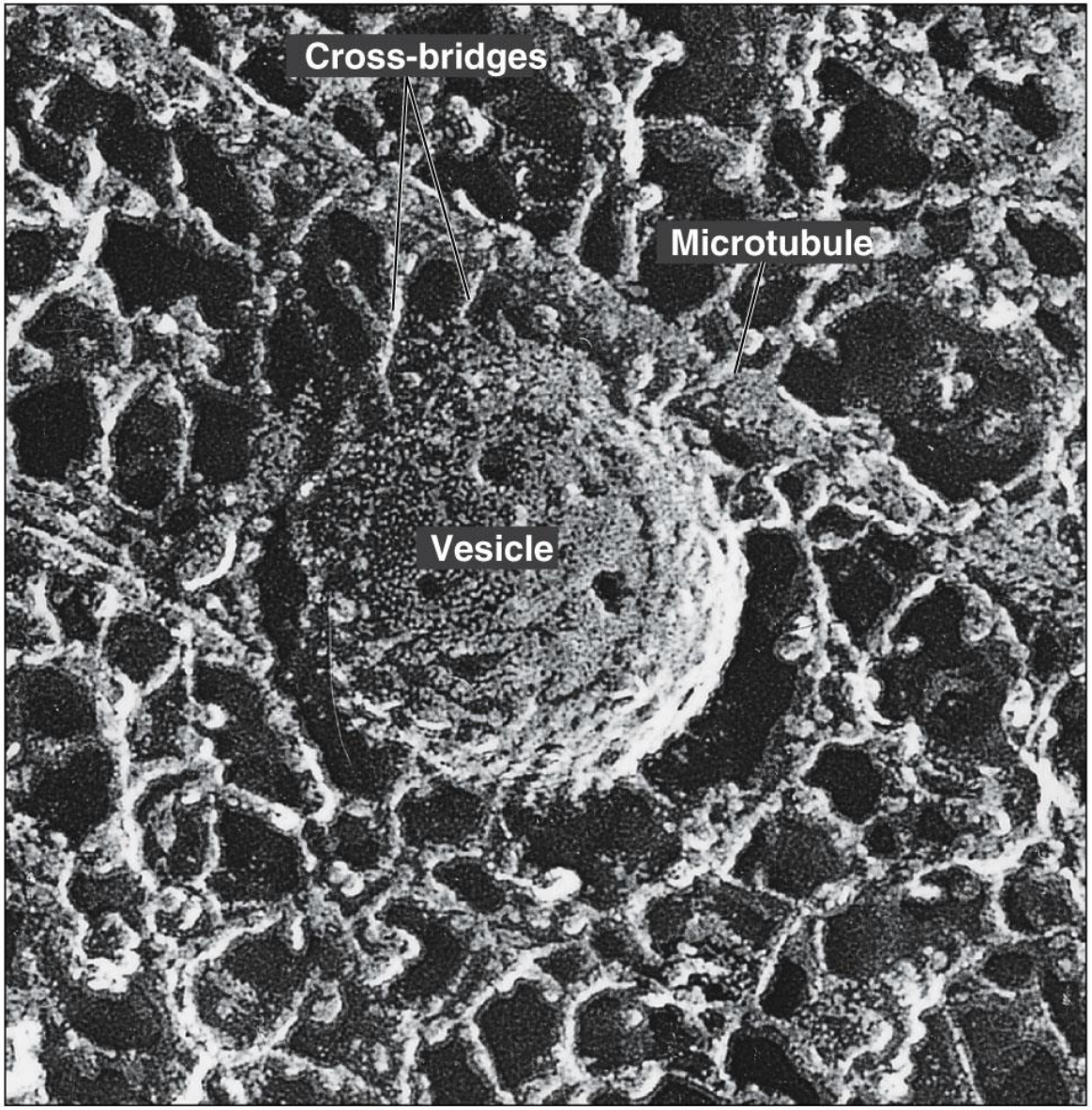
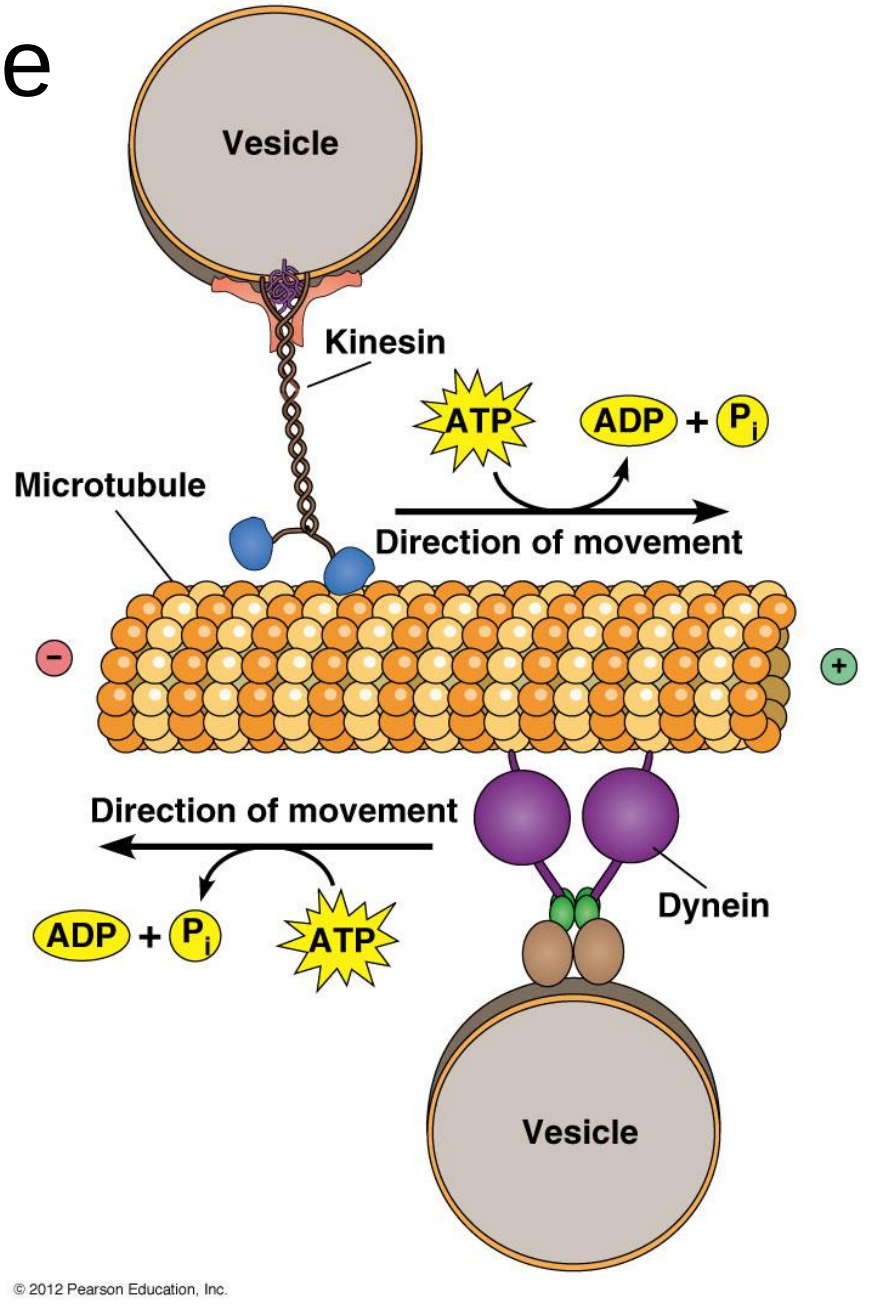


Figure 14-1



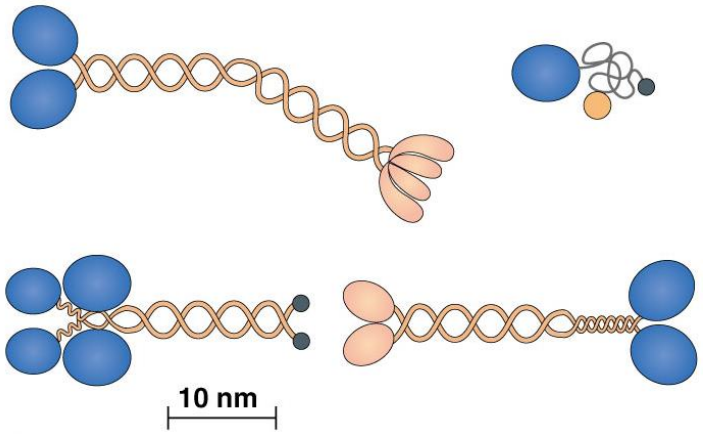
Two proteins responsible for fast axonal transport

- Kinesin I is involved in ATP-dependent transport toward the plus ends (away from the centrosome), called *anterograde (forward) axonal transport*
- Cytoplasmic dynein moves particles (*cargo*) in the opposite direction, called *retrograde (reverse) axonal transport*

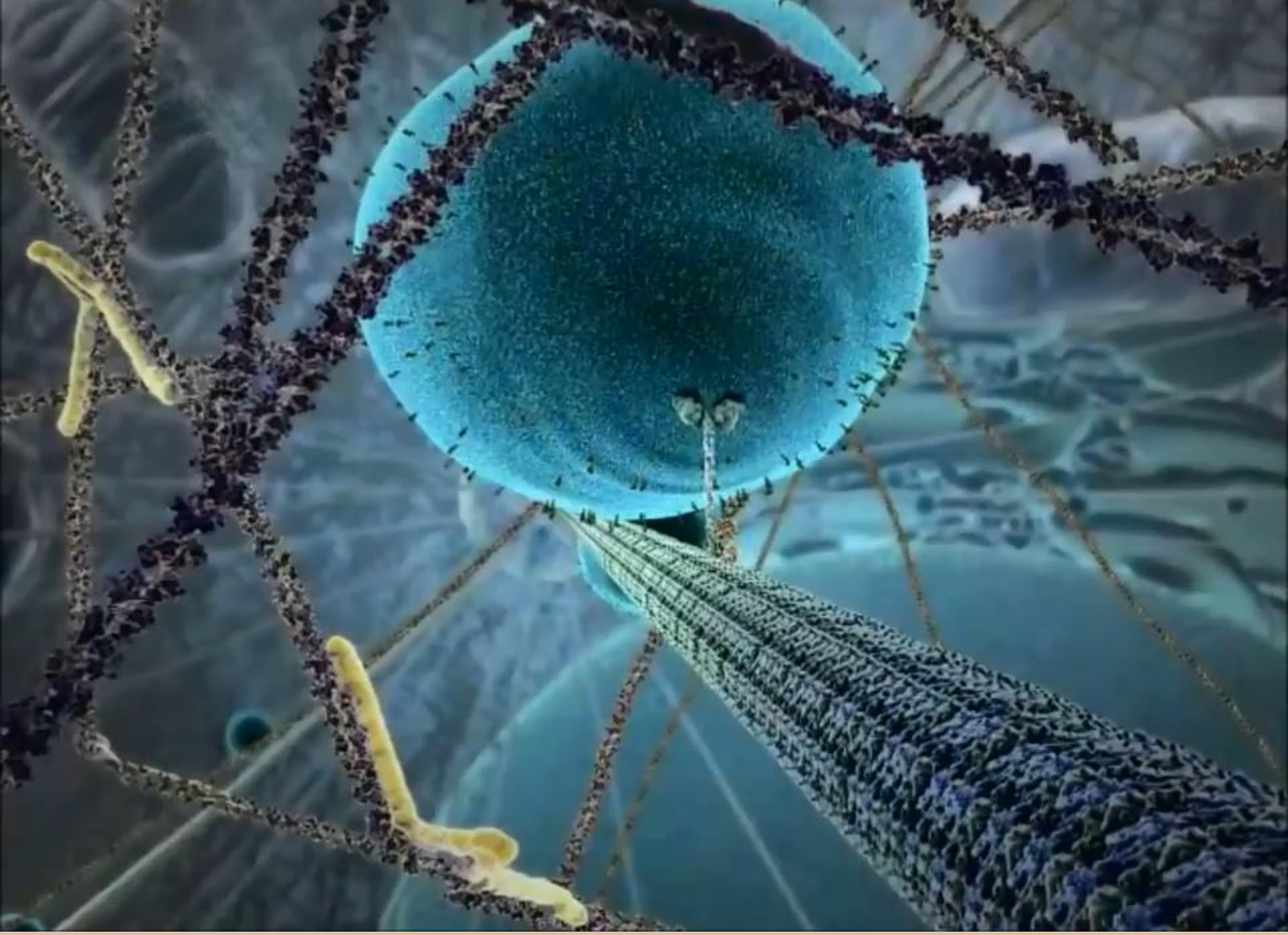


Classic Kinesins Move Toward the Plus Ends of MT

- Kinesins consist of three parts
  - A globular *head* region that attaches to MTs
  - A coiled helical region
  - A *light-chain* region involved in attaching the kinesin to other proteins or organelles



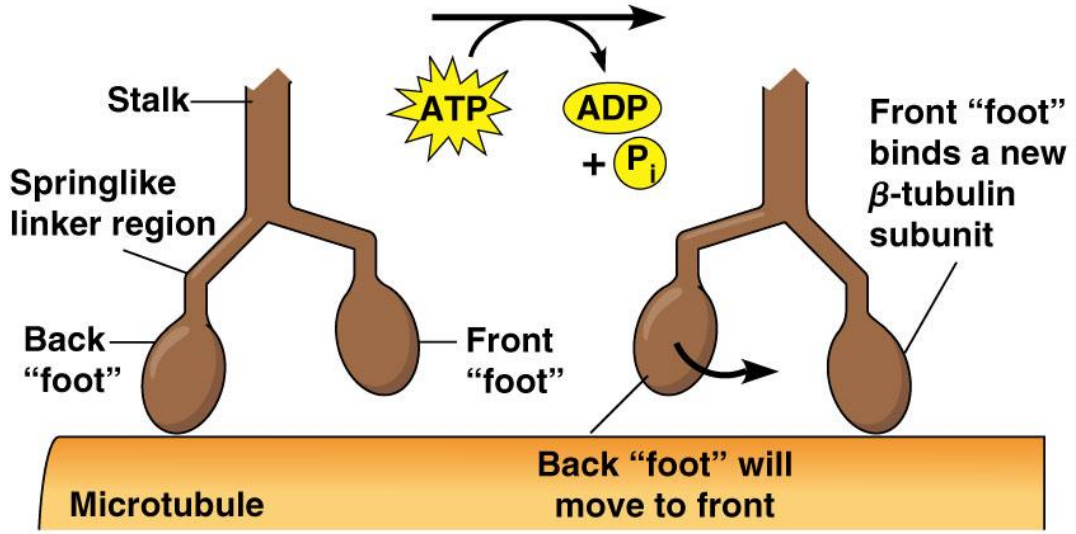
- Kinesins move along the MT in 8-nm steps; the movement is coupled to ATP hydrolysis



Kinesin movement along MTs

- Kinesin movement looks like "walking" with the two globular head domains taking turns as the front foot

- Each kinesin molecule exhibits *processivity;* it can move long distances along an MT before detaching from it by releasing bound ADP and acquiring a new ATP, so that the cycle repeats



(b)

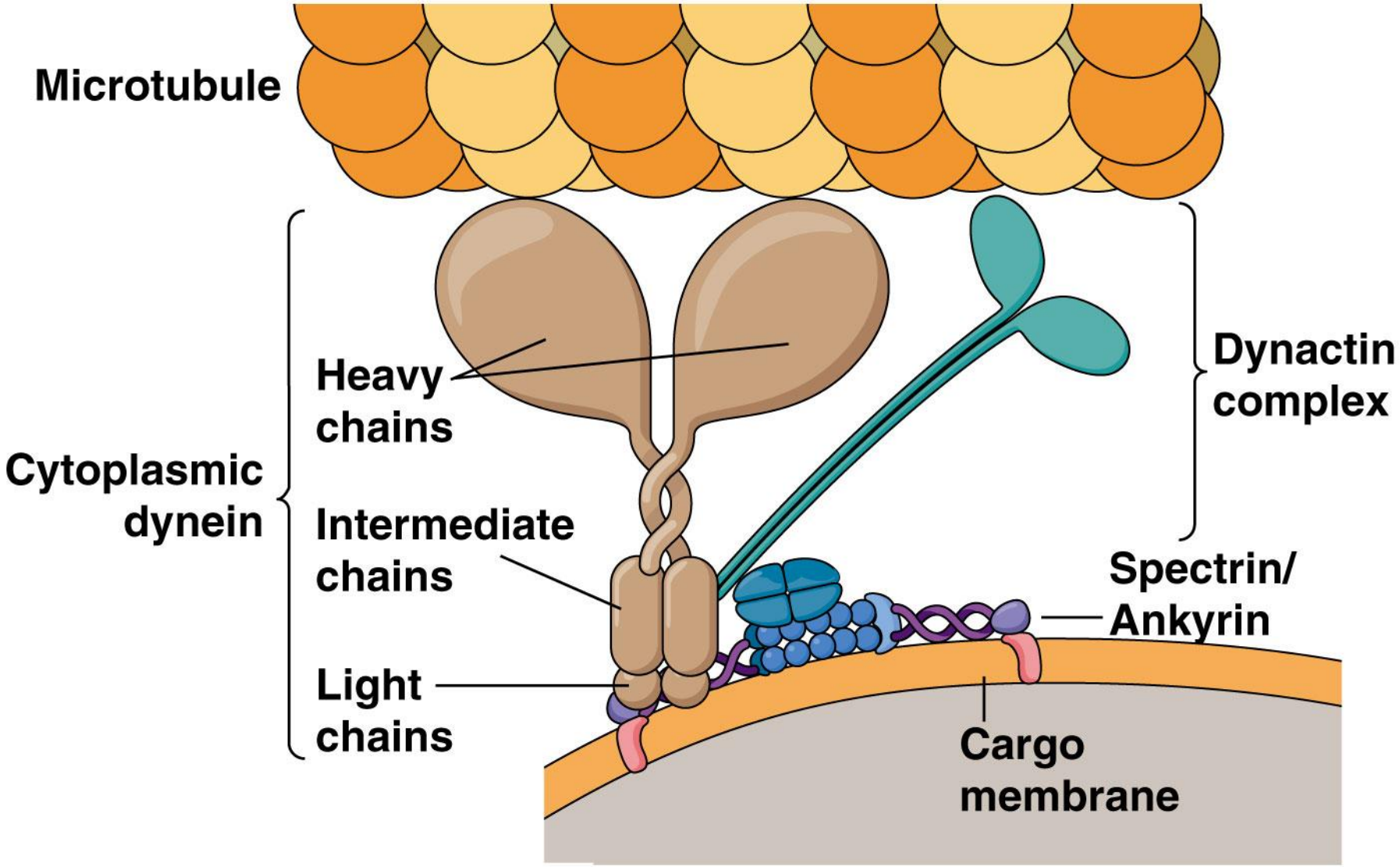
Kinesins Are a Large Family of Proteins

- Kinesins are classified into families based on their structures
- Some form homodimers; others heterodimers
- One family (kinesin 14) is minus-end directed motors
- They are involved in many different cellular processes

Dyneins Are found in Axonemes and the Cytoplasm

- **Cytoplasmic dynein** moves toward the minus ends of MTs
- It is associated with a protein complex called **dynactin**, which helps link it to cargo
- **Axonemal dyneins** include seven different types

Figure 14-5

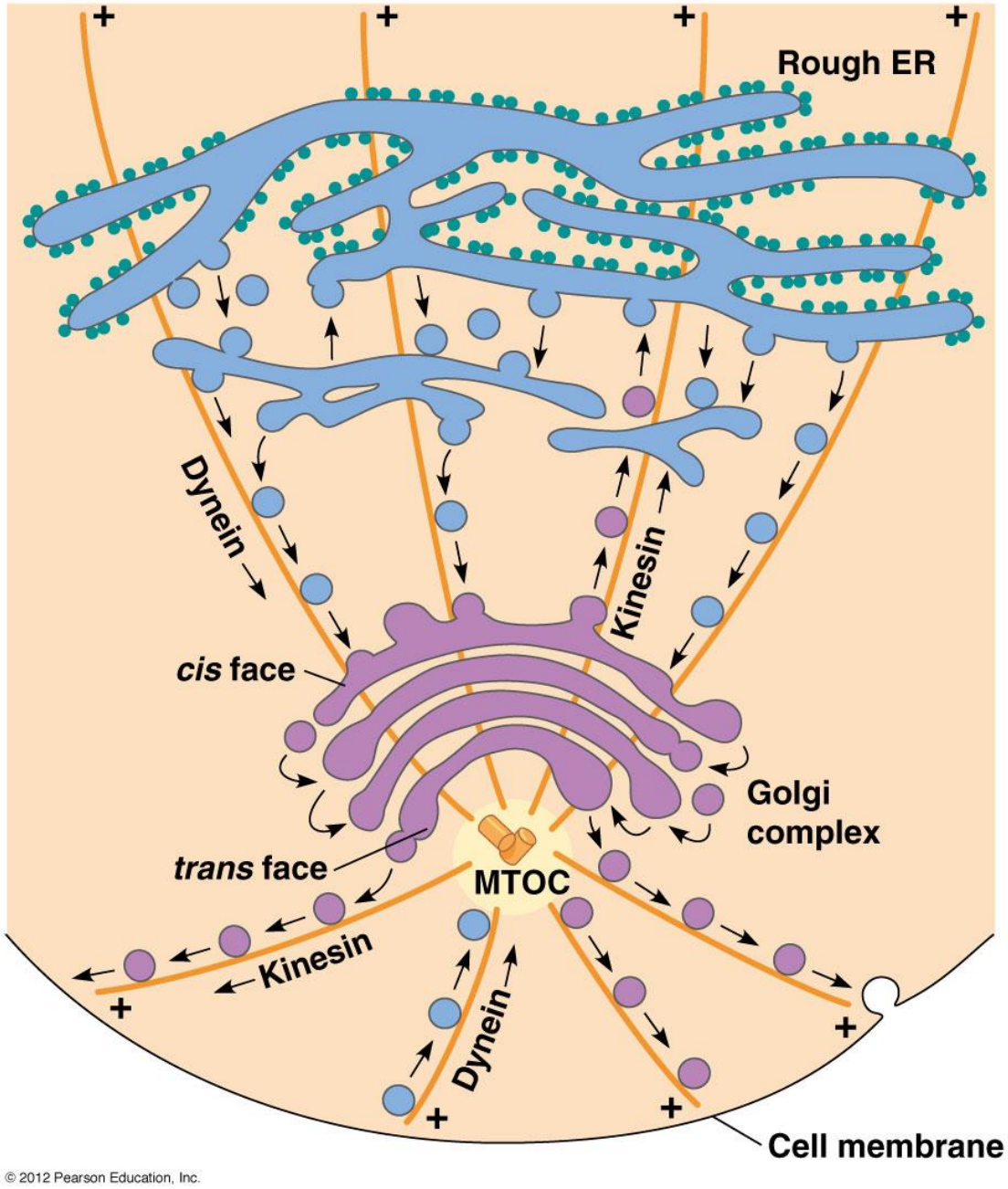


Microtubule Motors Direct Vesicle Transport and Shape the Endomembrane System

- MT motors are important for dynamically shaping the complicated endomembrane system

- The vesicles to and from the Golgi complex are carried by MT motors on microtubule tracks

Figure 14-6



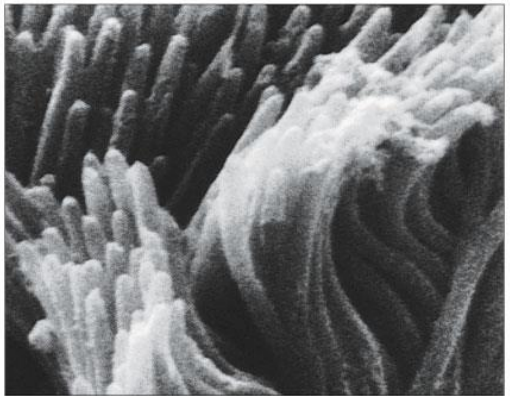
Microtubule-Based Cell Motility: Cilia and Flagella

- Microtubules are required for movements of cilia and flagella, the motile appendages of eukaryotic cells

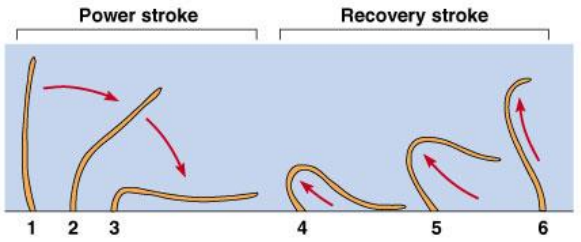
- The two appendages share a common structural basis

Cilia and Flagella Are Common Motile Appendages of Eukaryotic Cells

- **Cilia**: are about 2–10 μm long and occur in large numbers on the surface of ciliated cells
- They occur in both unicellular and multicellular eukaryotes
- Cilia display an oarlike pattern of beating, generating a force parallel to the cell surface



(a) Cilia on a mammalian tracheal cell

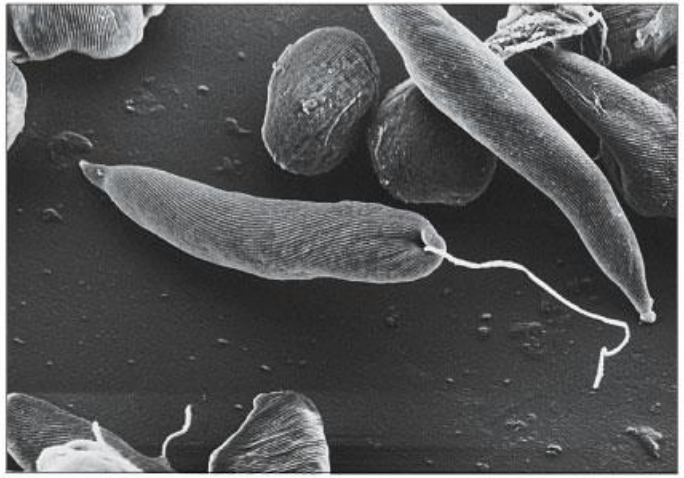


(b) Beating of a cilium

- **Flagella** move cells through a fluid environment

- They are the same diameter as cilia, but usually much longer (up to 200 μm)

- They are limited to one or a few per cell and move with a propagated bending motion



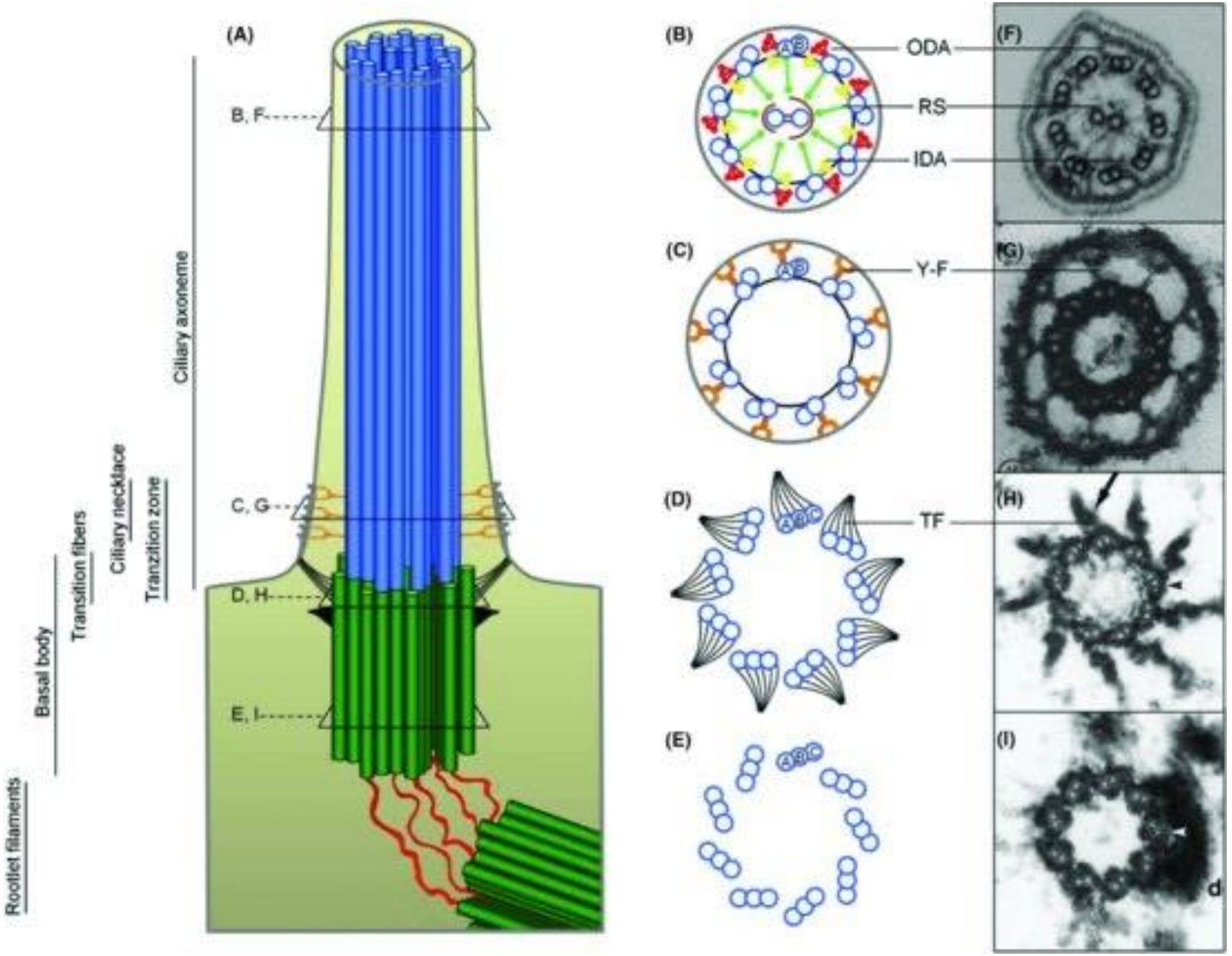
(c) Flagellum on unicellular alga *Euglena*

1 μm

(d) Movement of flagellated eukaryotic cell

Cilia and Flagella Consist of an Axoneme Connected to a Basal Body

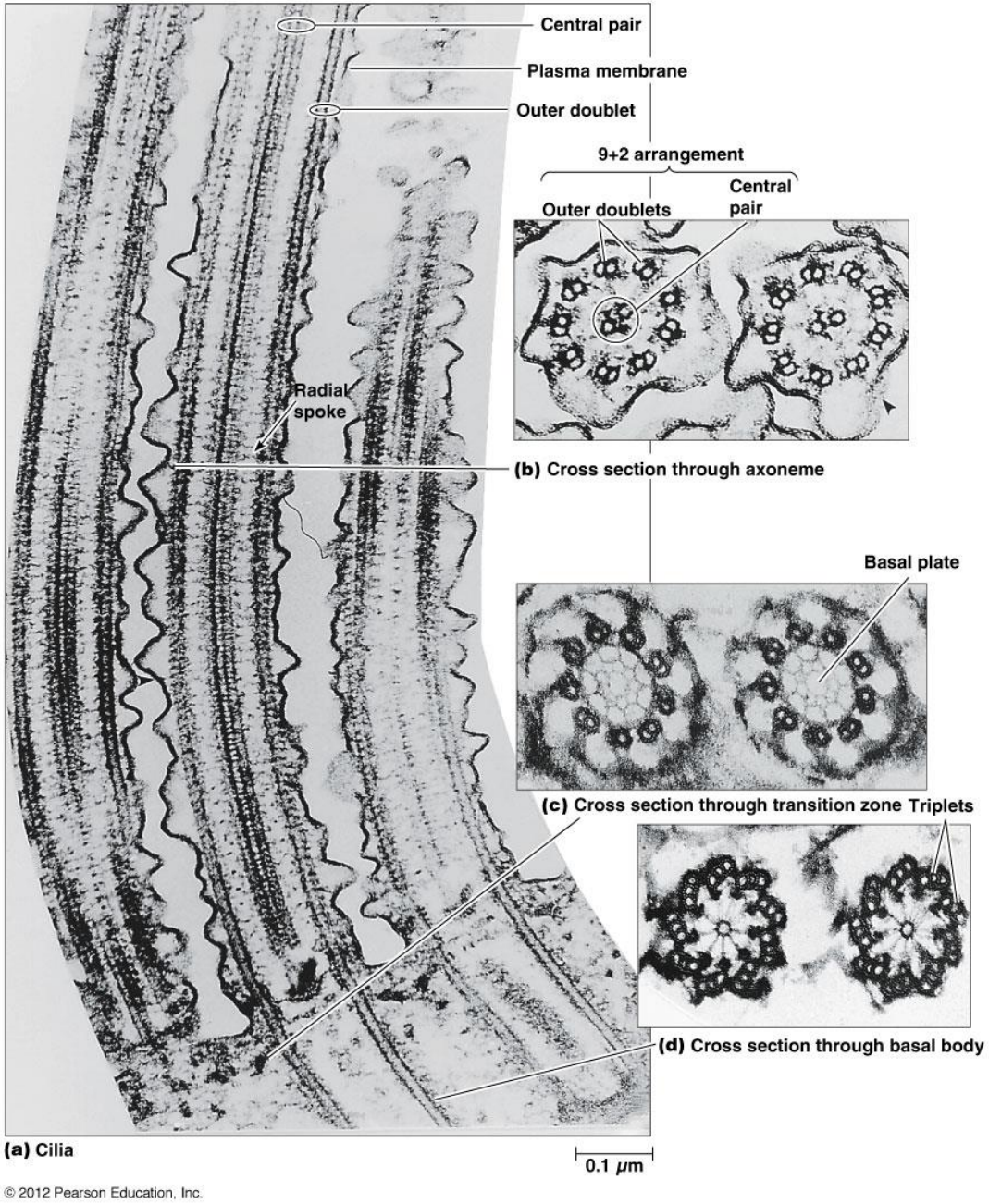
- Cilia and flagella share a common structure, the **axoneme**
- It is connected to a **basal body** and surrounded by an extension of the cell membrane
- Between the axoneme and basal body is a *transition zone* in which the MTs take on the pattern characteristic of the axoneme

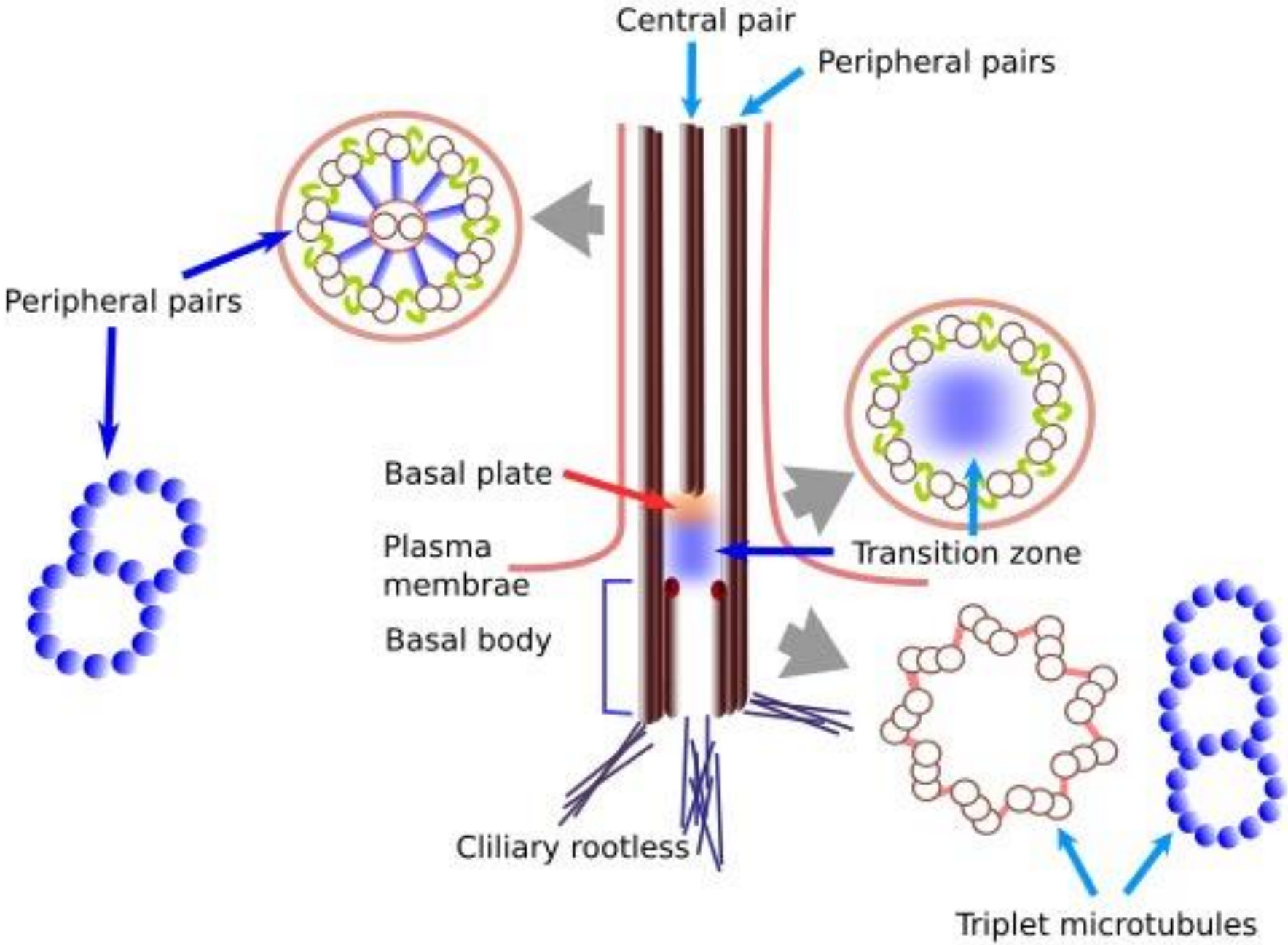


Structure of cilia and flagella

- The basal body looks like a centriole, with 9 sets of tubular structures around the circumference
- Each set is a *triplet* with three MTs that share common walls
- Axonemes have a characteristic "9+2" pattern, with 9 **outer doublets** and 2 MTs in the center, the **central pair**

Figure 14-8





Tubule structure

- Each A tubule has a set of **sidearms** that project from each of the outer doublets; these consist of axonemal dynein

- Axonemal dynein is involved in the sliding of MTs against each other, which bends the axoneme

Axonemal dynein

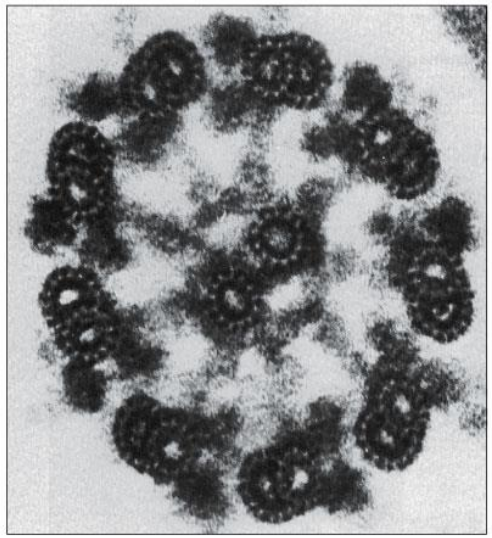
- The dynein arms occur in pairs, one inner and one outer arm
- Less frequently, adjacent doublets are joined by **interdoublet links** that limit the extent of relative movement of doublets, which are linked to each other by a protein called **nexin**

Radial spokes

- At regular intervals, **radial spokes** project inward toward the central pair

- The spokes are thought to be important in translating the sliding of MTs into the bending of the axoneme

Figure 14-9



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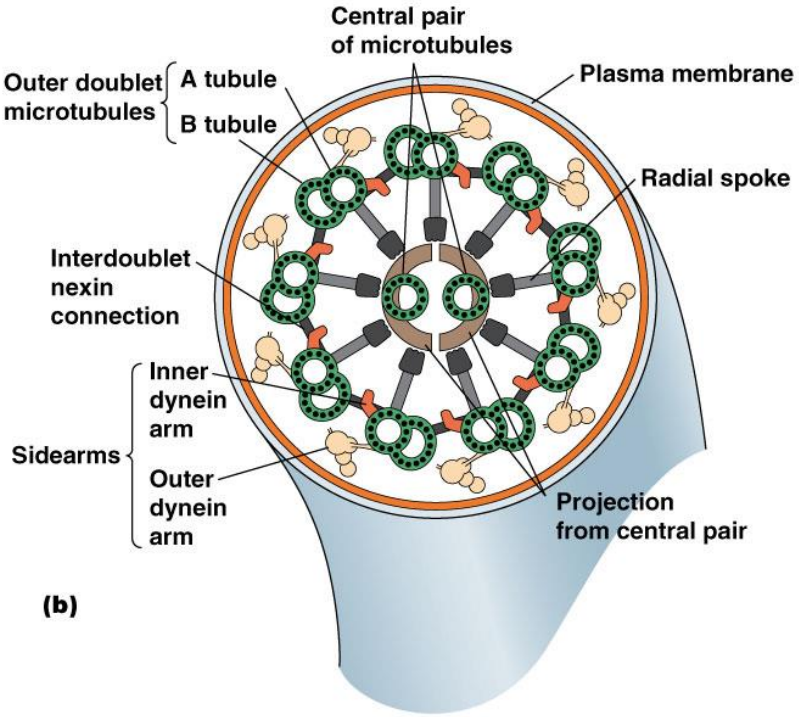

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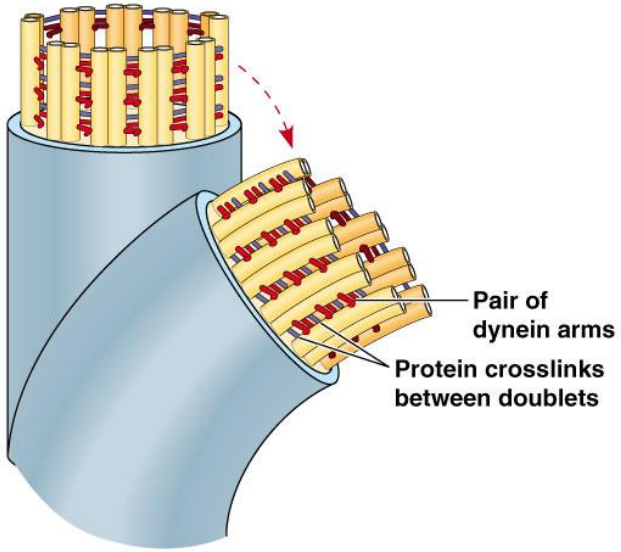

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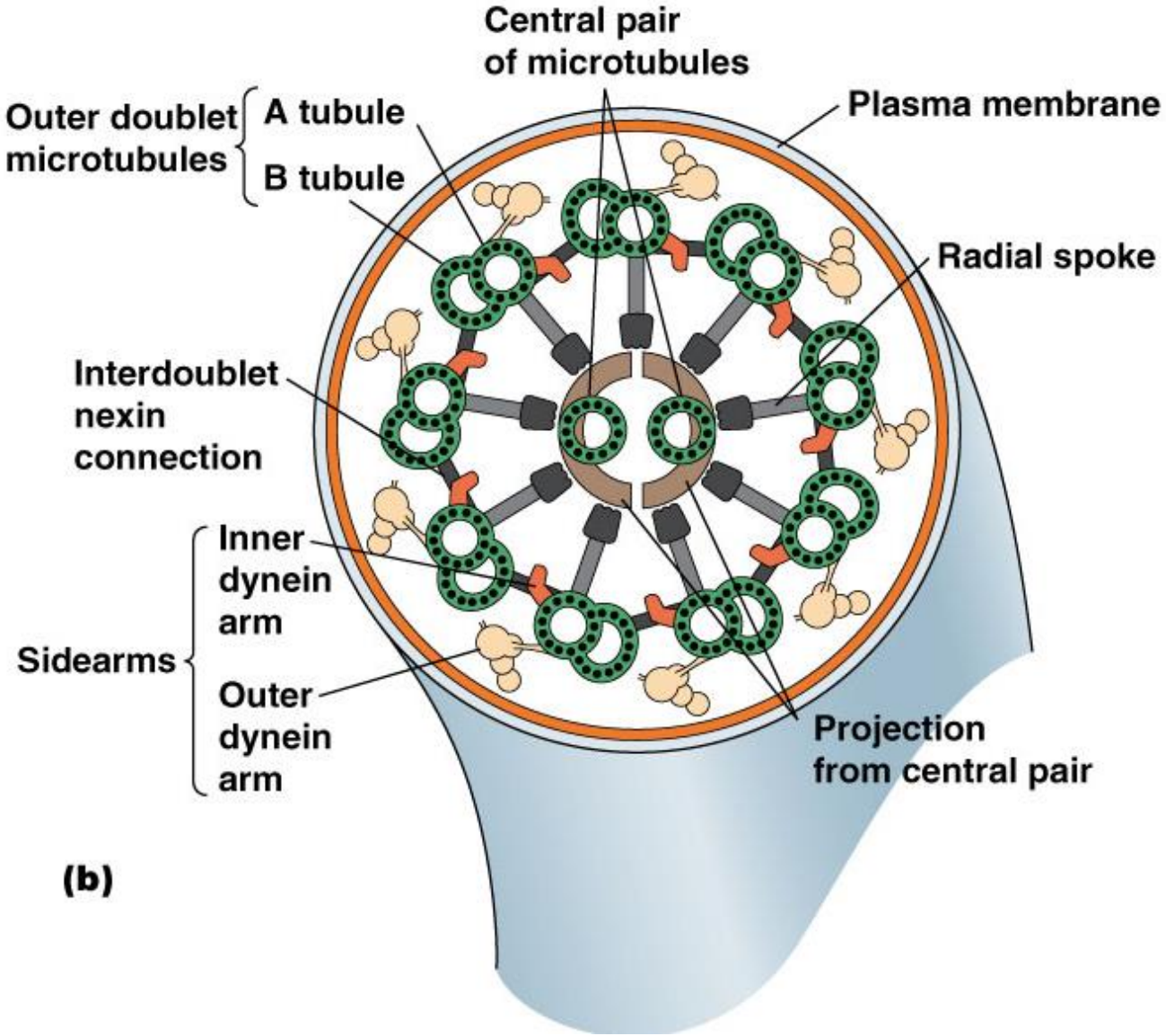


Doublet Sliding Within the Axoneme Causes Cilia and Flagella to Bend

- The **sliding-microtubule model** suggests that **sliding** of MTs relative to each other is converted into localized **bending** because the doublets are connected to the central pair and to each other
- Therefore, they cannot easily slide past each other
- The resulting bending takes the form of a wave

Dynein Sidearms Are Responsible for Sliding

- The driving force for MT sliding is provided by ATP hydrolysis
- Several lines of evidence suggest that dynein is responsible for the MT sliding; the dynein arm attaches to and detaches from the B tubule cyclically
- Axonemal dynein has multiple subunits, the largest three having ATPase activity

Crosslinks and Spokes Are Responsible for Bending

- Resistance in bending is provided by the radial spokes that connect the doublets to the central pair

- It is also possibly provided by nexin crosslinks between doublets

Intraflagellar Transport Adds Components to Growing Flagella

- Tubulin subunits are shuttled to and from the growing flagellum tip by both plus- and minus-end directed motor proteins
- This is known as intraflagellar transport (IFT)
- Kinesins move material to the tips of the flagella and dynein bring material back toward the base

Actin-Based Cell Movement: The Myosins

- Movements of molecules and other cellular components also occur along another system in the cell—the actin cytoskeleton

Myosins Are a Large Family of Actin-Based Motors with Diverse Roles in Cell Motility

- **Myosins** are ATP-dependent motors that exert force on actin filaments

- Currently there are 24 known classes of myosins

- The globular head binds actin and uses the energy of ATP hydrolysis to move along the filament
- Most move toward the plus-end

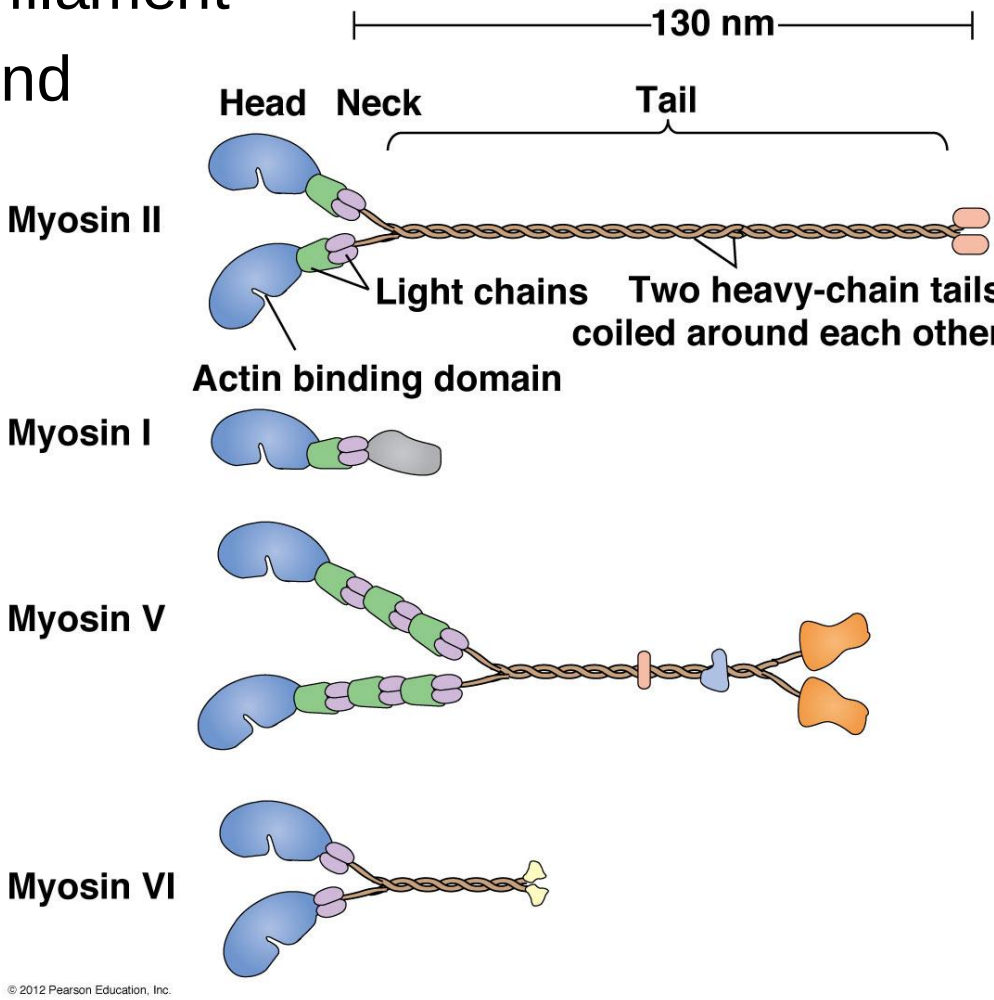


Table 16-1

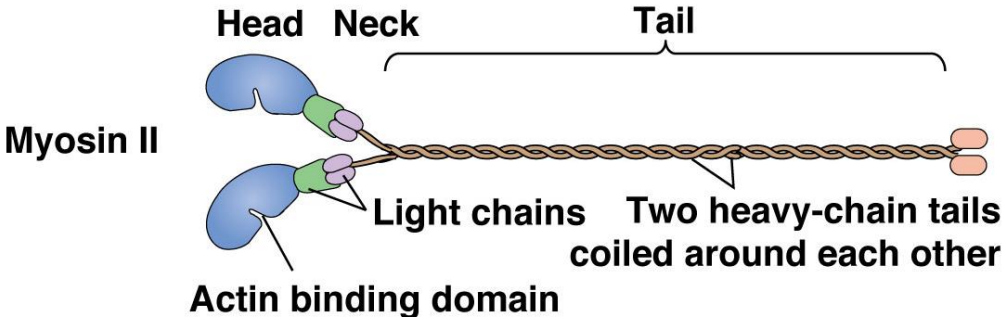
Microfilament (MF)-Associated Motors	
Myosins*	
Myosin I	Motion of membranes along MFs; endocytosis
Myosin II	Slides MFs in muscle; other contractile events such as cytokinesis, cell migration
Myosin V	Vesicle positioning and trafficking
Myosin VI	Endocytosis; moves toward minus ends of MFs
Myosin VII	Base of stereocilia in inner ear
Myosin X	Tips of filopodia
Myosin XV	Tips of stereocilia in inner ear

*Kinesins and myosins comprise large families of proteins. There are many families of kinesins and myosins.

Myosin functions

- Myosins function in a wide range of cellular events, including
  - Muscle contraction
  - Cell movement
  - Phagocytosis
  - Vesicle transport

Type II myosins



- They use ATP hydrolysis to cause actin filaments to slide past myosin molecules, resulting in contraction of a cell or group of cells

Many Myosins Move Along Actin Filaments in Short Steps

- Myosin II is an efficient motor that "walks" along actin like kinesin walks along microtubules
- Both have two heads that walk along a protein filament, and both use ATP hydrolysis to change their shape
- However, there are important differences

Kinesins vs. myosin

- Kinesins operate alone or in small numbers to transport vesicles over large distances (hundreds of nanometers)
- A single myosin II molecule slides an actin filament about 12–15 nm per power stroke
- Myosin II molecules move short distances but operate in large arrays, in some cases billions of motors working together to mediate muscle contraction

Filament-Based Movement in Muscle

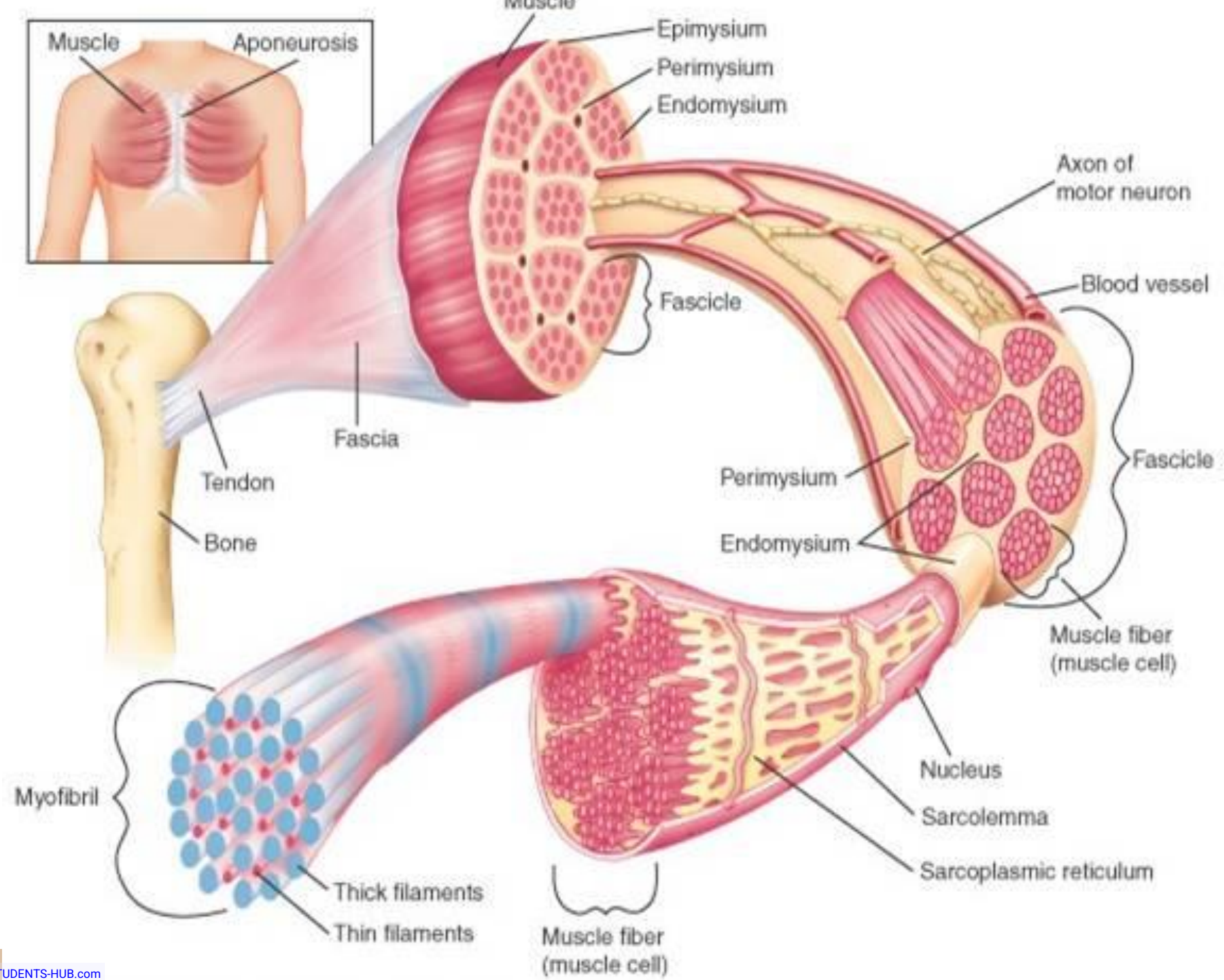
- Muscle contraction is the most familiar example of mechanical work mediated by intracellular filaments

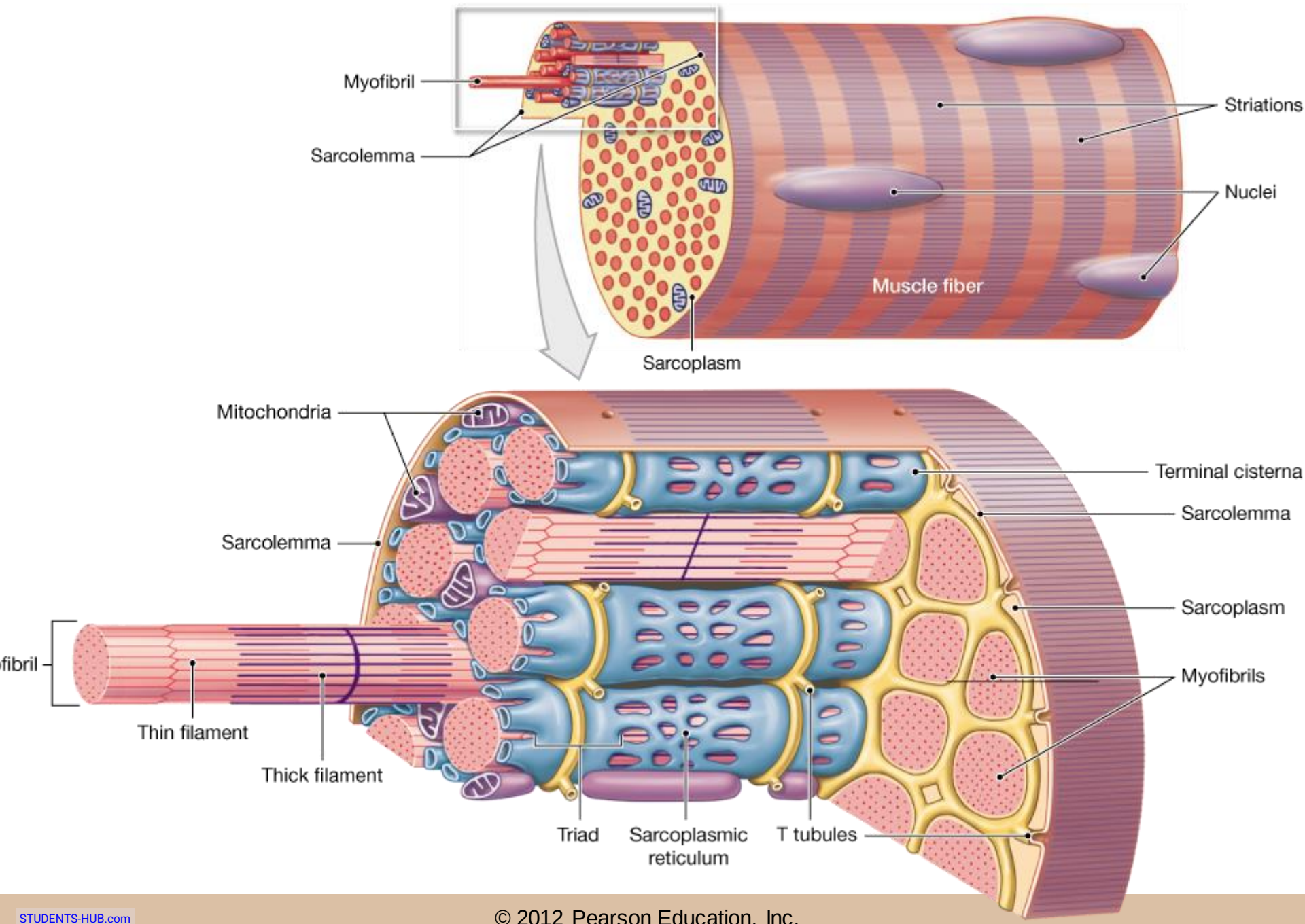
Skeletal Muscle Cells Contain Thin and Thick Filaments

- **Skeletal muscles** are responsible for voluntary movement
- A muscle consists of parallel **muscle fibers** joined by tendons to the bones that the muscles move
- Each fiber is a long, thin, highly specialized, multinucleate cell

Skeletal muscle cells

- Multinucleate cells arise from fusion of embryonic cells called *myoblasts*
- The multinucleate cell is called a *syncytium*

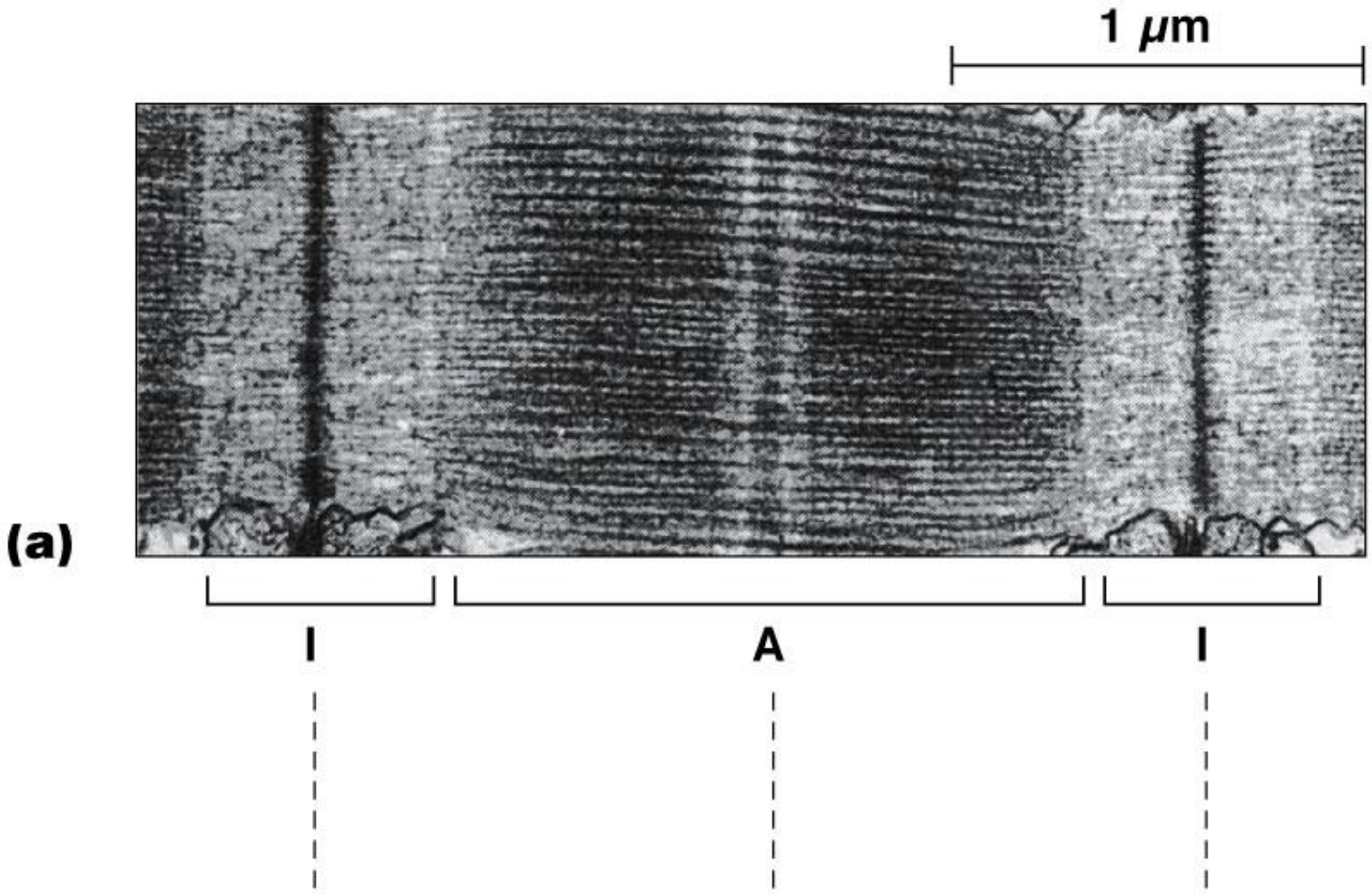




Striated muscle

- The filaments in skeletal muscle are aligned, giving myofibrils a pattern of alternating dark and light bands
- These *striations* are characteristic of cardiac and skeletal muscle, called **striated muscle**
- Dark bands are **A bands** and light bands are **I bands**

Figure 14-13a



Structure of A bands and I bands

- The lighter region in the middle of each A band is called the **H zone;** the **M line runs down the center**
- The M line contains *myomesin*, a protein that links myosin filaments together
- In the middle of each I band is a dense **Z line**; the distance from one Z line to another defines a sarcomere

Figure 14-13 (b)

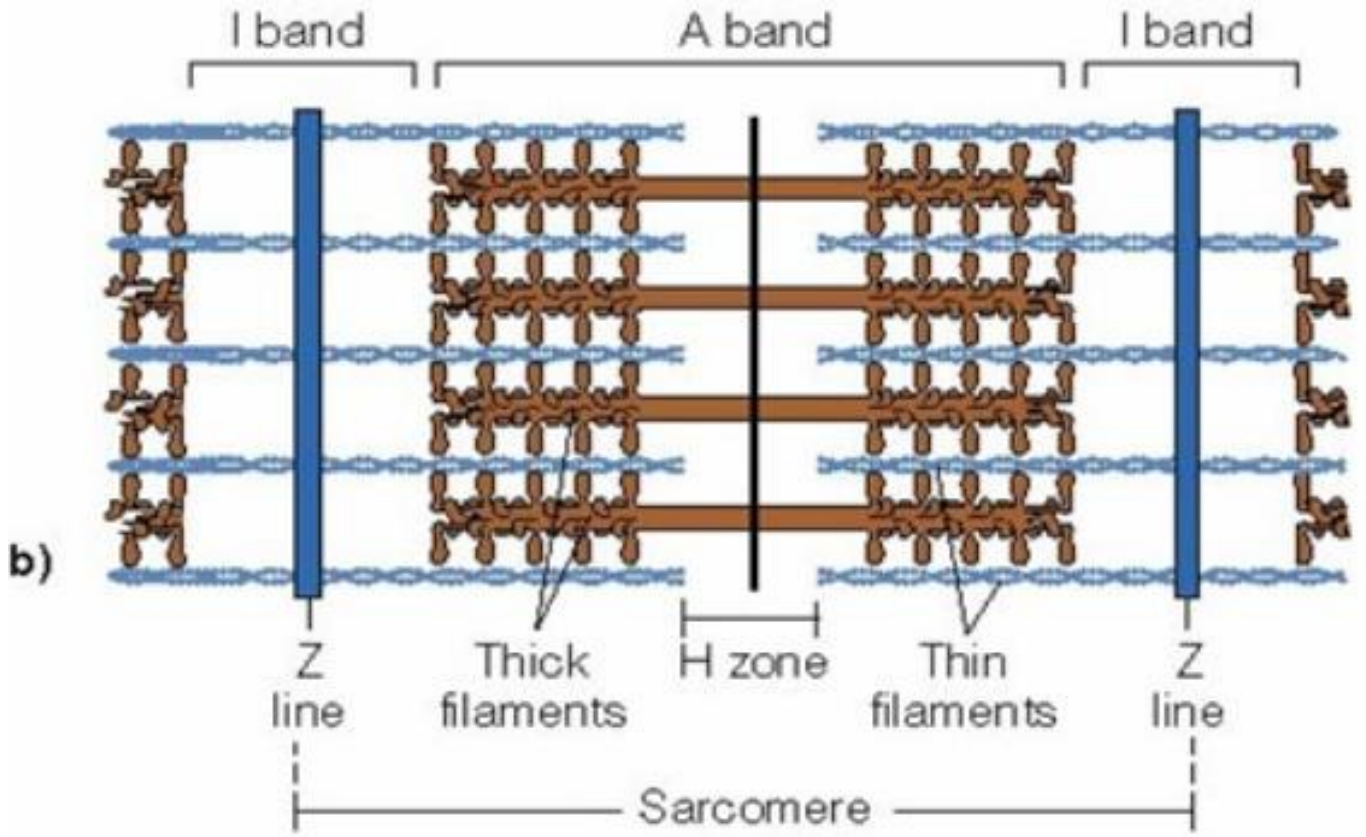
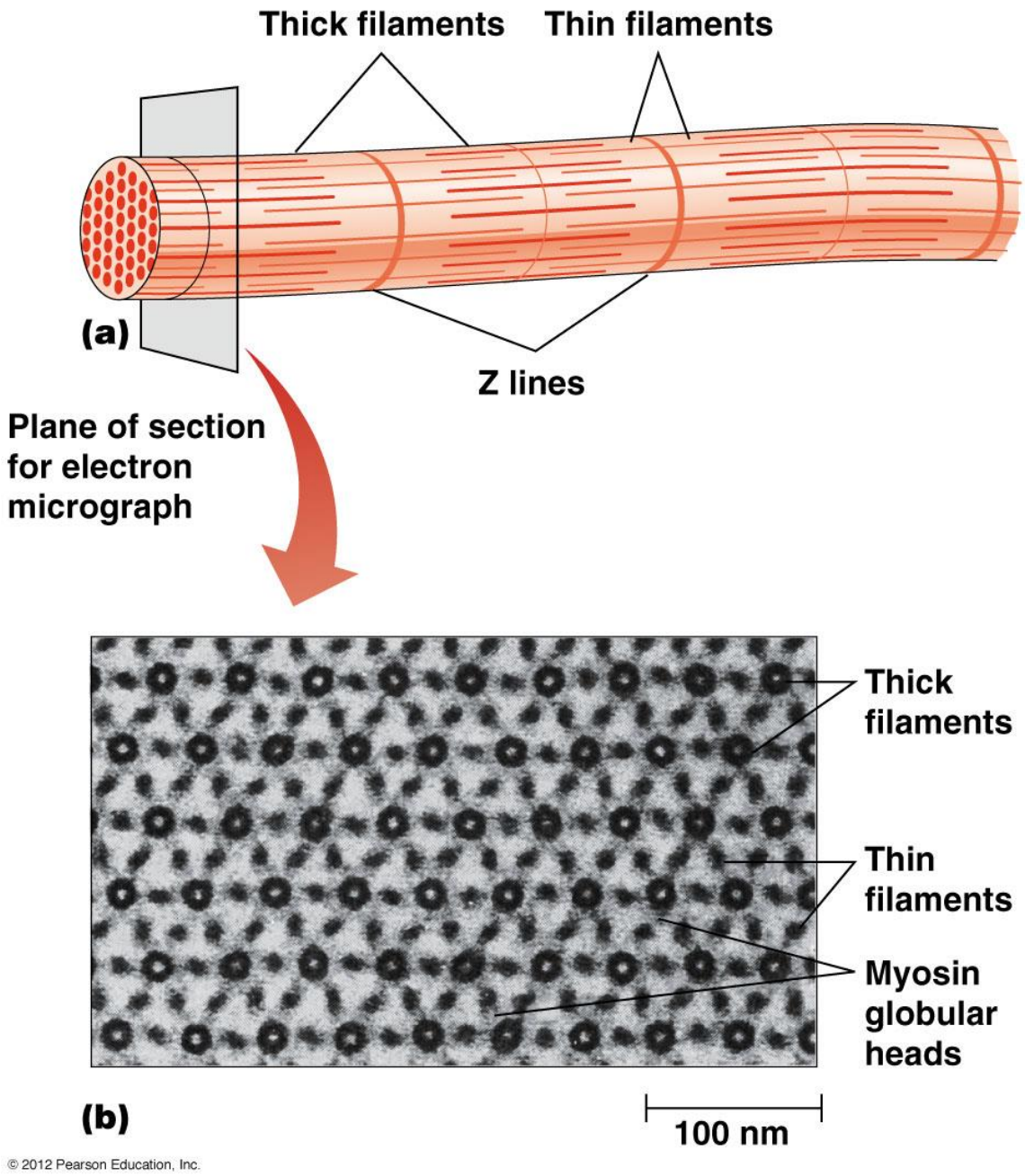


Figure 14-11



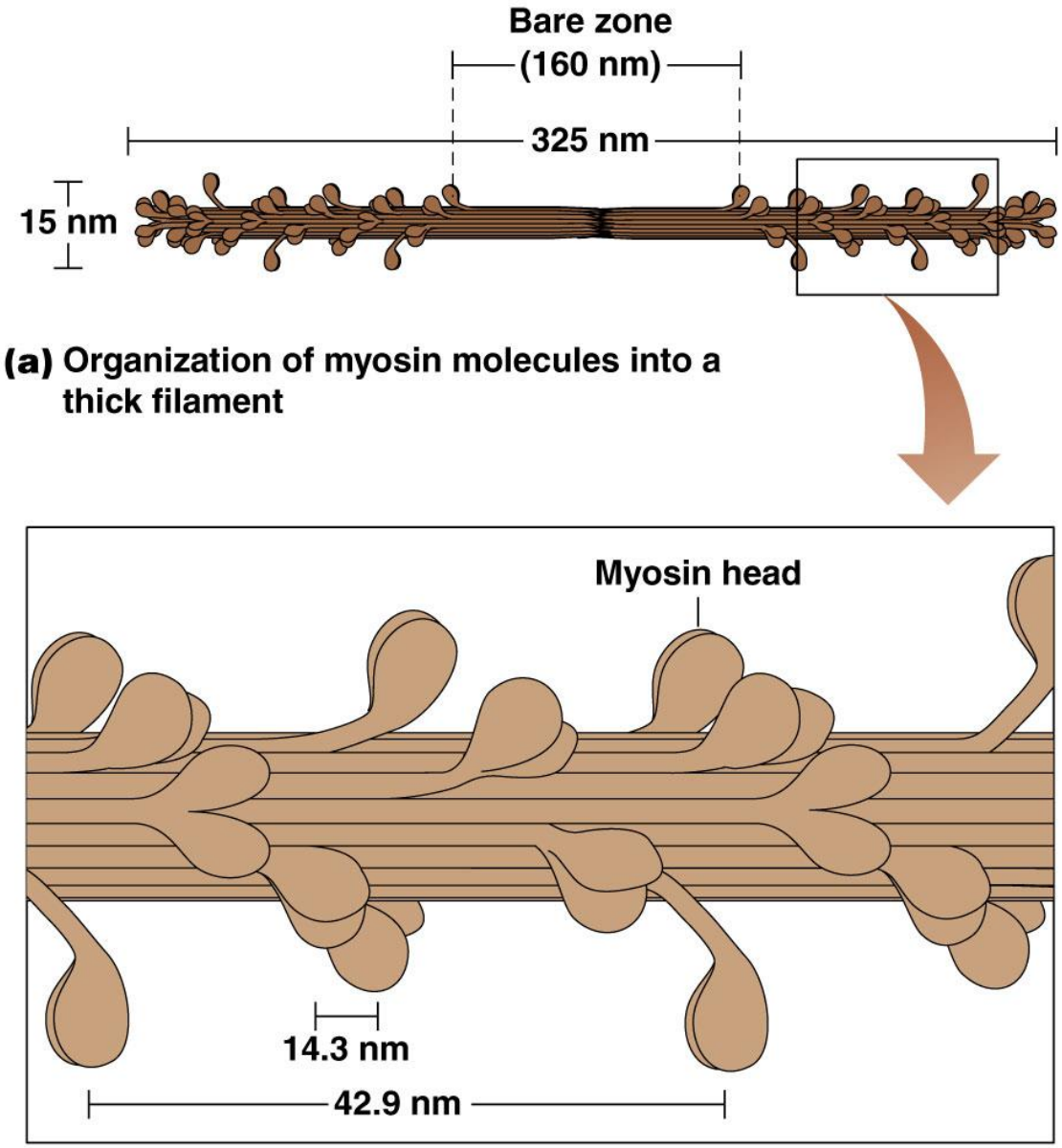
Sarcomeres Contain Ordered Arrays of Actin, Myosin, and Accessory Proteins

- The arrangement of thin and thick filaments in myofibrils gives rise to
  - The striated pattern of skeletal muscle
  - The observed shortening of sarcomeres during contraction

Thick Filaments

- Each thick filament consists of hundreds of molecules of **myosin**, oriented in opposite directions in the two halves of the filament
- The myosin is arranged in staggered fashion
- Protruding heads of myosin molecules contact the adjacent thin filaments, forming cross-bridges

Figure 14-12



(a) Organization of myosin molecules into a thick filament

(b) Portion of a thick filament

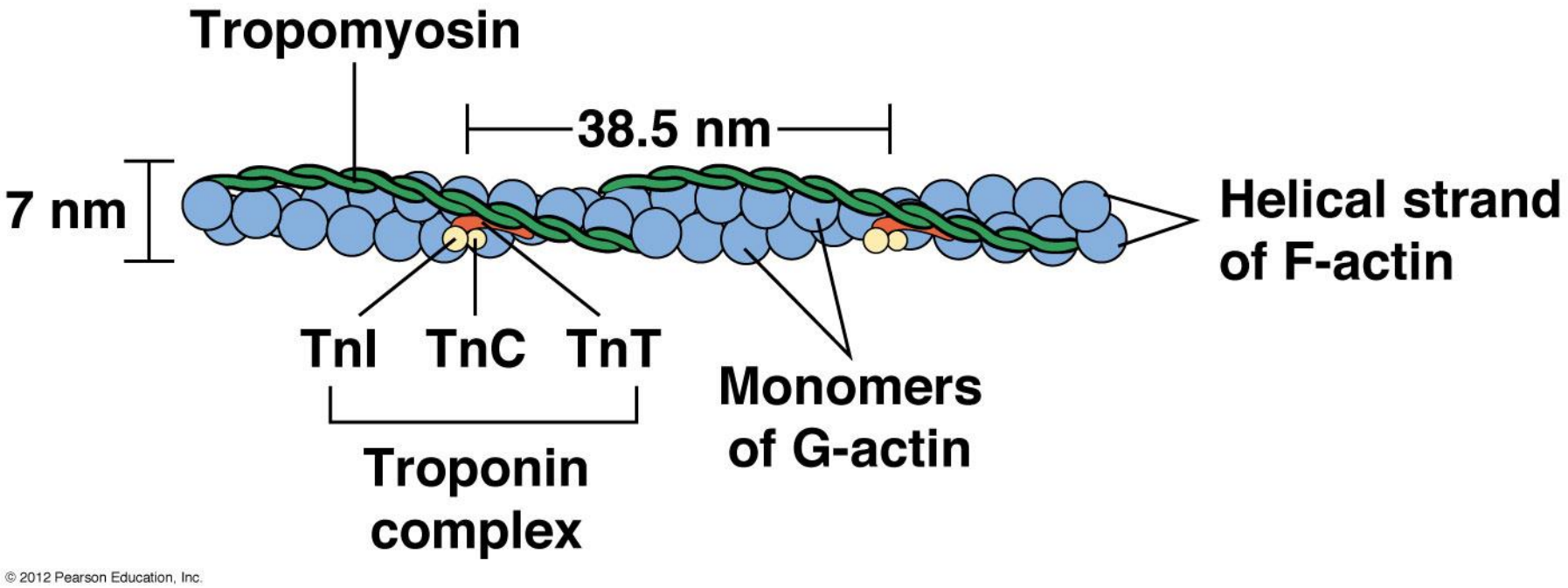
Thin Filaments

- Thin filaments interdigitate with the thick filaments
- Thin filaments contain three proteins: F-actin, intertwined with **tropomyosin** and **troponin**

Troponin and tropomyosin

- Troponin is composed of three polypeptides: *TnT*, *TnC*, and *TnI*
- One troponin complex associates with each tropomyosin
- Together they constitute a calcium-sensitive switch that activates contraction in striated muscle

Figure 14-15



Organization of Muscle Filament Proteins

- The actin in thin filaments is oriented so that all the plus ends are anchored at Z lines
- Myosin II moves toward the plus ends, so the thick filaments move toward the Z lines during contraction
- Structural proteins contribute to the architecture of muscle cells

Structural proteins associated with thin filaments

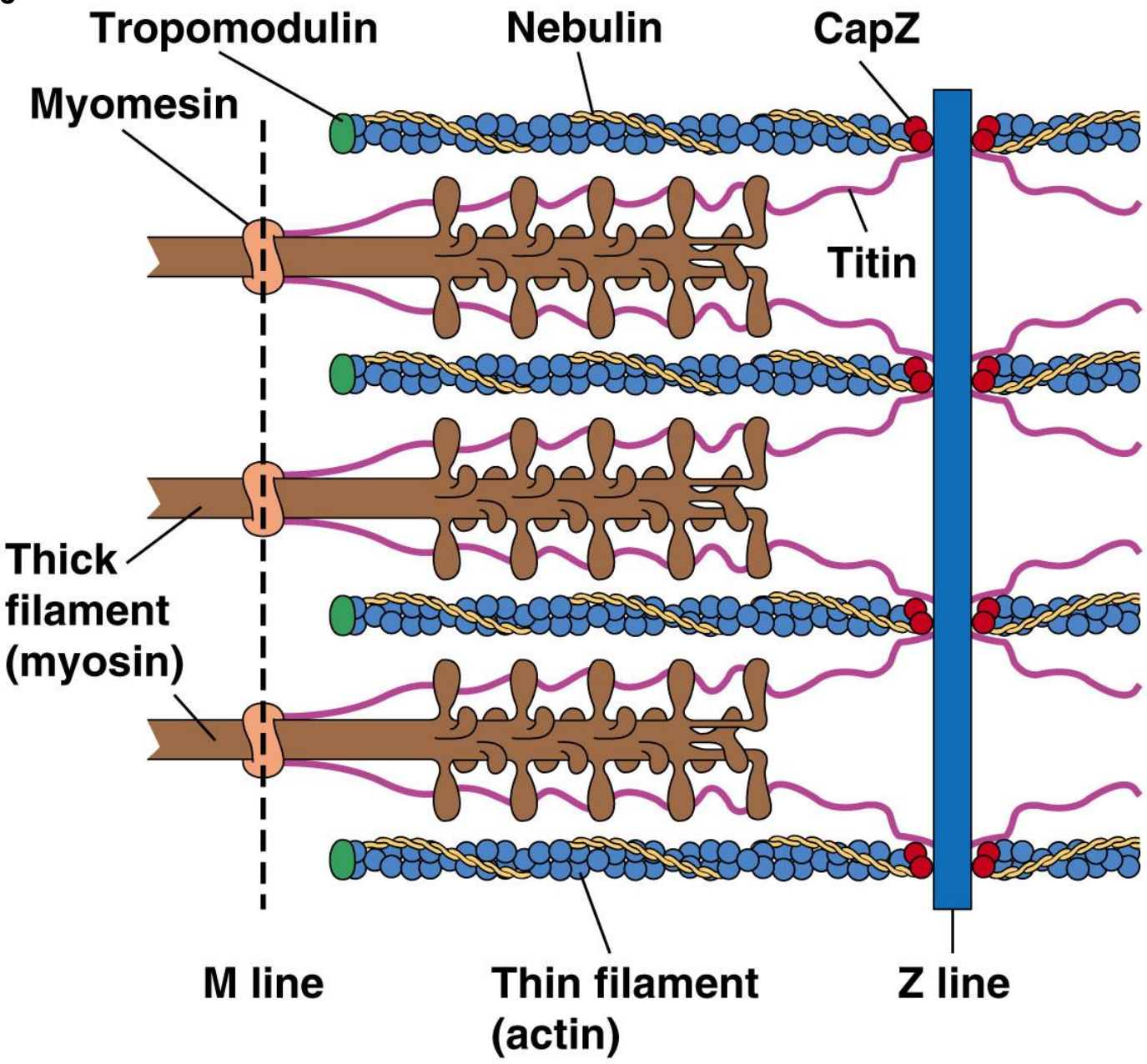
- *α-actinin* keeps actin filaments bundled into parallel arrays
- *CapZ* maintains the attachment of the plus ends to the Z line and caps the actin in the filaments
- *Tropomodulin* binds the minus ends of the filaments to maintain stability and *nebulin* stabilizes the thin filament organization

Structural proteins associated with thick filaments

- *Myomesin* is present at the H zone and bundles the myosin molecules

- *Titin* attaches the thick filaments to the Z lines and keeps thick filaments in correct position relative to thin filaments during contraction

Figure 14-16

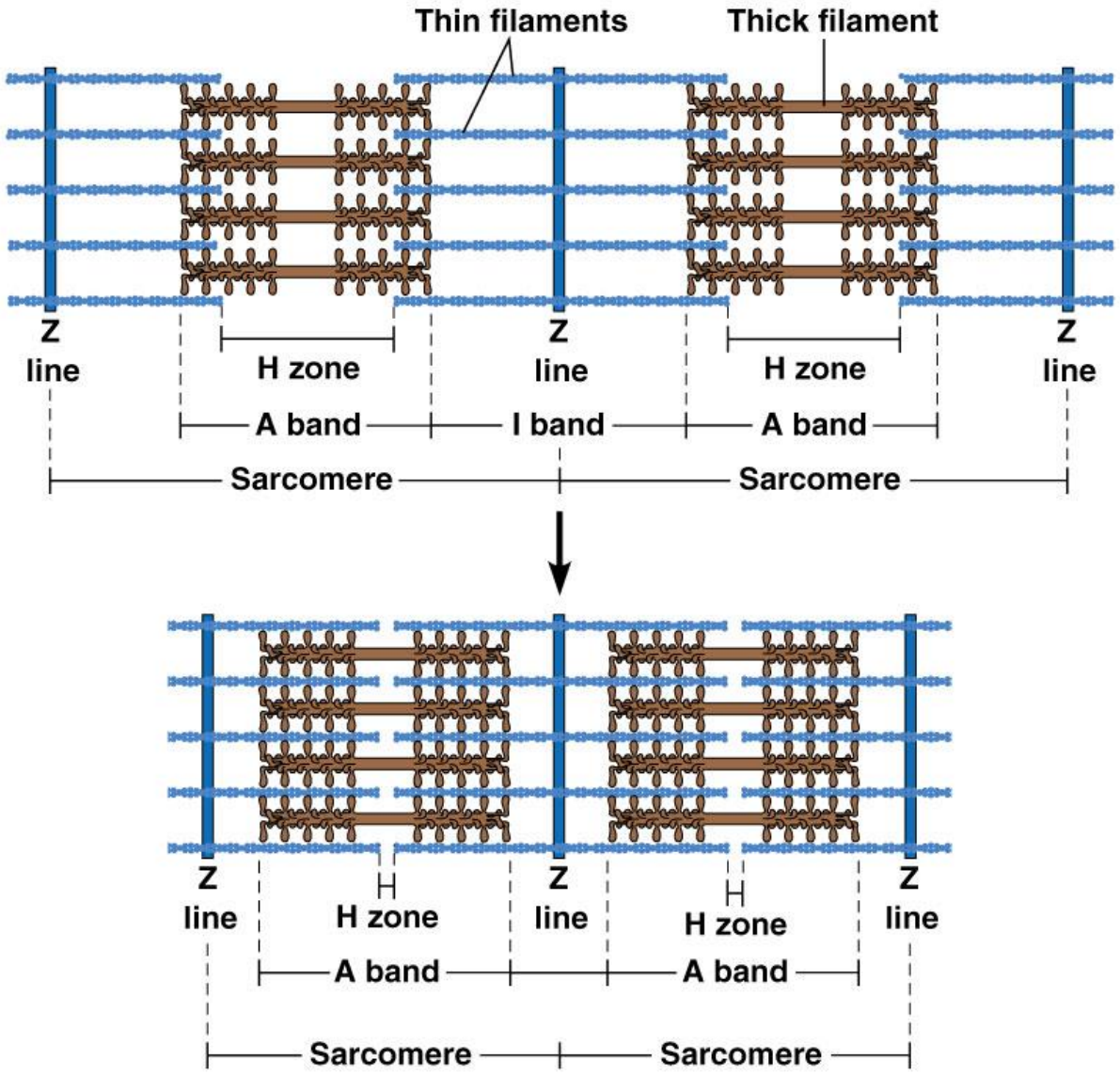


The Sliding-Filament Model Explains Muscle Contraction

- The **sliding filament model** was proposed in 1954

- According to the model, muscle contraction is due to thin filaments sliding past thick filaments, with no change in length of either

Figure 14-17A



(a) Sliding filament model

Muscle contraction

- Thin filaments slide such that they are drawn into the spaces between adjacent thick filaments, overlapping with them and narrowing the I band

- The amount of force generated during contraction depends on the number of myosin heads that make contact with the thin filaments

Cross-Bridges Hold Filaments Together and ATP Powers Their Movement

- Regions of overlap between thin and thick filaments are always characterized by the presence of transient **cross-bridges**

Cross-Bridge Formation

- Cross-bridges are formed from links between the F-actin of thin filaments and myosin heads of thick filaments
- Cross-bridges must dissociate repeatedly during contraction; each cycle of cross-bridge formation causes thin filaments to interdigitate further with thick filaments
- The result is shortening of sarcomeres and muscle fiber contraction

Muscle contraction

- Muscle contraction is the net result of a set of repeated events involving the myosin head
  - It binds to actin subunits on the thin filament
  - It undergoes an energy-requiring change in shape that pulls the thin filament
  - Then it breaks the association with the actin filament and associates with a site farther along the filament closer to the Z line

ATP and the Contraction Cycle

- The driving force for cross-bridge formation is ATP hydrolysis, catalyzed by the myosin heads
- The mechanism of muscle contraction is a four-step cycle

The contraction cycle

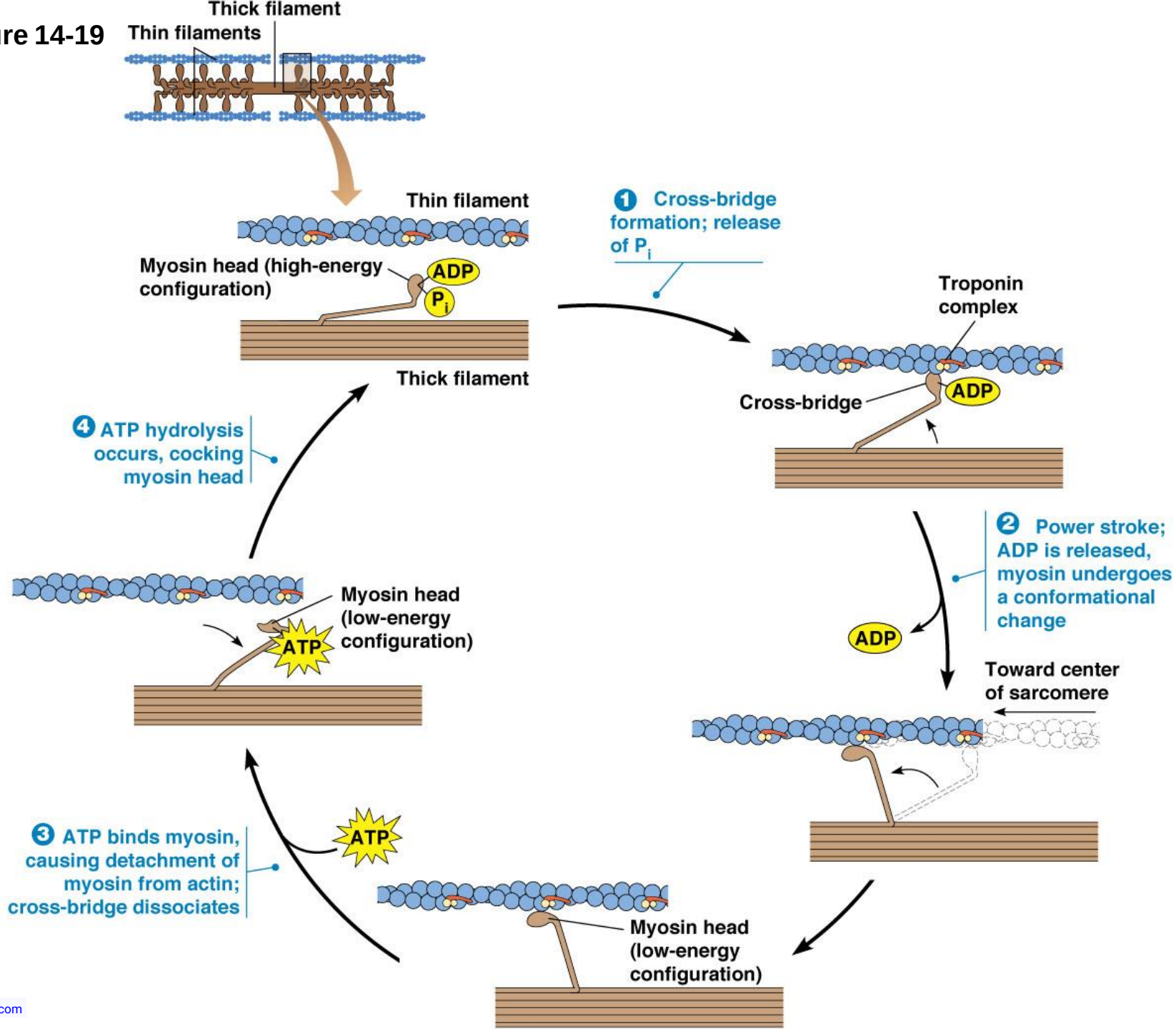
- A myosin head binds loosely to the actin filament in the high-energy configuration (ADP- and P_i-bound); this bond is tightened upon release of P_i (1)

- The myosin undergoes a conformation change releasing ADP; this causes the head to move, and the sliding of the thin filament with respect to the thick filament (the power stroke, 2)

The contraction cycle (continued)

- As ATP binds the myosin head, the cross-bridge dissociates due to a conformation change in the myosin (3)
- ATP hydrolysis occurs, returning the myosin head to the high-energy state, and it binds the thin filament again at a point closer to the Z line (4)

Figure 14-19



The Regulation of Muscle Contraction Depends on Calcium

- Most skeletal muscles spend more time in the relaxed state than in contraction

- Contraction and relaxation must be coordinated

The Role of Calcium in Contraction

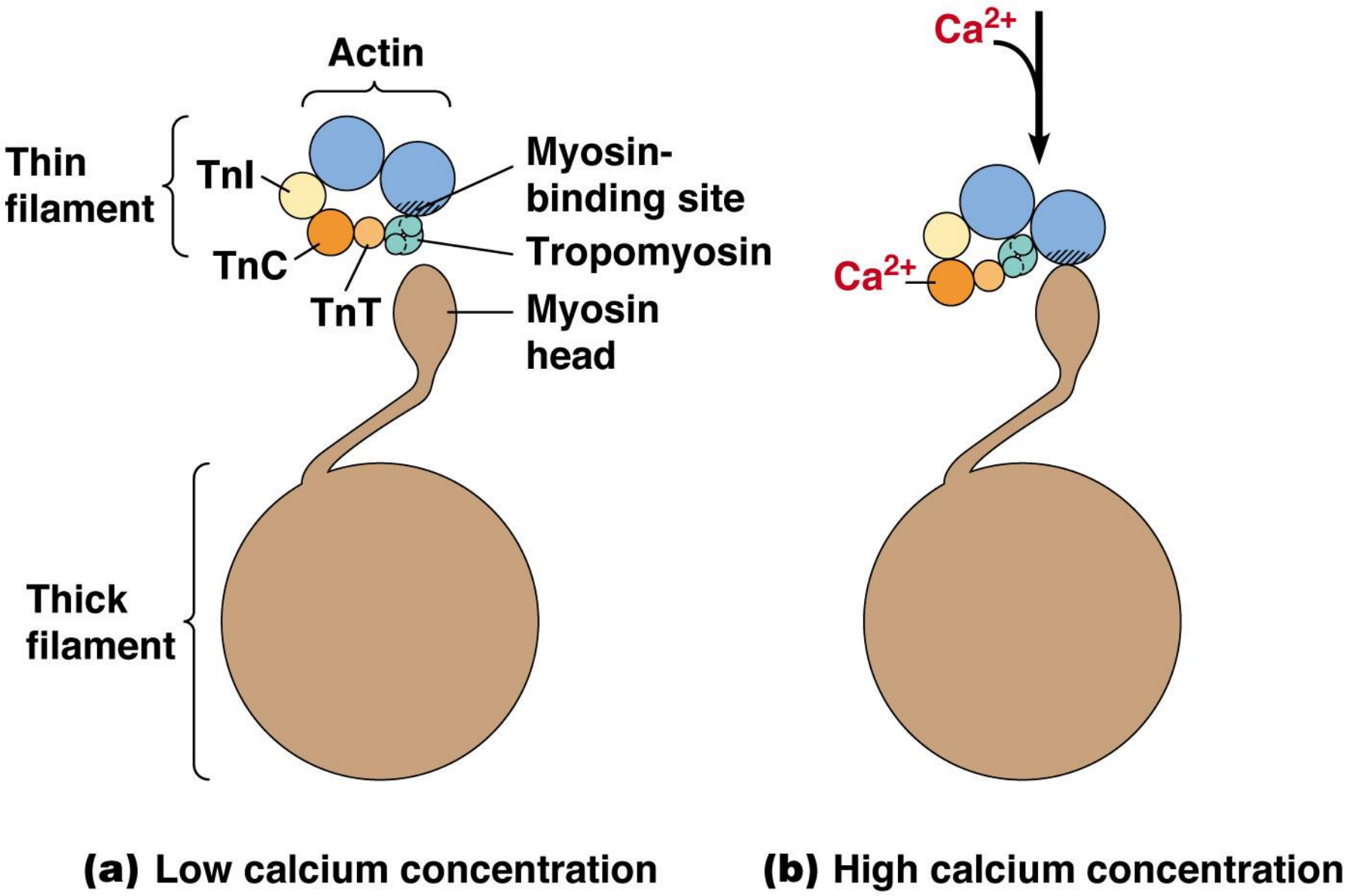
- The regulatory proteins *tropomyosin* and *troponin* regulate the availability of myosin binding sites on actin filaments in a calcium-dependent manner

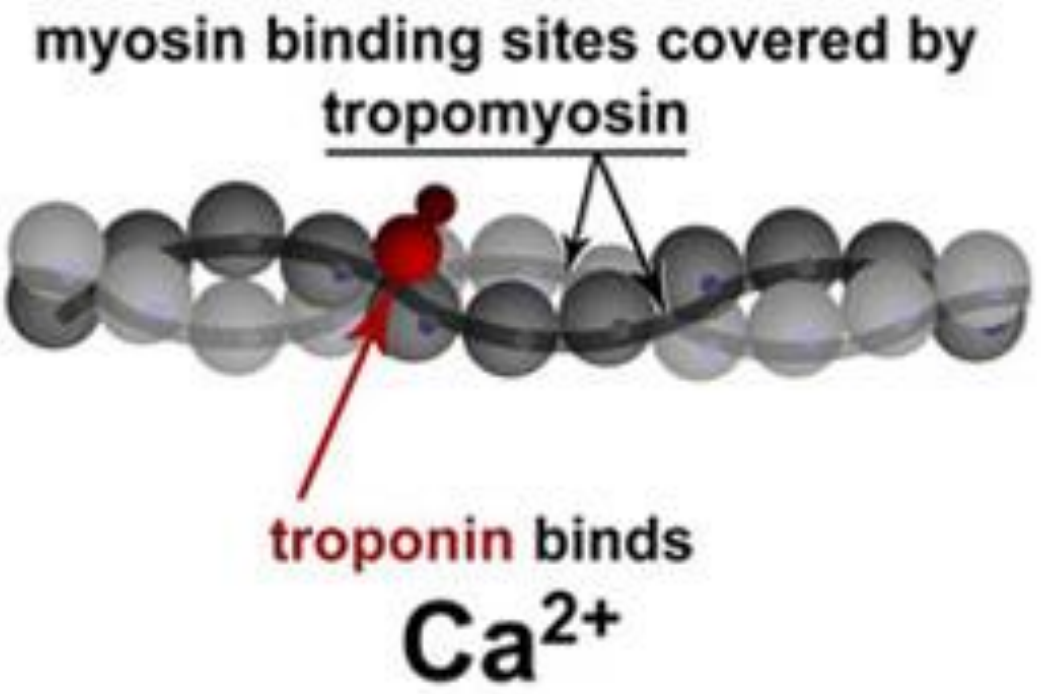
- Myosin binding sites on actin are normally blocked by tropomyosin, which must be moved if cross-bridges are to form

Contraction and calcium concentration

- When the calcium concentration is low, tropomyosin blocks the myosin binding sites on the actin filament, preventing interaction with myosin
- At higher concentrations, calcium binds TnC, causing tropomyosin to shift, and allowing myosin to bind

Figure 14-20





Regulation of Calcium Levels in Skeletal Muscle Cells

- Calcium levels are controlled by nerve impulses from *motor neurons*
- Muscle contraction is regulated by calcium ions in the *sarcoplasm* (cytosol of a muscle cell)
- Muscle cells have features that facilitate rapid changes in Ca^{2+} concentration

Events at the Neuromuscular Junction

- **Neuromuscular junction**: the site where a nerve contacts a muscle cell; conveying a signal to contract in the form of an *action potential*

- At the neuromuscular junction, *axon terminals* make contact with the muscle cell

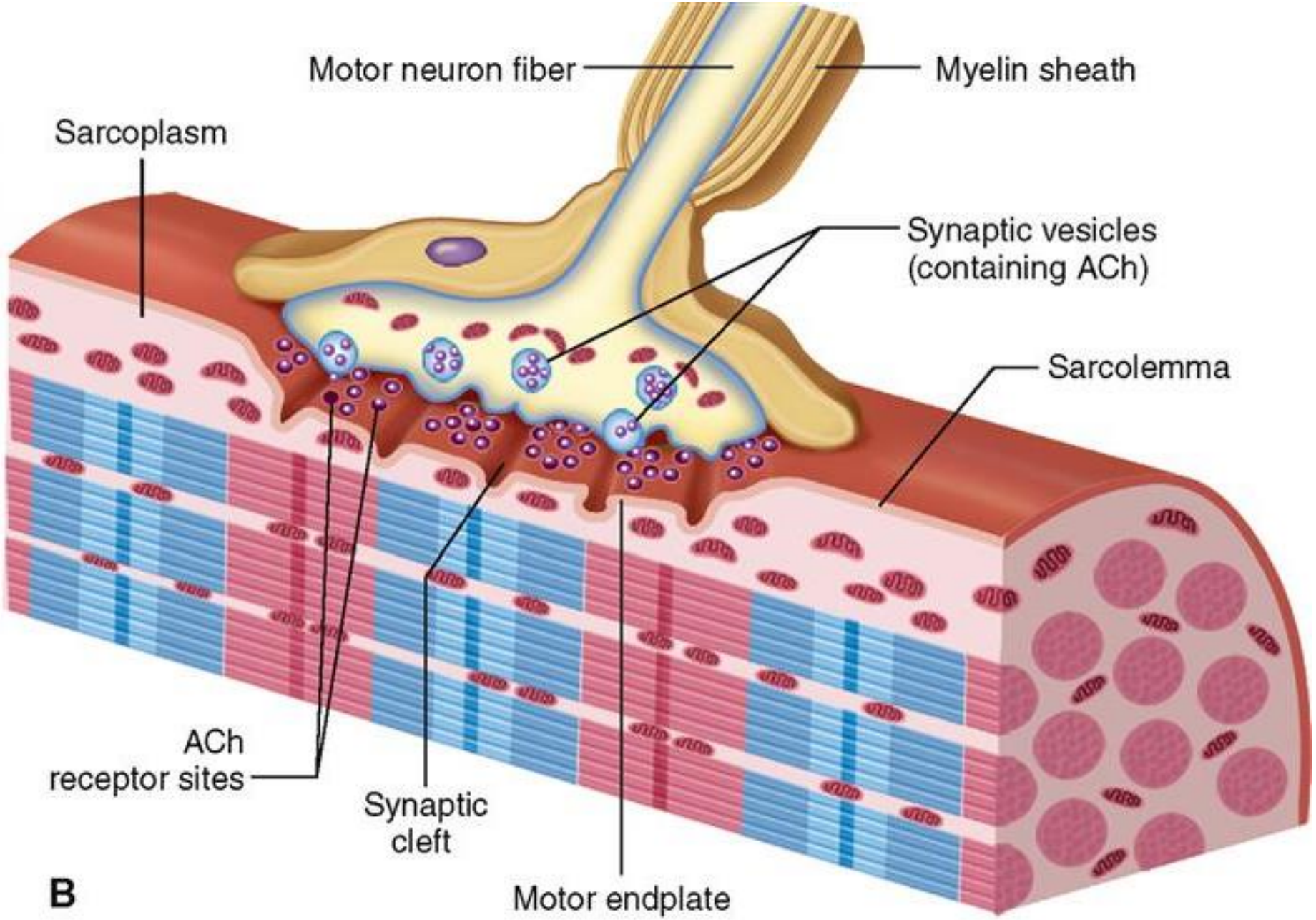
- These terminals store *acetylcholine,* which is released in response to an action potential

At the neuromuscular junction

- The *motor end plate* is the area of muscle cell membrane (*sarcolemma)* under the axon terminals
- There, acetylcholine receptors are clustered near axon terminals
- When a receptor binds acetylcholine it opens a pore in the membrane for inward Na^+ flow

At the neuromuscular junction (continued)

- The inward flow of sodium ions causes a membrane depolarization to be transmitted away from the sarcolemma at the motor end plate



Transmission of an Impulse to the Interior of the Muscle

- Membrane depolarization spreads through the sarcolemma via the **transverse** (**T**) **tubule system**

- Inside the cell the T tubule system contacts the **sarcoplasmic reticulum** (**SR**; similar to ER)

- The SR is poised to release calcium ions and then quickly remove them as needed

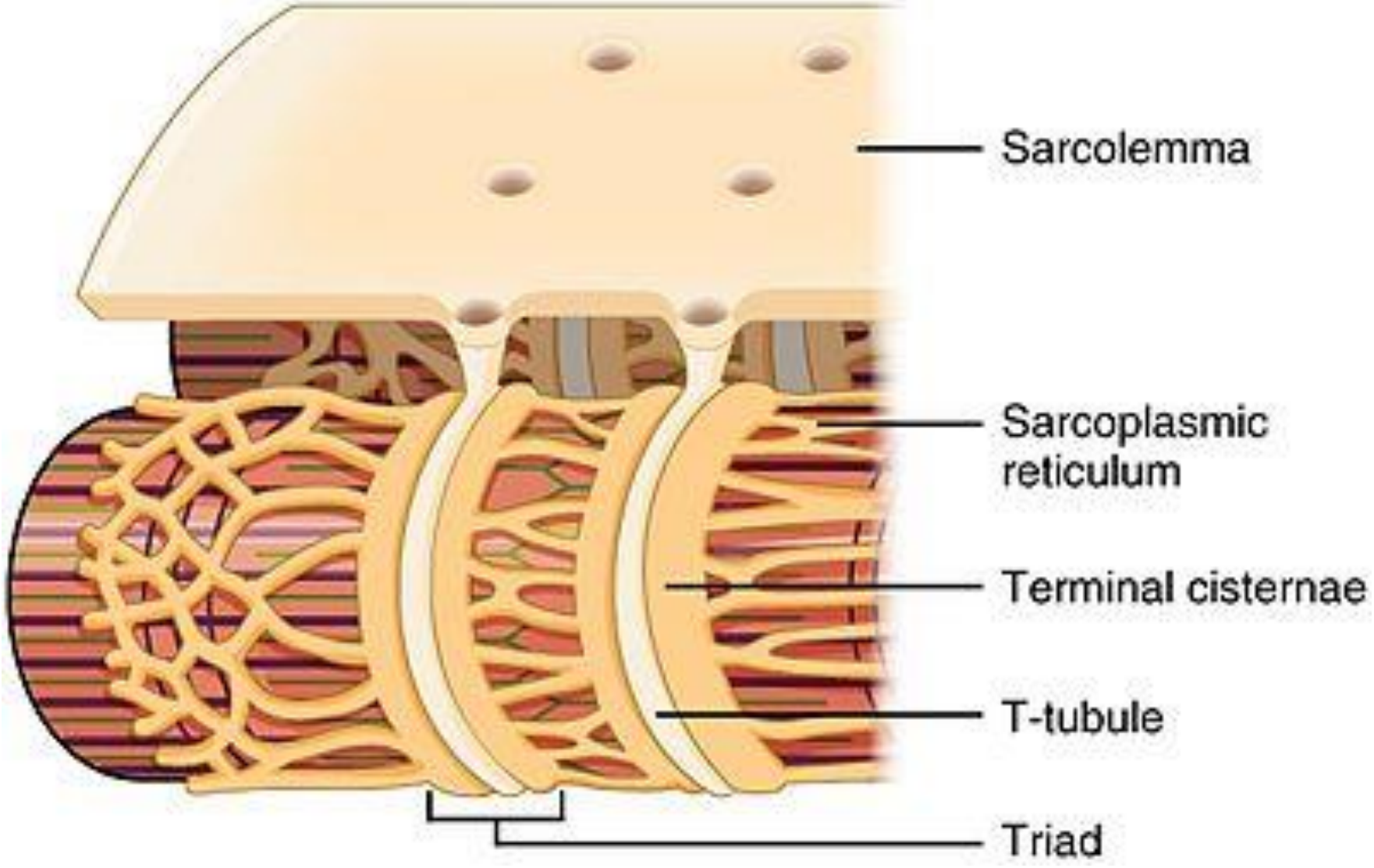
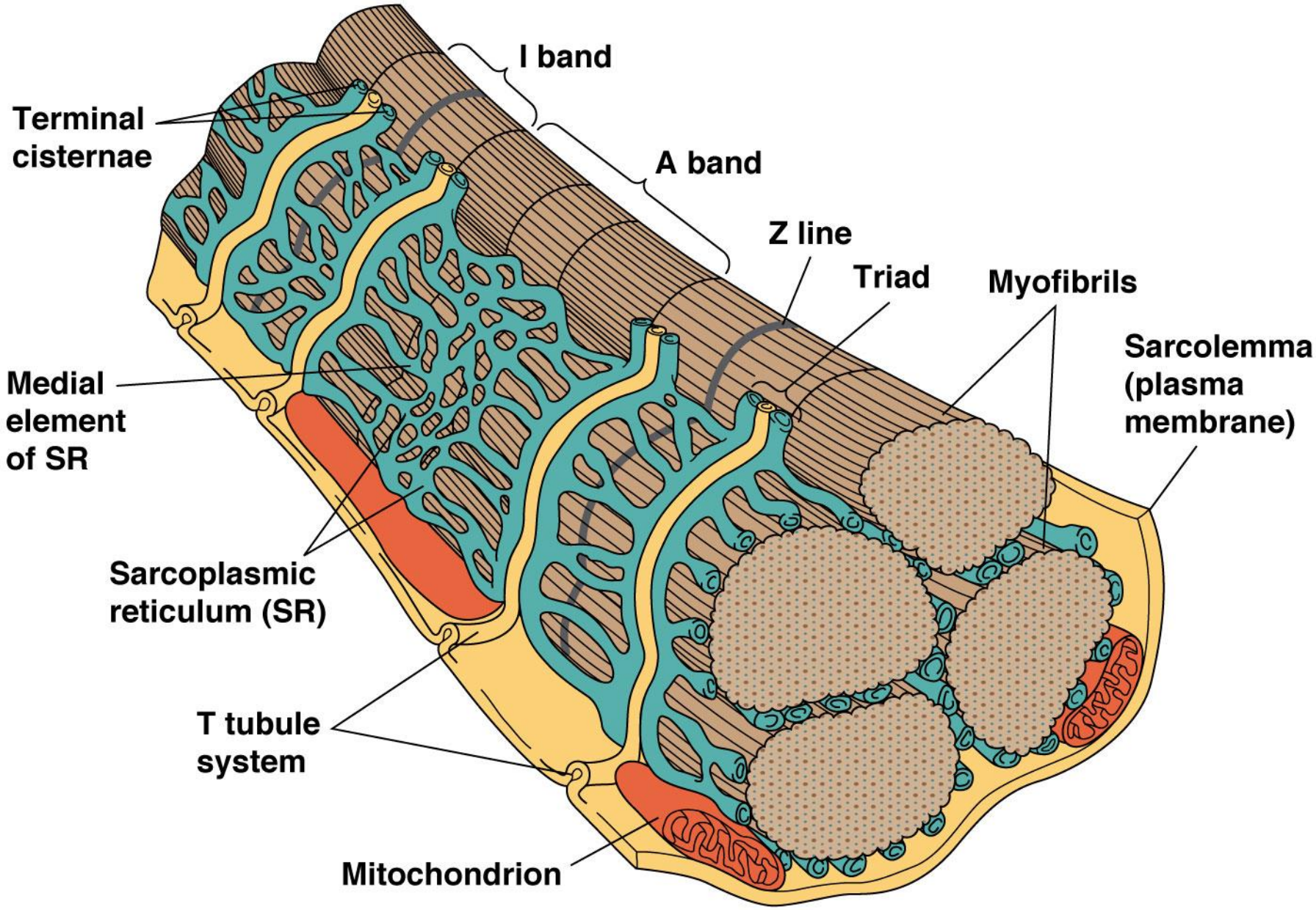
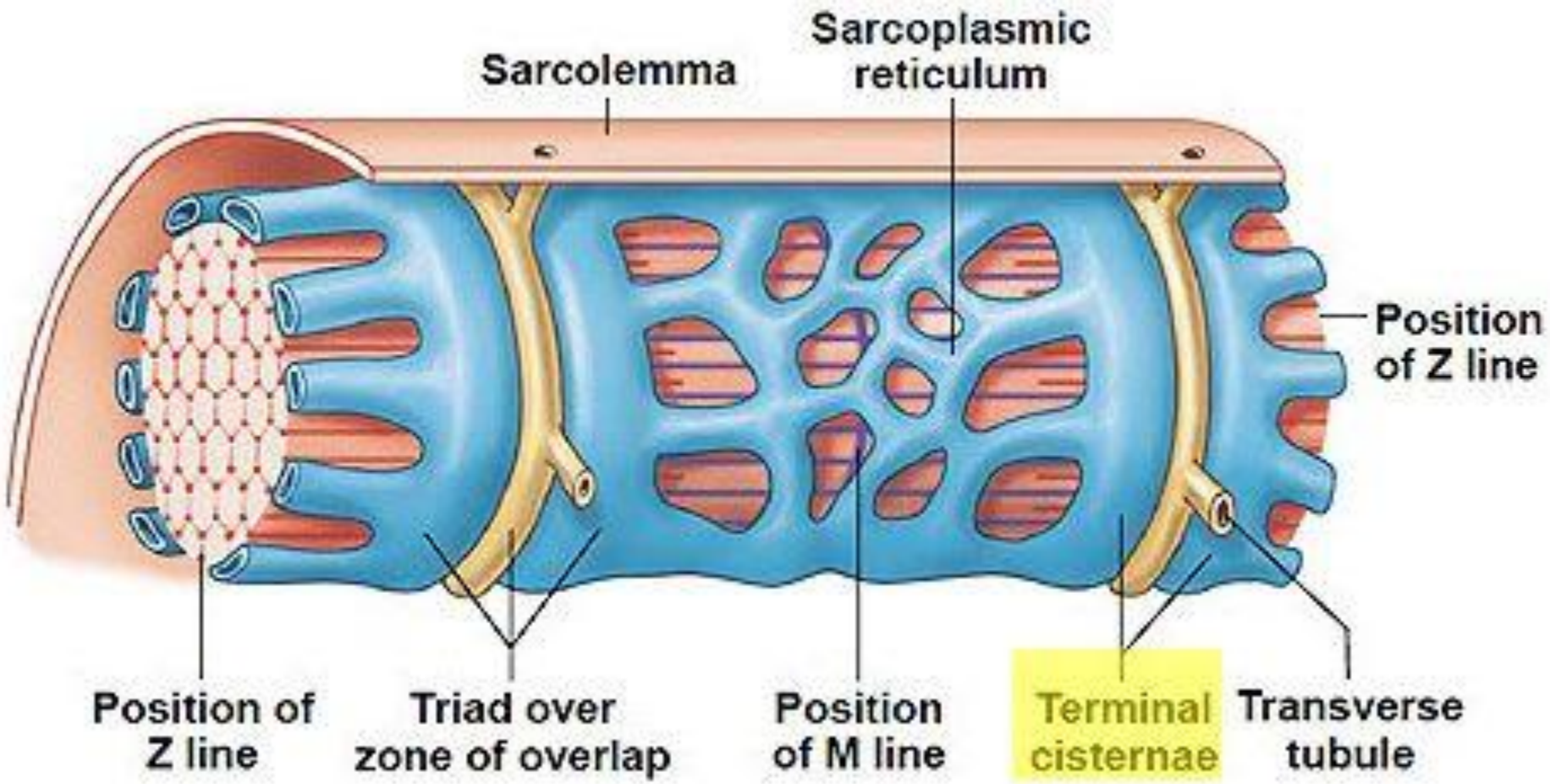


Figure 14-21



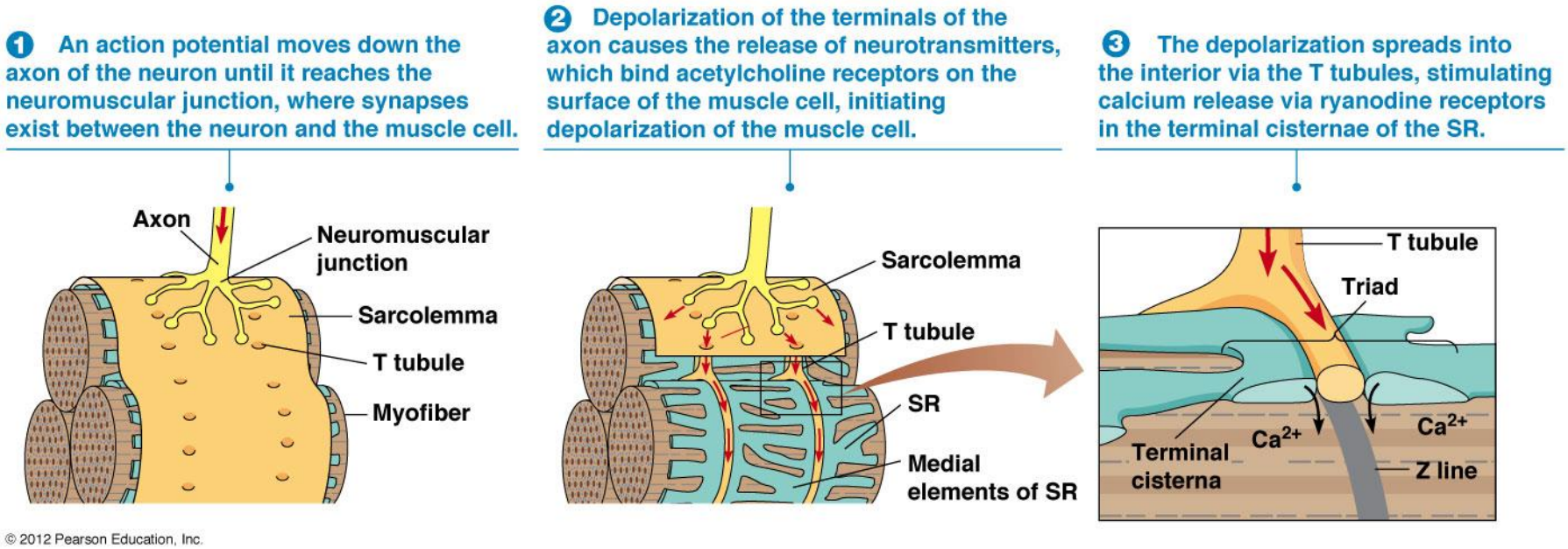


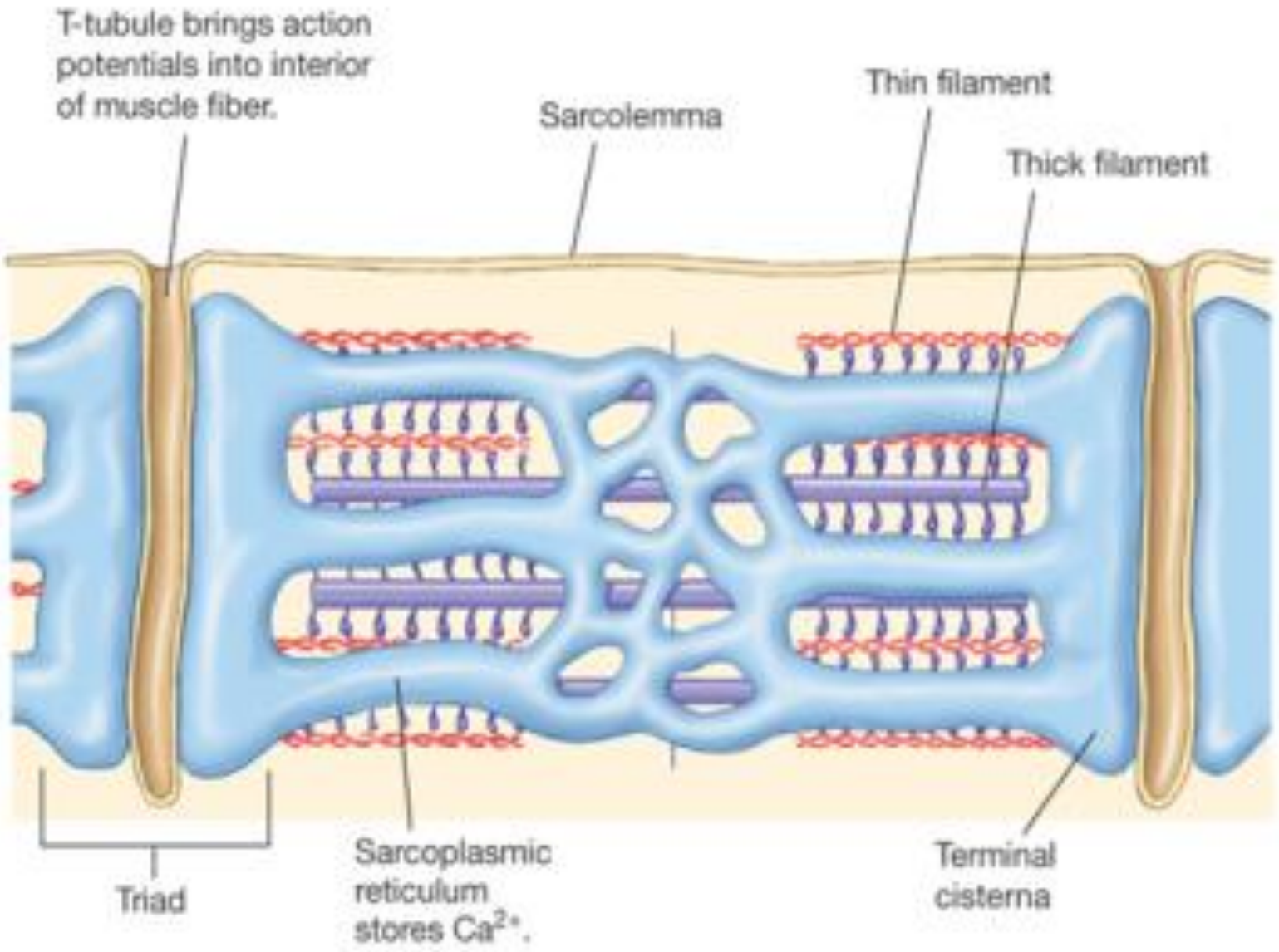
Terminal cisternae

- Terminal cisternae are generally found near a T tubule, forming a structure called a **triad**

- An action potential from the motor end plate spreads over the sarcolemma and enters a T tubule

Figure 14-22





Terminal cisternae and calcium

- As the action potential travels down the T tubule, it activates voltage-gated calcium channels in the tubule

- The receptor channels open and release calcium into the sarcoplasm, causing contraction

Muscle relaxation

- Released calcium triggers a contraction; for muscles to relax, calcium levels must decrease
- The SR membrane has a **calcium ATPase** to pump calcium into the SR cisternae