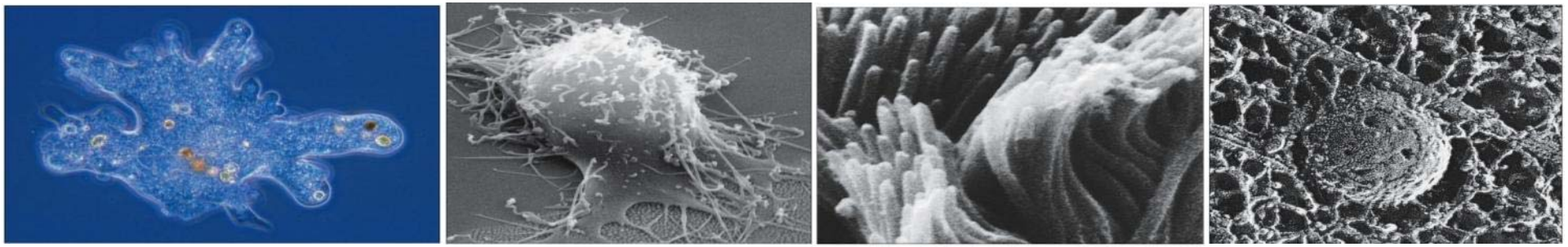
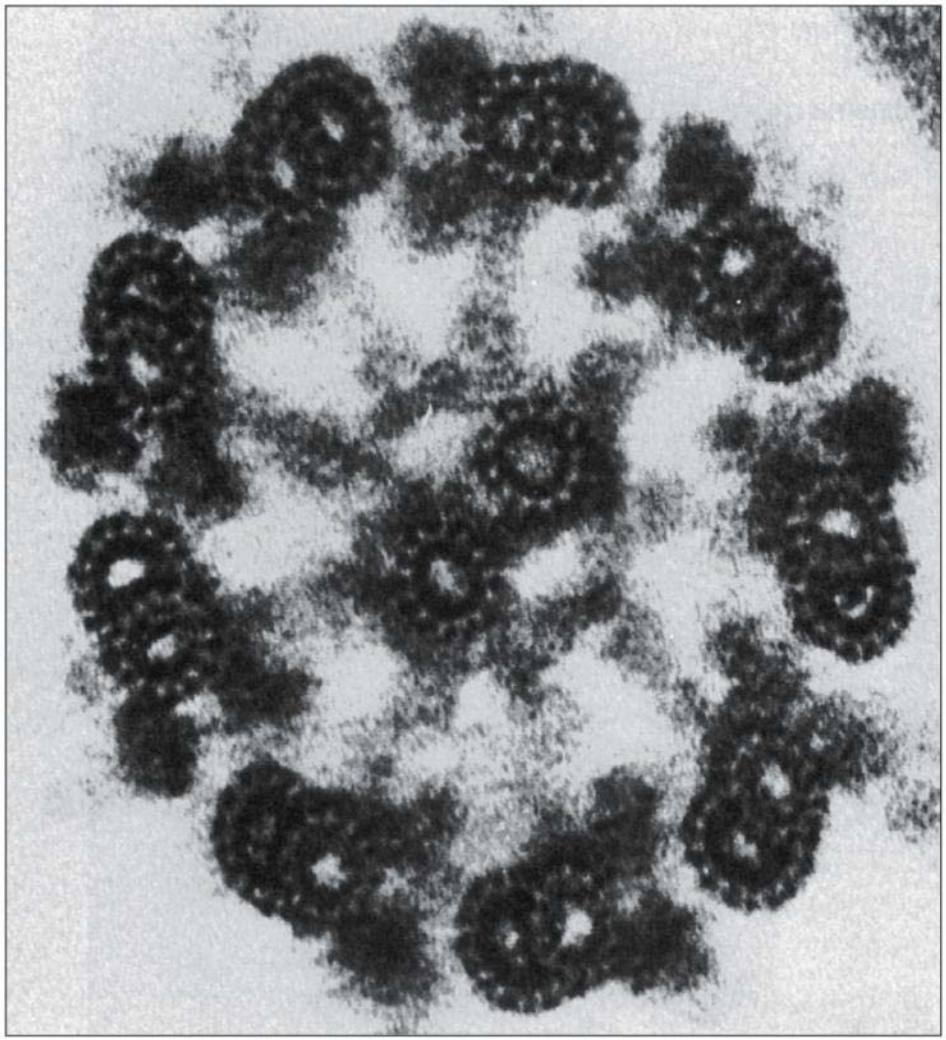


World of the Cell



Chapter 14: Cellular Movement

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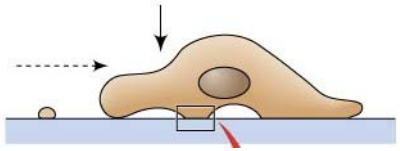
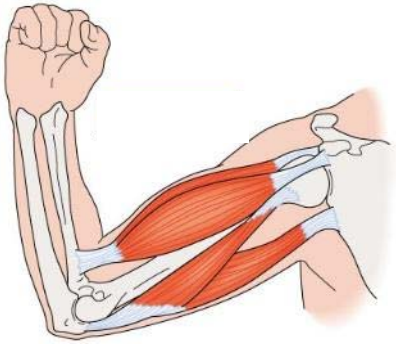
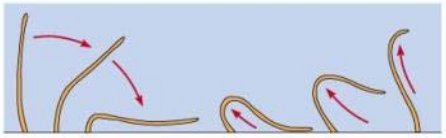
What is cellular motility?



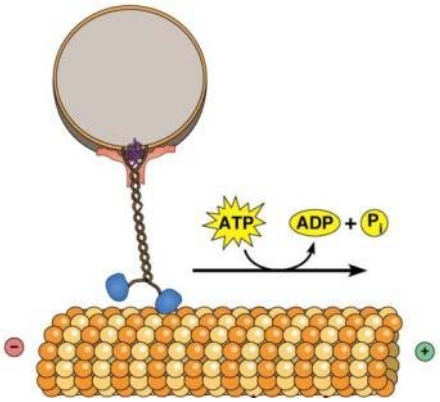
- Movement of single cells (macrophages, amoeba, cancer cells)



- Contractions of muscles (skeletal muscle or autonomous heartbeat and uterine contractions)

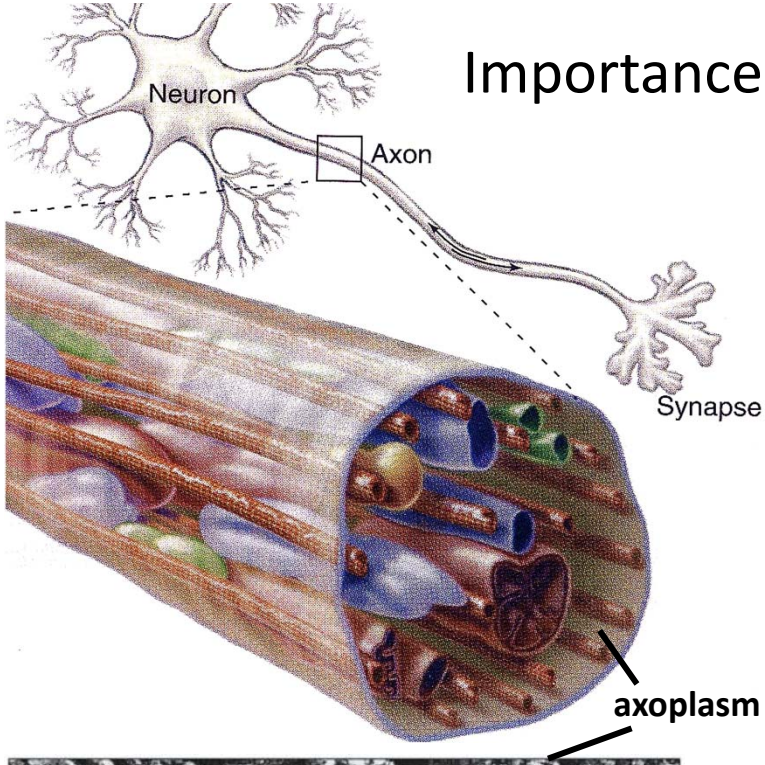


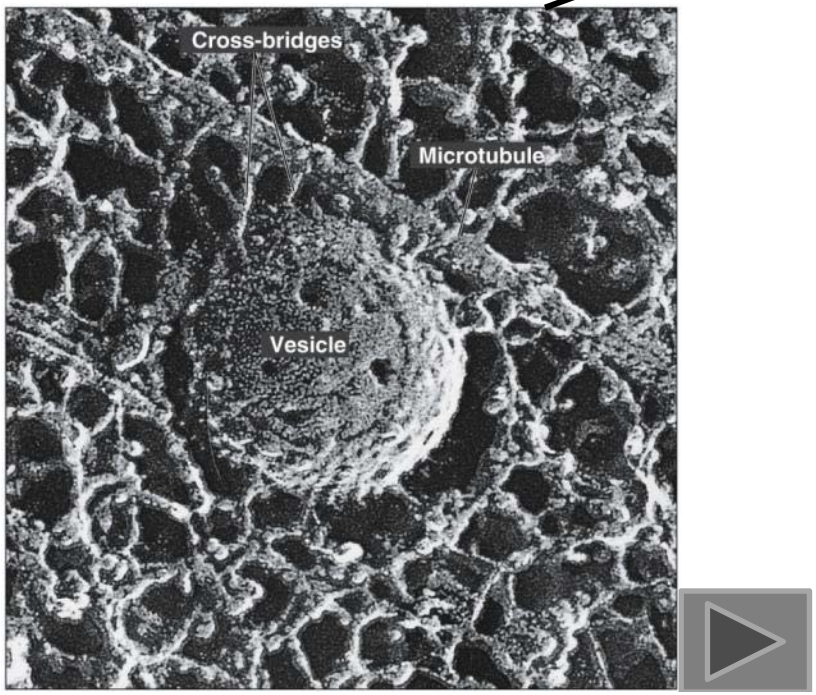
- Beating of cilia (mucus movements, *Paramecium*) and flagella (sperm)



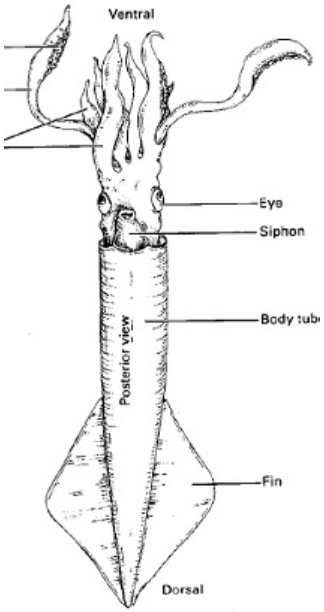
- Intracellular motility:
  - **Molecular motors** (kinesin, myosin): Movement of cargo (vesicles) on cytoskeletal tracks (microtubules, actin)
  - **Chromosome separation** during mitosis

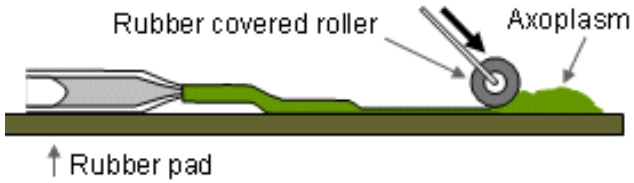
Importance of kinesin and dynein in neuronal transport





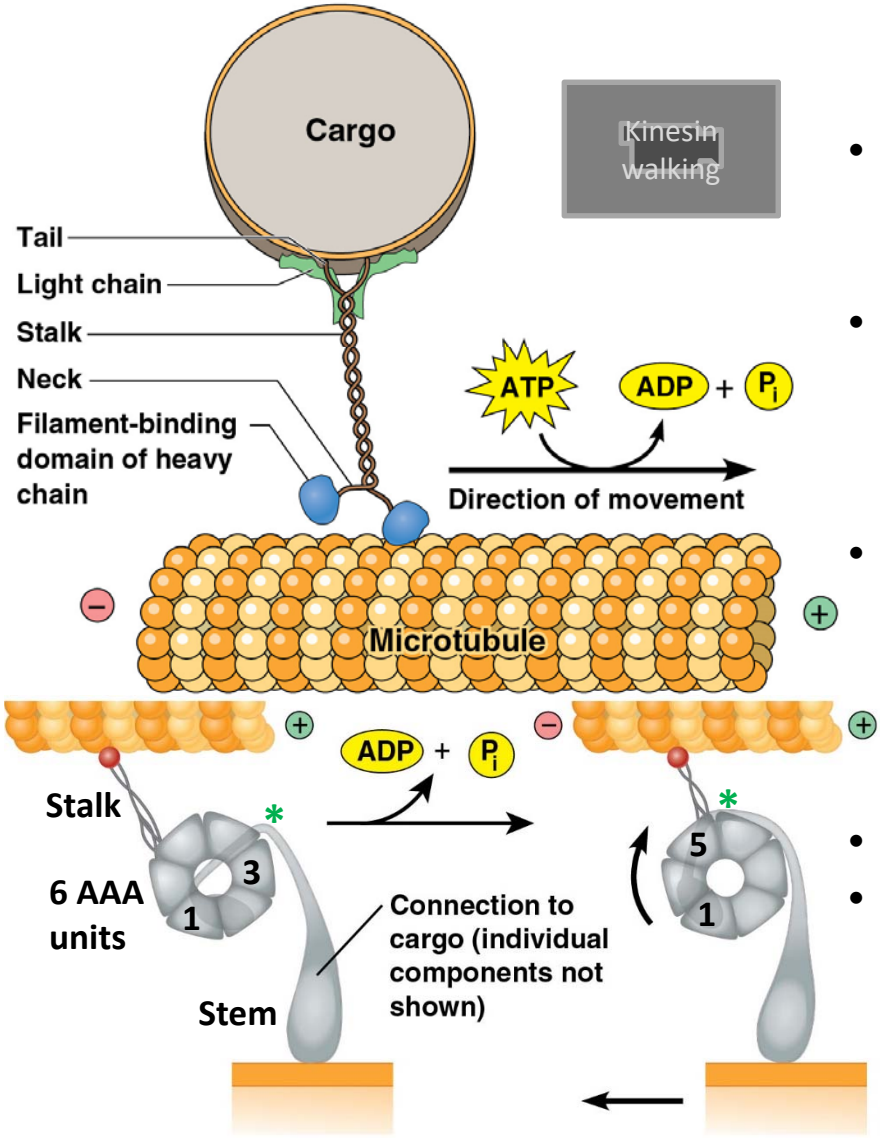
- Because no protein synthesis occurs at the synapse, neuron requires a transport system to move "cargo" (vesicles packed with proteins/neurotransmitters) from the cell body (soma) to the synapse
- In **axonal transport**, we can observe **anterograde** trafficking (from the soma to the synapse) of organelles, and **retrograde** transport of recycled vesicles (from the synapse back to the soma)
- Organelle movement (at speeds of **2 µm/s**) can be observed by simply squeezing out the **axoplasm** from a giant squid axon and observing it under DIC microscopy





Organelle movement

Basic mechanism of kinesin and dynein movement



1->3 to 1->5 movement of lever arm

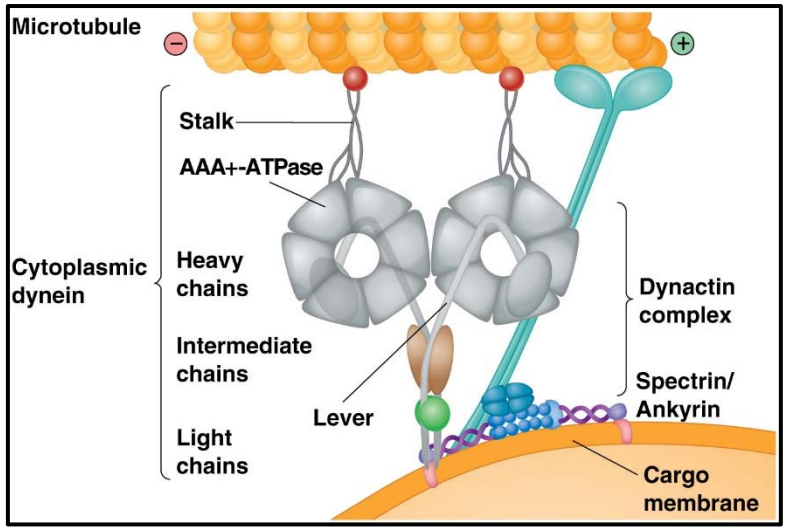
- **Axonal transport** is accomplished by two motor proteins that have a preferred direction (they recognize the polarity of microtubules): **kinesins** (move anterograde) and **dynein** (move retrograde)
- **Molecular motors** are able to convert chemical energy from ATP hydrolysis into mechanical work: they can "walk" along microtubules
- Each motor has a **globular head** that connects to the microtubule, a long **coiled coil region** and a (globular) **cargo binding domain** that recognizes specific cargo (vesicles, proteins, RNA etc.)
- When a kinesin "walks", the "**front foot**" (leading head) makes a step of 8 nm to the following tubulin subunit and at the same time the "**back foot**" (trailing head) detaches to make the next step (powered conformational changes).
- This process is coupled by ATP hydrolysis
- **Efficiency** of this motor to convert chemical energy into mechanical energy is very high (**60-70%**)

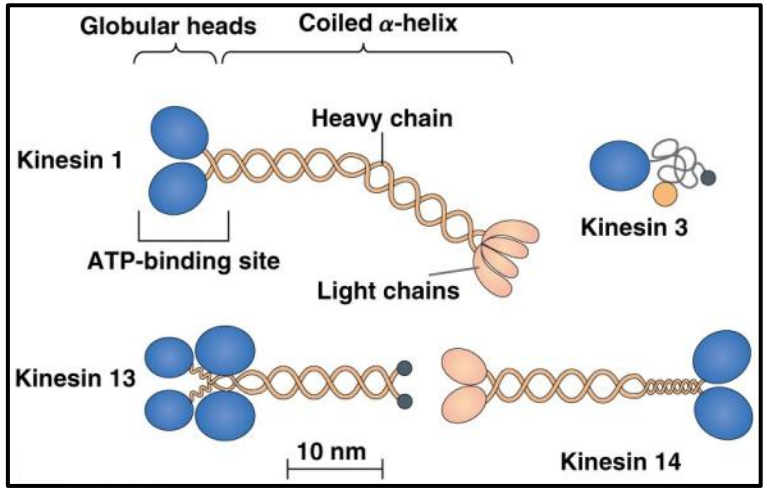
The variety of microtubule-associated motors

- 2 classes of dynein: **Cytoplasmic** (found in most cell types) and **axonemal** (found in cilia)
- Dynein requires a large adaptor protein **dynactin** to carry and transport cargo
- Kinesins are grouped in **families** based on their structure. Most of them are dimers but some are monomers (kinesin-3) or tetramers (kinesin-5). One kinesin depolymerizes MTs (kinesin-13, MCAK) and one kinesin moves into the opposite direction (kinesin-14).

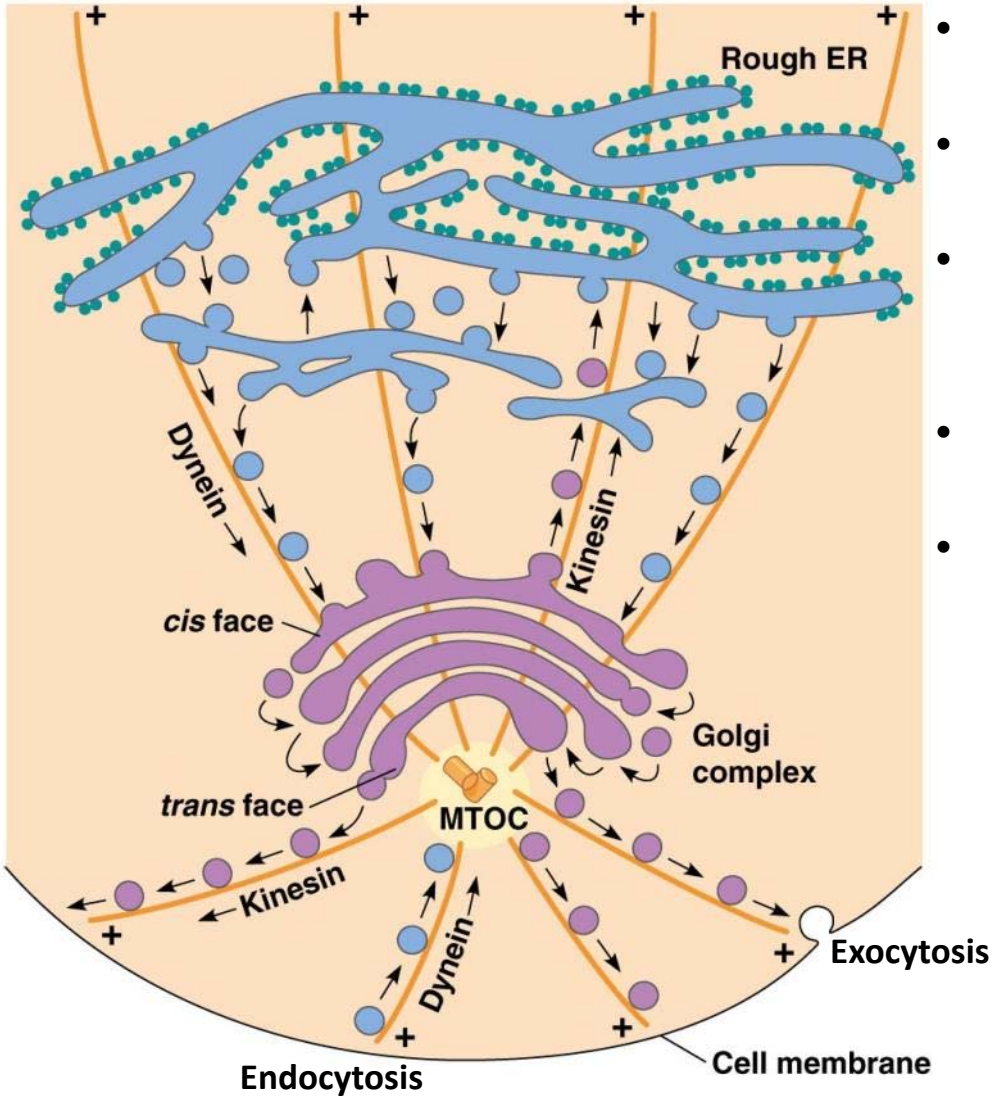
Motor Protein	Typical Function
Microtubule (MT)-Associated Motors	
Dyneins	
Cytoplasmic dynein	Moves cargo toward minus ends of MTs
Axonemal dynein	Activates sliding in flagellar MTs
Kinesins*	
Kinesin 1 (classic kinesin)	Dimer; moves cargo toward plus ends of MTs
Kinesin 3	Monomer; movement of synaptic 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

*selected examples of kinesin



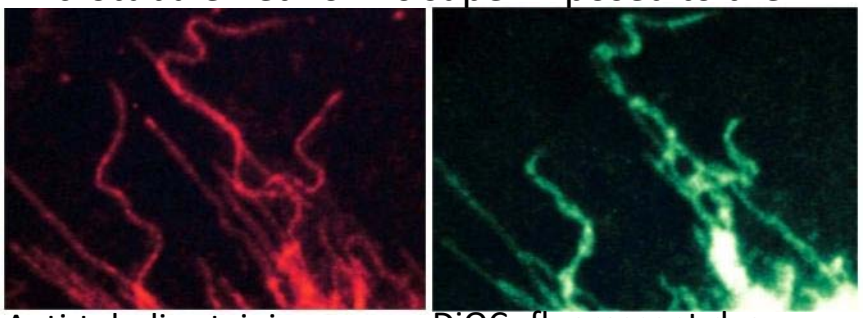


Microtubule motors shape the endomembrane system and power ER to Golgi transport



- Microtubules and their motors **position and shape** the ER and Golgi
- ER and Golgi are superimposed to the microtubules
- **Nocodazole** treatment (and disrupting motor function) results in breakdown of the endomembrane system
- **Dynein** is mostly responsible for the vesicle trafficking from the ER to the Golgi.
- **Kinesins** are mostly responsible for vesicle trafficking from the Golgi to the Cell membrane.

Microtubule network is superimposed to the ER

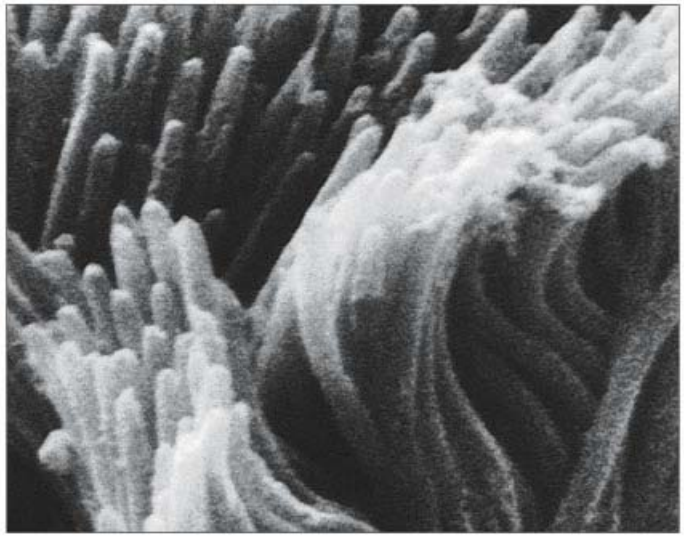


Anti-tubulin staining

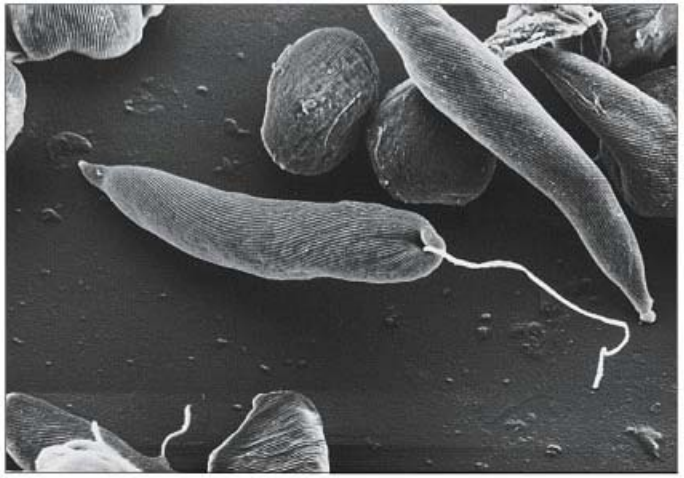
$DiOC_6$ fluorescent dye (stains the ER)

Cilia and flagella are the motile appendages of eukaryotic cells

- **Cilia**: appear in large numbers on surfaces and are **shorter** than flagella (2-10 µm)
- **Flagella**: appear usually as a single cell appendage and are **longer** than cilia (10-200 µm)
- Cilia can either **move** a unicellular **eukaryote forward** (*Paramecium*, a protozoa) or, e.g., **mucus**, dust, dirt on epithelial tissues in **respiratory tracts**.
- Long flagella on **sperm** or **algae** should not be confused with the bacterium flagellum (does not contain any microtubules and is constructed completely different)

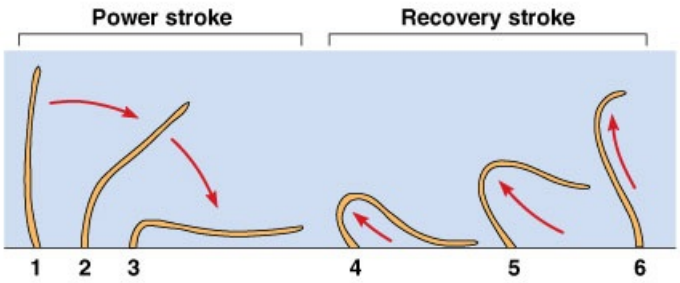


(a) Cilia on a mammalian tracheal cell



(c) Flagellum on unicellular alga *Euglena*

Cilia and flagella are surrounded by an extension of the plasma membrane but are still considered as an **intracellular structure**



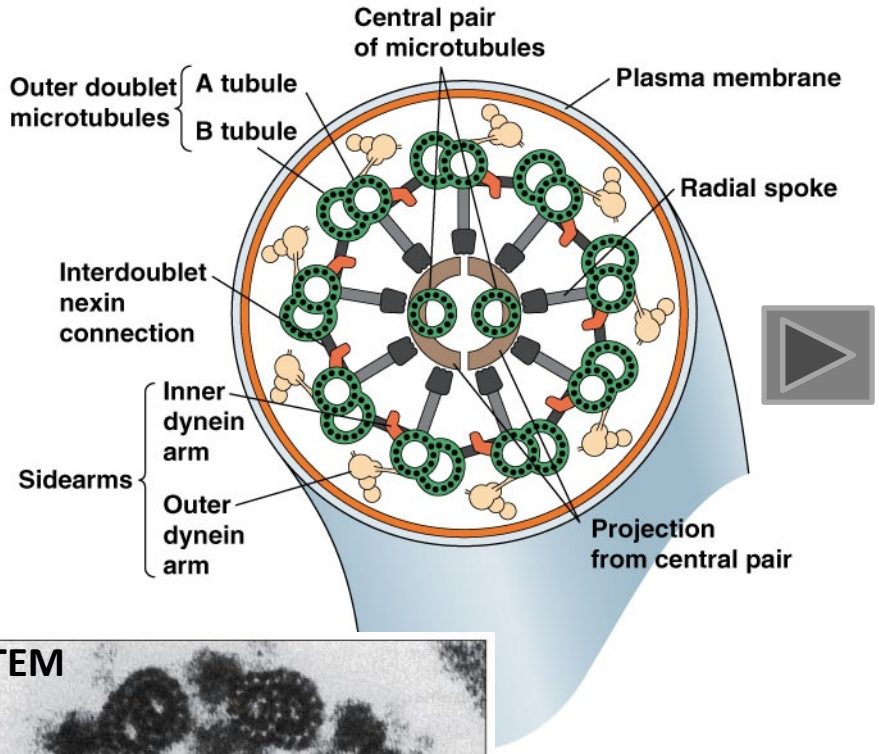
(b) Beating of a cilium

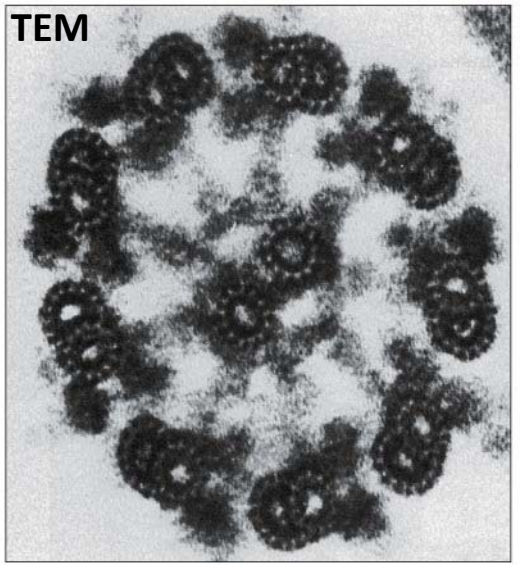
(d) Movement of flagellated eukaryotic cell

Cilia and flagella also differ in the way how they beat.

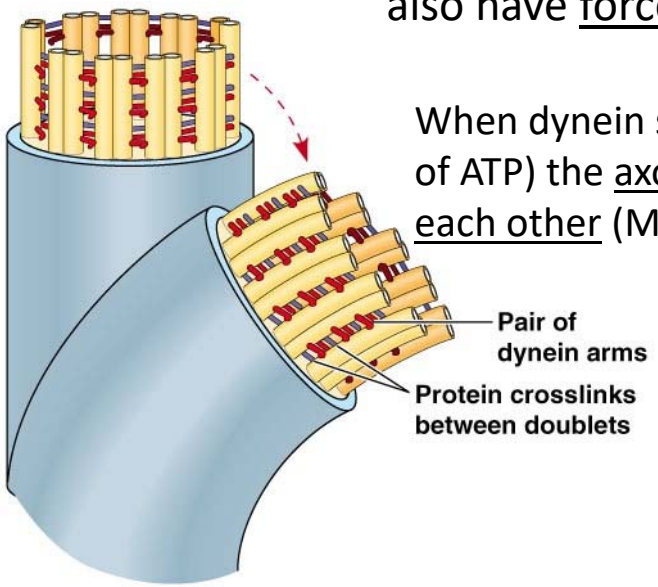
A cut bull sperm flagella still beats **autonomously** in the presence of ATP

The remarkable structure of cilia



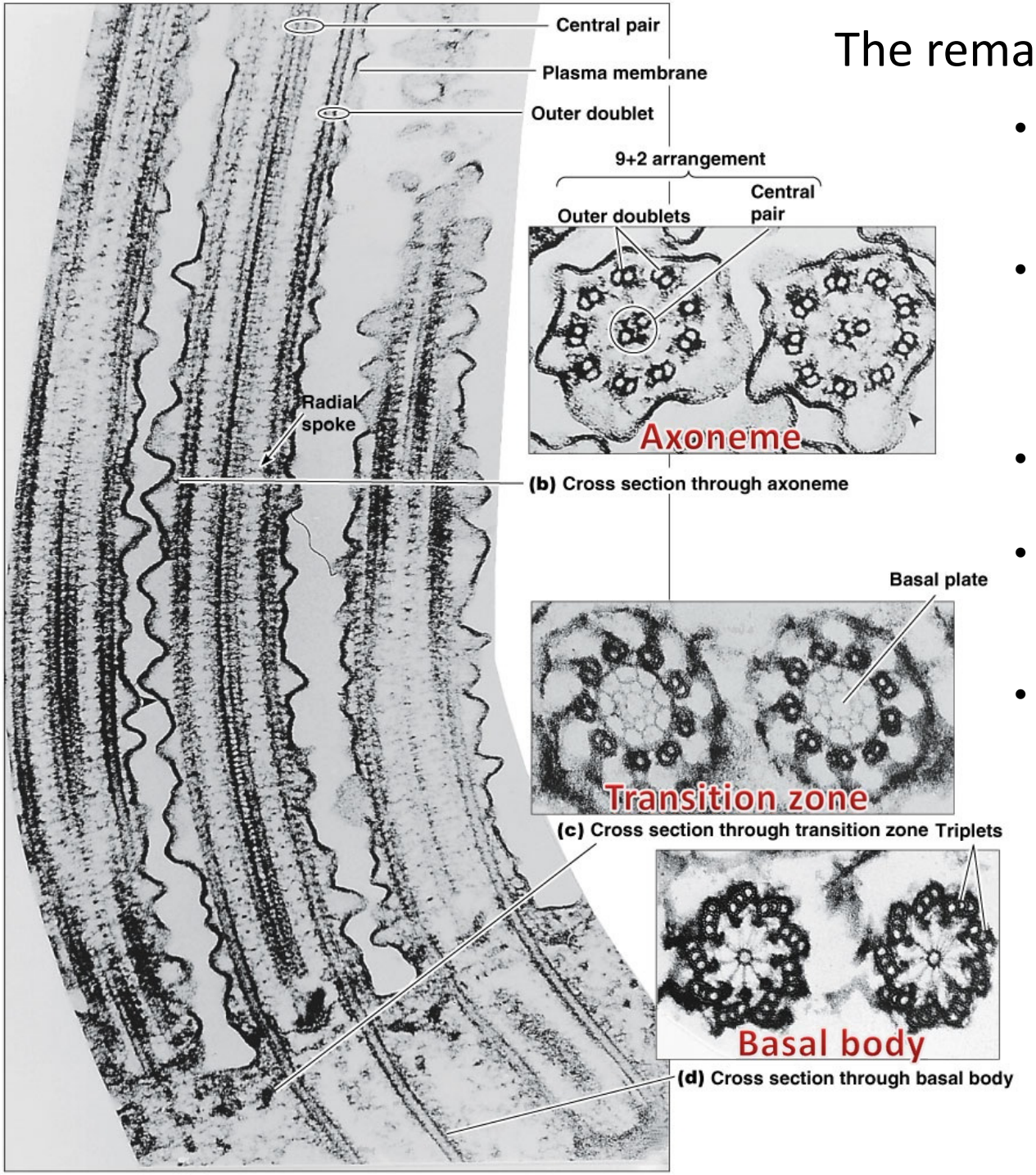


- The **axoneme** is the basic force-generating unit of the cilia
- It consists of **9 doublet microtubules** surrounding a **central pair of microtubules** (**9+2 pattern**)
- **Dynein** is attached to the **A tubule** and is able to move along the B tubule of the neighboring microtubule doublet
- The doublet microtubules are connected by a protein named **nexin**
- The outer 9 doublets are separated by the inner 2 singlets via **radial spokes** which also have force transmitting function

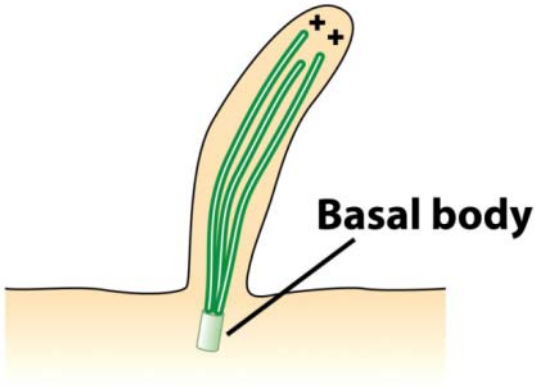


When dynein starts to move (in the presence of ATP) the axoneme bends as MTs slide pass each other (MT do not shorten)

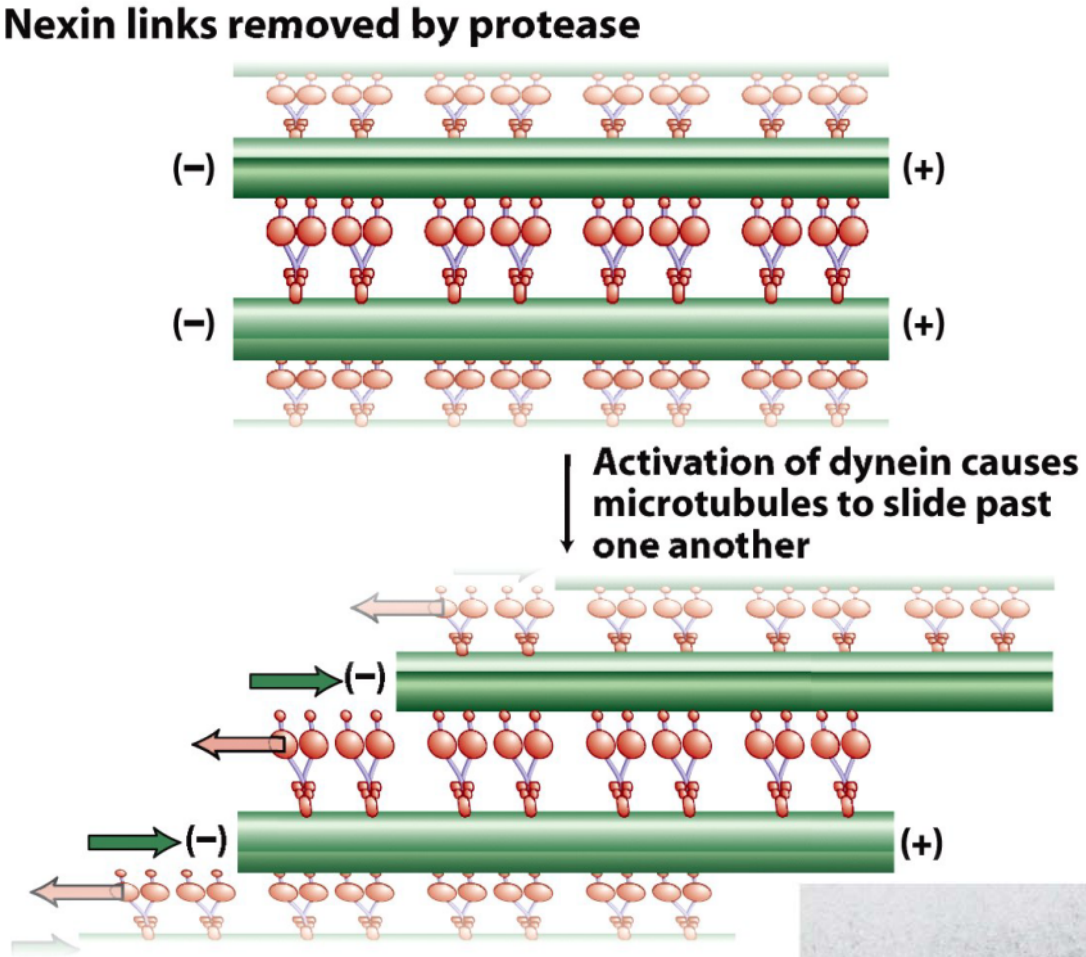
The remarkable structure of cilia



- The cilia emanates from a **basal body** that consists of 9 *triplet* microtubules
- Cilia development starts with a centriole that acts as a **nucleation site** for the axoneme microtubules
- The centriole is later referred to as a basal body
- The following **transition zone** lacks the central microtubule pair
- Besides tubulin, cilia microtubules contain another protein **tektin**

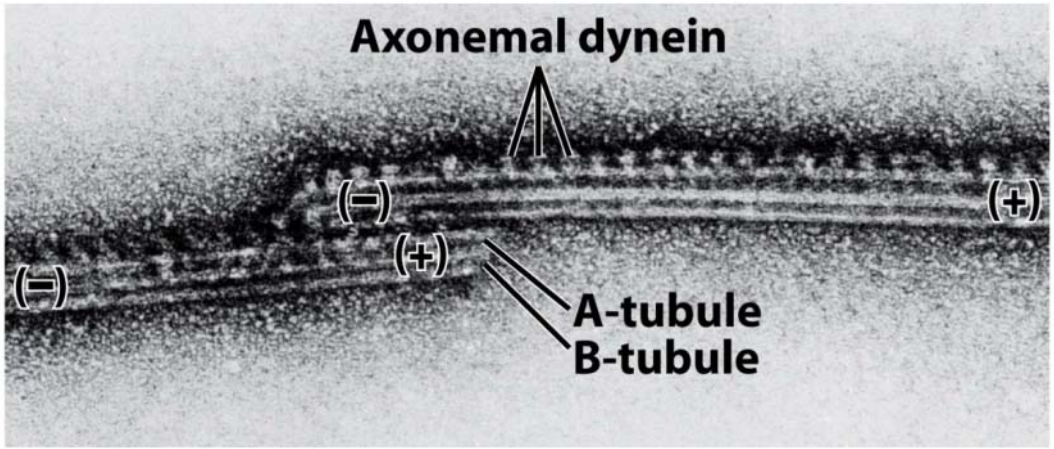


A classic experiment lead to the sliding MT model

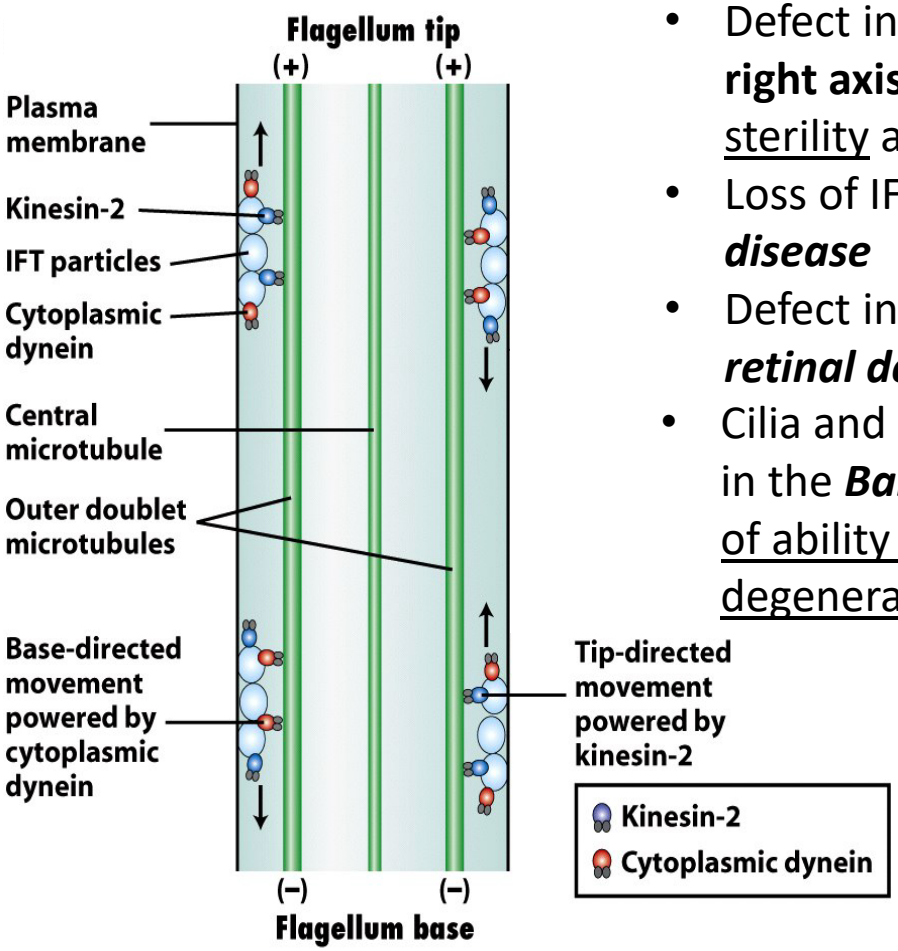


- Open the plasma membrane with **detergents**
- **Proteolysis** of cross-linking proteins as **nexin** (proteolytic cleavage)
- In the presence of ATP <u>doublet MT largely slide past each other</u> (visible in EM)
- <u>Dyneins</u> on the **A tubule** <u>walk along</u> the adjacent **B tubule** toward the (-) ends

Axoneme with removed plasma membrane and after proteolysis in TEM



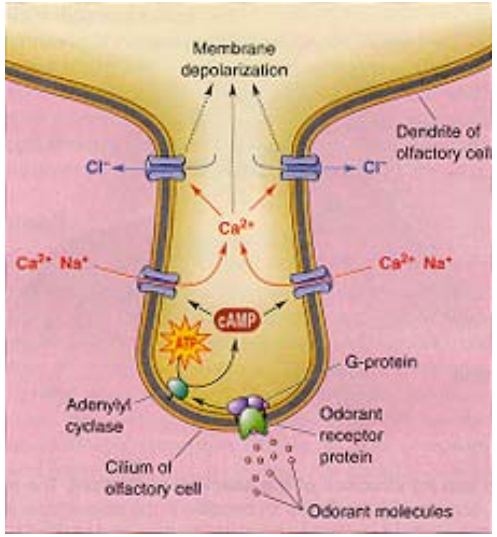
Intraflagellar transport (IFT) shuffles important molecules



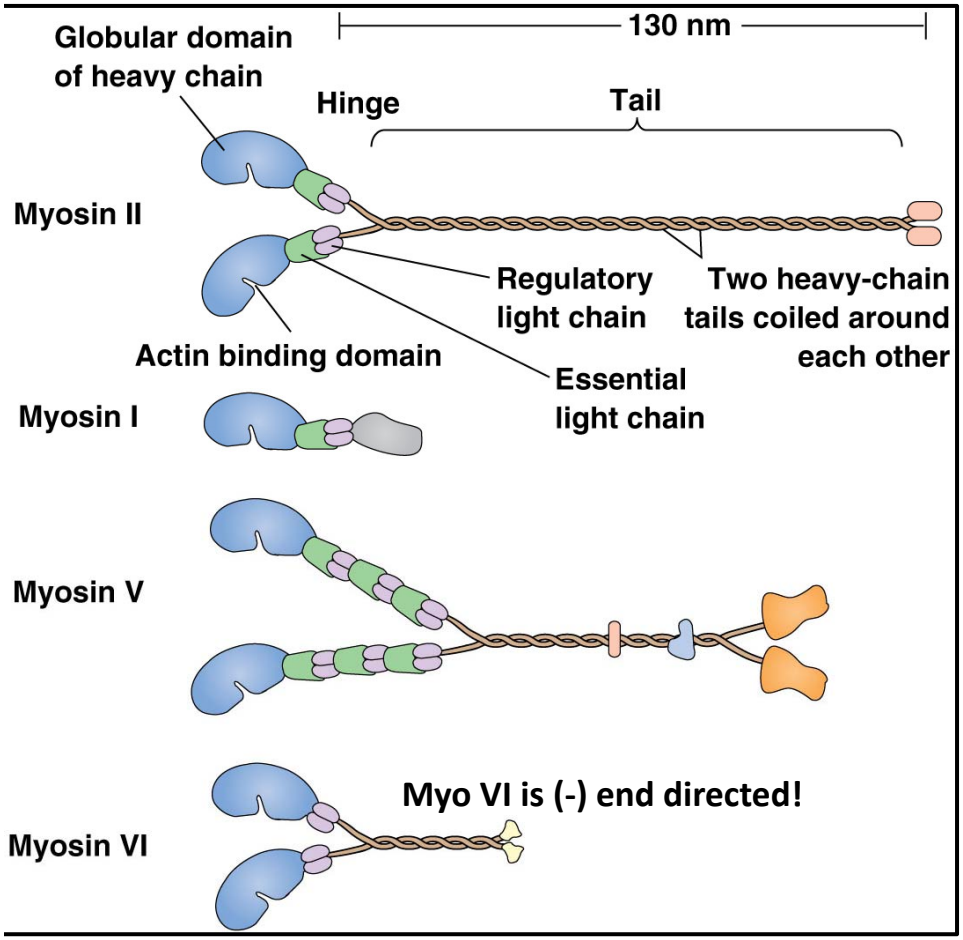
- Cilia of sensory neurons functions in **sensing the environment**, and important molecules need to be transported back and forth to the **tip** of these cilia
- **IFT particles** bind **kinesin** and **dynein** at the same time for fast shuttling of cargos in **two directions**

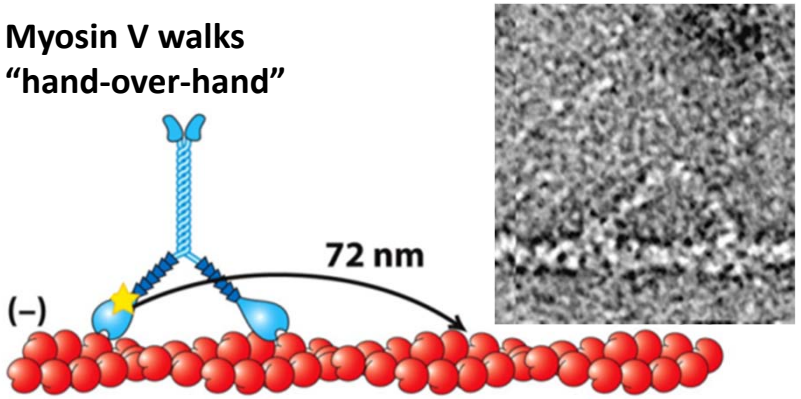
Cilia and diseases (ciliopathies):

- Defect in dynein's outer arms in cilia: **Reversal of left-right axis of organs** (***Kartagener's triad***) resulting in male sterility and bronchial problems
- Loss of IFT proteins can cause **PKD** = ***polycystic kidney disease***
- Defect in IFT transport of **photoreceptor cilia** can cause ***retinal degeneration***
- Cilia and basal bodies are affected in the ***Bardet-Biedl syndrom***: loss of ability to smell and retinal degeneration as well as obesity



Actin-based movements: the myosin motor



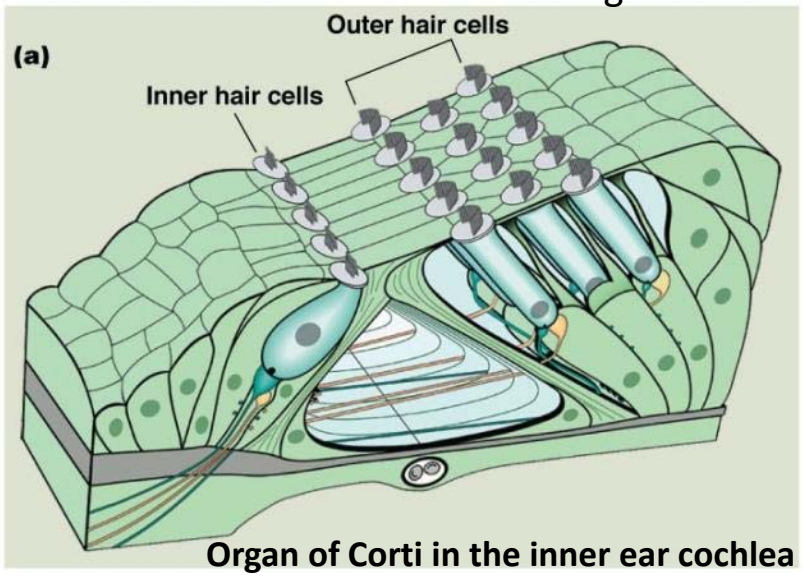


- Myosins are molecular motors that walk on actin filaments (powered by ATP hydrolysis)
- **Heavy chains** comprise the globular motor heads and the tail (coiled coils)
- Except myosin VI, all myosins move towards the plus-ends of F-actin
- Different from kinesins: **light chains** near the head region can be found with **regulatory activity**
- Tails of myosins are largely specialized because myosins functions in many ways
- **Myosin II** is important for muscle contraction; it can spontaneously assemble into so called **thick filaments**
- **Myosin I** and **VI** are involved in **endocytosis**
- **Myosin V** can make large steps on actin and transports vesicular cargoes
- **Myosin VII** and **XV** can be found in **stereocilia** in the ear; genetic defects can lead to ***Usher syndrome*** that results in **hearing loss** (**deafness**)

Stereocilia resemble microvilli but contain myosin

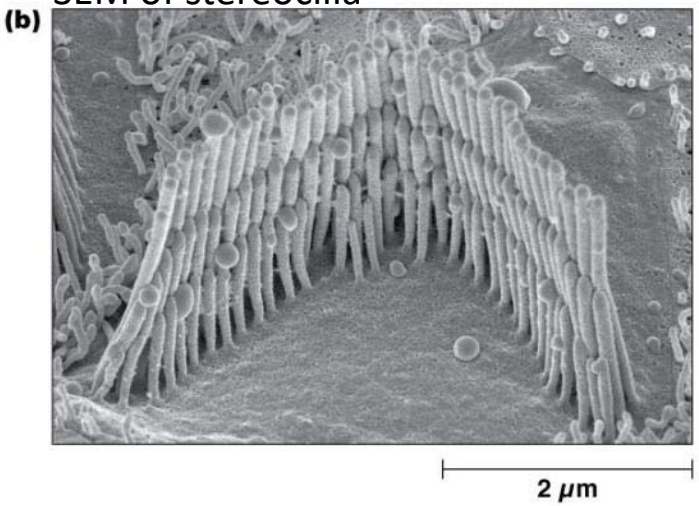
- Stereocilia are *not* related to axonemal cilia (they do not contain microtubules)
- They are more similar to microvilli but contain several types of myosins

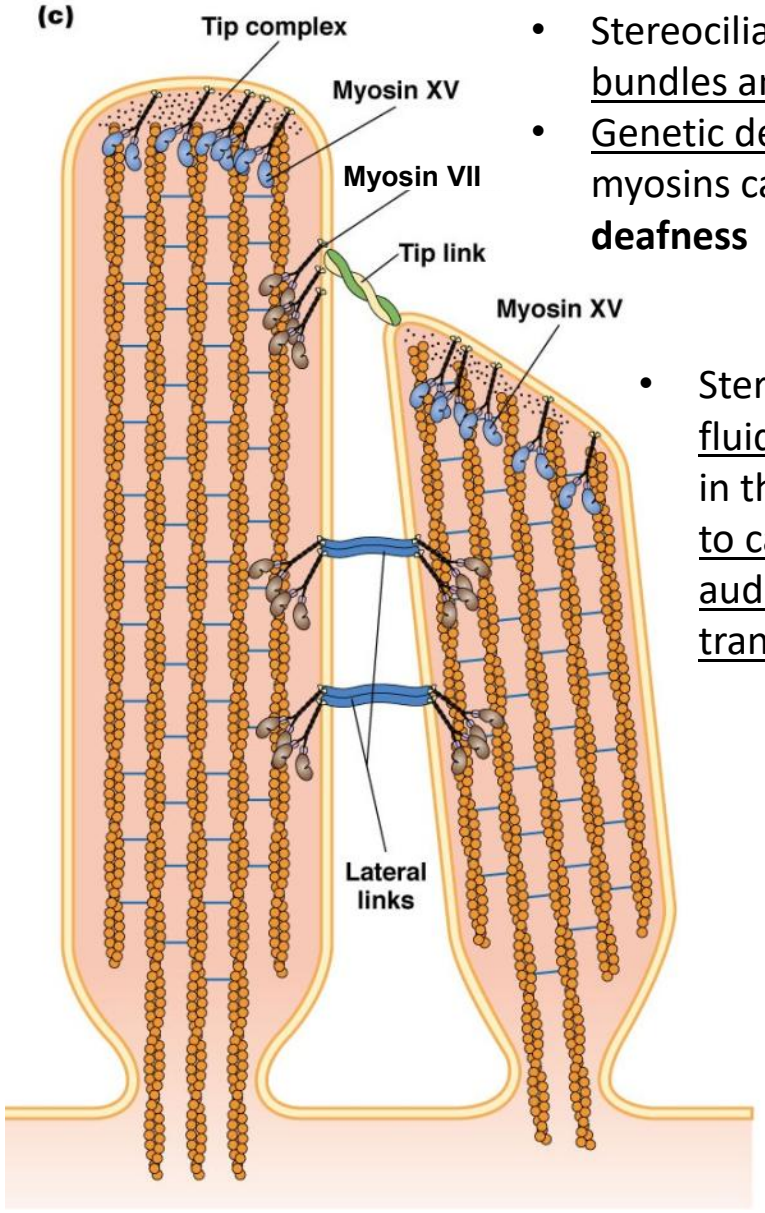
Hair cells in the inner ear containing stereocilia



Organ of Corti in the inner ear cochlea

SEM of stereocilia

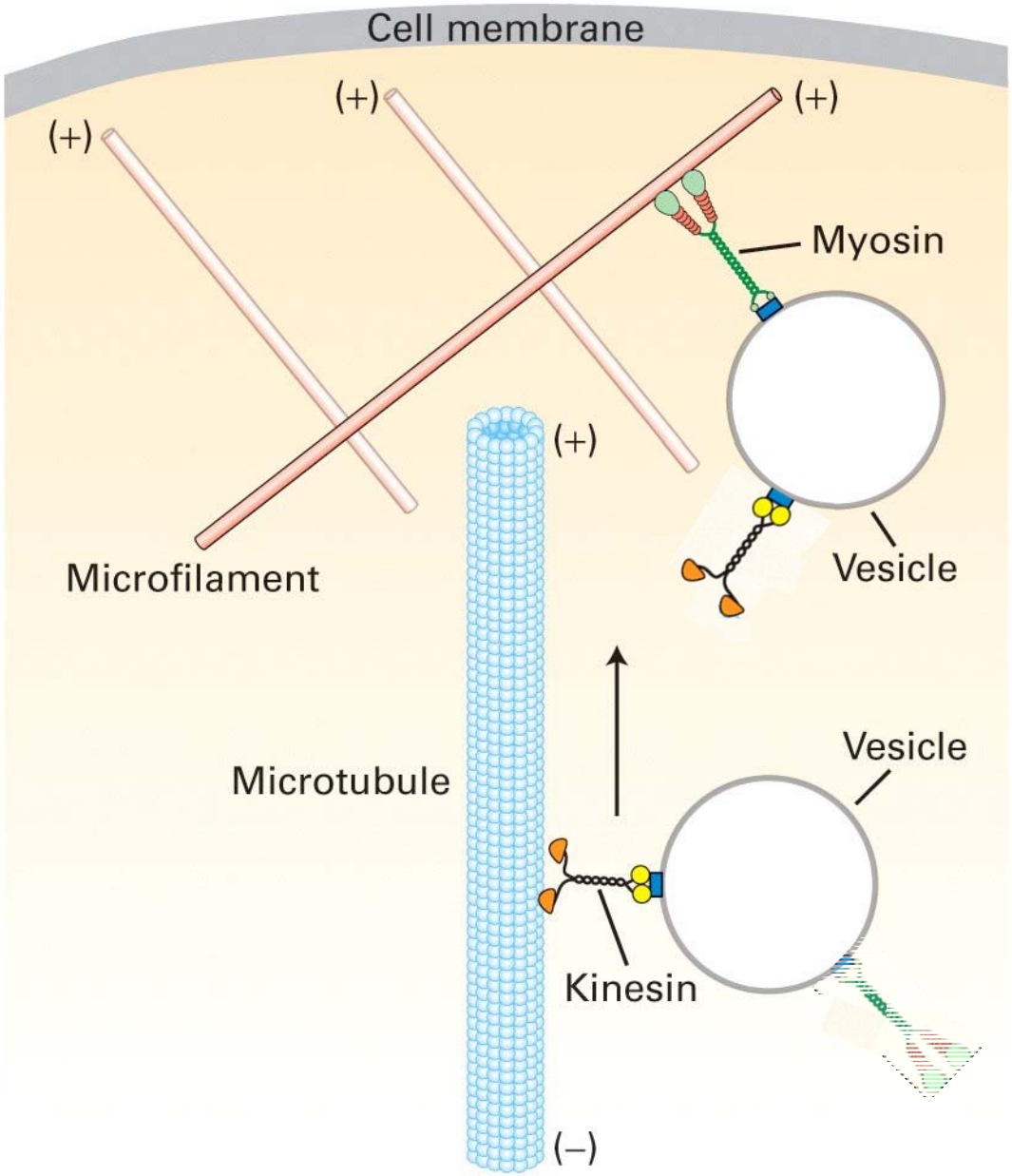




- Stereocilia with actin bundles and myosins
- Genetic defects in these myosins can lead to **deafness**

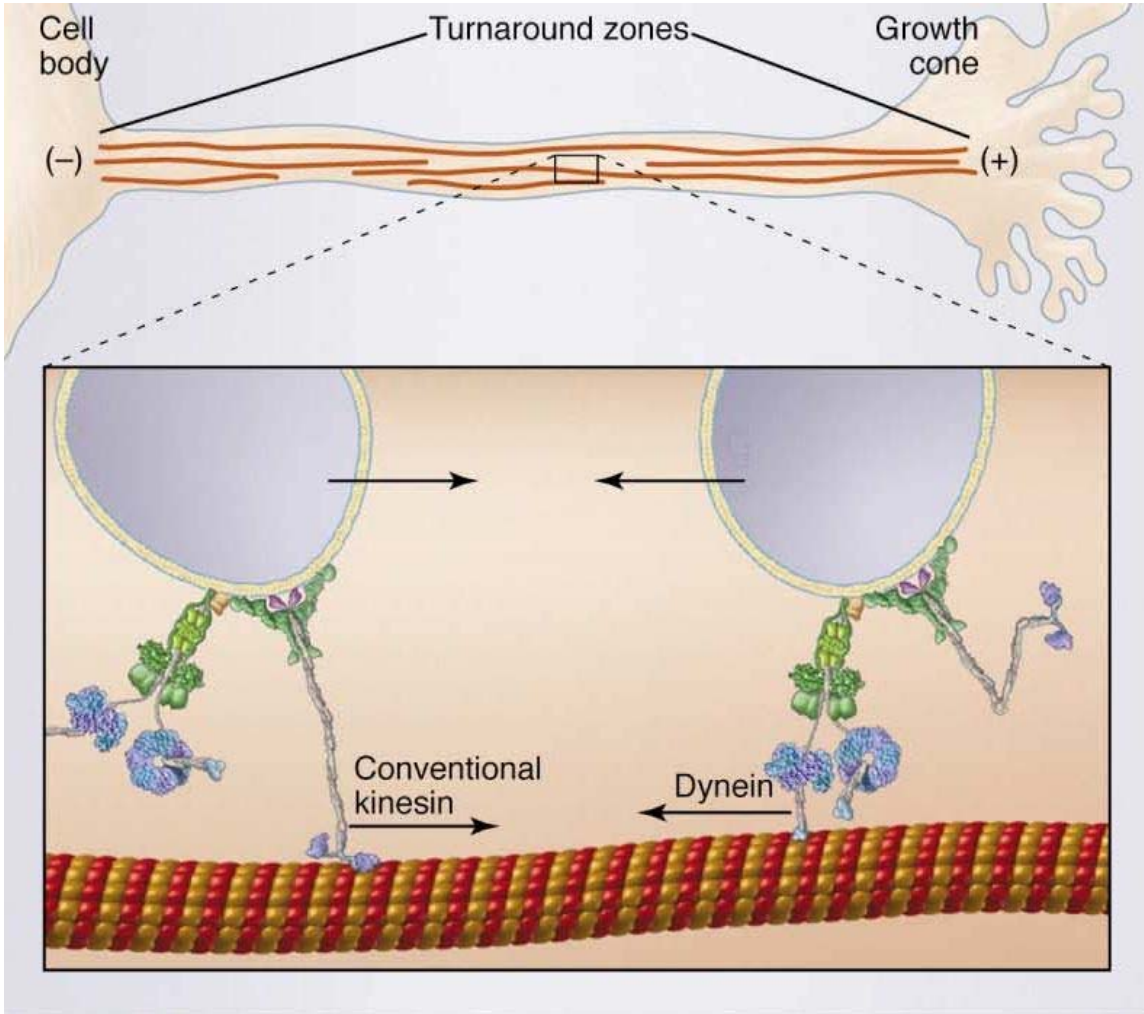
- Sterocilia sense fluid movements in the inner hear to carry out auditory transduction

Motors work in cooperation

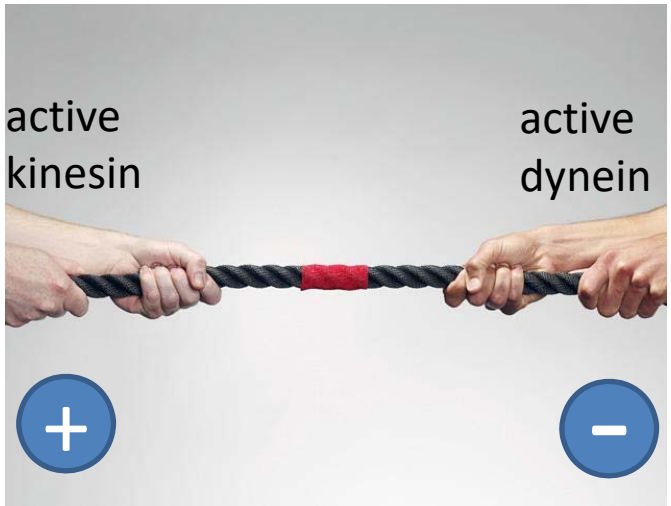


- In IFT, kinesins and dynein cooperatively transport IFT particles
- Another type of cooperation is between kinesin and myosins
- Here, vesicles have myosin (e.g., myosin V) and kinesin bound at the same time
- This enables the vesicle to **switch tracks** from a microtubule to an actin filament

Motors work in cooperation

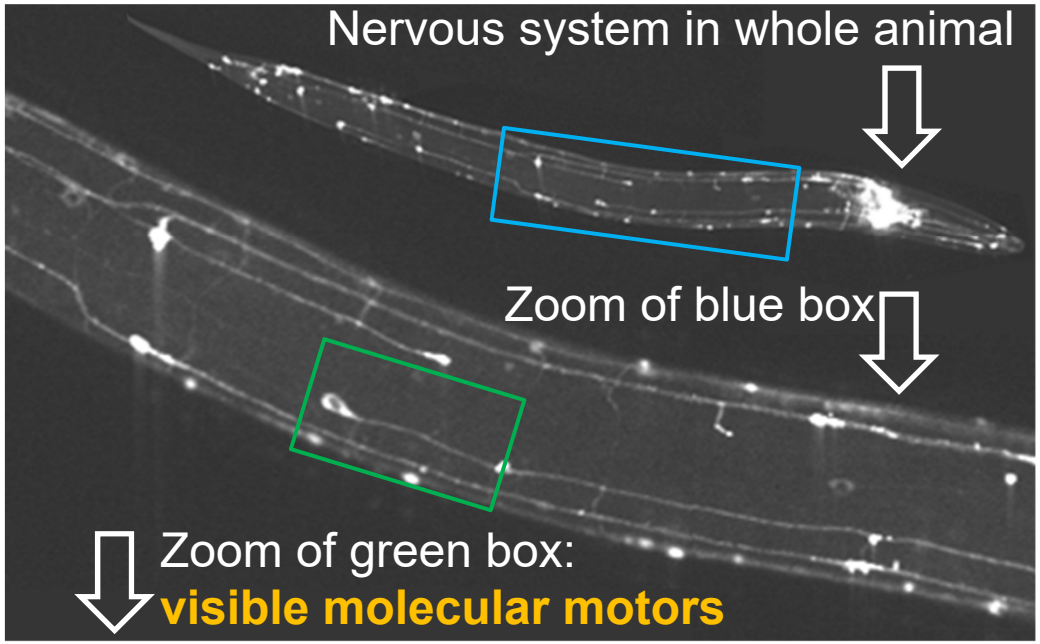


- Besides IFT in cilia, in neurons cooperation between kinesins and dynein might explain <u>fast directional switching</u> or **bidirectional movement**s of vesicles
- On the other hand, both kinesin and dynein might be <u>activated at the same time</u> and then pulling in opposite directions: **tug-of-war** (拔河) (oscillating vesicle)

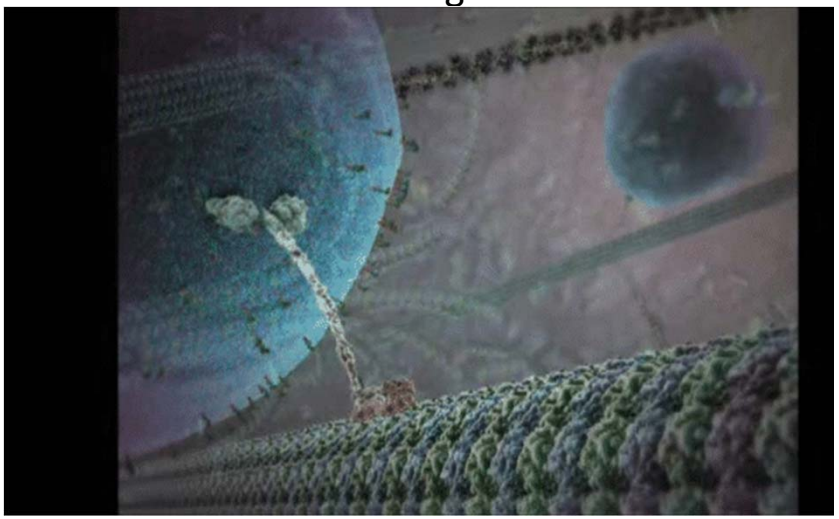


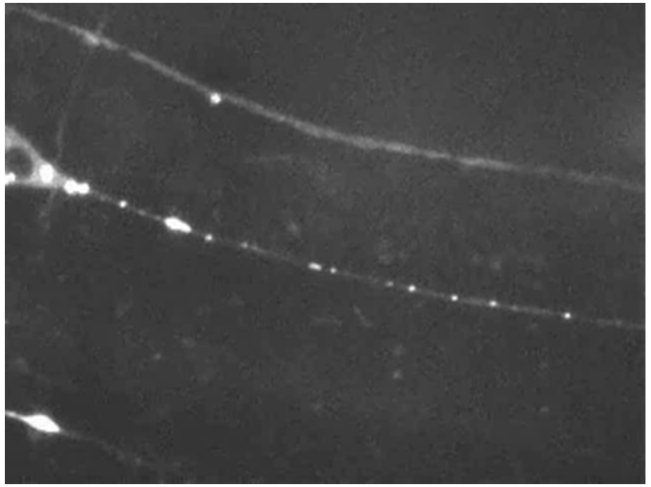
Wagner-Lab: How are molecular motors regulated in *C. elegans* neurons?

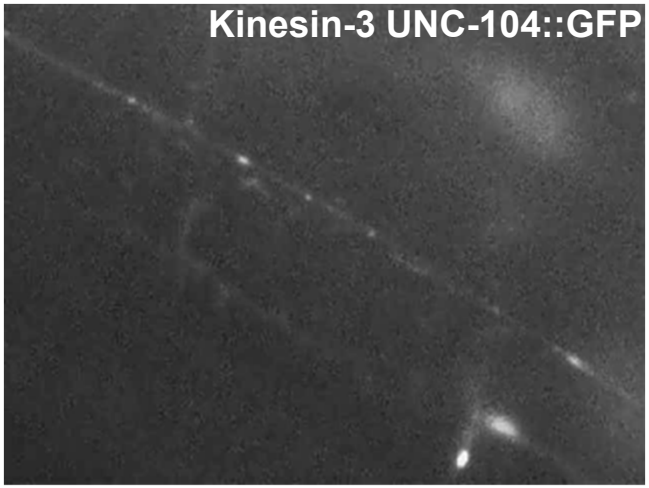
Current focus: Kinesin-3 (UNC-104 in *C. elegans*), the major transporter of synaptic vesicles



There is still little knowledge on how molecular motors are regulated

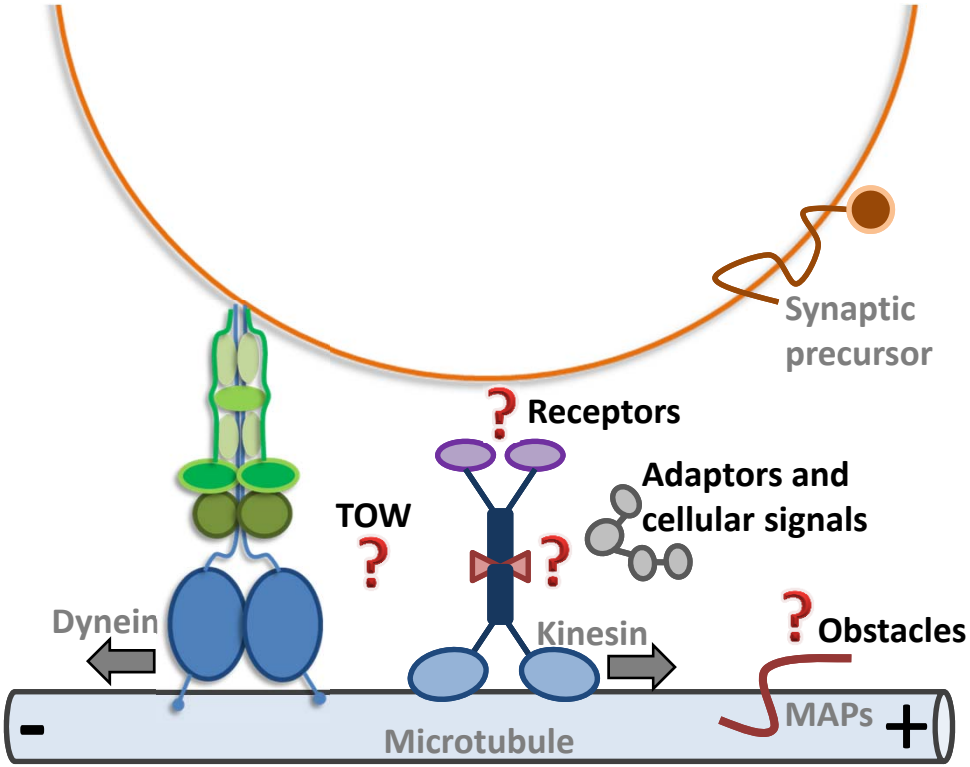






Single molecule tracking in living animals (using high resolution and high speed microscopy technology)

Wagner-Lab: How are molecular motors regulated in *C. elegans* neurons?



How do motors recognize their cargo?

- Membrane receptors?

How is cargo/vesicle transport regulated?

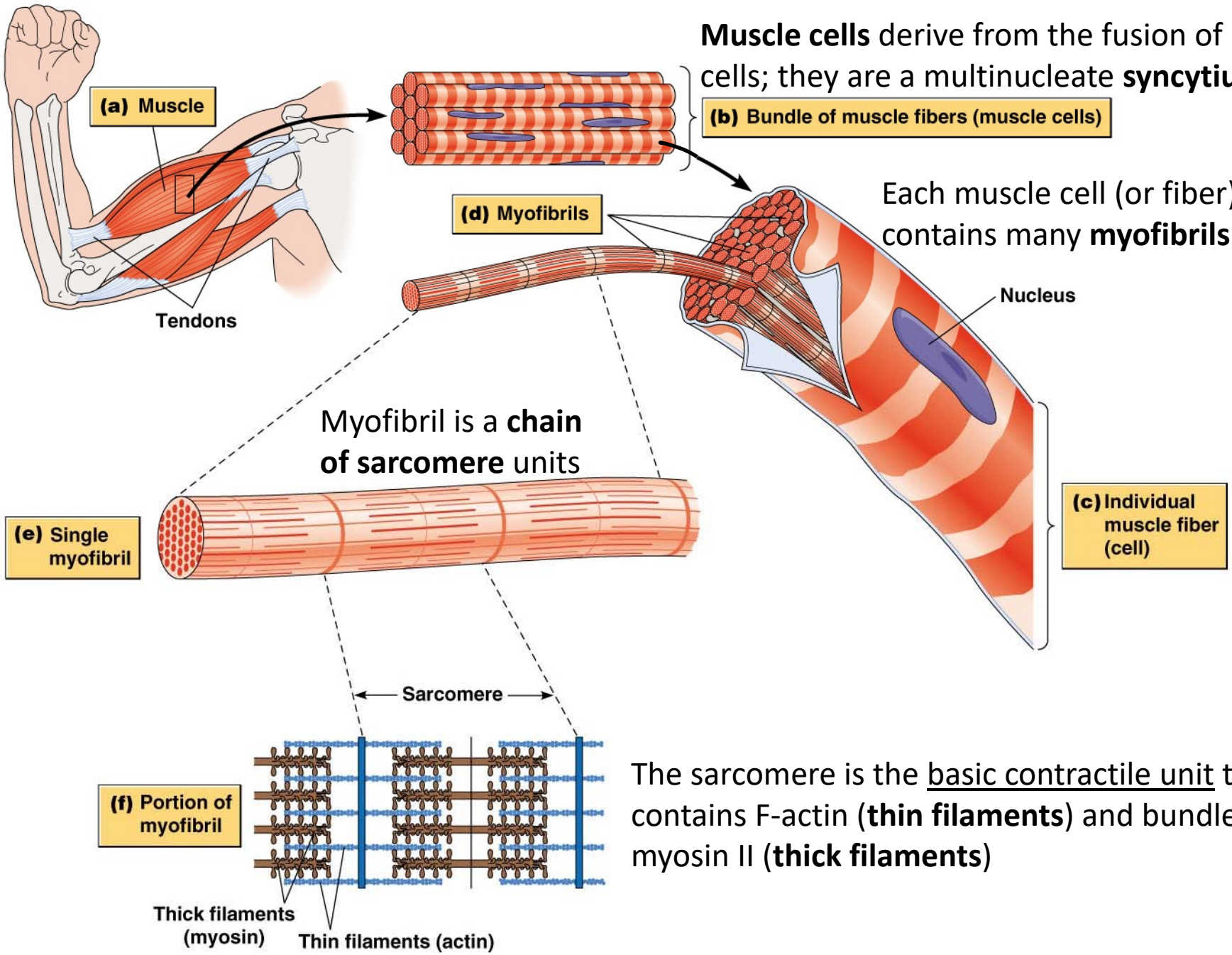
- Motor phosphorylation?
- Does cargo binding trigger motor activity and directionality?
- Small adaptor protein binding activates motors?
- Tug-of-war between opposing motors?

How do motors deal with obstacles?

- Do they slow down, stop, reverse or switch to other MTs?

http://life.nthu.edu.tw/~laboiw/

Myosins in muscle contraction



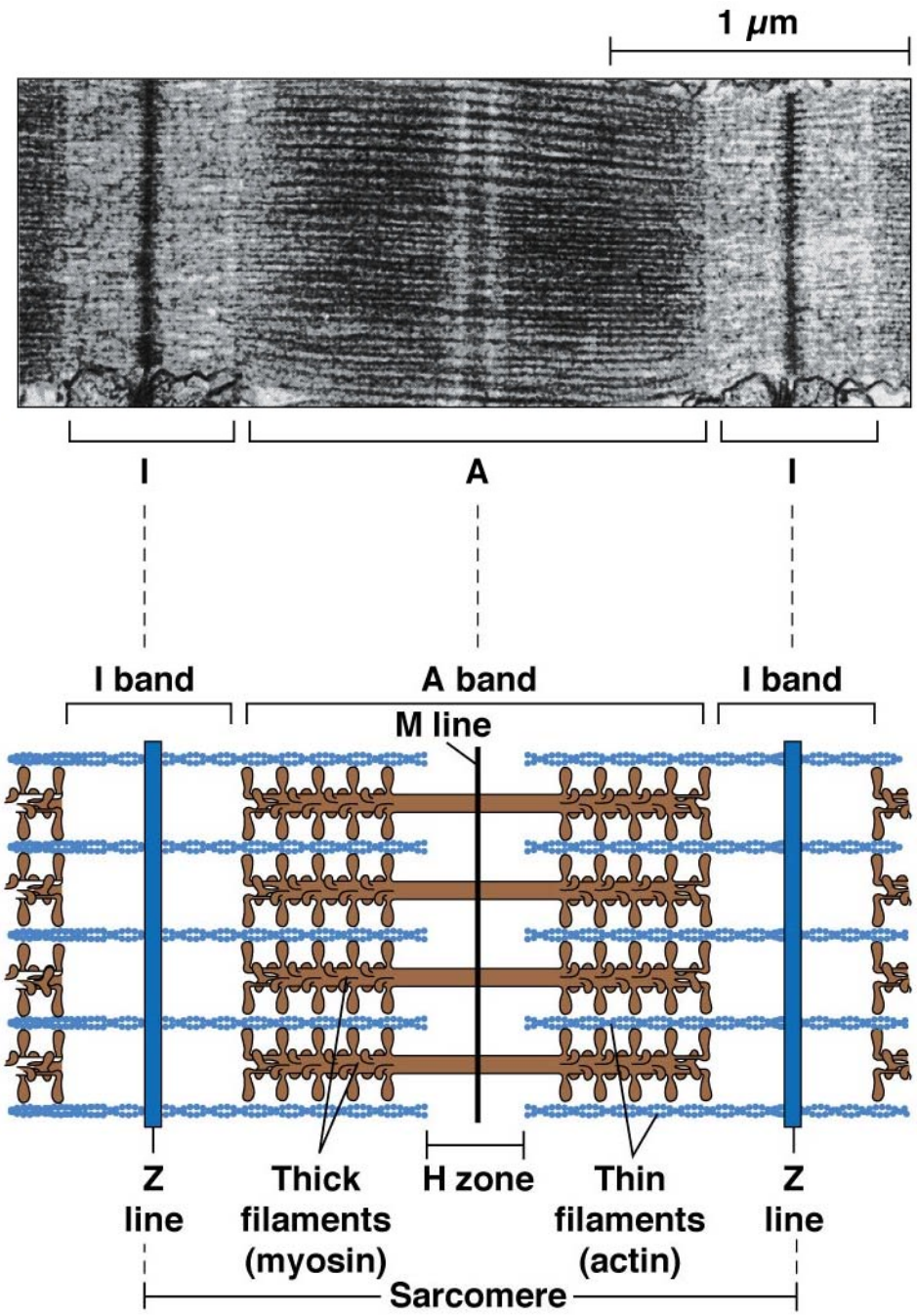
Muscle cells derive from the fusion of many cells; they are a multinucleate **syncytium**

Each muscle cell (or fiber) contains many **myofibrils**

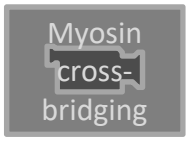
Myofibril is a **chain of sarcomere** units

The sarcomere is the <u>basic contractile unit</u> that contains F-actin (**thin filaments**) and bundles of myosin II (**thick filaments**)

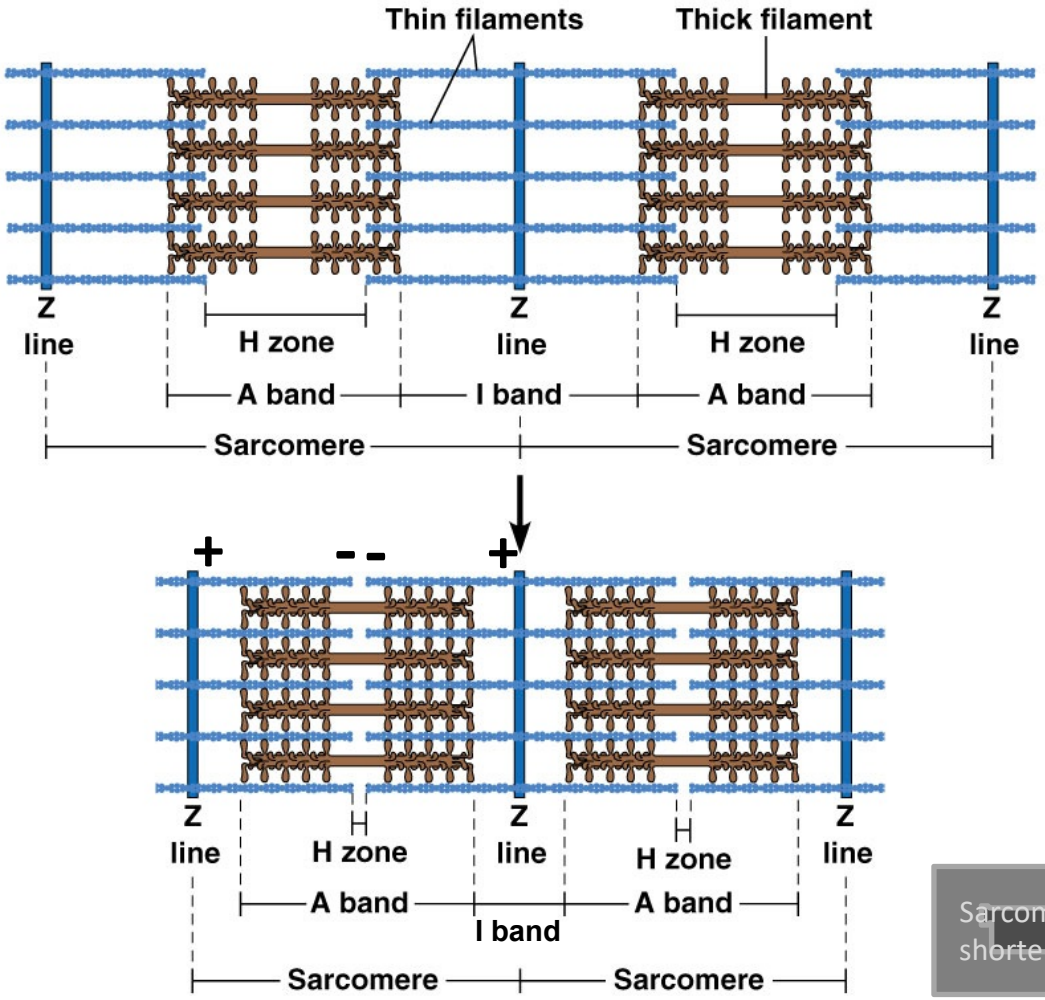
Sarcomere structure



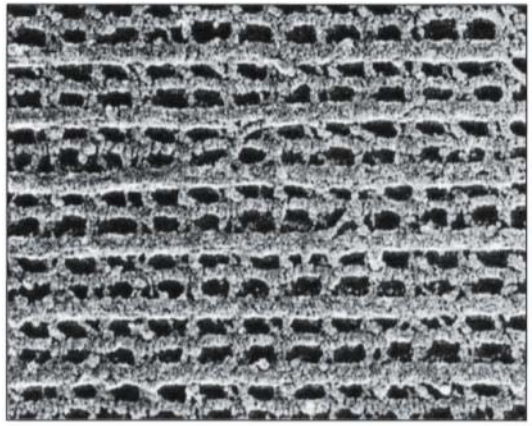
- In TEM, the different sarcomere zones are easily distinguishable based on their difference in electron density
- These patterns are called **striations**; skeletal and cardiac muscle are both **striated muscles**
- In **polarized light microscopy** different zones can be seen based on their *isotropic* (I band) or *anisotropic* (A band) appearance (depending on refractive indices, thus how the angle of polarized light is changed)
- **I band**: Mainly thin filaments
- **A band**: Mainly thick filaments
- **M line** = "*M*ittelscheibe" (*german*) = middle disc = only thick filaments
- **Z line** = "*Z*wischenscheibe" (*german*) = disc in between = actin anchor points
- **H zone** = "*H*ell" (*german*) = bright zone



During contraction the I- band and the H- zone shortens



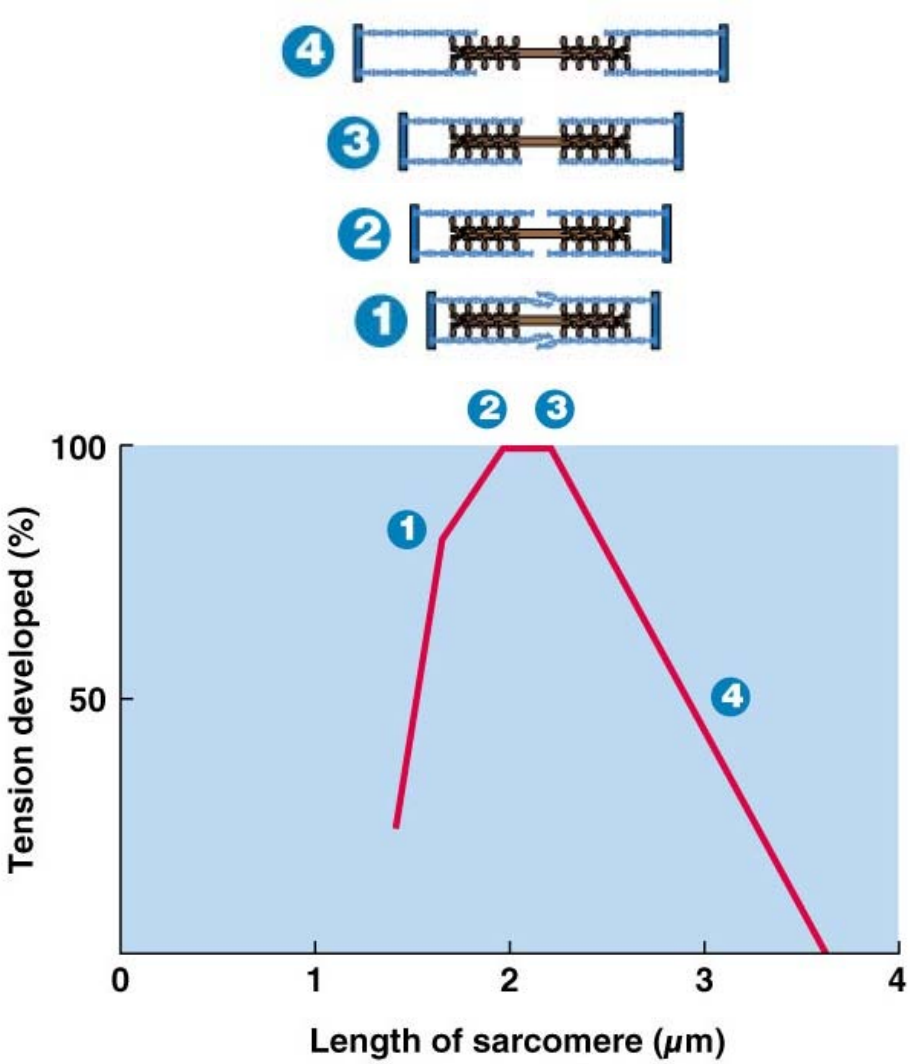
- By observing changes of the different zones and bands during muscle contraction, the **sliding filament theory** by Huxley and others was postulated in **1954**: "*Thin filaments slide past thick filaments and none of them change their length during contraction.*"
- During contraction the **I band** and the **H zone** clearly shortens



TEM of cross-bridges

Actin filaments all **face with their plus ends to the Z line** that forces the thick filaments to move towards the Z line (and the sarcomere shortens)

The sliding-filament theory fits well to observed tension development

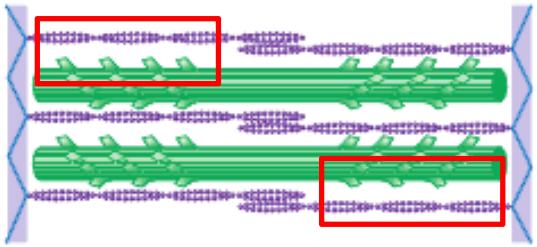


There is a point at which the sarcomere still shortens (2->1) but – interestingly - the tension is abruptly lost!

Length-tension diagram

4. Highly stretched sarcomere with little tension developed: **only few myosin heads interact with actin**
3. All myosin heads interact with actin: no further tension possible (**100%** reached)
2. Even though myosin heads "walk" further into the actin filament, no further tension possible (steady state)
1. Thin filaments start to crowed into one another and actin/myosin **interactions** are **disrupted** resulting in a **sudden drop of tension**

1

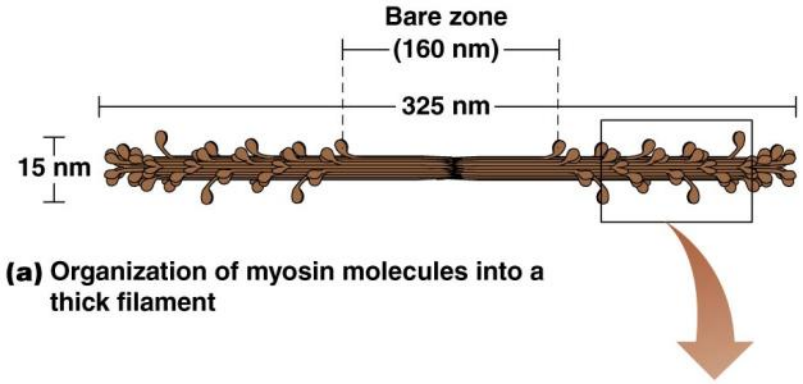


Disruption of actin/myosin interactions when thin filaments overlap

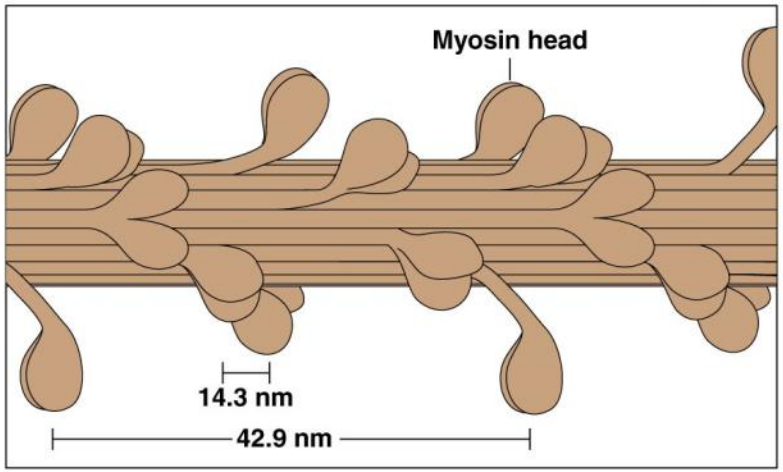
Details on thick and thin filaments

Thick filament

Bundle of hundreds of **tail-to-tail myosins** with heads protruding from the filaments. Myosin II heads are facing away from the center of the filament. **Bare zone**: only tails.



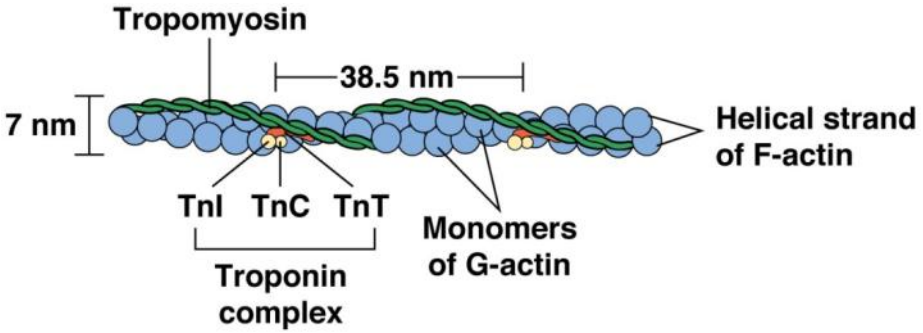
(a) Organization of myosin molecules into a thick filament

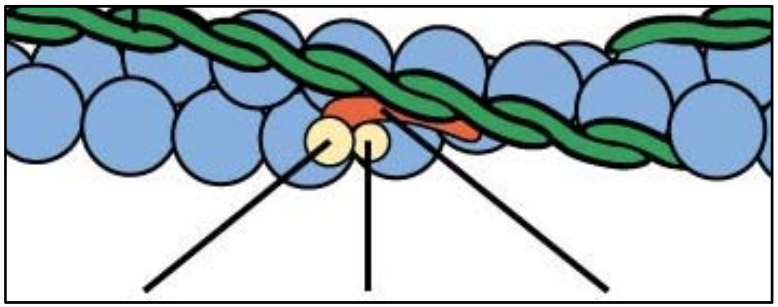


(b) Portion of a thick filament

Thin filament

Tropomyosin blocks binding sites for myosin heads. When **TnC** subunit of **troponin** (**Tn**) binds calcium, tropomyosin makes a small movement to free the myosin binding site.





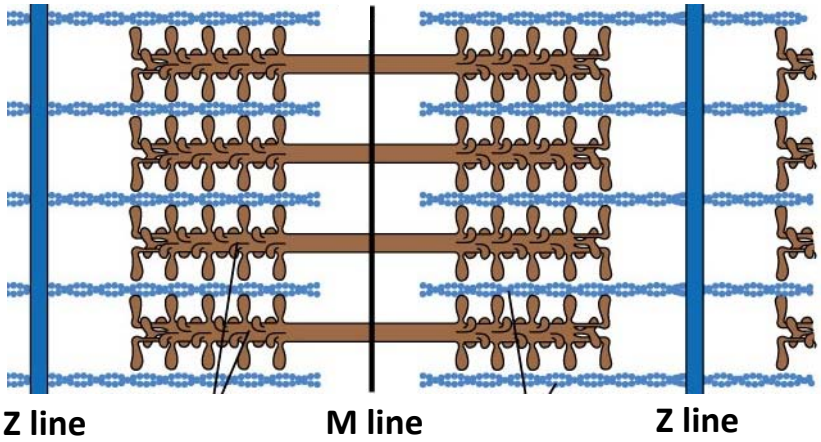
TnI has inhibitory functions

TnC binds calcium

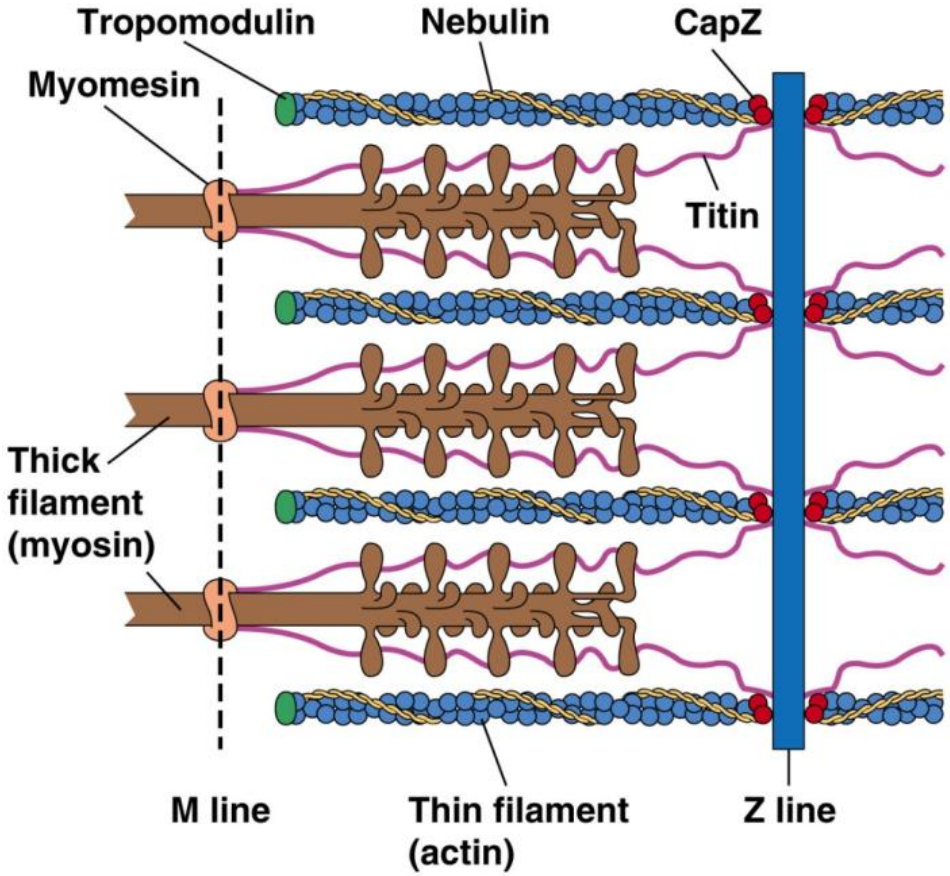
TnT binds tropomyosin

Stabilizing and integrating the filaments into the sarcomere

- How is the thick filament immobilized in the sarcomere?
- How is the thin filament fixed to the Z line?
- What prevents actin from further polymerizing?

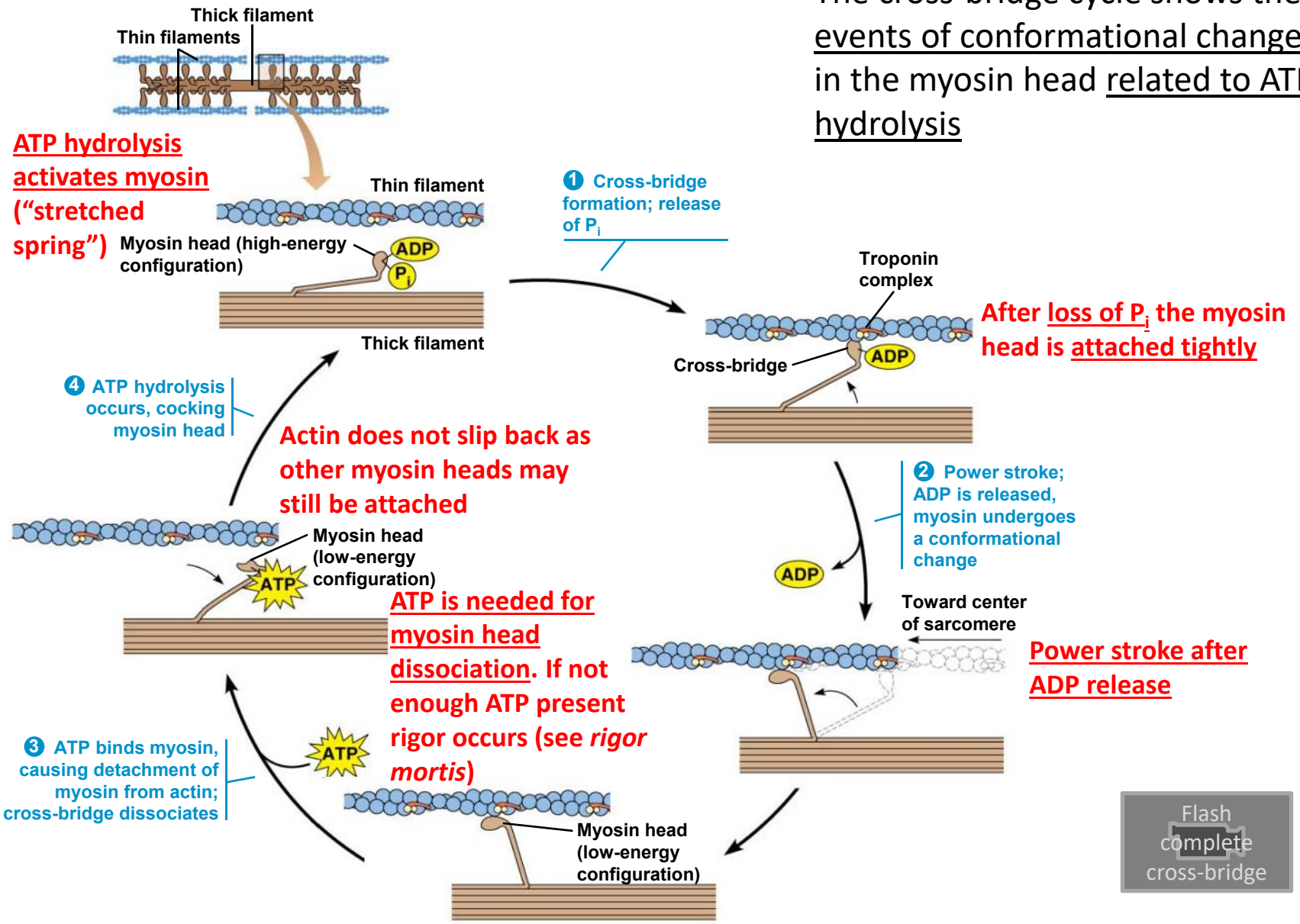


- Myosin is connected to the Z line via the large and very flexible molecule **titin** (2500 kDa!)
- Titin is fixed to **myomesin** which bundles myosin
- Actin is additionally stabilized by **nebulin**
- **Tropomodulin** and **CapZ** block further polymerization of actin

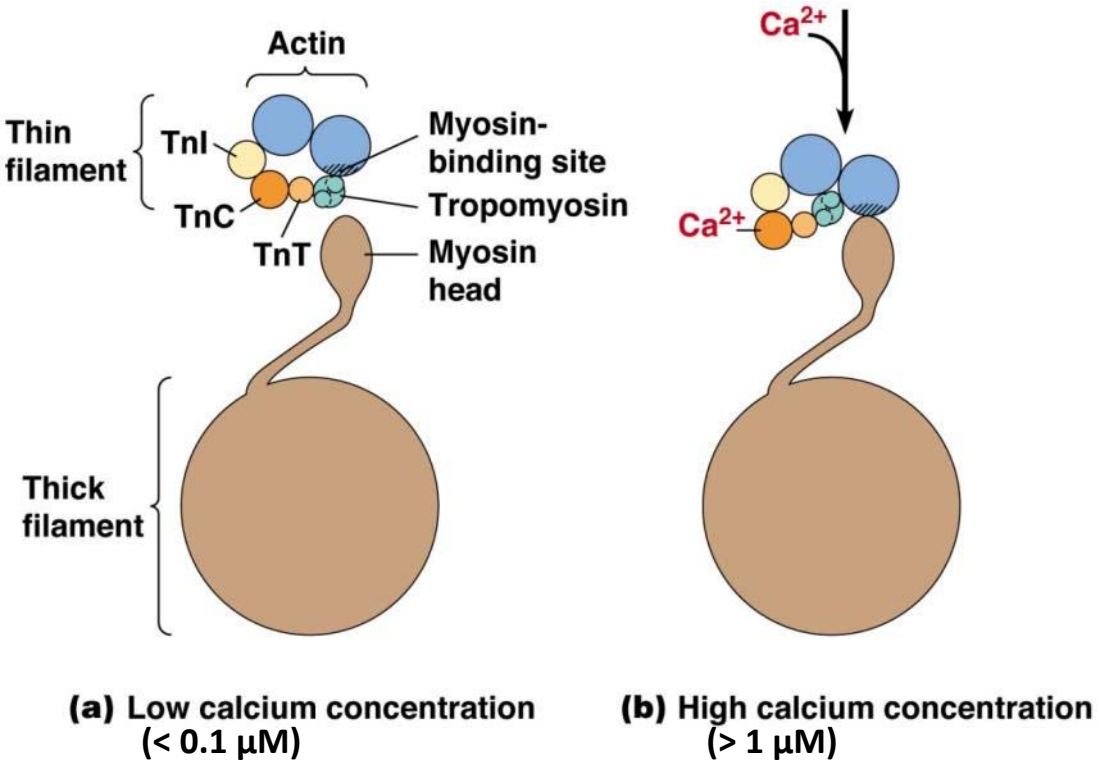


The cross-bridge cycle

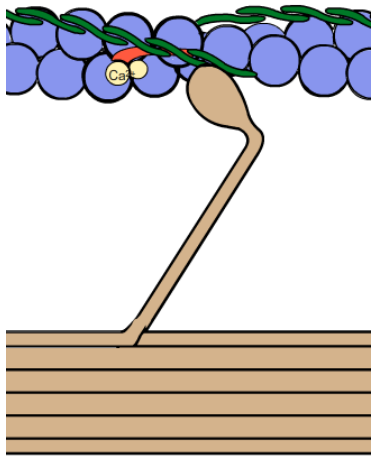
The cross-bridge cycle shows the events of conformational changes in the myosin head related to ATP hydrolysis



Regulation of myosin-actin interaction by tropomyosin

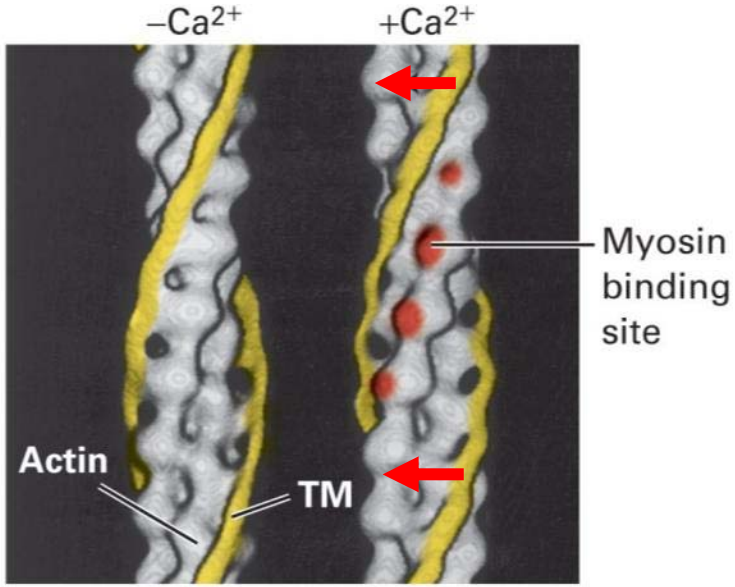


Lateral view



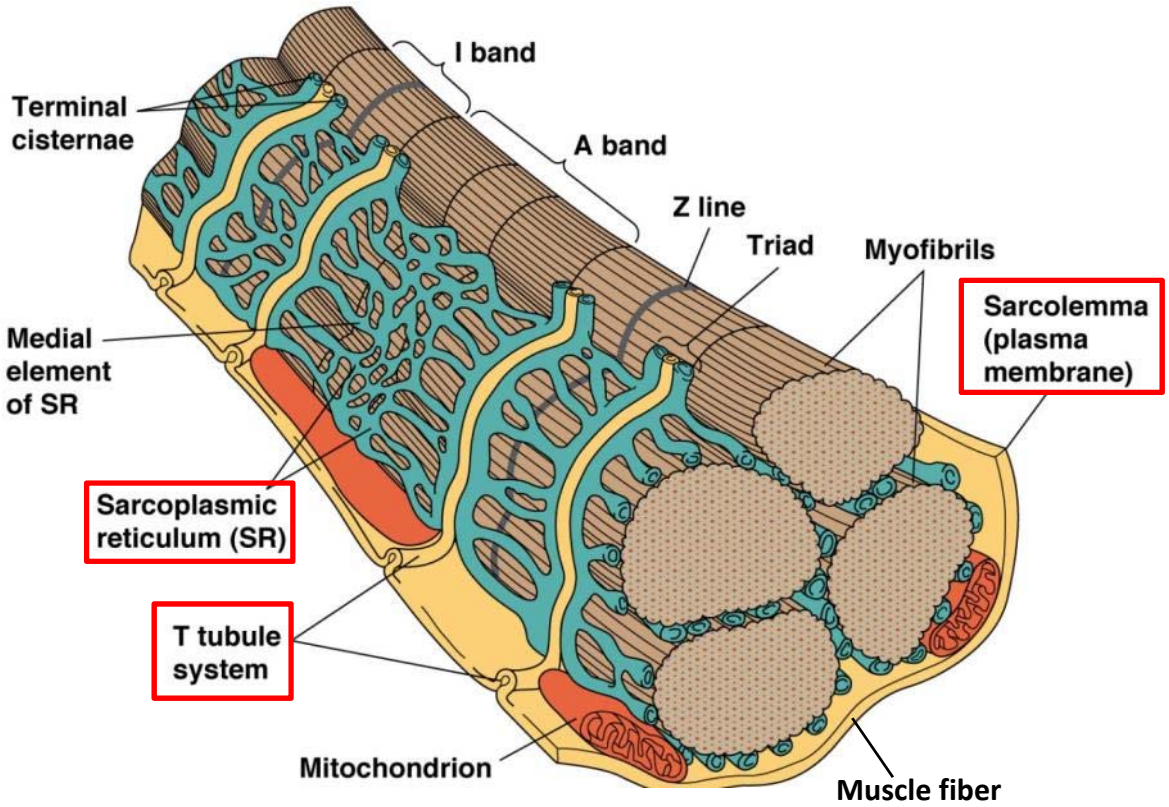
Ca^{2+} = yellow sphere
Mg^{2+} = white cone

- **Tropomyosin** (TM) <u>blocks the myosin binding</u> site on the **actin** filament
- TnI blocks acto-myosin ATPase (triggering of ATP hydrolysis by actin/myosin interaction)
- After **Ca^{2+}** <u>binds to</u> **TnC** conformation changes cause <u>tropomyosin to move away</u> from the myosin binding site



Electron tomography image

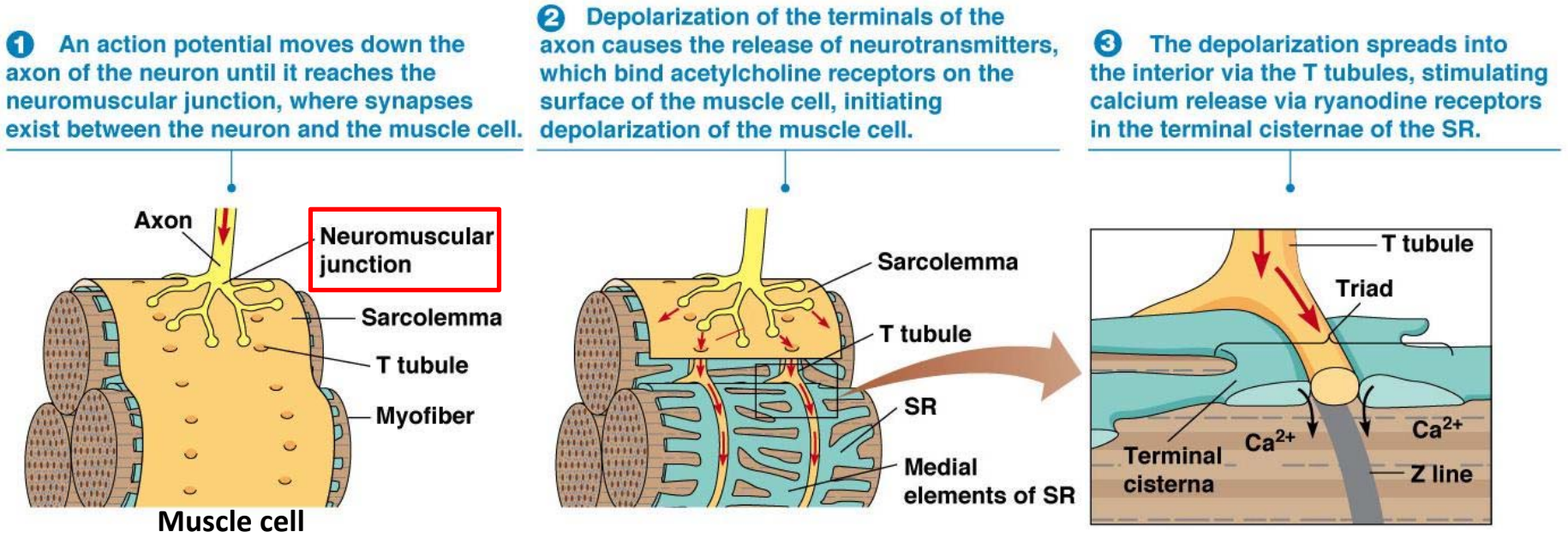
Where does the calcium for contraction come from?



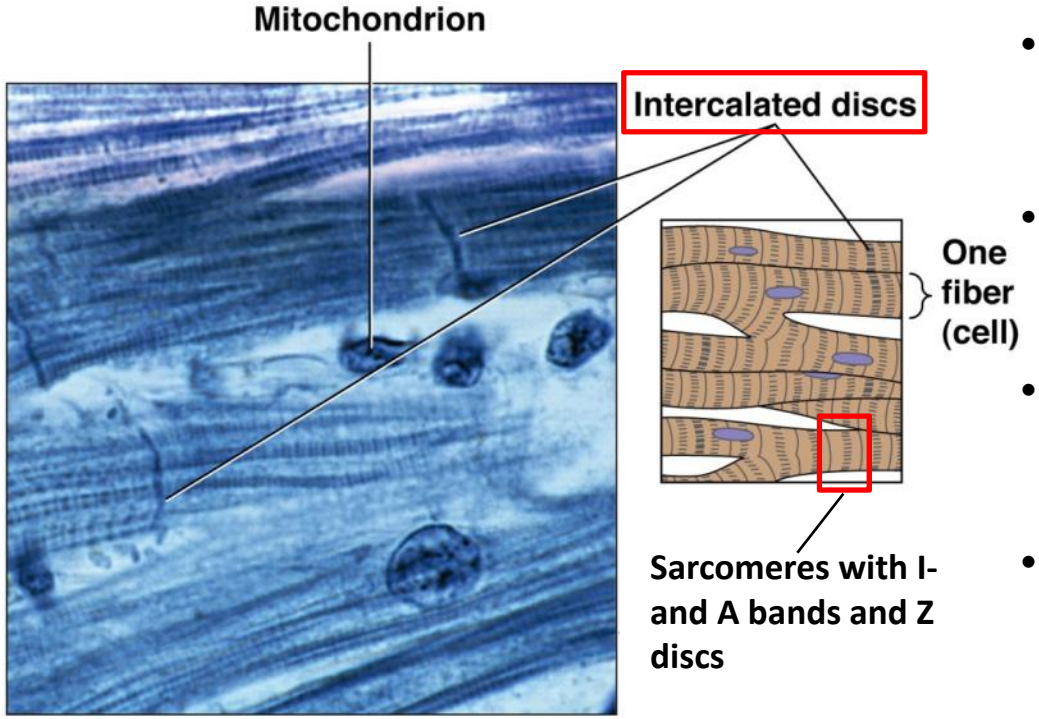
- Myofibrils are surround by a **tubular system** that stores calcium that is released by associated mitochondria
- This tubular system is called **sarcoplasmic reticulum** (**SR**)
- The **sarcolemma** has invaginations (inpocketings) forming the **T** (transverse) **tubule system**
- An incoming nerve impulse causes the muscle cell to **depolarize** which is conducted through the T tubules to the SR
- At the SR depolarization triggers then calcium release

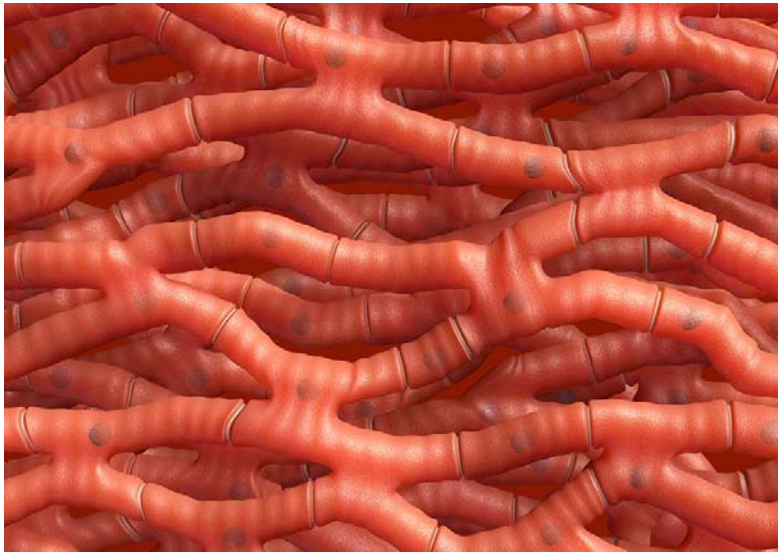
The neuromuscular junction is the electrical interface between a nerve and a muscle cell

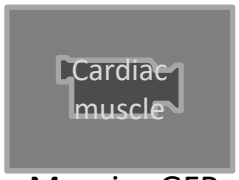
- The site where a neuron innervates a muscle cell is called **neuromuscular junction**
- An arriving action potential triggers the release of the neurotransmitter **acetylcholine** (ACh) from the **axonal terminals**
- Receptors in the **motor end plate** (muscle cell membrane under the axon terminals) bind the ACh that triggers the influx of sodium (Na^+) ions causing **muscle cell depolarization**
- The depolarization spreads deep into the T tubules triggering the **release of Ca^{2+}** from the closely associated SR (into the sarcoplasm) (via ryanodine receptors = **Ca^{2+}**-channels)
- For the muscle to relax Ca^{2+} is pumped back (from the sarcoplasm to the SR) via ATP dependent calcium pumps



Cardiac muscle cells also appear with striated pattern



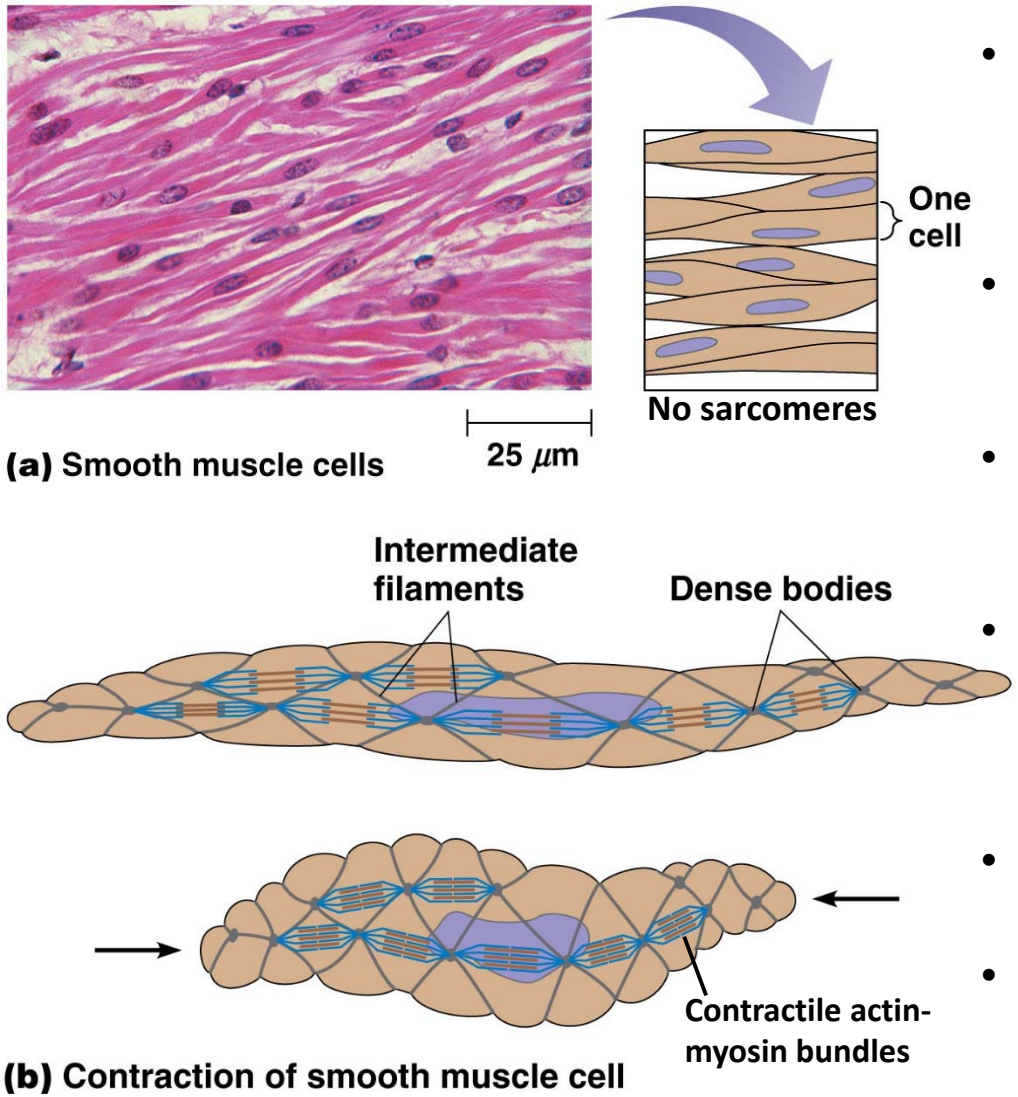




Myosin::GFP

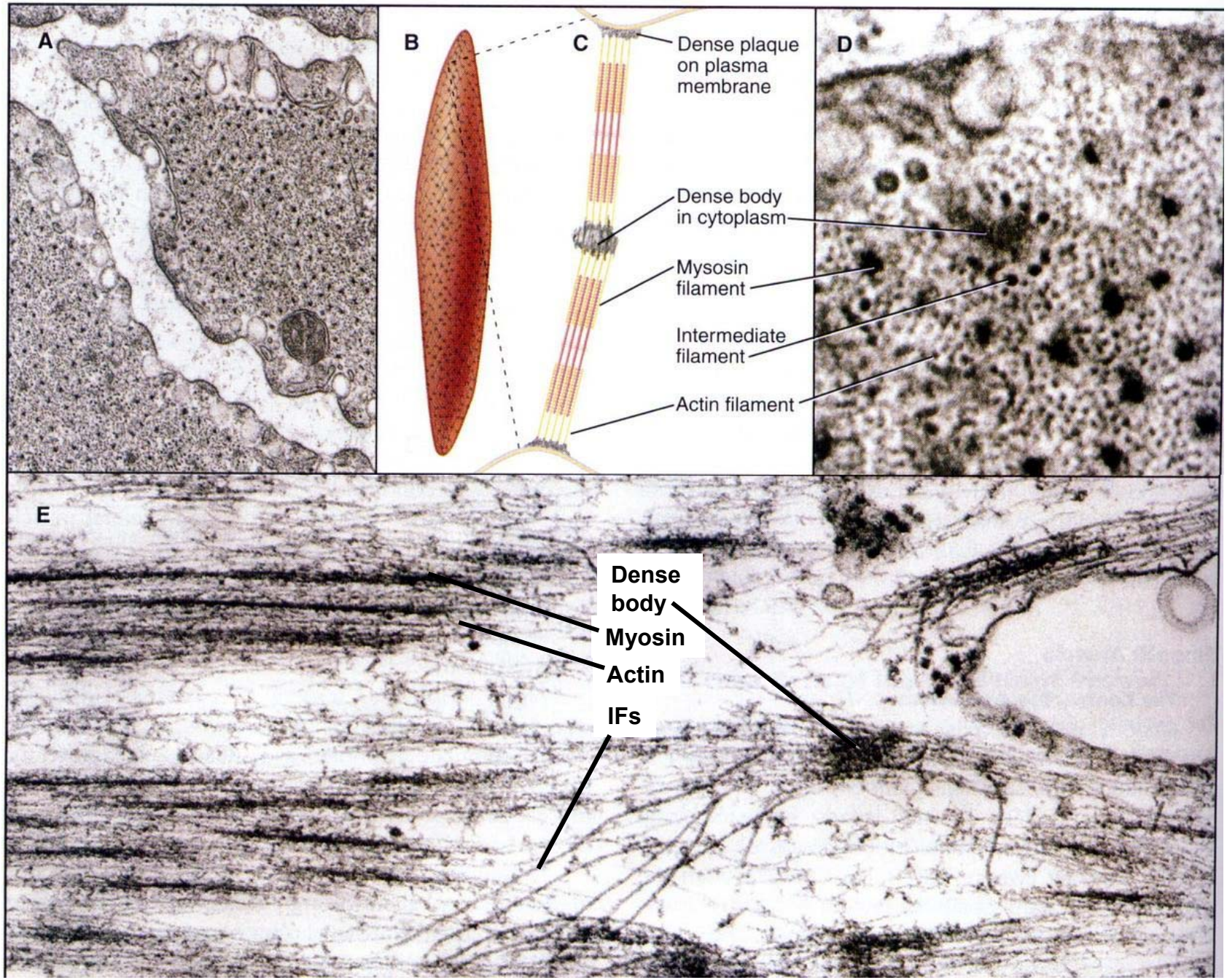
- Cardiac (heart) muscle cells <u>also have striated pattern</u> based on I bands, A bands and Z discs
- One <u>difference to striated skeletal</u> muscle is that they are **not multinucleate**
- Here, single nucleated cells are connected to each other (linear or in branches) via **intercalated discs**
- Intercalated discs are rich of **desmosomes** (cell adhesion junctions) and **gap junctions** (electrical coupling and ion/metabolite exchange)
- The <u>energy</u> used <u>for contraction</u> comes from **fat metabolism** rather than from glucose metabolism (skeletal muscle)
- **Heart attack** happens when blood flow to cardiac muscle cells is disturbed and cells die. Permanent heart dysfunction is the result (<u>stem cell therapy</u> is thought to partially cure dysfunction)

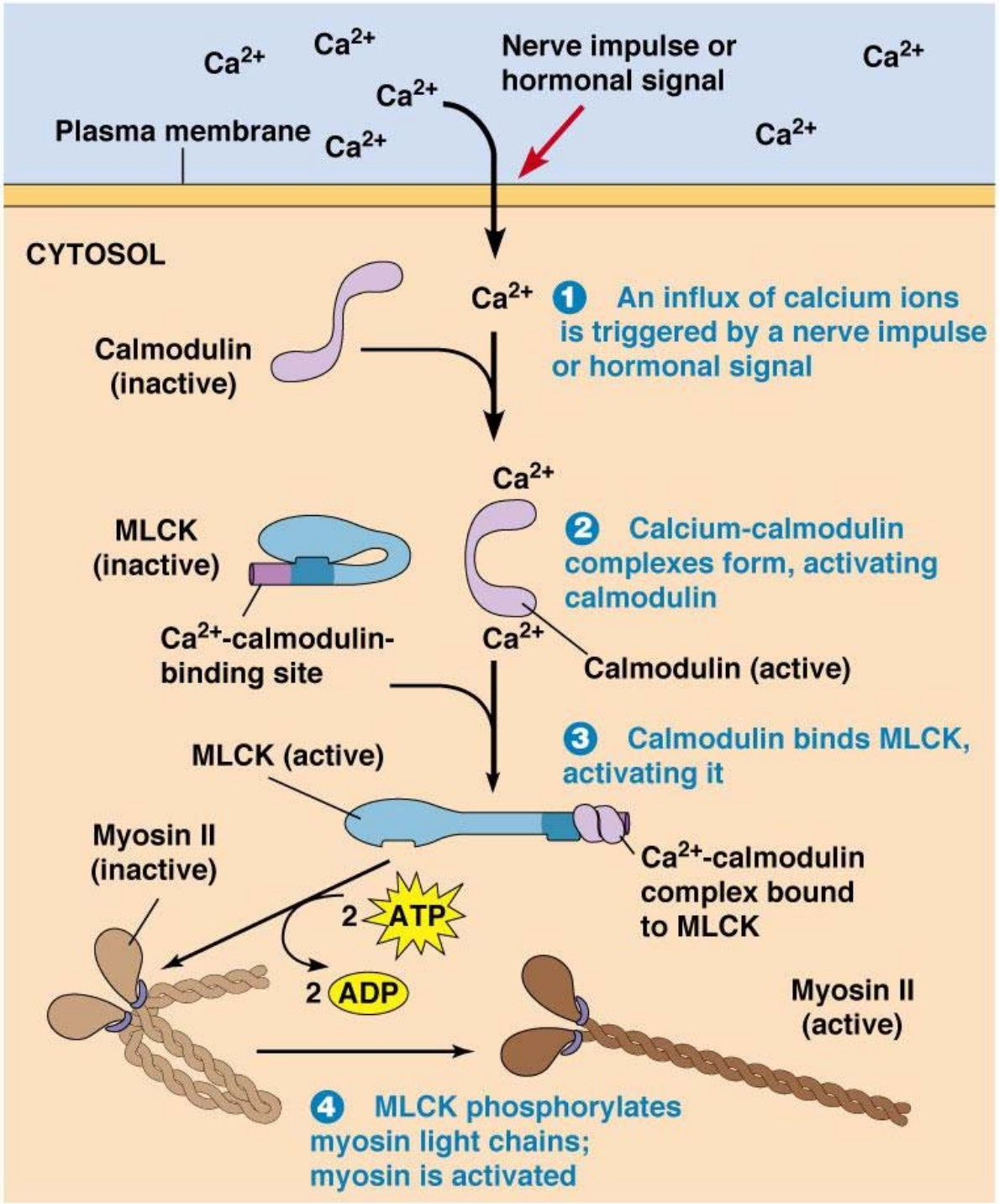
Smooth muscle cells are not striated and contract independently from our free will



- Smooth muscle cells are important for **involuntary contractions** in organs as stomach, intestines, uterus and blood vessels
- These contractions take more time to build up but they can last longer compared to skeletal muscle
- Smooth muscle cells are **not striated**, instead they have a **dotted appearance** in TEM reflecting dense bodies
- **Dense bodies** are the anchoring points for contractility units (comparable to the function of Z discs in skeletal muscle cells) composed of actin and myosin bundles
- Actin/myosin units are also cross-linked and stabilized by **intermediate filaments**
- When actin/myosin units contract they pull on the intermediate filaments and the cell contracts

Dense bodies and dense plaques anchor actin-myosin filaments

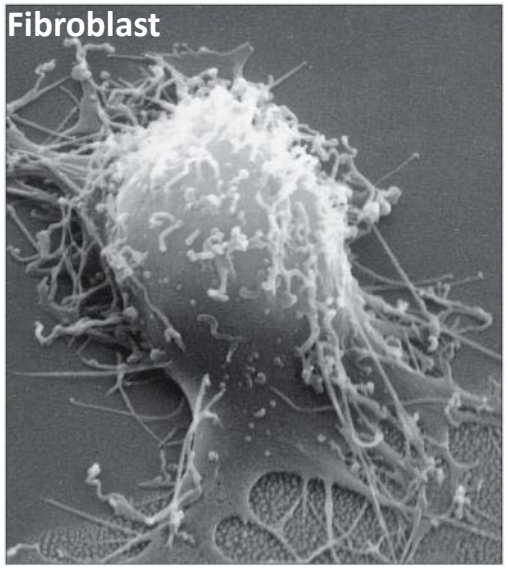


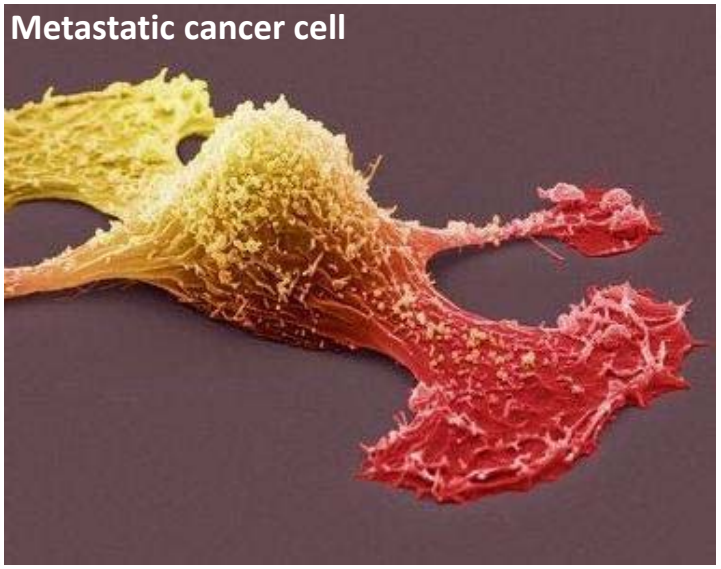


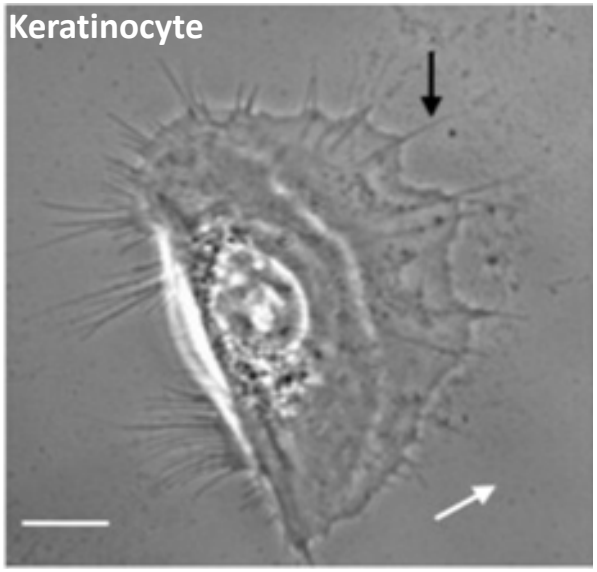
Why is the contraction of smooth muscles slow?

- Compared to skeletal muscle, the chain of events in smooth muscle cell contraction is more complex
- Especially it involves **phosphorylation of proteins** which is a slower process than a simple conformation change (as for troponin/tropomyosin)
- First, **calmodulin needs to be activated** by Ca^{2+} binding
- Second, **calmodulin binds to MLCK** (myosin light-chain kinase) which is then activated
- Third, activated **MLCK phosphorylates myosin II** (needs ATP)
- Forth, intramolecular folding of myosin is now released (so myosin can interact with actin)

Movement of whole cells





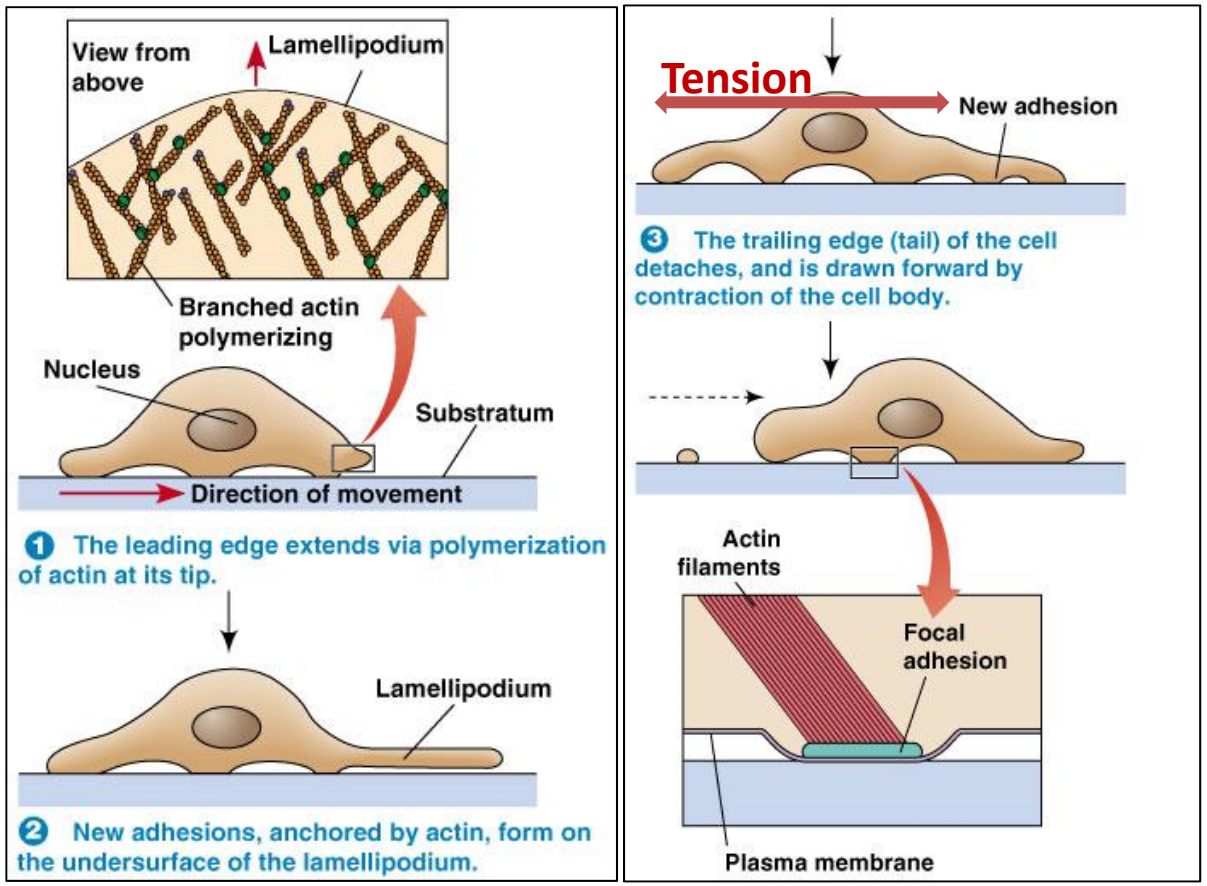


Keratinocyte (skin epidermis cell for wound healing)

Neuron (neurite extension)

- Using the **power of actin polymerization** motile cells form **protrusions** (lamellipodia or filopodia) into the direction of movement (leading edge)
- Polymerization at the **leading edge** is a branched-type of polymerization mediated by Arp 2/3
- Lamellipodia are **seeking new attachment points**
- This will **cause tension** in the cell
- Additionally **actin/myosin bundles** contract
- Eventually the back (tail or trailing edge) will detach and the whole cell moves “one step” forward

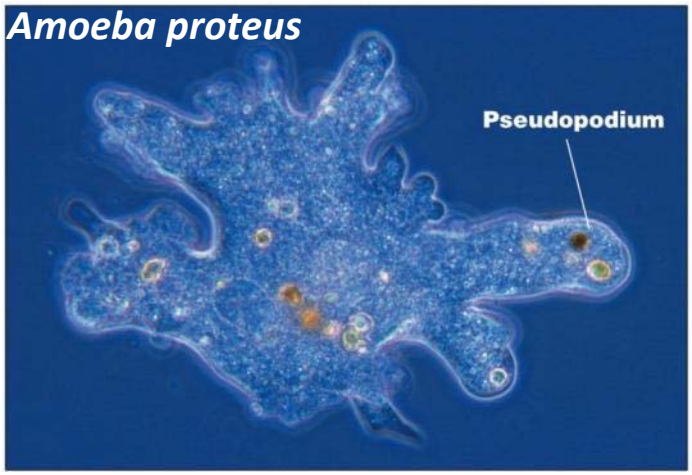
The critical steps of cell movement



- Cell movement usually begins with the <u>formation of a lamellipodia</u>
- <u>Branched actin polymerization</u> occurs that **extends the leading edge**
- At the same time used actin filaments are <u>transported rearward to the base</u> of the protrusion (**retrograde actin flow**)
- <u>Here they depolymerize</u> to make **new monomers available** for further polymerization at the front

- The extended <u>lamellipodium forms an new contact</u> with the substrate (**focal adhesion**)
- Whole cell is now **under tension** and <u>in addition the cell will start to contract</u>
- This will cause the <u>rear (back) of the cell to detach</u>
- <u>The cell made a net movement</u> and the whole cycle repeats
- Sometimes parts of the rear are attached too strongly and <u>will be left behind</u>:

Chemotaxis and amoeboid movement

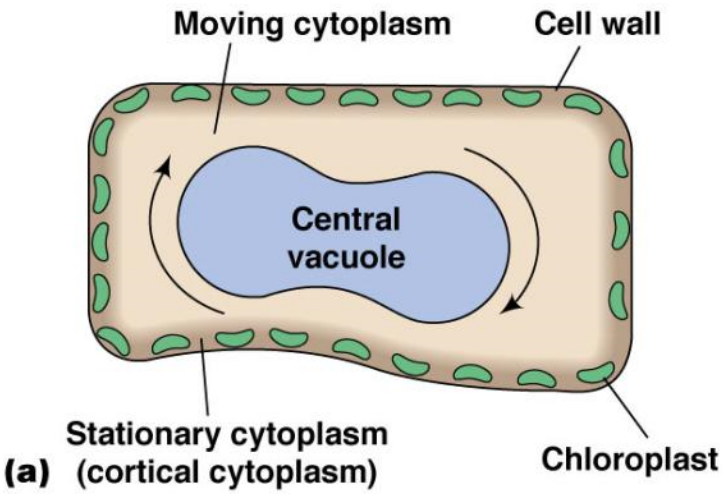


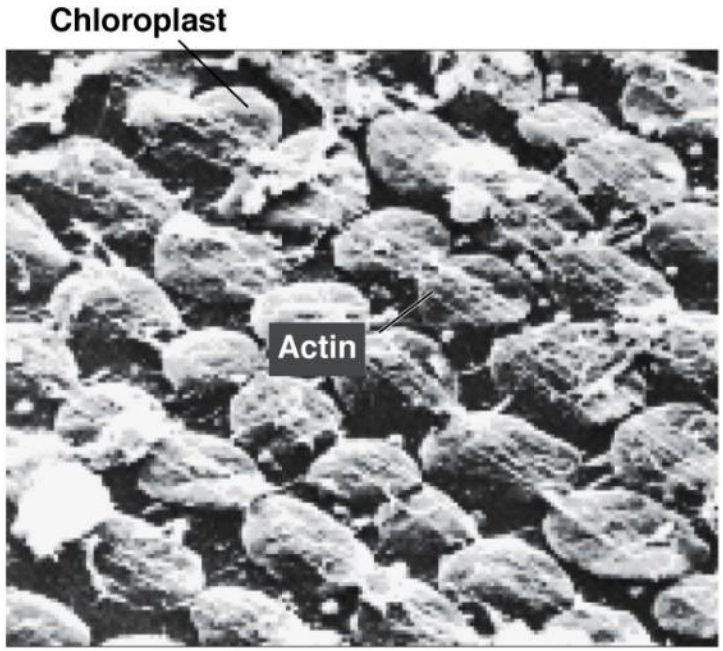
Dictyostelium moves towards gradients of cAMP (released from a pipette)

Neutrophil (white blood cell for first stage host defense) chasing and phagocytizing a bacterium

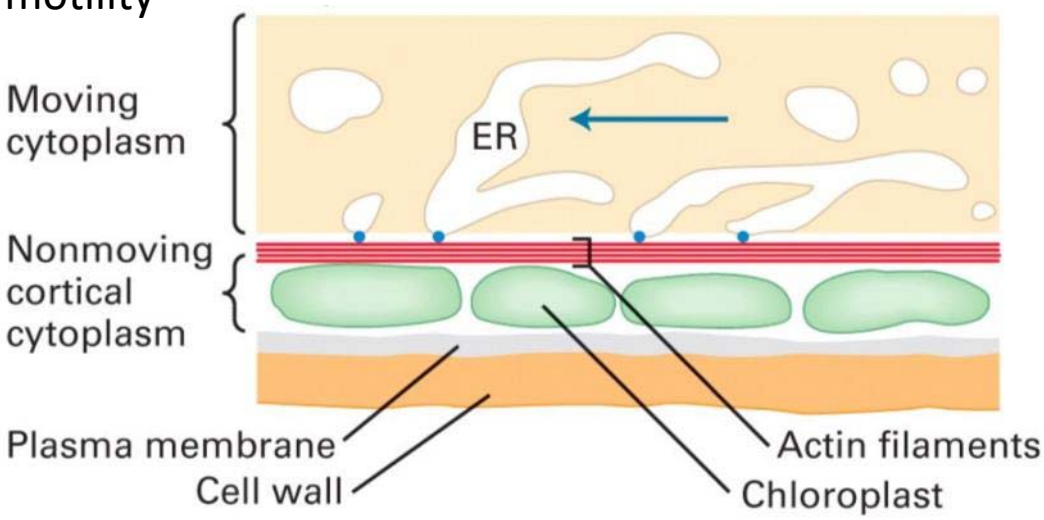
- Amoebas (e.g., **white blood cells**, ***Dictyostelium***) exhibit a crawling type of movement
- The protrusions here are called **pseudopodia**
- The cytosol of amoebas can be divided into two layers: an outer thick (**gel-type**) and an inner more liquid (**sol-type**) layer
- During movement fluid material is streamed into the front where it "freezes" (**gelation**, 凝結)
- At the same time in the rear the gel-type layer (actin rich) liquefies (**solation**) and streams forward
- It is thought that **gelsolin** is calcium activated during this *gel-sol* transition
- Many amoebas exhibit **chemotaxis**: directional movement towards a gradient of a small molecules (**chemoattractant**)
- Chemoattractants can be **cAMP** (for *Dictyostelium*) or **small peptides** (for neutrophils = white blood cells)
- Chemoattractants bind to **GPCRs** (G protein-coupled receptors) located in the plasma membrane
- This will result in increasing **phosphoinositide concentrations** known to remodel the cytoskeleton

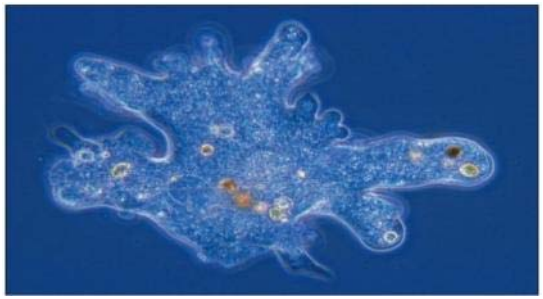
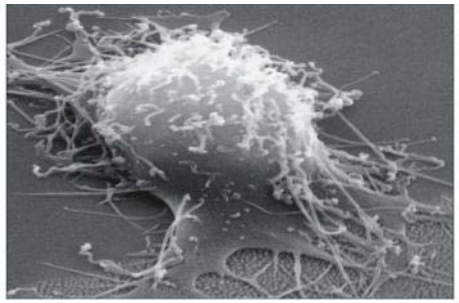
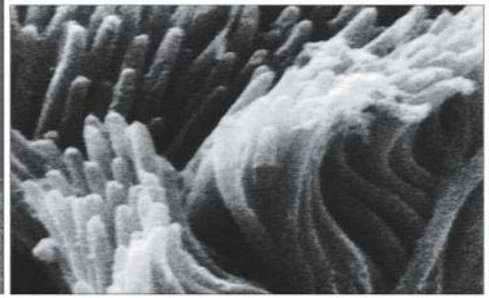
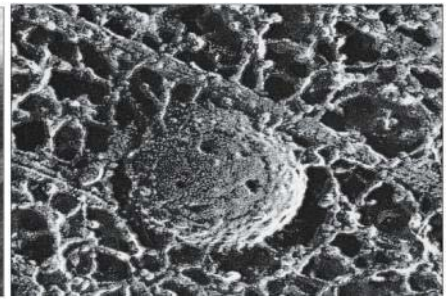
What about plant cells?



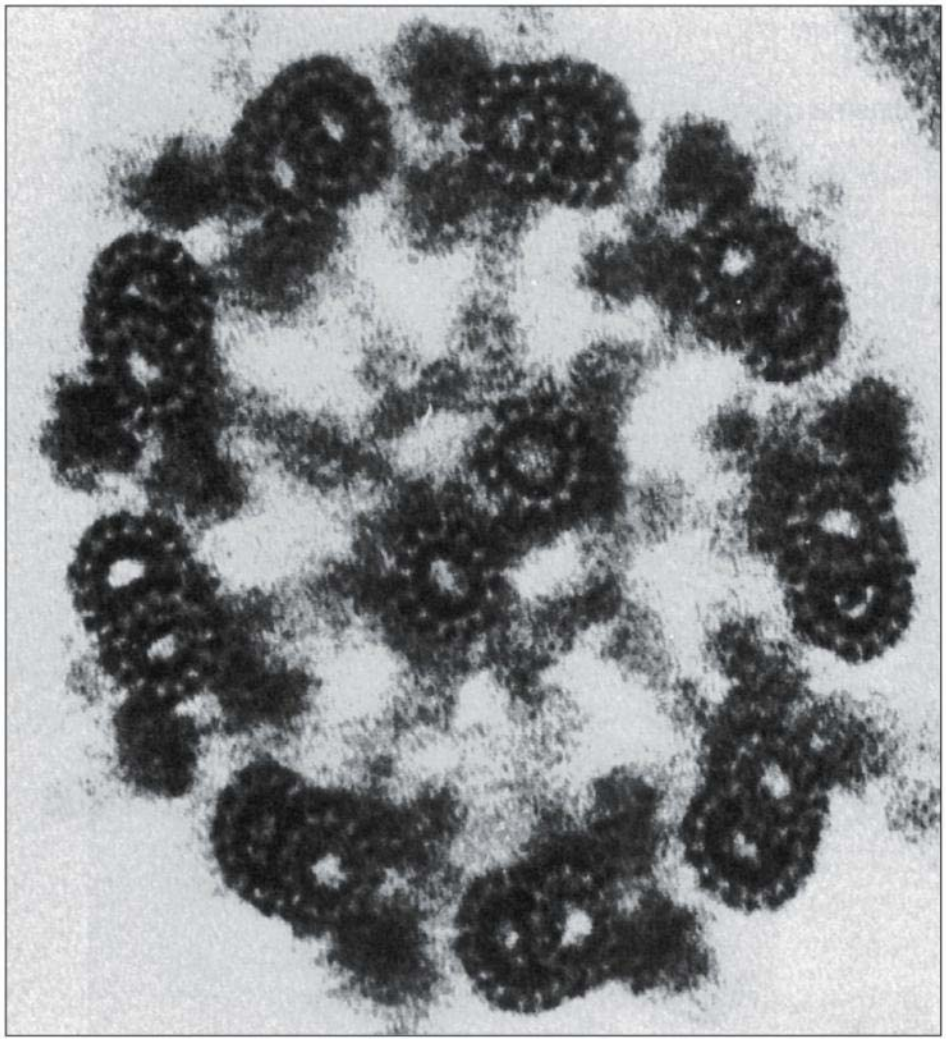


- Because of the rigid and thick cell wall, plant cells are usually unable to move
- However, inside the cell (for example in the algae *Nitella*) active flow of cytoplasm can be recognized
- This **cytoplasmic streaming** (or **cyclosis** in plant cells) is important for the spreading of metabolites throughout the cell (also in *slime molds*)
- Accomplished is this streaming by **myosin V** that moves on actin filaments (that are fixed to chloroplasts)
- Myosin V binds to large organelles (as the **ER**) that drags (拖曳) parts of cytosol with it during its motility



World of the Cell



The end of chapter 14!

Thank you!