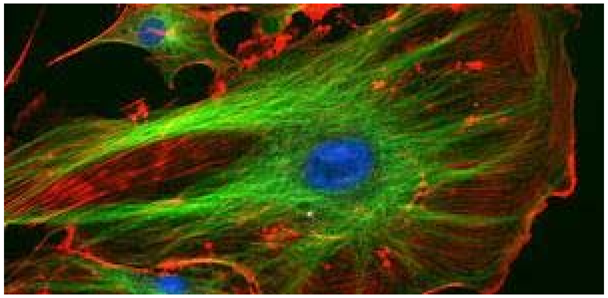
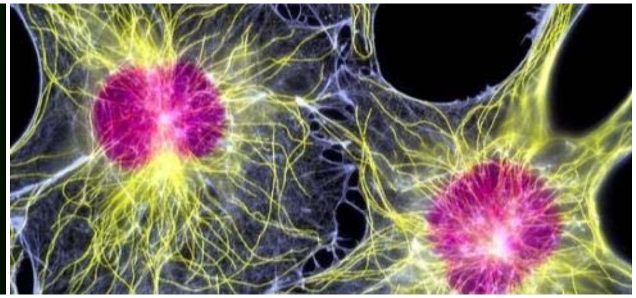
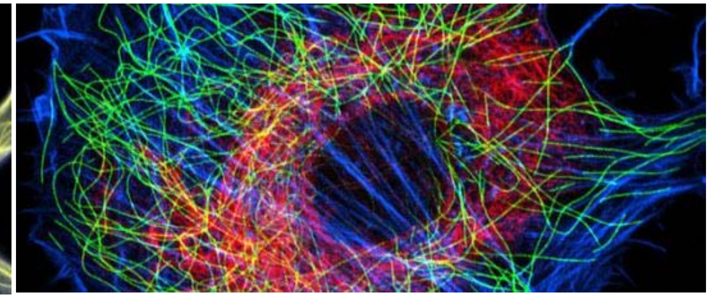
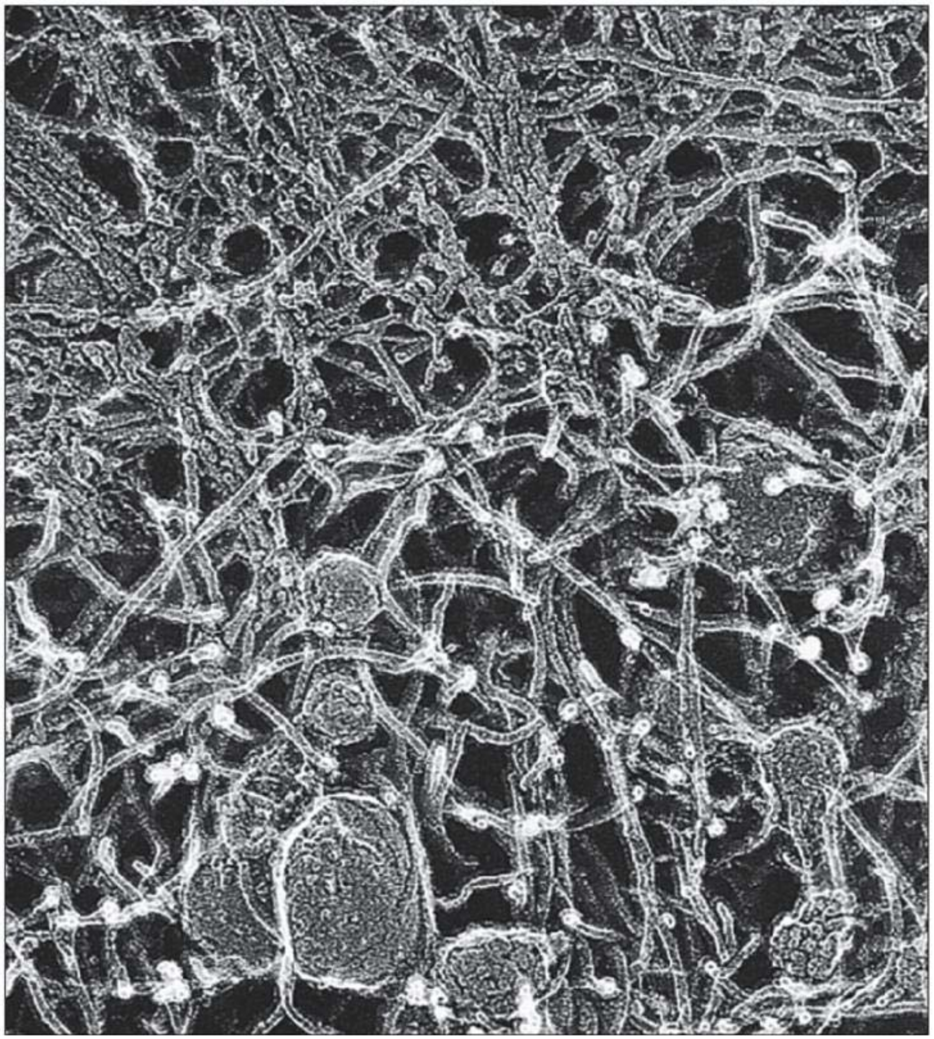




World of the Cell



Chapter 13: Cytoskeletal Systems

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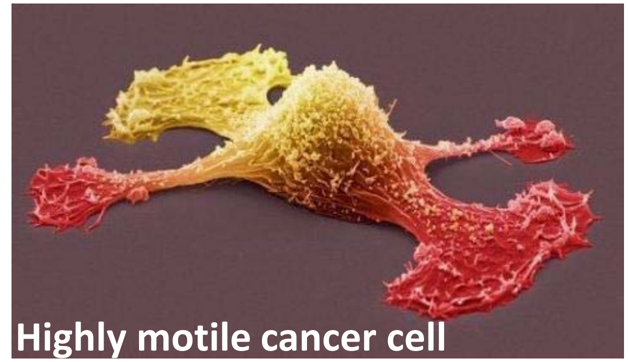
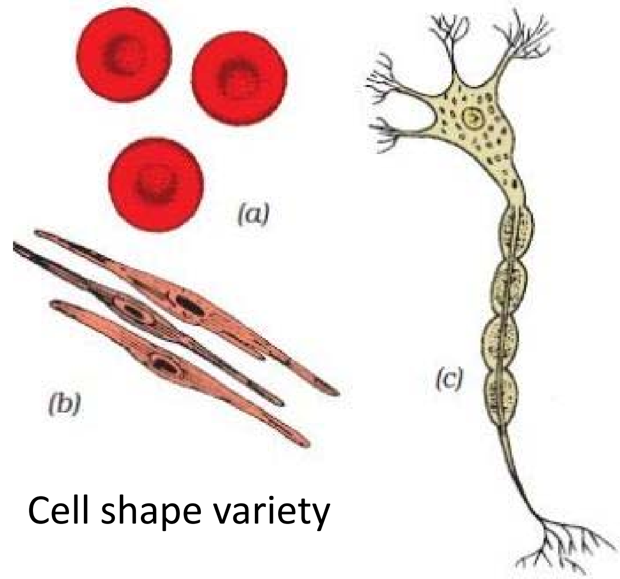
Importance of the cytoskeleton

The cytoskeleton:

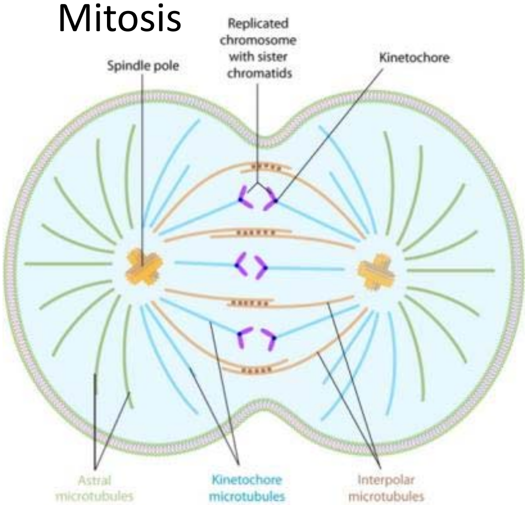
- <u>Allows cells to move</u>. Some movement is desired (**cell migration** during embryogenesis) and some movement is not desired (**cancer cell metastasis**).
- Provides the **cell stability** and its specific **shape** (e.g., compare red blood cells and neuron)
- Provides an <u>intracellular transport system</u> (molecular motors with “cargo” move on microtubules)
- **Positions organelles** as the nucleus, ER and Golgi
- Drives cell division (**mitosis**)
- Is <u>highly dynamic</u> (not static)


Highly motile cancer cell

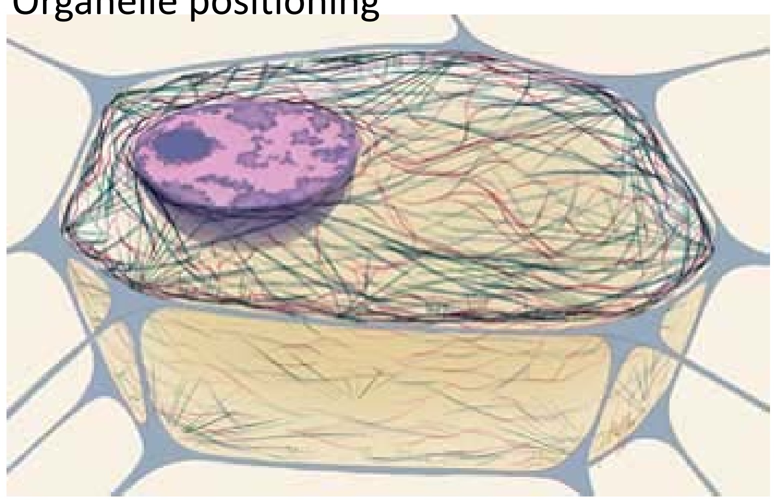


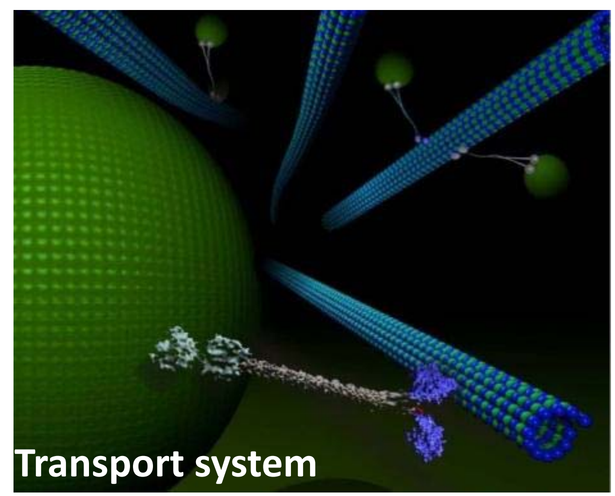
Cell shape variety

Mitosis

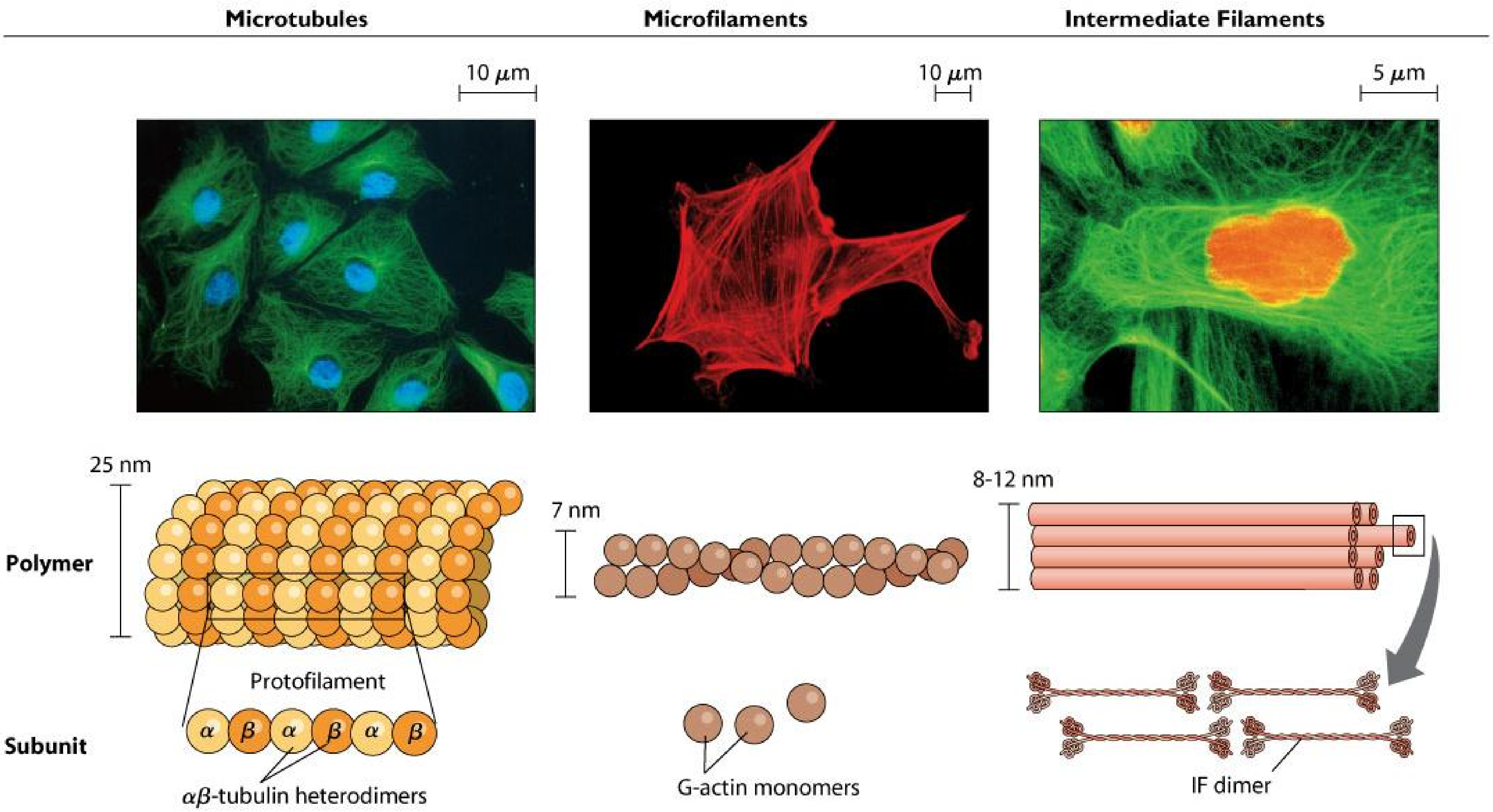


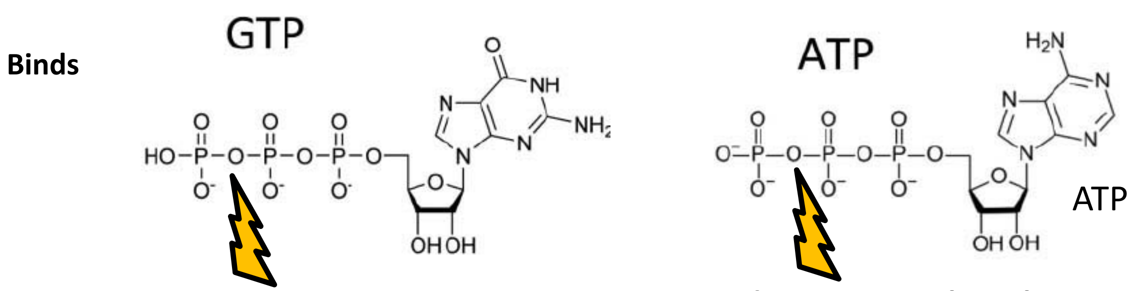
Organelle positioning




Transport system

Three major cytoskeletal elements exist

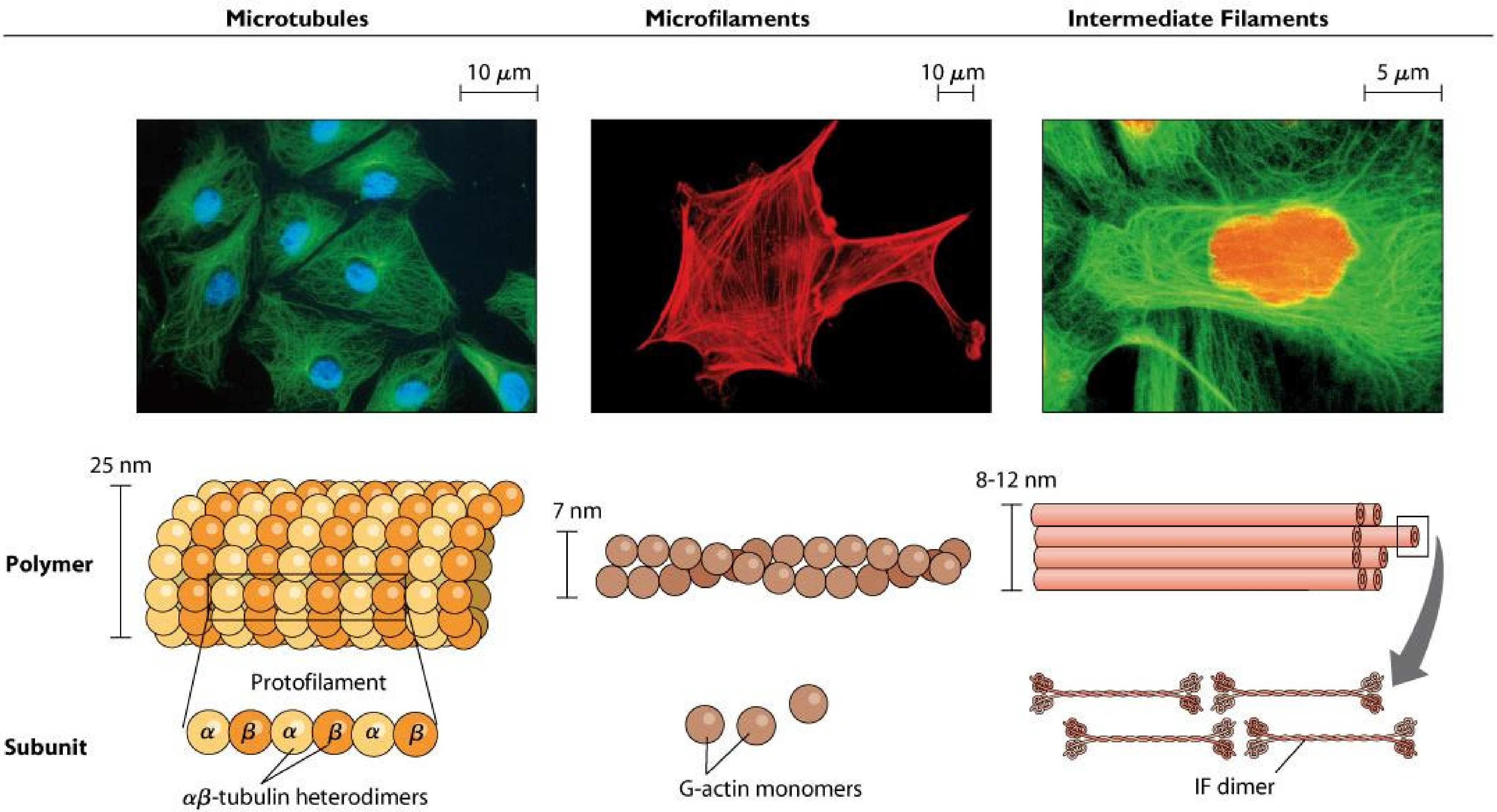




Guanosine triphosphate

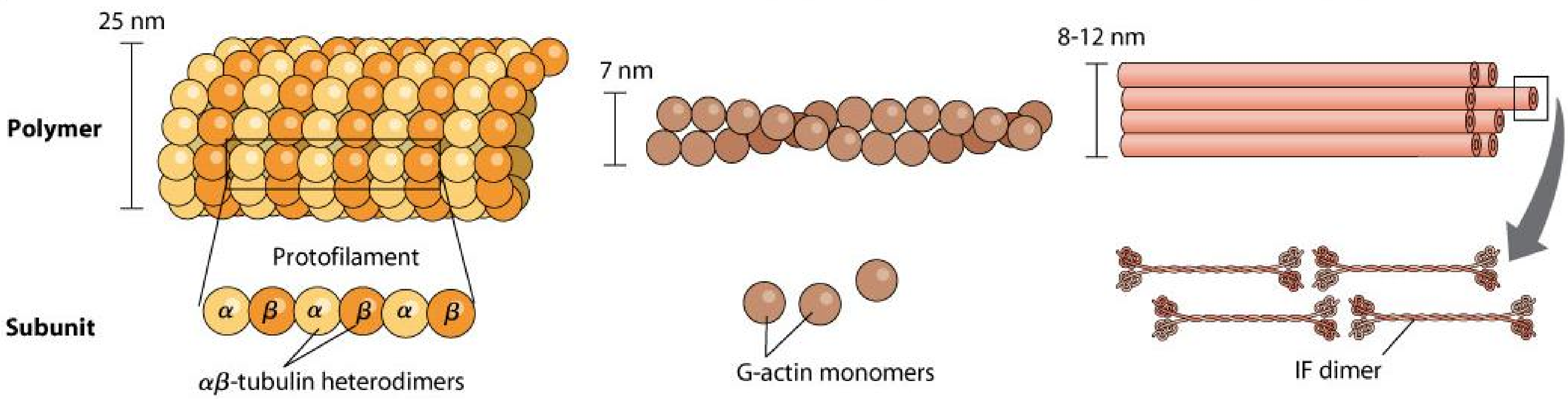
Adenosine triphosphate

$ATP - P_i = ADP + Energy$
(Adenosine diphosphate)



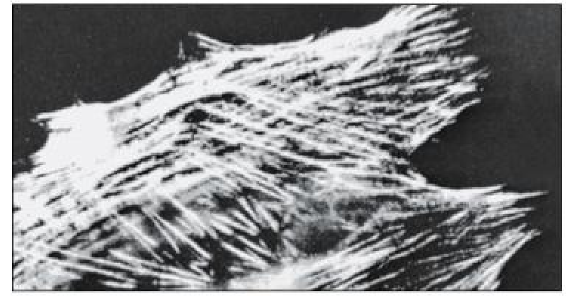
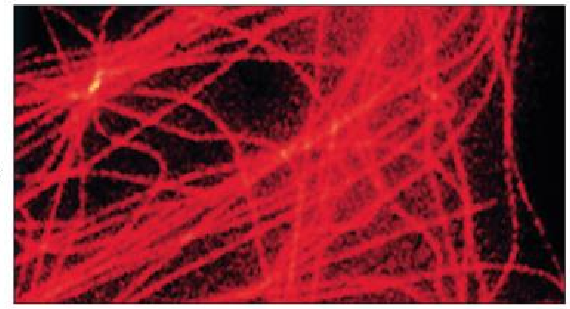
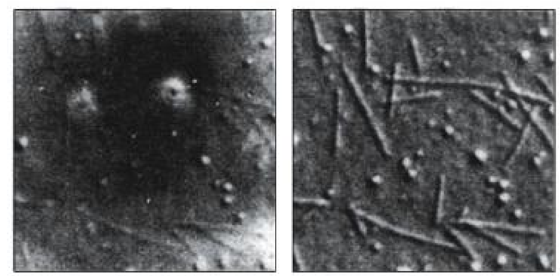
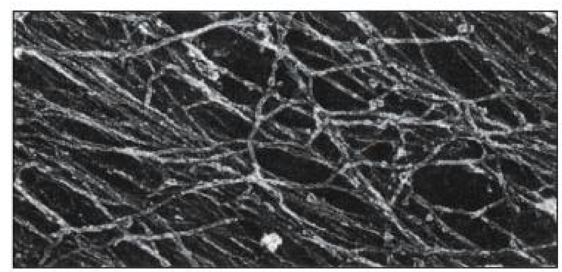
	Microtubules	Microfilaments	Intermediate Filaments
Structure	Hollow tube with a wall consisting of 13 protofilaments	Two intertwined chains of F-actin	Eight protofilaments joined end to end with staggered overlaps
Diameter	Outer: 25 nm Inner: 15 nm	7 nm	8–12 nm
Monomers	α-tubulin β-tubulin	G-actin	Several proteins; see Table 15-4
Polarity	(+), (−) ends	(+), (−) ends	No known polarity

Three major cytoskeletal elements exist



	Microtubules	Microfilaments	Intermediate Filaments
Functions	Cytoplasmic:	Muscle contraction	Structural support
	Organization and maintenance of animal cell shape and polarity	Cell locomotion	Maintenance of animal cell shape
	Chromosome movements	Cytoplasmic streaming	Formation of nuclear lamina and scaffolding
	Intracellular transport/ trafficking, and movement of organelles	Cytokinesis	Strengthening of nerve cell axons (neurofilament protein)
	Axonemal: Cell motility (sperm)	Maintenance of animal cell shape	Keeping muscle fibers in register (desmin)
		Intracellular transport/trafficking	

Techniques to visualize the cytoskeleton

Technique	Description	Example	
Fluorescence microscopy on fixed specimens*	Fluorescent compounds directly bind to cytoskeletal proteins, or antibodies are used to indirectly label cytoskeletal proteins in chemically preserved cells, causing them to glow in the fluorescence microscope.	A fibroblast stained with fluorescent antibodies directed against actin shows bundles of actin filaments.	
Live cell fluorescence microscopy* Microtubules (Alberts)	Fluorescent versions of cytoskeletal proteins are made and introduced into living cells. Fluorescence microscopy and video or digital cameras are used to view the proteins as they function in cells.	Fluorescent tubulin molecules were microinjected into living fibroblast cells. Inside the cell, the tubulin dimers become incorporated into microtubules, which can be seen easily with a fluorescence microscope.	
Computer-enhanced digital video microscopy	High-resolution images from a video or digital camera attached to a microscope are computer processed to increase contrast and remove background features that obscure the image.	Two micrographs showing several microtubules were processed to make them visible in detail.	 Unenhanced Enhanced
Electron microscopy	Electron microscopy can resolve individual filaments prepared by thin section, quick-freeze deep-etch, or direct-mount techniques.	A fibroblast cell is prepared by the quick-freeze deep-etch method. Bundles of actin microfilaments are visible.	

Going into details: Microtubules

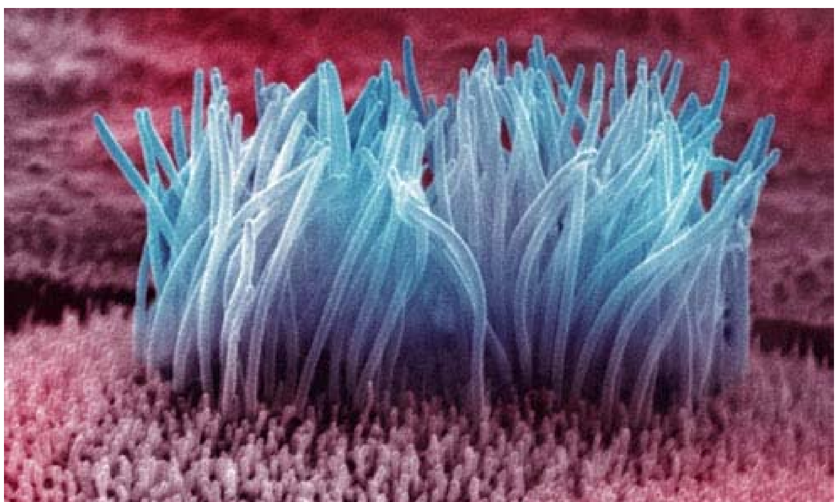
Two groups exist: **cytoplasmic** microtubules and **axonemal** microtubules

1) **Cytoplasmic** (inside the plasma of cells) microtubules

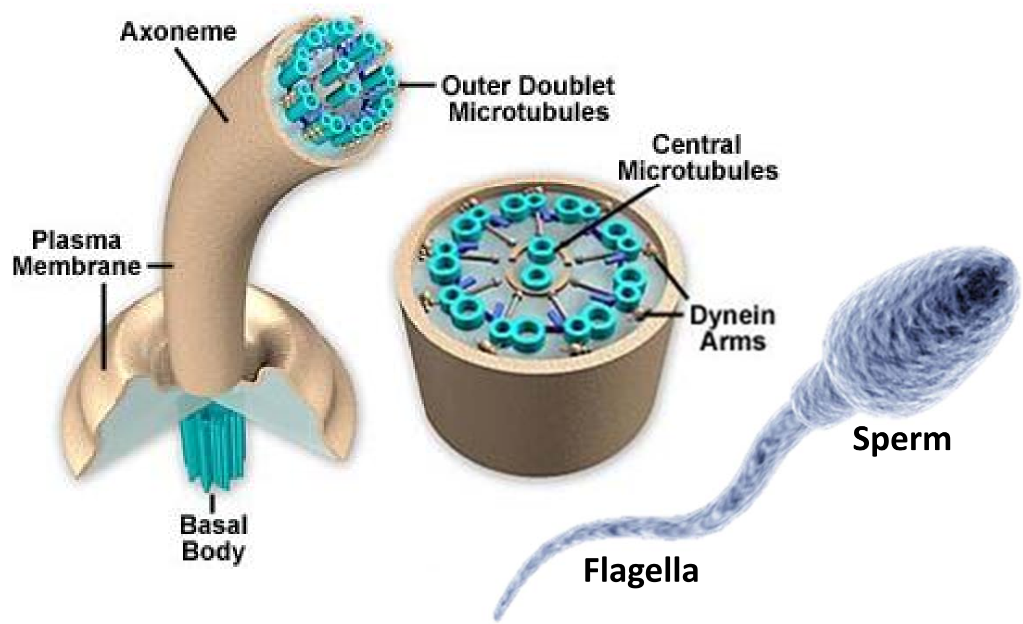
- Form a somehow **loosen but dynamic network** for providing cell form and shape
- Position the ER and the Golgi (MTs can be found superimposed to the ER and Golgi)
- Important to stabilize and maintain the long and thin axons and dendrites in neurons
- Form the **mitotic apparatus** (spindle) during mitosis and drive chromosome segregation
- Provide **tracks for molecular motors** to transport organelles and other cargo

2) **Axonemal** microtubules

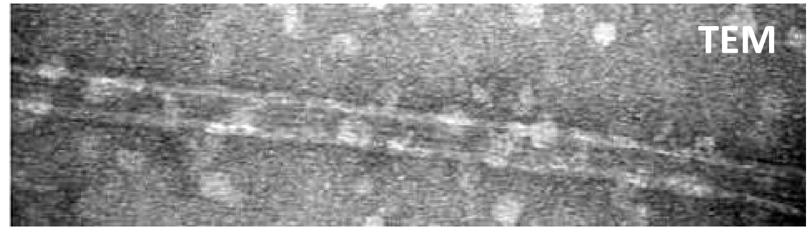
- **Stable and static** microtubules in cilia, flagella and basal bodies
- Form doublet and triplet structures with various associated proteins
- The **axoneme** is the central unit of cilia and flagella with a bundle of microtubules

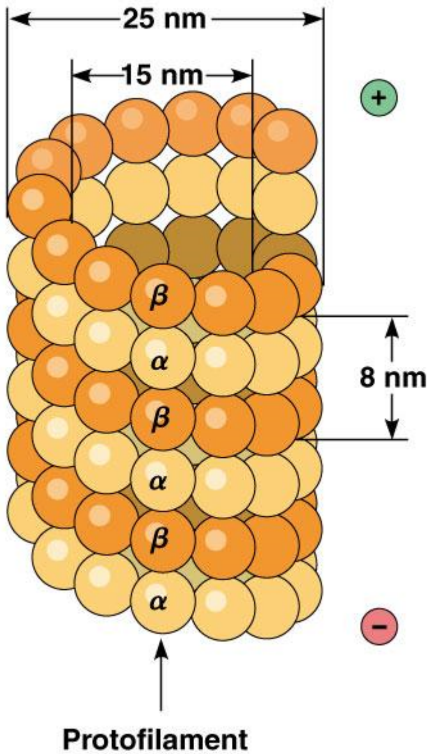


Motile cilia in airway epithelium sweeping mucus

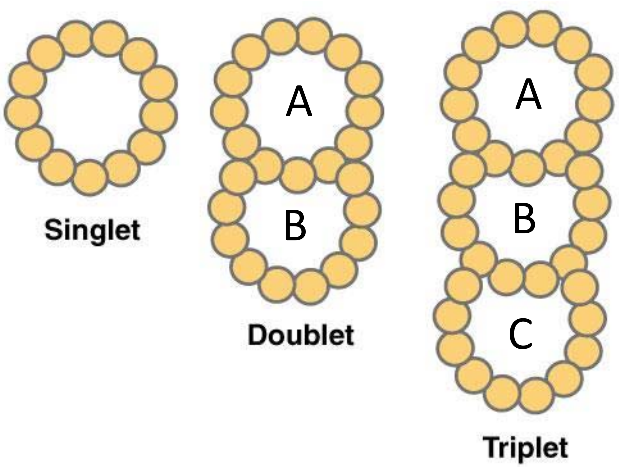


Fine structure of microtubules



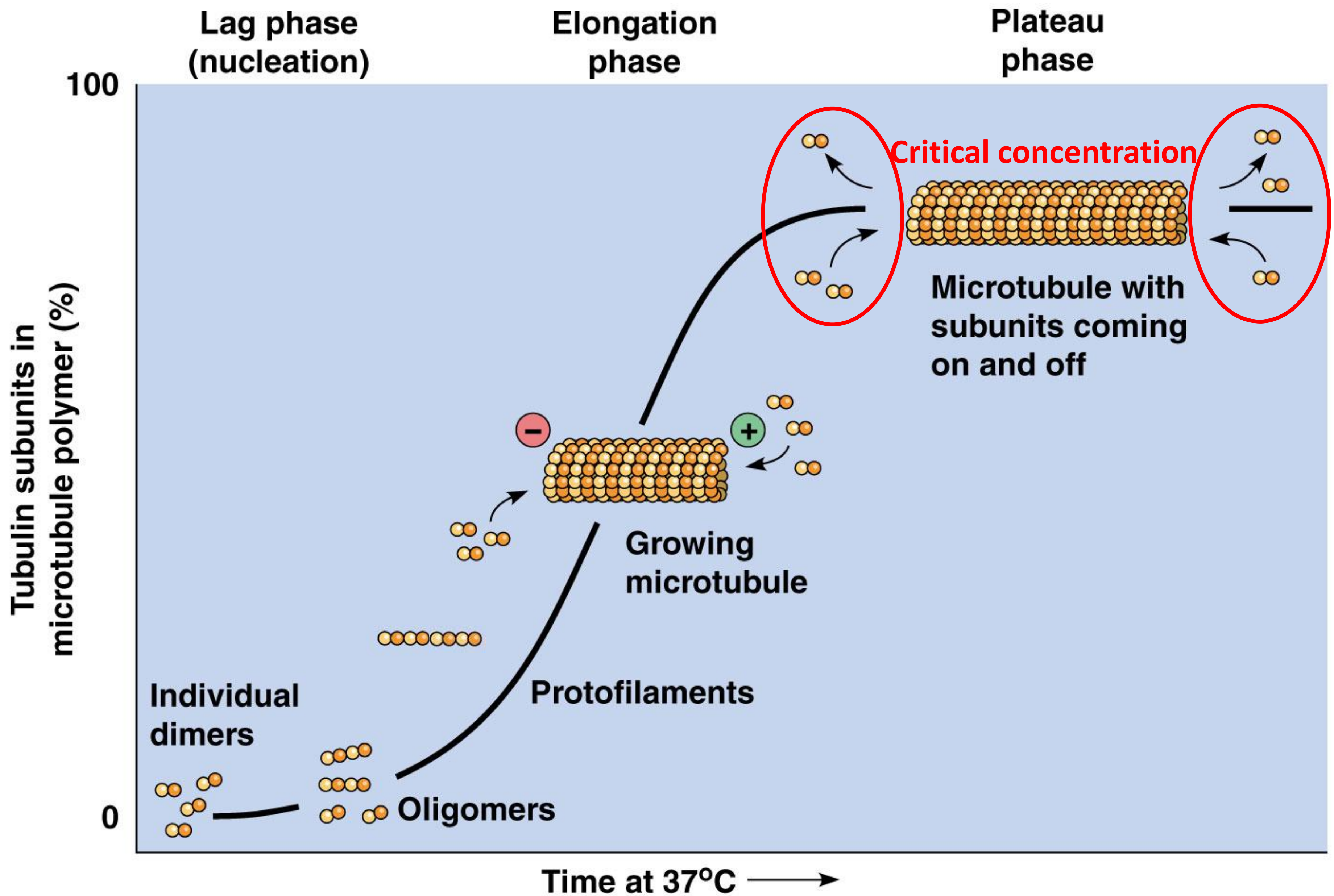


- The **diameter** of microtubules (MT) is **25 nm** and are thus the largest cytoskeletal elements
- MTs consist of **13 protofilaments** which are laterally assembled to form a hollow cylinder
- MTs are polymers and the basic subunit is a the **αβ-tubulin heterodimer** (covering 8 nm on the microtubule)
- Tubulin (55 kDa) **binds GTP** at its N-terminus and **MAPs** (microtubule associated proteins) at its C-terminus.
- The tubulin dimers have all the same orientation providing an intrinsic **polarity** of the microtubule
- The polymerization at the plus-end is faster compared to the minus-end



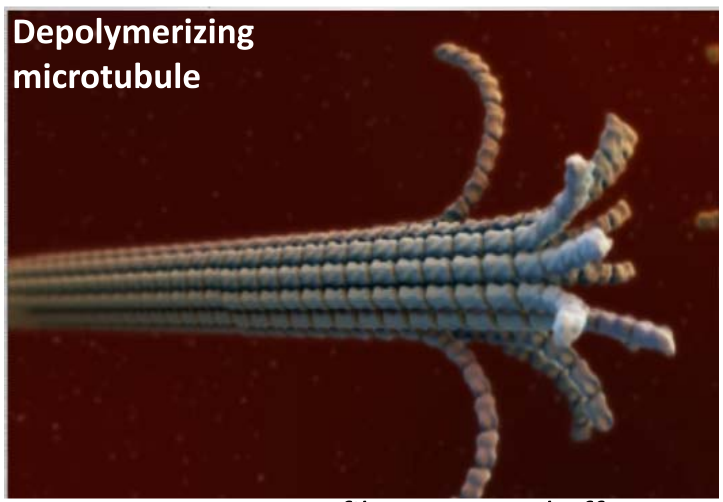
- In cilia and flagella microtubules appear as singlet and doublet structures.
- In basal bodies and centrioles microtubules appear as triplet structures
- In the doublet or triplet structure one ring is complete (A-ring with 13 protofilaments) while others contain only 10 protofilaments (B- and C-rings)

The polymerization of microtubules occurs at three phases

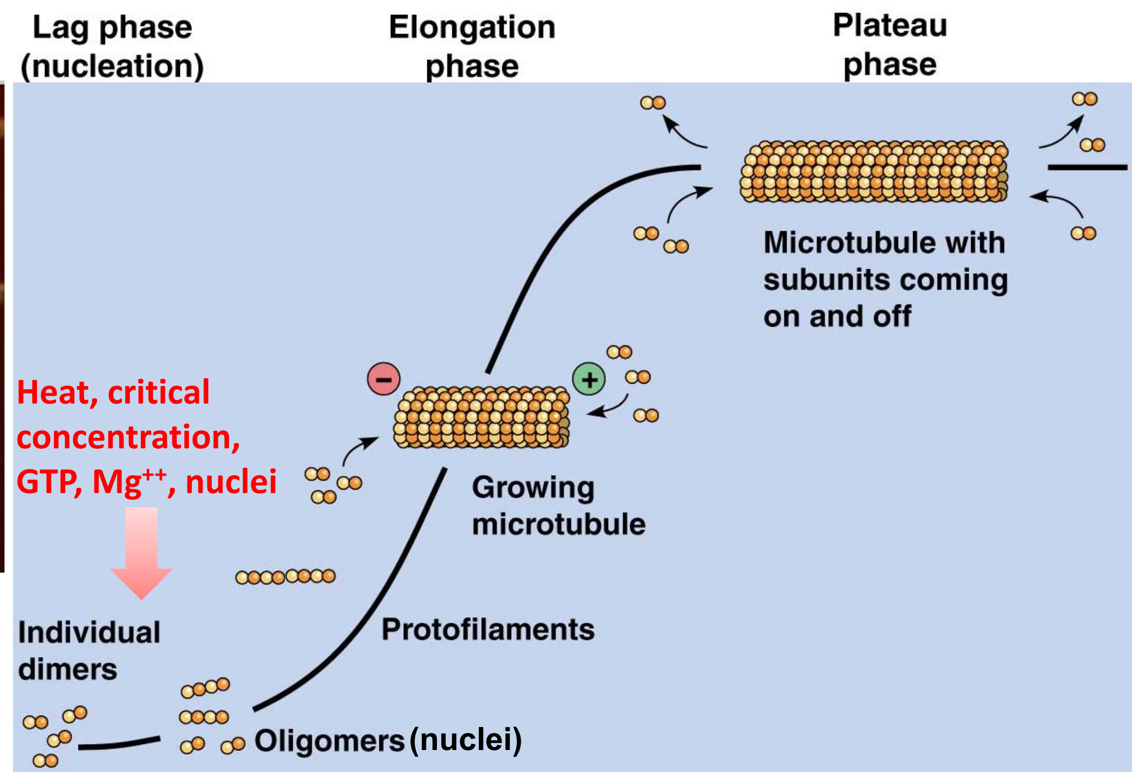


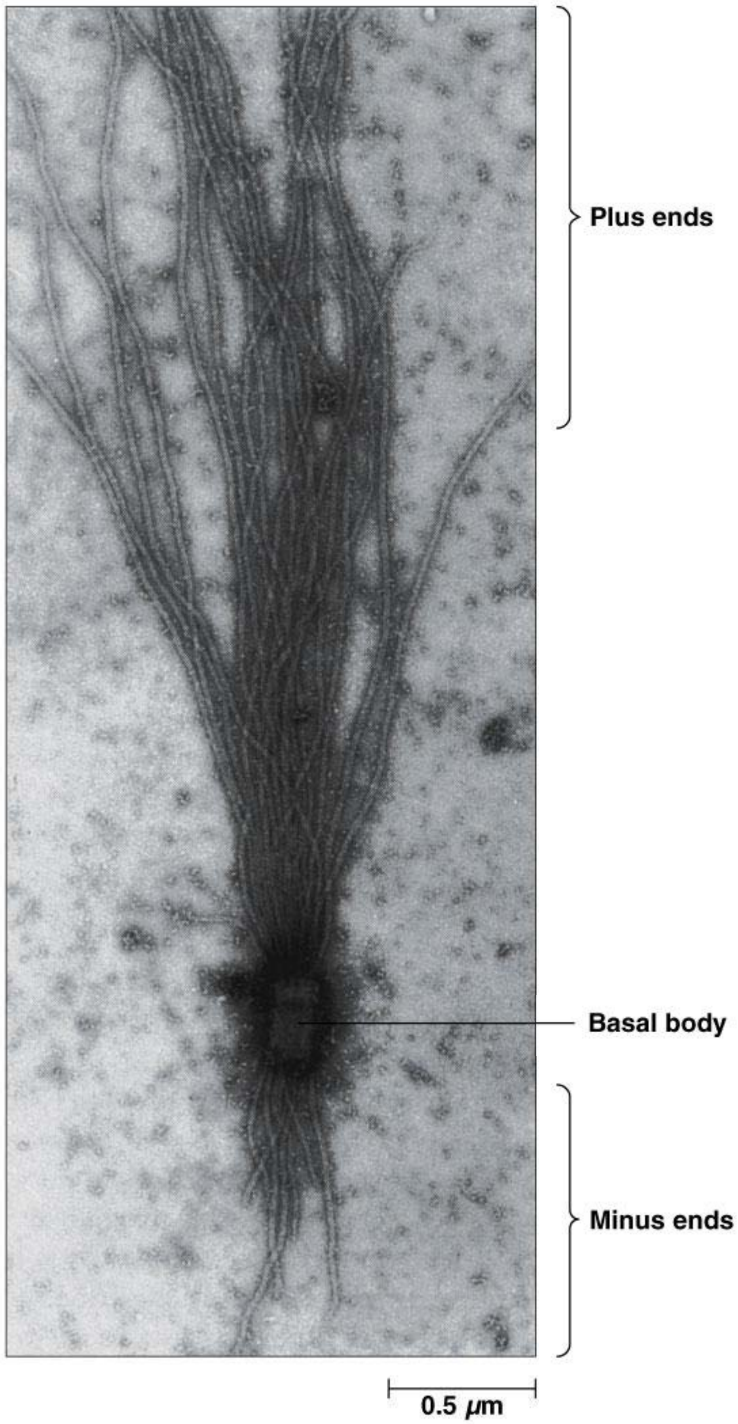
MT polymerization facts

- MT polymerization is **fastest at 37°C** and <u>does not occur in the cold</u> (e.g., 4°C)
- Besides **warmth**, polymerization requires **GTP** and **Mg^{2+}** and the formation of **oligomers**
- **Lag phase**: represents the <u>slow formation of oligomers</u> which serve as **nuclei** for the subsequent <u>fast</u> **elongation phase**
- **Plateau phase** (steady-state)**:** <u>concentration of free tubulin limits</u> further MT growth
- This concentration is also called the **critical concentration**
- On the other hand, if the <u>critical concentration</u> of free tubulin <u>falls below</u>, the MT will **depolymerize**



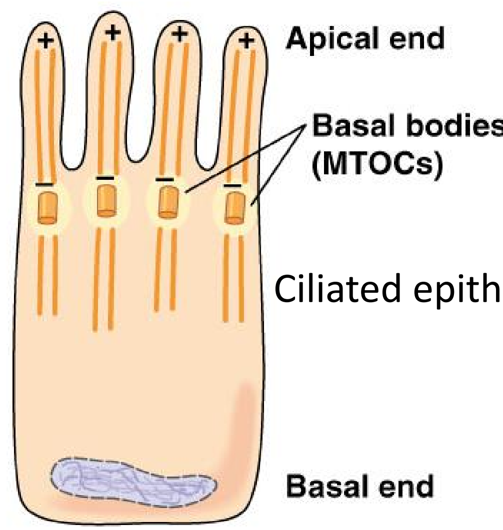
Protofilaments peel off



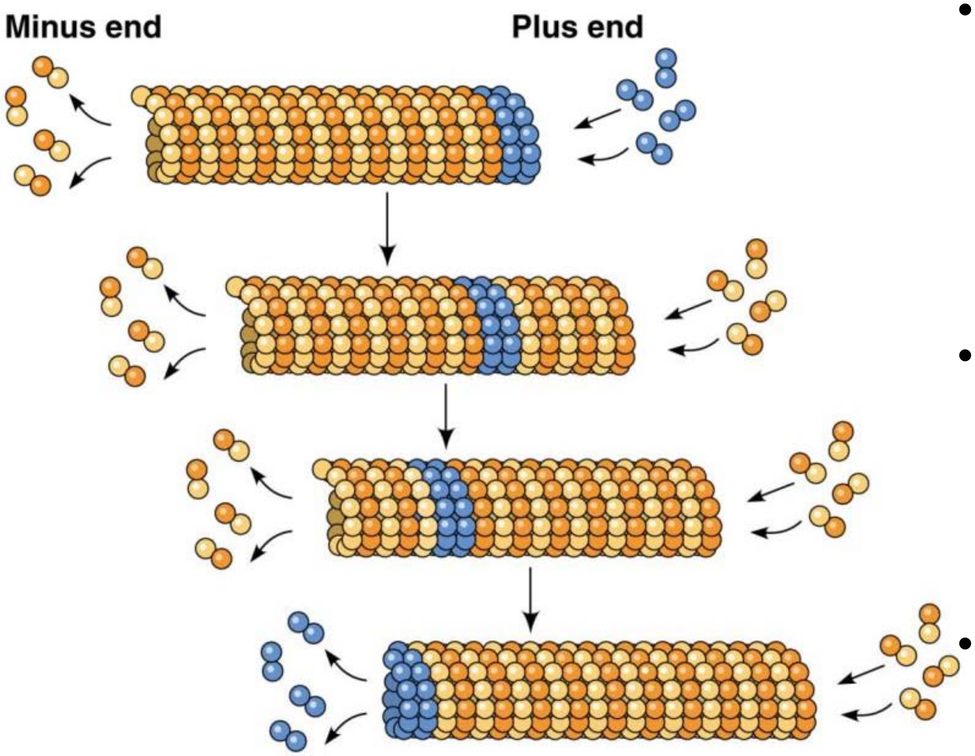


Microtubules grow faster at the plus end

- In a classic experiment a **basal body** was isolated and **used as a nuclei** to seed tubulin polymerization
- With the electron microscope it can be seen that more MTs grow at one end of the basal body and only few grew on the other end of the basal body
- Further investigation has shown that the end of the basal body with more MTs growing contain MTs with their plus-end out
- In ciliated epithelial cells the orientation of MTs is critical for metabolite transport



Reasons for the different growth rates and treadmilling



Marked tubulin dimers added at the plus-end progressively move through the microtubule and eventually fall of at the minus end

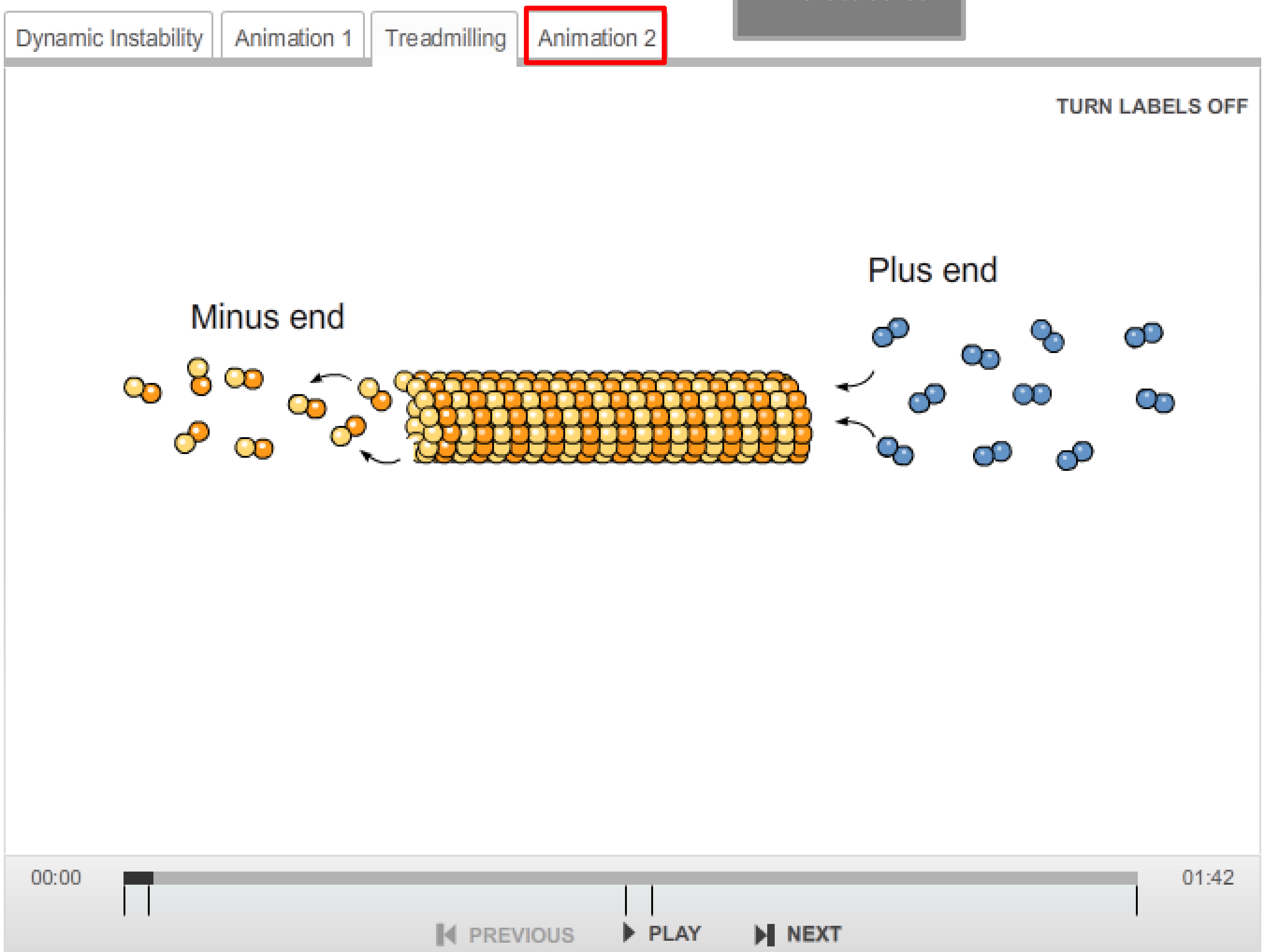
- The different growth rates at the two ends of a microtubule is related to the **different critical concentrations** (cc) for these ends: the **cc** at the **plus-end** is lower than the cc at the minus end (so plus-ends grow faster)
- Sometimes a phenomena called **treadmilling** can be observed: tubulin dimers add to the plus-end, travel through the filament and finally fall off at the minus end
- When does it happen? It happens when the free tubulin concentration is **above** the **cc** for the **plus end** but below the cc at the minus end:

$Cc^{(+)} <$ G-actin $< Cc^{(-)}$

A treadmill

Flash animation

Instability of microtubules



Drugs that affect microtubule stability

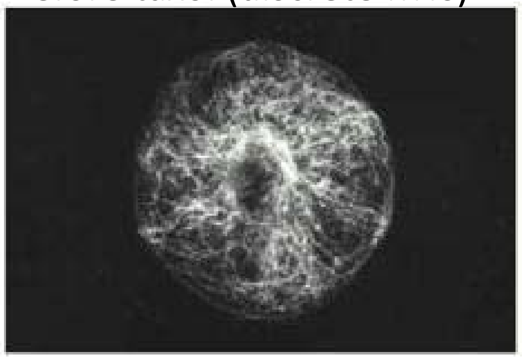
1) **Destabilizing drugs**: Prevent formation of mitotic spindle, thus mitosis is inhibited (used in treatment of rapidly dividing cancer cells)

- **Colchicine** (from plants): binds to tubulin dimers and inhibit their polymerization. Also depolymerizes existing MTs.
- **Vinblastine**, **vincristine** (from plants): aggregates tubulin and prevents MT growth
- **Nocodazole** (synthetic): similar to colchicine but effect is reversible (can be washed out)

2) **Stabilizing drugs**: Freezes mitotic spindle, thus inhibits completion of mitosis

- **Taxol** (from plants): Binds tightly to MTs and stabilizes them. Facilitates tubulin polymerization. Used in breast cancer treatment.

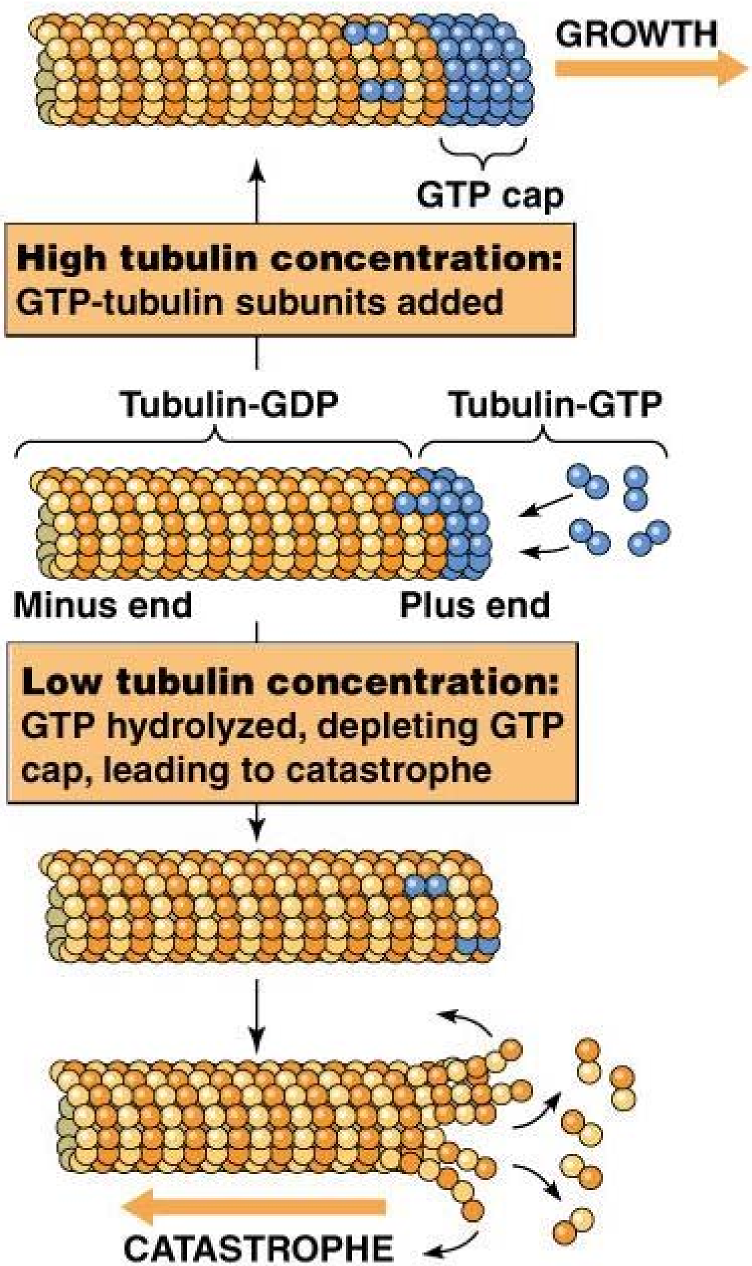
Before taxol (discrete MTs)



After taxol (thick MT bundles)

Drug	Source	Affect
Drugs Affecting Microtubules		
Colchicine, colcemid	Autumn crocus, *Colchicum autumnale*	Binds tubulin monomers, inhibiting assembly
Nocadazole	Synthetic benzimidazole	Binds β-tubulin, inhibiting polymerization
Vinblastine, vincristine	Periwinkle plant, *Vinca rosea*	Aggregates tubulin heterodimers
Taxol	Pacific yew tree, *Taxus brevifolia*	Stabilizes microtubules

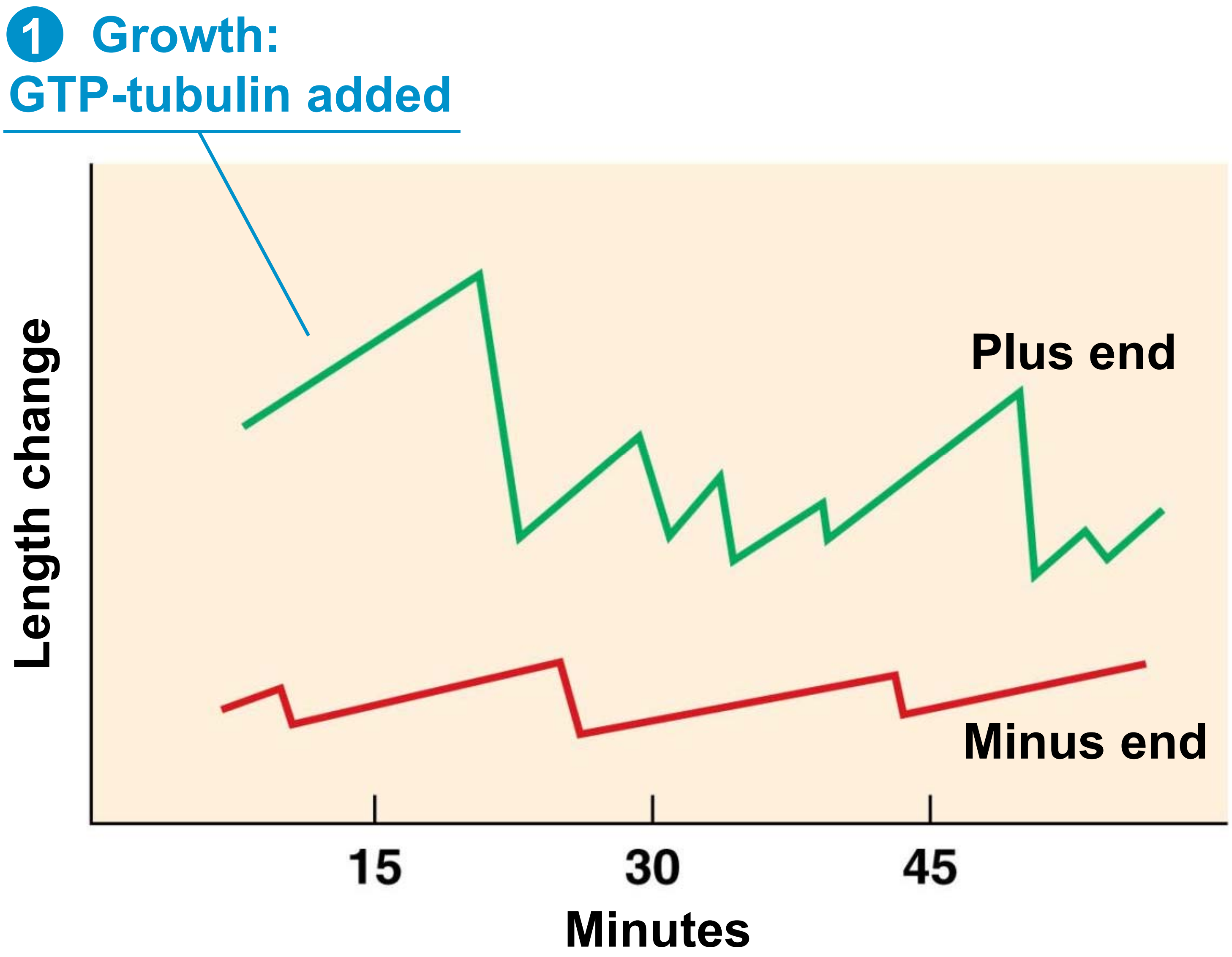
Dynamic instability of microtubules



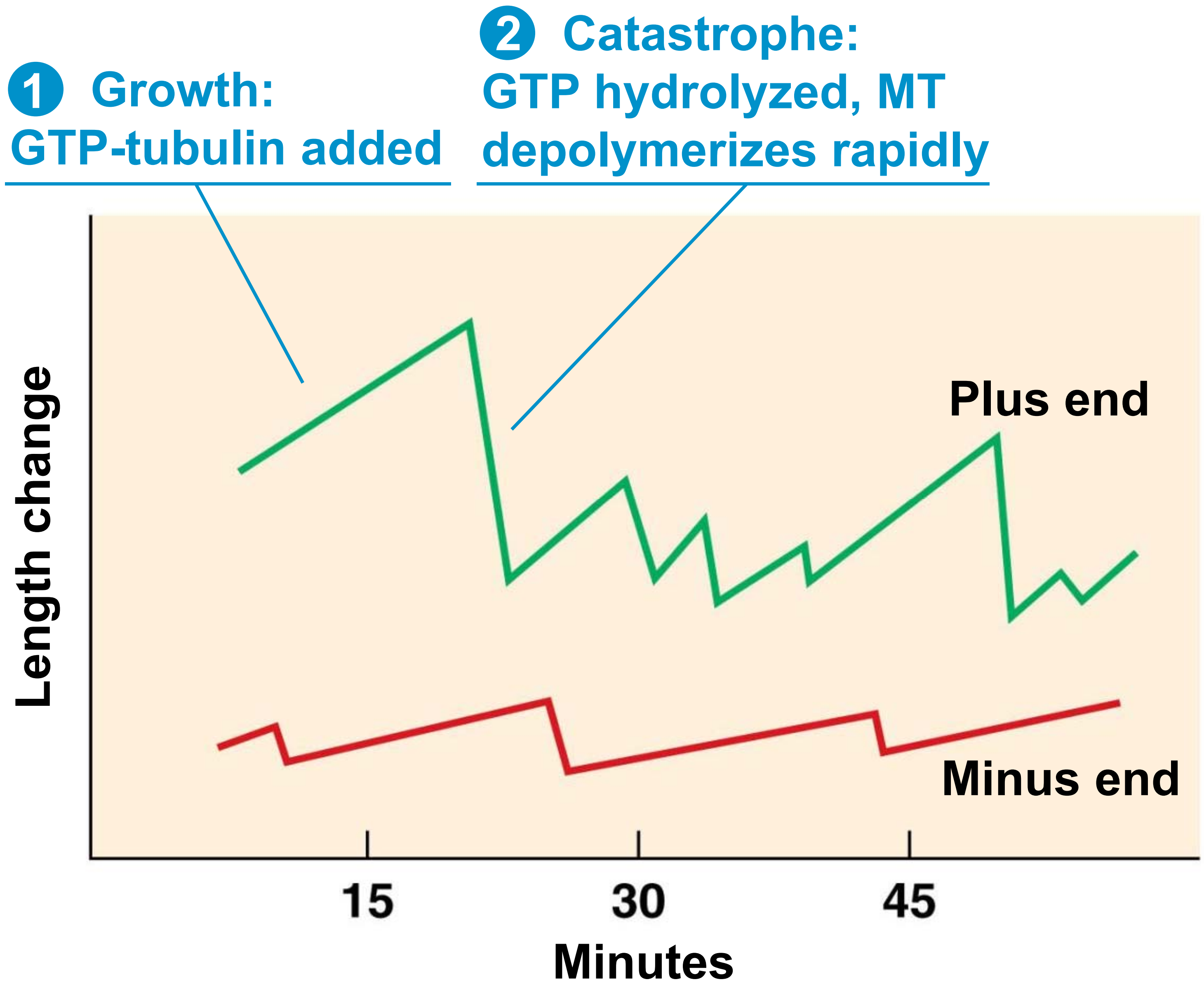
- In cells it can be observed that **some MTs slowly grow** while **others rapidly shrink** at the same time. The fast shrinking is also called catastrophe. Superresolution MTs
- Microtubule polymerization requires GTP bound to tubulin (**GTP-tubulin**)*.
- During polymerization the GTP bound to tubulin is hydrolyzed: the final MT contains lots of **GDP-tubulin**.
- The reason for **dynamic instability** is the presence or absence of GTP-tubulin: if enough GTP-tubulin is presence, a **protective GTP-cap** can form at the plus end preventing the MT from fast shrinking.
- If, however, GTP-tubulin becomes low, the cap disappears and catastrophe happens.

***Note**: Both α- and β-tubulin can bind GTP. However, only β-tubulin can hydrolyze GTP to GDP.

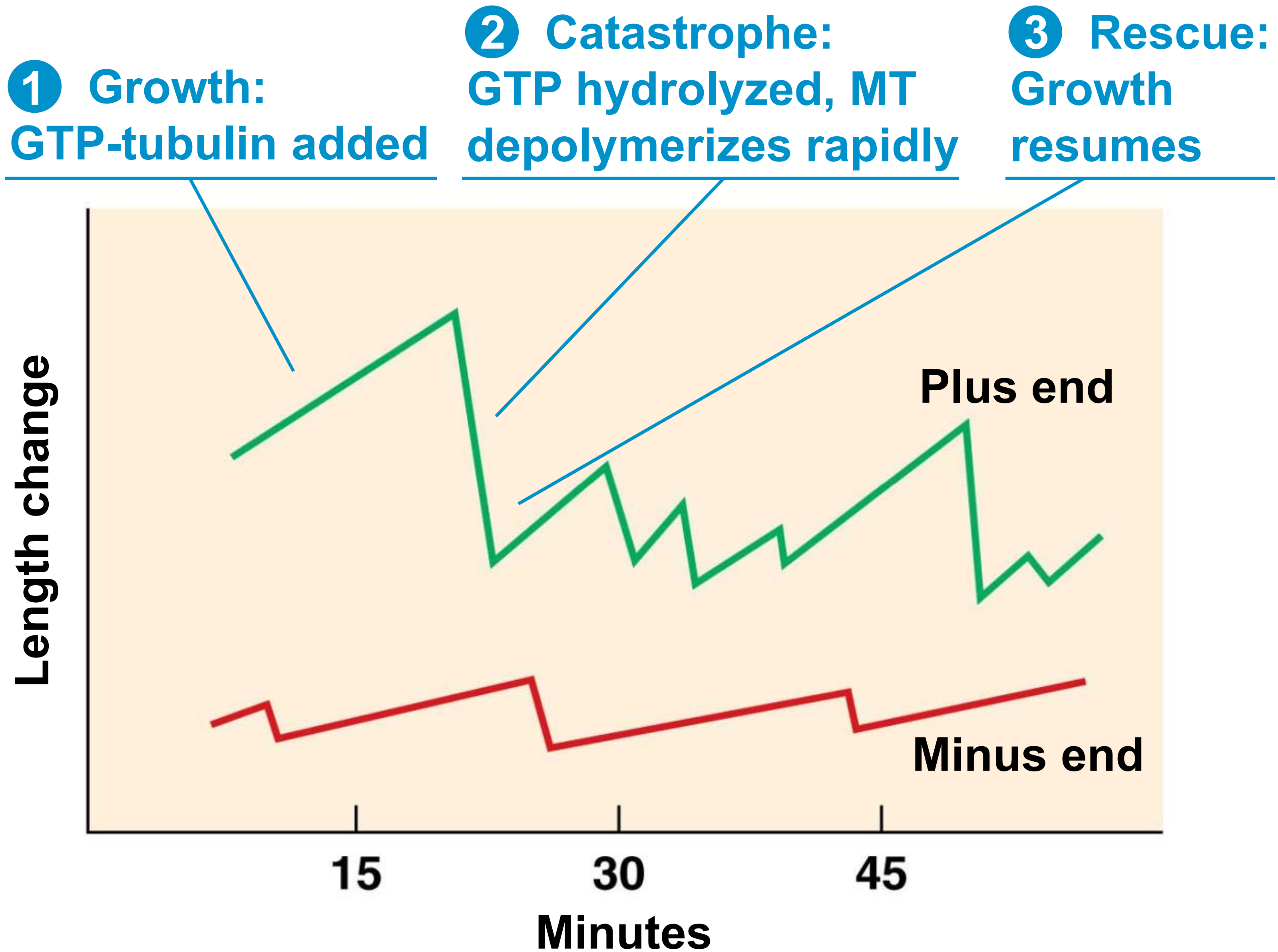
Dynamic instability of microtubules



Dynamic instability of microtubules

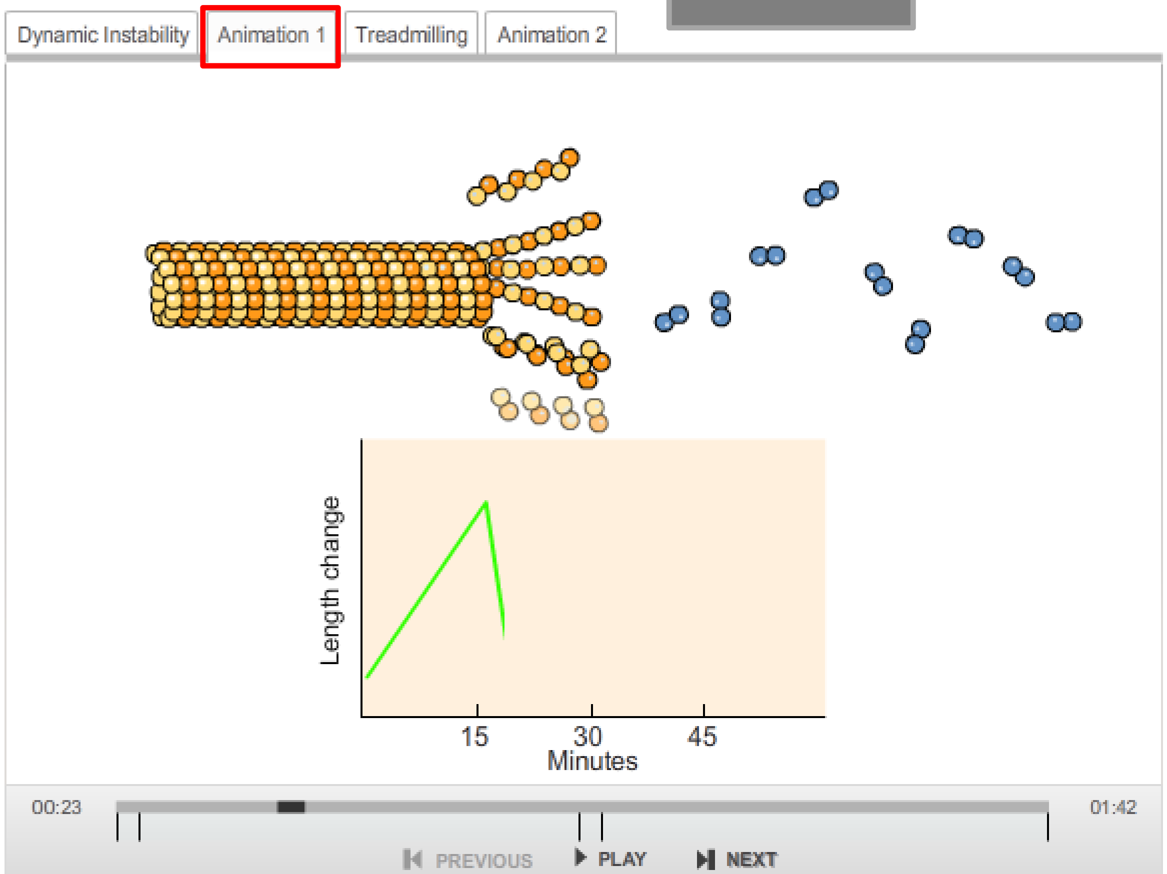


Dynamic instability of microtubules



Flash animation

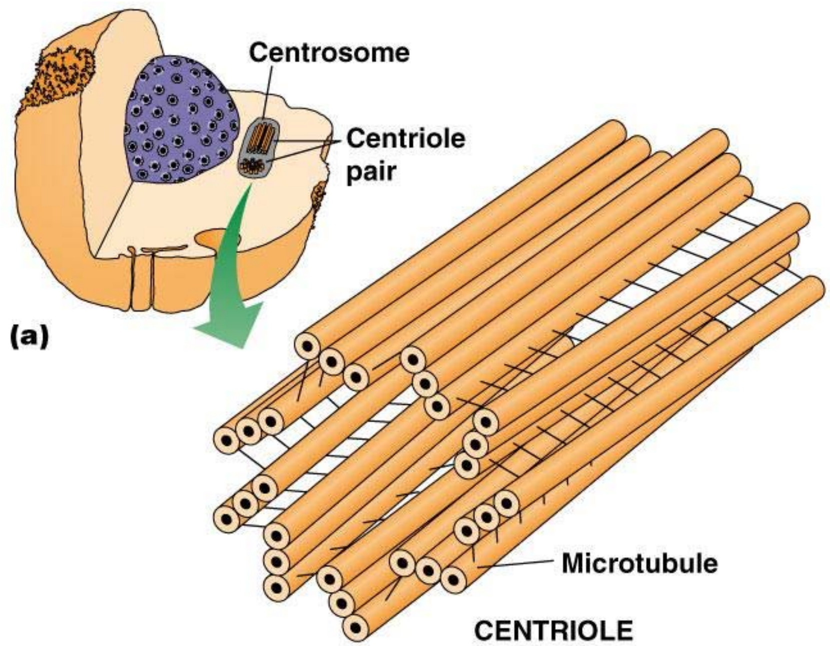
Instability of microtubules

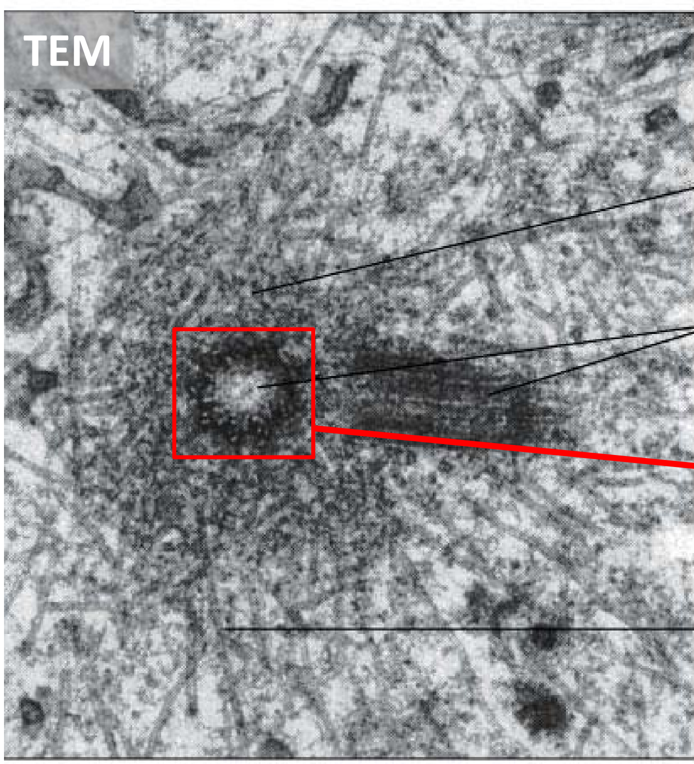


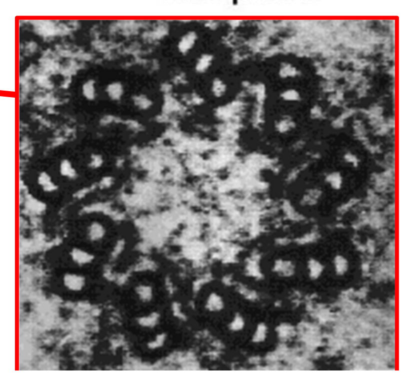
What does "protective" cap mean? Because GTP-tubulin dissociates much slower (4x) from the MT than GDP-tubulin (GTP-tubulin is more "sticky")

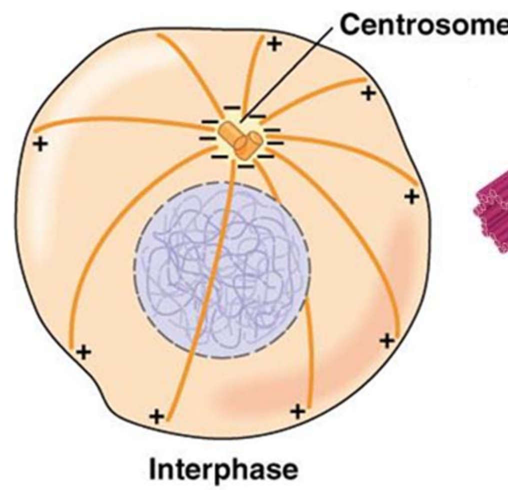
Microtubule organizing center (MTOC)

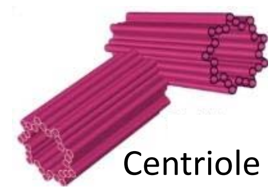
- The <u>m</u>icrotubule <u>o</u>rganizing <u>c</u>enter (MTOC) is <u>located near the nucleus</u>.
- MTOC is also called **centrosome**
- Microtubules are **attached with their minus ends to the MTOC** and <u>grow from there to the cell periphery</u>
- The MTOC contains <u>a pair of</u> **centrioles** which are composed of **9 triplet microtubules**







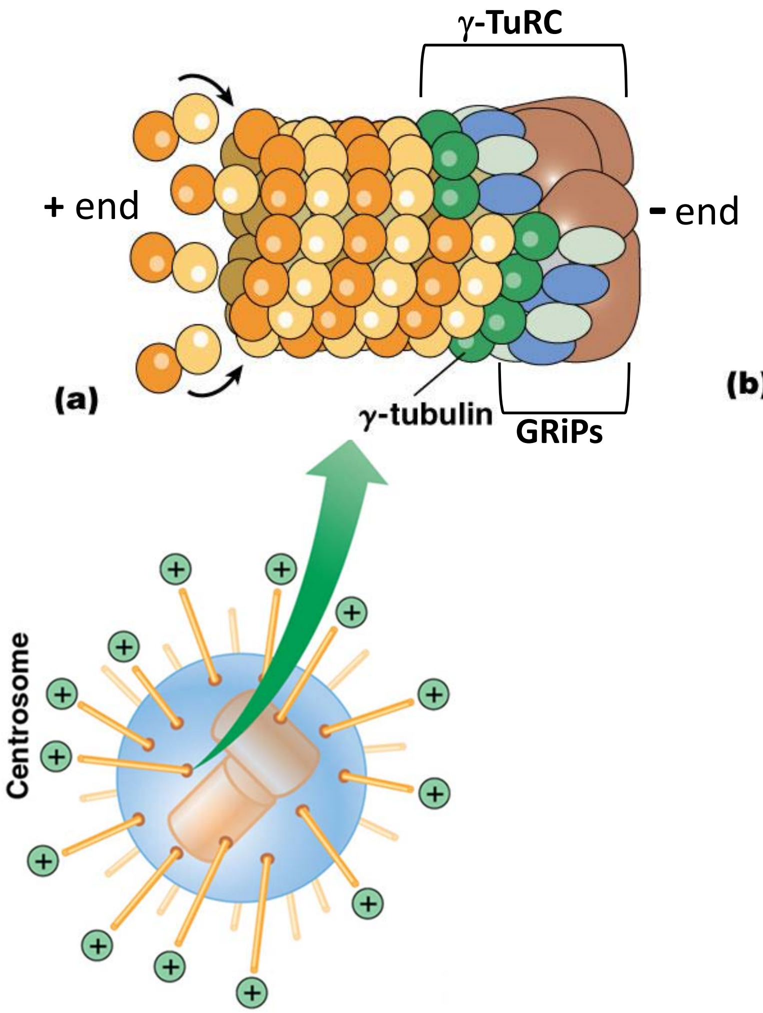


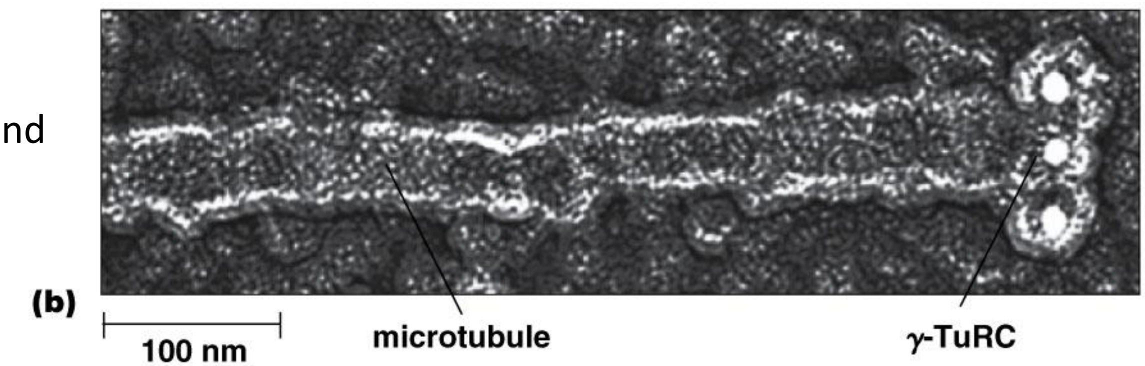


Centriole pair appears as a “T”

γ-tubulin is a nucleation factor found at MTOCs

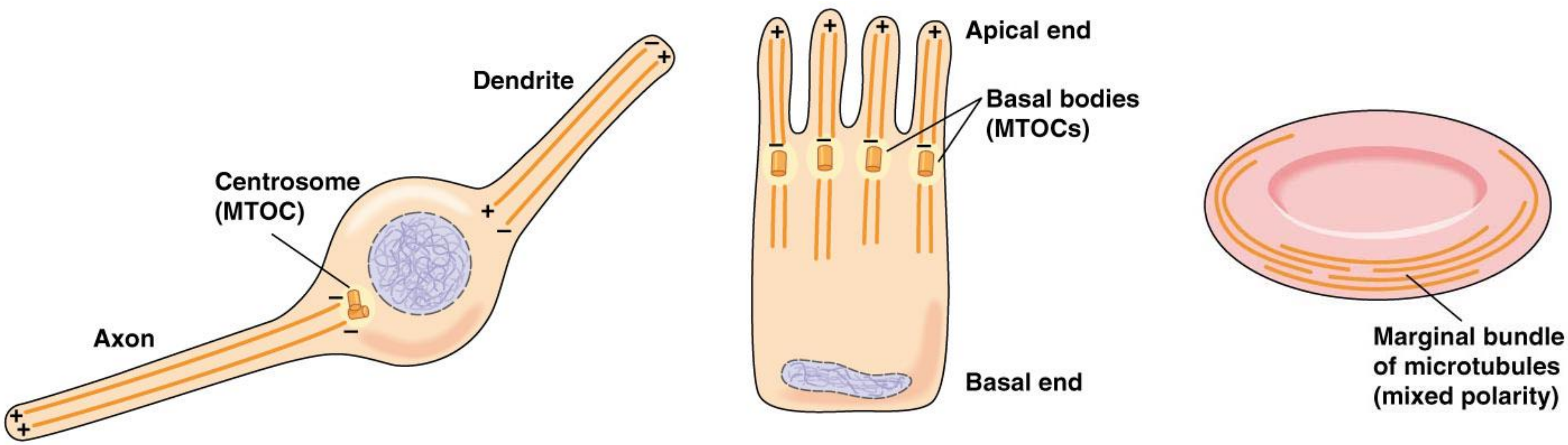
- The reason why microtubules preferably grow from MTOCs is because of the presence of the nucleation factor **γ-tubulin** in the pericentriolar matrix
- γ-tubulin also associates with other proteins named **GRiPs** (*g*amma tubulin *ri*ng *p*roteins)
- The GRiPs together with γ-tubulin form the so called γ-tubulin ring complex (**γ-TuRC**)





Antibody against an GRiP is coupled to gold beads (immuno-gold EM)

Centrosomes provide necessary microtubule order and polarity



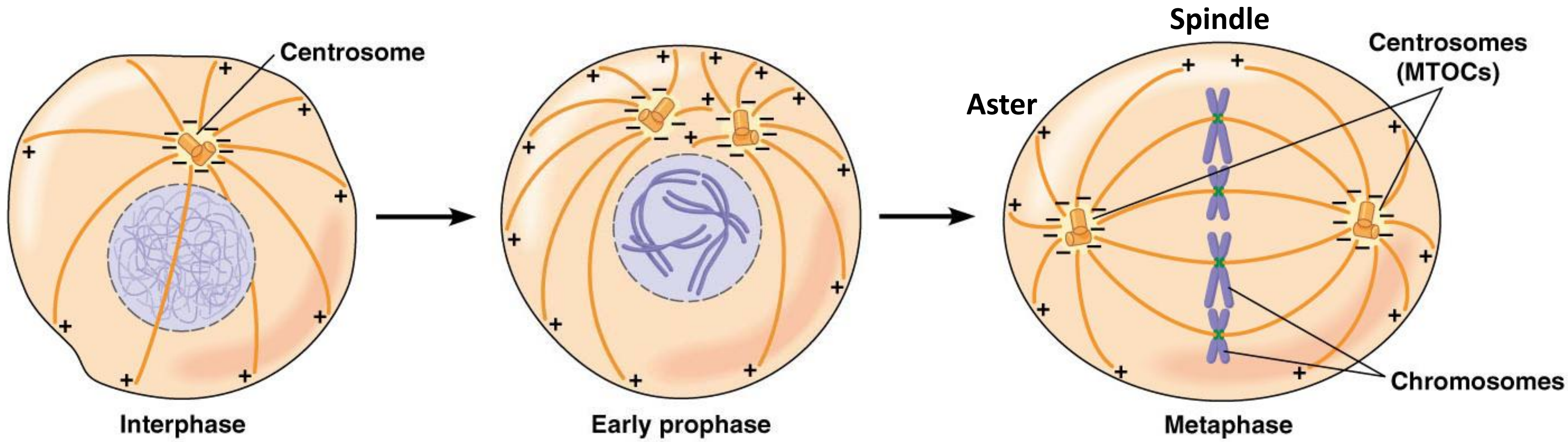
(a) Nerve cell **(b)** Ciliated epithelial cell **(c)** Red blood cell

- In **axons** MTs grow from an MTOC, thus the polarity is **uniform**. In **dendrites** MTs do not grow from an MTOC, thus the polarity in **mixed**.
- In axons it is important that synaptic vesicles are transported from the cell body to the distal synapses. In dendrites bidirectional transport is more important (e.g., receptor recycling)

- In ciliated epithelial cells the **MTOC** is a **basal body**.
- The uniform polarity of MTs is also important here as proteins need to be moved to the cilia tip (e.g., membrane receptors or tubulin)

- Red blood cells do not have MTOCs. Therefore, MTs appear as **mixed polarity**.
- The marginal bundle of MT stabilizes the blood cell.

Centrosomes duplicate and move to the poles during mitosis

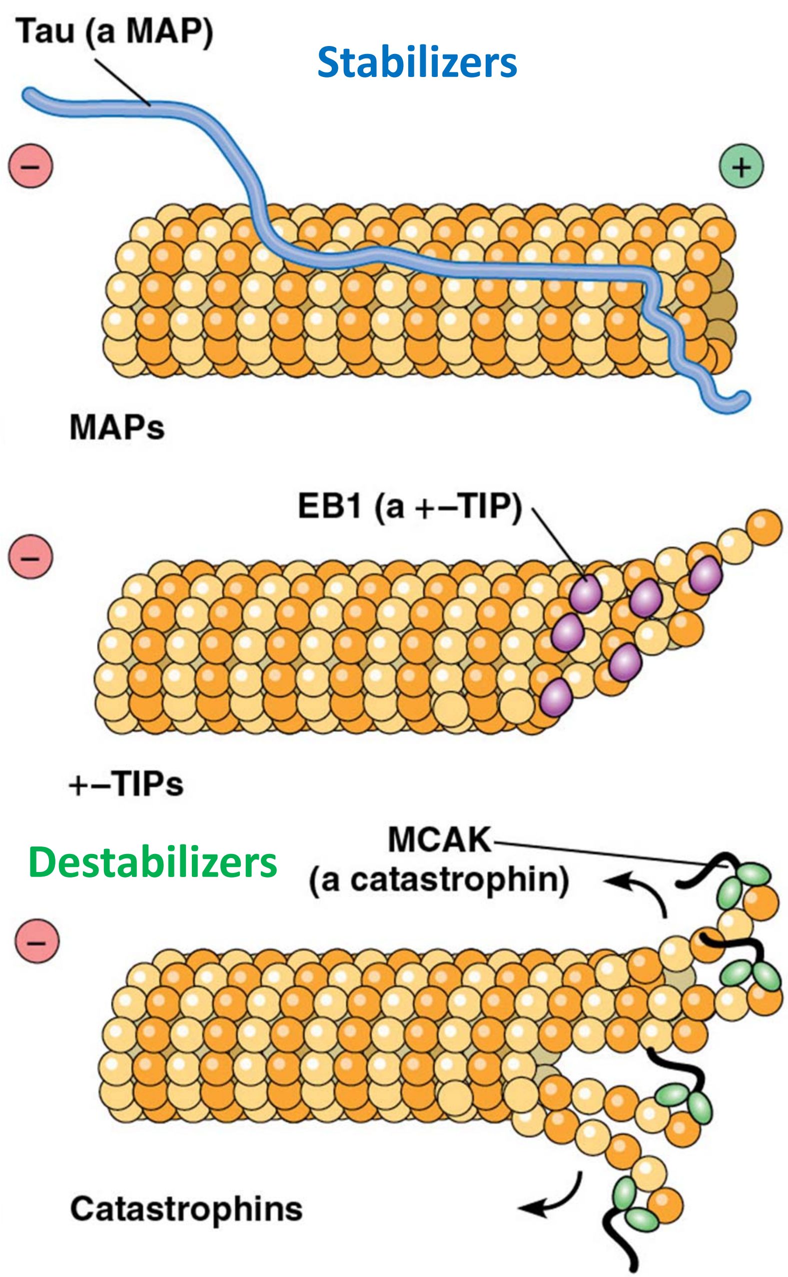


<u>Typical order</u> of MTs (interphase cell) <u>growing</u> from the MTOC (near the nucleus) to the <u>cell periphery</u>.

During cell division (mitosis) the **MTOC duplicates** (and chromosomes start to condense).

At a late stage of mitosis (metaphase) the <u>centrosomes form</u> the **spindle** apparatus from which <u>MTs grow to</u> **either** the **cell periphery** (**aster** formation) **or** to the **chromosomes** (in the equatorial plate).

Microtubule associate proteins (MAPs) regulate MT stability in cells

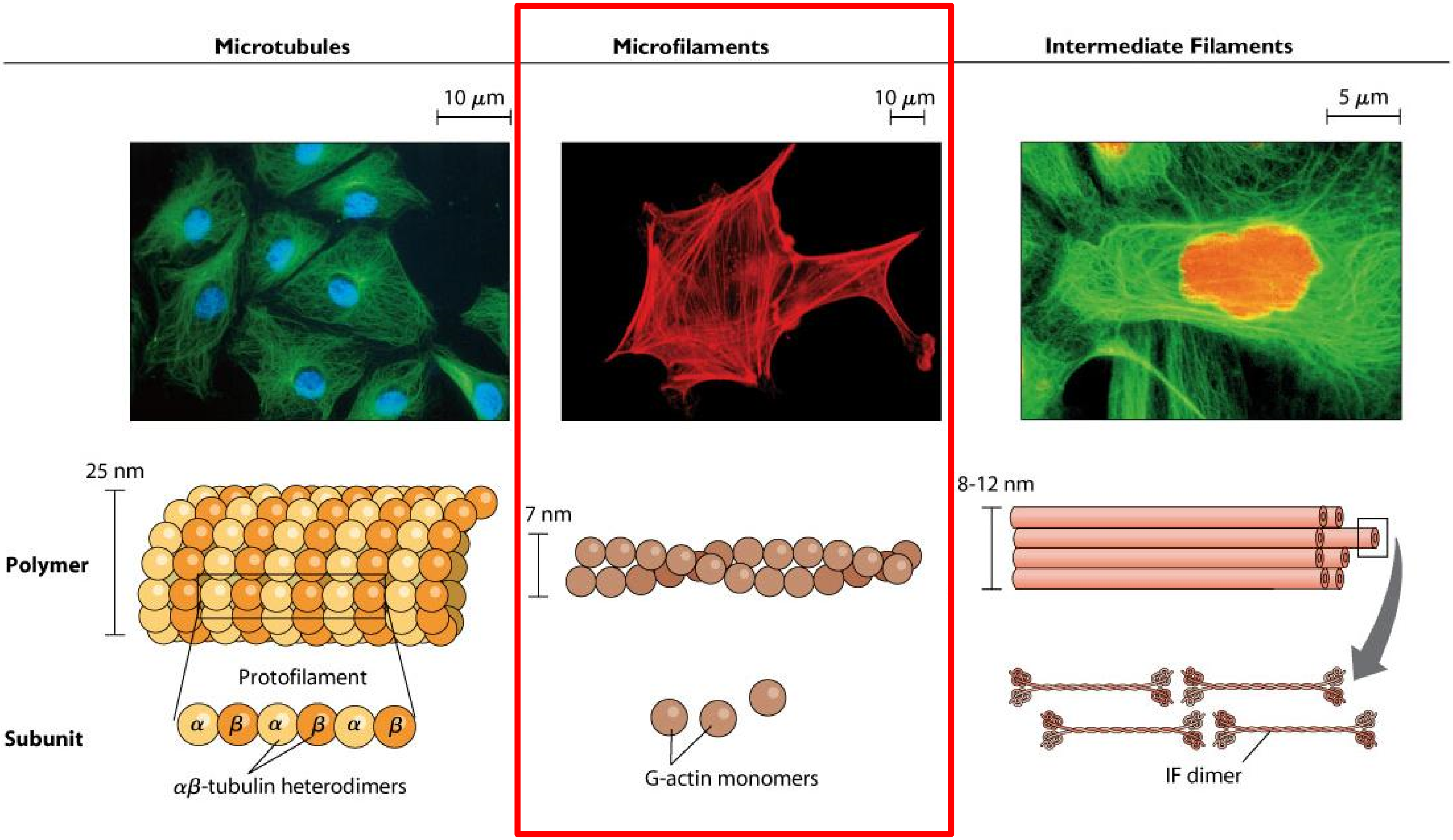


- MT dynamics are especially important for **mitosis** in which MTs have to catch the chromosomes in the equatorial plate (**short-lived MTs**)
- However, MTs in cilia or axons have to be **long-lived** thus very stable.
- This stability can be provided by **MAPs** (microtubule associated proteins). In neurons **tau** (axons) and **MAP2** (dendrites) are important for MT stabilization.
- Tau is also known to be involved in several neurodegenerative diseases, e.g., **Alzheimer's disease**. Here, *neurofibrillary tangles* (NFT) can be found in neurons that contain so called *paired helical filaments* (PHF) formed by **hyperphosphorylated tau**
- **+-TIP** proteins (+-end *t*ubulin *i*nteracting *p*roteins) stabilize the tip of the MT (e.g., EB1) and have similar function as the GTP-cap.
- **MCAK** is a catastrophin and promotes fast shrinkage of MT by peeling off the protofilaments.

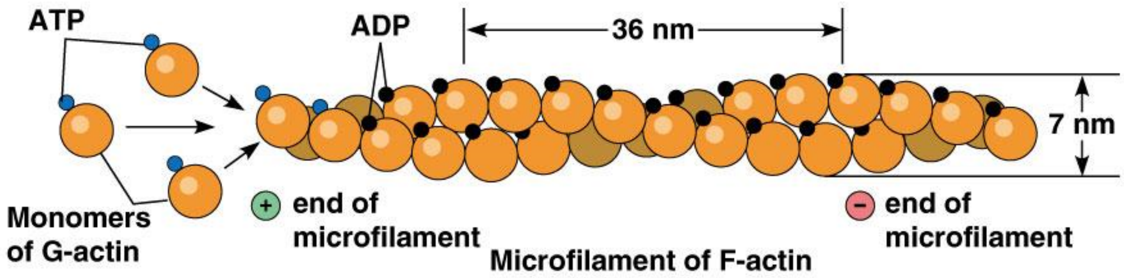
Other destabilizers

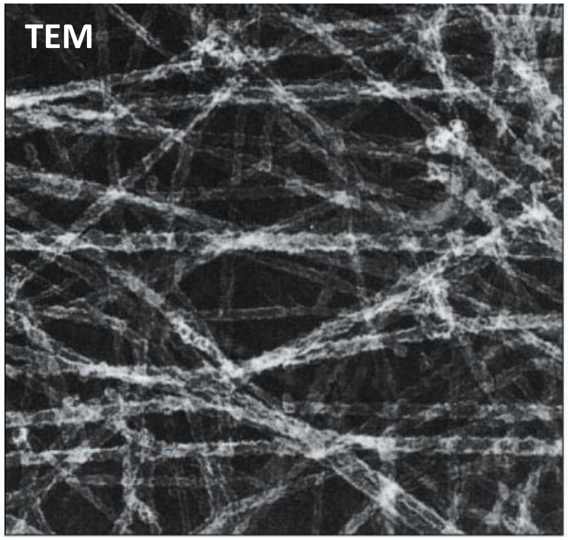
- **Stathmin/OP18** binds to tubulin heterodimers preventing their polymerization
- **Katanin** severs (cuts) microtubules

Going into details: Microfilaments (or F-actin)



The polymerization of actin requires ATP

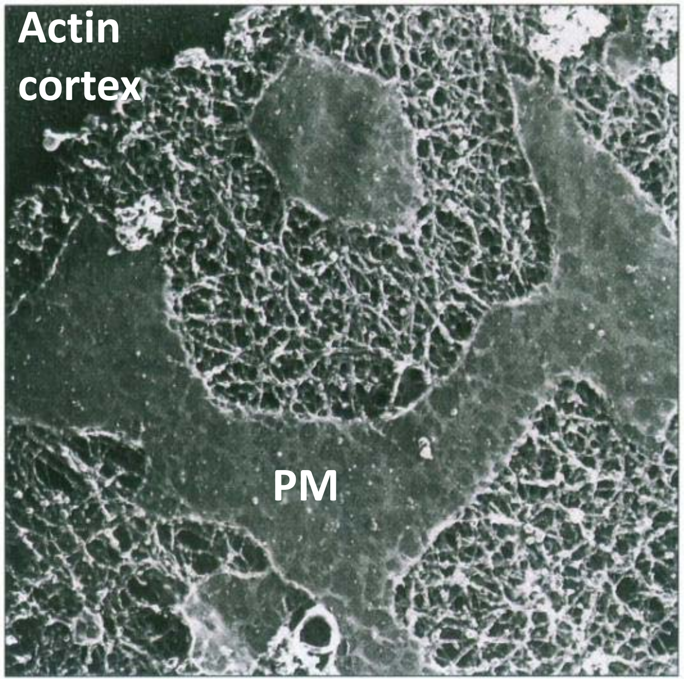


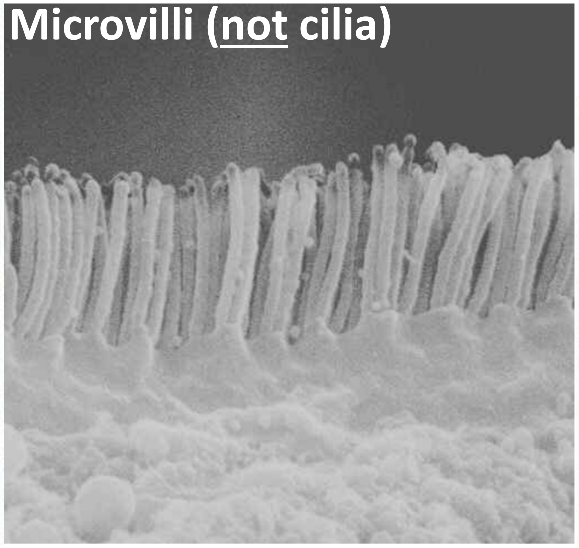


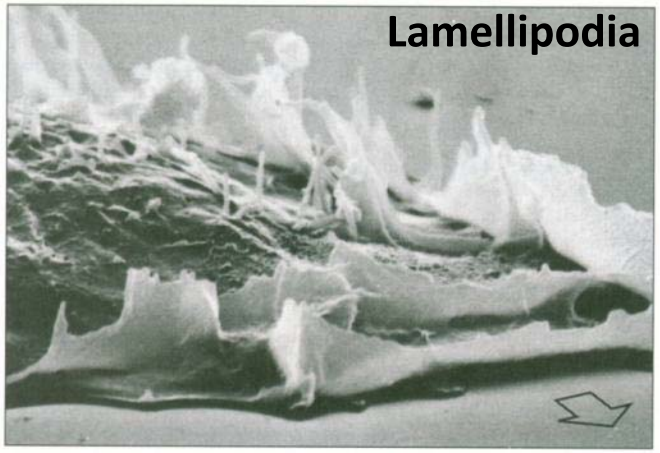
- Microfilaments (**MFs**) are the smallest cytoskeletal fibers with **7 nm** in diameter
- The basic subunit is **G-actin** (*g*lobular *a*ctin, 42 kDa) which polymerizes into **F-actin** (*f*ilamentous *a*ctin) in the **presence of ATP** (ATP is hydrolyzed to ADP during polymerization)
- The **pearl-string like** microfilament has a polarity (**ATP-actin cap** at the plus-end)
- Polymerization is faster at the plus-end and slower at the minus-end but does not depend on warmth
- Only if a **critical concentration** of G-actin is exceeded polymerization takes place
- Polymerization includes a slow nucleation phase, a fast elongation phase and a steady-state phase when free G-actin $\triangleq C_c$
- F-actin is composed of two linear strands of polymerized G-actin wound around each other in a helix (13.5 actin monomers per turn and a turn occurs every 36 nm)
- Three **isoforms** are known: α**-actin** (found in muscle cells) and β**-** and γ**-actin** (found in non-muscle cells)

Function and appearance of actin

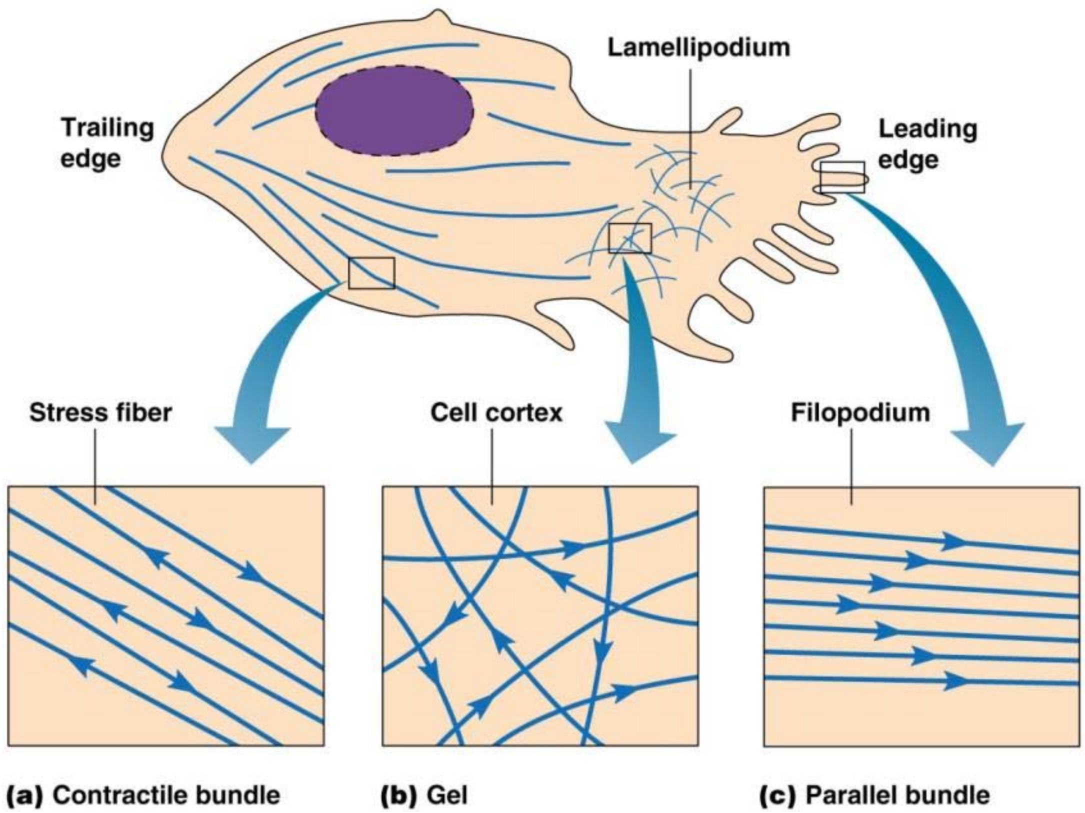
- Besides providing a cell's shape and mechanical resistance microfilaments are important for **muscle contraction** (together with myosin)
- MFs also provide tracks for myosin motors to transport cargo in cells
- Just below the plasma membrane an **actin cortex** can be found (to stabilize the membrane)
- Intestinal epithelial cells have finger-like extensions (**microvilli**) which are filled with tightly packed and in parallel arranged **actin filaments**.
- The polymerization of actin drives the formation of **lamellipodia** and **filopodia** important for cell locomotion

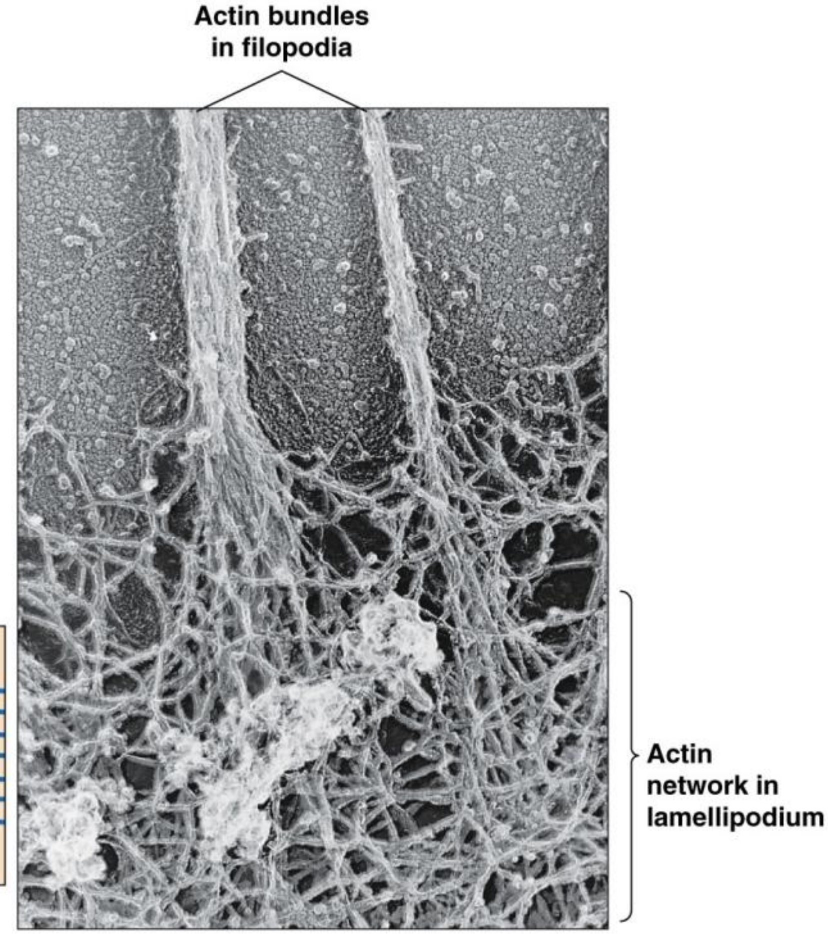






The variety of actin structures



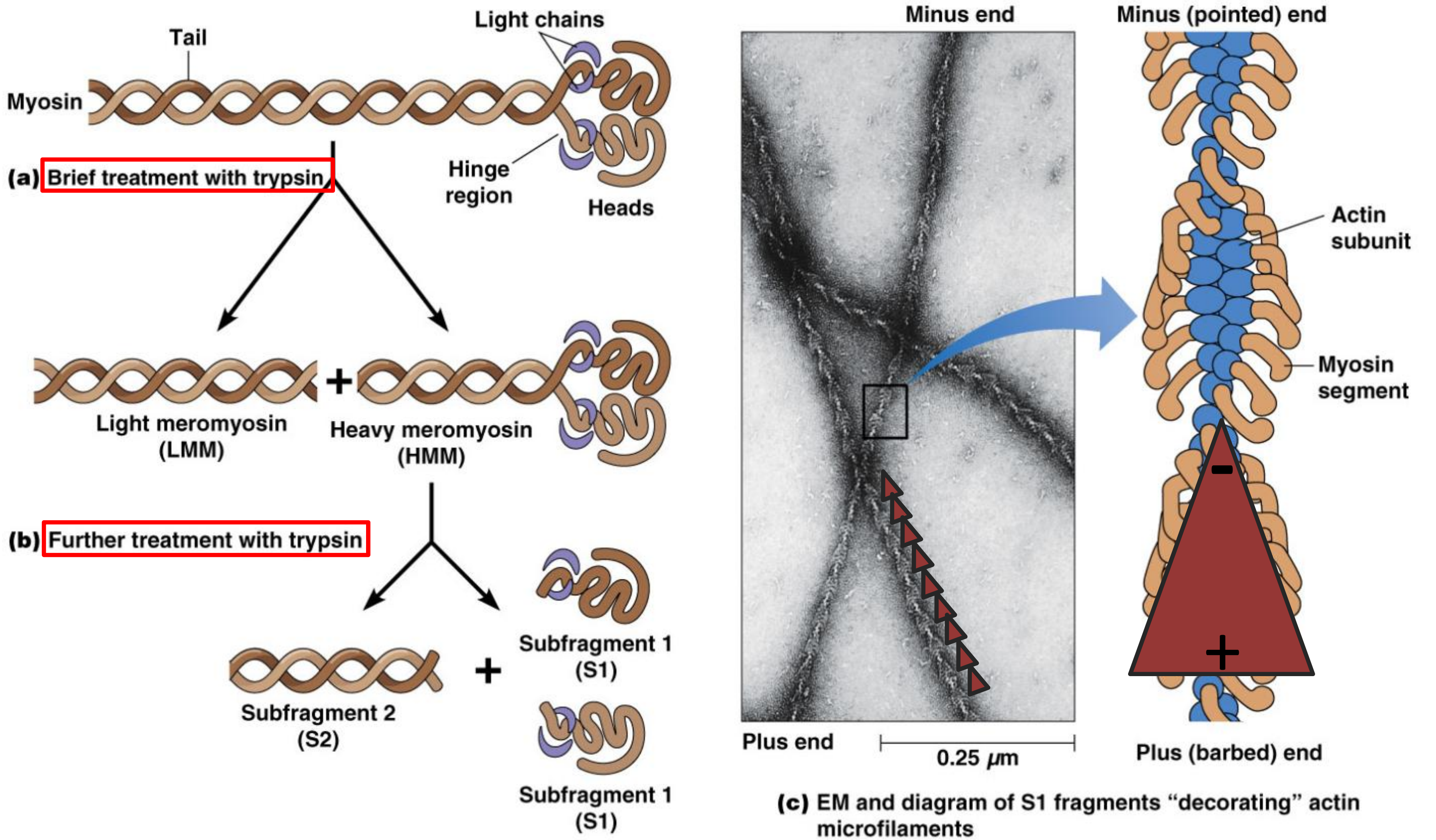


Stress fibers are contractile actin bundles mostly found in adhering cells

Cell cortex supports the fragile plasma membrane (dissolved in motile cells)

Lamellipodia are found at the leading edge of cells. They contain loosen actin networks.
Filopodia contain tightly bundled actin filaments with all their plus-ends facing to the tips of these “fingers”.

Myosin segment 1 (S1) decoration to determine F-actin polarity



Myosin **subfragment 1** (S1) is produced by successive proteolytic cleavage of myosin II. **Trypsin** is a serine protease found in the digestive system (produced in the pancreas).

Actin decorated with S1 **appears as a chain of arrowheads**. The **pointed end** of the arrow head faces to the **minus ends** and the **barbed end** faces to the **plus ends**.

Drugs that affect polymerization of actin

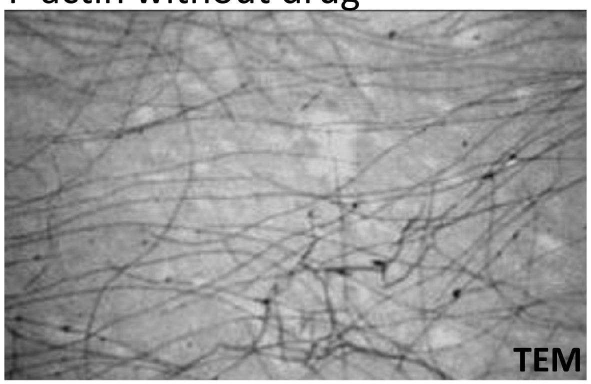
1) Microfilament **destabilizing drugs**

- **Cytochalasin B/D** (from fungus): Depolymerizes filaments (by capping the plus-end)
- **Latrunculin A** (from sponge): Sequesters ("quarantines") G-actin (which results in the prevention of F-actin assembly)

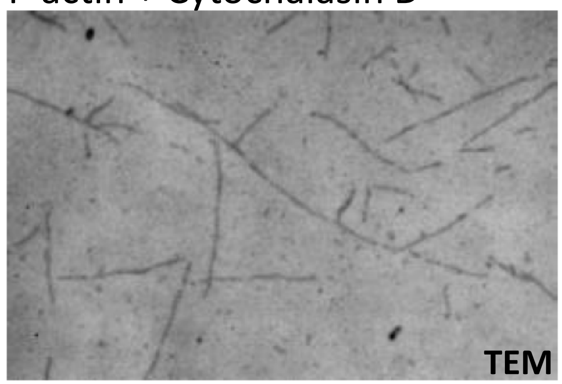
2) Microfilament **stabilizing drugs**

- **Phalloidin** (from fungus): Binds sidewise to F-actin and stabilizes the filament
- **Jasplakinolide** (from sponge): Promotes actin polymerization

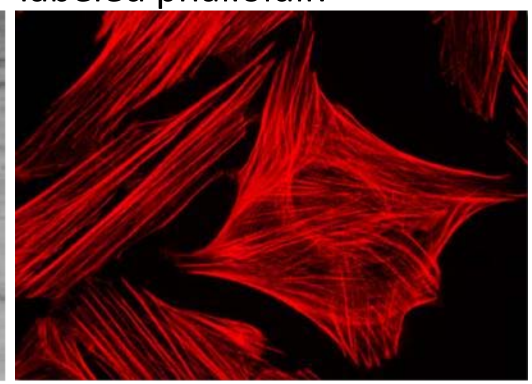
F-actin without drug

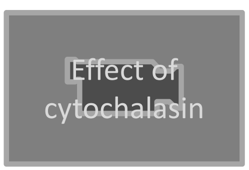


F-actin + Cytochalasin D



F-actin + fluorescently labeled phalloidin



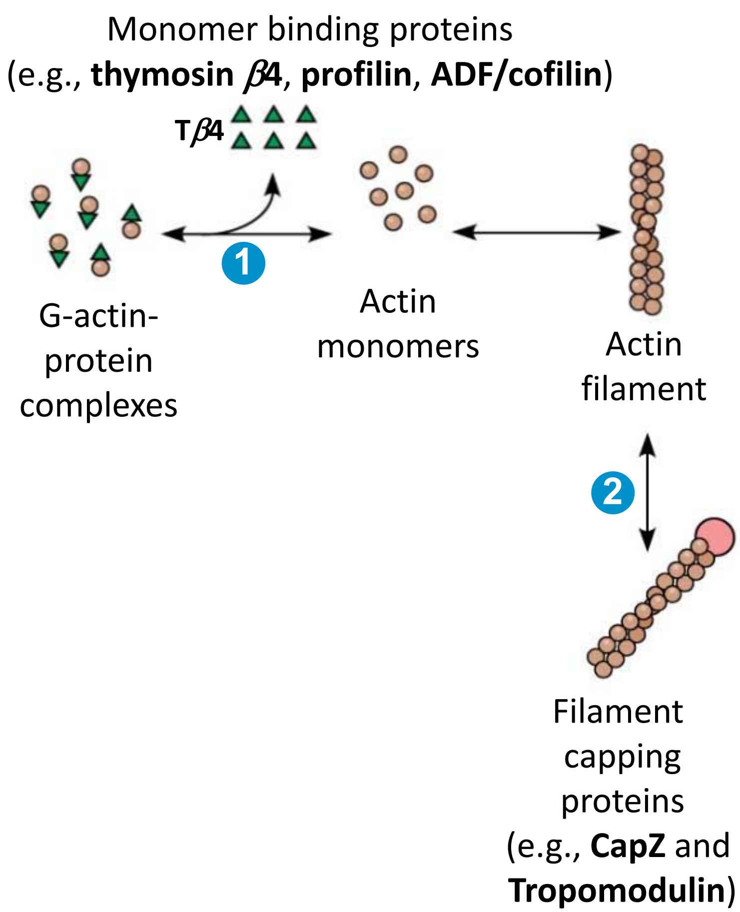


Cells with beads that attach to microfilaments:
The retrograde flow of actin is visible ("actin recycling").
Cytochalasin B effect is reversed by washing out the drug.

Drugs Affecting Microfilaments	Source	Effect
Cytochalasin D	Fungal metabolite	Prevents addition of new monomers to plus ends
Latrunculin A	Red sea sponge, *Latrunculia magnifica*	Sequesters actin monomers
Phalloidin	Death cap fungus, *Amanita phalloides*	Binds and stabilizes assembled microfilaments

Proteins that control actin polymerization and actin networks

Actin-binding proteins (ABPs) control the polymerization, length and crosslinking of actin



(1) ABPs that **bind to G-actin**

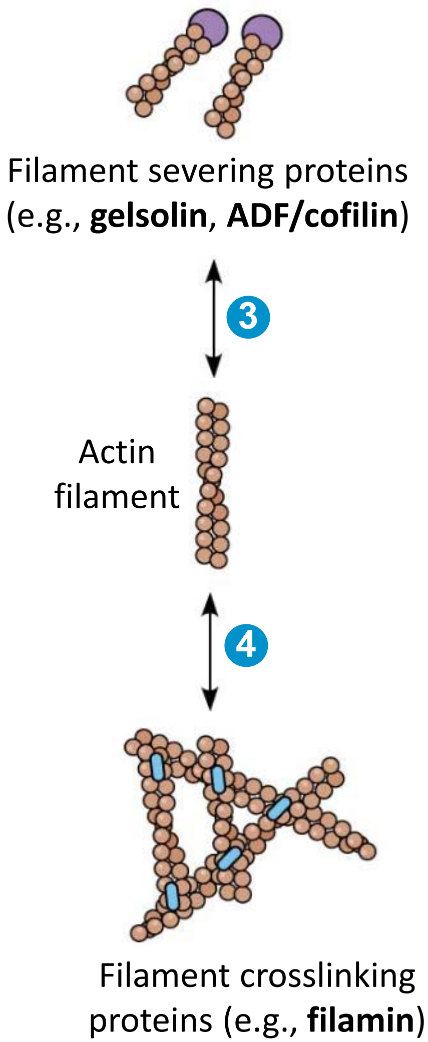
- **Thymosin β4**: Buffers (sequesters) G-actin (preferably ATP-G-Actin). G-actin bound to thymosin β4 cannot polymerize.
- **Profilin**: Binds G-actin and transports it to the plus-end of F-actin. It also helps the exchange of ADP to ATP on the G-actin molecule.
- **ADF/Cofilin**: Binds to and removes ADP-G-Actin from the minus-ends of F-actin. It also severs (cuts) F-actin.

(2) ABPs that **cap F-actin**

- **CapZ**: Binds to the plus-end of F-actin and stabilizes the filaments (as it prevents loss of G-actins at the plus-end).
- **Tropomodulin**: Binds to the minus-end of F-actin (e.g., muscle sarcomere) and stabilizes the filaments (as it prevents loss of G-actins at the minus-end).

Proteins that control actin polymerization and actin networks

Actin-binding proteins (ABPs) control the polymerization, length and crosslinking of actin

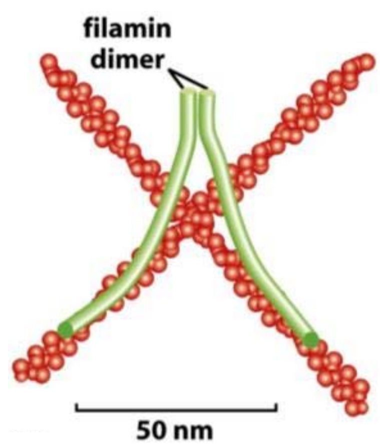


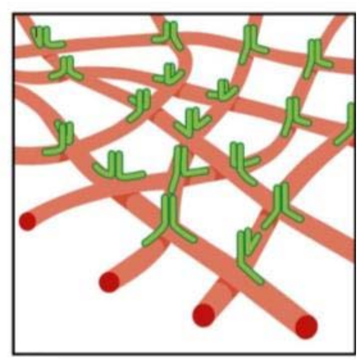
(3) ABPs that **sever** (cut) F-actin

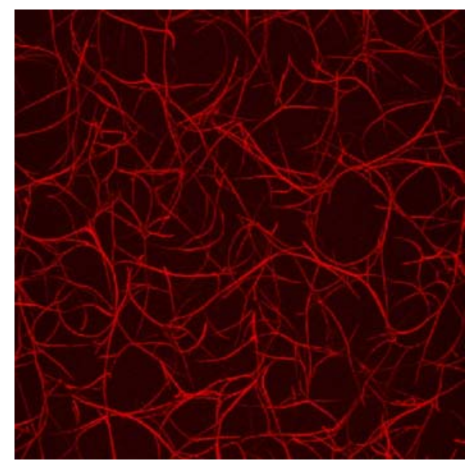
- **Gelsolin**: Cuts F-actin and caps the plus-end afterwards. For example the (strong/stiff) cortical network can be liquefied to make cells softer (for subsequent movements). Important for *gel-sol transitions* in amoeba.
- **ADF-cofilin**: Cuts preferentially at minus ends.

(4) ABPs that **cross-link** F-actin

- **Filamin**: A long molecule with two actin-binding sites at each end. Ability to splice two actin filaments together to form a loosen network.

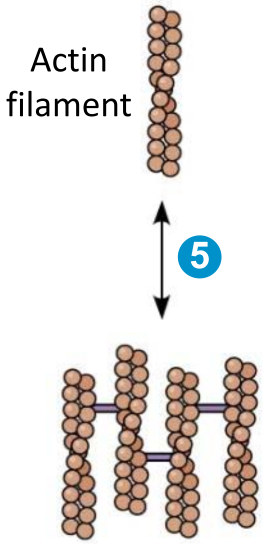





Actin + filamin
fluorescence image

Proteins that control actin polymerization and actin networks

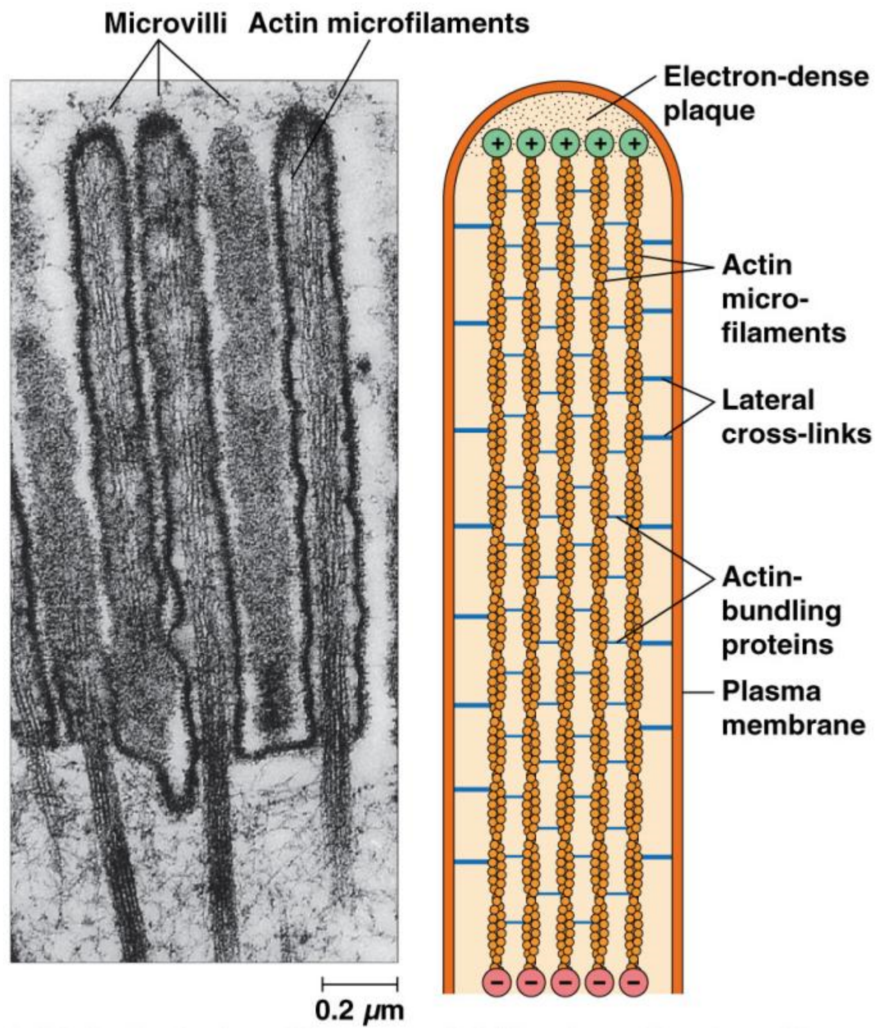
Actin-binding proteins (ABPs) control the polymerization, length and crosslinking of actin



Filament bundling proteins (e.g., α-**actinin**, **fimbrin**, **villin**, **fascin**)

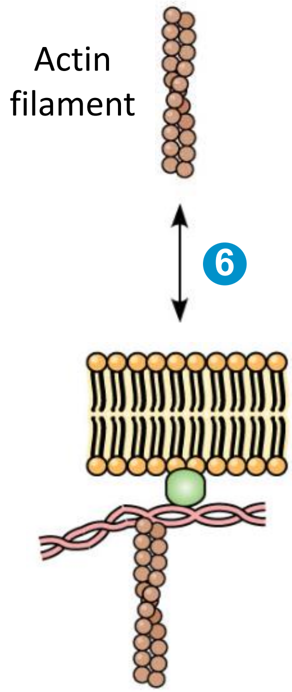
(5) ABPs that **bundle** F-actin

- **α-actinin**: A long, spacer-like molecule with two actin-binding sites at each end. Makes loosen bundles. Also part of **focal adhesions** (subs-trate attachment sites).
- **Fascin**: Makes very tight actin bundles in spike-like filopodia.
- **Fimbrin** and **villin**: Responsible for tight bundles in **microvilli**. Microvilli largely increase the surface of intestinal cells for food absorption purpose. Actin filaments face with their plus-ends to the tip and are fixed to the side-walls by myosin I (lateral cross-links).



Proteins that control actin polymerization and actin networks

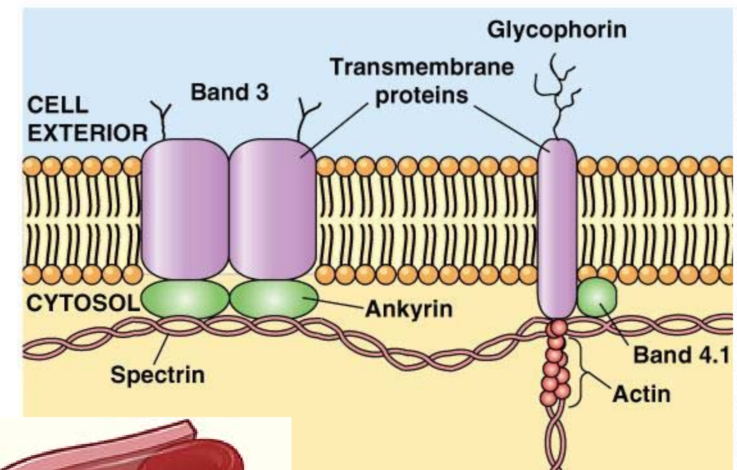
Actin-binding proteins (ABPs) control the polymerization, length and crosslinking of actin

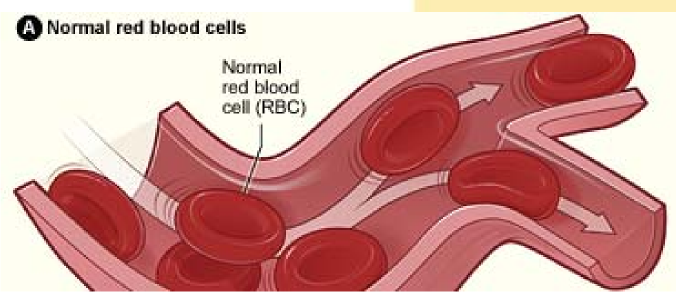


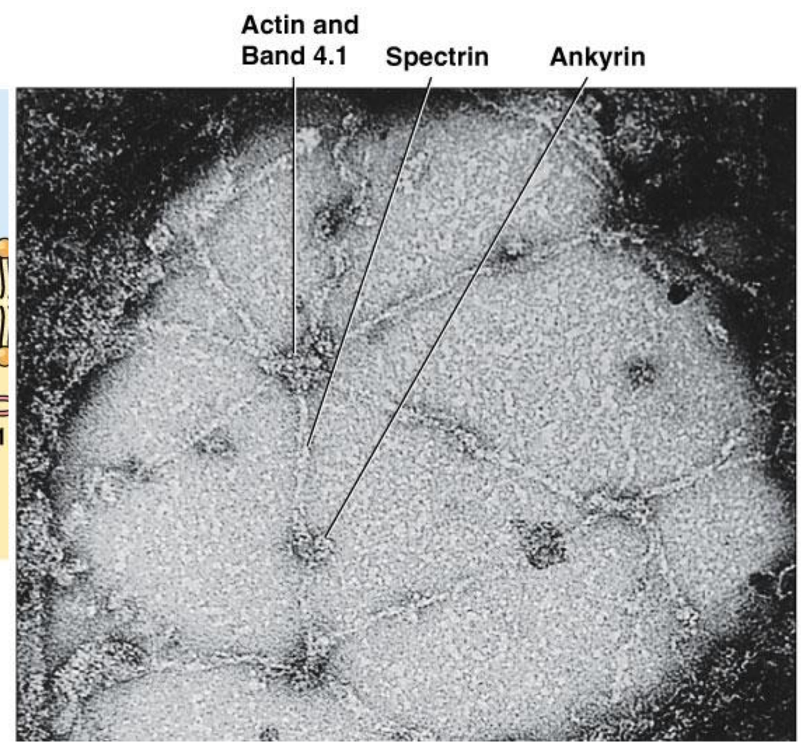
Filament anchoring proteins (e.g., **spectrin**, **ERM proteins**)

(6) ABPs that **link actin to membranes**

- **Ezrin**, **radixin** and **moesin** (ERM proteins): Connect F-actin to the plasma membrane. Important for the transmission of force generated from actin polymerization to the plasma membrane.
- **Spectrin**: Binds short **actin** polymers to form a loosen (hexagonal-type) network of (long) spectrin molecules below the plasma membrane of **erythrocytes**. **Ankyrin** and **band 4.1** are involved in direct membrane interactions.





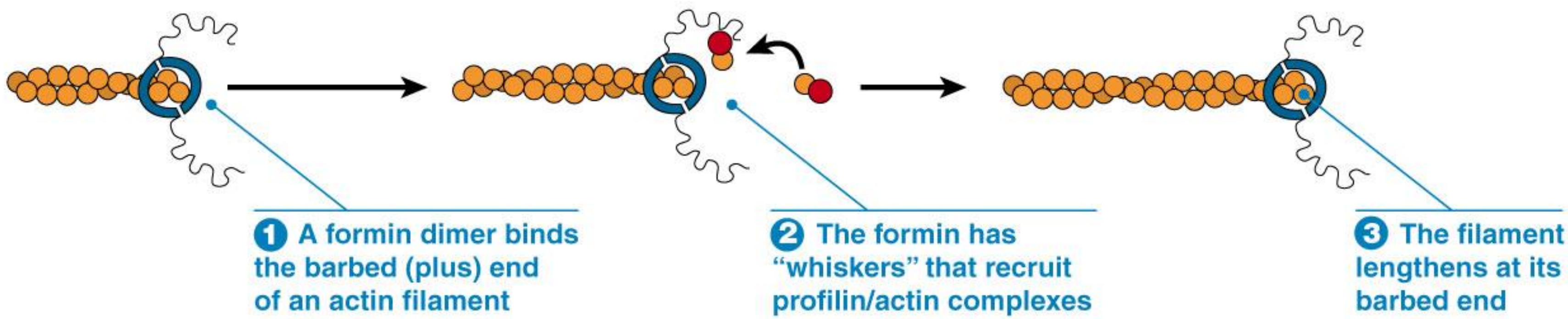


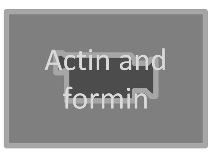
Proteins that control actin polymerization and actin networks

Actin-binding proteins (ABPs) control the polymerization, length and crosslinking of actin

(7) ABPs that **make long actin filaments**

- **Formins**: <u>Nucleation activity</u> at the plus-end of F-actin. Formins are dimers and resemble a <u>ring that processively moves</u> along the growing actin filaments. They have whiskers that recruit profilin-bound G-actin.



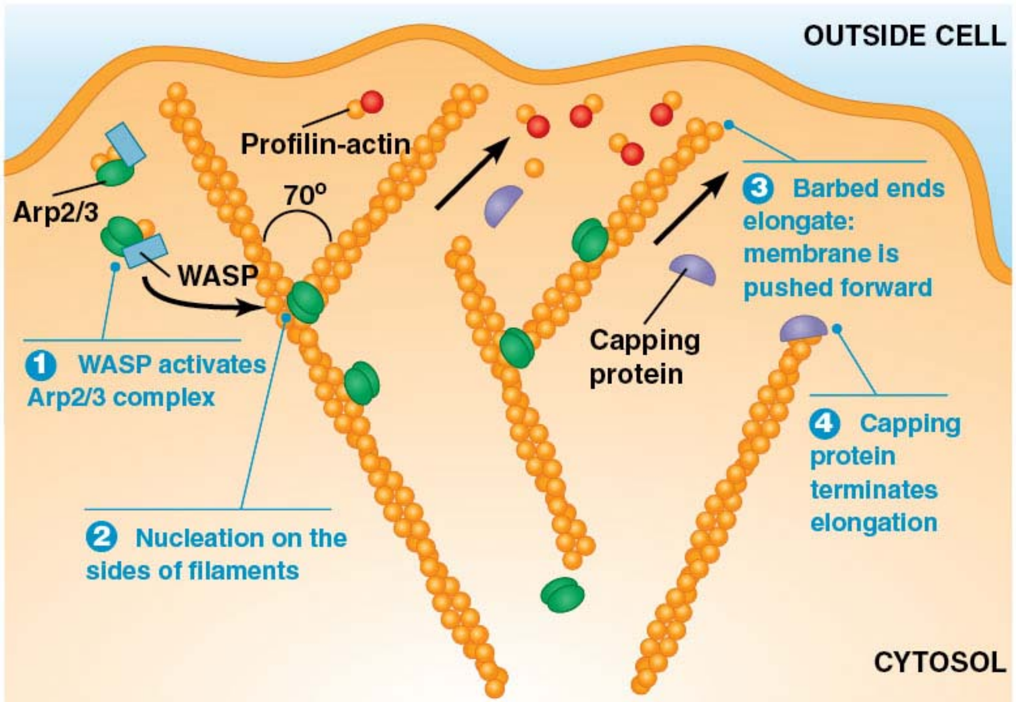


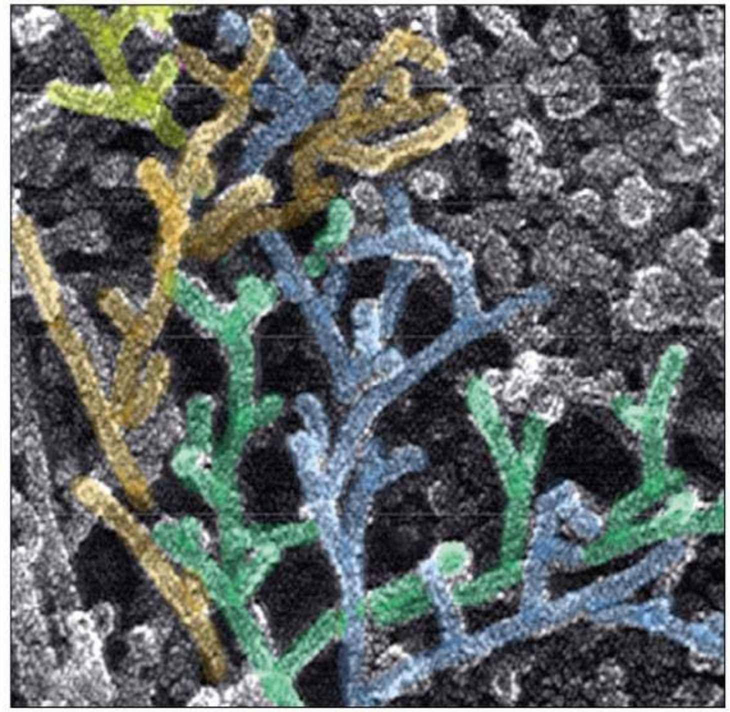
Proteins that control actin polymerization and actin networks

Actin-binding proteins (ABPs) control the polymerization, length and crosslinking of actin

(8) ABPs that **make actin branches**

Actin in crawling cell

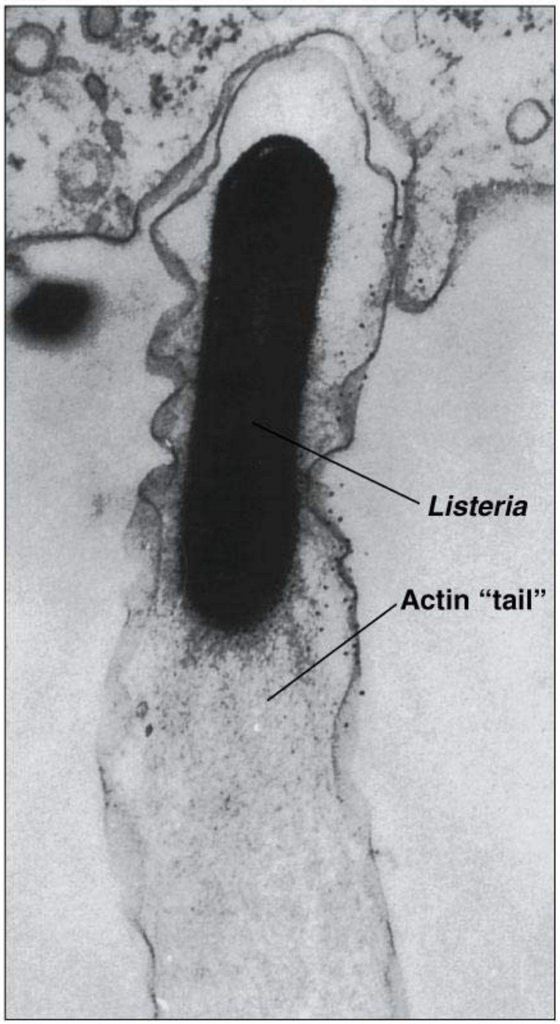




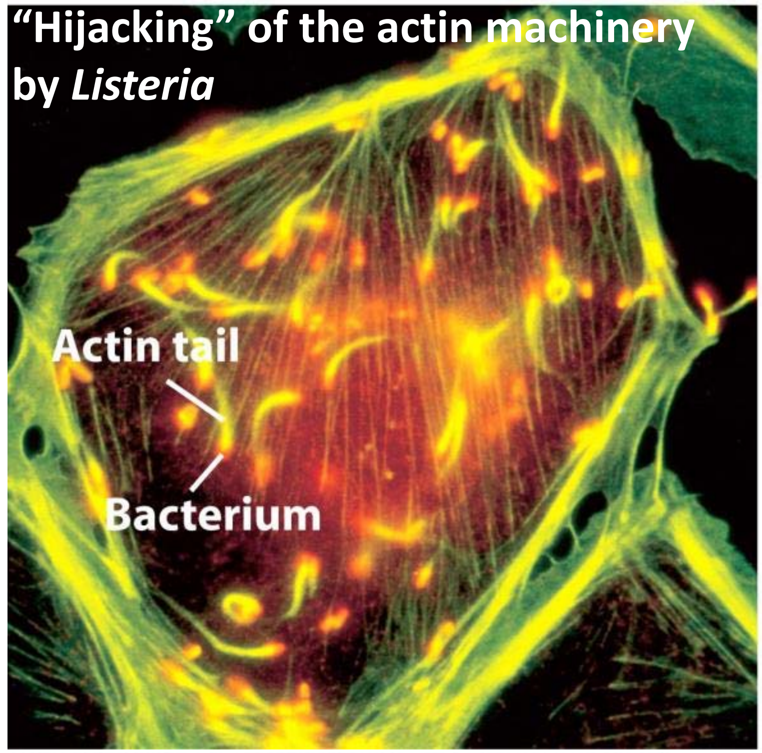
- **Arp2/3** (actin related protein) has the ability to bind side-wise to an actin filament and provides a (minus-end) nucleation side for an actin branch (with a precise 70° angle).
- **Profilin** is known to shuffle ATP-G-actin to the nucleation site and the final filament is capped by an actin **capping protein**.
- These dendritic-like (tree-like) networks are mostly found in lamellipodia.
- Arp2/3 needs to be activated by **WASP** (Wiskott-Aldrich syndrome protein) and WAVE/Scar (patients with WASP defects have platelets with altered shapes that affects blood clotting).

Arp2/3 is needed for *Listeria* movement in infected cells

- *Listeria monocytogenes* is a bacterium which propels thru the cytoplasm using the power of branched actin polymerization **stimulated by Arp2/3**
- Actin polymerizes into filaments at the base of the bacterium pushing it forward (propulsion)

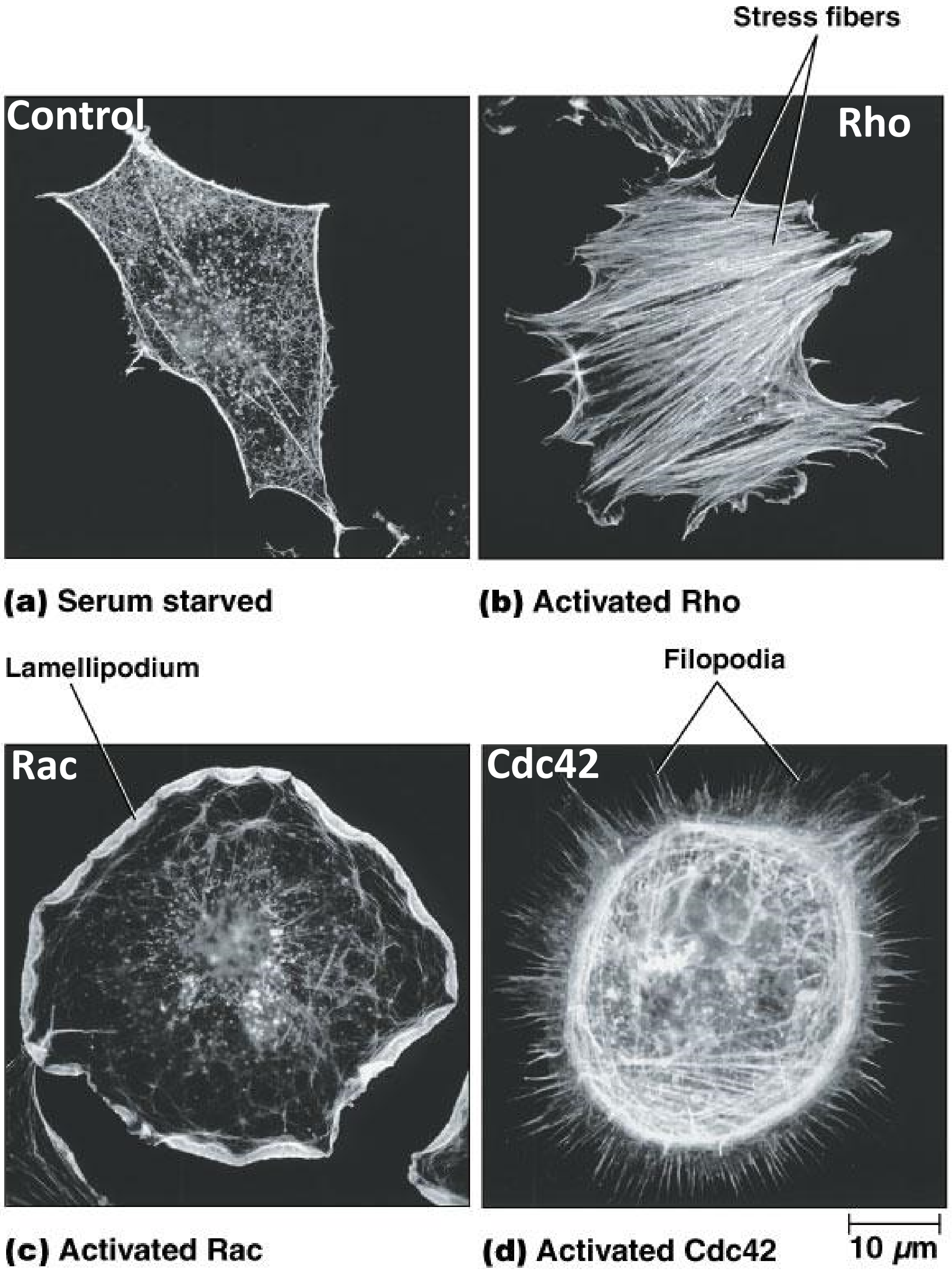


TEM high magnification



TEM low magnification

- Listeria is found in rotten food.
- Especially in not well prepared raw food as hamburgers or milk.
- Though it infects the **digestion system** it also leads to ***meningitis*** and 25% of patients death.

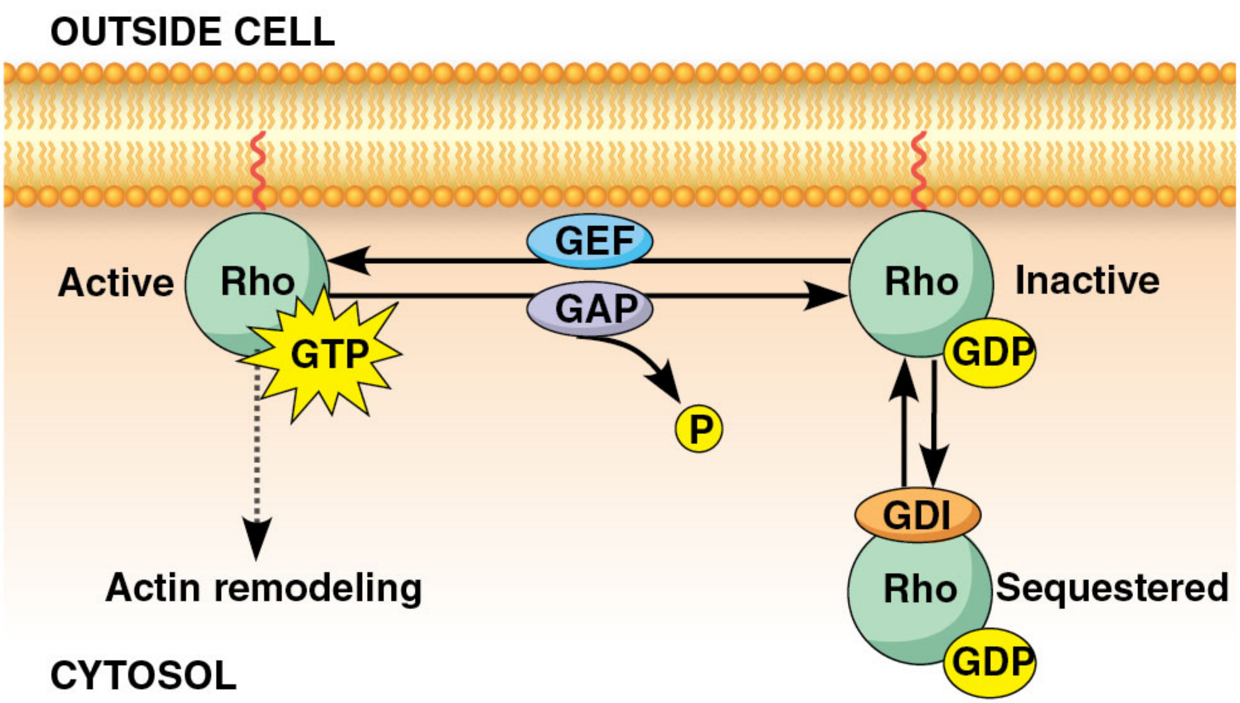


Cell signaling regulates complex actin-based structures

- ABPs directly control the diverse actin structures, while **ABPs themselves are regulated by cell signaling factors** such as **inositol phospholipids** (IPs) or **small G proteins** (of the Ras family).
- **PIP_2**: Regulates ezrin, profilin, gelsolin and CapZ. PIP_2 **uncaps** gelsolin and CapZ from the filaments.
- **Rho**: Responsible for **stress fiber** formation. Upstream of Rho is LPA (a phospholipid) and downstream formin.
- **Rac**: Responsible for **lamellipodia** formation. Upstream of Rac is PDGF (growth factor) and downstream WAVE (that activates Arp2/3).
- **Cdc42**: Responsible for **filopodia** formation. Upstream of Cdc42 are also growth factors and downstream is WASP (that activates Arp2/3).

Note: G proteins bind and are activated by GTP

G proteins themselves are controlled by other factors

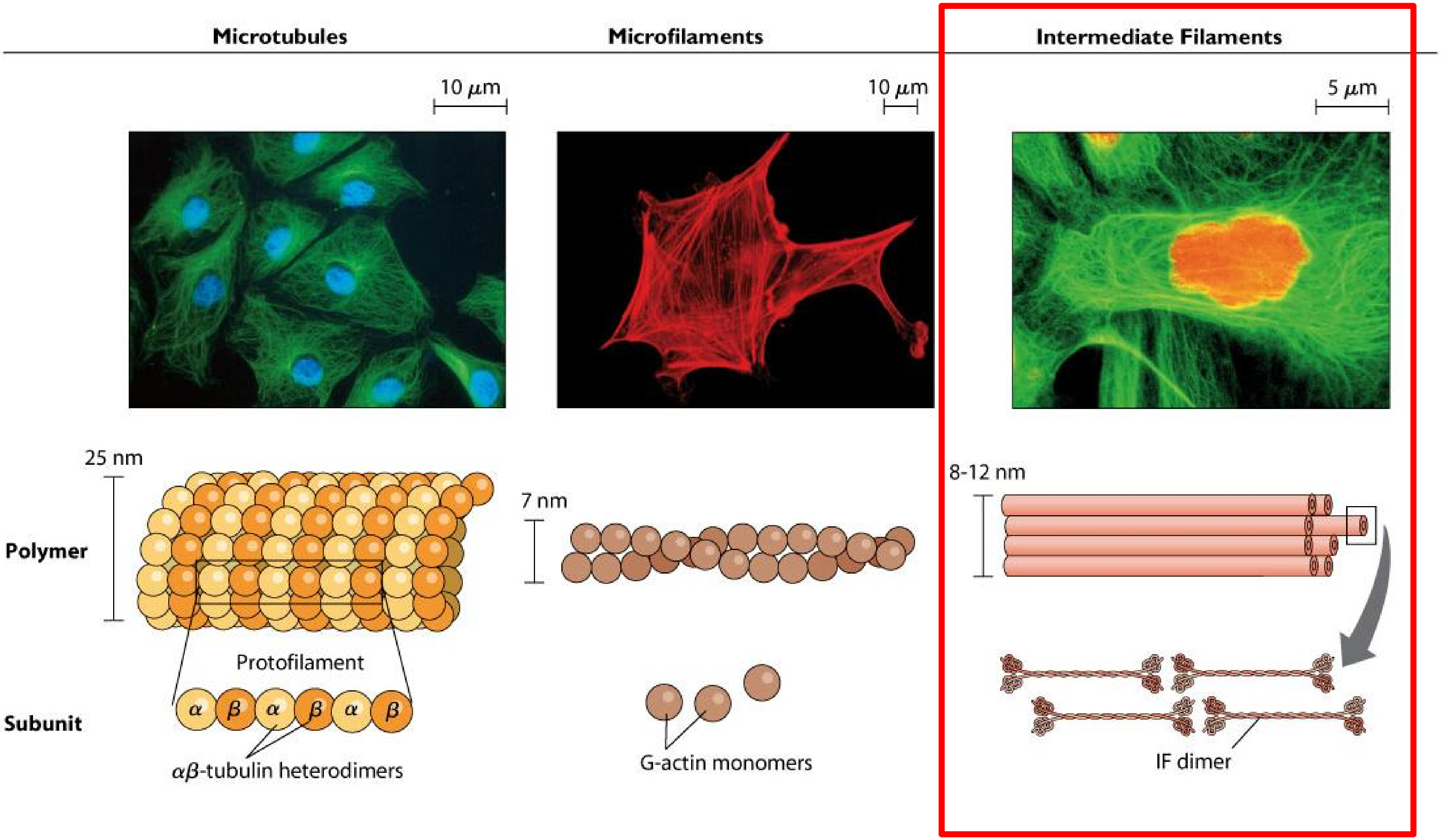


Switches and *feedback loops* are important for fine-tuning signaling events

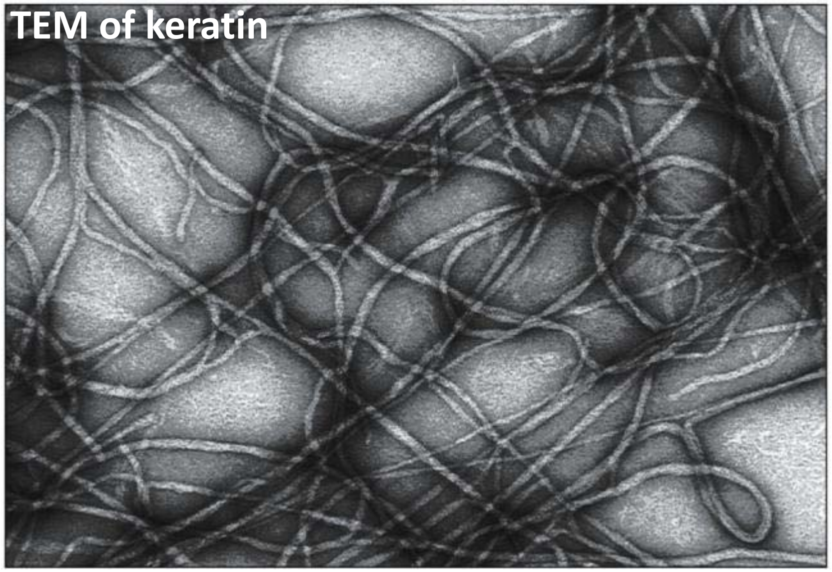
While actin is regulated by G proteins, different factors activate or inhibit G proteins:

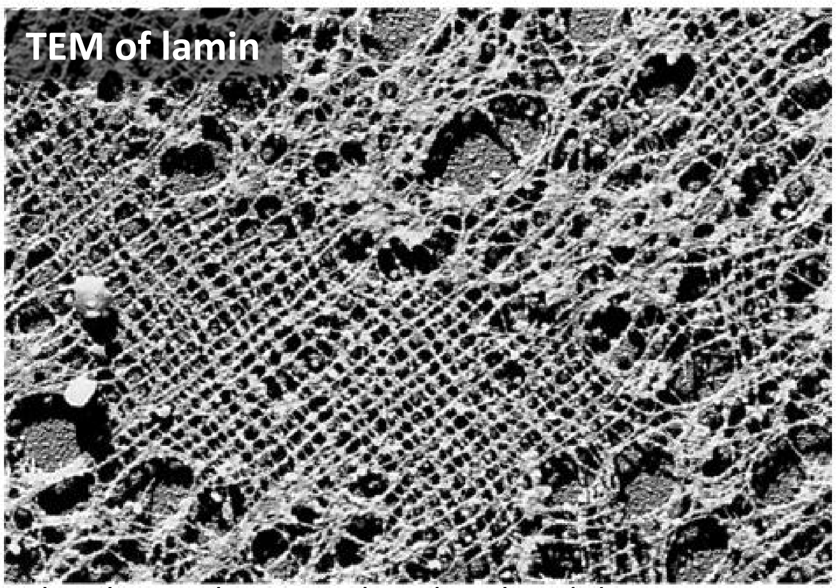
- **GEF**: Guanine-nucleotide exchange factors = stimulate the exchange of GDP to GTP on the G protein (activates G-proteins).
- **GAP**: GTPase activating proteins = stimulate the hydrolyzation of GTP to GDP which inactivates G-proteins.
- **GDI**: Sequesters the GDP-bound form of G proteins (inactivates G-proteins)

Going into details: Intermediate Filaments



Intermediate filaments provide mechanical strength of cells



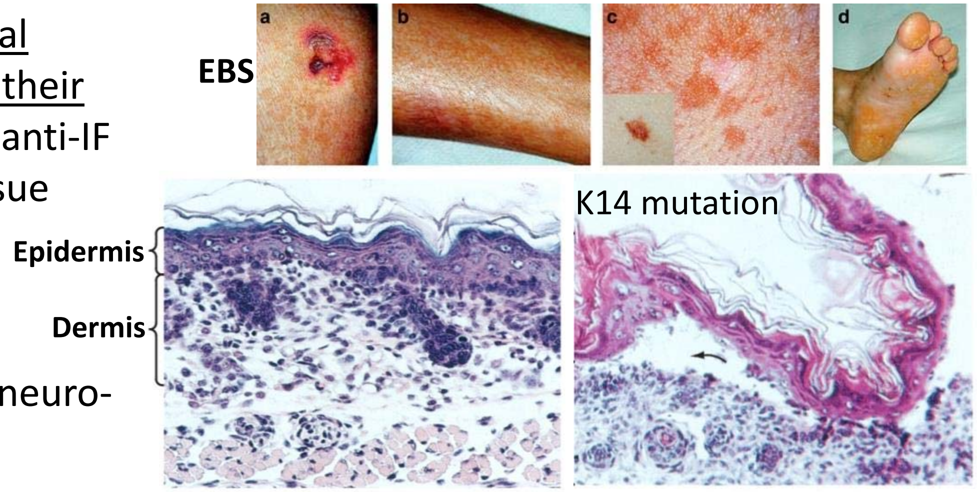


When lamins become phosphorylated during mitosis the whole network breaks down.

- Intermediate filaments (**IF**s) have a **diameter** between 8-12 nm. The diameter is in-between (intermediate) of the diameters of actin and microtubules.
- IFs do not have polarity, do not serve as tracks for molecular motors and do not assemble from globular subunits (but from long dimers).
- Important intermediate filaments are:
  - **Keratin**: Important for hard epithelial structures as hair, claws, fingernails, horn, feathers etc. **15 acidic** and **15 basic** (*neutral*) keratins exits.
  - **Vimentin**: In (soft) mesenchymal cells and fibroblasts.
  - **Desmin**: In muscle cells.
  - **GFA** (glial fibrillary acidic protein): Glial cells and astrocytes.
  - **Neurofilaments**: Provide mechanical strength for axons.
  - **Lamins**: Provide a dense and protective network around the inner nucleus membrane.

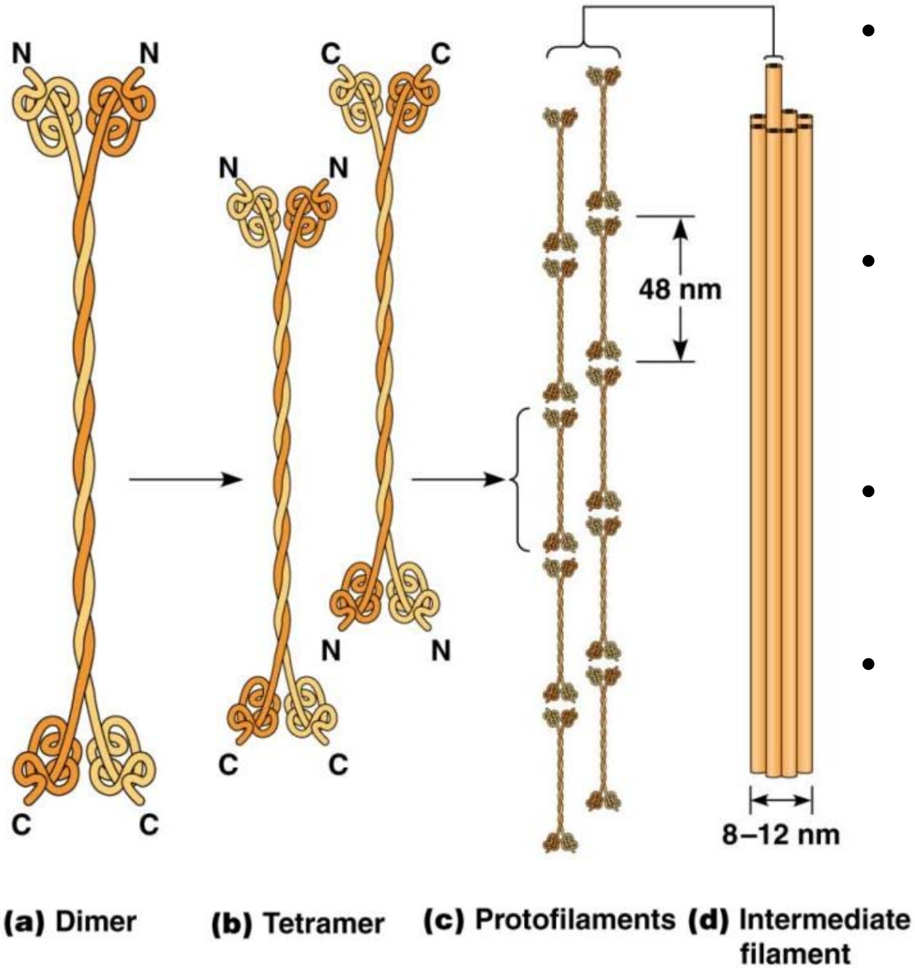
IFs can be grouped into 6 classes based on their cellular locations

- The **tissue specificity** of IFs is important for medical diagnostics. For example, cancer cells use to keep their original IFs, therefore, it is possible (using specific anti-IF antibodies) to find out the origin of the cancer tissue (especially for metastases).
- IFs are also involved in **skin diseases** such as **EBS** (*epidermolysis bullosa simplex*), and **neurodegenerative diseases** (brain diseases) such as **ALS** (neurofilament accumulations).

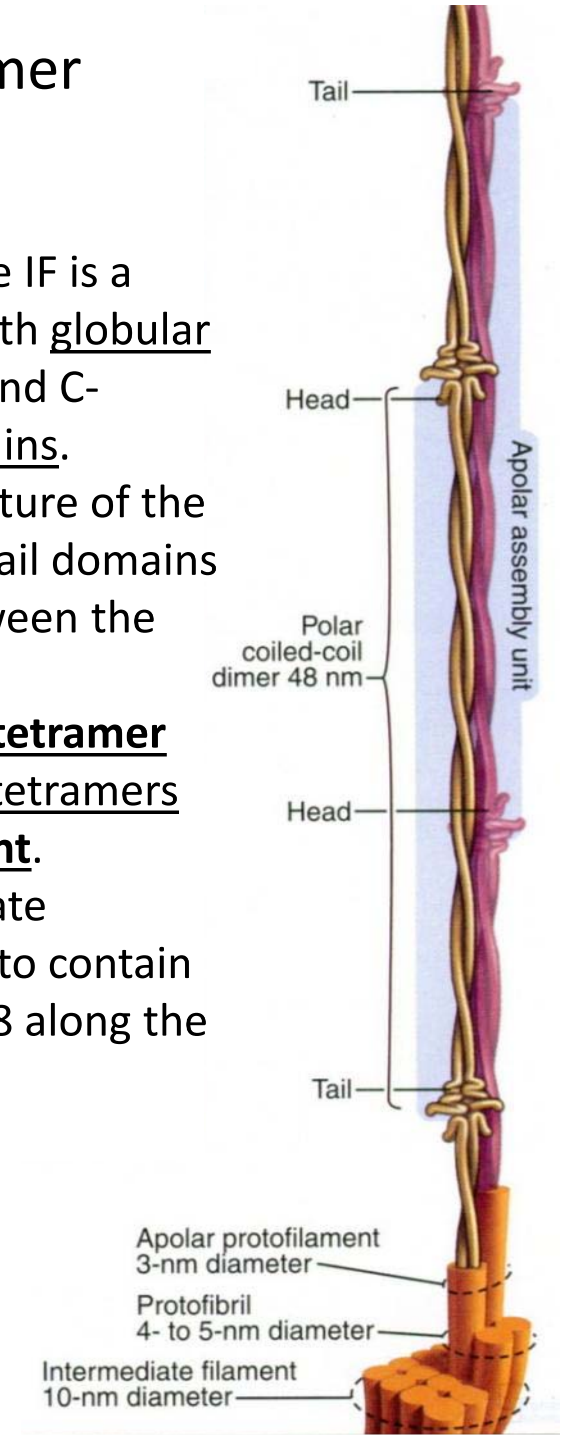


Class	IF Protein	Molecular Mass (kDa)	Tissue	Function
I	Acidic cytokeratins	40–56.5	Epithelial cells	Mechanical strength
II	Basic cytokeratins	53–67	Epithelial cells	Mechanical strength
III	Vimentin	54	Fibroblasts; cells of mesenchymal origin; lens of eye	Maintenance of cell shape
III	Desmin	53–54	Muscle cells, especially smooth muscle	Structural support for contractile machinery
III	GFA protein	50	Glial cells and astrocytes	Maintenance of cell shape
IV	Neurofilament proteins		Central and peripheral nerves	Axon strength; determines axon size
	NF-L (major)	62		
	NF-M (minor)	102	NF triplet proteins	
	NF-H (minor)	110		
V	Nuclear lamins		All cell types	Form a nuclear scaffold to give shape to nucleus
	Lamin A	70		
	Lamin B	67		
	Lamin C	60		
VI	Nestin	240	Neuronal stem cells	Unknown

The complex structure of the IF provides the polymer flexibility and strength

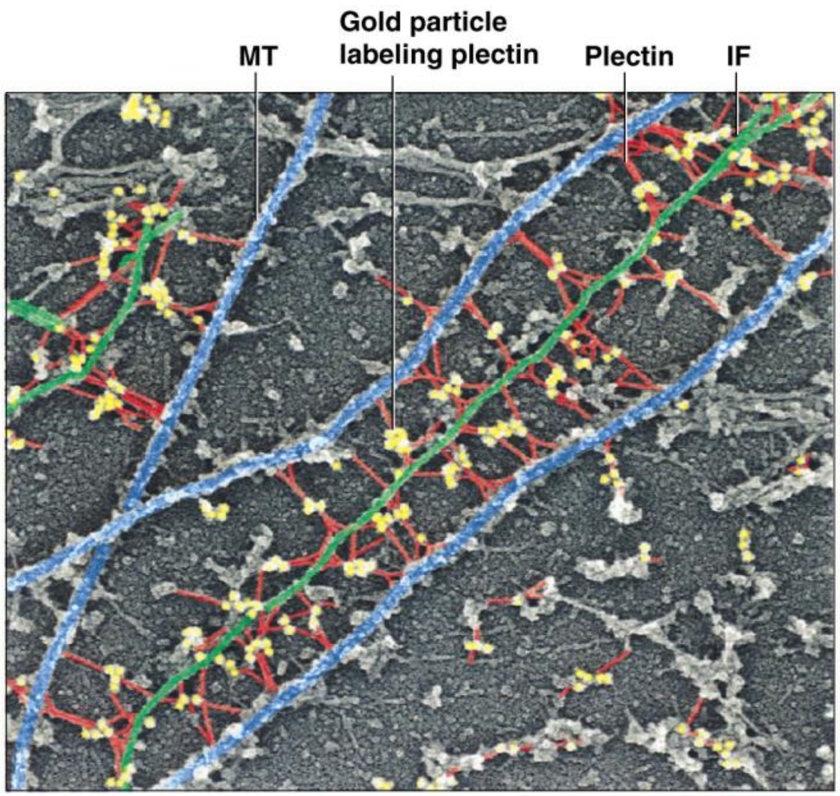


- The <u>basic unit</u> of the IF is a **coiled coil dimer** with <u>globular</u> N-terminal (head) and C-terminal (tail) <u>domains</u>.
- Sequence and structure of the globular head and tail domains strongly varies between the different IF types.
- <u>Two dimers form a **tetramer**</u> and <u>many of these tetramers form a **protofilament**</u>.
- The final intermediate filament is thought to contain <u>16 protofilaments </u>(8 along the axis).

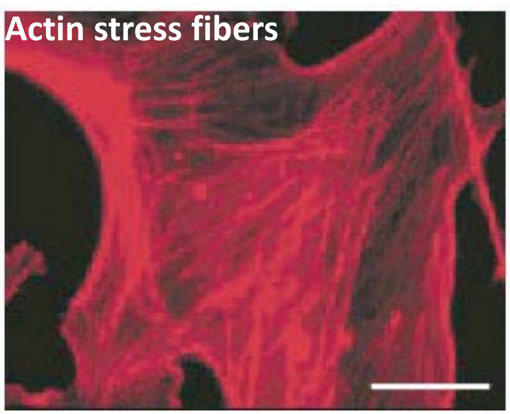


Coiled coil: The two α helices (of the two heavy chains) wrap (coil) around each other (based on interactions of their non-polar amino acid side chains)

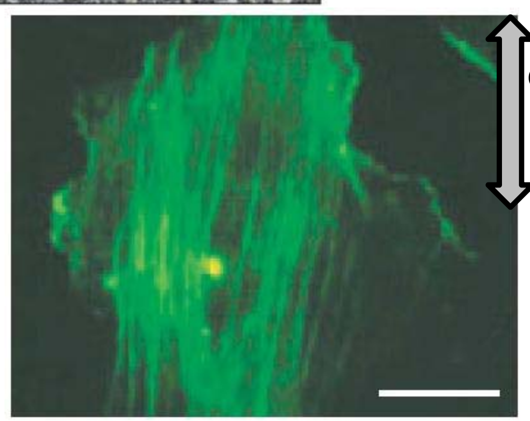
Plakins integrate all cytoskeletal elements into a single scaffold



- **Plectin** is a member of the plakin family and can bind to all three cytoskeletal elements
- The **integrated cytoskeleton** can resist large stretches and provides the cell mechanical resistance
- This **resistance** is especially important for cells that associate with smooth muscle cells (gut epithelial cells) or are exposed to high pressure (endothelial cells of the aorta)
- When endothelial cells are stretched for a period of time the stress bearing components align into the direction of the stretch

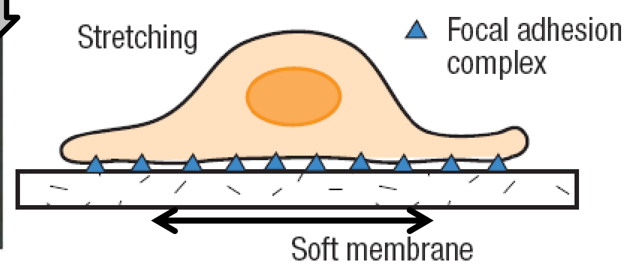


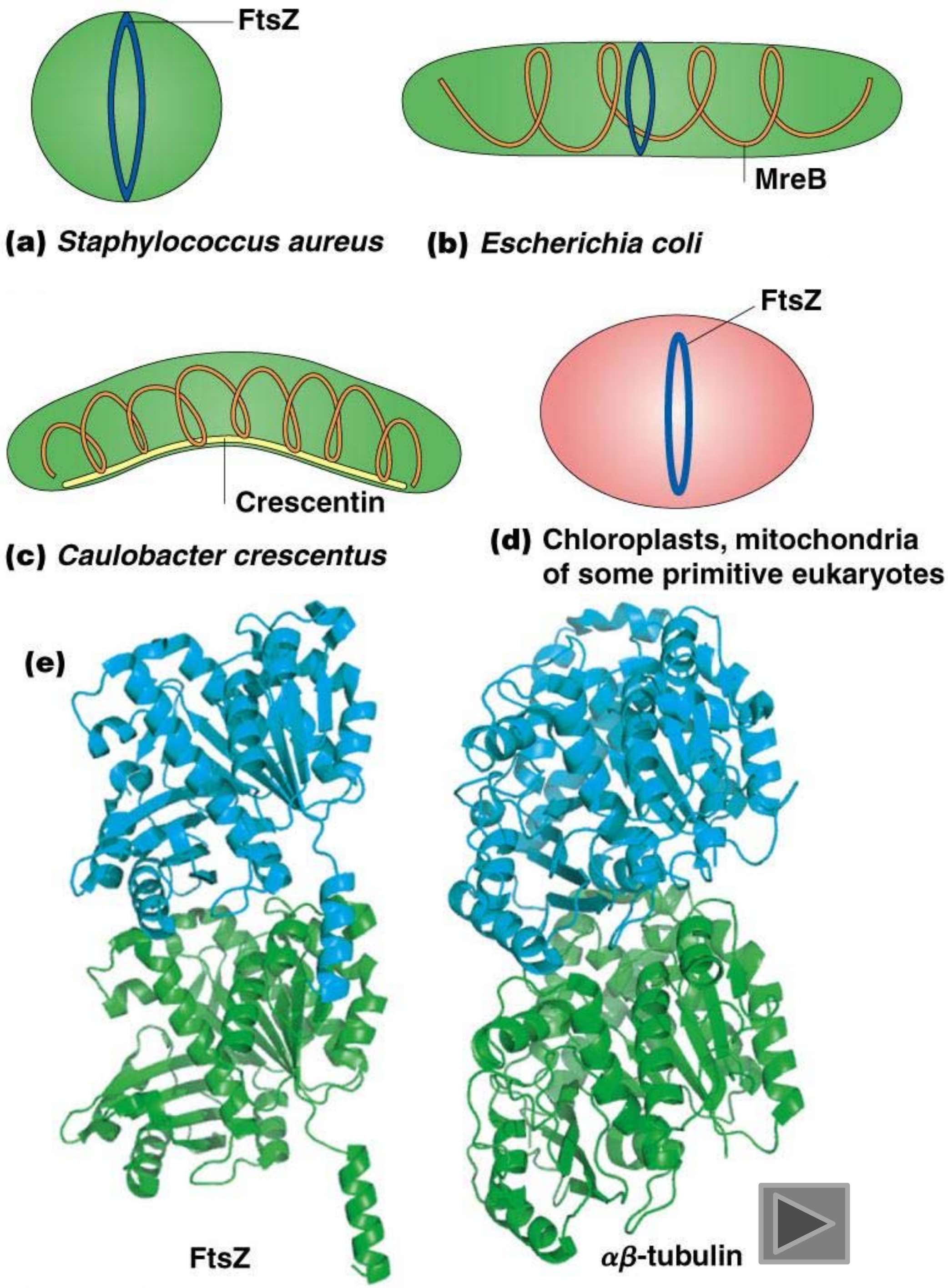
Endothelial cell before stretches



direction of stretch

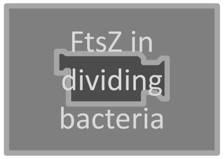
After several stretches





What are the bacterial analogs of the three (eukaryotic) cytoskeletal elements?

- Bacterial **FtsZ** is <u>similar to</u> **tubulin** in eukaryotes
- FtsZ can be also found in chloroplasts (plants) and mitochondria
- Bacterial **MreB** is <u>similar to</u> **actin** in eukaryotes
- Bacterial **crescentin** is <u>similar to</u> **intermediate filament** protein in eurkaryotes

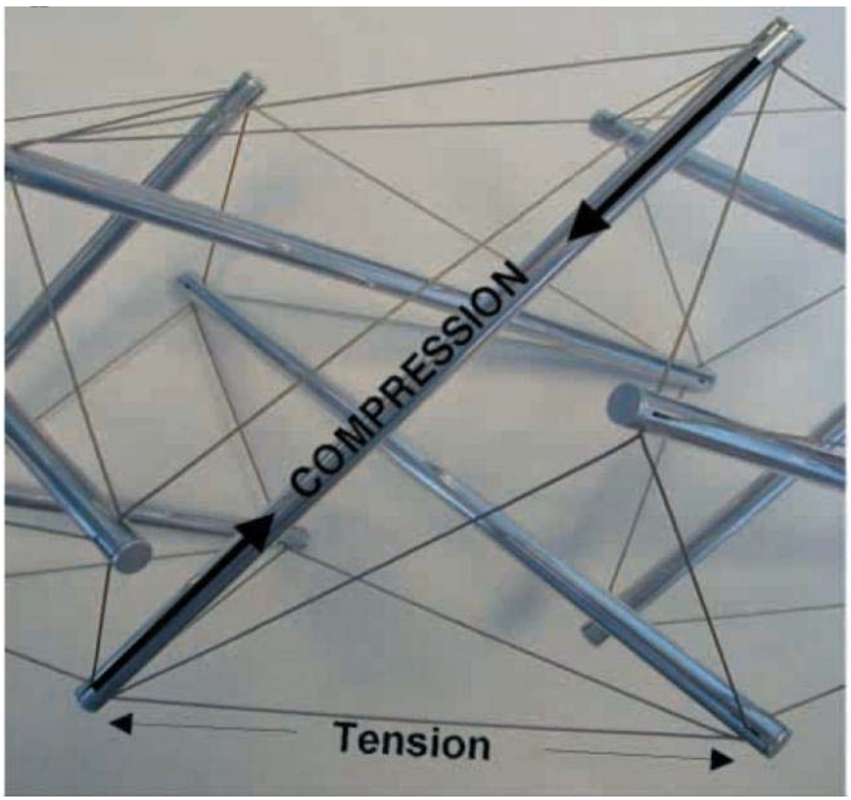


Bacterial cells expressing FtsZ-GFP. <u>FtsZ</u> can be seen to <u>accumulate at constriction sites</u>.

AtFtsZ1-1 in a chloroplast from a Arabidopsis leaf. Stack of optical sections rotated to visualize the 3D arrangement of the AtFtsZ1-1 ring.

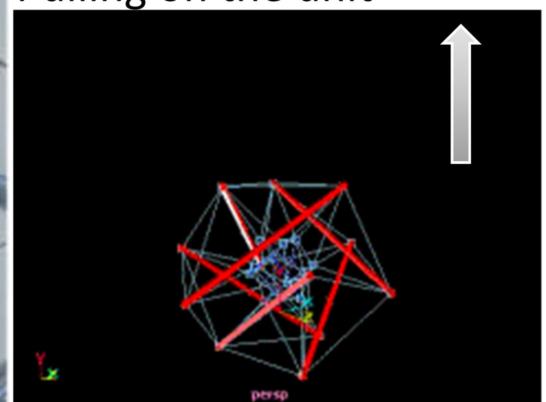
Tensegrity model: A balance between compression and tension

- **Tensegrity** means tensional integrity. Here, microtubules serve as **compression elements** (resist compression) and actin filaments serve as the **tension provider**
- Indeed, if we connect several metal rods ("microtubules") together with flexible strings ("actin") we receive a very shapeable unit that can be **stretched**, **compressed** and **sheared** (and always returns to its original shape after releasing the mechanical force).

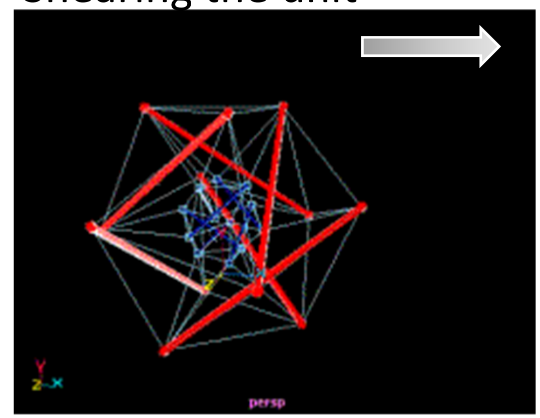


A highly shapeable unit only made of metal sticks and strings

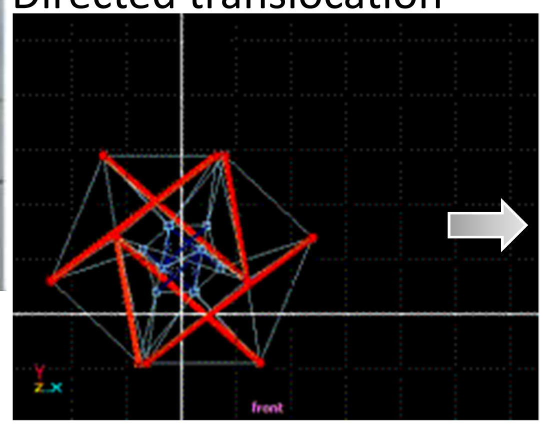
Pulling on the unit



Shearing the unit

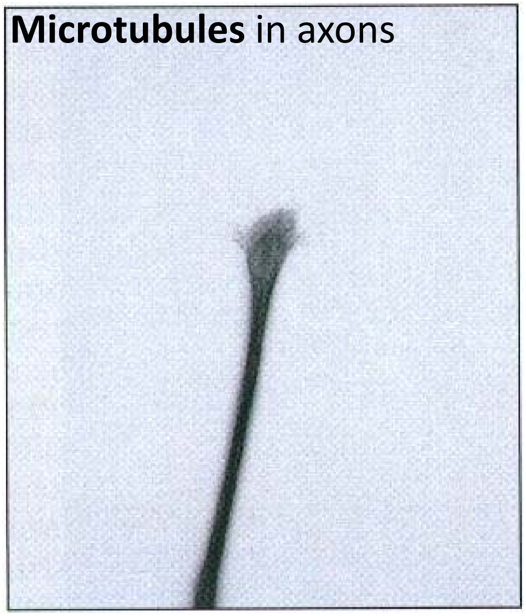


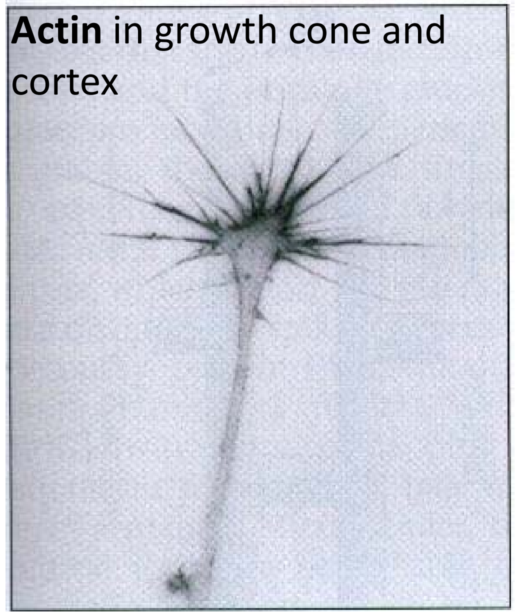
Directed translocation



Computer animation of the tensegrity model

Tensegrity model explains retraction of neurons after nocodazole treatment

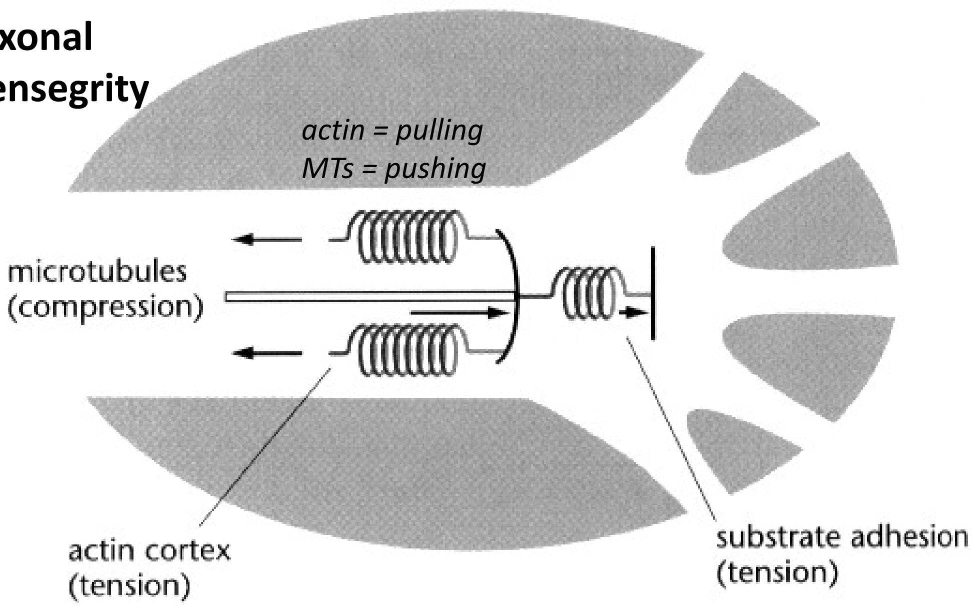


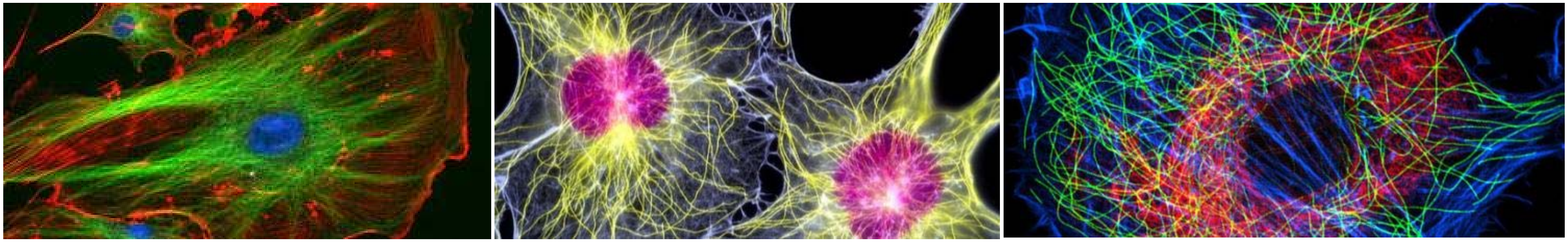


Neuronal cytoskeleton

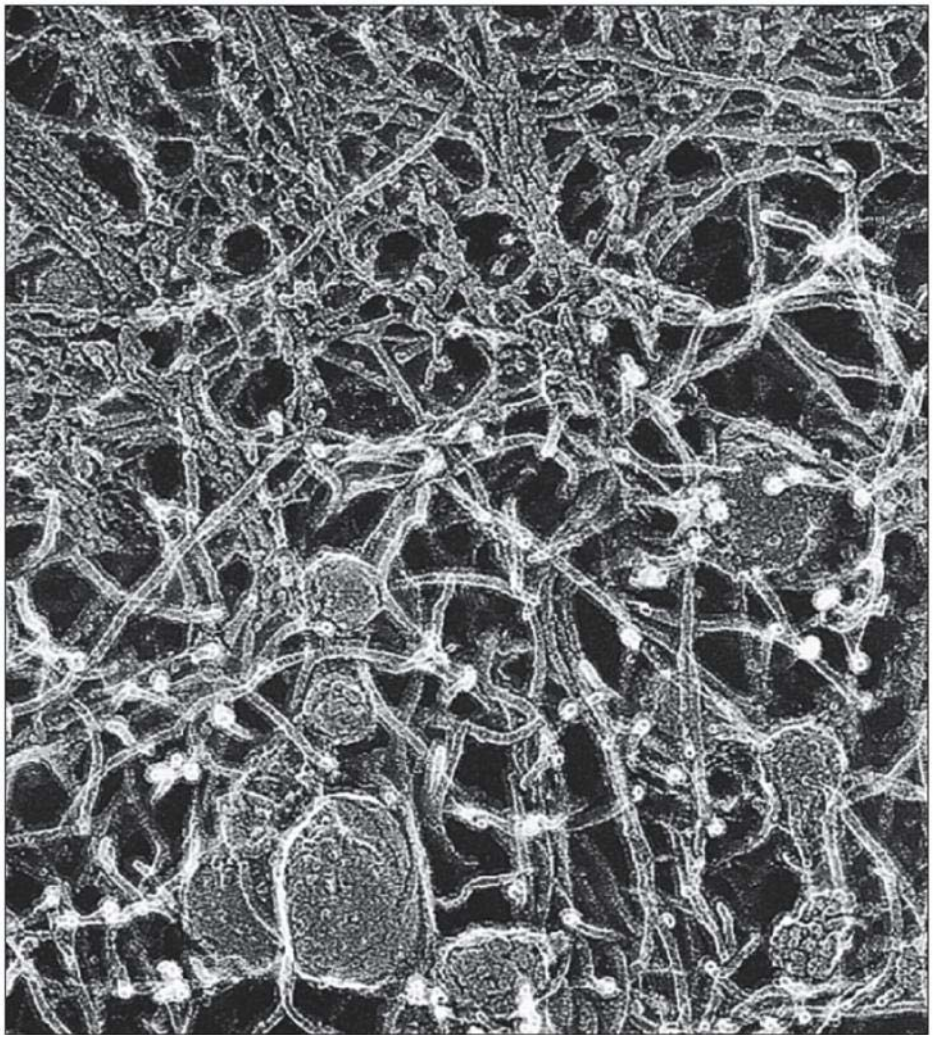
- The <u>axonal cytoskeleton</u> is composed of **microtubules**, **neurofilaments** and **actin**. With <u>actin only</u> at the cell <u>cortex</u> and at the <u>growth cone</u>.
- After **nocodazole** treatment we can observe **abrupt axon retraction**
- This behavior might be <u>explained by</u> the <u>tensegrity model</u>:
  - **Before** drug treatment, the axon is in a **mechanical balance** with <u>microtubules balancing the tension forces provided by actin</u>
  - **After** drug treatment, microtubules disappear and the <u>axon retracts based on the</u> **contraction of the actin network**

Axonal tensegrity





World of the Cell



The end of chapter 13!

Thank you for your attention!