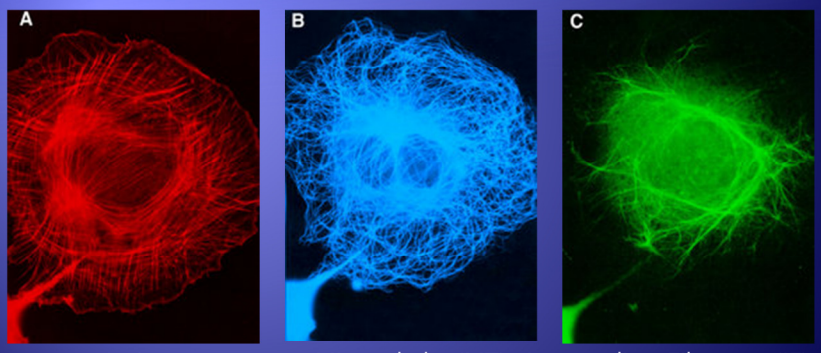
Session 5

CYTOSKELETON

Cytoskeleton

- Three fundamental cytoskeletal filaments



Actin Microtubules (MT) Intermediate Filaments (IF)

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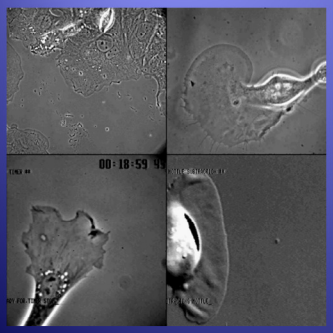
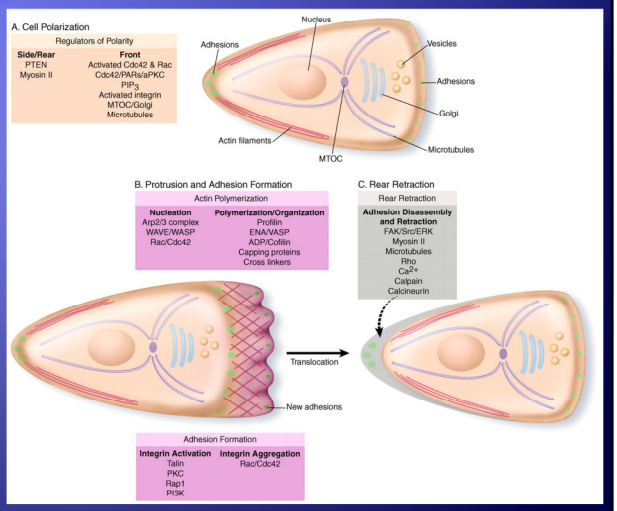
These three filamentous proteins are made up of subunits, actin monomer for actin filaments, alpha and beta tubulin for microtubules, and desmin or vementin for intermediate filaments.

Dynamic Structure

- Cell Crawling
- Phagocytosis
- Cytokinesis
- Muscle contraction
- Gastrulation



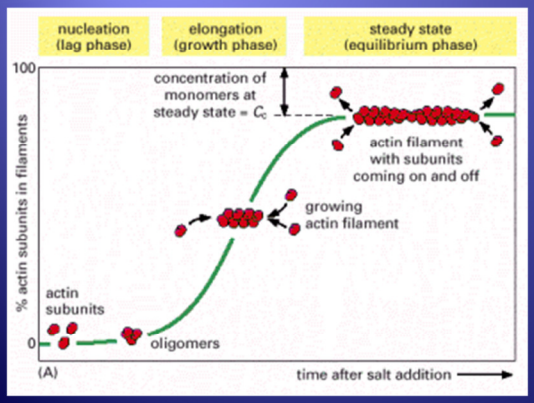


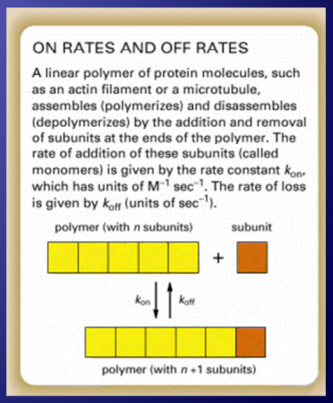
Top left: mouse fibroblasts in scratch wound assay (3h). Top right: mouse melanoma cell (20min) Bottom left: a chick fibroblasts (2h). Bottom right: trout epidermal keratocyte (4min).

These filamental proteins are amazingly versatile and participate in the movement of cells. The way that the filament proteins form and assembly is regulated by a host of enzymatic proteins that guide and direct the assembly process as well as special organelles that help to spatialy direct or stabilize the process.

Polymerization of Subunits

- Assembly/Disassembly of Monomers Subunits
- Treadmilling of monomers keeps a flux of subunits but a steady-stead length.





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These filaments are in constant flux of subunits (monomers) being added on or taken off. There are specially proteins that help add subunits, remove subunits, cleave a filament in the middle, or protect a filament from disassembly.

Chemical Kinetic .
Added to filament = k_{on} * Free Concentration (C)
Removed to filament = k_{off}

As filament grows, Free Concentration drops until it reaches a critical value, C_c (critical concentration). At this equilibrium:

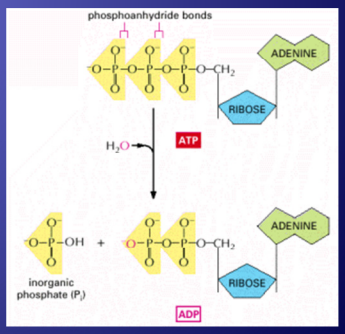
$k_{on} * C = k_{off}$

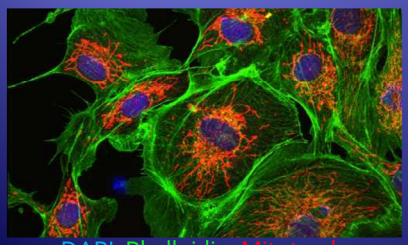
→ $Cc = k_{off} / k_{on}$

This is a steady-state of treadmilling where units are being added and removed, but the overall length remains at a steady-state. If you label an actin monomer, you can watch as it is added to the fast growing end and then eventualy falling off from the slow-growing end.

ATP/GTP Hydrolysis

- The energetically favorable reaction of ATP or GTP hydrolysis is coupled to many otherwise unfavorable reactions for cytoskeletal sythnesis.
- ATP + H_2O → ADP + Pi
- GTP + H_2O → GDP + Pi
- Mitochondrial biosynthesis





DAPI, Phalloidin, Mitotracker

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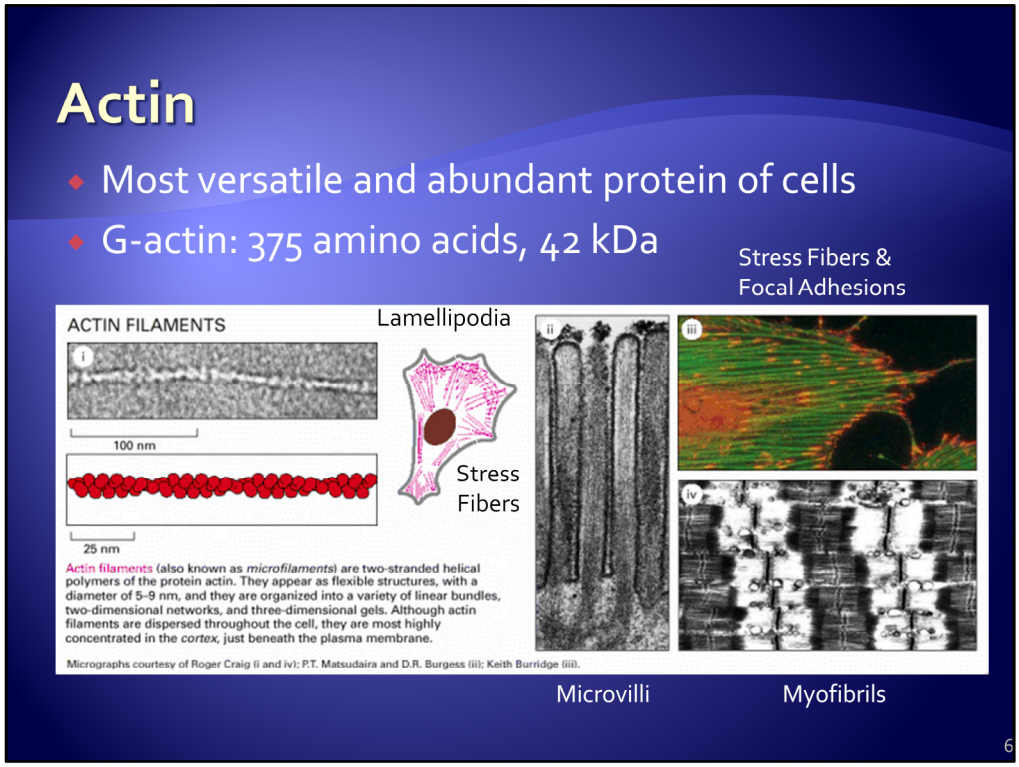
Recall that ATP is the primary energy carrier for cells and is generated by the metabolic activity of mitochondria. GTP is also generated by mitrochondira.

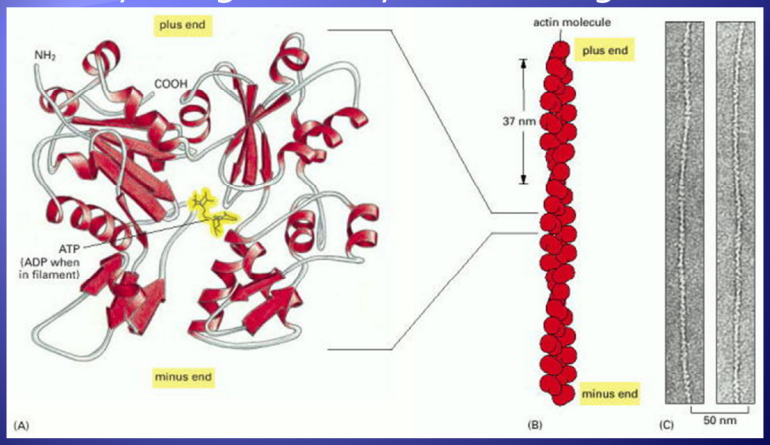


Initially believed to be only found in muscle cells, actin was later found to be in nonmuscle cells. In cells approximately 50% of actin is F-actin (filamentous actin), which is a helical double stranded structure. 50% is G-actin (globular actin), which is the free monomer.

Actin is highly versatile can form structures that are 2D or 3D. It is particularly important for cell migration where there is a rapidly polymerizing region of actin at the leading edge of a cell that pushed the cell membrane forward called the lamellipodia. The points of contact that a cell has with the extracellular matrix are called focal adhesions are like the feet of a cell that allow it to propel itself forward through the contraction of stress fiber structures. In muscle, there is a high density of parallel actin filaments that create much larger forces through the high degree of organization.

Actin

- Assembly is regulated by ATP binding



- Six major isoforms of actin found in different tissue that differ by their N-terminal sequence

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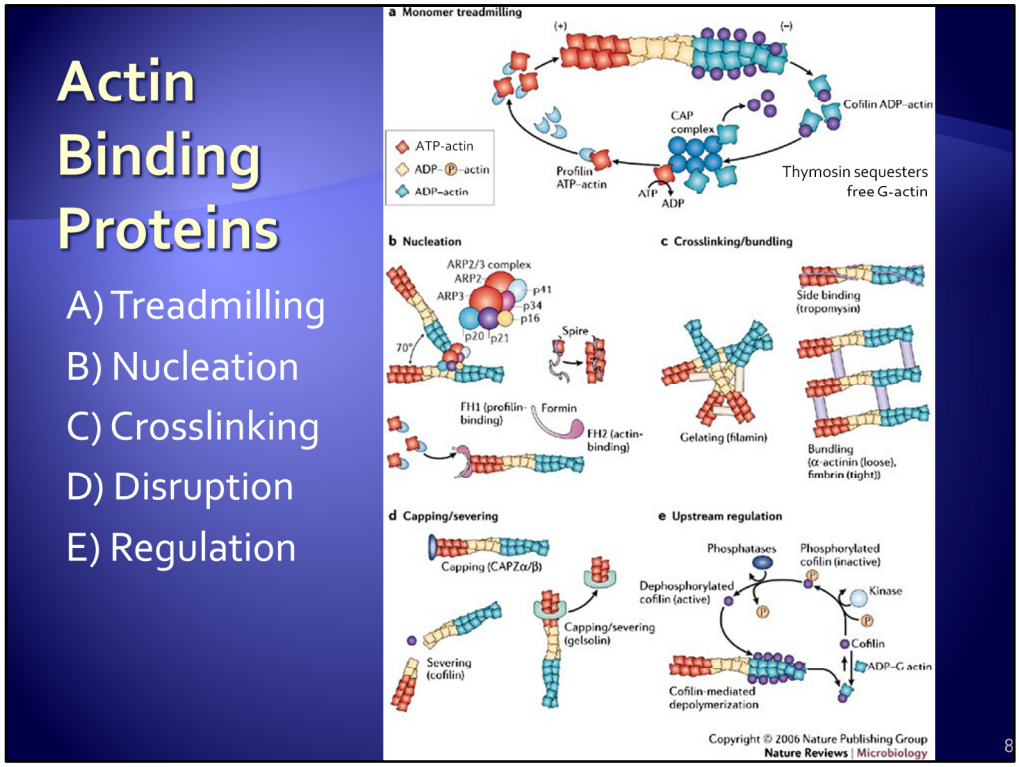
For the actin monomer, there is a cleft where ATP binds and it is called the minus end (slow growing end). The other side is the plus end (fast growing end). To assembly a subunits into a protofilament, the cleft-bound ATP undergoes ATP hydrolysis to yield ADP and Pi. The ADP molecule remains trapped inside the protofilament and stabilizes the structure. The rate at which ATP-bound actin is added is faster than the rate that ATP is hydrolysized into ADP so there is a ATP-cap region at the end of a growing filament.

The actin filament consists of two parallel protofilaments that twist around each other. Historically, because of the way that myosin binds to actin, the minus end was referred to as the pointed end and the plus end as the barbed end (direction of myosin movement). Like myosin, there are other proteins that recognize the one end or the other of an actin filament.

Eucaryotes had multiple isoforms of actin encoded by a family of actin genes and are present in different tissue: Alpha-actin (skeletal, cardiac, and smooth muscle variants), beta-actin (nonmuscle, “meshwork”), gamma-actin (smooth muscle and nonmuscle variants, “stress fibers”). The differences between the isoforms is at most 23 out of 375 amino acids. Interestingly these variants are indistinguishable in their ability to polymerize or interact with other proteins for the most part. It remains to be seen if there are special differences in their structure that affect the function of their specialized tissue.



A) Actin filaments grow by the addition of ATP-bound actin to the "barbed" plus end where profilin binding to ADP-actin causes the transfering out of ADP to be replaced by ATP and promote the addition of a new subunit to the filament. Once inside the filament, the ATP is hydrolyzed to ADP + Pi. The inorganic phosphate molecule (Pi) is slowly released from the subunits in the filament. A protein called cofilin can only specifically bind to ADP-actin, the older units towards the minus end. Cofilin binds to two actin monomers in the filament and causes a twist that destabilizes the filament and breaks it into short pieces. Thymosin sequesters large pools of ATP-actin monomer so that 50% of the actin pool is G-actin and 50% is F-actin.

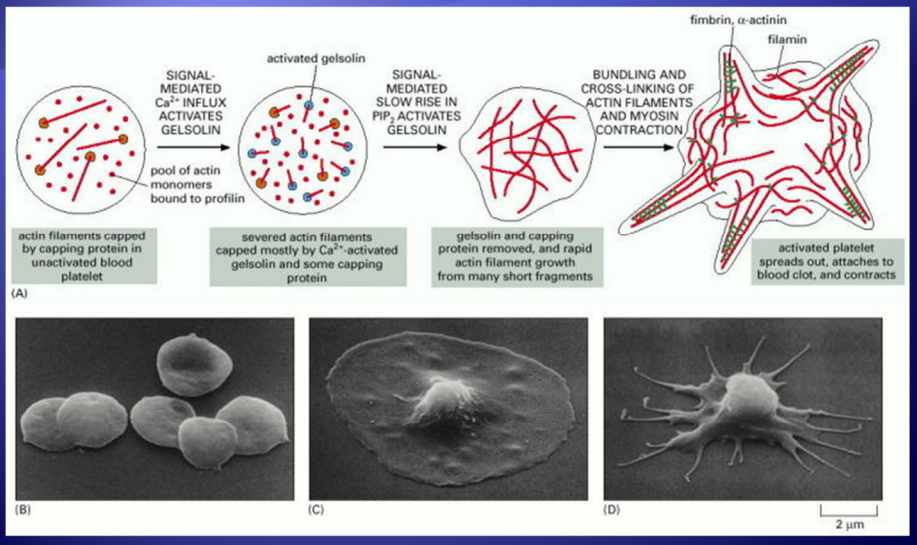
B) Arp2/3, formins, and Spire all work to nucleate new actin filaments de novo or as branch points from existing filaments. Actin solution will also nucleate spontaneously in the test-tube

C) Crosslinking proteins like alpha-actinin help to form the contractile stress fibers on nonmuscle cells, tropomyosin helps to stabilize the thin filaments of sarcomeres in muscle cells and filamen helps to form a 3D meshwork.

D) Capping proteins help to stabilize the growth of actin at the plus end while severing proteins can disassembly actin at midpoint along it's length. This allows for rapid chances in the actin cytoskeleton that are faster than depolymerization reactions by cofilin.

E) Enzymatic proteins called kinases and phosphatases regulate the activity of many actin binding proteins and control the growth rates of actin. For example, LIM Kinase 1 is a protein that adds a phosphate molecule to the serine residue of cofilin which changes the structure of the cofilin and renders it inactive. This will cause actin to continue to grow because cofilin's depolymerization activity is reduced. However, a phosphatase protein called Slingshot will undo the phophorylation of cofilin and remove the phosphate group to free cofilin to perform at its normal levels.

Rapid response of actin



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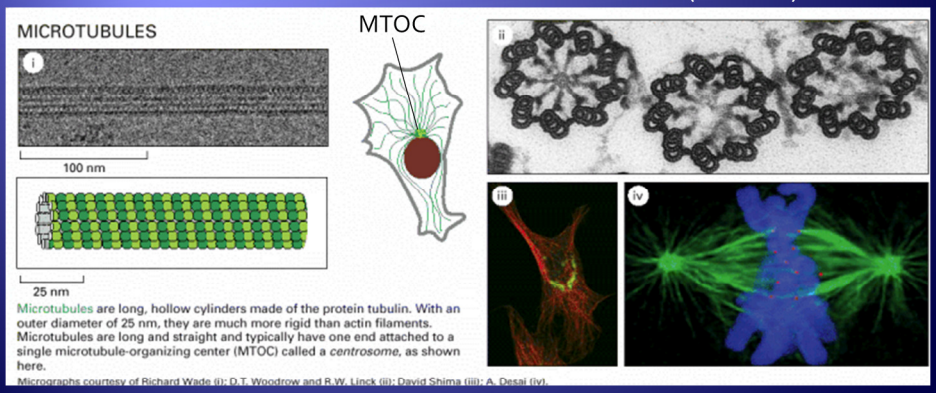
The process of platelet activation shows how a cell can regulate its actin accessory proteins that mediate severing, capping, and cross-linking to generate rapid and dramatic morphological changes. Platelets are tiny cells without a nucleus that circulate in the blood and help to form clots at sites of injury. The resting platelet is discoid in shape, and it contains short actin filaments capped by CapZ, surrounded by a large pool of actin monomer bound to profilin.

When the platelet is activated by physical contact with the edge of a damaged blood vessel or by a chemical clotting signal such as thrombin, a rapid, intracellular, signal transduction cascade results in a massive influx of Ca^{2+} into the platelet cytosol. The Ca^{2+} activates gelsolin, which cleaves the capped filaments into tiny fragments, each now capped by gelsolin.

With slower kinetics, the same signaling pathway causes a rise in PIP_2 levels, which inactivates both gelsolin and CapZ, removing them from the filament plus ends. The large numbers of free plus ends generated by severing and uncapping are then rapidly elongated by the monomeric actin pool, forming many long filaments. Some of these long actin filaments are cross-linked into a gel by filamin, while others are bundled by α-actinin and fimbrin. This causes the activated platelet to extend lamellipodia and filopodia and to spread itself across the clot, attaching to the clot by transmembrane adhesion proteins called integrins. Once the PIP_2 signal subsides, the CapZ returns to the ends of the filaments, rendering them stable against depolymerization and locking the platelet into its spread form. Finally, myosin II uses ATP hydrolysis to slide the long actin filaments relative to one another, causing a contraction of the platelet that pulls the edges of the wound together

Microtubules

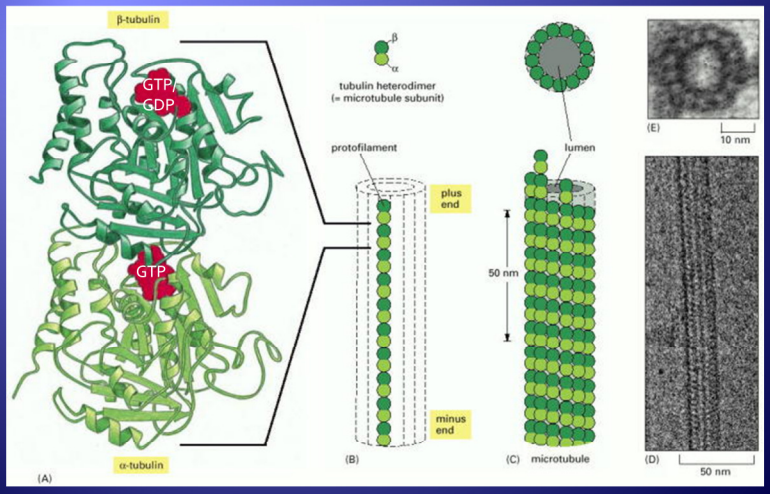
- Hollow, stiff filaments that direct traffic
- Tubulin: 50 kDa



By the hollow size, these structures are the stiffer of the cytoskeletal filaments. The radiate outward from about the center of the cell at the MTOC (microtubule-organizing center) and are typically 1-10 microns long. Kinesin and dynein are two motor proteins that use MTs as tracks for transport of vesicles or proteins. The tracks that MT form are especially important during cell division when the nuclear envelope is dissolved and the duplicate chromosomes need to be separated between the two daughter cells. The epithelial cells of our esophagus have hair-like structures called cilia whose internal structure is made up of several microtubules arranged in parallel called the axoneme.

Microtubule Assembly

- Heterodimers of α-tubulin and β-tubulin



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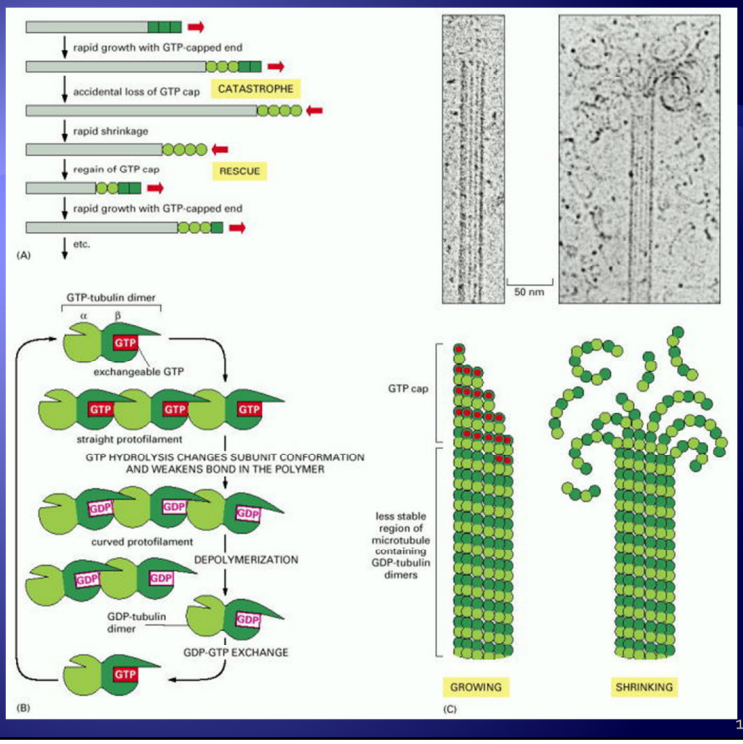
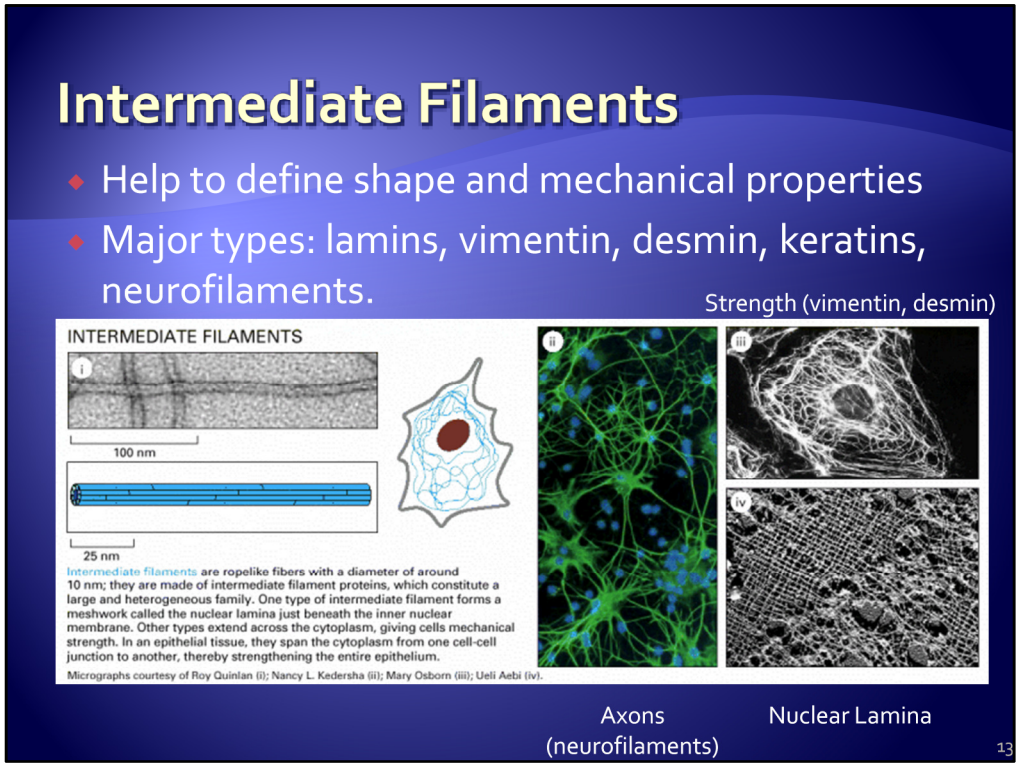
Microtubules are formed from protein subunits of tubulin. Each subunit is a heterodimer (two different parts) formed from alpha-tubulin and beta-tubulin. These subunits line up end-to-end to form a protofilament. Each microtubule is made of 13 protofilaments all with the same alpha/beta orientation. This results in distinct structural polarity with one end being of all alpha units (minus) and the other end being beta units (plus). The MTOC protect the minus ends through gamma-tubulins which nucleate microtubules.

Dynamic Instability

- Free ends abruptly alternate between assembly & catastrophe phases.

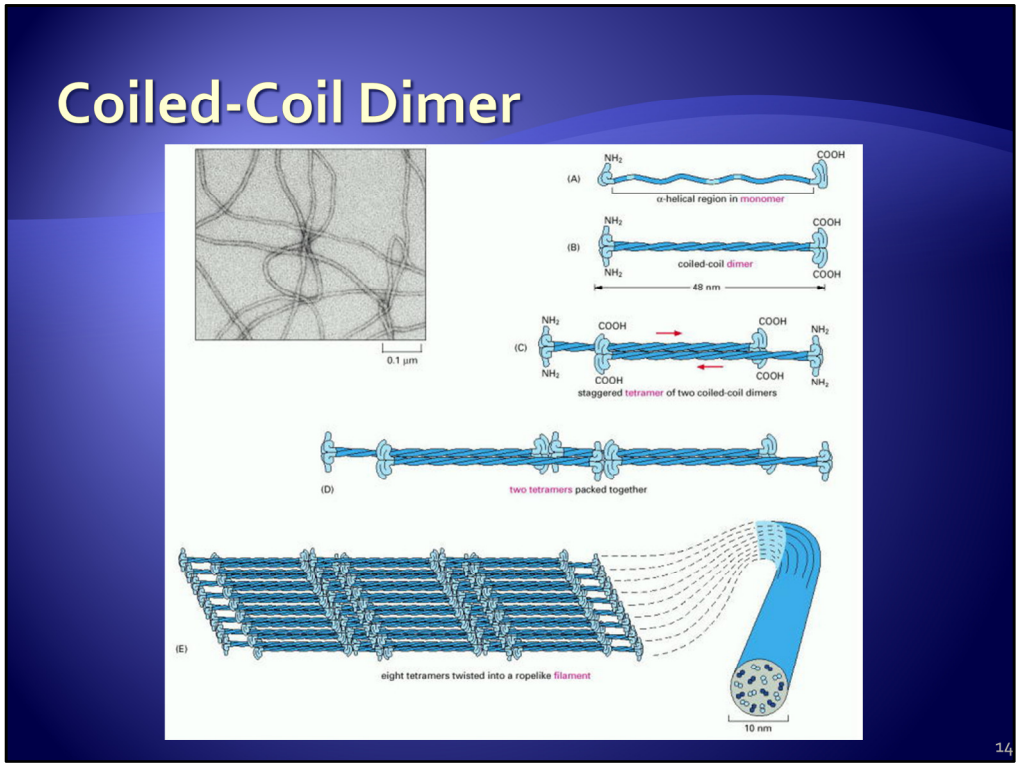


During rapid growth, the GTP-bound tubulin dimers are added faster than their GTP can be hydrolyzed. This creates an GTP-cap that stabilizes and promotes further growth. The reason being that GTP-bound tubulin is very straight but when GTP becomes GDP, the protofilament is weakened and is more flexible. This allows for the protofilaments at the end to become splayed apart and depolymerize. If these structures grow into a solid object, like the cell membrane, the local GTP-tubulin concentration drops and the GTP cap will shrink.



Take a look at your skin. This is made up of keratin which is an intermediate filament protein. The skin epithelial cells have a highly cross-linked cytoskeleton of intermediate filaments that provide toughness and mechanical integrity. Each cell is connected to its neighbor by intermediate filaments. When these skin cells die, they remain as a complex network structure.

Intermediate filaments are in between microtubules and actin in terms of size and hence this is where their name originates. In the cell, assembled IFs are resistant to the extraction with detergents and high-salt solution, under which the other two cytoskeletal systems (actin filaments and microtubules) are soluble. Therefore, they have long been viewed as a fixed architectural structure for cells that solely protects them against various mechanical stresses. However, recent studies from several research groups demonstrate that IFs undergo spatial reorganization in a variety of cell types in response to stimulation with physiological signals.

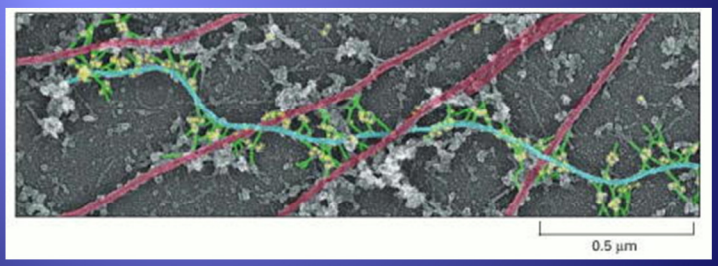


Less is understood about the mechanisms of assembly and disassembly of intermediate filaments than actin and microtubules, but IF are dynamic structures in most cell types. A monomer pairs with an identical monomer to form a dimer structure where they are aligned in parallel and wound about each other to form a coiled-coil dimer.

Two dimers then line up side by to form an anti-parallel tetramer. This is the soluble subunit of intermediate filaments. In the final 10nm rope-like filament, tetramers are packed together in a helical array of 16 dimers in the cross-section.

IF lead to CSK cross-linking

- Plectin (green), IF (blue), MT (red), anti-plectin labelled gold particles (yellow)



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Plectin is a cross-linking protein of the cytoskeleton. It links intermediate filaments to microtubules, actin filaments, and myosin filaments. It also connects the IF to adhesive structures in the cell membrane (desmosomes for cell-cell adhesion and hemidesmosomes for cell-ECM adhesion).

Phalloidin comes from the poisonous Death Cap mushroom that binds to three actin monomer in F-actin and stabilizes the structure from depolymerization. The antidote for eating a Death Cap mushroom is to eat lost of red meat. In fluorescent staining, phalloidin conjugated with a fluorescent probe to stain for actin.

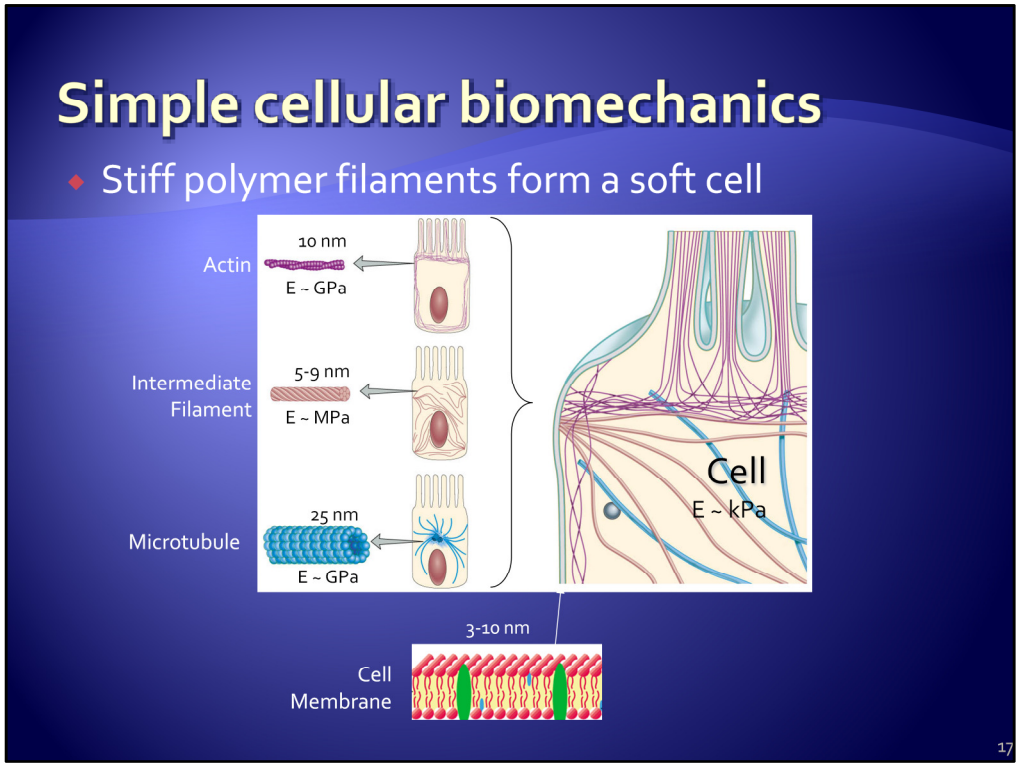
Cytochalasin a metabolite from mold that bind to the plus end of actin and prevent elongation.

Latrunculin is from the Red Sea sponge that directly depolymerizes actin and sequesters actin monomers.

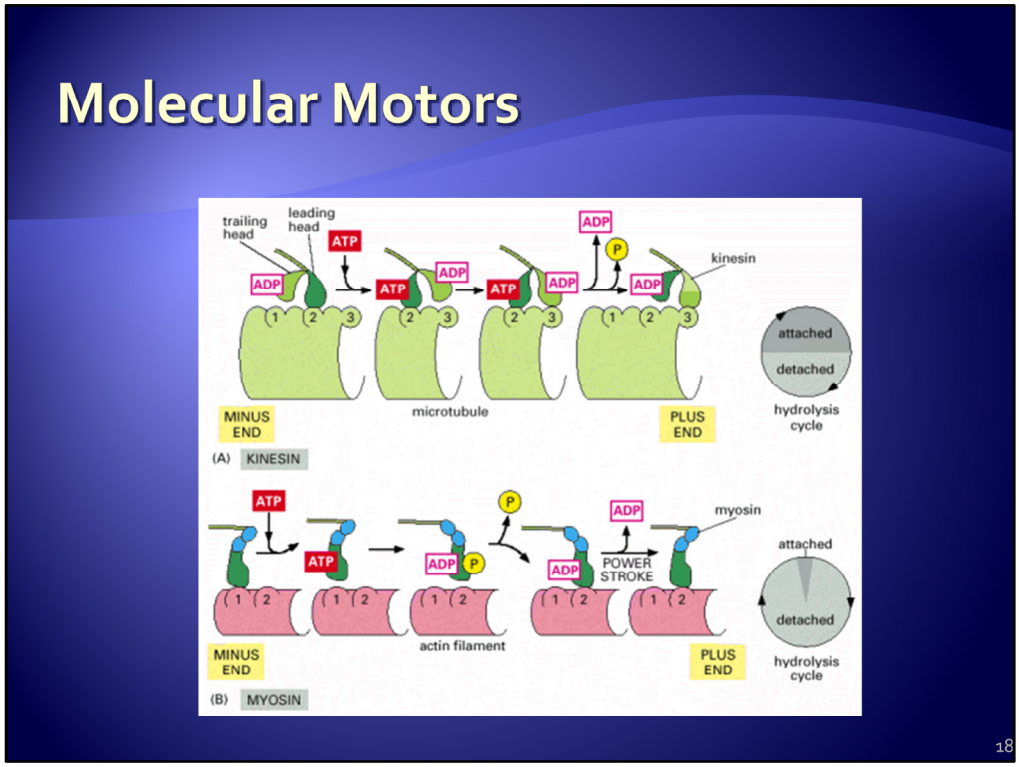
Taxol is from yew tree and stabilize microtubules against breakdown.

Colchicine is from autumn crocuses and binds to tubulin dimers and prevents polymerization.

Nocodazole is a synthetic compound that inhibits polymerization

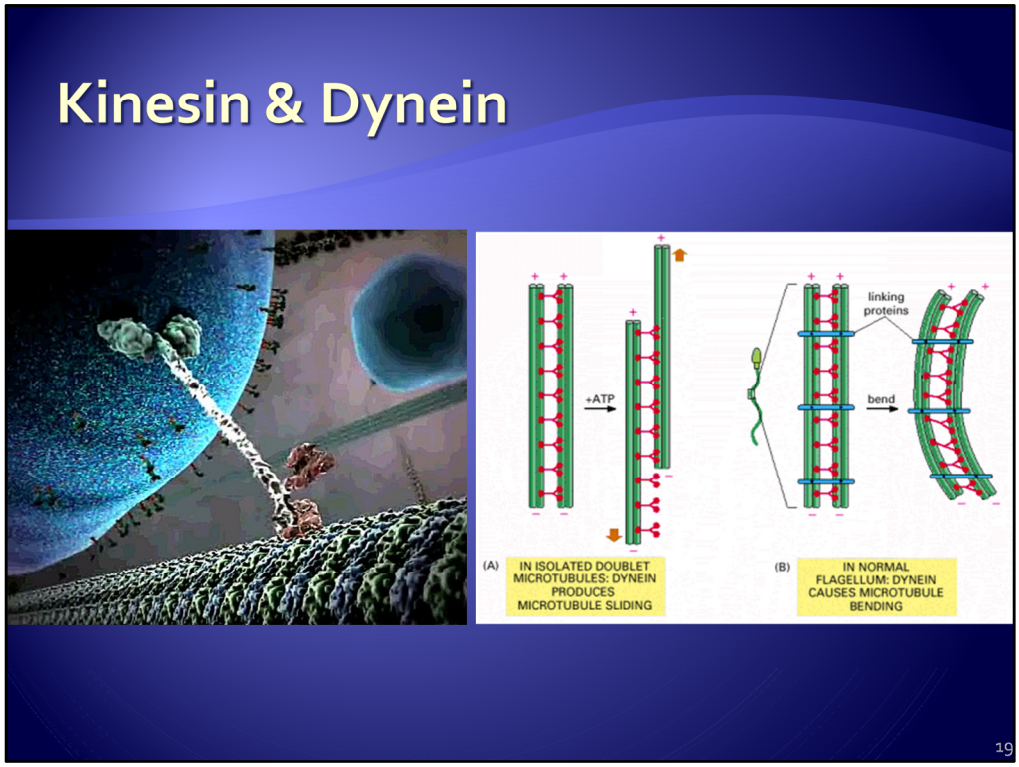


The bending stiffness of actin and microtubules is in the GPa range but for a cell, as we will see for mechanical testing of cells, their elastic modulus is in the kPa range.

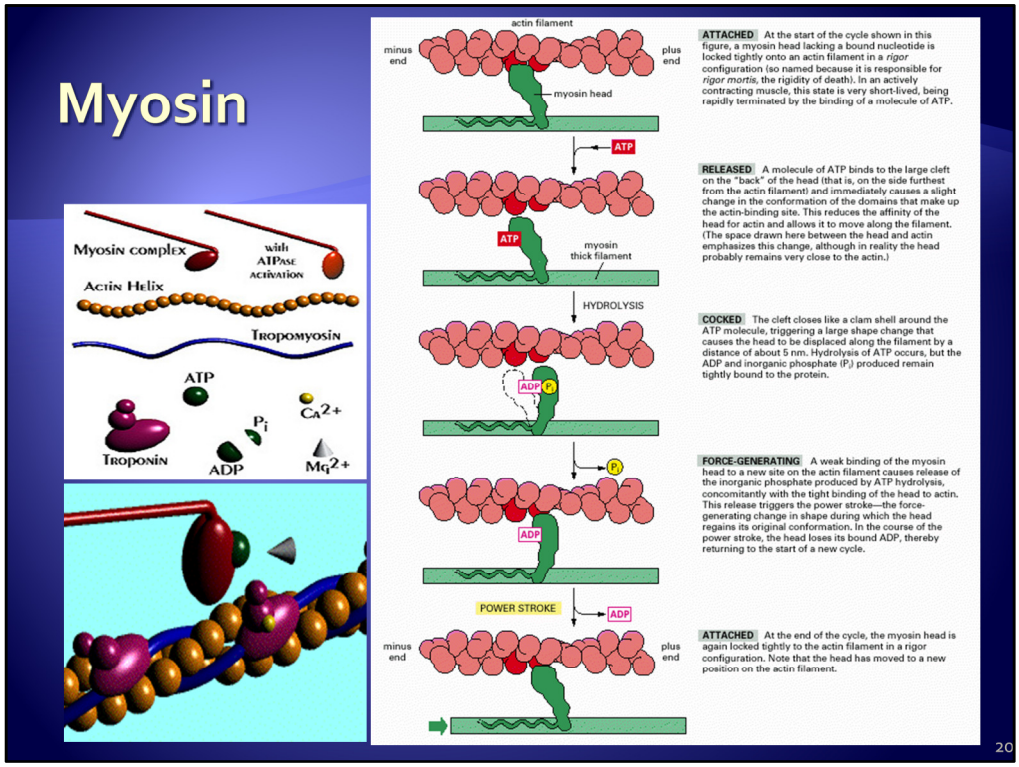


Both microtubules and actin filaments have their associated motor proteins. For microtubles, kinesin "walks" along a microtubule in a step-wise fashion to the plus end. Dynein is another motor protein that walk towards the minus end (back towards the MTOC).

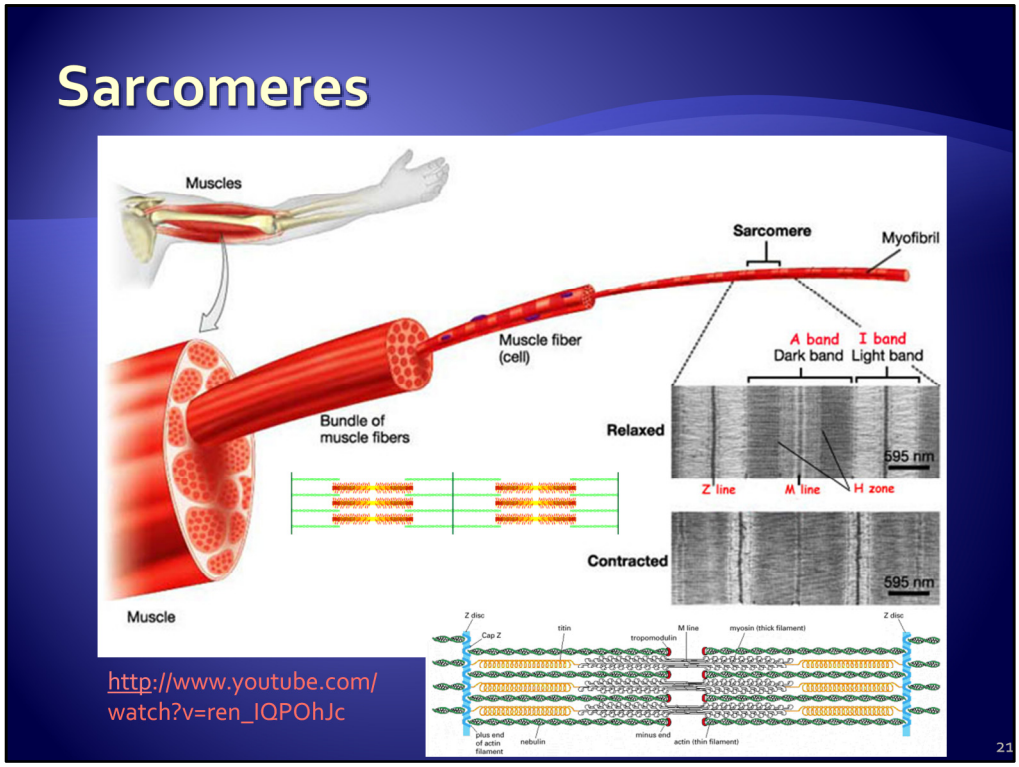
Myosin is motor protein associated with actin that has both muscle and nonmuscle isoforms. It undergoes an ATP driven powerstroke to pull on the actin filament and is essential for muscle contraction.



Kinesin transports a tethered vesicle to the plus end (away from the MTOC). They also push apart the two poles of a dividing cell to form the mitotic spindle. Dynein which is used by the flagellum of sperm to swim.



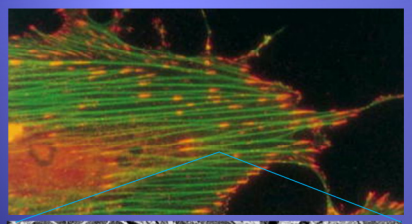
Myosin motor activity is through ATP hydrolysis and Ca2+ regulation of troponin.

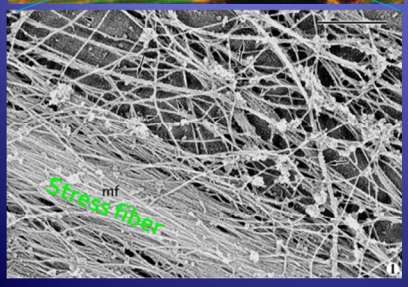


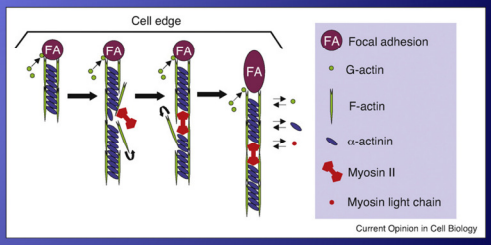
Actin, myosin, and associated actin-binding proteins control the structure and function of sarcomeres.

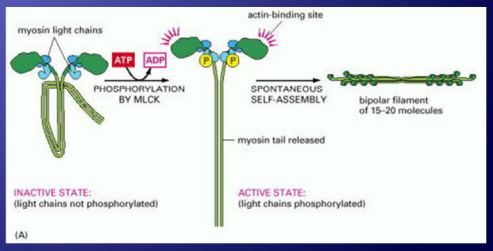
Stress Fibers

- Transient bundled structures









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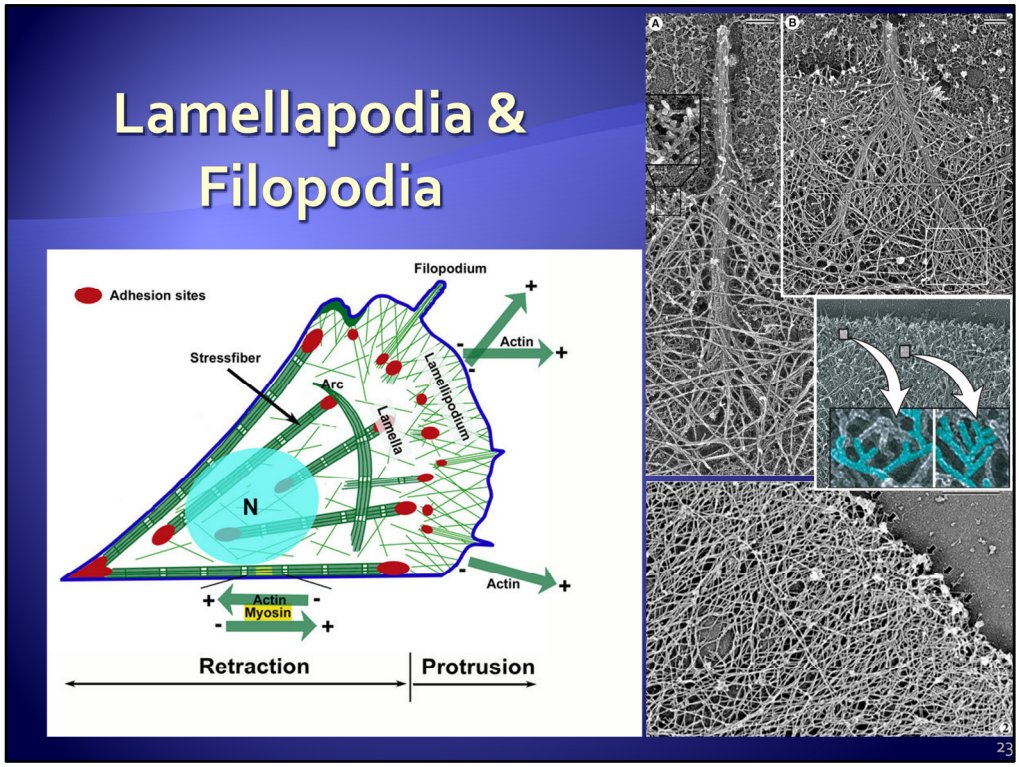
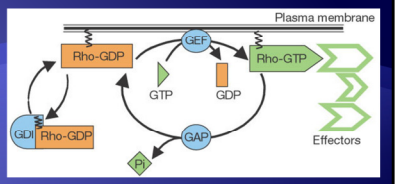
Stress fibers area loosely organized structure of actin, alpha-actinin, and myosin. Myosin needs to be phosphorylated on it's light chain elements in order to block the head-to-tail interaction and allow the protein to form bipolar thick filaments that contract stress fibers and deliver force to the focal adhesions.

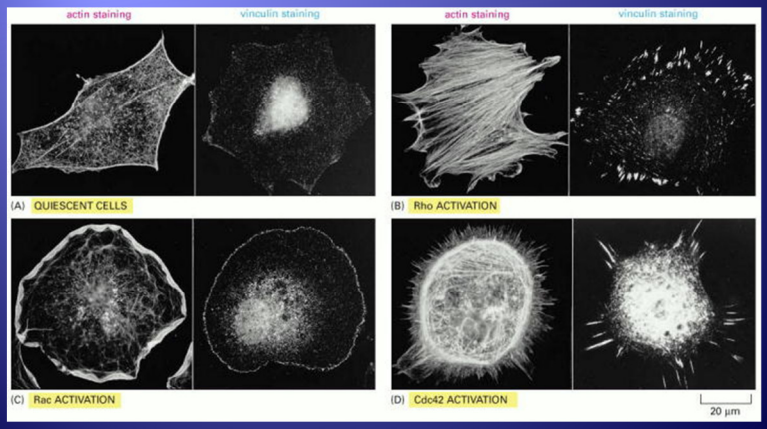


Filopodia are tightly-bound bundles of actin. Lamellipodia is a meshwork of actin that has branch points that are formed by Arp2/3 to protrude the membrane forward.

Rho Family GTPases

- An interesting discovery by Anne Ridley and Alan Hall while studying the oncogene *ras*...





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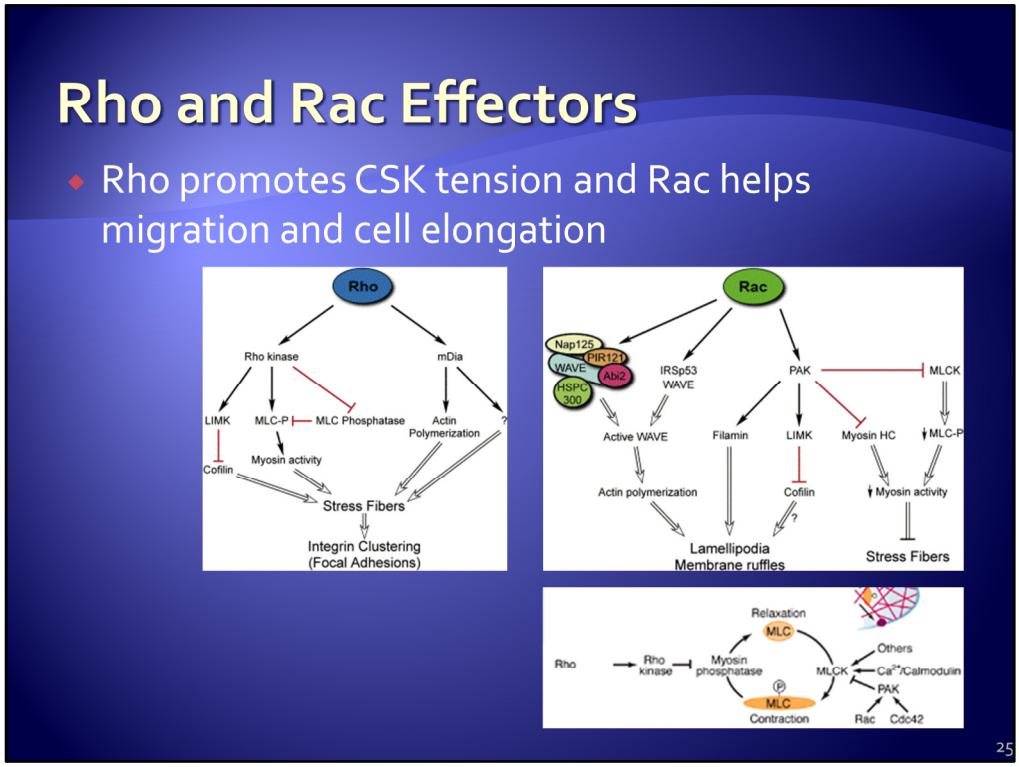
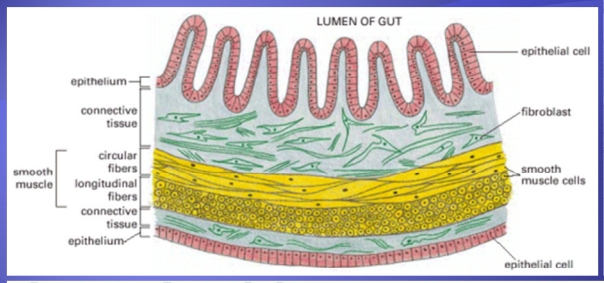
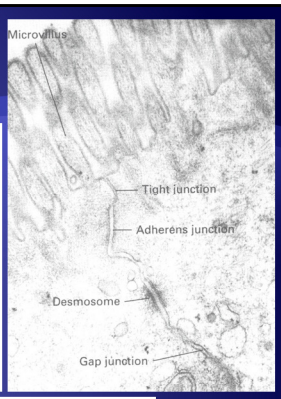
Hall's team had been struggling to decipher the function of the oncogene *ras* by injecting its protein into cultured cells. They set aside their studies on the protein ras and switched to its cousin rho. Their work revealed that rho and related GTPase proteins were key regulators of the cell's actin cytoskeleton. These protein flips between a GTP-bearing active state and a GDP-carrying inactive form through a molecular switch called the guanine nucleotide exchange factor (GEF). The researchers injected an always-on mutant of rho (constitutively active) and it's cousins. These mutationed proteins do not need GEF to stay on and cannot be turned off by GTPase activating protein (GAP). Expressing them in the cells caused interesting changes to the actin cytoskeleton, focal adhesions, and cell shape.

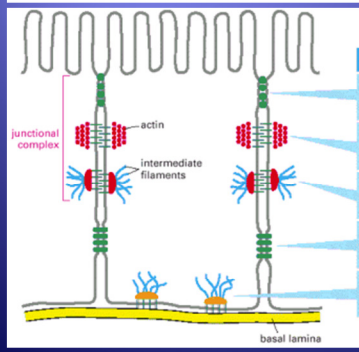


Since Ridley and Hall's initial discovery, the role of RhoGTPase in the cytoskeleton have been further explored and defined. Interestingly, Rho appears to regulate cytoskeletal tension through the promotion of actin polymerization and the upregulation of myosin motor activity through the inhibition of myosin phosphatase. Rac also promotes actin polymerization, but it has a negative effect on myosin activity. Take together, both Ca2+ signaling and the Rho/ROCK pathway can promote cytoskeletal tension.

Cell-Cell Adhesions







name	function
tight junction	seals neighboring cells together in an epithelial sheet to prevent leakage of molecules between them
adherens junction	joins an actin bundle in one cell to a similar bundle in a neighboring cell
desmosome	joins the intermediate filaments in one cell to those in a neighbor
gap junction	allows the passage of small water-soluble ions and molecules
hemidesmosome	anchors intermediate filaments in a cell to the basal lamina

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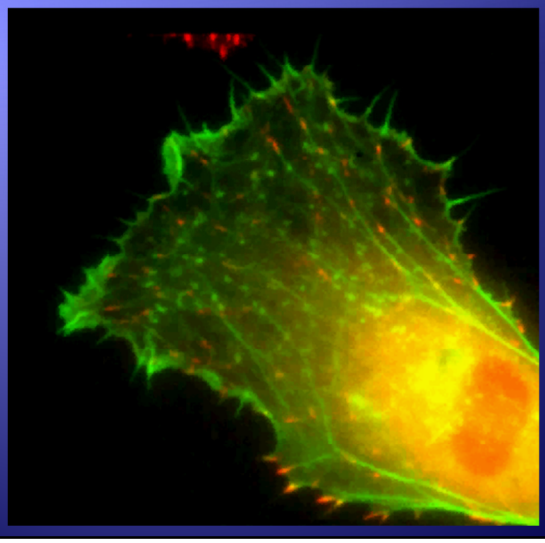
The cytoskeletons of cells are linked to each other in a tissue.

Focal Adhesions

- We will cover this more during the ECM session



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