Lecture 19. Microtubules, Motors and Movement

Objectives:

At the end of this lecture, students will be able to

1. *Cite several examples of what constitutes cell motility and the function of the cytoskeleton.*

2. *Identify the key molecular components of the cytoskeleton.*

3. *Described how the cytoskeleton's molecular components are arranged in a typical cell.*

4. *Discuss why biological systems use subunits and how linear filaments are assembled from these subunits.*

5. *Explain how the assembly of filaments can be regulated.*

6. *Compare and contrast the different types of myosin and discuss the implications of this for their possible functions in cells.*

7. *Explain how actin polymerization occurs and how it is regulated by actin-binding*

8. *Identify the processes that compose mitosis and discuss the molecular mechanism of those processes.*

9. *Describe how microtubules control the position and movement of membrane vesicles inside cells.*

10. *List the different classes of intermediate filaments and describe how they function in cells and tissues.*

Reading and Other Materials

Textbook Chapters

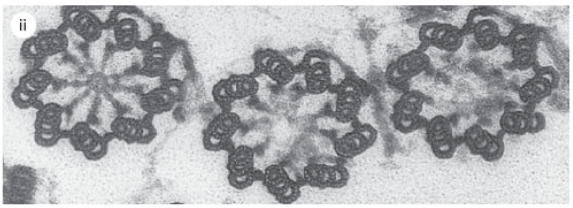
Alberts et al., Molecular Biology of the Cell, 6th ed., 2014, Garland. Chap. 16.
Lodish et al., Molecular Cell Biology, 7th ed., 2012, Freeman. Chaps. 17, 18.
Pollard & Earnshaw, Cell Biology, 2nd ed., 2007, Saunders. Chaps. 33-39, 44

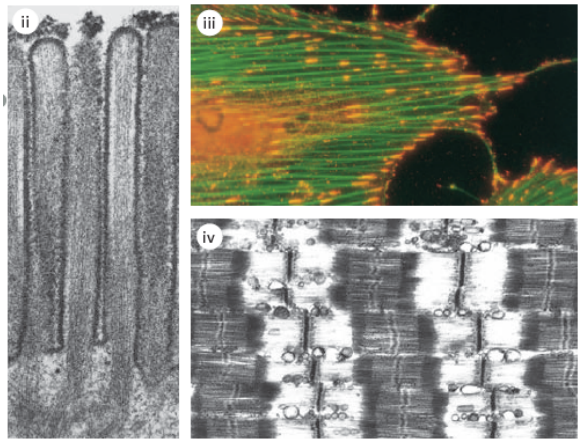
Movies and Videos Online

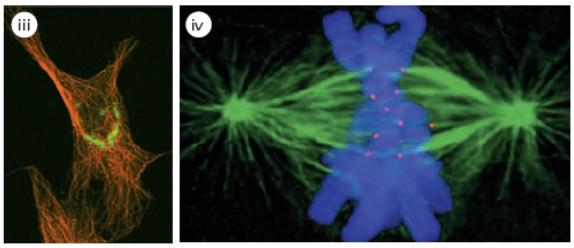
Nikon Microscope Company, http://www.microscopyu.com/galleries/
Swept Field Confocal Digital Video Gallery
Live-Cell Imaging Cell Motility
Ted Salmon Lab at Chapel Hill
http://labs.bio.unc.edu/Salmon/salmonlabmovies.html
Am Soc for Cell Biology
Videos - News and Education, http://www.ibiology.org/
Webinars on Imaging - http://www.ascb.org/webinars/

Microtubules, Motors and Movement: Cell Motility and the Cytoskeleton

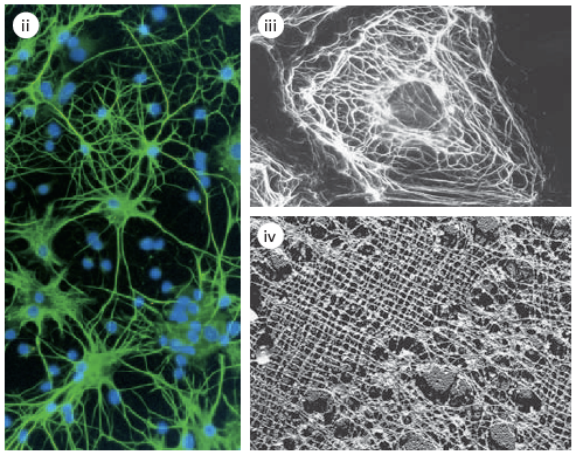
Microtubules are rigid, hollow cylinders that are 25 nm in diameter (at right). They are in all eukaryotic cells. They make both stable and dynamic structures.







Actin is a thinner filament and it is flexible (at left). The fact that microtubules are hollow makes them kind of brittle. Actin is more like threads. They are flexible and not all that strong. So, they often come together in bundles in order to function.

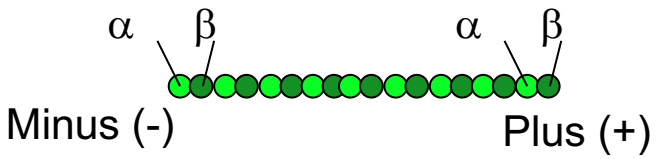


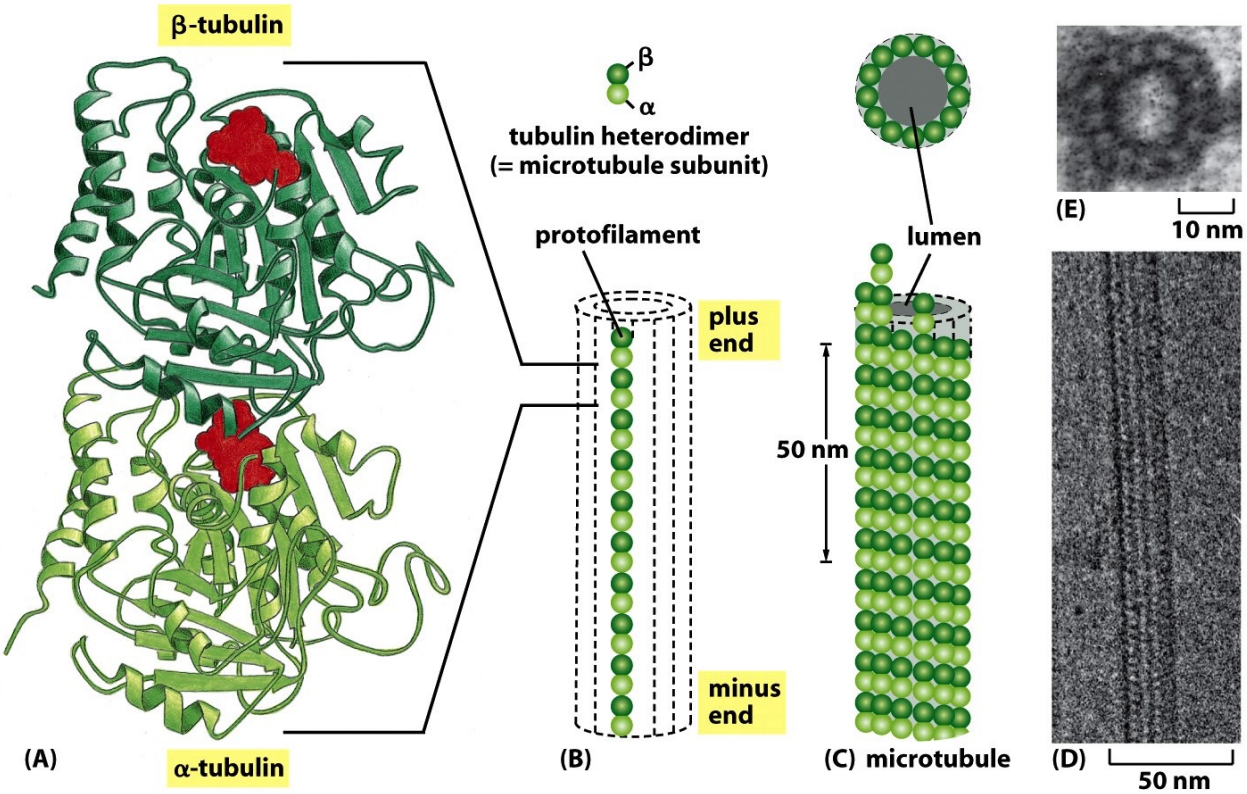
Intermediate filaments (left) are the third kind of cytoskeletal element. They are like ropes and they are in most metazoans. They often are used to hold the nucleus in the center of the cell.

There are some common features of the structure and assembly of microtubules and actin, so we will talk about them together. They are different from intermediate filaments.

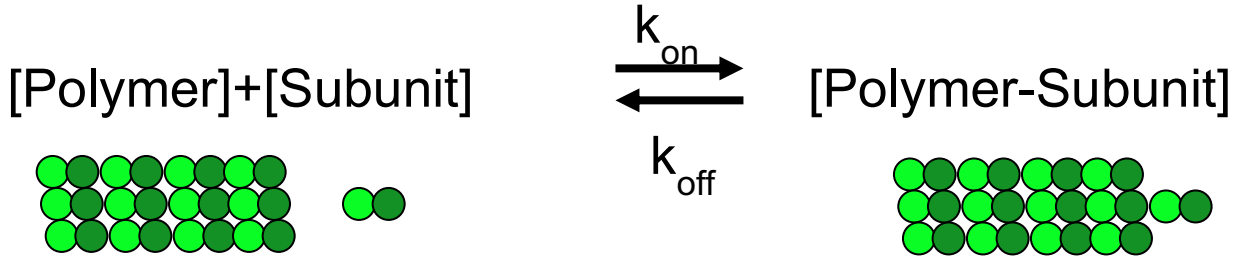
Microtubules are composed of a building block protein called tubulin, which is itself a heterodimer of an α and β subunit. This αβ unit assembles into a linear filament called a protofilament and the protofilaments come together to form a tube (see diagram next page). In fact, you probably have this tube at the outset, and the subunits add in this helical pattern going upwards. If you look down on the top of this tube, there are usually 13 of these protofilaments but there can be other numbers.

A key point is that the β subunit is up at the top and the α subunit is at the bottom. So, the microtubule itself has a polarity. The αβ subunits only go in in one direction. That's important because the two ends have different properties. In a typical cell, all the minus ends, where the α subunits are, cluster together in the cell center and the plus ends, where the β subunits are, are dispersed about the cell edge.





The plus ends are highly dynamic. In the slides, there is a movie of fluorescently labeled tubulin inside a living cell. If you follow one or two microtubules, you'll see that they have a remarkable property of constantly growing and shrinking. At first glance, this seems inefficient and pointless. However, this activity has value to the cell because the cell wants to connect its cell center to something on the periphery. So, you can think of the cell using microtubules for fly fishing to catch that something. The cell is willing to spend all this energy making the microtubules grow and shrink back, in order to find that target.

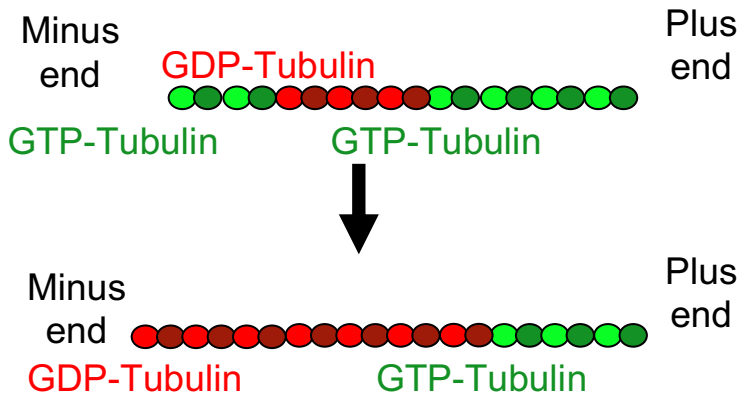


We can think about the mathematics of this. If we think about the polymer and the single subunits, there is a rate constant for the forward process and there is a rate constant for the backward process. If the polymer is left to its own devices with these subunits, it will grow until a critical concentration at which the net rate of polymerization is zero. That is, the rate of addition is the same as the rate of removal.

The critical concentration can be different at these two ends, i.e., the plus end and the minus end. The critical concentration is the concentration of subunits that an end wants to be in equilibrium

with. The plus end is more favored for polymerization. The rate of growth is faster, and it has more of a tendency to grow.

One key thing for microtubules, which gives them this dynamic property, is that each molecule of tubulin has a GTP on it when it is in solution as the subunit form. After the subunit adds to the microtubule, as time goes by, this GTP hydrolyzes to GDP. Because the plus end is growing faster than the minus end, and this GTP is constantly hydrolyzing, you ultimately arrive at a point where the minus end is going to have GDP-tubulin and the plus end is going to have GTP-tubulin. This is also important because GDP-tubulin just doesn't polymerize well at all. So, the minus end, once the GDP gets exposed, has a strong tendency to shrink. So, for certain concentrations of subunits, the minus end will shrink while the plus end will elongate.



This is a process called treadmilling. So, we have one filament that is at steady state and it is in solution with subunits. The subunits are going to add at the plus end, and they are going to leave from the minus end. If you were 100 yards away and you saw this one microtubule, it will look like its moving. From a distance, you can't tell if the whole thing is moving like a train or if it's this treadmilling mechanism. Experiments have been done to figure this out. People marked one position—one subunit. If this was a train, it would move over time. But if there is treadmilling, the subunit would not move. The data indicate that it is treadmilling.

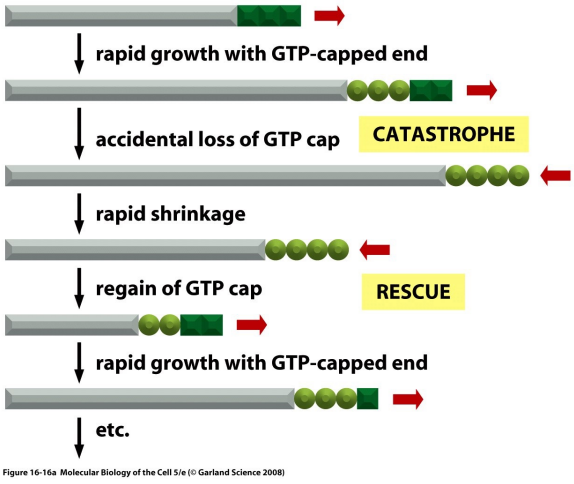
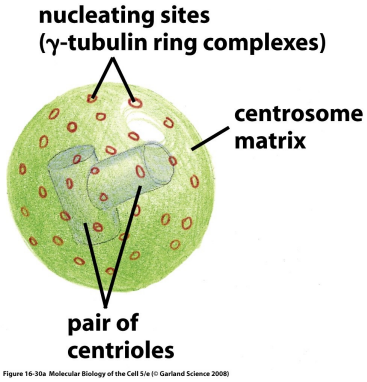
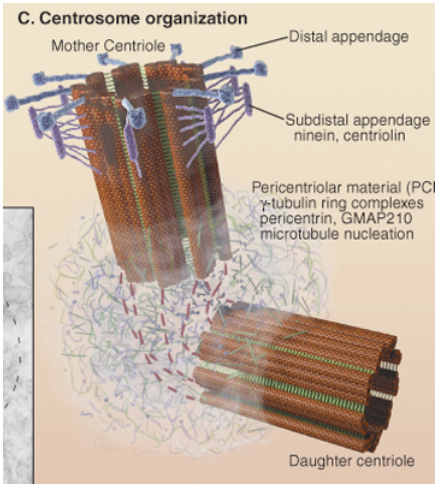


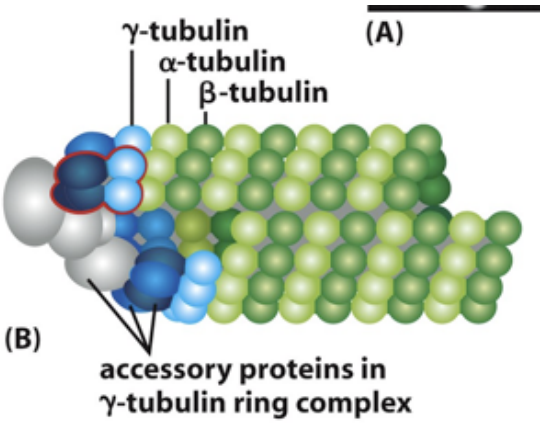
Figure 16-16a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Microtubules exhibit what is called dynamic instability, with repeating cycles of growth and shrinkage. One microtubule grows, then it undergoes a "catastrophe" and it starts to shrink. The shrinking can stop, which is called "rescue" and the microtubule starts to grow again. The growing phase ends with the loss of the GTP cap at the plus end which leads to a pause followed by rapid shrinking, i.e., "catastrophe". Catastrophe occurs about every 70 seconds. During shrinkage, 1000 dimers (0.5 µm of length) are lost per second. The shrinking phase ends with "rescue" which is a pause followed by growth. Rescue occurs about every 20 seconds. The molecular basis for catastrophe and rescue is not well understood, but it probably involves exchange of GTP and GDP for one another.

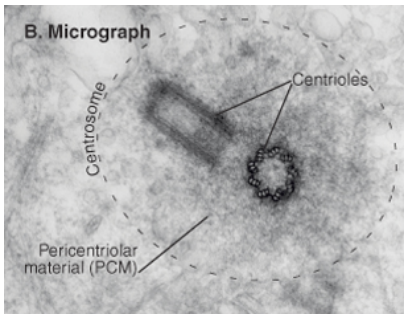


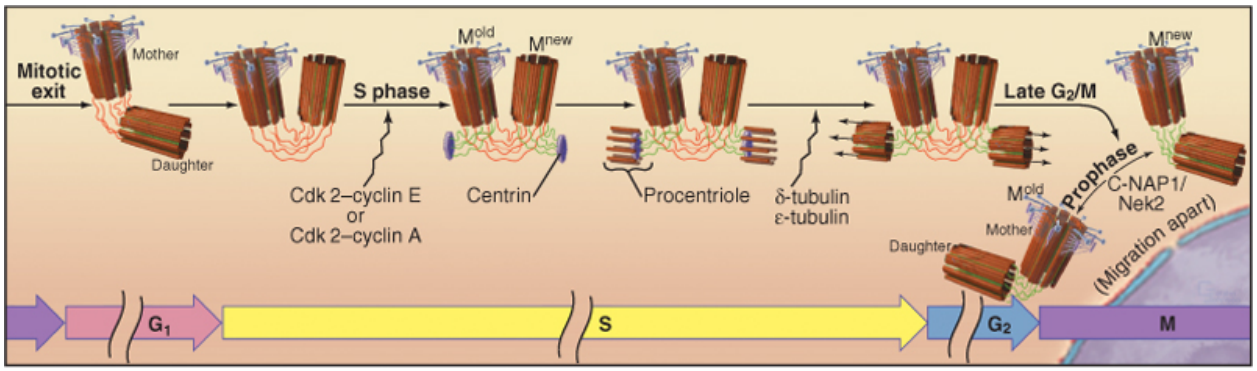
Microtubules are created from their minus end, inside a structure called the microtubule organizing center (MTOC) or centrosome. Within the centrosome, there is a special set of proteins, the γ-tubulin ring complex (γ-TuRC) that nucleates the microtubule. Once a microtubule gets started, it can grow and shrink from its free plus end while the minus end remains stable and anchored.





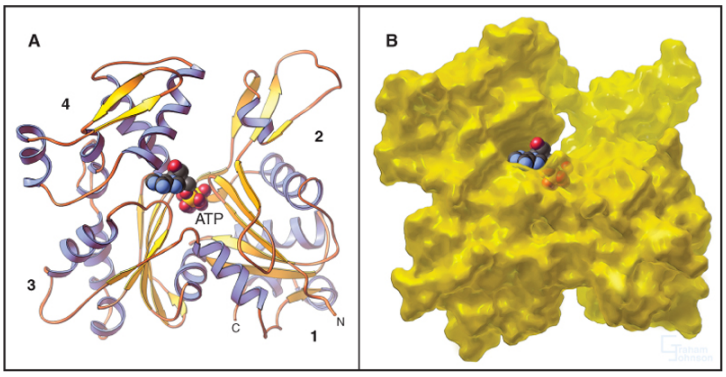
Inside the centrosome are two structures called centrioles, which are shown in the black and white picture at right. When the cell replicates, the cell has to make a copy of each of these. The curious way that it does this is that it takes these two structures and then splits them apart. It makes a daughter copy from the mother, using the mother as a template. This duplication is a case of epigenetics because the information about the structure of the centriole is inherited, from the mother, but the information is not in the DNA of the genome. The proteins themselves that are making the centriole could do this on their own, but it would take a very long time. Because they use the information that comes from the mother, the assembly happens at a much faster rate. Below is a diagram of how the centriole duplicates during the cell cycle.

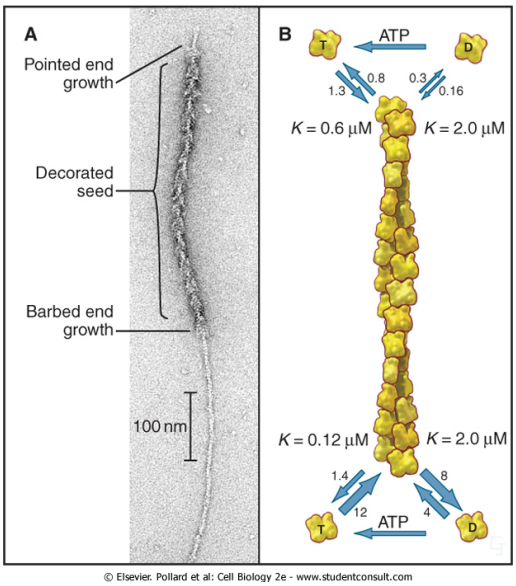




Part of why microtubules are important in medicine is that a number of drugs affect the ability of microtubules to polymerize, and some of these drugs are in clinical use. Taxol is a drug that has been used for breast cancer and a number of other solid tumors. Colchicine has been used for a very long time for gout. Vincristine and vinblastine were some of the first drugs used for leukemia. This is all based on their microtubule inhibition properties, and the effects of the drugs are mostly related to inhibition of mitosis or cell movement.

Moving on to actin, the actin subunit is just one protein; it is not an alpha-beta heterodimer like tubulin subunits. Actin binds ATP, not a GTP, in its center. The ATP is hydrolyzed when the actin subunit is incorporated into the actin filament. An actin filament is composed of two actin protofilaments. ATP hydrolysis occurs in the interior of the filament after the subunits add. So, like microtubules, actin can also potentially exhibit treadmilling and dynamic instability. But it is much less prominent than in microtubules.





In an actin filament, the subunits are all pointed in the same direction. So, the filament has two ends that are different. The actin filament has a "pointed" end that is analogous to the minus end of the microtubule. And there is a "barbed" end analogous to the plus end of the microtubule. The barbed end is the more dynamic end, it is more favored for polymerization, and it usually pointed towards the cell membrane.

Like microtubules, actin polymerization is nucleated in the cell (to avoid chaos). For actin, there is a group of proteins called the Arp2/3 complex, which gets activated and binds individual actin monomers. This creates a new filament. The actin monomers alone could nucleate a new filament, but it would take them too long and they would be forming anywhere – the cell doesn't want that.

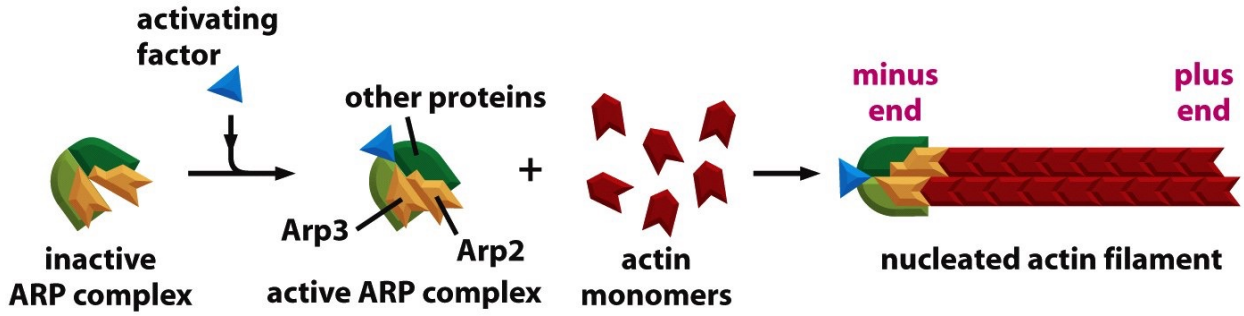


Figure 16-34b Molecular Biology of the Cell 5/e (© Garland Science 2008)

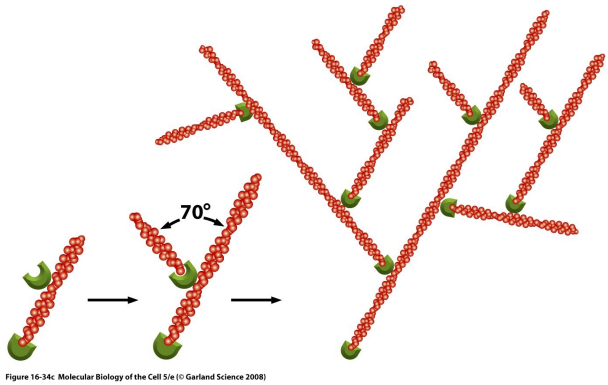
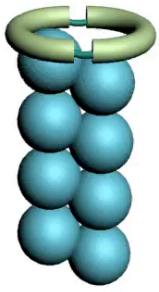


Figure 16-34c Molecular Biology of the Cell 5/e (© Garland Science 2008)

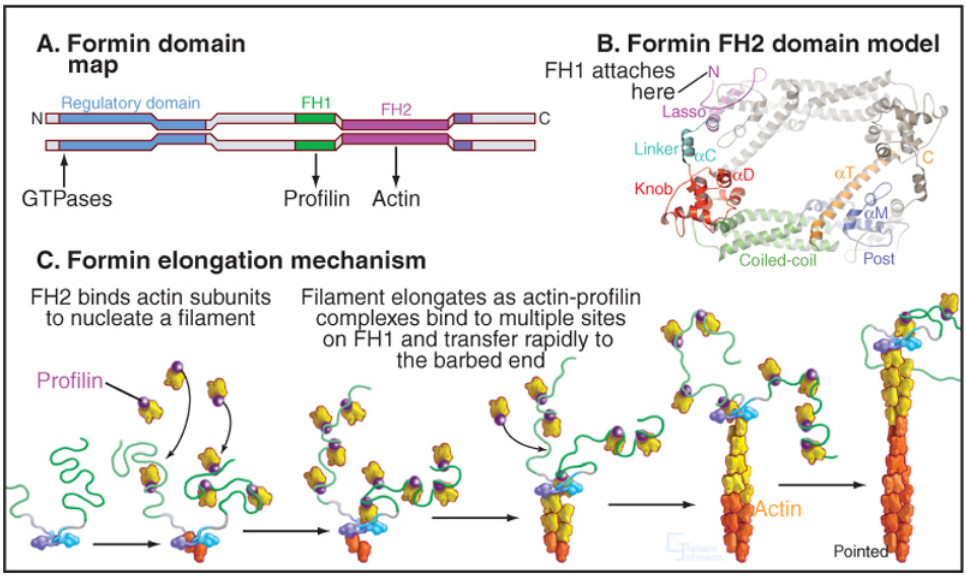
Actin filaments are often nucleated near the membrane, and Arp 2/3 complex is just one of the major nucleators that get actin filaments started. Arp 2/3 complex first binds to the side of an existing actin filament, often called the mother, and as it sits there it creates the daughter filament, which then grows. This nucleation process creates a densely branched network of actin filament. A branched network is important because its physical properties, as a meshwork, give it strength. Cell cytoplasm is not like soup – it is more like jello. If you push on a cell, it pushes back, meaning it has "elastic" properties.

This meshwork of actin filaments is a big part of what determines the physical properties of the cytoplasm. In a lot of electron micrographs, you see the cell cytoplasm composed of a dense meshwork of actin filaments. Things that are small, like a sugar molecule, can still diffuse around rather quickly, percolating through the mesh. But larger things, like mitochondria, ER, or any sort of vesicle, can't diffuse around. Those things need to be moved around. And the cell has specific mechanisms for that that we will discuss later.

Another way that actin filaments are nucleated is by a set of proteins called formins. Formins not only nucleate actin polymerization, but they also support filament elongation. The mechanism through which formins nucleate actin filaments is not well-understood. Much more is known about how they promote actin polymerization.

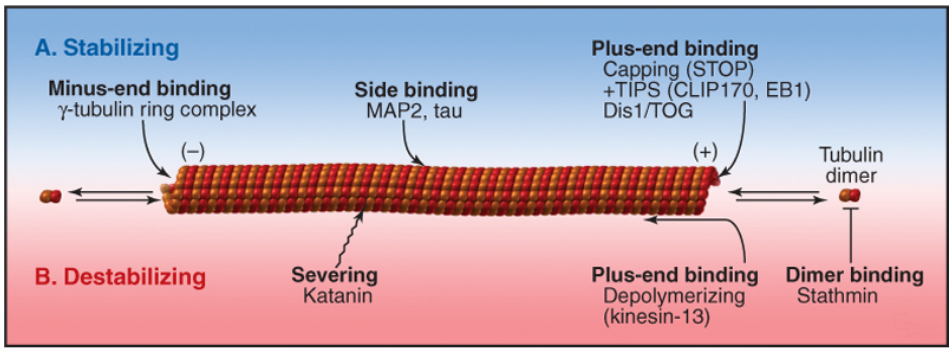


Formins exist at a barbed end of the actin filament and help that barbed end to keep growing. Formins form a donut-shaped ring that is a dimer that has a hole that is big enough so that one actin filament can come in or leave. As the filament grows, the formin stays with it and it keeps promoting the addition of actin subunits. So, the filament grows at a rate that appears to be faster than possible based on the diffusion of actin at the end. So formins are an efficient machine for helping the one end grow. But formins don't make branches, unlike Arp2/3 complex. Formins just make one filament continue to grow. There are many formins and they exist in many places. This allows actin filaments to have specific function at specific places. For example, there is one specific formin on the Golgi apparatus, helping the Golgi form and function properly.

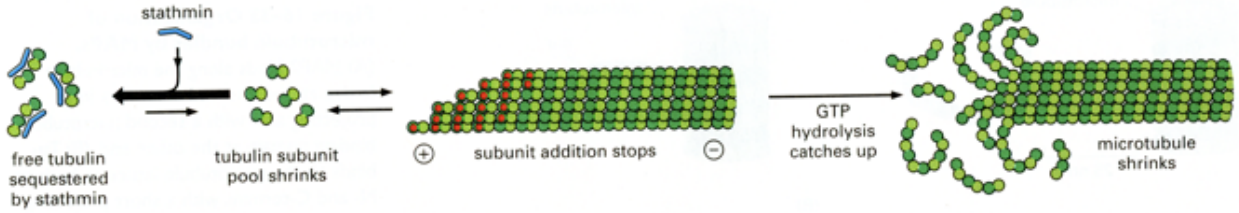


Microtubule and actin-binding proteins

Both microtubules and actin are associated with many different binding proteins that perform many different functions. There are proteins that bind to the monomers and create a buffered pool of subunits that the cell can use any time it wants. There are proteins that bind to the ends—they cap the ends and prevent that end from growing and shrinking. This helps keep the filaments short when that is necessary. There are other proteins that come along the side and break the filament. There are a bunch of proteins that tie the filaments together into spot welds or bundles. Depending on what the cell wants to do. All these sorts of proteins exist for microtubules as well as for actin.



An important monomer-binding protein for microtubules is stathmin. This protein binds monomers and helps make a pool of monomers available if the cell wants to rapidly grow or shrink the microtubules.



Stathmin is similar to thymosin (described below) for actin. Stathmin binds two tubulin dimers and slows down the rate of polymerization. This leads to an increased frequency of catastrophe because GTP hydrolysis catches up faster with the end.

For microtubules, there are a number of proteins that affect the ends. Plus-end binding proteins can either stabilize or destabilize those ends. They can lead to increased or decreased rates of catastrophe and rescue, which is critical for dynamic instability and how microtubules function in cells.

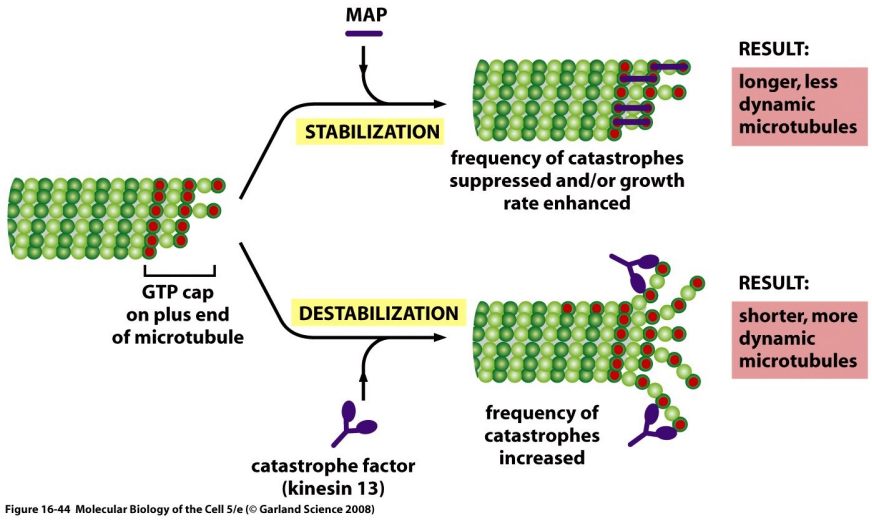
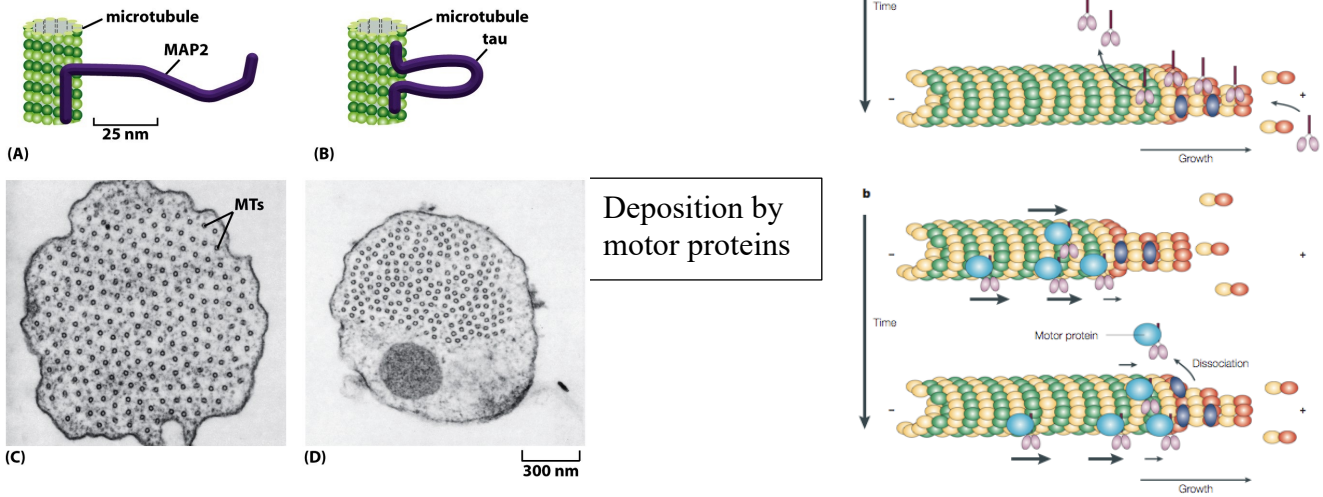


Figure 16-44 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Some plus-end binding proteins ride along with the plus end while it grows and shrinks. Examples of this are CLIP170 and EB1. These proteins provide a mark and allows the plus end to have specific interactions that other microtubules do not.

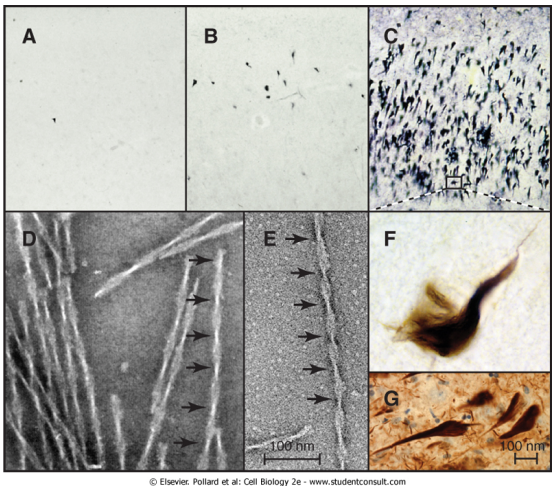
Deposition by treadmilling



Deposition by motor proteins

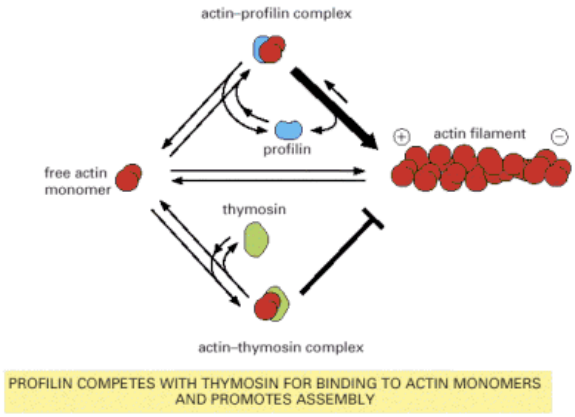
Microtubules have a number of side-binding proteins. Shown above is a cross section of an axon (left) and a dendrite (right). Axons are very long, and the cell transports a lot of things through the axon in order for the synapse to work. Many things are made in the cell body and transported down the line. Microtubules can be viewed as train tracks on which the cargo moves. These microtubules are held at defined distances apart from each other by side binding proteins, to prevent clogging. There are two different types of these. One, called MAP2, is in axons. Another, called tau, is in dendrites.

Tau is the first protein for which a mutation was associated with Alzheimer's disease. Phosphorylation of tau dissociates tau from microtubules. Free tau forms intracellular paired helical filaments. These filaments aggregate into neurofibrillary "tangles". The number of tangles correlates with disease severity. So, this is one of the proteins that can be mutated or form the abnormal aggregates when a person has Alzheimer's disease.



At right is a histology slide showing tangles. The tau, when it forms these aggregates forms these brown-black things. Tau has the ability on its own to just make filaments, to aggregate for reasons that we don't fully understand.

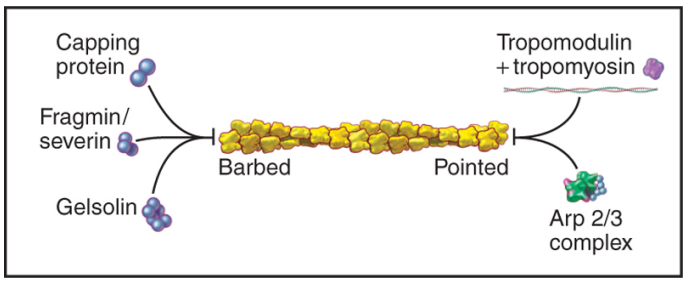
In cells, half of the actin is not polymerized (i.e. not in filaments). Based on the properties of pure actin, essentially all of the actin should be polymerized. The cell keeps half of its actin as monomers through the use of monomer-binding proteins. These un-polymerized subunits serve as a pool to grow new filaments when and where they are needed.



PROFILIN COMPETES WITH THYMOSIN FOR BINDING TO ACTIN MONOMERS AND PROMOTES ASSEMBLY

Thymosin and profilin are two small proteins that bind to actin monomers in a 1:1 stoichiometry. When thymosin is bound to an actin monomer, that monomer cannot polymerize. When profilin binds to an actin monomer, that actin subunit can add to the barbed end of a filament but not to the pointed end. Profilin competes with thymosin for binding to actin monomers, and profilin can promote assembly of actin filaments.

End-binding proteins for actin include gelsolin, CapZ and tropomodulin. Gelsolins (of which fragmin and severin are relatives) bind to the side and barbed end of actin filaments. Gelsolins block the association or dissociation of actin subunits at the barbed end. As discussed below, gelsolin can also sever actin filaments.

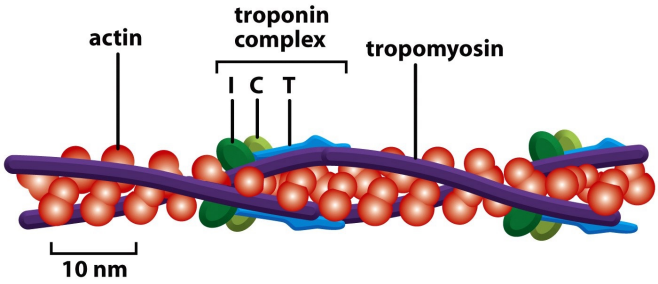


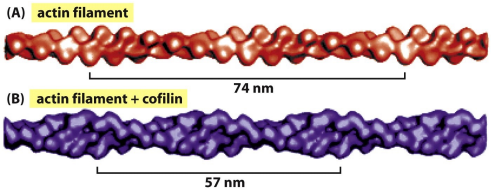
CapZ is a heterodimeric capping protein that binds to the barbed end of actin filaments in many cells. In the sarcomere, all the actin filaments are exactly the same length. And that's because the cell creates a molecular ruler, with caps at each end, to specify the length of the actin filament. The efficiency with which the sarcomere works depends on having filaments of uniform length. The barbed ends of the actin filaments

in sarcomeres of striated muscle are all bound to the Z disc. Those ends are bound by the capping protein, CapZ.

Tropomodulin caps the pointed end of stable actin filaments in cooperation with tropomyosin, a side-binding protein.

There are many side-binding proteins for actin filaments and a lot of them are seen in muscle. The protein tropomyosin binds along the side the actin filament and increases the tensile strength of actin, making stronger filaments. Another protein, called troponin, imparts the calcium sensitivity that you see in muscle.

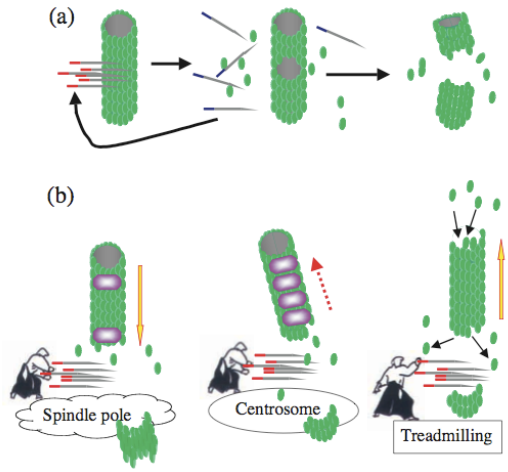


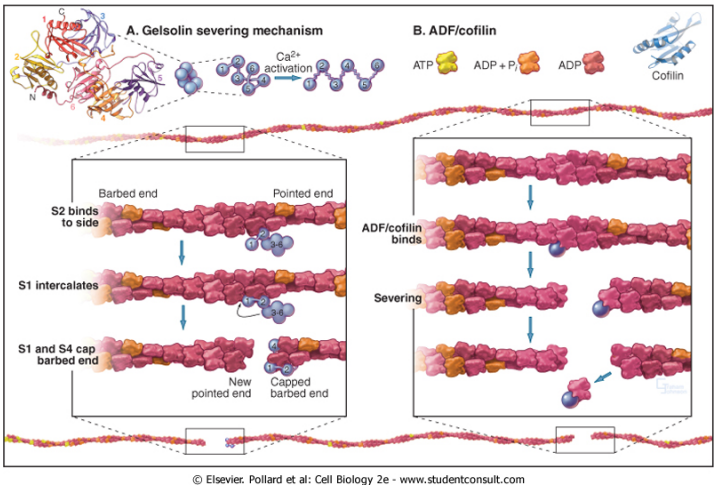


Cofilin is another side-binding protein for actin filaments. Cofilin increases the twisting of actin protofilaments thereby promoting severing.

Severing Proteins

One of the amazing things about microtubules and actin filaments is that it is possible to come up along the side of the filament and break it. You can think about the actin or microtubule microfilament as something that is constantly flexing. So, all the junction between the subunits are constantly under stress. So, if a protein inserts itself between the subunits, that is sufficient to act as a wedge and break these filaments.



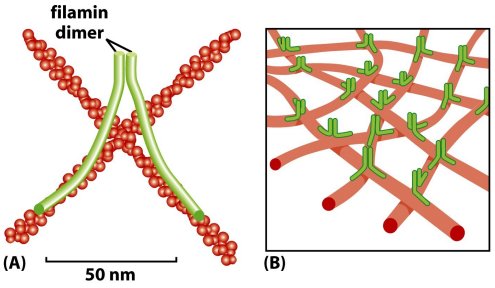


The actin severing proteins are gelsolin and cofilin. We have already seen gelsolin as a barbed-end capping protein. But gelsolin can also sever actin filaments, and it holds on to the barbed end after it breaks the filament. By contrast, cofilin severs the actin filament and then leaves, leaving the end free.

Microtubules also have a severing protein that has a great name from Japan – "katanin", which is a type of sword. This sword-like protein cuts microtubules. For the most part, severing is used in cases where the cell makes a quick decision that it is going to change all its microtubules from one state

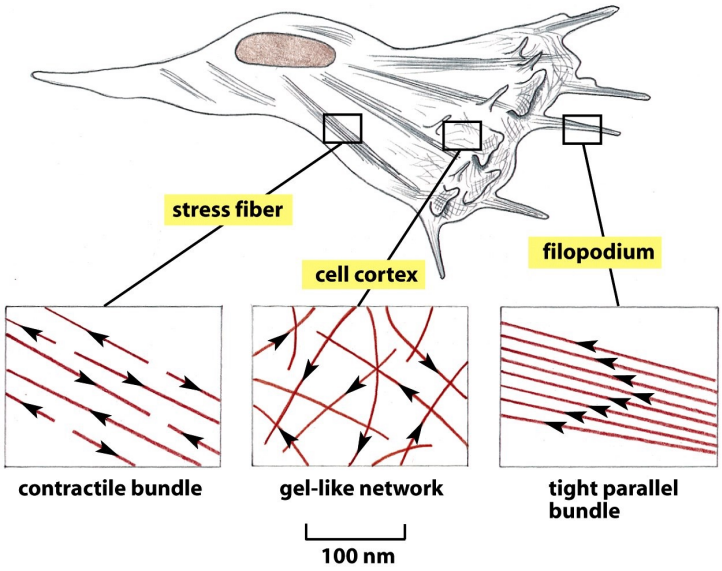
to another state and it needs to rapidly cut them into pieces and have the subunits diffuse to someplace else and make a different structure.

Higher-order Ultrastructural Organization of microtubules and actin filaments



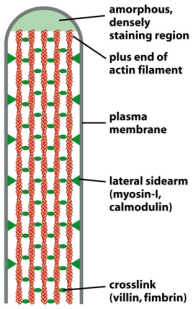
Both microtubules and actin come together to make large structures which do important work for us. Lamellipodia, for example, are found at the leading edge of migrating cells. At the very front, Arp2/3 complex creates a branching network and then proteins such as filamin create spot welds between overlapping filaments. Presumably, the point is to make the cytoplasm be strong and dense, providing a solid footing for protrusions at the front of the cell.

In a typical cell, if you look at the actin, it can either form bundles where all the filaments are pointing in the same direction or bundles with filaments of mixed direction. Often the barbed ends are oriented towards the cell plasma membrane, and this allows the cell to explore its space, by sending out a process as these barbed ends grow and push out the membrane.



Bundles with filaments of mixed (opposing) polarity are found in cells, and they are capable of mediating contraction, like a miniature sarcomere. Bundles with filaments of the same polarity are often tightly packed and serve structural roles. Among these actin-filament bundling proteins, there are several ones, and they impart distinct functional properties to the actin filament network. The names are not important.

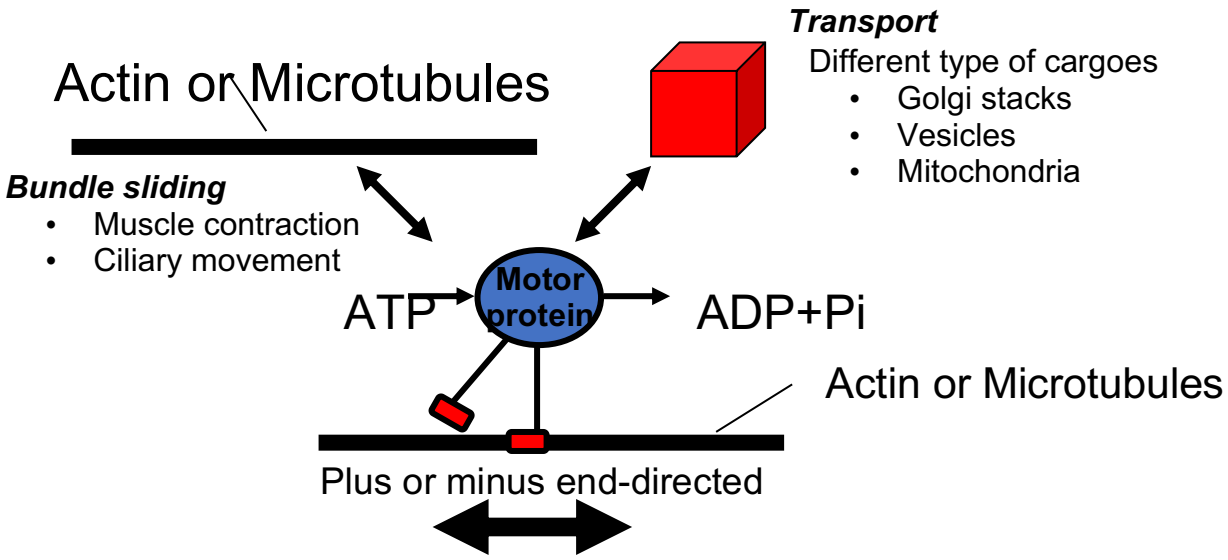
An important case of a tight parallel bundle with the same polarity is inside a microvillus, like you would see in the brush border of intestinal or kidney epithelial cells. The microvillus is important for absorption. The intestine needs a whole lot of surface area to absorb the stuff you just ate, and the kidney needs to resorb much of the water and ions in the glomerular filtrate. The cell makes this bundle of actin filaments and connects the cell membrane to the side of the bundle. This bundle is relatively stable but still somewhat dynamic.



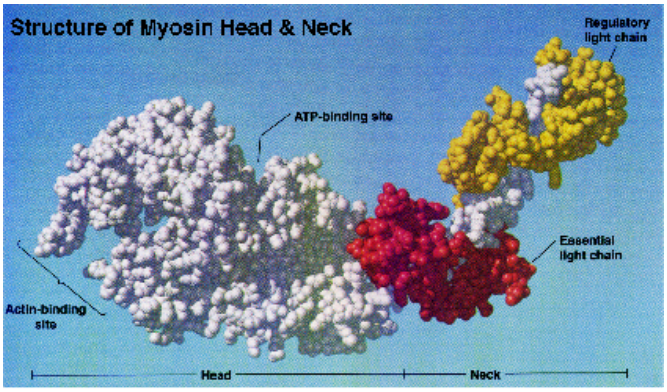
Motor Proteins

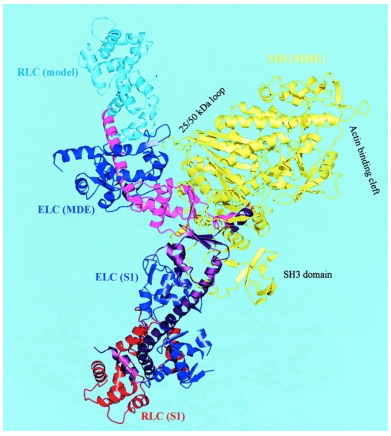
I want to introduce another class of proteins, called "motor proteins". For actin, motor proteins are called "myosins". For microtubules, they are "kinesins" and "dynein". Motor proteins convert ATP into mechanical work. In large part, they transport things along the actin filament or microtubule as though they were a train on a track. The filament is the train track, and the motor protein is the train.

The motor protein will walk along this track and carry some type of cargo with it. The other thing motor proteins can do is cause actin filaments or microtubulesto slide with respect to each other.



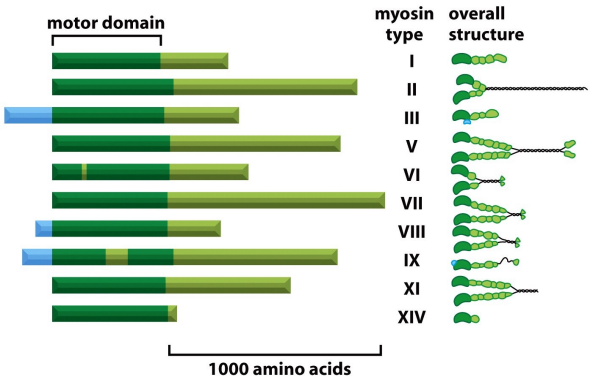
Here's an example of the atomic structure of one of these motor proteins, myosin. This motor has to bind to the actin filament, and it has to have a site where it binds ATP. The back part of the motor is attached to whatever cargo we're going to haul. With each step of this motor a molecule of ATP is hydrolyzed, and that energy induces a conformational change.





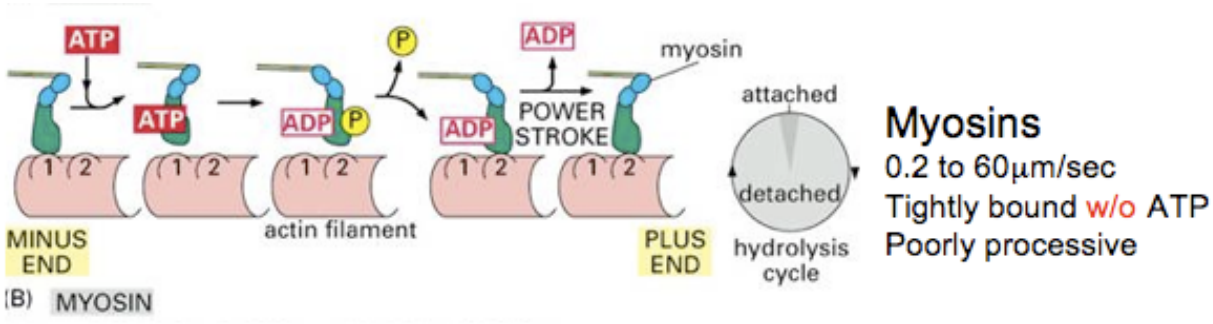
At left is an illustration of that conformational change. This figure is actually two structures, one of which has the ATP hydrolyzed and one with the ATP not hydrolyzed. The artist has overlain the motor head regions (yellow), and then they have shown you what the neck region looks like in the two structures. The neck swings significantly (compare the non-yellow regions) in a huge conformational change as a result of the ATP hydrolysis. So, this is how the transport works.

Mammalian cells have many different myosins, expressed from different genes. What all myosins have in common is a motor domain where they bind actin and hydrolyze ATP. In contrast, their tails, through which they bind a specific cargo, are completely different. That point is illustrated to the right. There are many different tails because there are many different cargoes. The tails are responsible for binding the cargoes – things which might need to move through the cytoplasm.

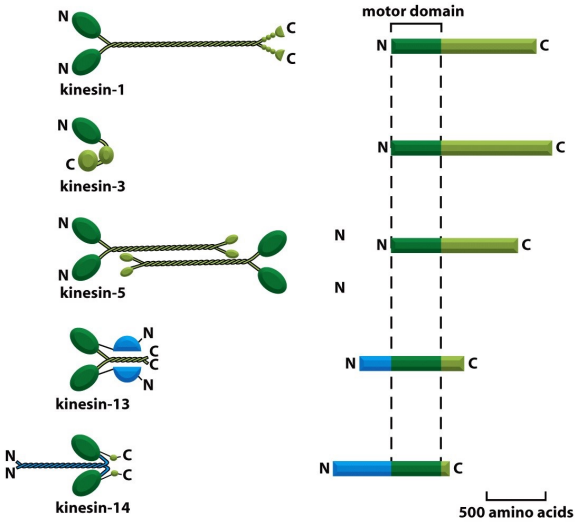


The lecture slides include a movie of one type of myosin, called myosin II. This is the myosin you know from skeletal and cardiac muscle. The movie illustrates what happens when ATP gets hydrolyzed and how that translates into motion. As the ATP is hydrolyzed, the big conformational change in the motor domain causes the movement of the actin "thin" filament with respect to the larger "thick" myosin filament. In the "thick" myosin filament, the myosin tails all bind to each other, and they make their own large filament, which has a distinctive bipolar structure necessary for sarcomere contraction. The left half of the thick filament points to the left, and the right half points to the right.

The way this machine works is based on cycle of ATP binding, hydrolysis and release. The motor is stepping along the actin filament (toward the barbed end), and the myosin motors are trying to push this actin filament in the opposite direction (towards its pointed end). Below is a biochemical diagram that illustrates the state of the ATP, as it goes through a cycle of ATP hydrolysis and release. The "power stroke" of the motor is labeled, coinciding with the release of ADP. If you are trying as hard as you can to lift something and you can't do it, the binding and hydrolysis of ATP is still going on, but there is no sliding. So even if the filament doesn't slide, the force is still being applied and ATP is being hydrolyzed.



Microtubules have analogous motor proteins. One type of microtubule motors are the kinesins. Much like myosin, there are many types of kinesin proteins, expressed from different genes. They have a common motor domain, which binds to the microtubules and hydrolyzes ATP. They have many different tails, and these tails can associate with each other or with organelles. The action of the kinesin motor causes the movement of this cargo with respect to a microtubule. Kinesins move (almost always) towards the plus end of the microtubule.

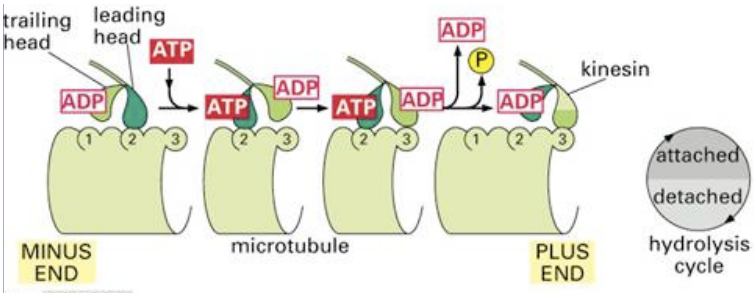


What we have come to realize is that there are some types of kinesins that are distinctive in their motor domain and maybe their tail isn't that important. Their motor domain binds to a microtubule and changes the dynamic assembly properties of the microtubule. This is one of the ways cells induce the catastrophe process leading to the shrinking of microtubules.

In the lecture slides, there is a movie similar to the myosin movie but with kinesin instead of myosin. The movie illustrates a key difference between kinesin and myosin. A single molecule of kinesin is a dimer. It is capable of walking by itself and not letting go of the microtubule. To accomplish this, the back head only detaches when it knows that the front head is firmly attached. This is called "processive" movement. If you watch one motor molecule over time, at no point does it completely let go. So, one kinesin molecule is able to move something through the cytoplasm (if it's strong enough).

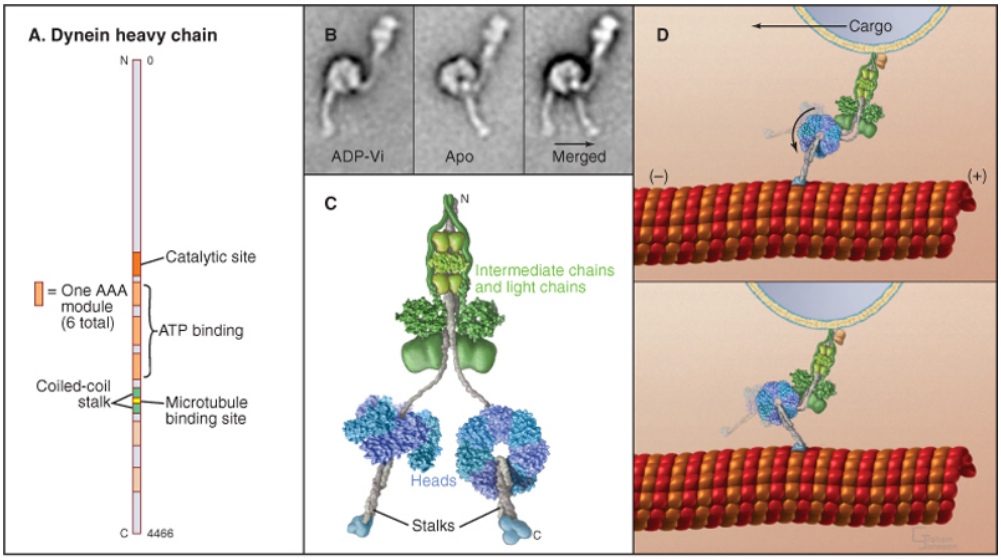
This processive movement is in contrast to the muscle myosin-II movie, where one myosin head comes off, and the other head is just waving around and not really doing anything. In this case, the two heads aren't communicating with each other, and they aren't working together. Within a sarcomere, there are probably a thousand of these motors working independently of each other. But not in a concerted way. This arrangement creates a lot of force, which is good for a muscle. But if you asked just one myosin to carry a load or to play tug of war, it would fail because these guys let go and nobody else is holding on. With kinesin, in contrast, the combined action of the two heads means that one dimer molecule never lets go of the microtubule. Once attached, a kinesin can run for a really long distance.

Here is the ATPase cycle diagram for kinesin, similar to the one for myosin, in which one head binds and hydrolyzes ATP, then one head lets go. In kinesin the two heads are coordinated and working together. How does one head know what the other head is doing? They must communicate, but how is a mystery.



For the cell, the key point is that kinesin is processive. One kinesin is able to move an object, like an organelle, a long distance. Kinesin takes a step, then holds still and then takes another step. This is important if you want to use only a few molecules to transport something a long distance – if you don't need a lot of force but you really want to never let go.

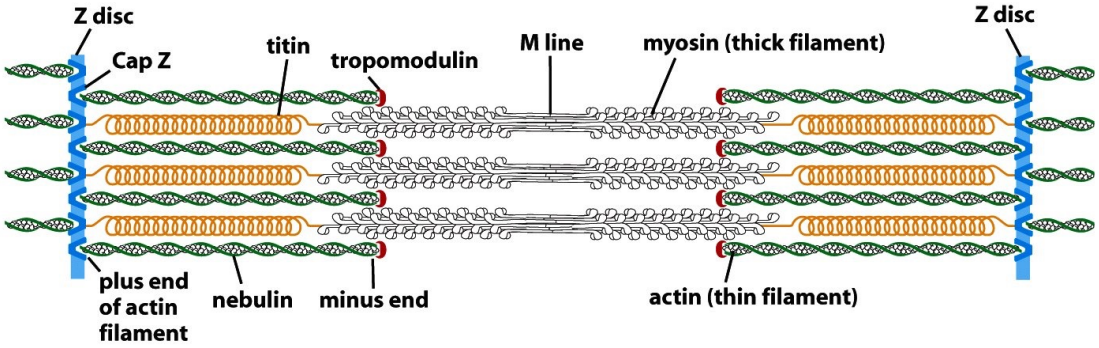
Dyneins are a second type of microtubule motor protein. Dyneins move towards the minus end of the microtubule. Dyneins are big proteins – ten times the size of kinesin. They have a lot of extra chains and associated proteins. Dyneins are one big motor, and they have many associated chains of different sorts, which then tie the motor to different cargoes and give it different properties.



Cytoplasmic dyneins are used for vesicle transport and positioning. They are also the motor component of cilia and flagella (see below).

Cellular Machines

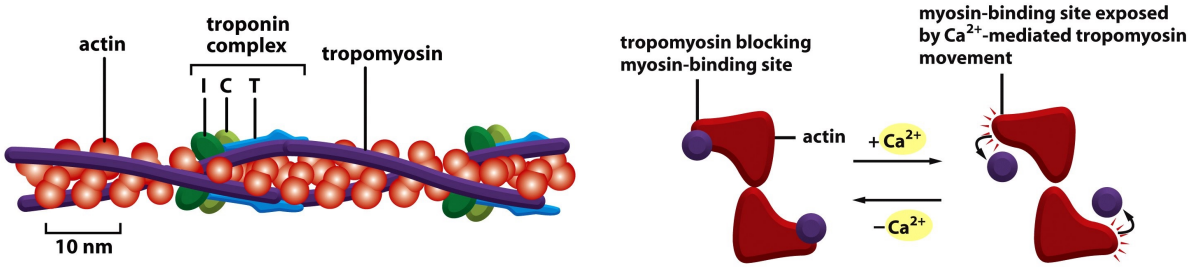
A. Skeletal and cardiac muscle – Actin works with Myosin II



Above is a picture of a sarcomere. It shows the thin actin filaments and the thick myosin filaments. The polarity of all the thin filaments is such that they all have their barbed ends at the Z-line (or disc). So, the sarcomere doesn't have a single polarity; the two sides are mirror images of each other. The myosin filaments form the thicker filament, and the heads all point out, away from the center. The myosin heads are always trying to walk towards the barbed end of the actin filament. Because the actin filaments on each side of the sarcomere have opposite polarity, the Z discs are going to be pulled together and that's how you get contraction.

Your heart and skeletal muscles need to turn on and then off, quickly. The sarcomeres of these striated muscles are turned on by calcium ions (Ca^{2+}) in the cytoplasm. The calcium is sensed by the troponins, which interact with tropomyosin. At rest, tropomyosin covers the binding site for the myosin on the actin filament, so the sarcomere is off. When you want to turn on contraction, the calcium signal comes through the troponin, and the tropomyosin rolls out of the way, allowing myosin to bind and the

muscle to contract. But then, when calcium concentration falls, the tropomyosin rolls right back to turn the signal off.

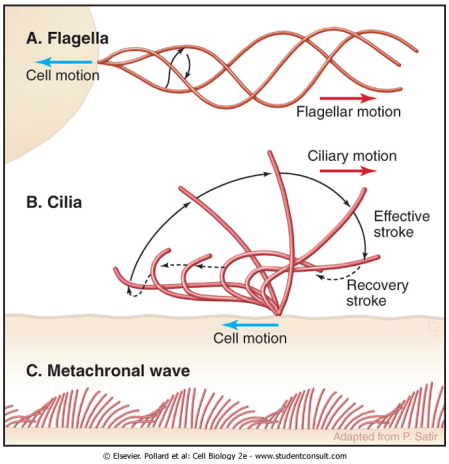


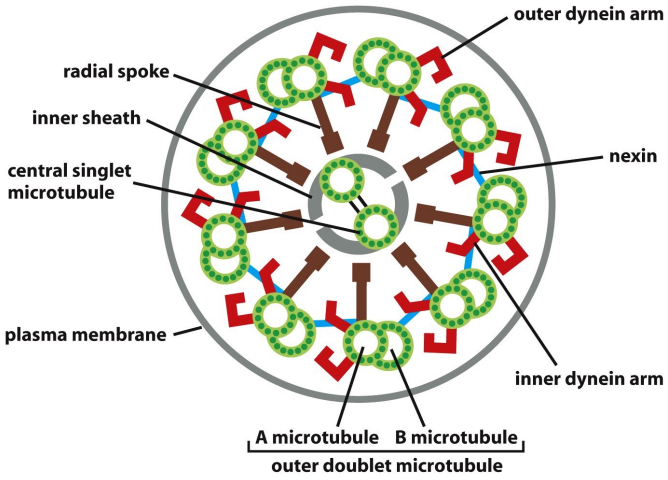
An interesting clinical correlate of sarcomere function is the various forms of familial hypertrophic cardiomyopathy. You've probably heard stories of young people who die suddenly, either in the middle of the night in their bed or when they are playing a game. A subset of those people have an inherited autosomal dominant disorder that affects proteins of the sarcomere. Mutations of the gene for the myosin heavy chain or one of many of the other sarcomere components, such as troponin or tropomyosin, can cause the disorder.

How can you live for 30 years and be apparently normal and then die? The reason is you have half normal and half mutated protein. If you had a mutation that completely removed both copies of the protein, that wouldn't be compatible with any life at all. More important, the mutations of familial hypertrophic cardiomyopathy have relatively subtle effects on the function of the protein in the ATPase and power cycle. The sarcomere still works but not quite as well as normal. In response to this subtle loss of function, the heart compensates by remodelling the architecture of its tissue, to allow it to continue to work well. The cells often rearrange their shape and position to make better connections. But the signal that causes the heart to contract is an electrical signal, and this signal moves through a defined conduction pathway and spreads out over the whole heart. Those pathways are very important, because they are critical to how the heart contracts in a synchronized way. At a certain point when the heart remodels itself, those conduction pathways can get disturbed or broken. The signal that starts in the sinus node might not make it to all of the heart muscle tissue in a way that causes an effective beat contraction. If this happens, the rest of the heart will spontaneously contract in a completely disorganized way. This desperate attempt is completely ineffective, and you get no pumping.

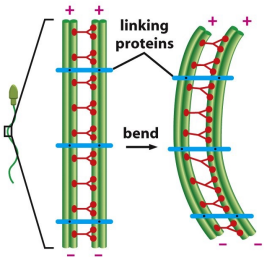
B. Cilia and flagella – a microtubule-based machine

Cilia and flagella are built using the same machine. Flagella are what moves sperm forward. One sperm has one flagellum, and it has a helical wave motion that propels the sperm forward. In contrast, there are usually many cilia on one cell – including epithelial cells lining the fallopian tube or the bronchial tree. Cilia beat with a swimmer's breast-stroke motion. All of the many cilia on one cell beat together, and this moves fluids over the cell, like the layer of mucus in your lungs, or it moves objects, like the egg cell. So, cilia are important for keeping your lungs clean and for moving eggs down the fallopian tube.



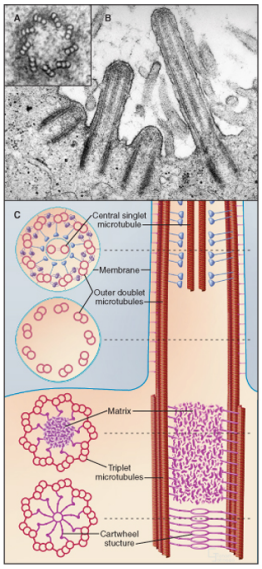


The machine on which both cilia and flagella are built is called the "axoneme." There are probably 800 different kinds of protein in an axoneme. If you cut an axoneme of these in cross section, it looks like this. You see individual microtubules, but they are special because they are all "doublet" microtubules. One microtubule looks round and normal – the "A" microtubule. The "B" microtubule looks to be cut open and then jammed onto the side of the A microtubule. There are always nine of these doublets around the outside, and there are two single microtubules in the middle. There are a bunch of connectors in here. The most important one is dynein which attaches to the A microtubule, reaches out and moves along the nearby B microtubule.



(B) IN NORMAL FLAGELLUM: DYNEIN CAUSES MICROTUBULE BENDING

Dyneins bind to the A microtubule and attempt to move. There is not a lot of movement but there is enough that it causes the microtubule to bend. Note that if we are going to create bending, we can only have dynein on one side of a microtubule. That causes the microtubules to bend in a certain way as it goes through its cycle. In order to get the characteristic motion, this cycle has to be regulated –going back and forth between sides. Also, that process has to propagate from the base to the very tip of the flagella or cilia to get the proper motion.



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At right is a diagram that shows how these dyneins attach at the A tubule and reach out to the B tubule. One of the important things is that the doublets have to be anchored at the base of this structure, and then they go up from there. The anchoring structure at the base is called the basal body. A basal body looks similar to a centriole of an MTOC or centrosome (discussed next). In order to create bending of the axoneme, which leads to the helical or swimming motion, we need for the dyneins to try to slide one microtubule with respect to another microtubule. But since the microtubules are linked together at their base, this attempt at sliding causes bending.

There are a whole host of these diseases caused by mutations of any one of these components of the axoneme. Disorders of the cilia affect your respiratory epithelium. It makes you prone to infections because you can't clean your lungs. Individuals with disorders of their cilia have too many or too severe infections. If the disorders affect your fallopian tubes or sperm it gives rise to infertility.

C. The mitotic spindle – a microtubule-based machine

In mitosis, there is an array of microtubules called the mitotic spindle. In the mitotic spindle, the microtubules are all anchored at their minus ends to two poles, each of which is called a centrosome. These centrosomes each have two centrioles. The microtubules of the mitotic spindle are anchored to the centrosome at their minus ends. In contrast, the plus ends are constantly growing and shrinking, performing "fly-fishing" in search of a target. Microtubules coming out the back of the mitotic spindle

are searching for something on the membrane to anchor themselves, which anchors the spindle. The microtubules in the middle are searching for an attachment to a chromosome. The job of the mitotic spindle is to attach to all the chromosomes and pull half of them to each of the daughter cells. A microtubule from the left side has to attach to the left side of a pair of sister chromatids. A microtubule from the right side has to attach to the right side of the sister chromatids. So, when the chromatids separate and the mitotic spindle splits, one chromatid is pulled to each side. These attachments and motions happen via the action of kinesins and dyneins. The mitotic spindle also contains special kinesins that help organize and assemble the mitotic spindle. These kinesins also help to pushing the poles of the mitotic spindle (the centrosomes) apart, and this is how the one set of chromosomes gets moved to each of the two daughter cells.

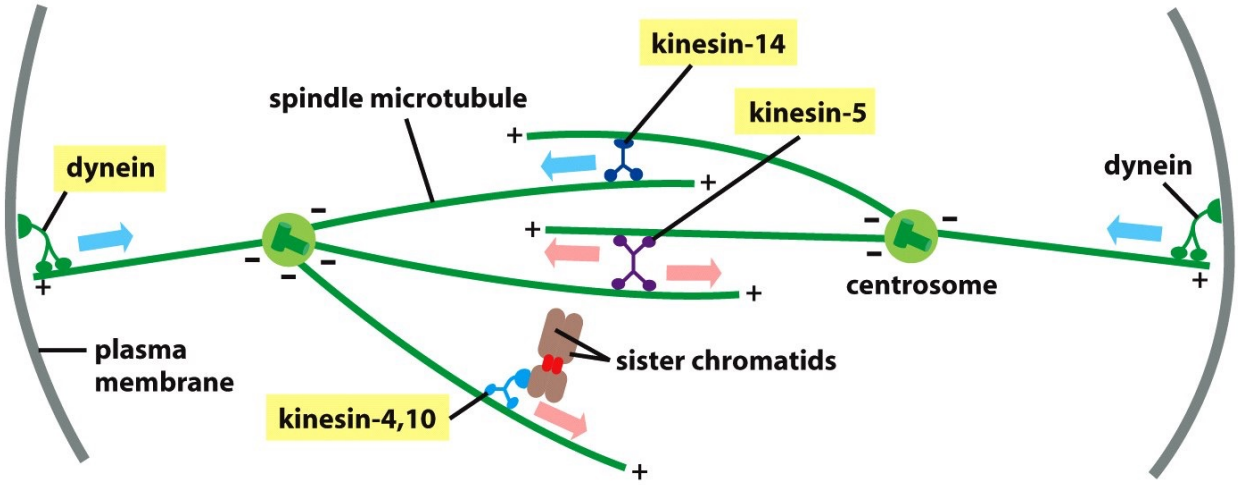


Figure 17-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

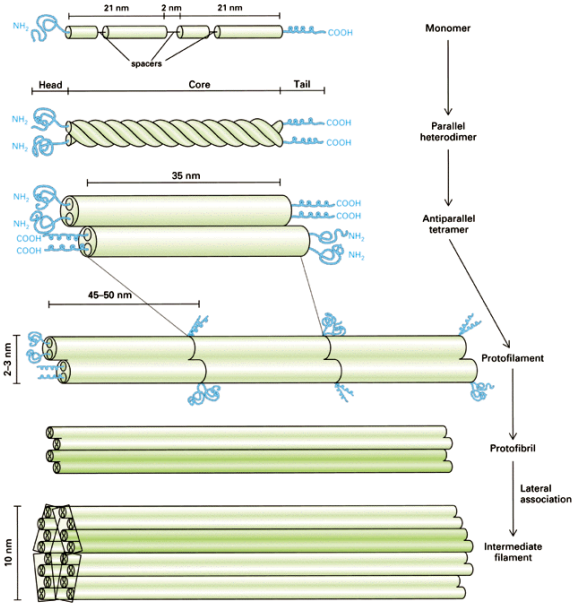
In cancer cells, the machinery of the mitotic spindle often doesn't work properly. Two chromatids may go to one cell, and the other cell will get no chromatid from that pair. That will either kill the daughter cell or alter the properties of that daughter cell. Having aneuploidy—abnormal numbers of chromosomes—is a hallmark of cancer cells.

Intermediate filaments

Intermediate filaments are the third major type of filament in the cell. They are called "intermediate" filaments because they are intermediate in width between the thick and thin filaments of the sarcomere. They are only present in metazoa and animals. There are 70 genes for intermediate filaments in humans, and they are expressed in different cells at different times.

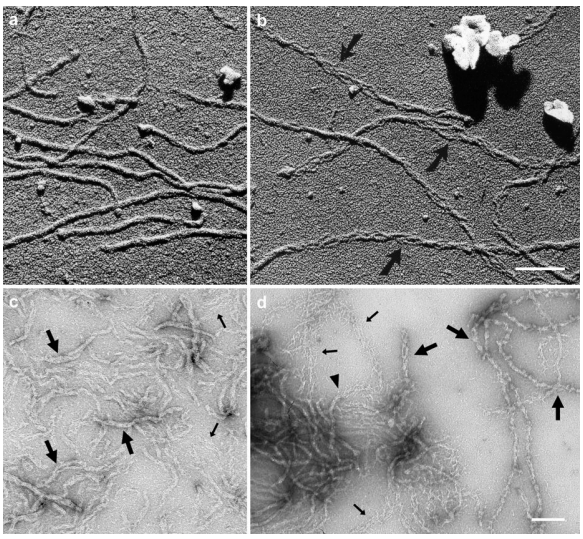
Intermediate filaments have biochemical properties very different from those of actin filaments and microtubules. For starters, intermediate filaments are very stable. The filaments exchange their subunits at a rate that is 10 or 100X slower than actin. They are also very strong, very hard to break. Microtubules are strong but brittle. Actin filaments are weak. By contrast, intermediate filaments essentially cannot be broken. Intermediate filaments make up the structures on the surface of your body like hair, finger nails, bird feathers and turtle shells, all of which the animal does not want to break. A typical living cell also uses intermediate filaments in their cytoplam as a way to give mechanical strength to the cells. The intermediate filaments often encircle the nucleus and hold it in the center of the cell.

With any layer of epithelia cells, such the epidermis layer of your skin, you don't want it to come off, even if you rub it. You want epithelial cells to be holding tight to each other. But it is not enough to have weak cells tightly stuck together. You also want the individual cells to be strong. That's what intermediate filaments do.



Intermediate filaments are very different in their assembly from actin and microtubules. They are composed of monomeric subunits. The first thing one monomer subunit does is associate with another subunit. The monomer has polarity, and this dimer is polar because the N-termini of the two subunits are on the same end when the monomers form the dimer. But in the next step, two dimers come together in an anti-parallel orientation. So now there are both N and C termini at each end. Once you make this anti-parallel tetramer, polarity is lost, and anything that is made afterwards from this building block cannot be polar. So, when these antiparallel tetramers assemble into filaments, those filaments don't have polarity. Interestingly, because intermediate filaments are not polar, this explains why there are no motors that move along intermediate filaments, because a motor protein requires a polar filament to create motion.

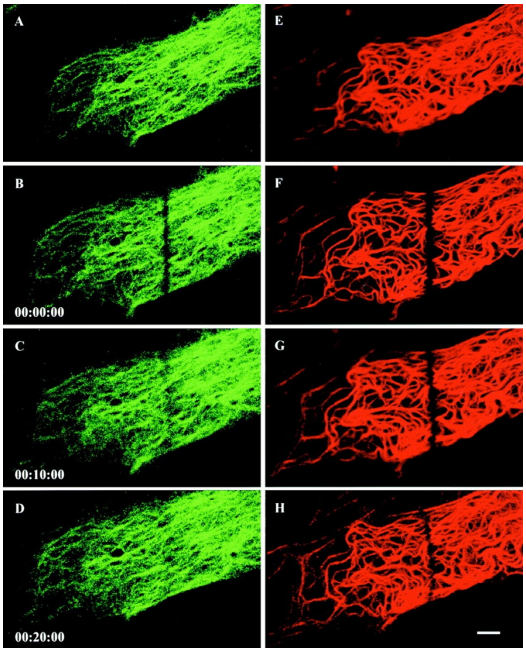
As intermediate filaments assemble, they associate laterally and make structures like those shown below.



There are five classes of intermediate filament proteins. None of the classes cross-polymerize with other classes. Some classes are homopolymers of one type of protein; other classes are heteropolymers with two or more types of protein subunits. Some of these proteins are important enough to become familiar with their names. For example, keratins are the intermediate filaments of epithelia. Here, the intermediate filaments are heteropolymers, and they contain one acidic and one basic keratin protein subunit. Keratins are found in your skin, nails, hair, and also intestine. Intermediate filaments of mesenchymal cells, including muscle cells, are made from proteins called vimentin and desmin. These form homopolymers, associating only with themselves. Neurons have their own type of intermediate filaments, called neurofilaments, composed of three different subunits. Inside every nucleus there is a set of intermediate filaments called lamins, which form both homo- and hetero-polymers. They line the interior of the nucleus to make it strong.

Class	Name	Cells	Number of Isoforms	Size (kD)	Polymers
I	Acidic Keratin	Epithelia	~15	40-60	Obligate Heteropolymers
II	Basic Keratin	Epithelia	~15	50-70	One acidic + one basic
III	Vimentin	Mesenchymal	1	53	
III	Desmin	Muscle	1	52	Homopolymers (single
III	Glial Fibrillary Acidic Protein (GFAP)	Glia	1	51	type of subunit) or co-polymers w/ each
III	Peripherin	Neurons	>1	58	other at varied ratios
IV	Neurofilament H	Neurons	1	135-150	
IV	Neurofilament M	Neurons	1	105-110	H & M each require
IV	Neurofilament L	Neurons	1	60-70	L for polymer
IV	Nestin	Glial scars, Early neurons & muscle	1	240	
V	Lamin A	All	1	60-75	Homopolymers or
V	Lamin B	All	1	60-75	Heteropolymer

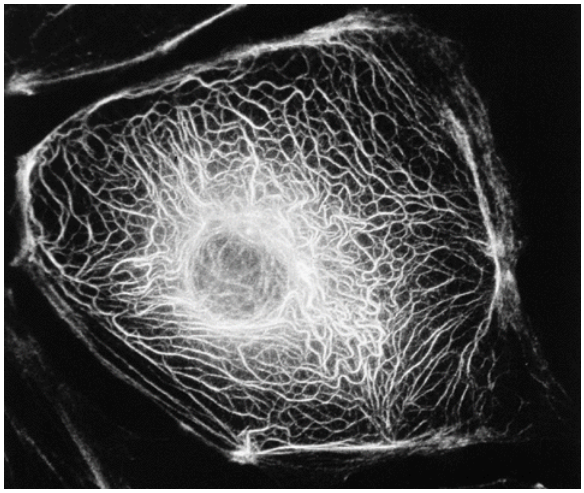
Intermediate filaments are notoriously stable. They do not bind nucleotides so there are no dynamics due to nucleotide hydrolysis. The filaments don't move very much. The subunits can and do move, however, in order to assemble filaments in this or that location. Intermediate filaments do need to disassemble at times, most notably during mitosis. The mitotic spindle needs to occupy a large central volume of the cell, and the intermediate filaments need to get out of its way. To do this, cells have a way to take intermediate filaments apart, based on phosphorylation by cyclin-dependent kinase.



At left is an illustration of how little the IF are dynamic. This is one cell that has two kinds of intermediate filaments, vimentin and keratin. One is green and one is red. The scientists bleached a line in the cell, and they asked how quickly the intermediate filaments exchange subunits allowing "repair" of the bleached line with new, unbleached subunits. Ultimately, this line does repair itself, but it takes 10-20 minutes for this to happen, not seconds like actin or microtubules. So, intermediate filaments are much more stable than actin and microtubules.

Intermediate filaments are more mobile and dynamic if you look at the edge of the cell where things are changing. If you fluorescently label the individuals subunits, you see individual dots dancing around, corresponding to small collections of a few subunits. So, the subunits are free to diffuse so the cell can grow longer intermediate filaments.

Vimentin is the intermediate filament that all cells have during early development. It forms a cage around the nucleus. These filaments are ropes that extend from the nucleus all the way out to the edge. So, if the cell crawls somewhere, the nucleus is going with it. This is important if you think you need your nucleus when you are crawling. Vimentins also interact with microtubules. Vimentin was one of the first genes that got knocked out and it was shocking that the mouse was normal. It turns out that when you look carefully, you can find abnormalities. But it is not as severe a phenotype as you might expect.

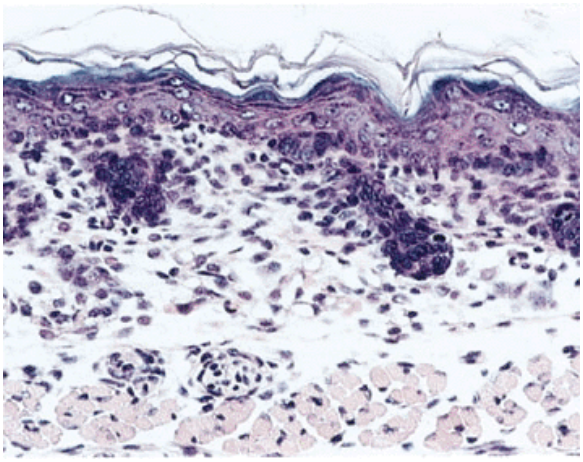


Desmin is a type of intermediate filament that is prominent in muscle. Part of what desmin does is to create elastic elements to prevent over-stretching. If you consider a sarcomere, the thing that makes it contract are the myosin motor heads binding to the actin. If you stretch the muscle and pull the Z lines so far apart that the myosin is no longer next to an actin, the sarcomeres can never contract. To prevent this, the cell builds in little rubber bands that pull the Z lines back to the resting state. The rubber bands are made by desmin which connects to the Z lines. Knockout of the gene for desmin does give a phenotype in the muscles.

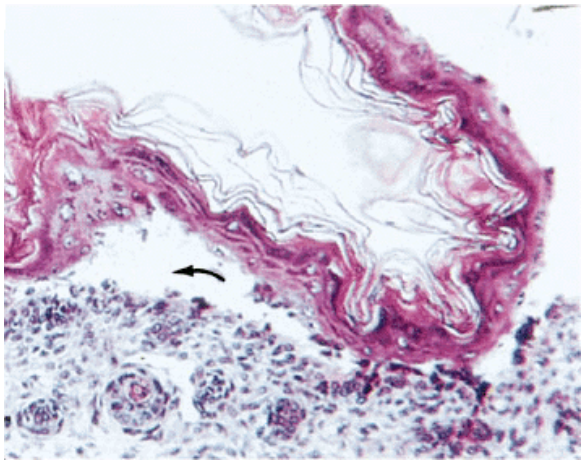
Keratins are expressed in epithelia. They connect to cell junctions called desmosomes and hemi-desmosomes which help tie one cell to another so the network of cells in an epithelium is one large interconnected sheet. Epidermal cells, the outer layer of skin, differentiate to produce massive amounts of keratins. This provides the outermost protection for your skin. If you do an electron micrograph of the outer layer of your epidermis, it is chocked full of these intermediate filaments. The cells are fully differentiated, and they ultimately die.

Some human skin diseases are caused by mutations in keratin. The epidermis of these patietnts doesn't mechanically hold together. What that looks like histologically is shown below. On the left is normal skin, showing epidermis and dermis. In the mutant, there is a white space, which is a blister. There is just fluid in here and when the sample was prepared, everything went away. The blister results from a rupture in a layer of the epidermis.

Normal



Mutant



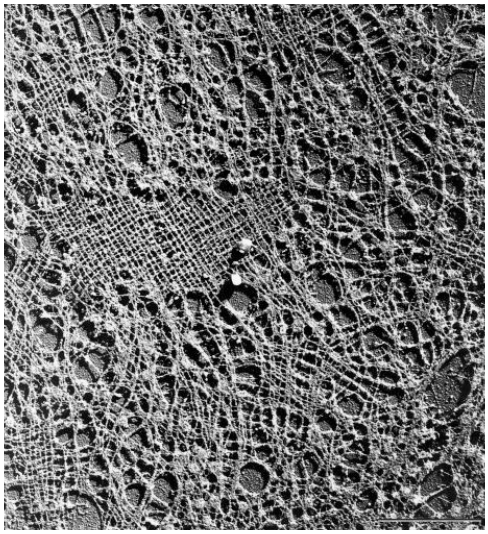
Different keratin genes are expressed in different layers of the epidermis, so different mutations can cause rupture in any one of these layers. One amazing thing was that when Elaine Fuchs, a cell biologist, made several keratin mutations and put them into cells and mice, the mice were born with blisters. She realized that there are a bunch of children born with skin-blistering diseases. These patients had diseases knowns epidermolysis bullosa simplex. The disease can be really bad depending on the mutation. Dr. Fuchs sequenced the genes of these kids and found that their genomes contained exactly the same mutations that she was making to figure out how keratins work. So, this is a great example of the usefulness of basic science.

What Elaine Fuchs found was that the EBS mutations disrupted up the whole keratin filament network, even when the mutant keratin was present at low levels compared to WT. How could this happen? Think of building a chain with links. Suppose that every 100 links, I put in one that is bad, that's weak. It still gets incorporated into the chain, and it still looks like a link. But when you pull on the chain, that link breaks. The cell needs keratin filaments that are 10,000 units long, for them to work.

Neurons have their own intermediate filaments. I have a neuron that extends from the base of my spine to my foot. If that axon breaks, I can't get it back. So, it is very important that that axon not break. To prevent this, the cell fills axons and dendrite with a type of intermediate filament called a neurofilament. Neurofilaments have heavy, medium and light subunits, and they prevent the axons from breaking. They are very stable, but one can see collections of subunits moving up and down the axons so there is some dynamics and turnover.

Clumps of neurofilaments are seen in many diseases where neurons die, including amyotrophic lateral sclerosis (ALS, aka Lou Gehrig's disease). Researchers hypothesized that the disease was caused by these clumps, which resulted from abnormal neurofilament assembly. However, other people found that mutations of an enzyme, superoxide dismutase, can cause ALS, including in mice. Using mice, they knocked out the neurofilament genes in these mice with ALS, so that the mice had no neurofilaments. The mice still got ALS. So, the clumps are an effect, not the cause, of the disease.

Lamins are present only in metazoans. Lamins include three proteins called A, B and C, and they come together to make a complex. Lamins assemble into filaments on the inner surface of the nuclear membrane where they form a square lattice. At right is a view of the inner surface of the nuclear membrane. The donut shaped things are nuclear pores. The square lattice of lamins is interspersed among them. It is a network which gives the nucleus strength. If this lamina is weak, the nucleus tears and that can tear the genomic DNA that is underneath it. The lamina has to break down during mitosis so that the chromosomes can come out and be split into the two daughter cells.



Progerias are diseases in which the patients exhibit accelerated aging. Progerias are caused by dominant mutations in lamins. As a result of the mutations, the nuclear lamina is weak and that makes the nucleus prone to tear. But we don't know how to connect the tearing of the nucleus to the symptoms of progeria patients.

Lecture 20. Cell-Cell and Cell-Matrix Interactions

Objectives:

At the end of this lecture, the student will be able to:

1. Define extracellular matrix and list its components

2. List the major classes of cell adhesion molecules and describe their structures

3. Compare and contrast outside-in and inside-out signaling by integrins

4. Discuss Glanzmann's thrombasthenia and its relationship to αIIb β3 integrin

5. Describe the role of integrins in bone remodeling and metastasis

Relevant Reading

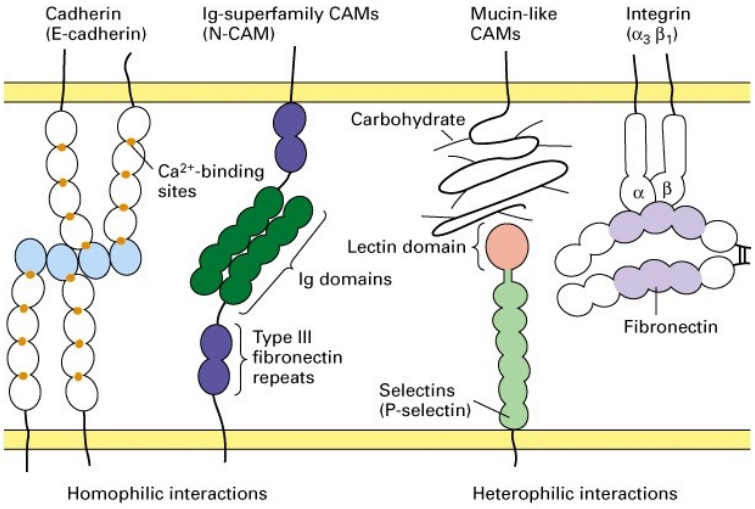
Alberts et al., Molecular Biology of the Cell, 6th ed., 2014, Garland.
Chap. 19 - Cell Junctions and the ECM.

Lodish et al., Molecular Cell Biology, 7th ed., 2012, Freeman.
Chap. 20 - Integrating Cells into Tissues.

Pollard & Earnshaw, Cell Biology, 2nd ed., 2007, Saunders.
Section VIII: Cellular Adhesion and the ECM. Chaps. 28-32.

Cell-Cell and Cell Matrix Interactions

Outside of cells there is not just nothing. There are a whole bunch of molecules that are sticky - they stick to cell surfaces and to each other. This is called the extracellular matrix (ECM) and cells are embedded and anchored in the ECM. Cell adhesion molecules are proteins on the outer surface of the cell that mediate interactions between cells or between a cell and the extracellular matrix.

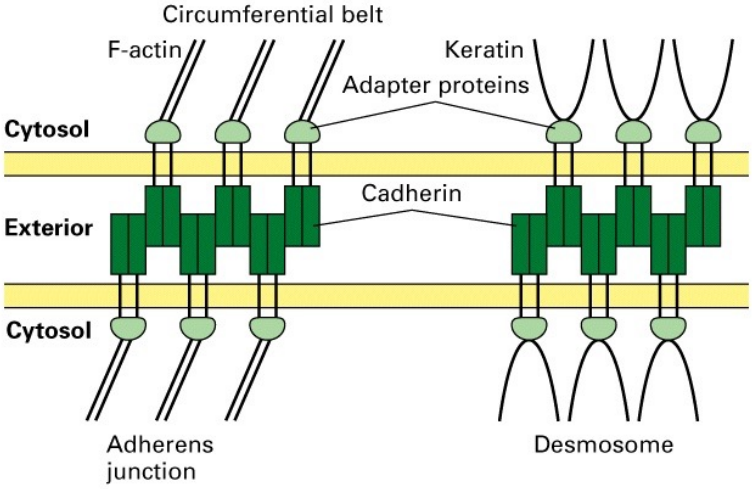


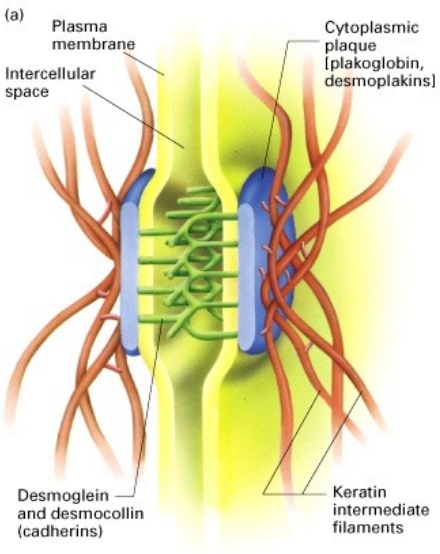
There are five classes of cell adhesion molecules, shown on the left. Cadherins and N-CAMS are two classes that work through homophilic interactions, i.e., they interact with other molecules like themselves. They are inserted into the membrane of opposing cells and their extracellular domains bind to each other. For cadherins, this binding is calcium-dependent. This is why you use EDTA (plus or minus trypsin protease) to remove tissue culture cells from their plates. You are breaking the cadherin-based connections allowing the cells to come free from each other.

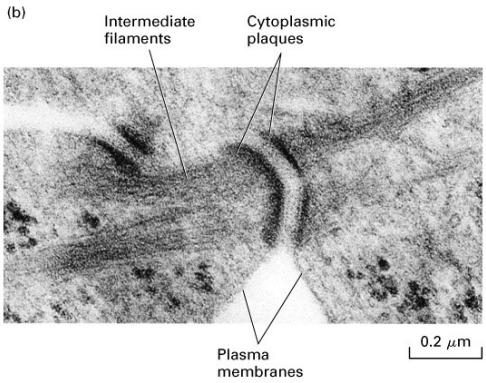
Other types of cell adhesion molecules make heterophilic interaction-- i.e. with other proteins. These are the selectins, the mucin-like CAMS and the integrins. In this lecture, we will focus on cadherins and integrins.

Cadherin-based junctions connect cells to each other. One type of cadherin junction is the adherens junction. Adherens junctions connect to actin filaments in the cytoplasm, and cadherins are used to glue the two cells together. Adherens junctions have a circumferential belt-like structure in epithelial cells, in contrast to the spot-weld structure that is characteristic of desmosomes.

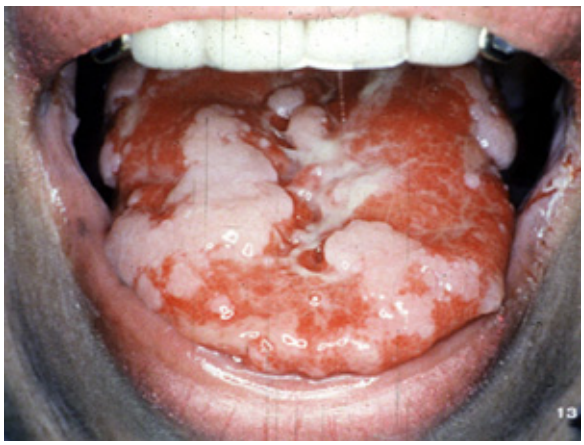
Desmosomes provide strength and rigidity to the epithelial layer, most notably the epidermis of the skin. Desmosomes act as spot welds between cells, and they use a particular form of cadherin. They connect the keratin intermediate filaments of the two cell cytoplasms, creating one large network among all the cells in a sheet.

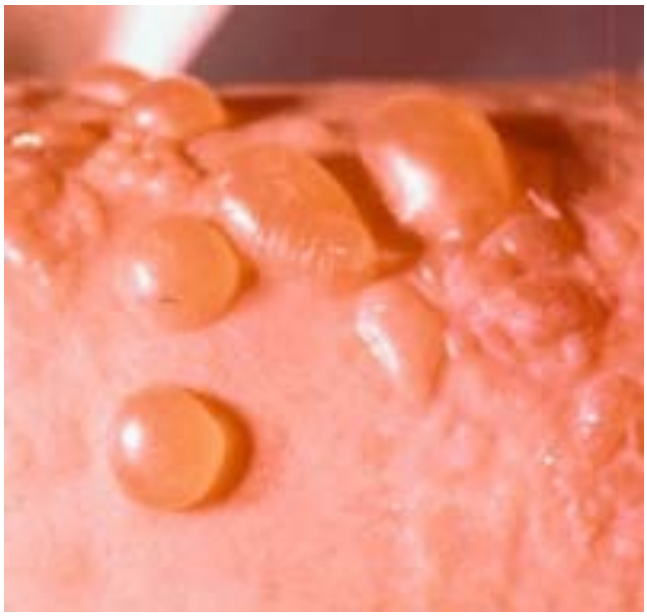




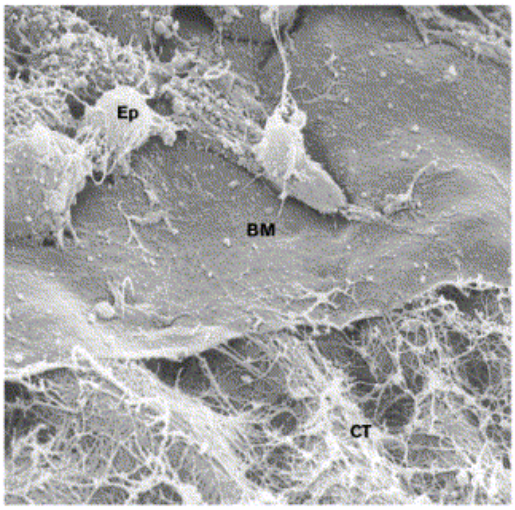


One human disease of the desmosomes is pemphigus vulgaris. This is a disease in which one of the cadherins, desmoglein, doesn't work because the patient has developed antibodies against it. The antibody disrupts the adhesion between epithelial cells, the epithelial layer ruptures and fluid fills the space. This gives rise to blisters on the skin and mouth.





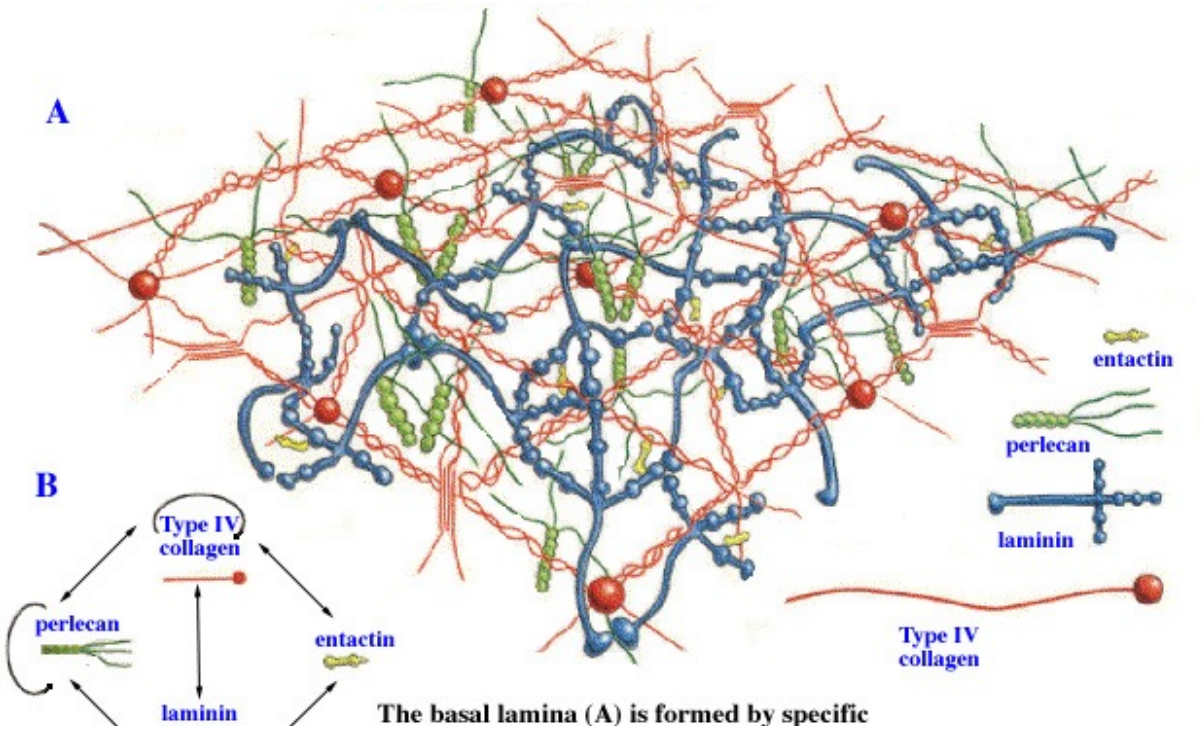
The Basement Membrane



Basement membranes are specialized layers of the extracellular matrix that surround or are adjacent to all epithelia, endothelia, peripheral nerves, muscle cells and fat cells. They often define the boundary between the layers, for example between the epithelium and the underlying interstitial tissue. The basement membrane was initially defined morphologically as a ribbon-like layer viewed in a histological section of tissue. The basement membrane can be crossed by cells and molecules in a controlled manner during normal physiological processes, but it can also break down and lead to disease.

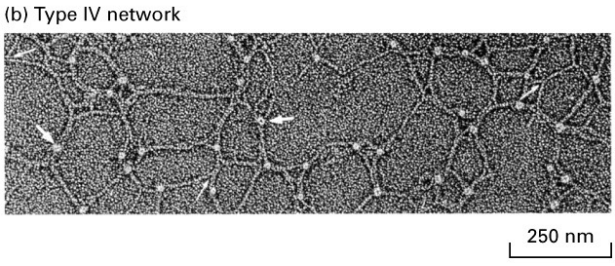
As seen in the electron micrograph at left, the basement membrane is a carpet-like structure. Sitting on top of the basement membrane are the epithelial cells (Ep). Underneath it is the connective tissue (CT) or interstitial tissue. The basement membrane is really just a tough densely-woven carpet.

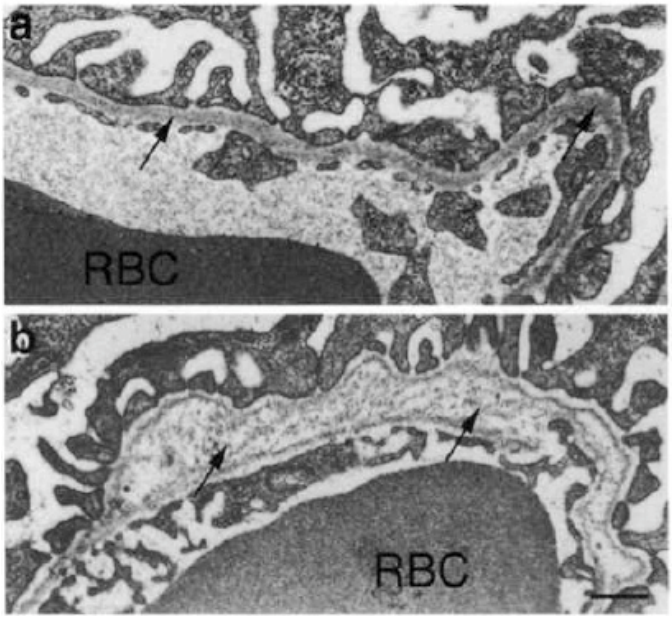
The basement membrane is made up of many different proteins. Below is an artist's drawing of the basement membrane. Two important molecules depicted are type IV collage and laminin. Type IV collagen is the red molecule shown as a ball with a long tail. Laminin (in blue) has 3 different subunits, alpha, beta and gamma. The laminin subunits can come together in different ways. Laminin is huge and forms an interesting cross-like structure. After the subunits come together in a heterotrimer, they interact with each other to make the carpet-like basement membrane structure. They are secreted as trimers, but they polymerize spontaneously into these larger networks. Laminin can interact with other molecules to help make the dense meshwork.

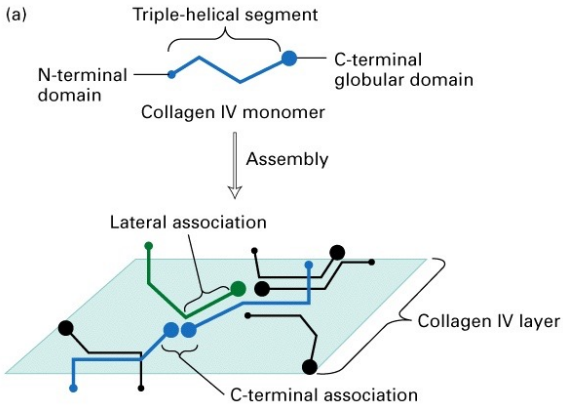


The basal lamina (A) is formed by specific

There are many different types of collagens. The one in the basement membrane is collagen type IV. Other types of collagens make up bones and skin. Collagen is arguably the most abundant protein in your body. Collagen molecules are large and interact with each other to make a network. There are a number of mutations of type IV collagen that cause human diseases.

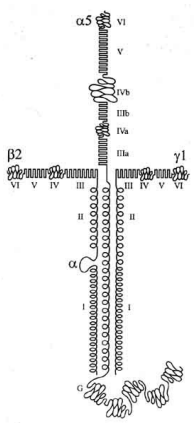






Here is a nice example of what happens to the basement membrane in diseases caused by mutations in collagen IV. This picture is of the kidney glomerulus. Panel a is normal. The basement membrane is the gray ribbon with the arrow pointing to it. On one side is blood (RBC). On the other side is what's going to be urine. The glomerulus is this whole apparatus, which filters blood in the first step of the kidney creating urine. Panel b is from a patient with a mutation in collagen IV. What's happened is that the basement membrane has gotten wider and is ripped apart. It is not as dense as it should be.

Laminins, as noted above, are heterotrimers of alpha, beta and gamma subunits. The chains are evolutionarily related, and they come in several types. There are 5 alphas, 4 betas and 3 gamma chains. They assemble with each other non-randomly into a cruciform structure. Fifteen heterotrimer combinations have been described to date.



Laminin trimers self-polymerize into a macromolecular network through short arm-short arm interactions. The alpha subunit "LG" domain is left free for interactions with cellular receptors. Laminins are not easy to study because they are so big, and it is difficult to do biochemistry on them.

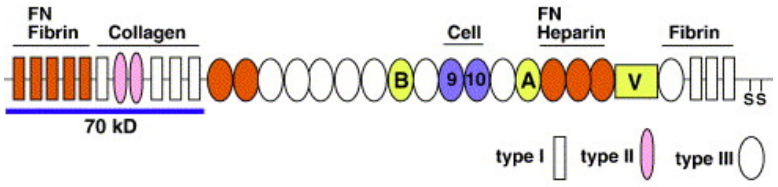
Humans have many genes for laminins, and individual genes are often expressed in specific places. If you have a mutation in one or another of them, then the consequences depend on where that laminin is being expressed. Some laminin functions are so fundamental that mutations are lethal. Some laminin mutations cause muscular dystrophy. Others cause a skin blistering disease. The consequences of these and other laminin mutations depend on where the genes are expressed.

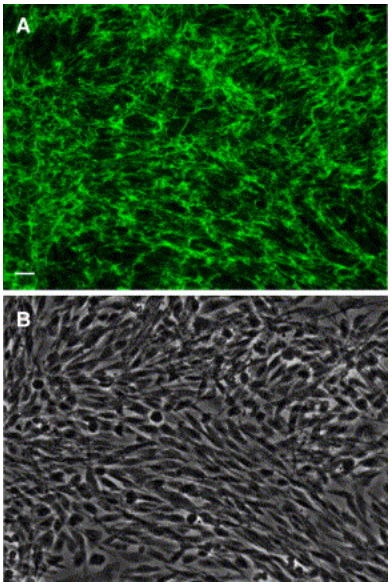
Fibronectin

Fibronectin is a glycoprotein that is associated with a lot of different extracellular matrices and is also present in blood. Fibroblasts synthesize fibronectin, secrete it, adhere to it and respond to its present. Tissue culture cells make fibronectin all the time. Fibronectins are solely extracellular. They are not inserted into the membrane.

Fibronectin is a long molecule with many different domains that mediate many different interactions. A major function of fibroblasts, the cells in the interstitium, is to synthesize fibronectin

and other extracellular matrix molecules. Fibroblasts also adhere to fibronectin, so they are a major part of the glue that is holding your tissues together. They help give shape to the tissues and organs.

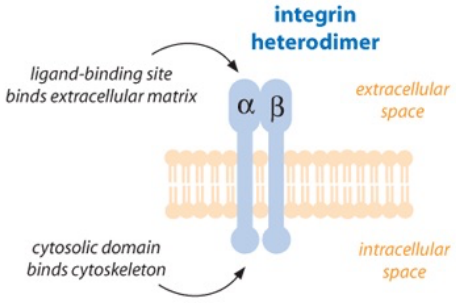




At right is a picture of the distribution of fibronectin in the extracellular matrix in green. One of the key things that the fibronectin protein contains is a motif called the RGD motif, which is named for the three amino acids – arginine, glycine, and aspartate, that comprise the motif. This motif binds to various integrins. An RGD motif is present in many other proteins besides fibronectin, allowing these other proteins to bind integrins. Knockout of fibronectin genes gives severe defects in vascular and heart development.

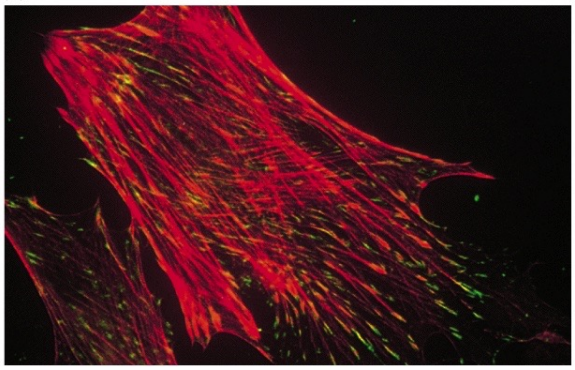
Integrins

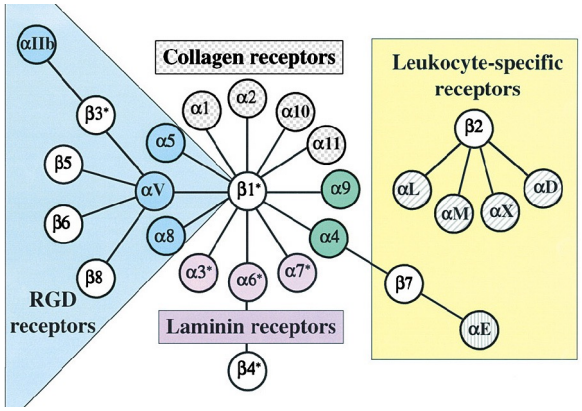
Integrins are transmembrane proteins on the cell surface. They have an extracellular part and an intracellular part. They are composed of an alpha and a beta subunit. There are 16 different alpha chains and 8 different beta chains that make a collection of 22 distinct heterodimers. The choice of which heterodimers form depends on the type of cell and the state of the cell. The cytoplasmic tails of both subunits generate cell signals in response to ligand binding to the extracellular domain.



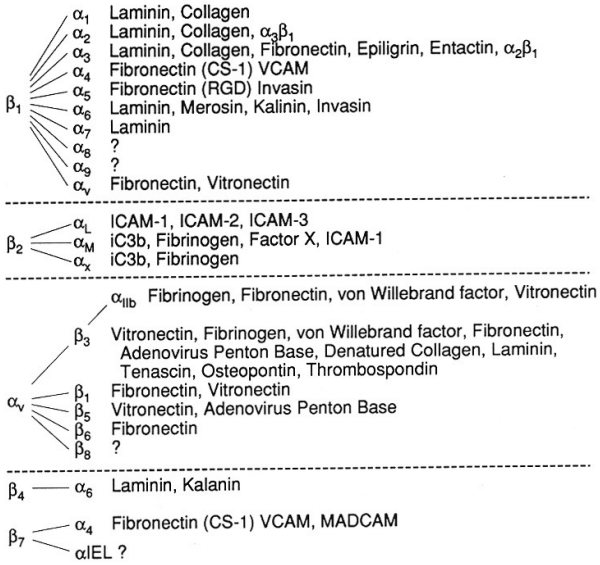
Integrins were discovered by researchers investigating how cultures cells interact with the extracellular matrix. This was done by generating antibodies that blocked the attachment of cells to the substrate and altered the organization of actin into stress fibers. The antibodies that did this were found to bind to the transmembrane proteins we now call integrins. The sites of attachment to the extracellular matrix in cultured cells are called focal adhesions. In this picture, actin stress fibers are red, and focal adhesions are green. Focal adhesions are different from desmosomes.

In an EM image of a focal adhesion, the plasma membrane is in the middle, with a bundle of actin filaments on the inside and a bundle of fibronectin fibers on the outside. The focal adhesion connects fibronectin fibers on the outside of the cell with actin fibers on the inside of the cells. This creates one continuous mechanical network, much like desmosomes and keratin fibers do in epithelial cells.

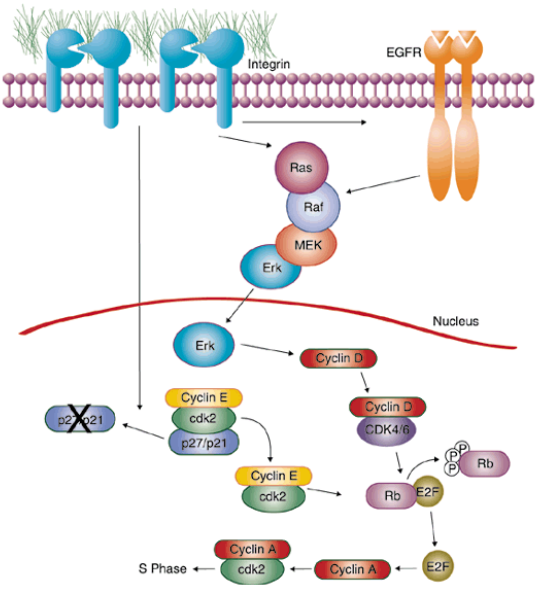


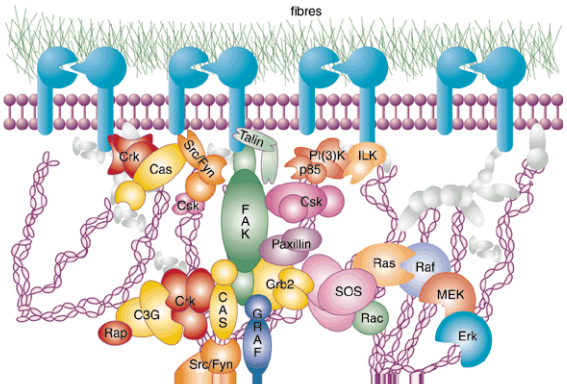


Integrins have an alpha subunit and a beta subunit. At left is a diagram of all the alpha subunits that interact with the different beta subunits. This also shows some of the potential interactions mediated by those alpha-beta pairs. For example, ß1 can interact with 12 different alphas.



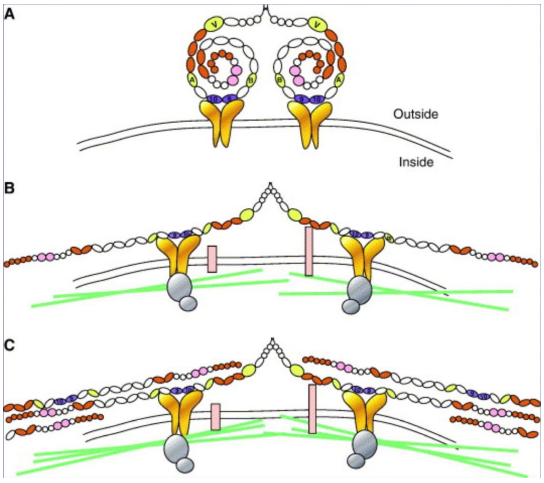
Integrins are really like cell surface receptors. They bind to proteins that contain the RGD motif, which includes proteins such as fibronectin, vitronectin and tenascin. Binding of these ligands requires divalent cations, specifically Ca^{2+}. A list of the different ligands bound by the different integrins is given at left. Binding of ligands to integrins result in the activation of a variety of signaling pathways.



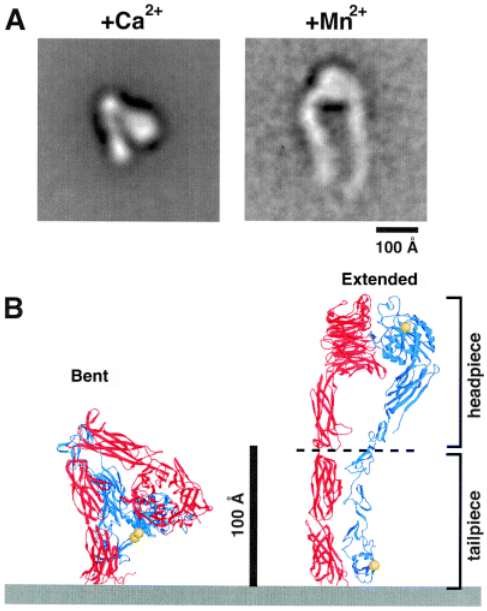


Signaling molecules such as FAK (focal adhesion kinase) become associated with integrins. FAK then recruits additional signaling molecules that create a complex signaling network that is intimately connected to the cytoskeletal network. In addition, integrins cooperate with receptor tyrosine kinases in cell cycle regulation. Both growth factors and cell adhesion are required for transmitting signals to the Ras/Raf/Mek/Erk signaling pathway.

One of the things that integrins do is to bind and compact the soluble fibronectin on the outside of the cell, organizing it into fibrils and fibers. Fibrils of fibronectin form through interactions between fibronectin molecules. On the inside of the cell, integrins bind to actin. The binding of fibronectin to integrins induces the reorganization of actin. Reciprocally, actin-based cell contraction pulls on fibronectin, promoting conformational changes that expose hidden binding domains.



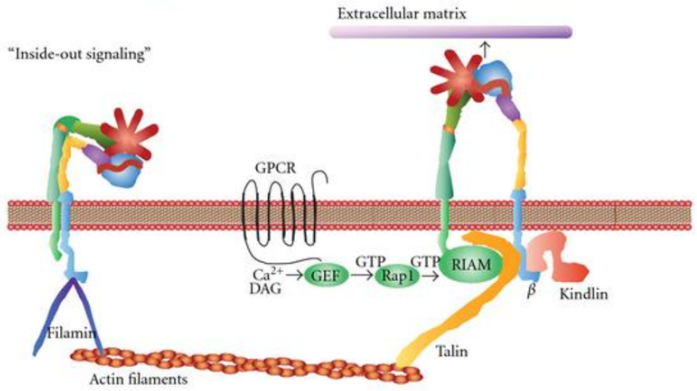
Fibronectins and integrins actually affect each other. Fibronectins cause integrins to signal on the inside of the cell. If the integrins on the inside of the cell can be either held in place or clustered together because of cytoskeletal elements like actin, that can influence what fibronectin does on the outside. So, there is this interplay between the elements on the outside of the cell and those on the inside of the cell. One of the ways to think about this is that the actin filaments on the inside of the cell are able to cause a contraction. They are able to move and exert force. When that happens, they pull on these integrin molecules, when you pull on the cytoplasmic part of integrin, it causes the outside to have a change in its function. So, you have outside-in and inside-out signaling.



Integrins exist in two major states: inactive and active. In the inactive state, the conformation of the extracellular part of the protein (both alpha and beta subunits), is bent and folded over, hiding the ligand binding site. This form is stabilized by Ca^{2+}. In the active state, the extracellular portion is unfolded and extended, exposing the ligand binding site. This form is promoted by Mn^{2+}.

Integrin signaling is outside-in and inside-out. "Outside-in" signaling refers to the situation in which a ligand, such as fibronectin, binds to the extracellular portion of an integrin and elicits a signal on the inside of the cell. This involves a huge group of proteins, many of which are signaling proteins that are kinases: FAK, Src, MEK, Erk, Raf. These kinases are found associated with the cytoplasmic domains of integrins in a domain called an attachment plaque. These signaling proteins interact with the EGF receptor and other tyrosine kinases and they cause changes which ultimately leads to changes inside the nucleus in the cell cycle. One of the things integrins do is to cooperate with the growth factor receptors and their signals come together and drive cell division. This is important for cancer.

"Inside-out" refers to activation of an integrin caused by signals from inside the cell. In this case, activation of a GPCR leads to the generation of the two intracellular second messengers, Ca^{2+} and diacylglycerol, that activate protein kinase C. This leads to the activation of a low-molecular-weight G protein called Rap1 which ultimately results in the actin fibers pulling on the integrin causing it to shift into its open, active state where it will bind fibronectin.

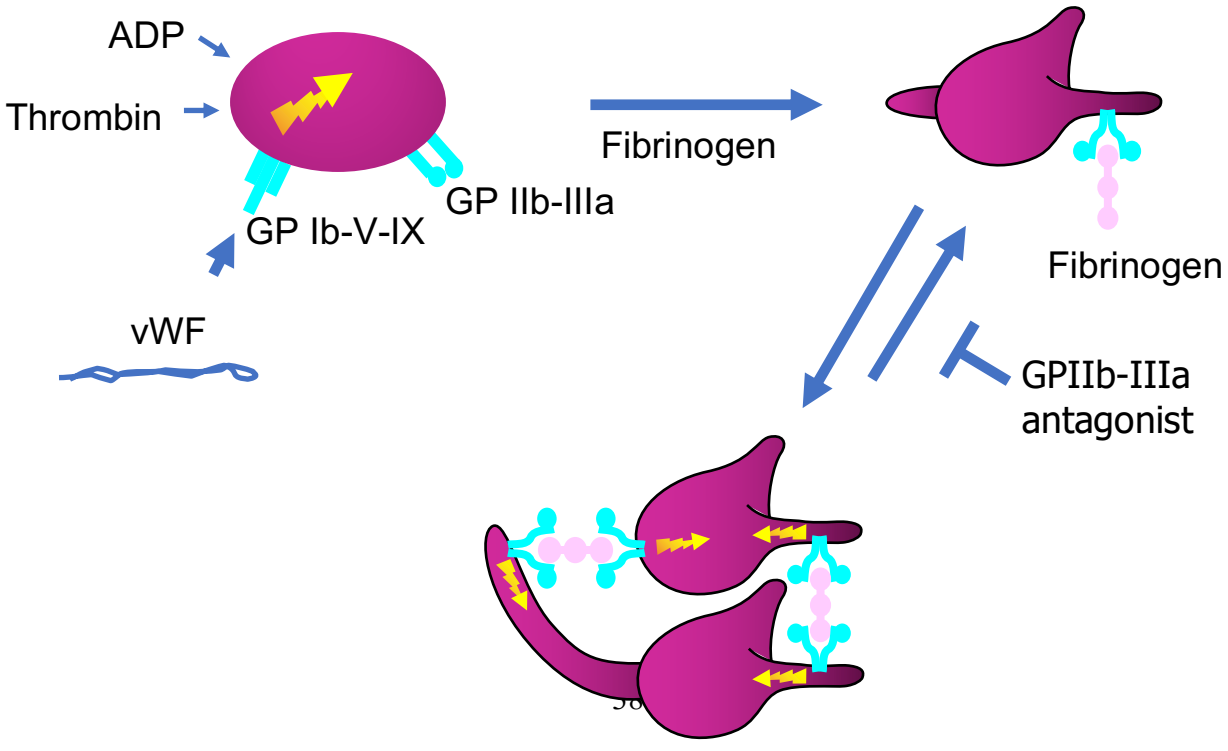


These processes are all going on at the same time. Outside-in signaling promotes inside-out signaling. The attachment plaques wind up being foci where this signaling process is amplified. So, the two types of receptor systems are cooperating with each other.

Integrins play important roles in a wide variety of cells. This includes physiological and pathological processes such as hemostasis, osteolysis, angiogenesis, hematopoiesis, inflammation, immune surveillance and metastasis. Integrin dysfunction can lead to disease. Altering the activity of integrins can be valuable treatment options for various diseases, including ones not primarily caused by abnormalities of integrins. Many of these diseases appear to involve excessive activity of the immune system. This includes: Crohn's disease, inflammatory bowel disease, ulcerative colitis, rheumatoid arthritis, ischemia-reperfusion injury, thrombosis, autoimmune diabetes, cancer and metastasis, occlusive stroke, stem cell mobilization in leukemias, psoriasis, multiple sclerosis, asthma, osteoporosis, graft vs host disease, atherosclerosis, transplant rejection, tumor-dependent osteoporosis leading to fracture.

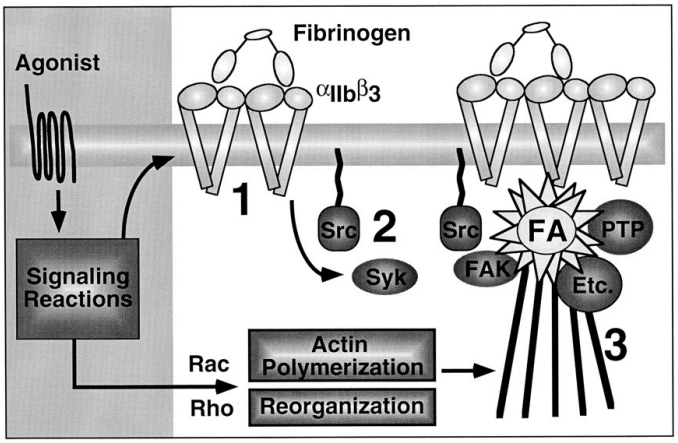
Platelet aggregation depends on integrins

Platelets aggregate in one of the first steps in blood clotting. Your platelets recognize that they are exposed to the interstitial tissue. They sense that they are not looking at endothelial cells anymore. They know when they see collagen or fibronectin, and they know that this is bad. So now it is time for the platelets to stick to the extracellular matrix and to each other. Then, they all contract and pull together, and this helps to contract the whole wound. They both plug the hole and then squeeze together to make the hole smaller.



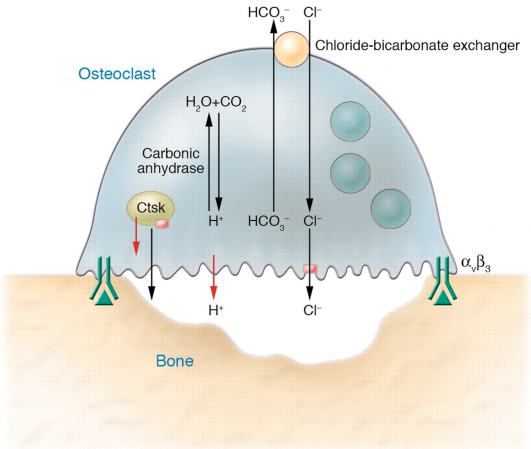
Platelets have an integrin on their surface called GPIIb-IIIa (αIIb/ß3). Several stimuli can start the process of platelet activation including ADP, thrombin and von Willebrand Factor (vWF). When this happens, platelets bind to fibrinogen which is in plasma. This helps link the platelets together. Your platelets have to run around with these receptors on their surface all the time and not be activated. But when called upon they have to undergo this explosive reaction. This has to be highly regulated. That's why we want this huge association of signaling proteins inside the cell which has a very positive feedback cycle so that it is off and when you push it over, it all goes over at once. This is not a linear response. This is a threshold response.

This is another drawing of how fibrinogen interacts with this one integrin on the platelet surface and how oftentimes this gets started by some other kind of agonist outside the cell. That can promote actin polymerization and reorganization. Actin interacts with these proteins which interact with the integrins on the inside of the cell. These are the traditional TKs – Src, Syk, Rac, and Rho – which are coming from transmembrane signaling. So, this is a huge positive feed forward cycle.

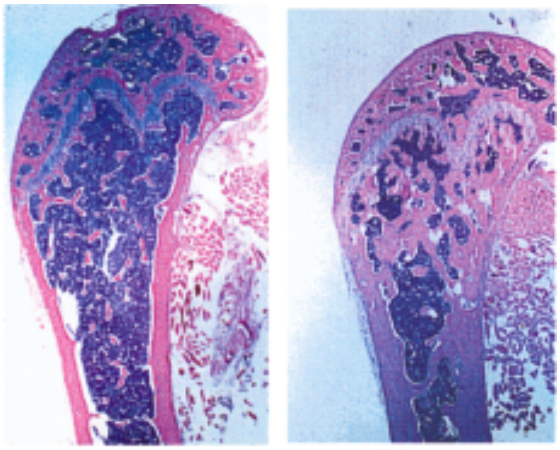


Glanzmann's thrombasthenia is a disorder of platelets that results in a low platelet count and a failure of the platelets to aggregate. As a result, patients with Glanzmann's thrombasthenia exhibit excessive bleeding upon wounding, during dental work or menstruation. In females, childbirth can be fatal. Classical Glanzmann's thrombasthenia is due to mutations in either chain of the platelet fibrinogen receptor—glycoprotein IIb/IIIa (or αIIb3) In the old days, we gave the patient platelets. The more modern way is to give the patient a bone marrow transplant because platelets come from megakaryocytes in the bone marrow and if you give the patient normal platelets, this will essentially cure the disease, albeit with the risk of having graft vs. host disease.

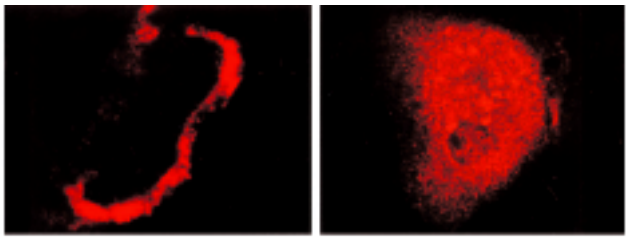
There is another major type of integrin, αvß3. In osteoclasts, αvß3 integrins are important for adhesion to bone and bone resorption. αvß3 is also important for the proliferation and survival of vascular endothelial cells during pathologic angiogenesis. Tumor cells use αvß3 integrins as they invade and go places where you don't want them to. Macrophages use αvß3 integrins to phagocytosed stuff and neutrophils use αvß3 integrins during extravasation and oxidative burst.



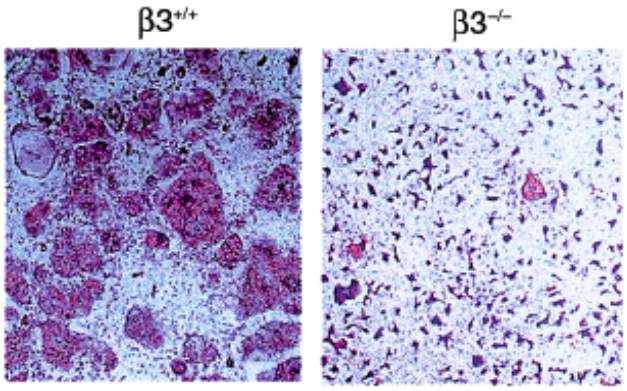
Osteoclasts sit on bone and form a very tight seal and secrete acid and proteases into this space. This tight seal is made by the action of this integrin binding to proteins that are in the extracellular matrix of the bone.



These histology slides are from the bones of wild type mice or mice that lack the ß3 subunit of integrins. This pink material on either side is bone. Pink means protein. Blue means cells—DNA taking up the blue stain. This section shows "trabecular" bone. Wt is on the left and mutant on the right. In the mutant, there is a lot more pink than blue. There's not as much space for bone marrow and it needs this space in order to work. When we don't have the ß3 integrin that makes the osteoclast work, there is too much bone.

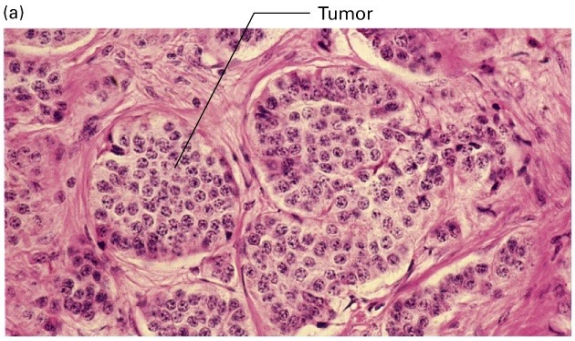


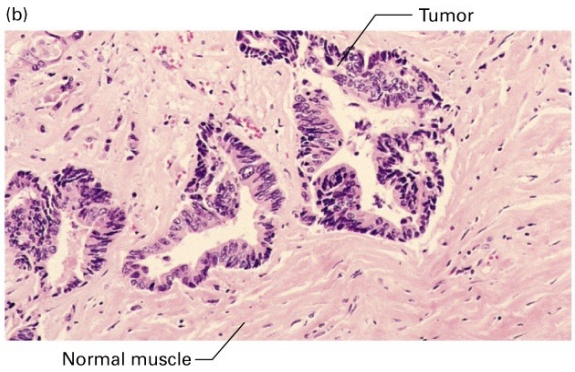
If you take one osteoclast and look down on it from the top, the integrins are associated with actin filaments—shown in red here. In a WT cell (left), there is a ring of actin filaments but in a mutant cell (right), there is actin but no ring.



If you just look at the osteoclast bone cells with and H and E stain, the normal cells get very large and multinucleated (wt on the left). In the mutant (on the right), the osteoclasts are there but are small and not doing anything. They are not interacting.

Integrins are also important in cancer. In the picture below, on the left, is a benign tumor. Benign means that the tumor is not going to migrate. Part of the reason that the pathologist can tell you that this tumor is benign is that these cells are surrounded by basement membrane. They respect the authority of the basement membrane and they don't violate it. So, a surgeon can go in and take this out and it won't come back.

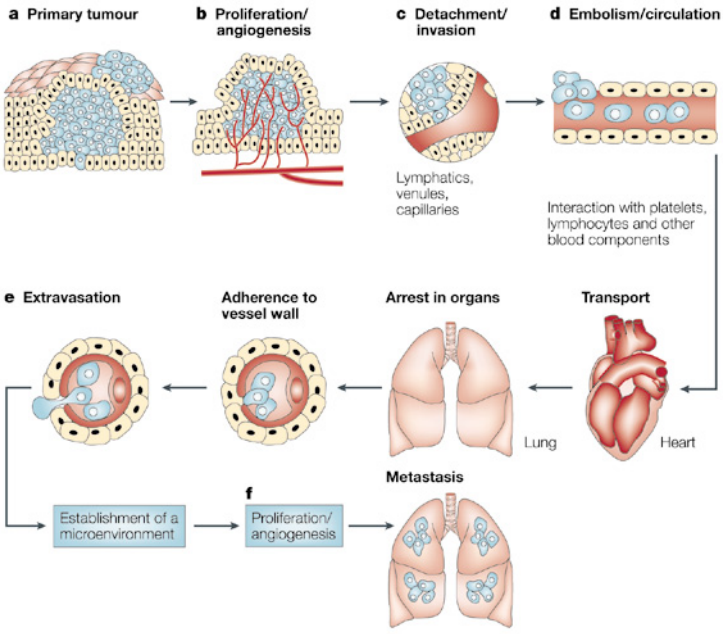




On the right is a malignant tumor, and its appearance is different. The malignant cells are all over the place, the cells don't have a uniform size and shape, and there is no basement membrane. Moreover, a number of the cells have escaped from the epithelium, and they are invading the surrounding interstitial tissue. So, this tumor is invasive.

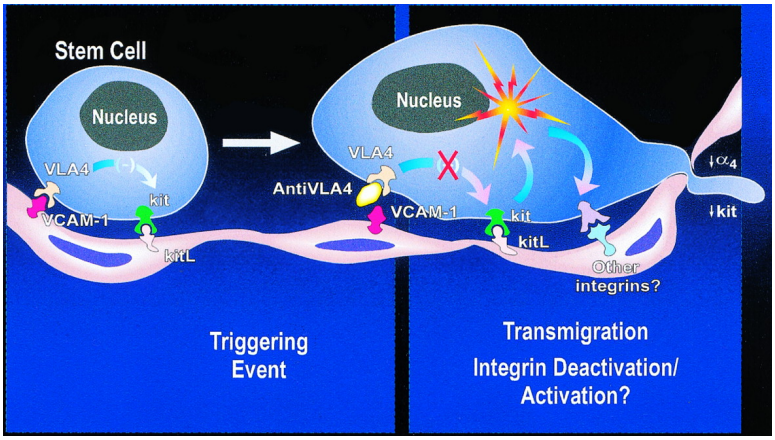
Integrins play many different roles in the cancer pathways that lead to metastases. As tumor cells grow and proliferate, one of the first things that tumors do is to make new blood vessels to feed themselves. Integrins are really important for how the blood vessels interact with the extracellular matrix as they grow out. So, this would be a great place to stop cancer if you could inhibit angiogenesis.

At some point, the tumor cells invade away from the initial tumor site and they enter the circulatory system or the lymphatic system. Here their interactions, which often include integrins, are important. You may know that different types of tumors tend to spread to different organs. Breast cancer tends to metastasize to bone or brain. Other tumors go to lungs or liver. The tumors cells are circulating through all the organs all the time. But there is something about their integrins and other adhesion proteins that makes those cells stick there. As the tumor cells go through all the different organs, they can adhere to a blood vessel wall and they infiltrate their way through the blood vessel wall and set up house where they don't belong and interfere with the function of the organ. So, if you could interfere with this, it would be a way to block metastasis.



A variety of proteins are involved in metastasis. Cadherins act as receptors in cell-cell interactions and are important for metastasis. Another set of extracellular matrix proteins are proteases. When cancer cells want to invade, they like to degrade the extracellular matrix and make a tunnel through there. There is a set of proteases called matrix metalloproteases (MMP) that carries out this function. As you've already learned, growth factors such as VEGF and FGF are important in proliferation and angiogenesis.

Integrins are important for bone marrow transplantation. One key variable as to whether an autologous (cells from the patient) bone marrow transplant works or not is how many normal stem cells you can get out of the patient. Your bone marrow has only so many stem cells. Stem cells are attached in the bone marrow via integrin-mediated interactions. John DiPersio in oncology here has developed ways to inhibit those integrin mediated interactions releasing more stem cells into the circulation which yields more stem cells and makes the probability that a transplant would work much higher.



So, in summary, cell adhesion molecules are important, playing essential roles in normal physiology and derangements of which cause disease. Cell-adhesion molecules (CAMs) involved with structural or surveillance roles are always "ON". This includes things like cadherins, mucins, and selectins. Integrins are a class of highly regulated CAM. They bind soluble or matrix-bound ligands, including fibrinogen, fibronectin, and thrombospondin, as well as cell-associated ligands of the IgG family like I-CAM and V-CAM. Integrins exist in active and inactive states. They can be activated by inside-out signaling. Mutations that affect integrin expression, activity and regulation cause a number of human diseases. Integrins are targets of mAbs and other therapeutics.