Cytoskeleton :

Structure and Movement

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

When a eukaryotic cell is taken out of its physiological context and placed in a plastic or glass Petri dish, it is generally seen to flatten out to some extent. On a precipice, it would behave like a Salvador Dali watch, oozing over the edge. The immediate assumption, particularly in light of the fact that the cell is known to be mostly water by mass and volume, is that the cell is simply a bag of fluid. However, the cell actually has an intricate microstructure within it, framed internally by the components of the cytoskeleton.

As the name implies, the cytoskeleton acts much like our own skeletons in supporting the general shape of a cell. Unlike our skeletons though, the cytoskeleton is highly dynamic and internally motile, shifting and rearranging in response to the needs of the cell. It also has a variety of purposes beyond simply providing the shape of the cell. Generally, these can be categorized as structural and transport. While all three major components of the cytoskeleton perform each of these functions, they do not do so equally, as their biophysical characteristics are quite different. With respect to structure, at some point in the life of every cell, it must change shape, whether simply increasing or decreasing in size, or a more drastic alteration like the super-elongated form of neurons with axons, the cytoskeleton must be able to respond by dynamically increasing and decreasing the size of the internal structures as needed. Structure also applies to the relative position of internal cellular elements, such as organelles or proteins, to one another. In many highly specialized cells, the segregation of particular structures within certain parts of the cell is crucial for it to function. Transport refers to the movement of molecules and organelles within the cell as well as movement of the cell as a whole. We just discussed intracellular movement of proteins and lipids by way of vesicles in the last chapter. Those vesicles, as we will see in this chapter, are not just floating from one place to another; they are moved purposefully and directionally along the cytoskeleton like cargo on highways or railroad tracks. With respect to whole cell movement, this can range from paddling or swimming by single-celled organisms to the stereotyped and highly coordinated crawling of many cells from their point of origin to their eventual destination during the development of a metazoan organism or the movement of fibroblasts to heal a cut in your skin.

Although the genes are not particularly well conserved, a combination of genetic similarity and protein structure have confirmed the presence of prokaryotic proteins that are related to eukaryotic cytoskeletal proteins in both form and function. Compared to the eukaryotic cytoskeleton, study of prokaryotic proteins is very recent, and for a long time, there was an assumption that prokaryotes did not have or need cytoskeletal architecture. FtsZ, the bacterial equivalent of tubulin, was discovered in 1980 but most of the work on it has occurred in the last decade. MreB is an actin-like protein, first compared to actin in 1992, and crescentin, an intermediate filament class protein, was only described in 2003. For comprehensive review of prokaryotic cytoskeleton proteins, see Graumann, P.L., *Ann. Rev. Microbiology* **61**:589-618, 2007.

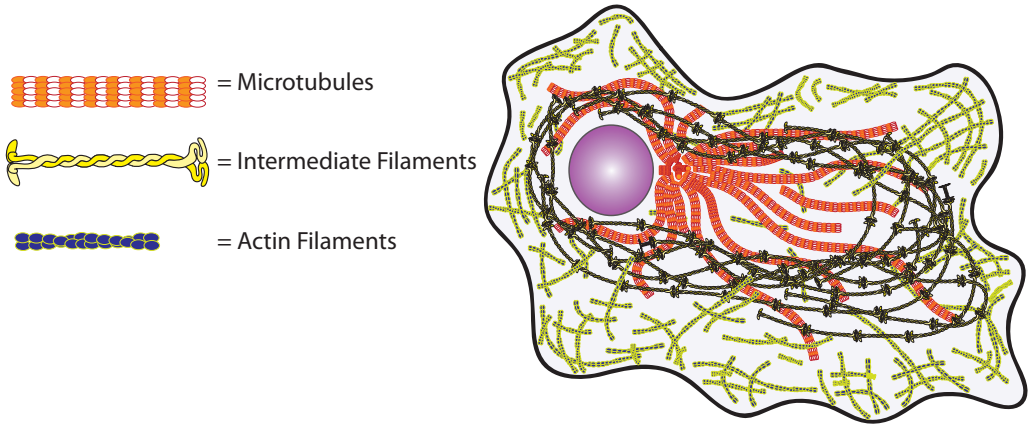

Figure 1. Cytoskeletal element distribution in a prototypical eukaryotic cell. The purple ball is the nucleus.

The three major components of the cytoskeleton are microtubules, microfilaments, and intermediate filaments. Each of these are polymers composed of repeating subunits in specific arrangements. With just a quick glance (fig. 1), it is very clear that the intermediate filaments will likely play a significantly different role from either microtubules or microfilaments. Because the IF's are made of long fibrous subunits that coil around one another to form the filament, there is clearly a great deal of contact (which facilitates formation of hydrogen bonds, aka molecular velcro^{tm}) between subunits providing great tensile strength. It is very difficult to break these subunits apart, and thus the IF's are primarily used for long-term or permanent load-bearing purposes. Looking at the other two components of the cytoskeleton, one can see that with the globular instead of fibrous shape of the subunits, the maximum area of contact between subunits is greatly limited (think of the contact area when you push two basketballs together), making it easier to separate the subunits or break the microfilament or microtubule. The cell can use this characteristic to its advantage, by utilizing these kinds of cytoskeletal fibers in dynamic situations where formation or destruction of intermediate filaments would take far too long. We now address these three groups of cytoskeletal elements in more detail.

Intermediate Filaments

"Intermediate filaments" is actually a generic name for a family of proteins (grouped into 6 classes based on sequence and biochemical structure) that serve similar functions in protecting and shaping the cell or its components. Interestingly, they can even be found inside the nucleus. The nuclear lamins, which constitute class V intermediate filaments, form a strong protective mesh attached to the inside face of the nuclear

Most intermediate filaments fall between 50-100 kDa, including keratins (40-67 kDa), lamins (60-70 kDa), and neurofilaments (62-110 kDa). Nestin (class VI), found mostly in neurons, is an exception, at approximately 240 kDa.

membrane. Neurons have neurofilaments (class IV), which help to provide structure for axons — long, thin, and delicate extensions of the cell that can potentially run meters long in large animals. Skin cells have a high concentration of keratin (class I), which not only runs through the cell, but connects almost directly to the keratin fibers of neighboring cells through a type of cellular adhesion structure called a desmosome (described in the next chapter). This allows pressure that might be able to burst a single cell to be spread out over many cells, sharing the burden, and thus protecting each member. In fact, malformations of either keratins or of the proteins forming the desmosomes can lead to conditions collectively termed *epidermolysis bullosa*, in which the skin is extraordinarily fragile, blistering and breaking down with only slight contact, compromising the patient's first line of defense against infection.

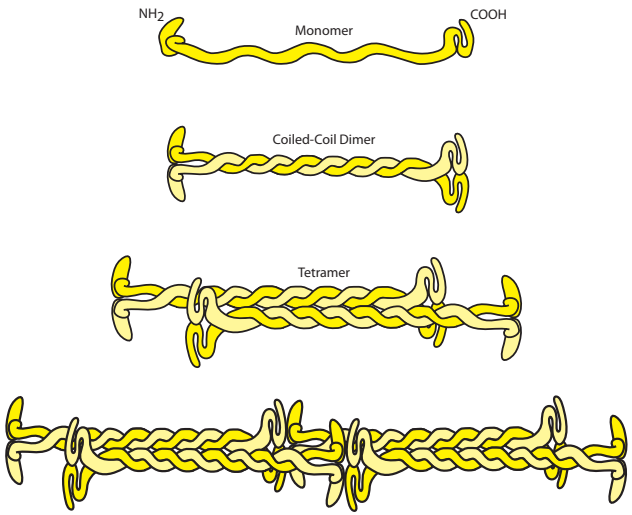

Figure 2. Intermediate filaments are composed of linear subunits that wrap around each other and interact very tightly.

Structurally, as mentioned previously, all intermediate filaments start from a fibrous subunit (fig. 2). This then coils around another filamentous subunit to form a coiled-coil dimer, or protofilament. These protofilaments then interact to form tetramers, which are considered the basic unit of intermediate filament construction. Using proteins called *plectins*, the intermediate filaments can be connected to one another to form sheets and meshes. Plectins can also connect the intermediate filaments to other parts of the cytoskeleton, while other proteins can help to attach the IF cytoskeleton to the cell membrane (e.g. desmoplakin). The most striking characteristic of intermediate filaments is their relative longevity. Once made, they change and move very slowly. They are very stable and do not break down easily. They are not usually *completely* inert, but compared to microtubules and microfilaments, they sometimes seem to be.

Epidermolysis bullosa simplex is a collection of congenital diseases caused by mutations to the keratin genes KRT5 or KRT14, or to the plectin gene PLEC1. These mutations either weaken the polymerization of keratin into filaments, or the interaction between keratin filaments. This leads to the inability of each individual cell to maintain structural integrity under pressure. Another type of EB, junctional epidermolysis bullosa (JEB), is caused by mutations to integrin receptors (b4, a6) or laminins. This includes JEB gravis or Herlitz disease, which is the most severe, often leading to early postnatal death. JEB is also related to dystrophic epidermolysis bullosa (DEB) diseases such as Cockayne-Touraine, each of which s due to a mutation in collagen type VII. The gene products involved in JEB and DEB are discussed in more detail in the next chapter. They play a role in adhering the cells to the basememnt membrane, and without them, the disorganization of the cells leads to incomplete connections between the epidermal cells, and therefore impaired pressure-sharing.

Some forms of *Charcot-Marie-Tooth disease*, the most common inherited peripheral nerve disease, are also linked to mutations of intermediate filament genes. This disease, also known as peroneal muscular atrophy or hereditary motor sensory neuropathy, is a non-lethal degenerative disease primarily affecting the nerves of the distal arms and legs. There is a broad variety of CMT types and causes, the most common being malformations of Schwann cells and the myelin sheath they form. CMT type 2 is characterized by malformations of the peripheral nerve axons, and is linked to mutations of lamin A proteins and of light neurofilaments. The causal mechanism has not yet been established; however, the neurofilaments are significant elements in maintaining the integrity of long axons.

Actin Microfilaments

Microfilaments are also known as actin filaments, filamentous actin, and f-actin, and they are the cytoskeletal opposites of the intermediate filaments. These strands are made up of small globular actin (g-actin) subunits that stack on one another with relatively small points of contact. You might envision two tennis balls, one fuzzy and the other covered in velcro hooks. Even if you push hard to mush them together, the area of contact between the balls (i.e. the area available for H-bonding between subunits) is fairly small compared to the overall surface area, or to the area of contact between IF subunits. They will hold together, but they can also fall apart with relatively little force. Contrast this with intermediate filaments, which might be represented as two ribbons of velcro hooks or loops. Considerably more work is required to take them apart. Because there are fewer H-bonds to break, the microfilaments can be deconstructed very quickly, making it suitable for highly dynamic applications.

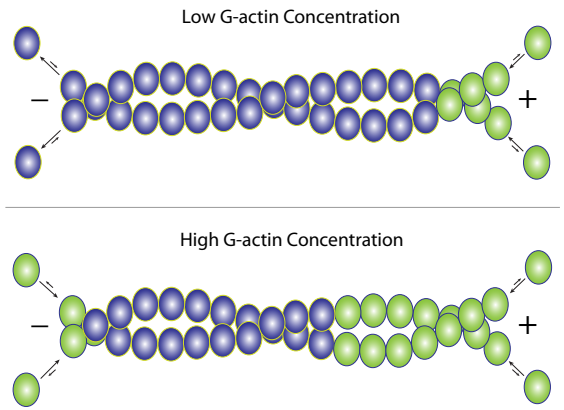

Figure 3. Actin microfilaments have a (+) and (-) end. When the free (globular) actin concentration is low, actin is primarily added to the (+) end, and lost from the (-) end. However at high levels of g-actin, new monomers can potentially add onto the filament from either end.

When the actin subunits come together to form microfilaments, they interact directionally. That is, subunits have a "top" and a "bottom", and the top of one subunit always interacts with the bottom of another. If we go to the "bottom"-most subunit of a filament, the open end is called the minus (-) end, while the opposite end, which incidentally sees more additive action, is called the plus (+) end. Microfilaments are also said to have polarity, but again this is only in the sense of having directionality, and has nothing to do with electrical charge. Individual microfilaments can exist, but most microfilaments in vivo are twisted pairs. Unlike DNA; however, microfilament pairs are not antiparallel: both strands have the same directionality.

The formation of filaments from g-actin is an ATP-dependent process, although not in the conventional sense of utilizing the energy released in hydrolysis. Instead, the globular actin subunits will only bind with another g-actin subunit if it has first bound an ATP. If the g-actin has bound ADP, then it must first exchange the ADP for ATP before it can be added onto a filament. This alters the conformation of the subunit to allow for a higher-affinity interaction. A short time later, hydrolysis of the ATP to ADP (with release of P_i) weakens the affinity but does not directly cause dissolution of the subunit binding. The hydrolysis is brought about by the actin itself, which has this ATPase enzymatic activity built in.

Although f-actin primarily exists as a pair of filaments twisted around each other, addition of new actin occurs by the addition of *individual* g-actin monomers to each filament (fig. 3). Accessory proteins can be used to help or hinder either the building or breakdown of the filaments, but the primary mechanism is essentially self-regulating. When free g-actin levels are high, elongation of actin filaments is favored, and when the g-actin concentration falls, depolymerization of f-actin predominates. Under average physiological conditions, though, what is often seen in actin microfilaments is an effect called treadmilling. Since actin is mostly added onto one end but removed from the other, the net effect is that any given actin monomer in a filament is effectively moving from (+) end to (-) end even if the apparent length of the filament does not change.

In most cell types, the greatest concentration of actin-based cytoskeletal structures is found in the periphery of the cell rather than towards the center. This fits well with the tendency of the edges of the cell to be more dynamic, constantly adjusting to sense and react to its environment. Clearly, the polymerization and depolymerization of actin filaments is much faster than for intermediate filaments. The big exception to the actin-in-periphery rule is found in muscle cells. Actin filaments, and the myosin motor proteins that work on them, are the basis for muscle cell contraction, and fill up most of the muscle cells, not just the periphery. We will discuss the role of actin in both types of cell movement later in the chapter.

Microtubules

Microtubules are made up of two equally distributed, structurally similar, globular subunits: α and β tubulin. Like microfilaments, microtubules are also dependent on a nucleotide triphosphate for polymerization, but in this case, it is GTP. Another similarity is that microtubules have a polarity in which the (-) end is far less active than the (+) end. However, unlike the twisted-pair microfilaments, the microtubules are mostly

Microtubule stability is temperature-dependent: if cooled to 4°C, microtubules fall apart into $\alpha\beta$-tubulin heterodimers. Warmed back up to 37°C, the tubulin repolymerizes if there is GTP available.

found as large 13-stranded (each strand is called a protofilament) hollow tube structures. Also, the α and β tubulin used for building the microtubules not only alternate, but they are actually added in pairs. Both the α-tubulin and β-tubulin must bind to GTP to associate, but once bound, the GTP bound to α-tubulin does not move. On the other hand, GTP bound in the β-tubulin may be hydrolyzed to GDP. GDP-bound αβ-dimers will not be added to a microtubule, so similar to the situation with ATP and g-actin, if the tubulin has GDP bound to it, it must first exchange it for a GTP before it can be polymerized. Although the affinity of tubulin for GTP is higher than the affinity for GDP, this process is usually facilitated by a GEF, or guanine nucleotide exchange factor. As the signal transduction chapter will show in more detail, this type of nucleotide exchange is a common mechanism for activation of various biochemical pathways.

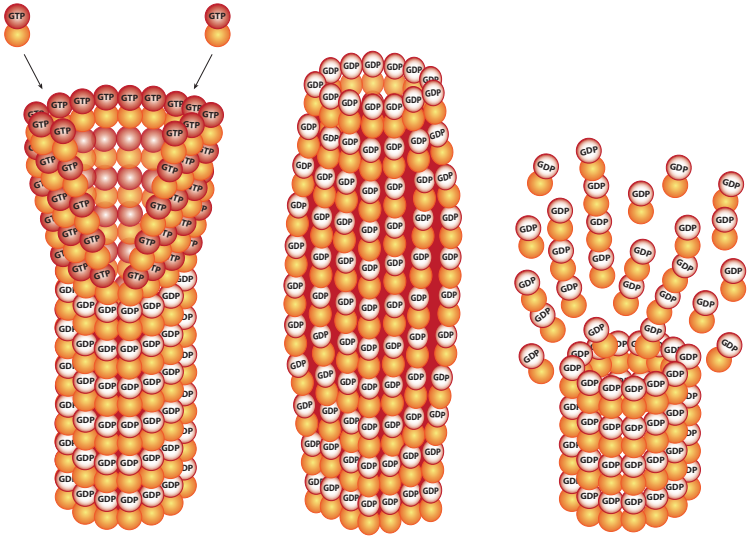

Figure 4. Microtubules exhibit dynamic instability. GTP-bound αβ-tubulin dimers are added onto the microtubule. Once the GTP is hydrolyzed, the conformational shift strains the microtubule, which will tend to break apart unless new tubulin dimers are added to stabilize the structure.

Again like actin, the tubulin itself has enzymatic activity, and over time, the GTPase activity hydrolyzes the GTP to GDP and phosphate. This changes the attachment between β-tubulin of one dimer and the α-tubulin of the dimer it is stacked on because the shape of the subunit changes. Even though it isn't directly loosening its hold on the neighboring tubulin, the shape change causes increased stress as that part of the microtubule tries to push outward. This is the basis of a property of microtubules known as *dynamic instability*. If there is nothing to stabilize the microtubule, large portions of it will fall apart. However, as long as new tubulin (which will have GTP bound) is being added at a high enough rate to keep a section of low-stress "stable"-conformation microtubule (called the GTP cap) on top of the older GDP-containing part, then it stabilizes the overall microtubule. When new tubulin addition slows down, and there is only a very small or nonexistent cap, then the microtubule undergoes a *catastrophe*

in which large portions rapidly break apart. Note that this is a very different process than breakdown by depolymerization, which is the gradual loss of only a few subunits at a time from an end of the microtubule. Depolymerization also occurs, and like with actin, is determined partially by the relative concentrations of free tubulin and microtubules.

From a physical standpoint, the microtubule is fairly strong, but not very flexible. A microfilament will flex and bend when a deforming force is applied (imagine the filament anchored at the bottom end standing straight up, and something pushing the tip to one side). The microtubule in the same situation will bend only slightly, but break apart if the deforming force is sufficient. There is, of course, a limit to the flexibility of the microfilament and eventually, it will also break. Intermediate filaments are slightly less flexible than the microfilaments, but can resist far more force that either microfilaments or microtubules.

Microtubule Organizing Centers

Microtubules, like microfilaments, are dynamic structures, changing in length and interactions to react to intra- and extra-cellular changes. However, the general placement of microtubules within the cell is significantly different from microfilaments, although there is some overlap as well as interaction. Microfilaments do not have any kind of global organization with respect to their polarity. They start and end in many areas of the cell. On the other hand, almost all microtubules have their (-) end in a perinuclear area known as the MTOC, or microtubule organizing center and they radiate outward from that center. Since the microtubules all radiate outward from the MTOC, it is not surprising that they are concentrated more centrally in the cell than the microfilaments which, as mentioned above, are more abundant around the periphery of the cell. In some cell types (primarily animal), the MTOC contains a structure known as the *centrosome*. This consists of a centriole (two short barrel-shaped microtubule-based structures positioned perpendicular to each other) and a poorly defined concentration of pericentriolar material (PCM). The centriole is composed of nine fibrils, all connected to form a cylinder, and each also connected by radial spokes to a central axis. The electron micrograph in figure 5 shows a cross-section of a centriole. In it, each fibril is shown to actually be a fused triplet of microtubules.

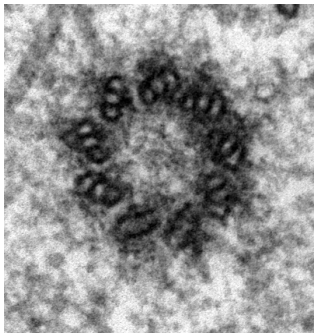

Figure 5. An electron micrograph depicting the cross-section of a centriole in an embryonic mouse brain cell. L. Howard and M. Marin-Padilla, 1985

Inhibition of γ-tubulin function by antibody blocking, RNA interference of expression, and gene knockout confirm that without γ-tubulin function, the microtubule structures did not form. In addition, it appears to be play roles in coordination of late mitosis (anaphase onwards).

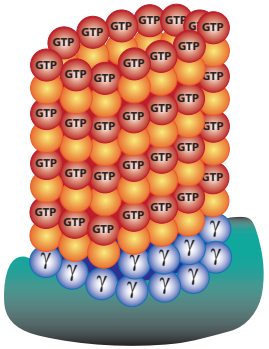

Figure 6. γ-tubulin ring complex facilitates microtubule nucleation.

However, in each triplet, only one is a complete microtubule (designated the A tubule), while the B and C tubules do not form complete tubes (they share a wall with the A and B tubules, respectively). Interestingly, the centrioles do not appear to be connected to the cellular microtubule network. However, whether there is a defined centrosome or not, the MTOC region is the point of origin for all microtubule arrays. This is because the MTOC contains a high concentration of γ-tubulin. Why is this important? With all of the cytoskeletal elements, though it is most pronounced with microtubules, the rate of nucleation, or starting a microtubule is significantly slower than the rate of elongating an existing structure. Since it is the same biochemical interaction, the assumption is that the difficulty lies in getting the initial ring of dimers into position. The γ-tubulin facilitates this process by forming a γ-tubulin ring complex that serves as a template for the nucleation of microtubules (fig. 6). This is true both in animal and fungal cells with a single defined MTOC, as well as in plant cells, which have multiple, dispersed sites of microtubule nucleation.

Transport on the Cytoskeleton

While it can be useful to think of these cytoskeletal structures as analogous to an animal skeleton, perhaps a better way to remember the relative placement of the microtubules and microfilaments is by their function in transporting intracellular cargo from one part of the cell to another. By that analogy, we might consider the microtubules to be a railroad track system, while the microfilaments are more like the streets. By the same analogy, we can suggest that the microtubule network and microfilament network are connected at certain points so that when cargo reaches its general destination by microtubule (rail), then it can be taken to its specific address by microfilament. Let's extend this analogy a bit further. If the microtubules and microfilaments are the tracks and streets, then what are the trains and trucks? Ah, an astute question, Grasshopper. On the microtubules, the "trains" are one of two families of molecular motors: the kinesins and the dyneins.

We can generalize somewhat and say that the kinesins drive towards the (+) end (toward periphery of cell) while the dyneins go toward the (-) end (toward the MTOC). On actin microfilaments, the molecular motors are proteins of the myosin family. At this point, the analogies end, as the functioning of these molecular motors is very different

from locomotion by train or truck. Finally, one might question the biological need for such a transport system. Again, if we analogize to human transport, then we could say that transport via simple diffusion is akin to people carrying packages randomly about the cell. That is to say, the deliveries will eventually be made, but you wouldn't want to count on this method for time-critical materials. Thus a directed, high-speed system is needed to keep cells (particularly larger, eukaryotic cells) alive.

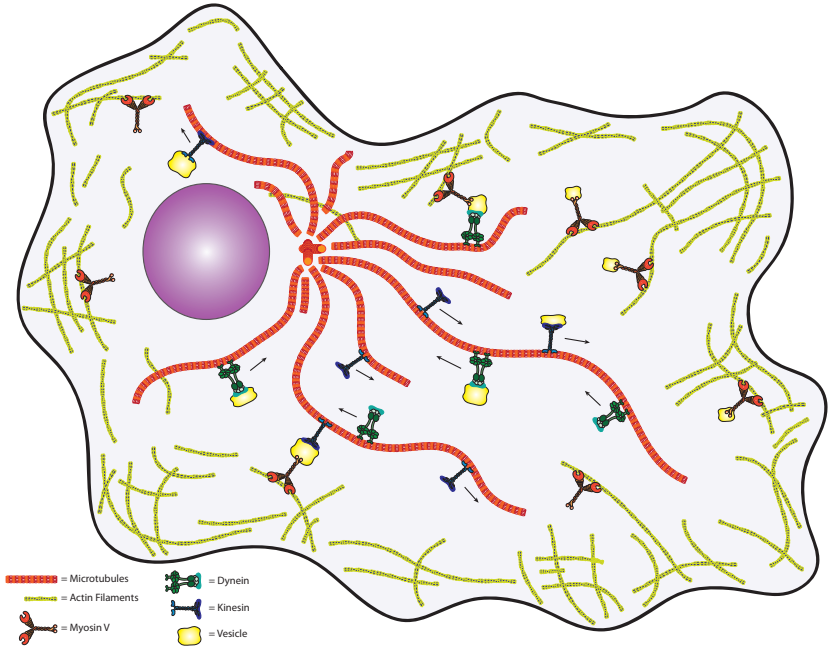

Figure 7. Transport on microtubules and microfilaments.

All of the kinesins and dyneins have a few key commonalities. There is a catalytic energy-releasing "head" connected to a hinge or neck region allowing the molecule to flex or "step", and there is a cargo-carrying tail beyond that (fig. 8). The head of a kinesin or dynein catalyzes the hydrolysis of ATP, releasing energy to change its conformation relative to the neck and tail of the molecule, allowing it to temporarily release its grip on the microtubule, swivel its "hips" around to plant itself a "step" away, and rebind to the microtubule (fig. 9). On the actin microfilaments, the myosins, of which there are also many types (some depicted in fig. 10) are the molecular motors. Their movement is different from dyneins and kinesins, as will be described in the next section, but also uses the energy of ATP hydrolysis to provide energy for the conformational changes needed for movement. We have introduced the motors, but considering the enormous diversity in the molecules that need to be transported around a cell, it would be impos-

Although this type of transport occurs in all eukaryotic cells, a particularly well-studied case is axonal transport (also called axoplasmic transport) in neurons. Here, the transport of materials from the cell body (soma) to the tips of the axons can sometimes traverse very long distances up to several meters in larger animals, and must do so in a timely manner. Axonal transport is generally classified as anterograde (from soma to axon terminal) or retrograde (from terminals back). The types of material transported in these two directions is very different: much of the anterograde transport is protein building blocks for extending the axon or synaptic vesicles containing neurotransmitters; retrograde transport is mostly endocytic vesicles and signaling molecules. Axonal transport is also categorized as fast and slow. Slow transport is primarily the movement of proteins directly bound to the motors, and they can move from from 100 μm per day (SCa, slow component a) up to 3mm/day (SCb). In comparison, fast transport is generally movement of vesicles, and can vary from 50 to 400 mm/day. The mechanism of slow transport had been debated for over a decade until 2000, when direct visualization of fluorescently labeled neurofilaments in transport showed that the actual movement of the proteins was very similar to the movement in fast axonal transport, but there were many pauses in the transport, a "stop and go" mechanism rather moving from source to destination continuously.

sible for the motors to directly bind to all of them. In fact, the motors bind to their cargo via adapter molecules that bind the motor on one side, and a cargo molecule or vesicle on the other. Further examination of the cargo and the routing of the cargo by address markers (SNAREs) was discussed in the vesicular transport chapter.

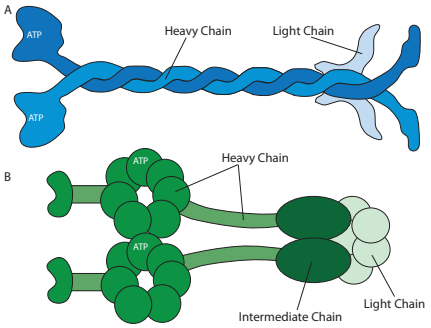

Figure 8. Kinesin (A) and Dynein (B) are motor proteins that move along microtubules. Generally, kinesins move to the (+) end while dyneins move to the (-) end. Their motor function requires ATP hydrolysis. ATP binding sites are marked in white.

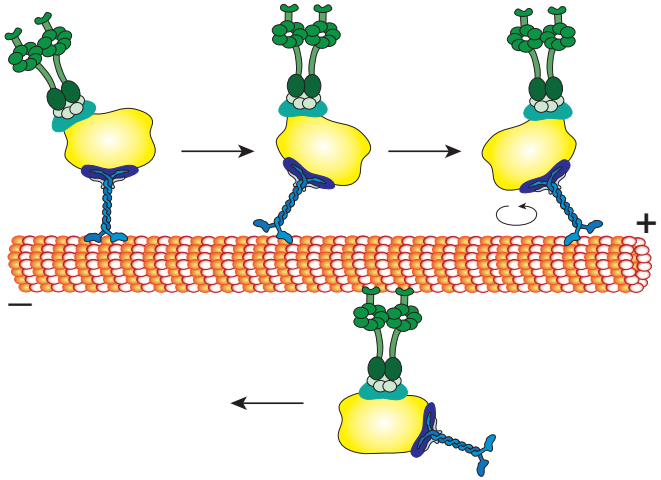

Figure 9. A cargo vesicle (yellow) can be simultaneously bound by dynein (green) and kinesin (blue) via adapter proteins. This top side also depicts the movement of the kinesin, in which binding of ATP causes one "foot" to release, and hydrolysis of ATP causes the molecule to swivel the other foot in front.

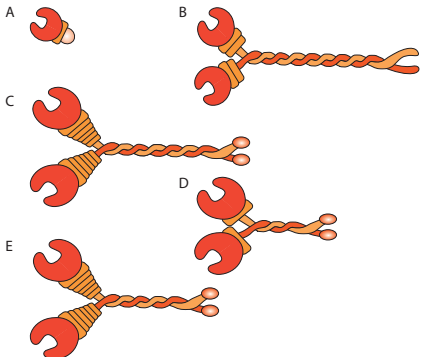

Figure 10. Selected Myosins. (A) Type I myosin, primarily for binding membranes to f-actin, including endocytic vesicles. (B) Type II myosin, binds f-actin on both ends to slide filaments against each other. (C) Type V myosin, used in vesicular transport. (D) Type VI myosin, used in endocytosis. (E) Type XI myosin, a fast myosin used in cytoplasmic streaming in plant cells.

Actin - Myosin Structures in Muscle

The motor proteins that transport materials along the acting microfilaments are similar in some ways, such as the globular head group that binds and hydrolyzes ATP, yet different in other ways, such as the motion catalyzed by the ATP hydrolysis. Much of the f-actin and myosin in striated and cardiac muscle cells is found in a peculiar arrangement designed to provide a robust contractile response over the entire length of the cell. The sarcomere is an arrangement of alternating fibers of f-actin (also known as "thin fibers" based on their appearance in electron micrographs) and myosin II (or "thick fibers"). Although we do not normally think of the motor protein as a fiber, in this case the tails of the myosin II molecules intertwine to form a continuous fiber of myosin molecules. As the contractile cycle proceeds, the myosin molecules grip the adjacent actin fibers, and move them. In fig. 11, you can see that a sarcomere is constructed so that the stationary myosin fibers are located centrally, with two parallel sets of actin fibers interspersed between the myosin fibers, to the left and the right of the center. Note that the actin fibers do not cross the center line, and that at the

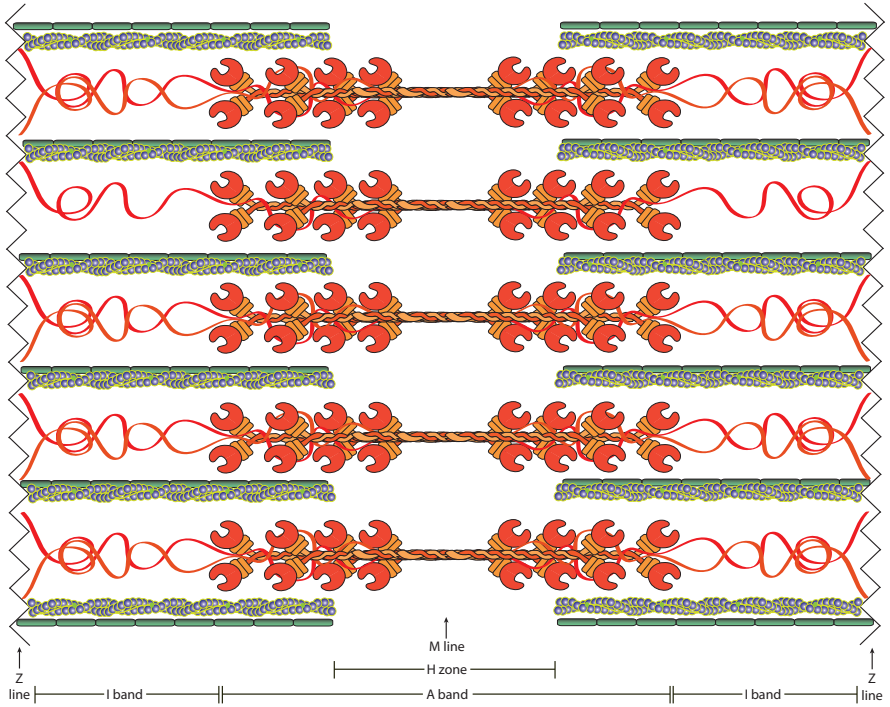

Figure 11. Sarcomere. Myosin II is depicted as in fig. 9, but here entwined with other myosins to form the thick filament. They are supported and anchored by titin (shown as long tangled orange ribbons). The myosin heads act on the actin filaments (blue), pulling them towards each other in a contractile movement.

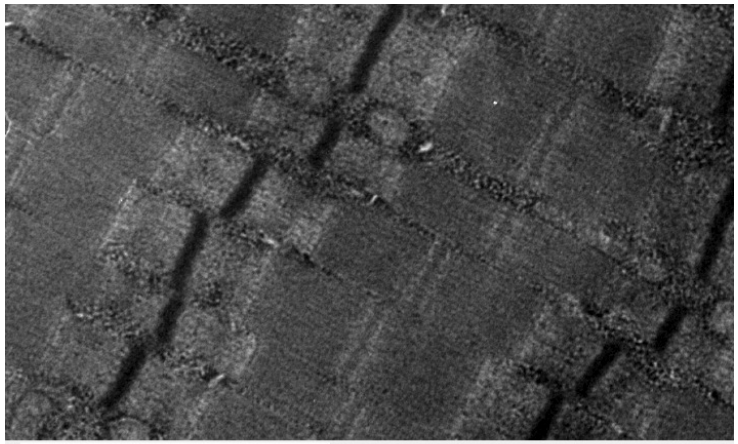

Figure 12. Human skeletal muscle is organized into sarcomeres. The dark Z lines are a clear reference point in comparing this to diagram in fig.11. This electron micrograph placed in the public domain by L. Howard.

center, the myosin molecules switch orientation. The physiological effect of this is that the actin filaments are all pulled inwards toward the center of the sarcomere. The sarcomere in turn, is merely one of many connected together to form a myofibril. The myofibrils extend the length of the muscle cell.

When the myosin head is in its resting state, it is tightly attached to the actin filament. In fact, *rigor mortis* occurs in dead animals because there is no more ATP being made, and thus the sarcomeres are locked into place. Rigor begins approximately 2-3 hours after death in humans, after reserves of ATP are depleted. When the body relaxes again in about 3 days, it is due to the decomposition and breakdown of the actin and myosin

OK, maybe you've watched CSI or Bones, etc enough to already know this, but pretty neat nonetheless, right?

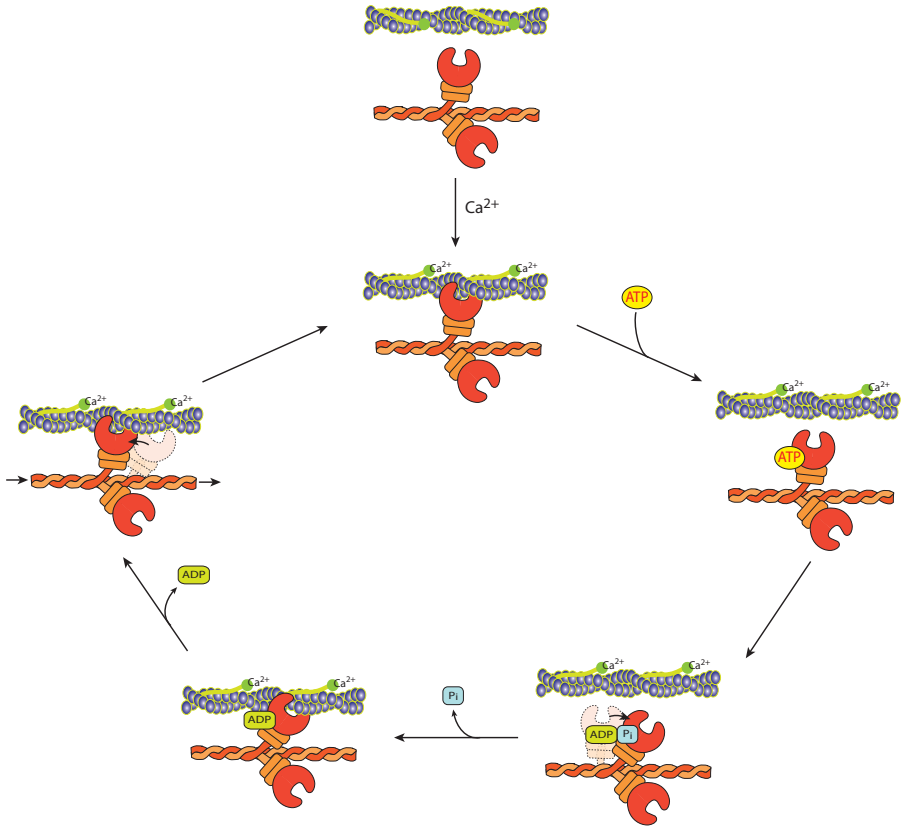

Figure 12. The myosin power stroke. Myosin can only attach to f-actin if there Ca^{++} available to bind troponin (green) and move tropomyosin (yellow) out of the binding groove. When ATP binds to the myosin head, it releases the f-actin. Hydrolysis of the ATP leads to cocking of the myosin head (moving it relative to the f-actin). As P_i leaves the myosin head, it reattaches to the f-actin, but slightly displaced from its original binding site. ADPis then released and the myosin undergoes a power stroke in which it springs back to its original position, moving the f-actin along with it.

proteins. However, while they are still living animals, ATP is generally available, and it can bind to the myosin head, causing it to lose affinity for the f-actin, and let go (fig. 12). At this point, no significant movement has occurred. Once the ATP is hydrolyzed though, the myosin head can reattach to the f-actin a little further down the filament than it had originally. The energy released is stored in the neck region. The ADP and P_i are still attached to the myosin head as well. The next step is for the P_i to drop off the myosin, leading to the power stroke. The neck of the myosin swivels around, leading to a translocation of the head by approximately 10 nm for myosin II. The distance of translocation varies depending on the type of myosin, but it is not yet clear whether the length of the neck is proportional to the displacement of the head. Finally, the ADP drops off the myosin head, increasing the affinity of the head for the f-actin.

The sarcomere structure described in the first paragraph was incomplete in order to place the major players clearly in their roles. There are other proteins in the sarcomere with important structural and regulatory functions. One of the key regulatory components is tropomyosin. This is a fibrous protein that lies in the groove of an actin microfilament and blocks access to the myosin binding site. Tropomyosin attaches to the microfilament in conjunction with a multi-subunit troponin complex. When Ca^{++} is available, it can bind to troponin-C, leading to a conformational change that shifts the position of tropomyosin to reveal the myosin binding site. This is the primary point of control for muscle contraction: recall that intracellular Ca^{++} levels are kept extremely low because its primary function is in intracellular signaling. One way that the Ca^{++} levels are kept that low is to pump it into a reservoir, such as the endoplasmic reticulum.

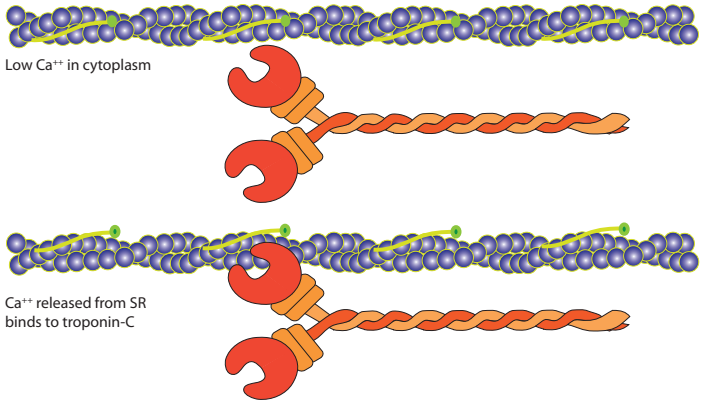

Fig. 13. In low Ca^{++} conditions, tropomyosin (yellow line) is held in the myosin-binding groove of f-actin (blue) by a tripartite troponin complex (light green). Once Ca^{++} levels increase, it can bind to troponin-C, causing a conformational shift that moves the tropomyosin out of the way so that myosin (orange) can bind the actin microfilaments.

In muscle cells, there is a specialization of the ER called the sarcoplasmic reticulum (SR) that is rich in Ca^{++} pumps and Ca^{++}. When a signal is sent from a controlling nerve cell to the muscle cell, it causes a depolarization of the muscle cell membrane. This consequently depolarizes a set of membranes called the transverse tubules (T-tubules) that lie directly on parts of the sarcoplasmic reticulum. There are proteins on the t-tubule surface that directly interact with a set of Ca^{++} channel proteins, holding the channel closed normally. When the t-tubule is depolarized, the proteins change shape, which changes the interaction with the Ca^{++} channels on the SR, and allows them to open. Ca^{++} rushes out of the SR where it is available to troponin-c. Troponin-C bound to Ca^{++} shifts the tropomyosin away from the actin filament, and the myosin head can bind to it. ATP can bind the myosin head to start the power stroke cycle, and voila, we have controlled muscle cell contraction.

In addition to the "moving parts", there are also more static, structural, proteins in the sarcomere (fig. 11). *Titin* is a gigantic protein (the largest known, at nearly 3 MDa), and can be thought of as something of a bungee cord tether to the myosin fiber. Its essential purpose is to prevent the forces generated by the myosin from pulling the fiber apart. Titin wraps around the myosin fiber and attaches at multiple points, with the most medial just near the edge of the H zone. At the Z-line, titin attaches to a telethonin complex, which attach to the Z-disk proteins (antiparallel α-actinin). Titin also interacts with obscurin in the I-band region, where it may link myofibrils to the SR, and in the M-band region it can interact with the Ca^{++}-binding protein calmodulin-1 and TRIM63, thought to acts as a link between titin and the microtubule cytoskeleton. There are multiple isoforms of titin from alternative splicing, with most of the variation coming in the I-band region.

Of course in an actual muscle (fig. 14), what happens is that nerves grow into the muscle and make synaptic connections with them. At these synaptic connections, the nerve cell releases neurotransmitters such as acetylcholine (ACh), which bind to receptors (AChR) on the muscle cell. This then opens ion channels in the muscle cell membrane, triggering a voltage change across that membrane, which also happens to affect the nearby membrane of the transverse tubules subsequently opening Ca^{++} channels in the SR. The contraction of sarcomeres can then proceed as already described above.

The SR is a specialization of part of the endoplasmic reticulum, and contains a high concentration of Ca^{++} ions because the SR membrane is embedded with Ca^{++} pumps (ATPases) to keep the cytoplasmic concentration low and sequester the Ca^{++} ions inside the SR. This is regulated by phosphorylation and $[Ca^{++}]$ via a regulatory protein such as phospholamban (in cardiac muscle). Phospholamban is an integral membrane protein of the SR that normally associates with and inhibits the Ca^{++} pump. However when it is phosphorylated, or as cytoplasmic Ca^{++} levels rise, the phospholamban releases from the Ca^{++} pump and allows it to function.

Disturbances to the proper formation of the titin-based support structure can be a cause of dilated cardiomyopathy, and from that, congestive heart failure. Some 20-30% of cases of dilated cardiomyopathy are familial, and mutations have been mapped to the N-terminal region of titin, where the protein interacts with telethonin. Defects in titin are also being investigated with respect to chronic obstructive pulmonary disease, and some types of muscular dystrophy.

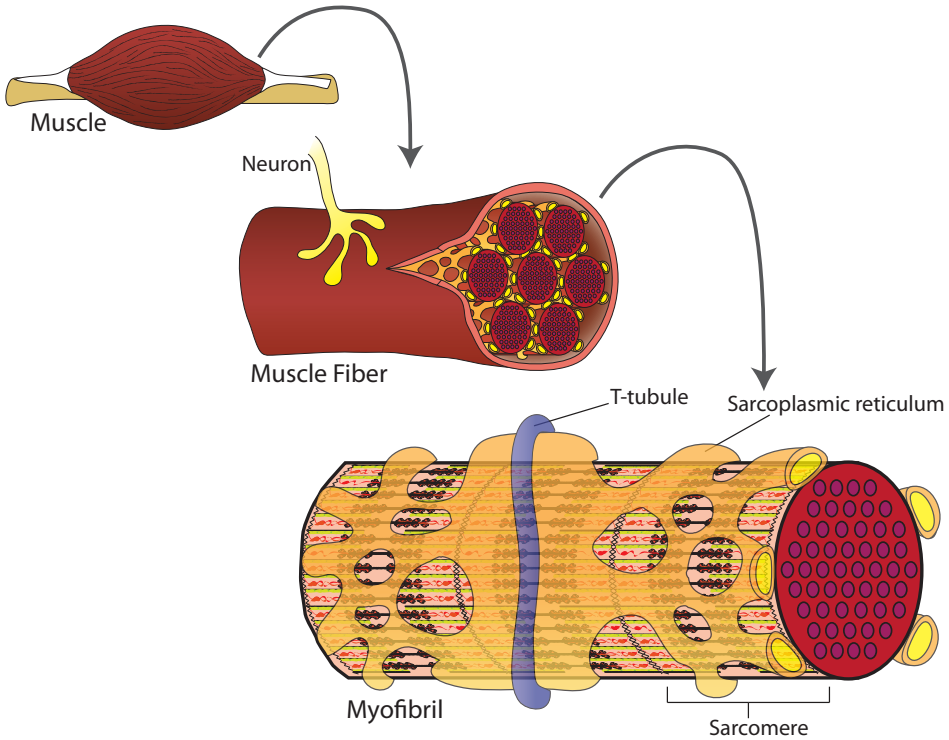

Figure 14. The sarcomere in context. The sarcomere of figures 11 and 12 is one tiny contractile unit within an array that forms a myofibril. The myofibril is one of many within a muscle cell, surrounded by the sarcoplasmic reticulum, a specialized extension of the ER that sequesters Ca^{++} until T-tubule excitation causes its release.

Cytoskeletal Dynamics

In the early development of animals, there is a huge amount of cellular rearrangement and migration as the roughly spherical blob of cells called the blastula starts to differentiate and form cells and tissues with specialized functions. These cells need to move from their point of birth to their eventual positions in the fully developed animal. Some cells, like neurons, have an additional type of cell motility - they extend long processes (axons) out from the cell body to their target of innervation. In both neurite extension and whole cell motility, the cell needs to move first its attachment points and then the bulk of the cell from one point to another. This is done gradually, and uses the cytoskeleton to make the process more efficient. The major elements in cell motility are changing the point of forward adhesion, clearing of internal space by

myosin-powered rearrangement of actin microfilaments and the subsequent filling of that space with microtubules.

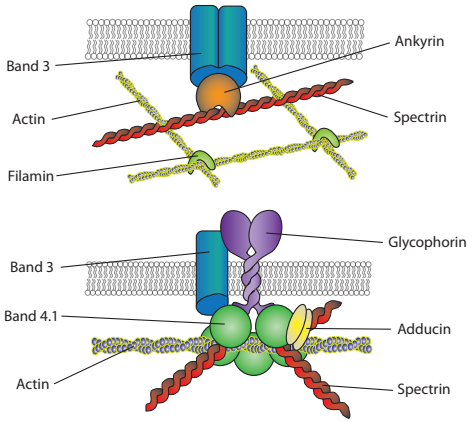

Figure 15. Membrane to microfilament linkage complexes in erythrocytes involve spectrin.

For force to be transmitted, the membrane must be attached to the cytoskeleton. In fact, signaling (chap. 14) from receptors in the membrane can sometimes directly induce rearrangements or movements of the cytoskeleton via adapter proteins that connect actin (or other cytoskeletal elements) to transmembrane proteins such as integrin receptors. One of the earliest experimental systems for studies of cytoskeleton-membrane interaction was the erythrocyte (red blood cell). The illustrations at right (fig. 15) show some of the interactions of an extensive actin microfilament network with transmembrane proteins. Ankyrin and spectrin are important linkage proteins between the transmembrane proteins and the microfilaments. This idea of building a protein complex around the cytoplasmic side of a transmembrane protein is ubiquitous, and scaffolding (linking) proteins are used not only in connecting the extracellular substrate (via transmembrane protein) to the cytoskeleton, but also to physically connect signaling molecules and thus increase the speed and efficiency of signal transduction.

Accessory proteins to actin filaments and microtubules were briefly mentioned earlier. Among other functions, they can control polymerization and depolymerization, form bundles, arrange networks, and bridge between the different cytoskeletal networks. For actin, the primary polymerization control proteins are profilin, which promotes polymerization and thymosin β_4, which sequesters g-actin. The minus end capping proteins Arp 2/3 complex and tropomodulin, and the plus end capping proteins CapZ, severin, and gelsolin can stabilize the ends of f-actin. Finally, cofilin can increase depolymerization from the (-) end.

Profilin has two activities that promote polymerization. First, it is a nucleotide exchange factor that removes ATP bound to g-actin, and replaces it with ADP. This sounds counterintuitive, but keep reading through to the next paragraph. Second, when bound to a g-actin, it increases the rate of addition to actin microfilaments. It does so by binding to the end opposite the ATP-binding site, leaving that site and that side open to binding both ATP and the (+) end of a microfilament. Profilin can be found

both in the cytoplasm at large, and associated with phospholipids (PIP_2) and membrane proteins, to control such processes as leading edge remodeling of f-actin cytoskeletal structures.

Thymosin β_4 regulates microfilament assembly by controlling the available pool of g-actin. We already stated that greater concentrations of g-actin can increase polymerization rates. However, because of the highly dynamic nature of the actin cytoskeleton, the time constraints of degrading and producing new actin would prevent the fast-response control necessary. Therefore, the optimal mechanism is to maintain a large pool of g-actin monomers, but regulate its availability by tying it up with a sequestering protein - thymosin β_4. Thymosin β_4 has a 50x higher affinity for g-actin-ATP than for g-actin-ADP, so here is where profilin comes back into the picture. Profilin exchanges the ATP of a $T\beta_4$-g-actin-ATP complex for an ADP. The result is that the $T\beta_4$ releases the g-actin-ADP, allowing it to enter the general pool for building up filaments.

Increased depolymerization and slowing or cessation of polymerization can gradually break down f-actin structures, but what if there is a need for rapid breakdown? Two of the capping proteins previously mentioned, gelsolin and severin, have an alternate mode of action that can sever actin microfilaments at any point by binding alongside an actin filament and altering the conformation of the subunit to which it is bound. The conformational change forces the actin-actin interaction to break, and the gelsolin or severin then remains in place as a (+) end capping protein.

Gelsolin is inhibited by the phospholipid PIP_2. Phospholipase C, which breaks down PIP_2 can also increase cytosolic Ca^{++}, which is an activator of gelsolin. Thus it is possible to rapidly upregulate gelsolin activity by PLC signaling.

On the microtubule side of things, due to dynamic instability, one might think that a severing enzyme is not needed, but in fact, spastin and katanin are microtubule-severing proteins found in a variety of cell types, particularly neurons. There is also a $T\beta_4$-like protein for tubulin: Op18, or stathmin, which binds to tubulin dimers (not monomers), acting to sequester them and lower the working concentration. It is regulated by phosphorylation (which turns off its tubulin binding). Microtubule-associated proteins MAP1, MAP2, and tau (τ) each work to promote assembly of microtubules, as well as other functions. MAP1 is the most generally distributed of the three, with tau being found mostly in neurons, and MAP2 even more restricted to neuronal dendrites. These and some other MAPs also act to stabilize microtubules against catastrophe by binding alongside the microtubule and reinforcing the tubulin-tubulin interactions.

Mutations in spastin are linked to 40% of those spastic paraplegias distinguished by degeneration of very long axons. The severing ability of spastin appears to be required for remodeling of the cytoskeleton in response to neuronal damage.

Tau has a complicated biomedical history. Its normal function is clear - assembling, stabilizing, and linking microtubules. However, it is also found in hyperphosphorylated neurofibrillary tangles that are associated with Alzheimer's disease. A cause for Alzheimer's is not yet known, so it is still unclear whether the tau protein tangles are play a major role in any of the symptoms.

Finally, with respect to microfilament and microtubule accessory proteins, there are the linkers. Some of the aforementioned MAPs can crosslink microtubules either into parallel or mesh arrays, as can some kinesins and dyneins, although they are conventionally considered to be motor proteins. On the microfilament side, there are many known proteins that crosslink f-actin, many of which are in the calponin homology

domain superfamily, including fimbrin, α-actinin, b-spectrin, dystrophin, and filamin. Although they all can bind to actin, the shape of the protein dictates different types of interaction: for example, fimbrin primarily bundles f-actin in parallel to form bundles, while filamin brings actin filaments together perpendicularly to form mesh networks.

Cell Motility

There are a number of ways in which a cell can move from one point in space to another. In a liquid medium, that method may be some sort of swimming, utilizing ciliary or flagellar movement to propel the cell. On solid surfaces, those mechanisms clearly will not work efficiently, and the cell undergoes a crawling process. In this section, we begin with a discussion of ciliary/flagellar movement, and then consider the more complicated requirements of cellular crawling.

Cilia and flagella, which differ primarily in length rather than construction, are microtubule-based organelles that move with a back-and-forth motion. This translates to "rowing" by the relatively short cilia, but in the longer flagella, the flexibility of the structure causes the back-and-forth motion to be propagated as a wave, so the flagellar movement is more undulating or whiplike (consider what happens as you waggle a garden hose quickly from side to side compared to a short piece of the same hose). The core of either structure is called the axoneme, which is composed of 9 microtubule doublets connected to each other by *ciliary dynein* motor proteins, and surrounding a central core of two separate microtubules. This is known as the "9+2" formation, although the nine doublets are not the same as the two central microtubules. The A tubule is a full 13-protofilaments, but the B tubule fused to it contains only 10 protofilaments. Each of the central microtubules is a full 13 protofilaments. The 9+2 axoneme extends the length of the cilium or flagellum from the tip until it reaches the base, and connects to the cell body through a *basal body*, which is composed of 9 microtubule triplets arrange in a short barrel, much like the centrioles from which they are derived.

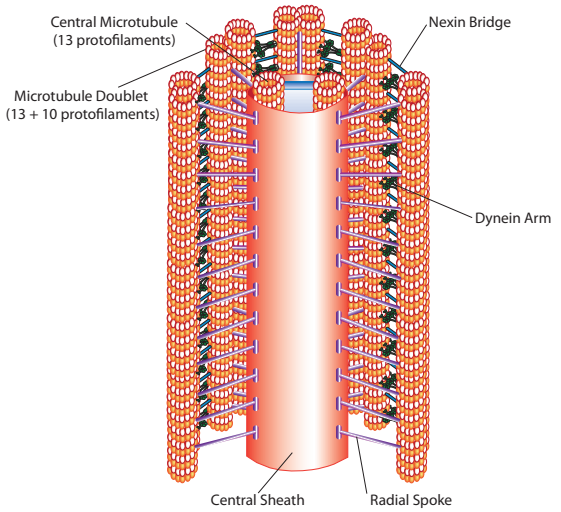

Figure 16. Partial (cutaway) diagram of an axoneme, the central bundle of cilia and flagella.

FG Syndrome is a genetically linked disease characterized by mental retardation, enlarged head, congenital hypotonia, imperforate anus, and partial agenesis of the corpus callosum. It has been linked to mutations in several X chromosome genes, including filamin A (FLNA, FLN1, located Xq28).

Mutations in dystrophin, which is a major muscle protein of the CD-domain superfamily, can result in Duchenne Muscular Dystrophy or the related but less severe Becker Muscular Dystrophy. The most distinctive feature is a progressive proximal muscular degeneration and pseudohypertrophy of the calf muscles. Onset of DMD is usually recognized before age 3 and is lethal by age 20. However, symptoms of BMD may not present until the 20s, with good probability of long-term survival. Although it is primarily a muscle-wasting disease, dystrophin is present in other cell types, including neurons, which may explain a link to mild mental retardation in some DMD patients. Like FLNA, the dystrophin gene is also located on the X chromosome (Xp21.2).

The ciliary dyneins provide the motor capability, but there are two other linkage proteins in the axoneme as well. There are *nexins* that join the A-tubule of one doublet to the B-tubule of its adjacent doublet, thus connecting the outer ring. And, there are *radial spokes* that extend from the A tubule of each doublet to the central pair of microtubules at the core of the axoneme. Neither of these has any motor activity. However, they are crucial to the movement of cilia and flagella because they help to transform a sliding motion into a bending motion. When ciliary dynein (very similar to cytoplasmic dyneins but has three heads instead of two) is engaged, it binds an A microtubule on one side, a B microtubule from the adjacent doublet, and moves one relative to the other. A line of these dyneins moving in concert would thus slide one doublet relative to the other, if (and it's a big "if") the two doublets had complete freedom of movement. However, since the doublets are interconnected by the nexin proteins, what happens as one doublet attempts to slide is that it bends the connected structure instead (fig. 17). This bend accounts for the rowing motion of the cilia, which are relatively short, as well as the whipping motion of the long flagella, which propagate the bending motion down the axoneme.

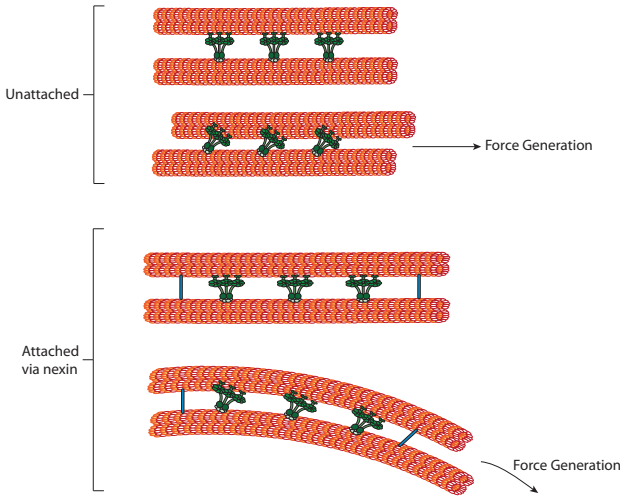

Figure 17. The nexin bridges connecting adjacent microtubule doublets transform the sliding motion generated by the ciliary dynein into a bending motion.

Although we think of ciliary and flagellar movement as methods for the propulsion of a cell, such as the flagellar swimming of sperm towards an egg, there are also a number of important places in which the cell is stationary, and the cilia are used to move fluid past the cell. In fact, there are cells with cilia in most major organs of the body. Several ciliary dyskinesias have been reported, of which the most prominent, primary ciliary dyskinesia (PCD), which includes Kartagener syndrome (KS), is due to mutation

This section refers only to eukaryotes. Some prokaryotes also have motile appendages called flagella, but they are completely different in both structure and mechanism. The flagella themselves are long helical polymers of the protein flagellin, and the base of the flagellin fibers is connected to a rotational motor protein, not a translational motor. This motor (fig. 18) utilizes ion (H^+ or Na^+ depending on species) down an electrochemical gradient to provide the energy to rotate as many as 100000 revolutions per minute. It is thought that the rotation is driven by conformational changes in the stator ring, nestled in the cell membrane.

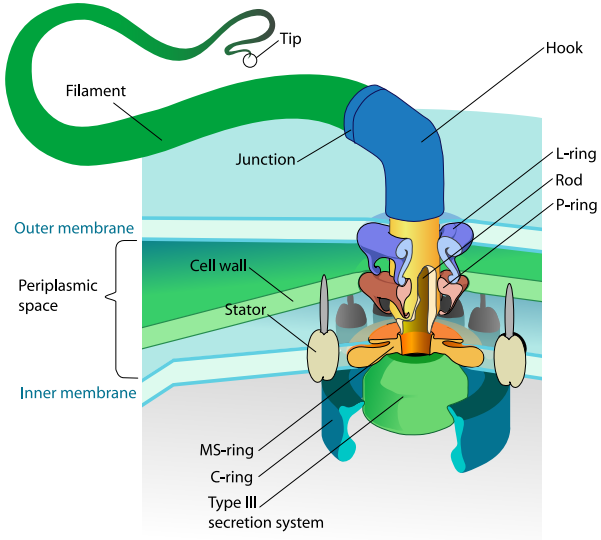

Figure 18. The bacteria flagellum is completely different from eukaryotic flagella. It is moved by a rotary motor driven by proton or Na^+ ion flow down the electrochemical gradient. Illustration released to public domain by M.R. Villareal.

of the DNAI1 gene, which encodes a subunit (intermediate chain 1) of axonemal (ciliary) dynein. PCD is characterized by respiratory distress due to recurrent infection, and the diagnosis of KS is made if there is also *situs inversus*, a condition in which the normal left-right asymmetry of the body (e.g. stomach on left, liver on right) is reversed. The first symptom is due to inactivity of the numerous cilia of epithelial cells in the lungs. Their normal function is to keep mucus in the respiratory track constantly in motion. Normally the mucus helps to keep the lungs moist to facilitate function, but if the mucus becomes stationary, it becomes a breeding ground for bacteria, as well as becoming an irritant and obstacle to proper gas exchange.

Situs inversus is an interesting malformation because it arises in embryonic development, and affects only 50% of PCD patients because the impaired ciliary function causes randomization of left-right asymmetry, not reversal. In very simple terms, during early embryonic development, left-right asymmetry is due in part to the movement of molecular signals in a leftward flow through the embryonic node. This flow is caused by the coordinated beating of cilia, so when they do not work, the flow is disrupted and randomization occurs.

Other symptoms of PCD patients also point out the work of cilia and flagella in the body. Male infertility is common due to immotile sperm. Female infertility, though less common, can also occur, due to dysfunction of the cilia of the oviduct and fallopian tube that normally move the egg along from ovary to uterus. Interestingly there is also a low association of hydrocephalus internus (overfilling of the ventricles of the brain with cerebrospinal fluid, causing their enlargement which compresses the brain tissue around them) with PCD. This is likely due to dysfunction of cilia in the ependymal cells lining the ventricles, and which help circulate the CSF, but are apparently not completely necessary. Since CSF bulk flow is thought to be driven primarily by the systole/diastole change in blood pressure in the brain, some hypothesize that the cilia may be involved primarily in flow through some of the tighter channels in the brain.

Cell crawling (fig. 19) requires the coordinated rearrangement of the leading edge microfilament network, extending (by both polymerization and sliding filaments) and then forming adhesions at the new forward-most point. This can take the form of filopodia or lamellipodia, and often both simultaneously. *Filopodia* are long and very thin projections with core bundles of parallel microfilaments and high concentrations of cell surface receptors. Their purpose is primarily to sense the environment. *Lamellipodia* often extend between two filopodia and is more of a broad ruffle than a finger. Internally the actin forms more into meshes than bundles, and the broader edge allows for more adhesions to be made to the substrate. The microfilament network then rearranges again, this time opening a space in the cytoplasm that acts as a channel for the movement of the microtubules towards the front of the cell. This puts the transport network in place to help move intracellular bulk material forward. As this occurs, the old adhesions on the tail end of the cell are released. This release can happen through two primary mechanisms: endocytosis of the receptor or deactivation of the receptor by signaling/conformational change. Of course, this oversimplification belies the complexities in coordinating and controlling all of these actions to accomplish directed movement of a cell.

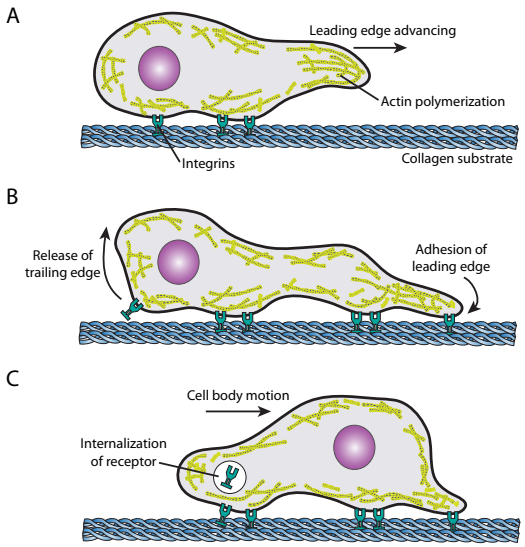

Figure 19. Cells crawl by (a) extending the leading edge primarily through remodeling of the actin cytoskeleton, (b) forming new adhesive contacts at that leading edge while releasing adhesions to the rear, and (c) bulk internal movement forward to "catch up" with the leading edge.

Once a cell receives a signal to move, the initial cytoskeletal response is to polymerize actin, building more microfilaments to incorporate into the leading edge. Depending on the signal (attractive or repulsive), the polymerization may occur on the same or opposite side of the cell from the point of signal-receptor activation. Significantly, the polymerization of new f-actin alone can generate sufficient force to move the membrane forward, even without involvement of myosin motors! Models of force generation are being debated, but generally start with the incorporation of new g-actin into a filament at its tip; that is, at the filament-membrane interface. Even if that might technically be enough, in a live cell, myosins are involved, and help to push and arrange filaments directionally in order to set up the new leading edge. In addition, some

One model of microfilament force generation, the Elastic Brownian Ratchet Model (Mogilner and Oster, 1996), proposes that due to Brownian motion of the cell membrane resulting from continuous minute thermal fluctuation, the actin filaments that push out towards the edges of the membrane are flexed to varying degrees. If the flex is large enough, a new actin monomer can fit in between the membrane and the tip of the filament, and when the now longer filament flexes back, it can exert a greater push on the membrane. Obviously a single filament does not geneate much force, but the coordinated extension of many filaments can push the membrane forward.

filaments and networks must be quickly severed, and new connections made, both between filaments and between filaments and other proteins such as adhesion molecules or microtubules.

How is the polymerization and actin rearrangement controlled? The receptors that signal cell locomotion may initiate somewhat different pathways, but many share some commonalities in activating one or more members of the Ras-family of small GTPases. These signaling molecules, such as Rac, Rho, and cdc42 can be activated by receptor tyrosine kinases (see RTK-Ras activation pathways, Chap. 14). Each of these has a slightly different role in cell motility: cdc42 activation leads to filopodia formation, Rac activates a pathway that includes Arp2/3 and cofilin to lamellipodia formation, and Rho activates myosin II to control focal adhesion and stress fiber formation. A different type of receptor cascade, the G-protein signaling cascade (also Chapter 14), can lead to activation of PLC and subsequent cleavage of PIP_2 and increase in cytosolic Ca^{++}. These changes, as noted earlier, can also activate myosin II, as well as the remodeling enzymes gelsolin, cofilin, and profilin. This breaks down existing actin structures to make the cell more fluid, while also contributing more g-actin to form the new leading edge cytoskeleton.

In vitro experiments show that as the membrane pushes forward, new adhesive contacts are made through adhesion molecules or receptors that bind the substrate (often cell culture slides or dishes are coated with collagen, laminin, or other extracellular matrix proteins). The contacts then recruit cytoskeletal elements for greater stability to form a focal adhesion (fig. 20). However, the formation of focal adhesions appears to be an artifact of cell culture, and it is unclear if the types of adhesions that form in vivo recruit the same types of cytoskeletal components.

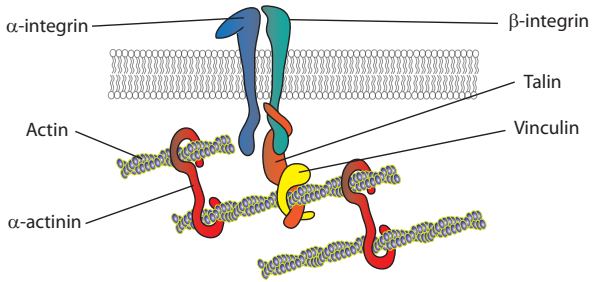

Figure 20. Focal adhesions form when integrins bind to an artificial ECM surface in cell culture dishes.

The third step to cell locomotion is the bulk movement of the cellular contents forward. The mechanisms for this phase are unclear, but there is some evidence that using linkages between the actin cytoskeleton at the leading edge and forward parts of the microtubule cytoskeleton, the microtubules are rearranged to form an efficient

transport path for bulk movement. Another aspect to this may be a "corralling" effect by the actin networks, which directionally open up space towards the leading edge. The microtubules then enter that space more easily than working through a tight actin mesh, forcing flow in the proper direction.

Finally, the cell must undo its old adhesions on the trailing edge. This can happen in a number of different ways. In vitro, crawling cells have been observed to rip themselves off of the substrate, leaving behind tiny bits of membrane and associated adhesion proteins in the process. The force generated is presumed to come from actin-myosin stress fibers leading from the more forward focal adhesions. However, there are less destructive mechanisms available to the cells. In some cases, the adhesivity of the cellular receptor for the extracellular substrate can be regulated internally, perhaps by phosphorylation or dephosphorylation of a receptor. Another possibility is endocytosis of the receptor, taking it off the cell surface. It could simply recycle up to the leading edge where it is needed (i.e. transcytosis), or if it is no longer needed or damaged, it may be broken down in a lysosome.

Much of the work on microtubule-actin interactions in cell motility has been done through research on the neuronal growth cone, which is sometimes referred to as a cell on a leash, because it acts almost independently like a crawling cell, searching for the proper pathway to lead its axon from the cell body to its proper synaptic connection (A.W. Schaefer et al, Dev. Cell 15: 146-62, 2008).