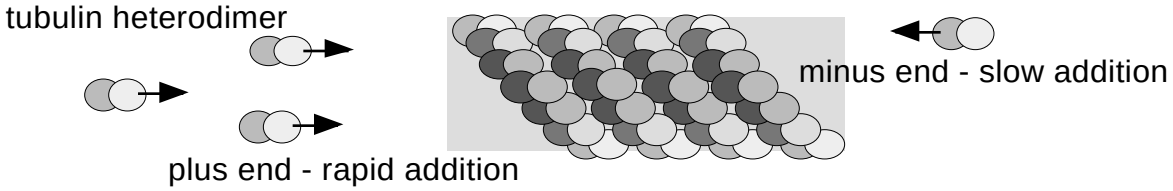
PHYS 101 Supplement #2 - Movement and motors in the cell

Cells are more than just passive objects responding to external stresses: they can actively change shape or move with respect to their environment. A very familiar example of cellular shape change is the contraction of our muscle cells. Less familiar, but very important to our health, is the locomotion of cells such as macrophages, which work their way through our tissues to capture and remove hostile cells and material. Another example of cell movement is the rotation of flagella (Latin plural for the noun *whip*) which extend from some cells and provide them with propulsion in a fluid medium. Structurally related to flagella are cilia (Latin plural for *eyelash*), which occur on the surfaces of some cells and wave in synchrony like tall grass in the wind, creating currents in their fluid environment. All of this motion involves, at least in part, two of the three common filaments in the cytoskeleton - actin filaments (8 nm diameter) and microtubules (25 nm diameter). We first describe the filaments and then the principal motor proteins involved with their movement.

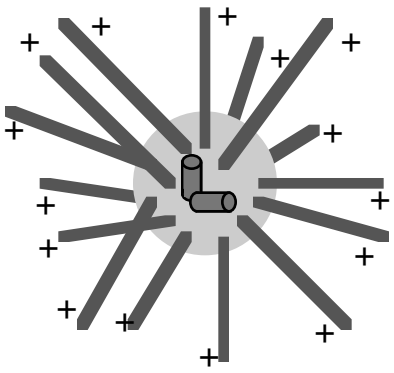
Actin and tubulin filaments

Actin and tubulin are dynamic polymers: their fundamental protein building blocks (*G*-actin or the tubulin heterodimer) can both polymerize and depolymerize, changing the length of the filament in the process. Further, each end of the filament grows and shrinks at a different rate: the rapidly growing end is called the plus end, while the slowly growing end is minus. The growth of dynamic filaments such as microtubules affects cell shape and motility. As shown in the cartoon on the next page, most animal cells have a nucleation region from which literally hundreds of microtubules radiate at any given instant. The filaments grow and shrink continuously, with their rapidly-growing plus ends extending out towards the cell periphery. Thus, the filaments can exert a collective pressure on the plasma membrane in some cases, and, as a result, can push the nucleation region towards the center of the cell. In addition, microtubules provide a mechanism for dragging chromatids away from each other during cell division.

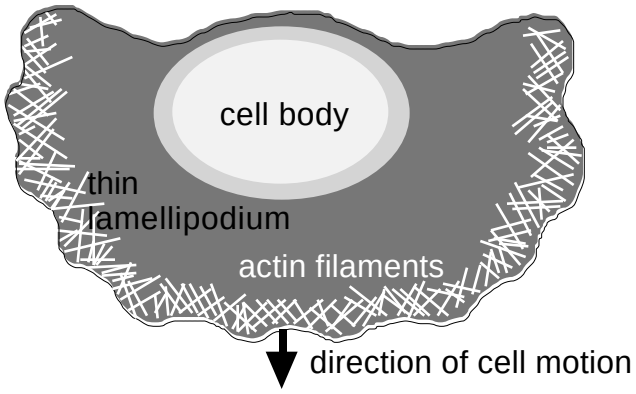
Filament growth also plays a role in cell locomotion. For example, fibroblasts and other cells can move along a substrate, adhering to it by spreading a sheet-like structure (the lamellipodium) across the surface. Other types of cells create extensions called pseudopods (literally *false feet*) to enable them to crudely walk over a surface. Observations of fluorescently labelled filaments show that the leading edge of such cells is actin-rich, with filament growth occuring at the cell boundary.



Microtubule filaments can grow or shrink through the gain or loss of individual tubulin heterdimers.



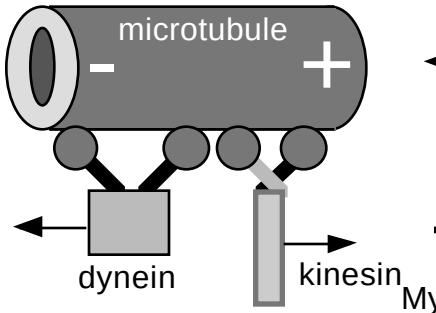
Long microtubules radiate from the cell's centrosome , their rapidly-growing (+) ends extending towards the cell boundary.

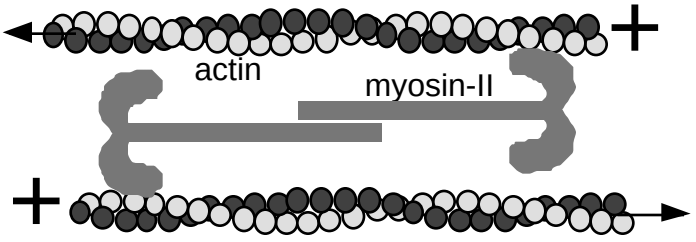


Actin filaments occur at high density within several microns of the leading edge of a moving cell on a substrate. In profile, the thin lamellipodium hugs the substrate and only the cell body has an appreciable thickness.

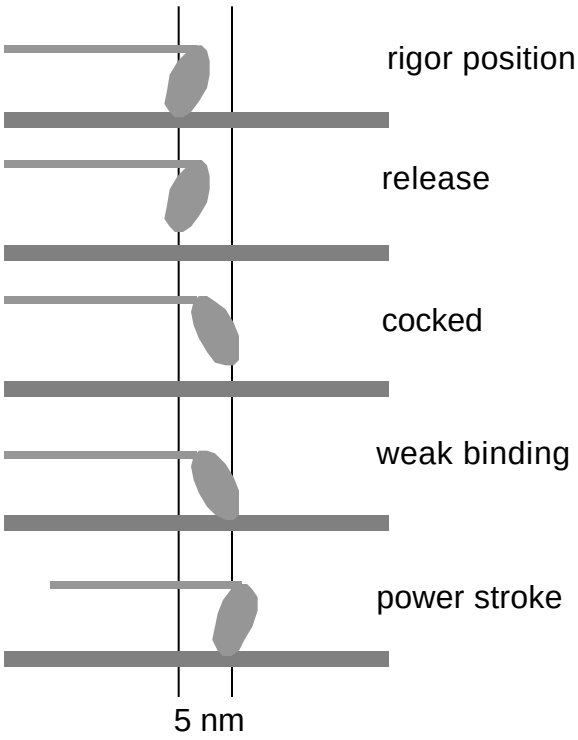
Motor proteins

Specialized proteins can slide along actin and tubulin filaments, the energy for their motion being provided by ATP hydrolysis. These so-called motor proteins can be grouped into three families: myosins associate with actin, while kinesins and dyneins associate with microtubules. Because actin filaments and microtubules have physically distinct plus and minus ends, the motor proteins have a preferred direction to their movement. Kinesin and dynein together provide a mechanism for transport in either direction from the nucleus with modest speeds at cellular length scales. Motors on axonal microtubules can walk at speeds up to 2-5 microns per second towards the end of the axon, a rate which permits a chemical cargo of neurotransmitter, for example, to be transported in 2-6 days from a production site in the brain to the end of a motor neuron a meter away.





Myosin walks towards the plus end of actin; with two myosins attached tail-to-tail, the minus ends of the actin filaments are pulled towards each other.



Proposed mechanism for a myosin motor protein to crawl along an actin filament.

Actin / *myosin systems*

The relative speed of actin and myosin falls in a range from 10^{-2} to 1 µm/s, depending on conditions in the cell. If the myosin head in the cartoon above moves forward at about 5 nm per completed step, and if 10^{1} - 10^{2} steps can be completed per second then the motor protein can advance at a rate of 0.05 to 0.5 µm/s. Although this speed appears slow, our muscles employ actin/myosin bundles joined end-to-end to amplify this motion to reach centimeters per second. The force generated by a single myosin motor has been measured by several techniques, generally yielding values in the 3 to 8 pN range. We can estimate the force generated in a single step knowing that the energy provided by the hydrolysis of an ATP molecule is about 20 $k_B T$. If 50% of the energy released results in a single 5 nm step of the motor, the resulting force must be $0.5 \bullet 20 k_B T$ / 5 nm = 8 pN from [*work*] = [*force*]•[*distance*], consistent with the measured values.

Motor / *microtubule systems*

The motor proteins kinesin and dynein walk (towards opposite ends of a microtubule) at a similar range of speeds, from 1 to 4 µm/s. Given that the polarity of microtubules in most cells has the plus end towards the cell boundary, these two molecular motors permit the transport of cargo-laden vesicles or organelles to and from the protein manufacturing sites near the cell's nucleus. Vesicle transport over long distances is particularly important for nerve cells, where chemical neurotransmitters produced in the cell body must be delivered to a synapse

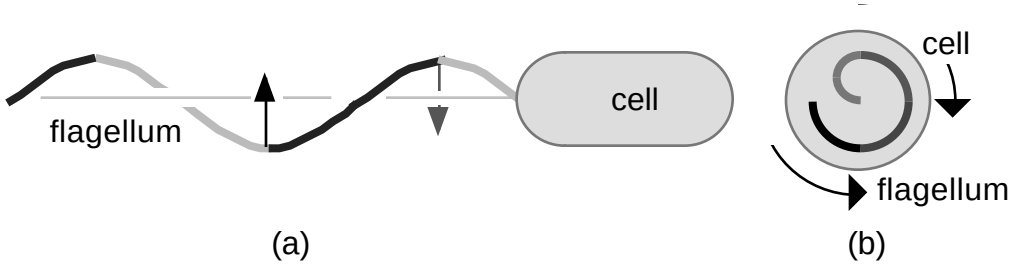
(anterograde motion) and waste products must be returned to the cell body for recycling (retrograde motion). The cargo carried by the motor proteins may range from small vesicles to mitochondria, which provide ATP to molecular motors themselves as they crawl along axonal microtubules.

The force generated by a motor on a single microtubule has been measured by several independent experiments to be 3-6 pN. Our general expectation of the force, based on nucleotide hydrolysis as we discussed for myosin, is 5 pN for 20 $k_B T$ released per hydrolysis resulting in a step of 8 nm at 50% efficiency.

Flagella

A common propulsion mechanism for swimming bacteria employs whiplike flagella, which can extend from both ends of a bacterium. Flagella have a typical length of 10 µm, although examples ten times this length are known, such that their length is usually several times that of the main body of the bacterium. Both torque and thrust are generated by a flagellum as it rotates about its axis, driven by a rotary motor. The filament executes a helical motion as its rotates, and acts like a propeller.

As illustrated in the cartoon below, the flagellar helix moves through a fluid at an angle with respect to the symmetry axis of the bacterium. The resistive force from the fluid can be resolved into two components: one generates a thrust along the symmetry axis while the other results in a torque around the axis. As shown, the rotation of the flagellum is balanced by the slower counter-rotation of the cell proper. The figure displays the rotation of *E. coli*: normally the filament rotates counter clockwise (CCW) as viewed looking along the axis towards the cell. When the appropriate flagella rotate CWW cooperatively, the cell can move forward at speeds of 20 µm/s in a mode of motion called a "run". However, the motor has a switch permitting it to run in reverse, or clockwise (CW); flagella rotate CW independently, resulting in no net thrust on the cell such that it "tumbles", losing its orientation. For *E. coli* under common conditions, a typical run lasts 1 second, and a typical tumble lasts 0.1 seconds with much variation.



(a) A flagellum adopts a helical shape as it rotates about the symmetry axis of a rod-shaped bacterium. (b) Looking along the axis towards the cell, the flagellum rotates counter clockwise to provide thrust, and the cell slowly rotates clockwise in response. Darker regions are closer to the viewer.

As a bacterium swims, its flagella may rotate at 100 revolutions per second (Hz) or more. Such rotational rates are comparable to an automobile engine, which runs comfortably at 30 Hz (or 2000 rpm) and reaches its operating limit at ~100 Hz; also like a car engine, flagellar motors fail catastrophically if driven too hard. A single flagellum attached to a fixed substrate can cause the cell body to rotate at 10 Hz, a lower rotation rate than the free flagellum because of viscous drag on the cell. Extensive measurements have been made of the torque generated by the *E. coli* flagellar motor; the torque decreases monotonically to zero at several hundred revolutions per second, depending on conditions. The magnitude of the torque lies in the range 2-6 x 10^{-18} N•m in a number of cells investigated to date.