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# Physical Principles in Biology

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## Chapter 7
## Molecular Motors

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Chapter 7

# Molecular Motors

The cells of nearly all (if not all) organisms contain protein complexes that are able to convert chemical energy into mechanical work. These motors include:

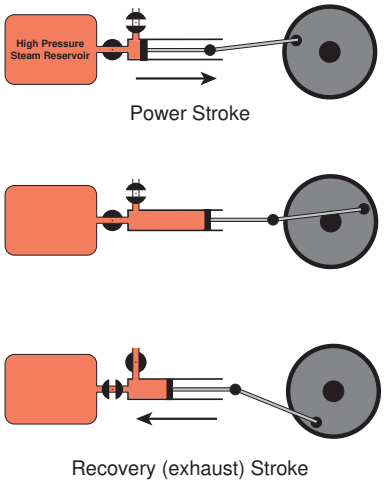
- Myosin, which generates force by interacting with actin in muscle and other cells.
- Kinesin and dynein, which both move along microtubules, but in opposite directions.
- The ATP synthase that produces ATP from ADP in mitochondria and aerobic bacteria, using the electrochemical potential of a $H^+$ gradient. Force generation is not part of the normal function of this protein, but its catalytic activity is coupled to rotary motion.
- The bacterial flagellar motor, which uses electrochemical gradients to rotate flagella and enable swimming.

In all of these motors, conformational changes in protein complexes are coupled to chemical changes, either ATP synthesis/hydrolysis or motion of ions across membranes.

## 7.1 Some Basic Principles

I. Steam engines

In thinking about how molecular motors might work, it is useful to consider first a simple engine on a larger scale, such as a steam engine.




Note the following important features of the engine illustrated above:

- The energy source for the engine is a pressure difference, which is created by a temperature difference.
- The free energy of the steam (its ability to do work) is lost as it expands, and its entropy increases.
- Expansion of the gas is coupled to movement of the piston and, in turn, turning the wheel and shaft.
- After the powerstroke, the momentum of the flywheel returns the engine to its starting state so that the cycle can be repeated.
- If the coupling between the steam expanding and the mechanical motions is disrupted, the free energy of the steam is wasted.
- The valves that control the flow of steam are essential, and their function must be coupled to the movement of the piston to ensure that each one opens and closes at the correct time in the cycle.

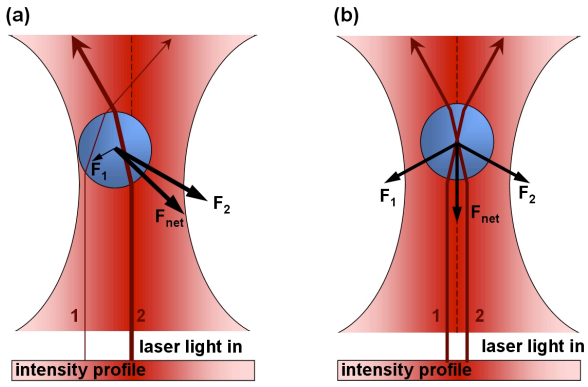
Some of these features can be found in molecular motors. Perhaps most important are the mechanisms that couple a highly favorable process with an unfavorable mechanical process. Molecular motors also function in a cyclical fashion. On the other hand, there are important differences between molecular motors and steam engines:

- There cannot be significant temperature differences at the molecular scale.
- The molecular structures have no significant inertia, so that a flywheel cannot restore the motor to its initial state.

II. Measuring forces at the molecular scale and stretching a DNA molecule

One of the technical advances that has enabled the study of molecular motors is the development of instruments capable of measuring forces at the molecular level. In the previous chapter, we briefly discussed one such instrument, the atomic force microscope (pages 189–190). Another instrument used to measure molecular forces and manipulate individual molecules is called an *optical trap* or *optical tweezers*. This type of instrument can be used to manipulate objects as small as individual atoms, but when used in studies of biological macromolecular strategies, the usual strategy is to attach the molecules to small glass or silica beads, typically about 1 $\mu$m in diameter, which are then manipulated. The optical trap is generated by focusing a beam of light into a narrow spot. When viewed from the side, this beam has an hour-glass shape with a narrow waist, as illustrated below[1]:

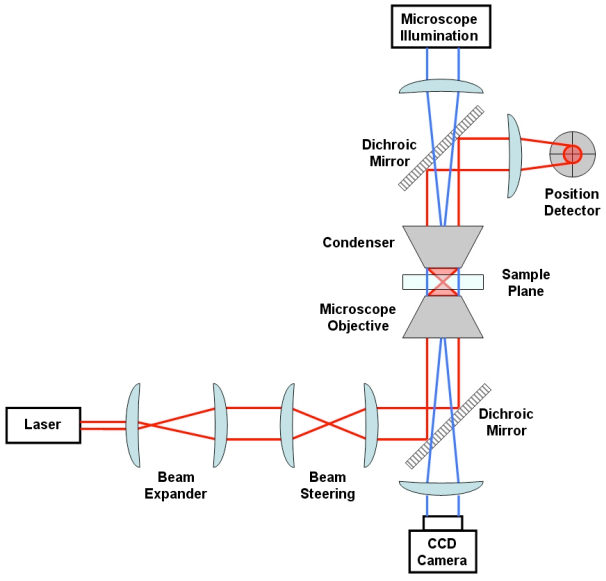
[1]Illustrations of the optical trap device are from https://en.wikipedia.org/wiki/Optical_tweezers




When a transparent bead is placed within the light beam, it acts as a small lens and refracts, or bends, the light. This effect represents a force of the bead acting on the light, and, by Newton's third law of motion, there must be an equal and opposite force acting on the bead. As shown in the diagram above, light rays entering the bead at different points generate forces in different directions, and the magnitudes of these forces are determined by the intensity of light at different postions in the beam. All of these forces, along with the gravitational force acting on the bead, are balanced when the bead is in the center of the beam and slightly above the beam waist, as shown in the right-hand side of the illustration.

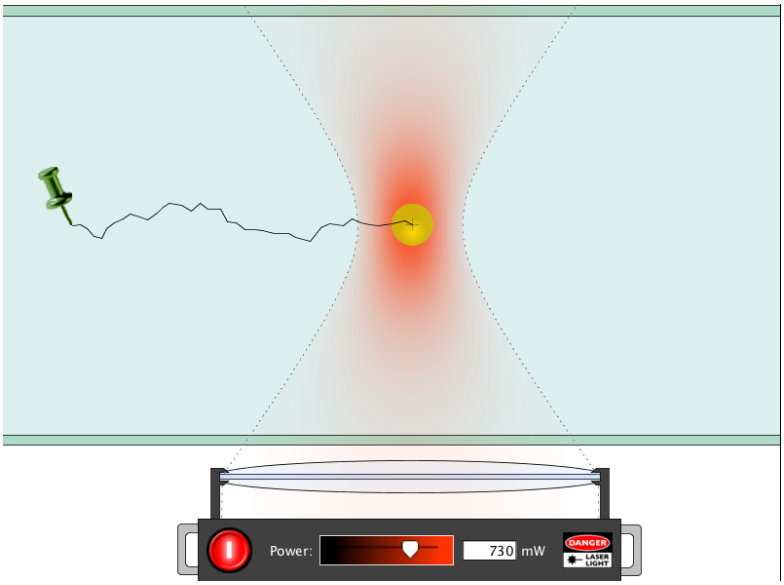
If an outside force acts on the bead to move it away from the center of the beam, the forces from the focused light will try to return the bead to the center. The magnitude of this restoring force increases as the bead is displaced from the beam center, until the outside force becomes great enough to pull the bead out of the beam altogether. The beam thus acts like a spring, and the displacement indicates the magnitude of the outside force, much like a spring scale, as found in a grocery store.

The construction of an optical trap device is quite intricate and requires very precise engineering. The schematic diagram below shows the main elements:



The apparatus is typically built around a conventional optical microscope, and the trapping beam is generated by a laser and focused by the microscope objective. In addition, there are lenses that are used to move, or steer, the beam. After passing though the sample, which contains the glass bead, the beam is focused on a position detecter, which can very precisely record the position of the beam as it is steered. In addition, illumination from the top of the microscope allows the position of the glass bead to be monitored from an image created in a camera below.

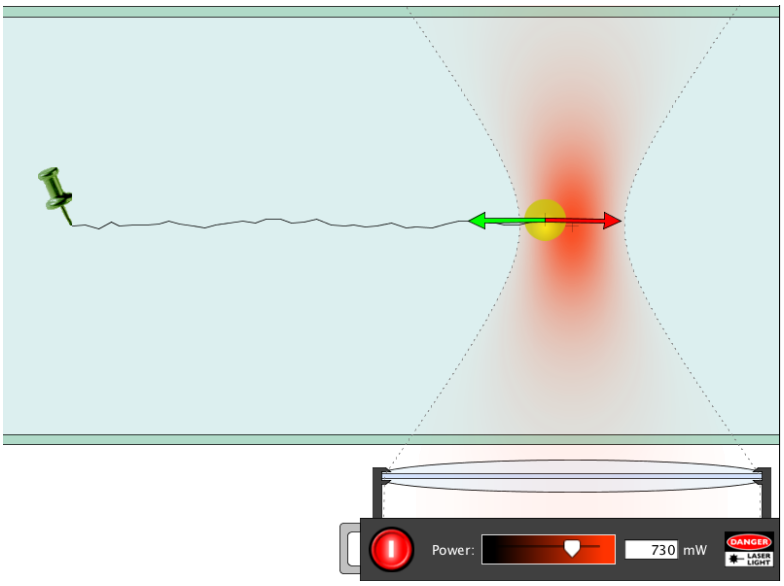
One application of optical traps has been to measure the forces exerted on DNA molecules as their ends are pulled appart. This is conceptually very similar to the protein stretching experiment described in the previous chapter. A highly schematic representation of the experimental apparatus is shown below:




This figure is a screen capture from a very clever educational simulation that allows the user to manipulate the virtual optical trap and follow the effects on the trapped bead and DNA molecule[2]. In this kind of experiment, one end of the DNA is somehow fixed to the microscope slide (as represented by the thumbtack), and the other is attached to the transparent bead. The focused laser beam is then manipulated to capture the bead and then move it about.

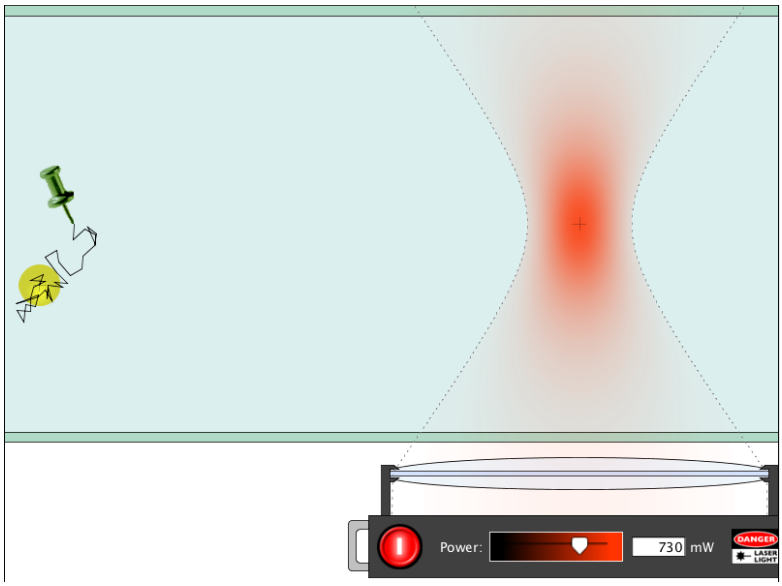
As the bead is moved away from the point at which the other DNA end is attached, the forces acting on the bead increase, as shown by the red and green arrows in the illustration below:

[2]https://phet.colorado.edu/en/simulation/legacy/stretching-dna




The green arrow represents the force exerted by the DNA, and the red arrow represents the net force generated by the optical trap as the bead is pulled away from the center of the beam.

If the bead is pulled too far, the force exerted by the DNA will exceed the maximum force of the optical trap, and the bead will break away. Rather than staying in one position, or moving randomly by brownian motion, the bead will gradually (via a biased random walk) move towards the fixed end of the DNA, as shown below:




As discussed in the context of the protein-stretching experiment, the force generated by the DNA is entropic in nature. As the ends of the molecule are separated, the number of possible conformations (microstates) is reduced and the free energy increases. Although this argument offers a thermodynamic explanation for the work, and therefore force,

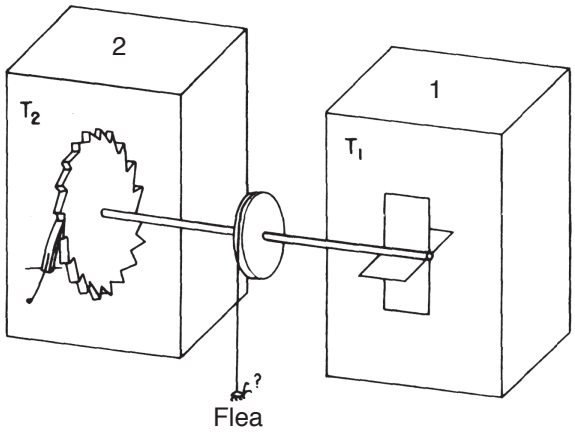
required to stretch the molecule, it is worth giving some thought to exactly what causes the force and net motion of the bead when it is released from the optical trap.

It might seem that some of the force could come from the stretching of covalent bonds in the DNA molecules. However, the forces generated in this experiment are far smaller than those required to stretch a bond by even a very small amount. The actual motions of the DNA and the bead are due to thermal motion and the collisions with surrounding water molecules, that is Brownian motion. Over time, a very large number of the possible conformations of the DNA are sampled due to these motions, and all of them have roughly equal energies. However, many more of the possible conformations are ones where the two ends are relatively close together than far apart. This is very similar to what we discussed in the context of two- and three-dimensional random walks, and exactly the same mathematics can be used to describe the entropic stretching force.

The protein- and DNA-stretching experiments demonstrate that thermal motion can generate macromolecular forces and net motion along a single direction. But, can these motions be used in a cyclic motor?

III. A Brownian ratchet and Maxwell's demon

To help understand the requirements for capturing thermal energy to produce mechanical energy, it is helpful to consider a hypothetical heat engine designed to capture the thermal energy of gas molecules. One version of this engine, sometimes called a "Brownian ratchet", was described by Richard Feynman in his classic book of physics lectures [3], as shown below:




In this device, there are two isolated compartments. In compartment 1, there is a paddle wheel surrounded by gas molecules at temperature $T_1$. The gas molecules randomly collide with the paddles and can, in principle, cause periodic rotations in either direction. But, the paddle is connected via a shaft to a ratchet and pawl mechanism in compartment 2, which contains a gas at temperature $T_2$. Because of the shape of the

[3]Figure from Feynman, R. P., Leighton, R. B. & Sands, M. (2013). *The Feynman Lectures on Physics*, volume I, chapter 46. Basic Books, new millennial edition. http://www.feynmanlectures.caltech.edu/I_46.html

teeth on the ratchet wheel, the wheel can rotate in the clockwise direction, as viewed in the drawing, but counter-clockwise rotation is blocked by the pawl, which is held in position by a spring. It would appear that this mechanism can capture the thermal energy of the gas molecules in compartment 1 to cause rotation in the clockwise direction, perhaps even doing a little bit of work, such as lifting the flea in the drawing.

One statement of the second law of thermodynamics is that work can only be obtained from thermal energy when there is a net flow of heat from a warm object to a cooler one. In the Brownian ratchet, however, it is not so obvious why work could only be obtained if $T_1$ is greater than $T_2$. Nor is it obvious how heat could flow from compartment 1 or 2. The key to both paradoxes lies in the ratchet mechanism itself. In order for the wheel to move in the clockwise direction, the force generated by the collision of the gas molecules on the paddles must be great enough to lift the pawl. However, if the temperatures of the two sides of the apparatus are equal, then this amount of thermal energy is also available to lift the pawl directly, which will allow the wheel to turn in either direction.

The notion that the ratchet mechanism could allow rotation in either direction with equal probability may still seem rather counterintuitive, since it certainly looks as though it is much harder to lift the pawl to allow counter-clockwise rotation. And, anyone who has used a wrench with a ratchet mechanism knows that a ratchet does, indeed, allow rotation in one direction but not the other. This, however, is a case in which our intuition based on the macroscopic world fails us when we move to the microscopic scale, where motions are determined by random collisions. If we examine the mechanism closely, it is apparent that the clockwise rotation involves a gradual lifting of the pawl along the slope of the teeth. Since the collisions of gas molecules on the paddle wheels cause only very small movements, moving the ratchet wheel to the next stopping point requires a series of microsteps in the same direction. Otherwise, the force of the pawl will move the wheel back to its starting point. Probabilistically, this is equivalent to flipping a coin 10 times, say, and seeing 10 heads. This is unlikely, but not impossible. On the other hand, for the pawl to be lifted by thermal energy all the way up to allow a counter-clockwise step is equivalent to many unlikely events all at once. This would be equivalent to flipping 10 coins all at once and, again, seeing 10 heads. This is unlikely, but no more so than 10 heads from 10 sequential coin flips.

The only way in which net work can be extracted by this apparatus is if the two parts, the paddle wheel and the ratchet, are isolated and the temperature of the paddle wheel chamber ($T_1$) is greater than that of the ratchet chamber ($T_2$). Under these conditions, more thermal energy is available to move the wheel than to spontaneously lift the ratchet pawl, thus favoring the forward direction. During this process, the temperature of the paddle chamber will decrease, as the gas molecules lose kinetic energy. In the ratchet chamber, the temperature will increase because each time that the ratchet pawl falls back on the wheel heat is generated. Thus, there is a net flow of heat associated with the work generated, as required by the second law. Eventually the two chambers will reach the same temperature, and no further work can be extracted.

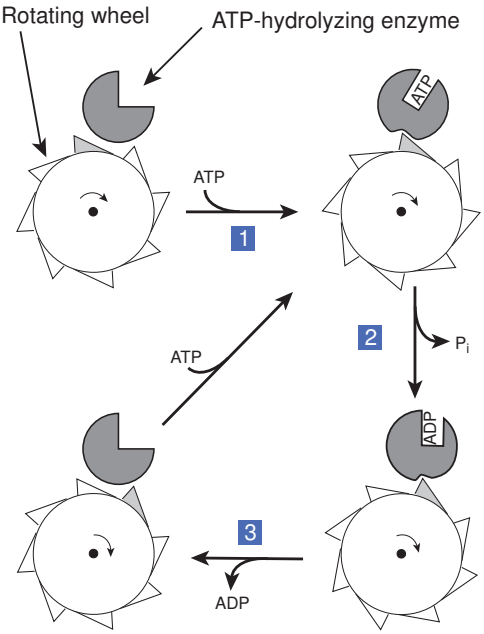
The one way in which work could be obtained with the ratchet and paddle at the

same temperature is if some "intelligent" being could monitor the direction of the small fluctuations and allow the wheel to go in one direction, but not the other. This mythical creature is usually referred to as "Maxwell's demon".

The original version of the demon, proposed by George Clerk Maxwell in 1871, was poised near an opening between two chambers containing a gas. By allowing only the faster gas molecules to move in one direction, and the slower ones in the opposite direction, the demon is able to take a system at thermal equilibrium and raise the temperature of one side and lower that of the other. The key, however, is that the demon must, herself, expend energy to create this temperature difference.

IV. A hypothetical ATPase ratchet

To consider what features might be important for an ATP-driven motor, we can design an imaginary motor in which an ATP-hydrolyzing enzyme plays the role of Maxwell's demon. One possible scheme is drawn below:




Note the following features of this device:

- The motor has two components:
  - A rotating wheel with teeth like those on a ratchet wheel.
  - An ATPase enzyme that undergoes conformational changes as it binds and hydrolyzes ATP.
- The teeth on the rotating wheel have an asymmetric structure, so that they interact with the ATPase differently depending on which direction the wheel moves.
- The ATPase has three different conformations, depending on whether ATP or ADP, or neither is bound.

In one forward cycle of the motor, the following steps take place:

1. The enzyme binds ATP and changes conformation; The wheel rotates clockwise.
2. The ATP is hydrolyzed, phosphate is released and the enzyme changes conformation; The wheel rotates clockwise.
3. ADP is released and the enzyme returns to its starting conformation; The wheel rotates clockwise.

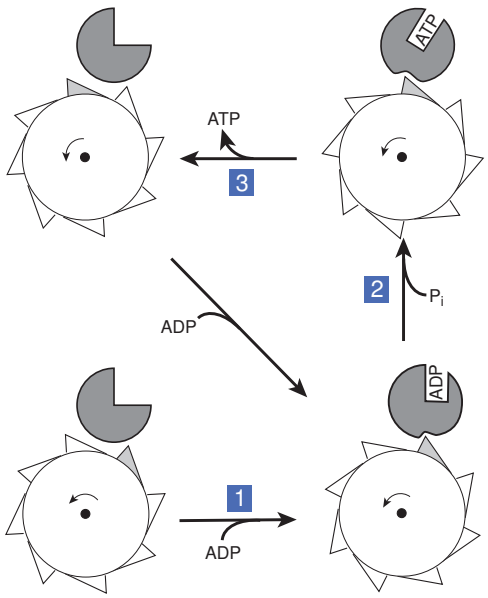
At the end of these steps, the motor is in its original state, except that the wheel has rotated by one full step from its starting position. The cycle can then begin again with the binding of another ATP molecule.

Each of the steps outline above actually includes three kinds of events:

- Nucleotide binding or release, or catalysis.
- A conformational change.
- Rotation of the wheel.

The three events could, in principle, occur in a specific order or in a concerted process. For instance, the first step might begin with the Brownian motion that brings a tooth of the wheel into contact with the ATPase, which then causes a change in the enzyme's conformation, which then favors ATP binding. Or, the process could begin with ATP binding, followed by the conformational change and rotation. Dissecting the details of such processes is an important aspect of the study of real molecular motors.

What is most important about this scheme is that there is an order to the conformational changes, and this order is determined by the order of steps in the chemical reaction. If the concentrations of ADP and $P_i$ are much higher than that of ATP, then the reverse chemical reaction will be favored thermodynamically. Under these conditions, the open enzyme is more likely to bind ADP than ATP, leading to rotation in the counter-clockwise direction, as illustrated below:



Each step in the counter-clockwise cycle is the exact reverse of the corresponding step in the clockwise cycle, including nucleotide binding or release, catalysis, conformational change and rotation.

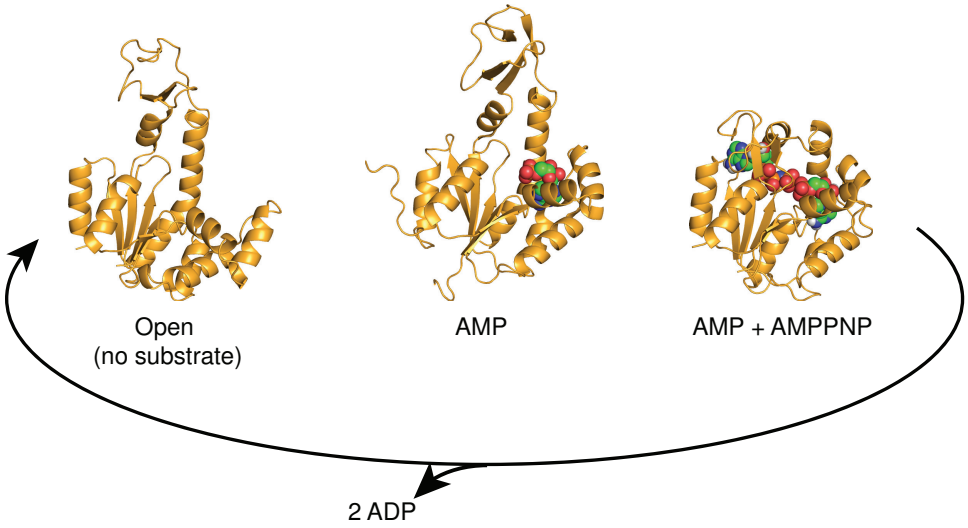
Another critical aspect of the motor is that the catalytic cycle and the rotation are tightly coupled, so that the wheel cannot rotate without the enzyme catalyzing the reaction in one direction of the other. Conversely, the conformational change of the enzyme and rotation of the wheel cannot occur in the absence of the catalytic cycle. It should then be possible for mechanical energy to be used to make ATP from ADP and $P_i$. This is what, indeed, happens in the ATP synthase that we will discuss later. In this case, rotation of one portion of the enzyme is driven by the movement of $H^+$ ions down a concentration gradient, and this mechanical motion is coupled to ATP synthesis, by a mechanism similar in principle to that suggested above.

## 7.2 Adenylate kinase: Coupling a chemical reaction to conformational change

Before discussing some real molecular motors, we will consider an enzyme that illustrates how a cyclical structural change can be coupled to a chemical reaction. The reaction catalyzed by adenylate kinase is the transfer of a terminal phosphate group from one adenosyl nucleotide to another:

$$\mathrm{ATP} + \mathrm{AMP} \rightleftharpoons \mathrm{ADP} + \mathrm{ADP}$$

This reaction has an equilibrium constant close to 1. The major physiological role of adenylate kinase is in muscle tissue, where the reverse of the reaction drawn above regenerates a reserve of ATP, from ADP, when ATP levels are depleted. The structure of the enzyme has a cleft in which the substrates bind, and the protein undergoes large structural changes upon substrate binding, as illustrated in the drawings shown below:



These drawings are based on crystal structures of adenylate kinase with no substrate bound (PDB entry 4AKE), AMP bound (PDB entry 2AK3) and AMP and a non-hydrolyzable ATP analog (AMPPNP) bound (PDB entry 1ANK). The non-hydrolyzable substrate was used to trap the enzyme in the conformation presumed to exist when it is about to carry out the catalytic reaction.

As illustrated above, the cleft closes tightly around the substrates, and it is believed that the major role of this structural change is to exclude water, which otherwise could act to hydrolyze the ATP. Once the chemical reaction has taken place, in either direction, the cleft must open up again to allow release of the products. One could imagine how this motion might, if connected to other structures, be used to carry out mechanical work, and it turns out that the structural motif found in adenylate kinase is present in several ATP-driven molecular motors, including myosin, kinesin and ATP synthase.

Consideration of adenylate kinase also raises a bit of a paradox. Suppose that ATP, ADP and AMP were all present, along with the enzyme, and the reaction had reached equilibrium. Under these conditions, the free energy change for the reaction is zero, and no work should be available. However, the enzyme will continue to catalyze the reaction in both directions, undergoing a continuous opening and closing process. It seems as though it is generating work without consuming energy. This would be equivalent to the Brownian ratchet performing work when the two compartments are at the same temperature, which we have argued violates the second law of thermodynamics. However, as soon as we try to couple the enzyme to some other structure, in the hopes of producing mechanical work, the direction of the reaction will be biased in one direction or the other. Only if the reactant and product concentrations favor the opposite reaction will we be able to produce motion against the force acting on the enzyme.

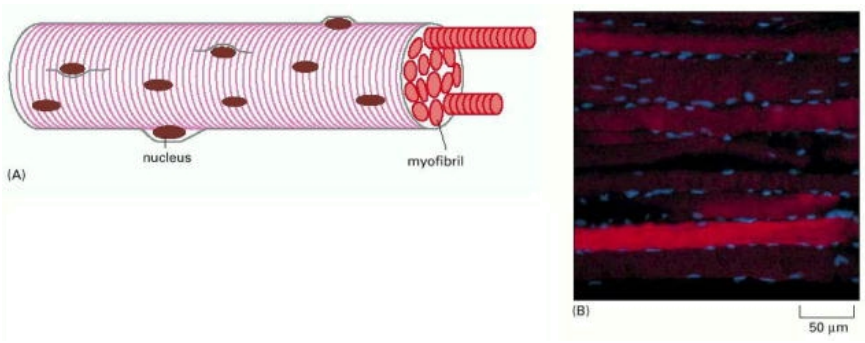
## 7.3 Myosin and Muscle Contraction

The molecular motors with which we have the most readily visible experience are found in our muscle cells. The primary components of these motors are two proteins, myosin and actin, which interact with one another and link ATP hydrolysis to a relative motion of one with respect to the other. Before considering the molecular details of these motors, it is useful to describe the larger structures in which they are found.

I. The structure of muscle fibers

The force-generating cells of muscles are called *myocytes* and extend the full length of the muscle. During development, these cells are generated by the fusion of multiple precursor cells, *myoblasts*, and the nuclei of the individual precursors are retained in the myocytes, as illustrated in the figures below[4]:

[4]Figure from Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. https://www.ncbi.nlm.nih.gov/books/NBK26888/#A3065




As shown in the diagram in Panel A, the nuclei are located at the periphery of the cell, and the central part of the cell is filled with roughly cylindrical structures, which extend along the full length of the cell (and the muscle), called *myofibrils*. Panel B shows a fluorescent micrograph of muscle tissue stained with a blue-fluorescent dye that binds to DNA and highlights the nuclei.

The figure below shows several parallel myofibrils as visualized by electron microscopy at relatively low resolution[5]:

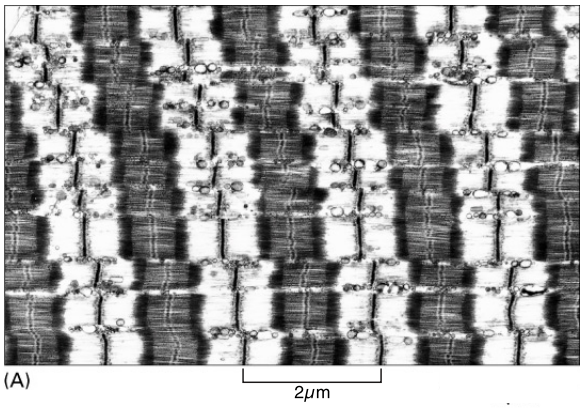



Figure 16–69 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

The sample used for this micrograph was stained with a heavy-metal compound that reacts with protein molecules, so that the darker regions represent the most protein-dense regions. The proteins are organized into repeating bands of high and low density. These repeating structures are called *sarcomeres* and have a length of about $2\,\mu$m in this sample.

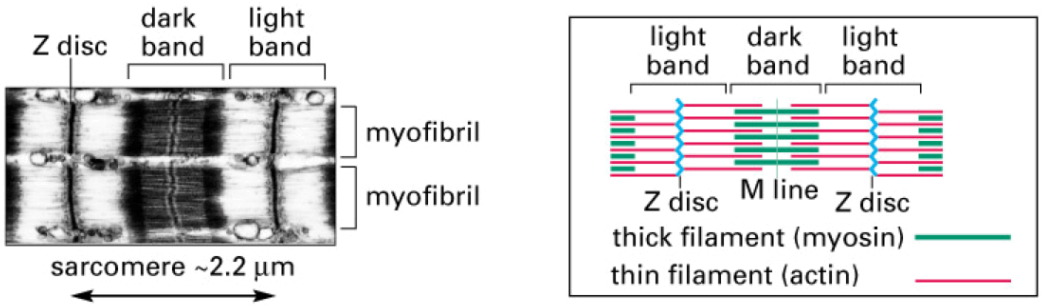
The protein composition of myofibrils was extensively studied in the 1930s and 40s, particularly by Albert Szent-Györgyi and his colleagues at the the University of Szeged, Hungary. These studies identified the two major protein components of muscle cells:

- Actin, with a molecular mass of 42000 Da.
- Myosin, a much larger protein, with a molecular mass of about 500000 Da.

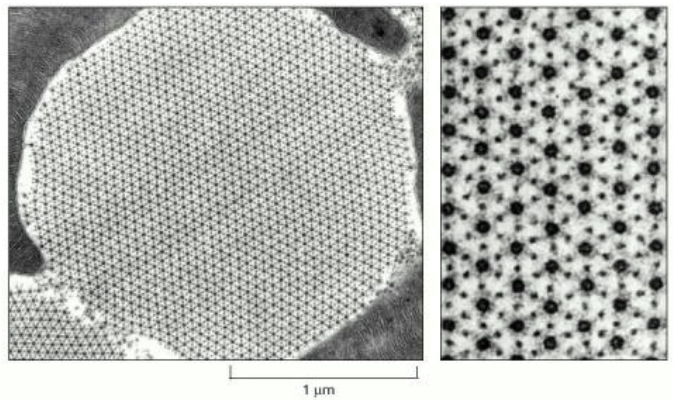
[5]Figure from Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. https://www.ncbi.nlm.nih.gov/books/NBK26888/#A3065

Actin can be isolated as a monomer, but readily assembles into long, thin fibers. Myosin can also be isolated in a soluble form, but assembles in to filaments that are much thicker than those formed by actin. Myosin was also discovered to have an ATP hydrolyzing activity that is stimulated by the presence of actin filaments.

Further studies, in the 1950s, established that the darkly staining regions of the sarcomeres contained predominantly the thick filaments formed by myosin, and that the lightly staining regions contained actin thin filaments. The figure below shows, in an electron micrograph and a diagram, the organization of the sarcomere and the locations of the thick and thin filaments[6]:




A better sense of how the filaments are arranged in three dimensions can be gained from a cross-sectional view of a myofibril, as shown below for an insect flight muscle:



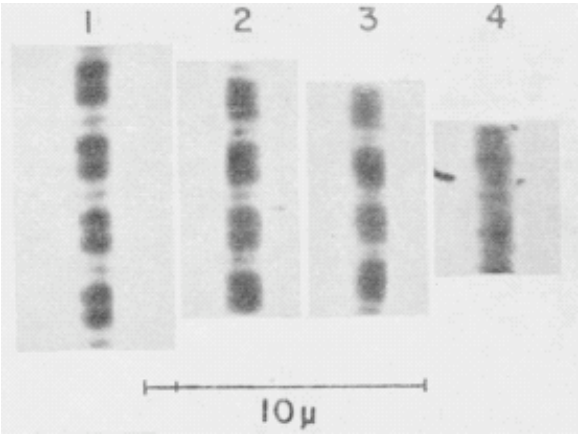

The myofibrils of insect flight muscles are more highly organized than most, with the thick and thin fibers in overlapping hexagonal arrays, but the same interdigitation of the two types of fibers is found in all muscle cells.

The year 1954 brought a major advance in the understanding of muscle contraction, based on studies by two groups, who published their findings in side-by-side articles[7]

[6]Both figures on this page are from Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. https://www.ncbi.nlm.nih.gov/books/NBK26888/#A3065

[7]Huxley, A. & Niedergerke, R. (1954). Structural changes in muscle during contraction: Interference microscopy of living muscle fibres. *Nature*, 173, 971–973. http://dx.doi.org/10.1038/173971a0 and

Both groups used special forms of light microscopy to enhance the contrast of images of myofibrils, allowing the alternating patterns of high and low protein density to be visualized as the fibrils underwent contraction. A set of images from the paper by Huxley and Hanson is shown below:




As in the electron micrographs shown earlier, the dark bands in these images represent the protein rich regions containing the myosin thick filaments, and the light bands contain primarily actin. The sequence of images, labeled 1 through 4, represent the same four sarcomeres during contraction induced by the addition of ATP. The important observation, reported by both groups, was that the light bands became progressively shorter during contraction, while the dark bands remained largely unchanged. Importantly, there was no thickening of the fibrils during contraction, as might have been expected from the familiar bulging of muscles when they contract.

From these observations, both pairs of authors proposed what came to be known as the *sliding-filament* model of contraction, which is diagrammed below[8]:

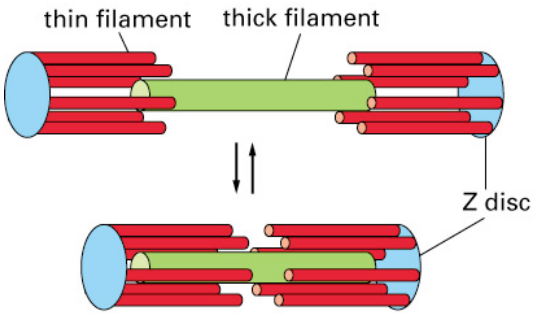


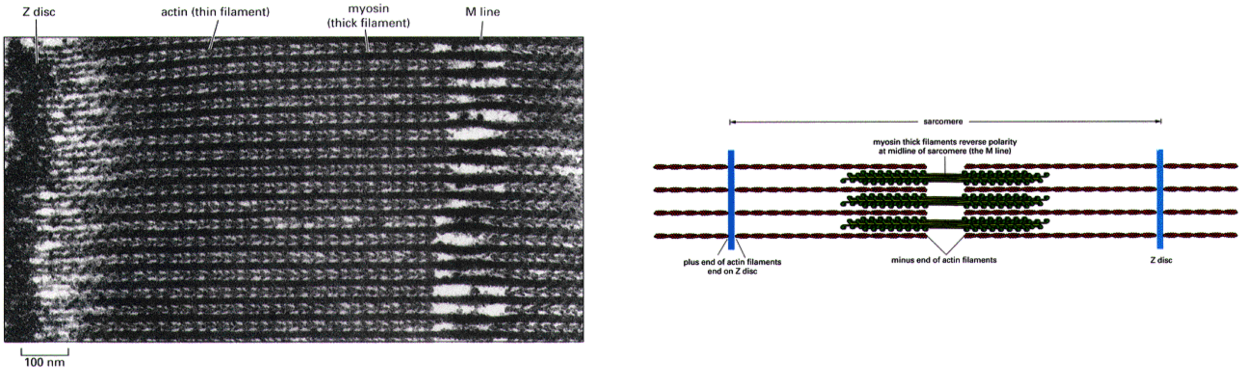

Figure 16–71. Molecular Biology of the Cell, 4th Edition.

Huxley, H. & Hanson, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature*, 173, 973–976. http://dx.doi.org/10.1038/173973a0 (The two Huxleys are unrelated, but both made major contributions to physiology in the 20th century.)

[8]Figure from Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. https://www.ncbi.nlm.nih.gov/books/NBK26888/#A3065

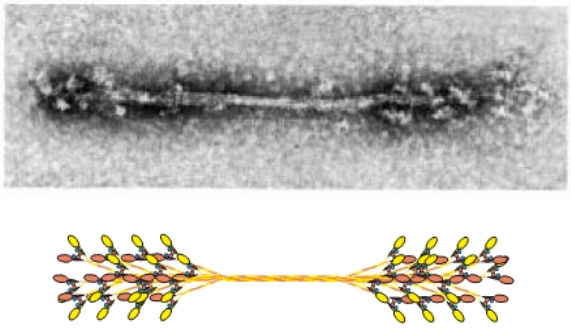
The major feature of the model is that contraction is based on the sliding of the thin filaments past the thick filaments, thus accounting for the shortening of the light regions in the micrographs. In addition, the actin filaments are attached the densely staining structures, called *Z disks*, that separate the sarcomeres, thus linking the sarcomeres together.

Following the 1954 studies, the major question to be resolved was what generates the force that moves the filaments past each other. Again, a major source of insight was electron microscopy. In the figure below[9] , the left-hand panel shows myofibrils stained in a way so that the proteins appear light against a dark background (negative stain).




A schematic interpretation of the electron micrograph is shown on the right. A prominent feature visible in the micrograph is the array of globular protrusions located between the thick and thin filaments. If you look closely, you will see that these protrusions are angled and that they point in opposite directions on the two sides of the region identified as the M line.

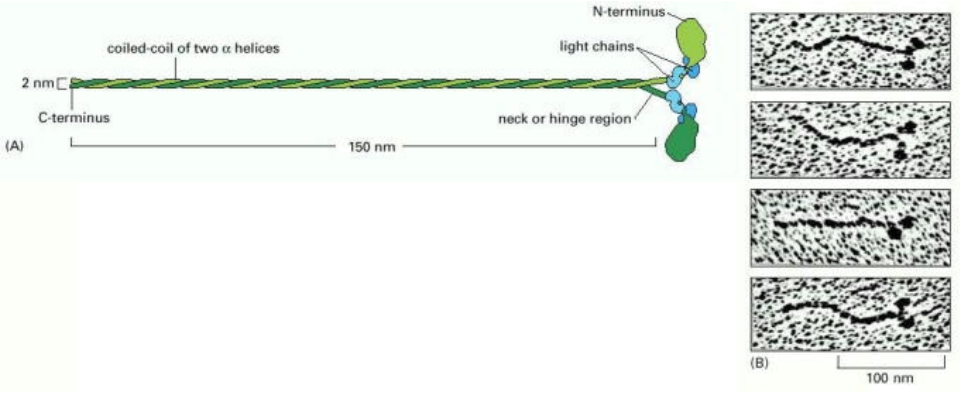
Based on micrographs like the one above, Hugh Huxley proposed that these cross-bridges were the location of ATP hydrolysis and force generation. This interpretation was supported by electron micrographs of isolated thick filaments, as shown below[10], as well as biochemical experiments.



[9]This figure and the one at the top of the following page are from Aberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. (1994). *Molecular Biology of the Cell*. Garland Publishing, $3^{rd}$ edition.

[10]Figure from Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th edition. New York: W H Freeman; 2002. https://www.ncbi.nlm.nih.gov/books/NBK22418/

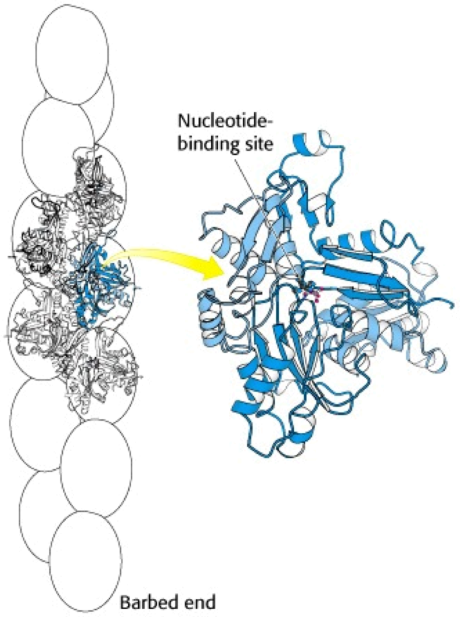
The micrograph of the isolated filament shows that the cross-bridges are restricted to the outer thirds of the filament and that the middle third is relatively thin. The cartoon representation below the micrograph reflects the structures of individual myosin molecules, which are shown in more detail in the next figure.




Each myosin molecule contains two very large and identical polypeptide chains (called the heavy chains and composed of about 2,000 amino acid residues each) and four smaller polypeptides, called light chains. The C-terminal portions of the heavy chains form $\alpha$-helices, and the helices of the two chains wrap around one another to form a structure known as a coiled coil. The coiled-coil regions of individual myosin molecules assemble further to form the thick filaments. The N-terminal regions of the heavy chains fold into a globular structure. The coiled-coil tails of the molecules are linked to the globular heads by a hinge region that includes two light chains bound to each heavy chain. The ATPase activity is located in the globular heads.

The thin filaments, composed of actin, have a structure very different from that of the thick filaments. Actin folds into single globular domain and assembles into filaments as illustrated below[11]

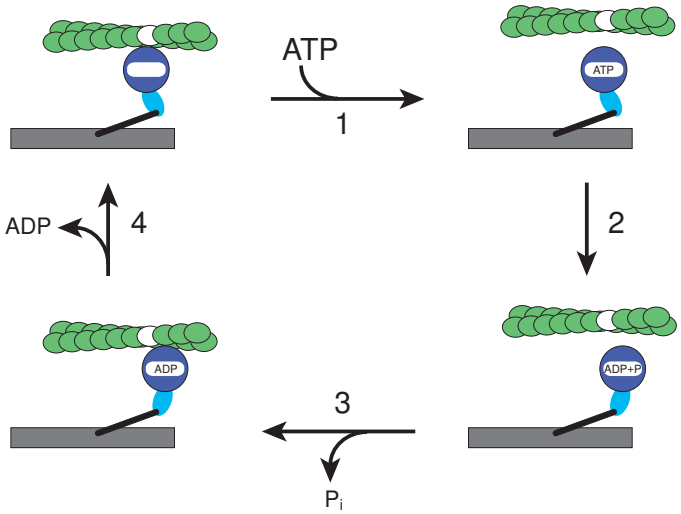
[11]Figure from Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th edition. New York: W H Freeman; 2002. https://www.ncbi.nlm.nih.gov/books/NBK22418/




Interestingly, the actin monomers contain a nucleotide binding site, but do not posess catalytic activity. Nucleotide binding helps to control the polymerization of actin, but does not appear to play any role in force generation.

II. The ATPase cross-bridge cycle

In addition to structural studies, our understanding of the mechanism of muscle contraction (and other molecular motors) comes from extensive biochemical studies, including kinetic experiments in which the rates of the catalytic reaction have been studied using a range of substrate concentrations and in the presence or absence of actin. Work by Edward Taylor and his colleagues[12], in particular, along with subsequent work, has led the model diagrammed below:




[12]Lymn, R. W. & Taylor, E. W. (1971). Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry*, 10, 4617–4624.
http://dx.doi.org/10.1021/bi00801a004

In this figure, one of the actin monomers is left uncolored to highlight the movement of the filament with respect to the myosin during the cycle, which consists of the following steps:

1. Binding of ATP to the enzyme, which is initially bound to the actin filament. Binding of ATP is coupled to the release of the actin by myosin.

2. Hydrolysis of ATP, which is coupled to a conformational change in the myosin and a change in the position of the myosin head with respect to the thin filament and the rest of the thick filament.

3. Release of the inorganic phosphate, which is coupled to rebinding of the myosin head to the actin filament.

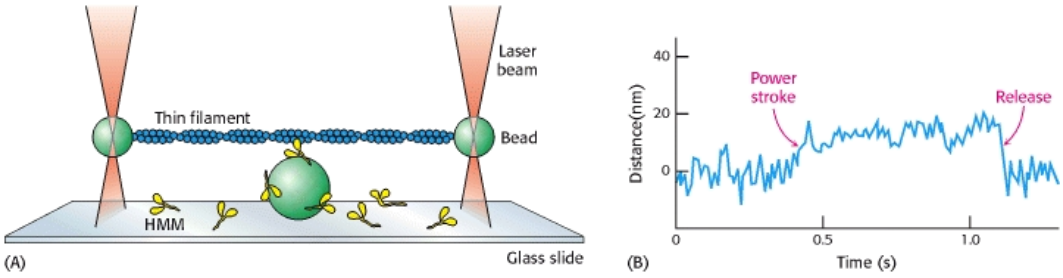
4. Release of ADP, which is coupled to a conformational change in the myosin, which leads to a shift in the relative positions of the two filaments.

The last step described above is called the powerstroke and is the point in the cycle in which motion and force are generated. Note also the critical role of the conformational change associated with the ATP hydrolysis step. Although no force is generated in this step, the conformational change positions the myosin head so that when it rebinds it is most likely to do so at a position further along the thin filament. As in the hypothetical ATPase ratchet discussed on pages 202–204, the catalytic cycle is coupled to both conformational changes of the ATPase and binding and release of the second component of the motor, in this case the actin filament. The actual motions produced by myosin and actin arise from thermal Brownian motions of the molecules. But, the motion is biased by the conformational change which increases the probability that a head will rebind further along the filament.

Contraction of a myofibril requires the action of millions of myosin heads, and one could imagine that there is some mechanism to coordinate them all. However, the myosin heads all act independently. This independence requires a limited degree of elasticity in the filaments, which comes primarily from the connections between the myosin heads and the core of the thick filament. The elasticity allows the fibril to continue contracting as individual myosin heads move through their catalytic cycles independently. Each myosin head only spends about 5% of the time bound to actin.

The action of individual myosin molecules can be studied using the optical trapping method described on pages 196–198. One such experiment is illustrated below[13]:

[13]This figure is from Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th edition. New York: W H Freeman; 2002. https://www.ncbi.nlm.nih.gov/books/NBK22418/ and is adapted from:
Finer, J. T., Simmons, R. M. & Spudich, J. A. (1994). Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature*, 368, 113–119. http://dx.doi.org/10.1038/368113a0




In this experiment glass beads were attached to both ends of a thin filament and each was held in an optical trap. A fragment of myosin, containing the head domains and enough of the stalks to hold the two heads together, was attached to the surface of another glass bead, so as to hold it in place above the surface of the glass slide. The optical traps were then steered to move the actin filament into the proximity of the bead with myosin heads and the actin and myosin bound to each other in the absence of ADP or ATP. When ATP was added, the myosin underwent its catalytic cycle and exerted force on the actin filaments. During this process, the displacement of the glass beads attached to the actin was monitored, to generate plots such as shown in the right-hand side of the figure, which represents a single cycle.

From analysis of the distance versus time plots, the investigators estimated that the average step size was about 10 nm and the average force was 3–4 pN. (1 piconewton = $10^{-12}$ N). From these values, the work done by the motor is:

$$\begin{aligned} w &= \int F dx \approx F_{\text{ave}} \times \text{distance} \\ &= 3\,\text{pN} \times 10\,\text{nm} = 30\,\text{pN}\cdot\text{nm} \\ &= 3 \times 10^{-12}\,\text{N} \times 10^{-8}\,\text{m} = 3 \times 10^{-20}\,\text{N}\cdot\text{m} \\ &= 3 \times 10^{-20}\,\text{J} \end{aligned}$$

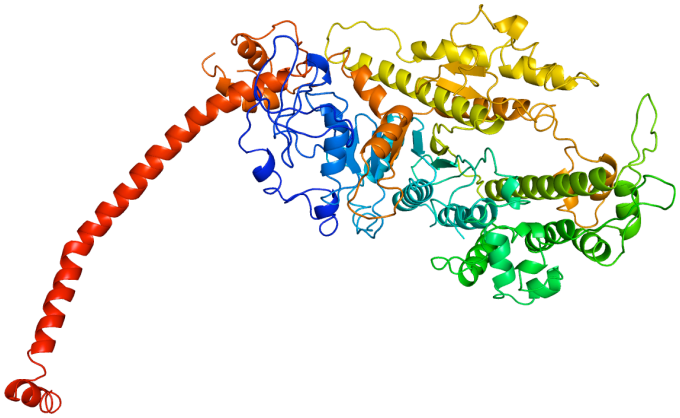
It is instructive to compare this amount of work with the standard free energy change for hydrolysis of a single molecule of ATP:

$$\begin{aligned} \Delta G^\circ &= -30 \times 10^3\,\text{J/mol} \div 6.02 \times 10^{23}\,\text{molecules/mol} \\ &= 5 \times 10^{-20}\,\text{J/molecule} \end{aligned}$$

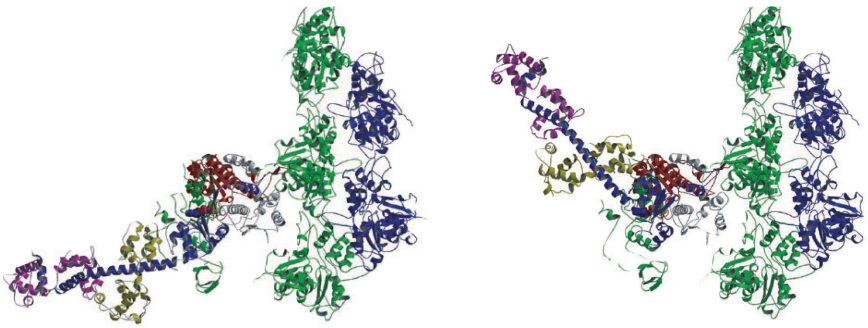
This would appear to be remarkably consistent with the work generated by the myosin, which is expected to be at least somewhat less than the theoretical energy available from ATP hydrolysis. It is important to note, however, that $\Delta G^\circ$ represents the free energy change when the reactant and products are all present at 1 M concentrations. In the single-molecule experiment, only ATP was added to the reactions, at concentrations of 1 $\mu$M to 2 mM. From this information, it is difficult to estimate the reaction quotient, $Q$, or the actual free energy change, $\Delta G$, under the experimental conditions. But, forces on the order of a few piconewtons and distances of a few nanometers appear to be common feature of molecular motors, leading to the use of the pN $\cdot$ nm as a common unit of work and energy in the field of molecular motors.

III. Atomic resolution structures of myosin and actin

In the early 1990s, a high-resolution structure of a myosin head and stalk region was determined by x-ray crystallography, providing a much more detailed view of the mechanism of muscle contraction[14]. A ribbon diagram of this structure is shown below:



As can be seen, the structure confirms the overall structure deduced earlier from electron microscopy. At about the same time that this structure was determined, the structure of actin monomers was also determined by x-ray crystallography and was used, in conjunction with EM images, to construct models of the thin filament, as shown in the figure on page 211. These structures were used to construct models of the actin-myosin complexes in different states of the catalytic cycle. The figures below represent models of the complex before and after the powerstroke[15].



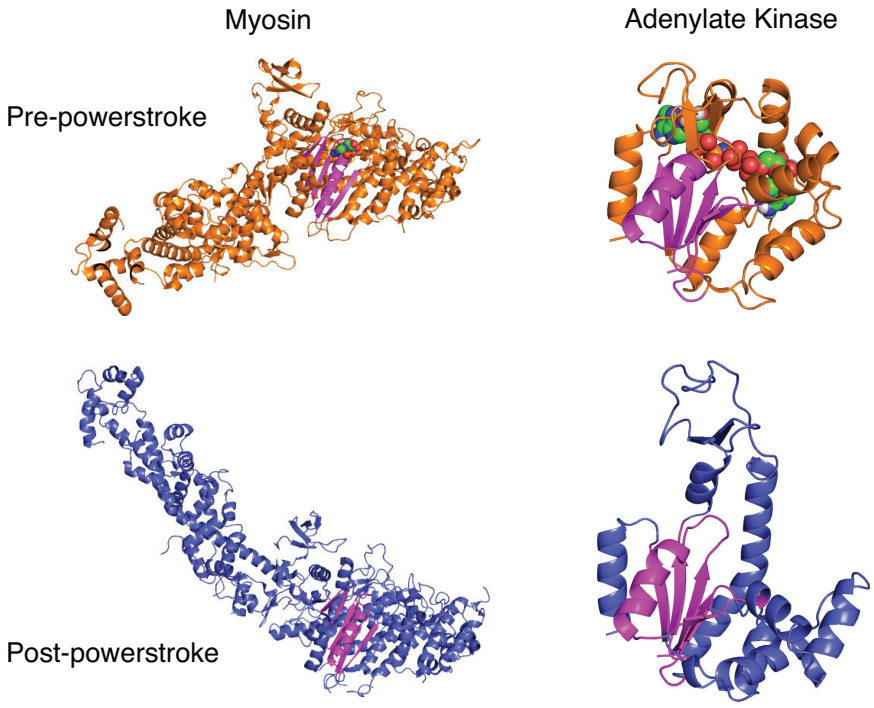

As can be seen in the figure, the position of the myosin head remains essentially fixed relative to the actin filament during the powerstroke, but the orientation of the head with respect to the myosin stalk changes dramatically. If the other end of the stalk

[14]Rayment, I., Rypniewski, W. R., Schmidt-Base, K., Smith, R., Tomchick, D. R., Benning, M. M., Winkelmann, D. A., Wesenberg, G. & Holden, H. M. (1993). Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science*, 261, 50–58. http://dx.doi.org/10.1126/science.8316857

[15]Figure from Geeves, M. A. & Holmes, K. C. (1999). Structural mechanism of muscle contraction. *Ann. Rev. Biochem.*, 68, 687–728. http://dx.doi.org/10.1146/annurev.biochem.68.1.687

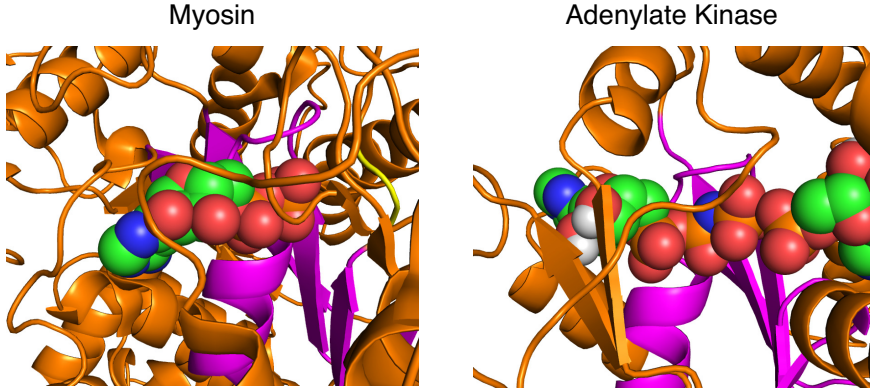
were fixed, as in an intact thick filament, the conformational change would lead to a motion of the thin filament with respect to the thick one.

Earlier, we considered the conformational changes of adenylate kinase as an example of how a catalytic cycle can be coupled to molecular movement. In fact, the relationship between adenylate kinase and myosin is more than just a formal one. The two proteins appear to be evolutionarily related and show significant conservation, particularly in the nucleotide binding site. The ribbon diagrams below highlight this relationship:




In all four drawings, the nucleotide binding region is colored magenta, and the molecules are oriented to emphasize the similarity of the structures. In both proteins, the conformational changes are centered at and near the binding sites and are propagated over long distances, though the nature of the propagated motions are different.

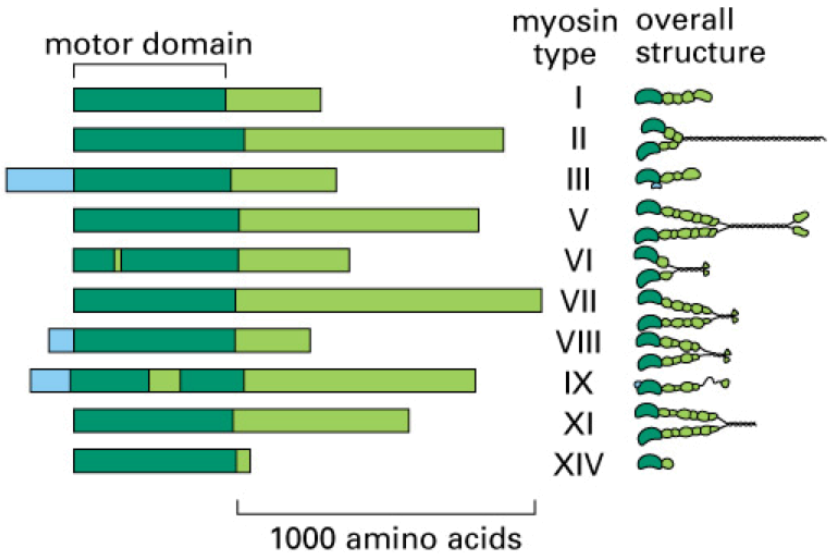
The similarity between the binding sites is further illustrated in the close-up drawings below:



The drawing on the left represents myosin in the pre-powerstroke state, with ADP bound, and the one on the right is of adenylate kinase with AMP and an ATP analog bound. The similarity of these structures, and the ubiquitous occurrence of adenylate kinase (in eukaryotes, bacteria and archaea), indicates that the structural motif at the core of myosin is ancient and has evolved to carry out very different functions.

IV. Non-muscle myosins

Although the form of myosin found in skeletal muscle cells (called type II) is probably the best known and best studied, it is only one of a large family of myosins, which display a wide range of structural organization and carry out a variety functions. The figure below diagrams the sequence relationships among the heavy chains of the major myosin types[16]:

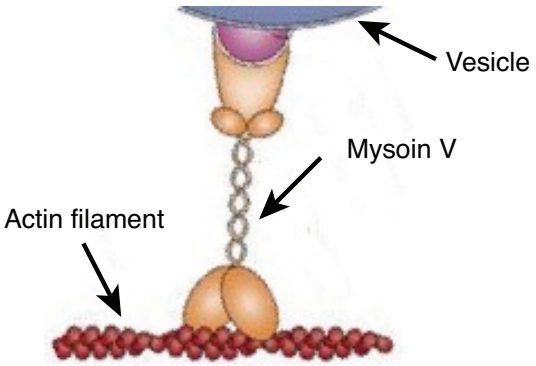



In all of these myosin types, the motor domain (in the N-terminal portion of the heavy chain) is highly conserved, but there is great variety in the C-terminal regions. Only type II myosin possesses the extended coiled-coil structure that leads to assembly into muscle thick filaments. In most, but not all, of the others, the C-terminal region leads to dimerization via a coiled-coil, but the coiled-coil regions are shorter than in type II myosin, and other domains are present in the other types.

One of the better studied of the non-muscle myosins is the type V form. This myosin functions to move intracellular cargoes, such as vesicles along actin filaments, as illustrated in the diagram below[17]

[16]Figure from Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. https://www.ncbi.nlm.nih.gov/books/NBK26888/#A3065

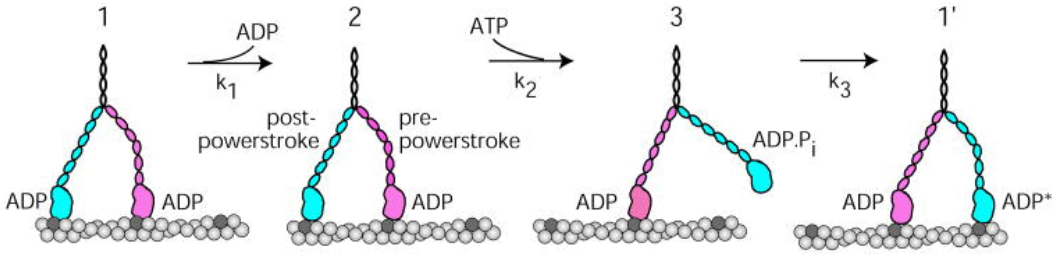
[17]Figure adapted from Soldati, T. & Schliwa, M. (2006). Powering membrane traffic in endocytosis and recycling. *Nature Rev. Mol. Cell. Biol.*, 7, 897–908. http://dx.doi.org/10.1038/nrm2060




As shown in the diagram, myosin V interacts with the actin filament via its two head domains, just as myosin II does in muscle cells, and the two myosin heavy chains are dimerized via a coiled-coil. However, the C-terminal region of the heavy chain has a specialized function to interact with proteins linked to the transport vesicle.

As discussed earlier, the heads of myosin II are bound to the thin filaments only about 5% of the time during muscle contraction. This is described as a low *duty cycle* and is a requirement to minimize the potential interference among the thousands of myosin molecules in a sarcomere. For a myosin molecule engaged in vesicle transport, however, a light duty cycle would cause the vesicle to fall off of the actin filament. In this context, it is essential that one or both of the myosin heads be bound to the actin almost all of the time, resulting in a high duty cycle.

A model for the myosin V mechanism for moving along actin filaments is diagrammes=d below[18]:




Each of the myosin heads undergoes the ATPase catalytic cycle, which is coupled to conformational changes and binding to and release from the actin filament. In this representation, the stalk of the myosin head in the pre-powerstroke state (with ADP bound) is bent, whereas the stalk is straight after the powerstroke. The powerstroke leads to the movement of the other myosin head along the filament in a hand-over-hand fashion. As in the muscle cross-bridge cycle, ATP binding is required for release of the myosin heads after the powerstroke.

In contrast to myosin II, the catalytic cycles of the two myosin V heads must be tightly coordinated in order to ensure that the complex does not fall of the filament and that

[18]Trybus, K. M. (2008). Myosin V from head to tail. *Cell. Mol. Life Sci.*, 65, 1378–1389. https://dx.doi.org/10.1007%2Fs00018-008-7507-6

the motion proceeds directionally. In particular, the powerstroke of the leading head is coordinated with the ATP binding, filament release and forward motion of the trailing head. The detailed molecular mechanism of this coordination remains to be elucidated.