THE BIOCHEMICAL BASIS OF MUSCLE CONTRACTION[1]

Summary: Early microscopic data concerning the appearance of relaxed and contracted muscles is reviewed. This is correlated with modern biochemical studies that have investigated the nature of the proteins involved in contraction -- both their structures and functions. Finally, the actual molecular events and bioenergetics of a contraction cycle are explained.

I. Movement and Muscles: general overview:

A. Three principal types of mechanisms related to locomotion have evolved in eukaryotic animals. In some organisms these are the only means of locomotion, while in others, multiple means of movement are employed. In large eukaryotes, such as most animals, all three types of movement are used, but only in cells where a particular type of movement is most appropriate.

1. **Amoeboid motion**: characterized by the presence of projections called **PSEUDOPODS** that extend from the cell in the direction of movement. Much the motion is apparently due to the forced flow of relatively low viscosity cytosol (endoplasm) through a more rigid, viscous, contracting tube (ectoplasm). Many cells in large organisms use this type of locomotion, ex: leucocytes

2. **Ciliary Motion**: characterized by the use of beating cilia or flagella (which are essentially long cilia); they tend to work either like oars (cilia) or pushing or pulling propellers (flagella). Besides obvious examples where they are used in large eukaryotic organisms (such as sperm), these are also used to create fluid currents around cells that themselves are fixed; ex: ciliary cells in lungs and nasal passages, choanocytes in sponges, ciliary combs in ctenophores, and feeding cilia in a large number of different invertebrates.

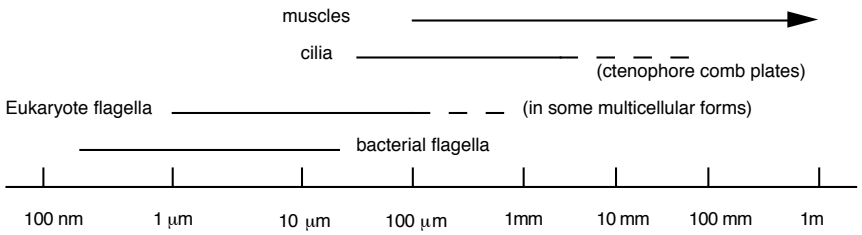
3. **Muscles**: Since these involve very large structures, they are capable of producing the most force and are therefore most suitable for movement of large organisms. The following points should be kept in mind:

a. Muscles express the conscious (skeletal muscles) and unconscious mind (smooth and cardiac muscles -- their activity is modified by certain other factors unlike that of the skeletal muscles).

b. About 45 to 60% of the mass of most animals is made up of muscles (the figure is much lower in some animals (such as Cnidaria)).

c. Active muscles require by far the largest energy expenditures of any cells in the body. As a result, **many of the support systems in the body (such as the circulation and respiration) have evolved principally to meet the maximum demands of the muscles.**

4. Organism size and system(s) used for movement:



After Schmidt-Nielson, Animal Physiology, 1st ed.

B. Muscles:

1. **Types**: **STRIATED:** (includes **SKELETAL** and, in most higher animals, **CARDIAC**) and **SMOOTH**.

2. Here is a comparison between the two types of striated and smooth muscles; note that cardiac muscle sits in the middle in terms of its properties:

Type	Size	Appearance	Control	Contraction
Skeletal	large, syncytial	regular banding	somatic ns (exogenous) (voluntary)	short duration (<10 ms) but many single contractions can fuse to tetany
Cardiac	single cells	regular bands	endogenous but heavily influenced by the autonomic ns	variable, usually less < 1 s.
Smooth	small	no banding, spindle-shaped single cells	endogenous but heavily influenced by the autonomic ns	often longer than many seconds.

Note: **ENDOGENOUS** refers to the fact that under the right conditions these cells all have **PACEMAKER** activity. That is, they spontaneously discharge and this results in a contraction. Cells that are endogenously controlled are strongly influenced by the activity of the **AUTONOMIC NERVOUS SYSTEM** that **MODULATES** (modifies) their activity. Also, cardiac and smooth muscle cells (endogenous cells) tend to be **COUPLED VIA ELECTRICAL SYNAPSES** with each other and as a result they produce an **ELECTRICAL SYNCYTIUM (as compared to an actual cellular syncytium in skeletal muscles**). More about this later .

Since skeletal muscle cells are simpler to understand with the background that we have, we will start with them.

II. Skeletal muscle morphology:

A. ***Gross morphology***:

1. Connective tissues: (see diagram below)

a. outer covering is called the **FASCIA** or **EPIMYSIUM**

b. Internal connective sheaths that separate relatively large portions of muscle tissue are called the **PERIMYSIUM**

c. Internal connective sheaths that separate individual groups of muscle cells are called the **ENDOMYSIUM**.

d. connective tissues that connect the muscle to bones are called **TENDONS**

2. **Contractile tissue**:

a. **MUSCLE FIBER BUNDLE** or **FASICULUS**, the largest subdivision of contractile tissue, it is a group of muscle fibers that are enclosed by perimysium and is sub-divided by endomysium.

b. **MUSCLE FIBER** or **"MUSCLE CELL"**: a multinucleate **SYNCYTIUM** (redundant terminology!), this "cell" runs the length of the muscle fiber and is the result of the fusion of many individual embryonic cells.

3. ***HISTOLOGY OF A MUSCLE FIBER*** (see diagram, p4)

a. We can functionally divide the cell into three components, the **CONTRACTILE FIBRILS**, the **MEMBRANE SYSTEMS**, and the **SUPPORT SYSTEMS**.

b. **MEMBRANE SYSTEMS**:

1. **SARCOLEMMA**: the plasma membrane of the muscle cell, **it conducts a surface action potential** just like what we have studied in neurons. The motor endplates are found on the sarcolemma as are numerous openings to the t-tubules, an internal membrane system.

2. **T-tubules** (**transverse tubules**), a system of tubules that are continuous with both the sarcolemma and with the internal membrane system (the sarcoplasmic reticulum). They pass from one side of the cell to another as essentially a little tube that is filled with extracellular fluid.

3. **SARCOPLASMIC RETICULUM (SR)**: the internal membrane system of the cell, **it is connected extensively to the t-tubules and any AP that moves along the sarcolemma will cause APs to enter the T-tubules and will set-of a general depolarization of the SR.** The SR that is associated with the T-tubules is called the **T-SYSTEM.**

4. **CISTERNAE**: specialized regions of the SR that concentrate large amounts of Ca^{++} by removing it actively from the cytosol ("sarcoplasm"). Thus:

a. they contain a Ca^{++} - ATPase (Ca^{++} pump).

b. They also contain voltage-dependent Ca^{++} channels that can release Ca^{++} back into the sarcoplasm whenever the SR is depolarized.

c. **CONTRACTILE SYSTEM:** a complex of four types of proteins that are arranged in bundles called MYOFIBRILS. These are the entities that actually produced contractile force. On the microscopic level they are of two types, **THICK (MYO)FIBRILS** and **THIN (MYO)FIBRILS**. We will consider them in more details starting shortly.

(Please see the illustration in the next page)

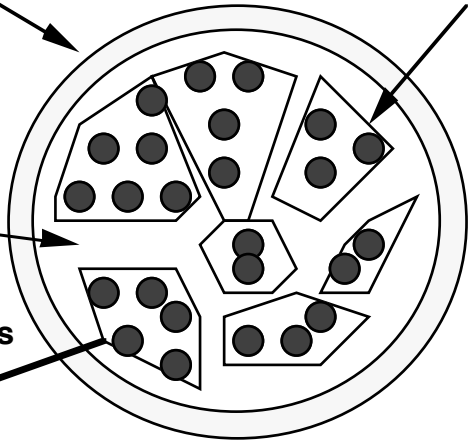
Gross Muscle Morphology -- The Cellular, Subcellular and Connective Tissue Components of a Muscle

***Fascis* (*epimysium*) -- the outer covering of connective tissue.**

Cross Section of a Muscle

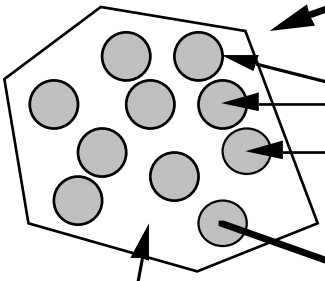
Fiber bundle or *Fasciculus*

The connective tissue running towards the center of the muscle is called *Perimysium*



Cross-Section of a Fasiculus

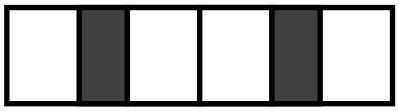
Fiber bundle or *Fasciculus:*



Muscle fibers or "cells" (recall that they are the result of the fusion of many embryonic muscle cells (myoblasts) into one large syncytium. Each of these contains many Myofilament bundles and there are many of these in each muscle "cell".

View of small length-wise section of a myofilament bundle. Many more of these untis join end to end and in parallel to produce the characteristic banding of skeletal muscles.

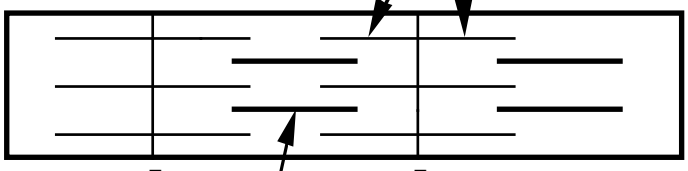
Endomysium **(the connective tissue that surrounds each muscle fiber (muscle "cell")**



striations (banding)

The same view as above but expanded somewhat

thin myofibril



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thick myofibril

A single sarcomere -- many of these repeat in each myofilament bundle; they give the muscle "cell" its characteristic striations.

Please note that these diagrams are only schematic and are greatly limited by my "artistic ability"

d. Support system: the portions of a cell that are responsible for **(i) producing the ~P needed for contractions; (ii) serving as an avenue whereby Ca^{++} can flow from its storage area in the cisternae to the thin fibrils; (iii) general support functions for the cell (protein synthesis, etc.)**.

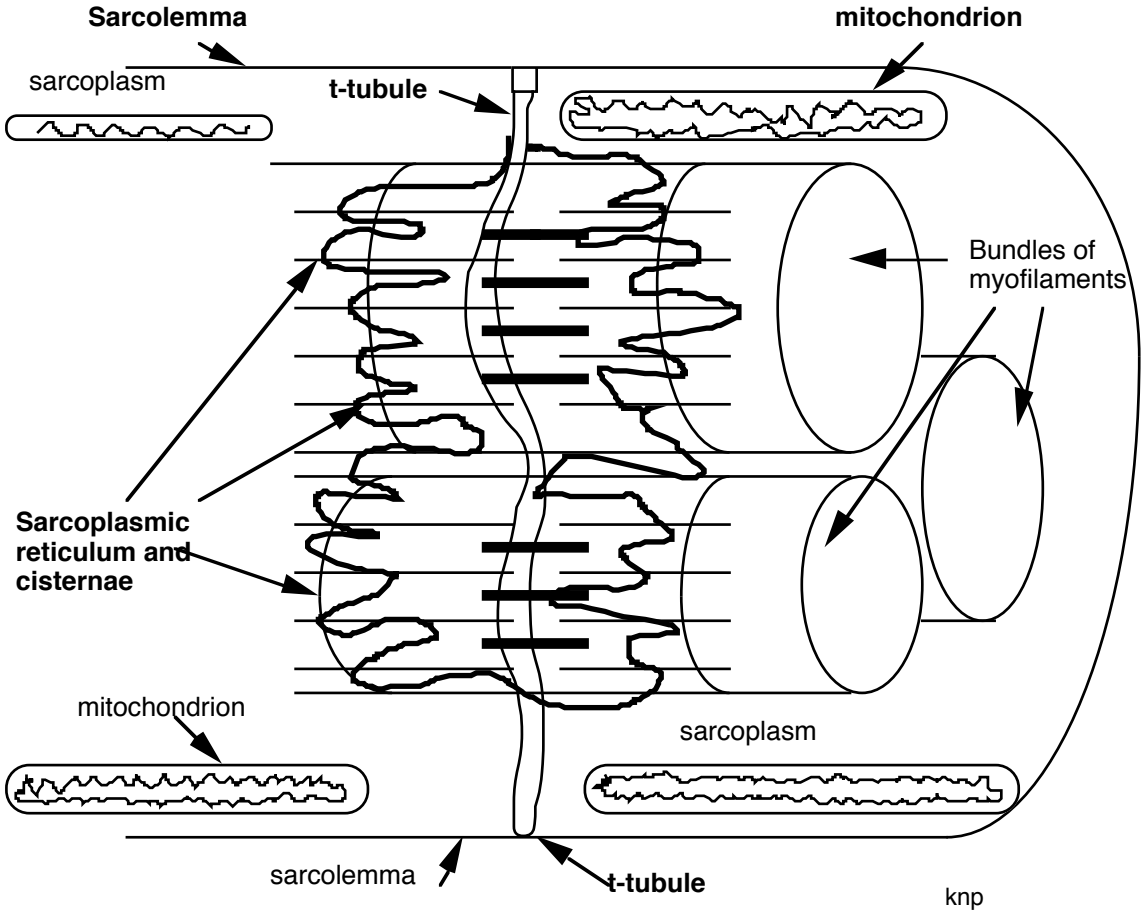
1. **sarcoplasm**: the cytosol, it contains stores of ~P in the form of **ATP** and **phosphagen** (creatine phosphate in vertebrates), it is the seat of:

a. **the glycolytic reactions for forming ~P from carbohydrates**

b. glycogen stores are located in the sarcoplasm, etc.

2. **mitochondria**: generally located right next to the fibrils, they are present in all muscle cells but they vary tremendously in number. In some muscles that depend heavily on oxygen for ~P generation they may make up 50% of the cell volume while in cells that are mainly anaerobic they make up less than 1% of the cell volume.

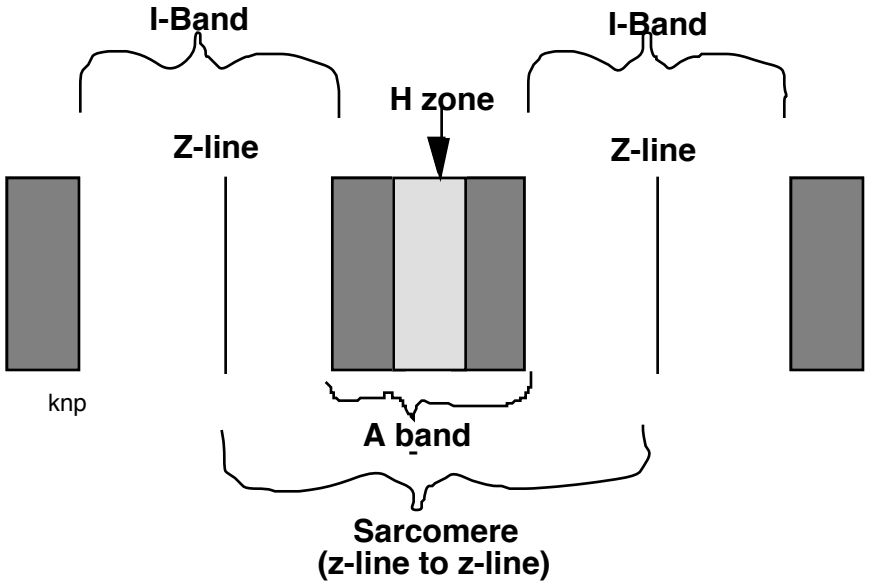
Some of the Histological Elements of a Single Skeletal Muscle Cell (Muscle Fiber)



A section of a single muscle cell. Please note that in most cells there would be many more bundles of myofibrils in parallel with each other. Note also that each bundle is composed of large numbers of sarcomeres lined up in series -- this is a very small length of the overall muscle cell. Note that near the center of sarcomeres, t-tubules cross the cell. They are closely associated with the sarcoplasmic reticulum but they are actually tubular invaginations of the sarcolemma. Specialized areas of the SR called cisternae sequester and release Ca^{++}.

e. Appearance of relaxed and contracting muscle cells. The most conspicuous thing about any skeletal cell is the banding pattern. Thus, it was one of the first things described about muscles and the terminology to describe the banding patterns is useful:

1. ***relaxed:***



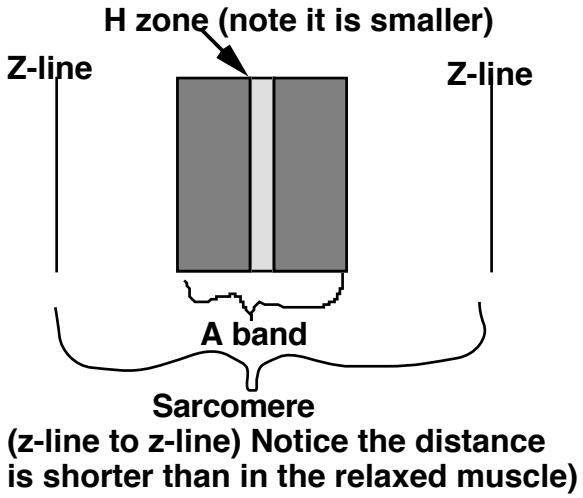
(i) the striations consist of alternating light (**I or isotopic bands**) and dark regions (**A or anisotropic bands**).

(ii) the A bands at rest have a distinctly lighter colored central region, called the **H-band**.

(iii) the middles of the I bands have transverse lines that extend through them called **Z-LINES**.

(iv) The area between adjacent Z-lines is called a **SARCOMERE**.

2. ***CONTRACTING***



(i) the length of the sarcomere shortens

(ii) as the sarcomere shortens, the size of the H-band decreases and if the contraction is extensive enough, the H-band completely disappears.

3. After having observed this behavior, biologists sought to learn the identity of the materials in the different bands.

a. the I-bands were composed mostly of a complex of proteins that together make up the **THIN FILAMENTS**. They are anchored to the Z-lines and are arranged in a lattice. Each Thin filament was found to be made up of three types of protein that are directly connected to contraction. By weight from most common to least, these proteins are:

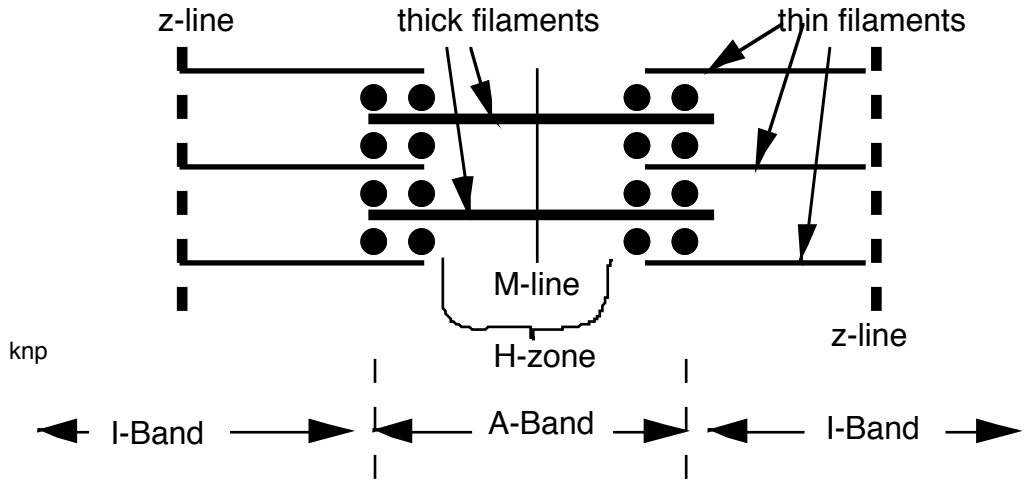
1. **ACTIN**
2. **TROPOMYOSIN**
3. **TROPONIN**

! In addition, it is known that many of the enzymes of glycolysis are complexed onto the thin filaments (they attach to actin). The result of this is that the glycolytic enzymes and therefore reactions are concentrated in the parts of the cell where the ATP produced via glycolysis is most needed -- the contractile proteins. See G. Somero 1990, Phys. Zool review paper.

b. the H-band was made mainly of THICK FILAMENTS composed of mainly one type of protein, **MYOSIN**.

c. the A - bands were composed of both thick and thin filaments, this is why they were the darkest. Since the H-bands had only the thick fibers they were less dark and the I bands were the lightest since they contain only thin filaments.

Relaxed



d. During contraction, the thin filaments move along the thick filaments and move further and further into the H zone, slowly obliterating it. Since the thin filaments also are anchored to the Z-lines, the sarcomere decreases in length.

4. The original theory of muscle contraction grew out of these observations and was called the **"SLIDING FILAMENT THEORY"**. However, it was soon modified since no one could think of a way whereby one filament could glide past another and produce tension. When better microscopic techniques became available, it became obvious that **during contraction only, there were physical connections being formed between the thick and thin filaments**; these were called **CROSS-BRIDGES** and the cross-bridge theory supplanted the sliding filament theory.

B. Biochemical Basis of Contraction:

1. Introduction: much of the evidence for how muscles actually produce contractions has come over the last 20 years. (I should mention that another Huxley from the same family as the one who worked on APs was involved in the discovery of how muscles contract). Three important types of studies have been used and have been integrated into an overall picture of how muscles contract:

a. X-ray crystallography: to see how various proteins' shapes (conformations) changed with respect to different conditions

b. Protein biochemistry: elucidate the chemical properties of the proteins involved in contraction.

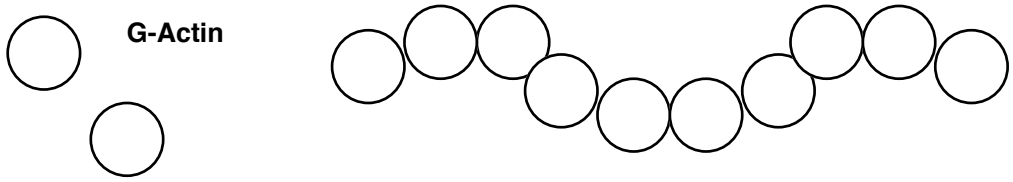
c. Histochemistry: very sophisticated new techniques that have allowed the testing of ideas about how muscles worked (that were based on simplified chemical systems) on real cells. These included techniques where dyes are injected to change color according the amount of Ca++ present (aqueoin) in the sarcoplasm or according to the voltage.

2. The **THIN FILAMENTS**:

a. **ACTIN**

1. The majority of the thin filament is a double helix of two actin polymers (**F- ACTIN POLYMERS**).

2. Each these polymers are made up a large number of single actin molecules. These individual molecules are globular proteins (i.e., **G-ACTIN**) with MW=45,000.



F- actin (helical polymer chain of G-action)

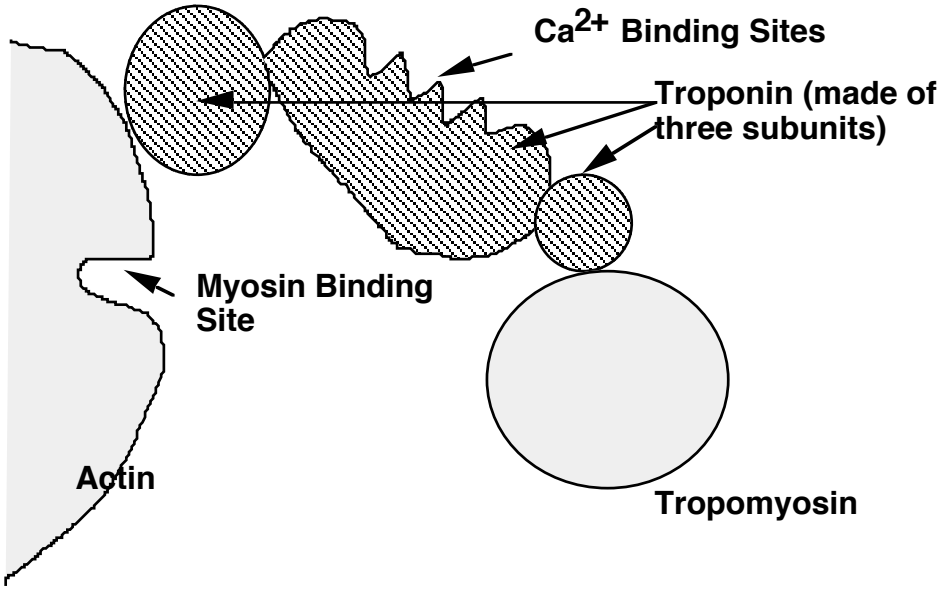
3. Each G-ACTIN can bind to:
   a. other G-actins to form a polymer
   b. to troponin (see below)
   c. under certain circumstances, to myosin (see below)

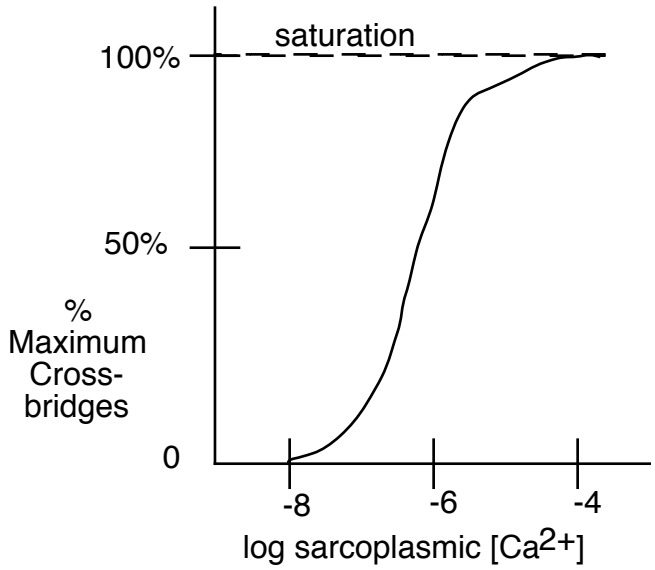
b. **TROPONIN**

1. It is the least common protein on the thin filament, it is the **FILAMENT-LEVEL REGULATOR OF CROSSBRIDGE FORMATION.**

2. It has quaternary structure; it is made of three subunits.
   a. the first subunit binds to actin and to the middle subunit, (which is called troponin-c).
   b. **TROPONIN-C** is the middle subunit, it can bind up to 4 Ca^{++}.
   c. the other subunit binds the troponin-c to Tropomyosin:

3. The overall troponin molecule is **ALLOSTERIC**, that is, it can change shape in response to the amount of Ca^{++} that has bound to it. The allosteric effect is very tightly controlled by the [Ca^{++}]; the curve is very steep meaning that a slight change in [Ca^{++}] will cause a large effect on troponin's shape.





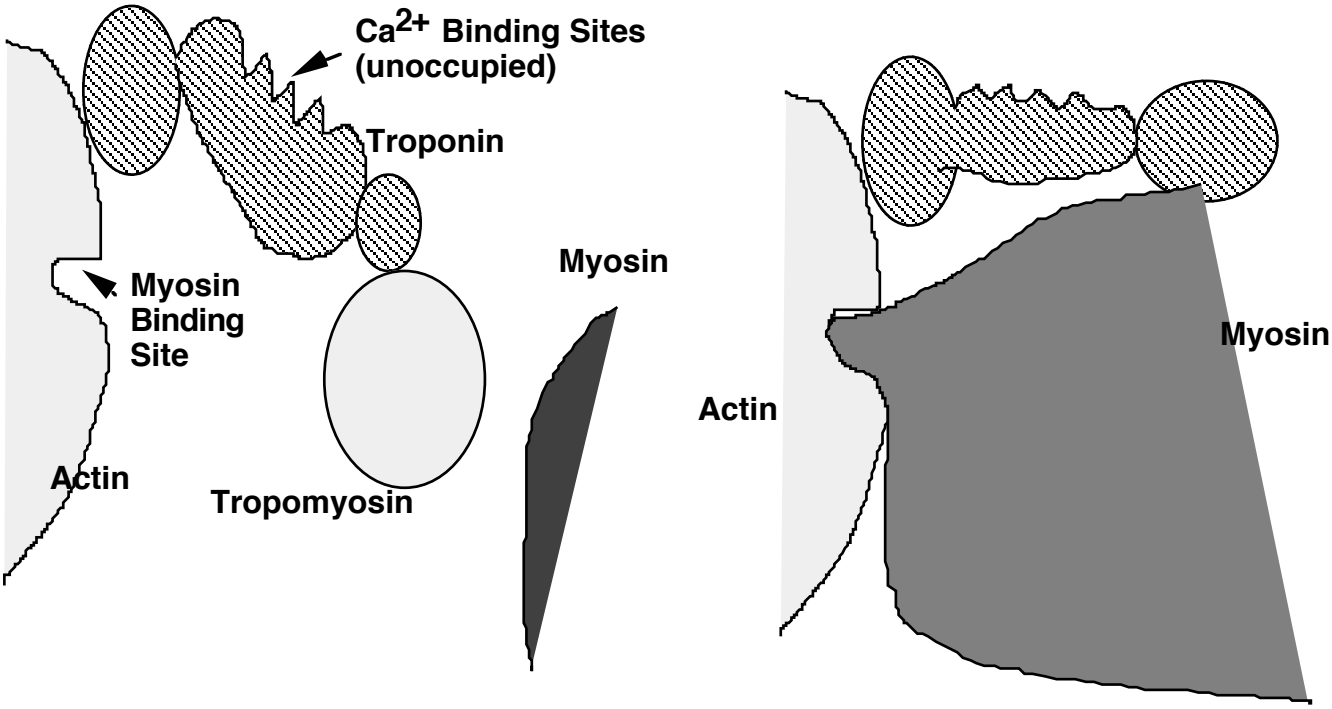
c. **TROPOMYOSIN**

1. this is a rod-shaped molecule that is attached to troponin.

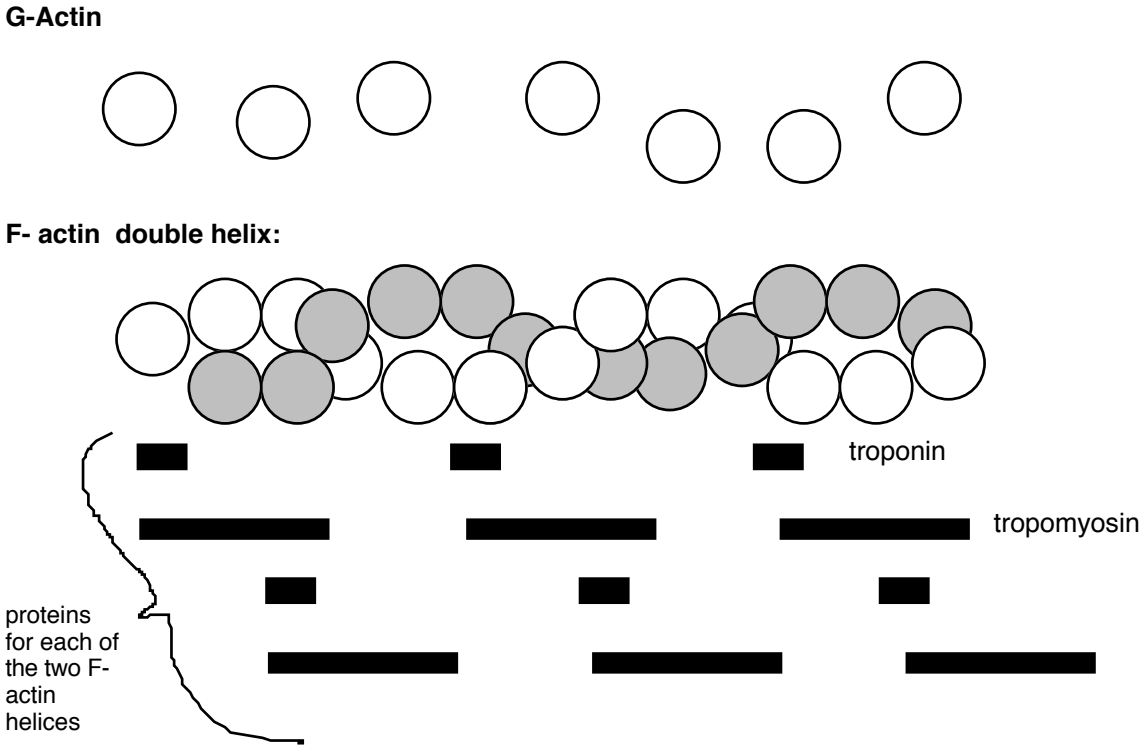
2. Under relaxed conditions, the tropomyosin is held over the myosin binding sites on the actin.

3. When the sarcoplasmic [Ca^{++}] is high (as during a contraction), tropomyosin is moved away from the myosin binding sites and it is possible for myosin to bind to actin.

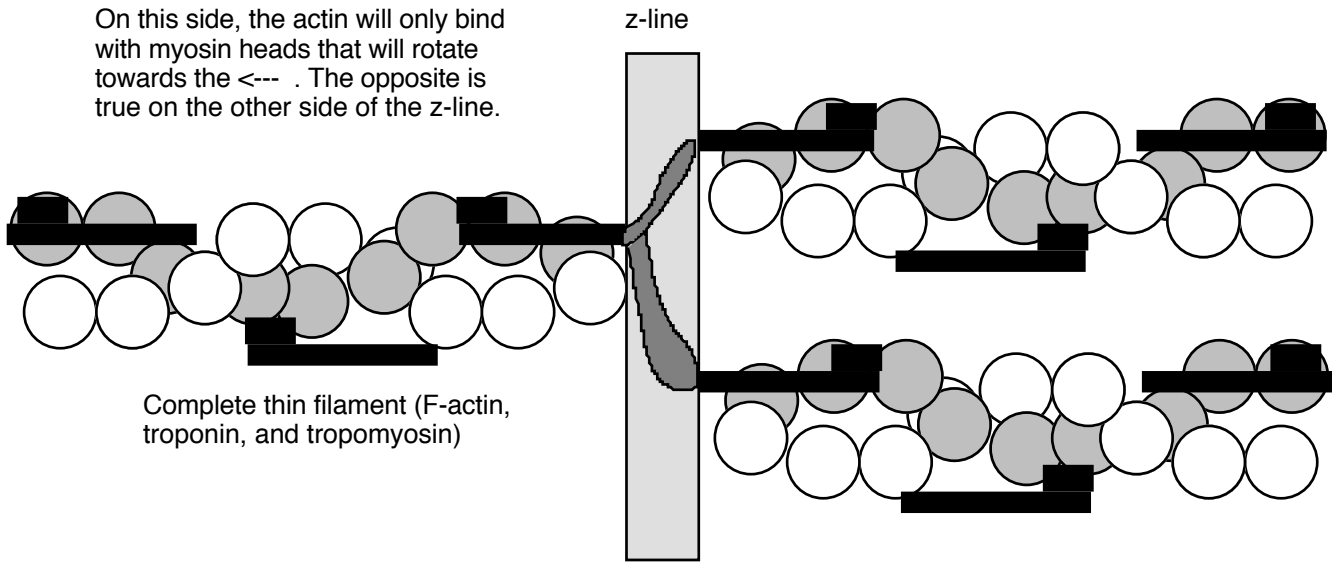
4. Thus, the role of tropomyosin is that it can create **STERIC HINDRANCE** or (**STERIC INHIBITION)** to the binding of myosin to actin.



e. Overall configuration of the thin filaments:



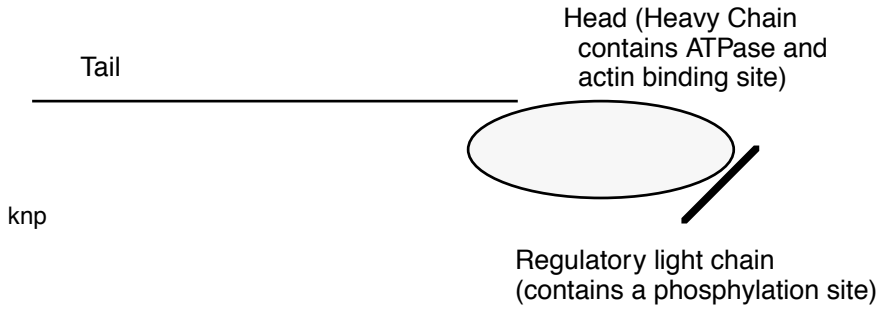
f. The thin filaments exhibit a **POLARITY**, that is, they will only bind with myosin heads that point in a certain direction. This will be more obvious in a moment. For now, simply **remember that thin filaments have polarity and that it reverses at each side of the Z-line.**



2. **THICK FILAMENTS**:

a. these are composed entirely of MYOSIN, a very large (MW= 470,000), quaternary (6 chains), allosteric protein.

b. Each myosin consists of two identical units that are largely wrapped around each other. These units can be dissected with proteases and it is found that each unit consists of a HEAVY CHAIN and TWO DIFFERENT LIGHT CHAINS:



COMPONENTS OF THE HEAVY CHAIN:

1. **TAIL**, these are long, very insoluble units that polymerize with each other to form a very thick filament that is essentially what is viewed as the thick filament.

2. **NECK**: more of the material similar to that making up the tails except that it is more water soluble and is flexible

3. **GLOBULAR HEADS**: these are large and water soluble, they are one end portion of the "heavy chain".

a. they contain binding sites for actin. Thus, together with the necks, they make up the cross-bridges.

b. *they also contain an ATPase*.

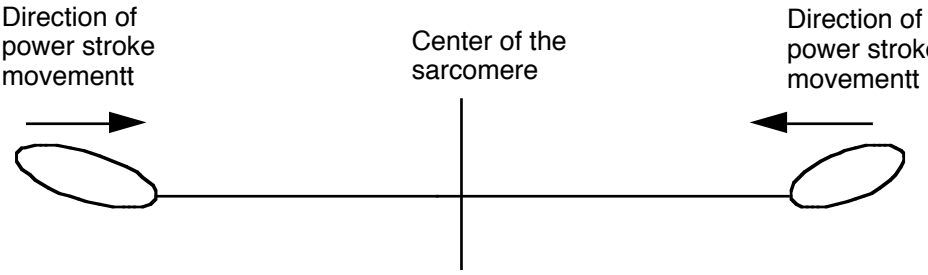
LIGHT CHAINS: these are attached to the Globular Heads of the heavy chains, one of these has important regulatory functions that will be discussed later.

c. **POLARITY**: like the thin filaments, thick filaments also possess polarity. In their case, polarity refers to the way that myosin will attach to the actin and the direction that the myosin will move during a contraction.

1. myosin polarity reverses at the center of each sarcomere.

2. movement of the myosin head is always towards the center of the sarcomere.

3. polarity is a consequence of the way that the myosin tails are connected together; its result is an orderly contraction where all myosin heads on one side of the center of a sarcomere move in the same direction. Their movement will result in the pulling of thin filaments towards the center of the sarcomere:



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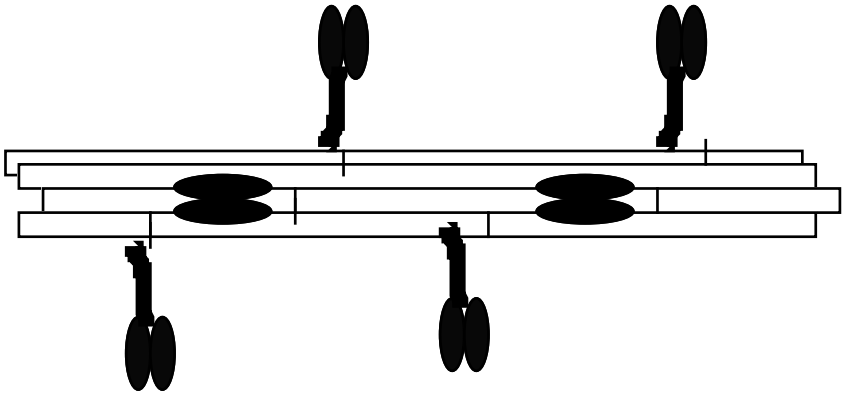
Notice that on opposite sides of the center of the sarcomere, the heads of different myosin molecules rotate in opposite directions with the effect that both rotate towards the center of the sarcomere.

4. Finally, each thick and thin filament is organized in a three-dimensional lattice that permits each filament to form cross-bridges with several others:



Simplified Diagram of a Thick Filament Showing a Number of Individual Myosins whose heads are regularly spaced all around the fiilament such that they can interact with the thin filaments that surround them.

3. ENERGETICS OF CONTRACTION:

a. Muscle contraction is dependent on the hydrolysis of ~P from ATP. However, the exact relationship between the hydrolysis of the terminal ~P on ATP and contraction is complex and is not what most would expect.

b. Studies were made on cell-free solutions that contain thick and thin filaments and where ATP could be added or removed. Also, x-ray crystallography has revealed much about the different conformations of myosin the presence and absence of ATP.

c. THE CROSS BRIDGE CYCLE AND ENERGY:

1. **Cross bridge cycling** refers to the **formation of a bond between actin and a myosin head (cross-bridge), the rotation of the myosin head to cause shortening, and then the breaking of this bond**.

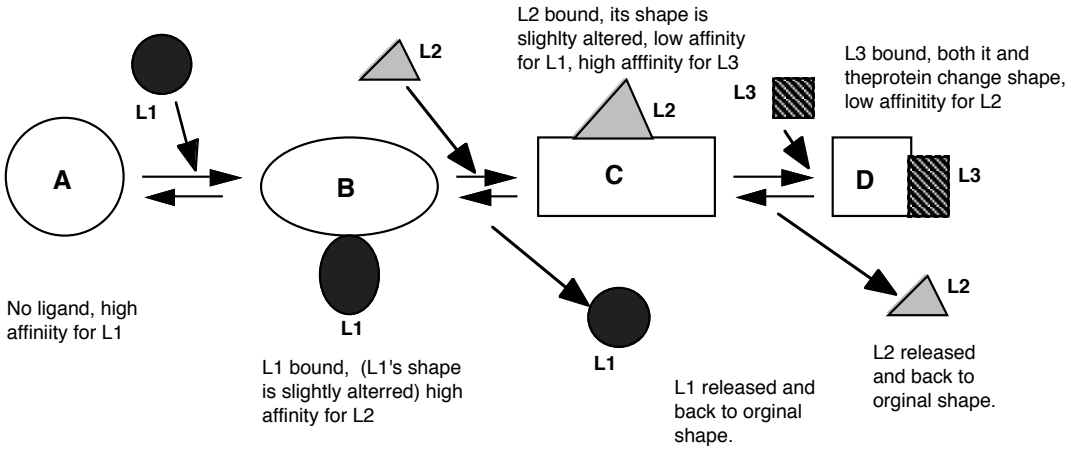
2. Since mechanical work is done, obviously energy will be required. This energy comes from ATP. However, the way the energy is provided is probably different than you thought.

a. The entire process will be one cycle.

b. Each stage of the cycle will be a molecule with:

1. **a unique structure (shape)**

2. **a certain affinity for other molecules**, this affinity is a function of the particular shape the molecules has at that point in time. **Thus, the entire cycle can be viewed as a series of reactions each characterized by proteins of a certain shape and affinity; these reactions are all linked tightly to each other such that the equilibrium for any one affects all others.**



A General Schematic Illustrating the Allosteric Properties of Certain Proteins, for instance, most of those found in the myofilamants: Four Shapes of the same protein (denoted A to D), each determined by the particular ligands to which the protein is bound. Each step is freely reversible under cellular conditions -- this implies that the most prevelant form will be determined by the relative abundances of the various ligands. *This is a general model for allosteric proteins.* Note that each time that a ligand binds, both the protein and tthe ligand undergo a shape change; when the ligands are released, tthey return to their original shape. Note that the previous binding influences the affinity of the protein for the next ligand.

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3. **With the exception of the step that involves the hydrolysis of ATP, each stage is easily reversible to the previous one.** That is, the structure of a given compound can relatively easily change to one of two other alternatives, depending on the mass-action ratio for a particular reaction compared to its equilibrium ratio.

a. Note that the mass action ratio is determined in part by the abundance of a particular form (allosteric shape) of a protein -- this is determined in part by the abundance of other ligand (see the illustration above).

b. Thus, this is a rather complicated equilibrium .process -- since we are dealing with a series of linked reactions, the exact calculation of the relative amounts of each form is often a difficult problem.

4. Movement will be the result of an orderly sequence of change in structure. Some of these changes are the actual movements of myosin head, and they result in pulling the thin filaments towards the center of the sarcomere. Other changes involve the head's release of the actin, its "re-setting" and eventually its re-attachment.

c. Items #3 and #4 above are the crux of the problem. If all the steps are easily reversible, then motion in a constant direction (as occurs in contraction) is not possible unless one of the steps is made irreversible.

1. **ESSENTIALLY,** one step is made such that:

A --> B is likely while B --> A is very unlikely.

2. The result is that the entire cycle is forced to move always in one direction, provided that conditions for this one particular step continue **... REMEMBER, without one irreversible step to force the cycle to move in a single direction, movement in one direction is as likely as the other.**

d. Steps become irreversible when they are greatly displaced from equilibrium in a direction that causes them to proceed in a certain direction. This step and all linked steps will continue to move in that same direction as long as the step remains well out of equilibrium**.**

d. **In cross-bridging, the step that makes the process move in a predicted direction is the hydrolysis of ATP.**

! The fact that the muscle cell maintains the [ATP] a long way from equilibrium means that ATP will tend only to be hydrolyzed, not synthesized in this process (in other words, the step will move in only one direction).

In cross-bridging, the following are the actual steps:

3. If the following rules are learned, understanding the sequence is easy:

a. myosin that is bound to actin (crossbridged, **ACTO-MYOSIN**) has a high affinity for ATP.

b. **If ATP binds to acto-myosin, the affinity of myosin for actin if lowered**. Thus, myosin will release actin. The process of release will affect the structure of myosin.

c. **Myosin that binds ATP will use its ATPase to partially hydrolyze the ATP.** The hydrolysis products are not released and the energy from the hydrolysis is stored in the myosin head:

myosin * ATP (myosin-ATP complex) --> M^* * ADP * Pi

where M^* represents a myosin intermediate that is of different shape from the form of myosin previous to its binding of ATP..

d. this new complex (M^* * ADP * Pi) has a high affinity for actin and thus will bind with it if it is available (when would that be?) and thereby create a cross bridge:

M^* * ADP * Pi + Actin ---> A - M^* * ADP * Pi

e. the formation of the actin-myosin complex lowers the affinity of myosin for the ADP and Pi and they are released.

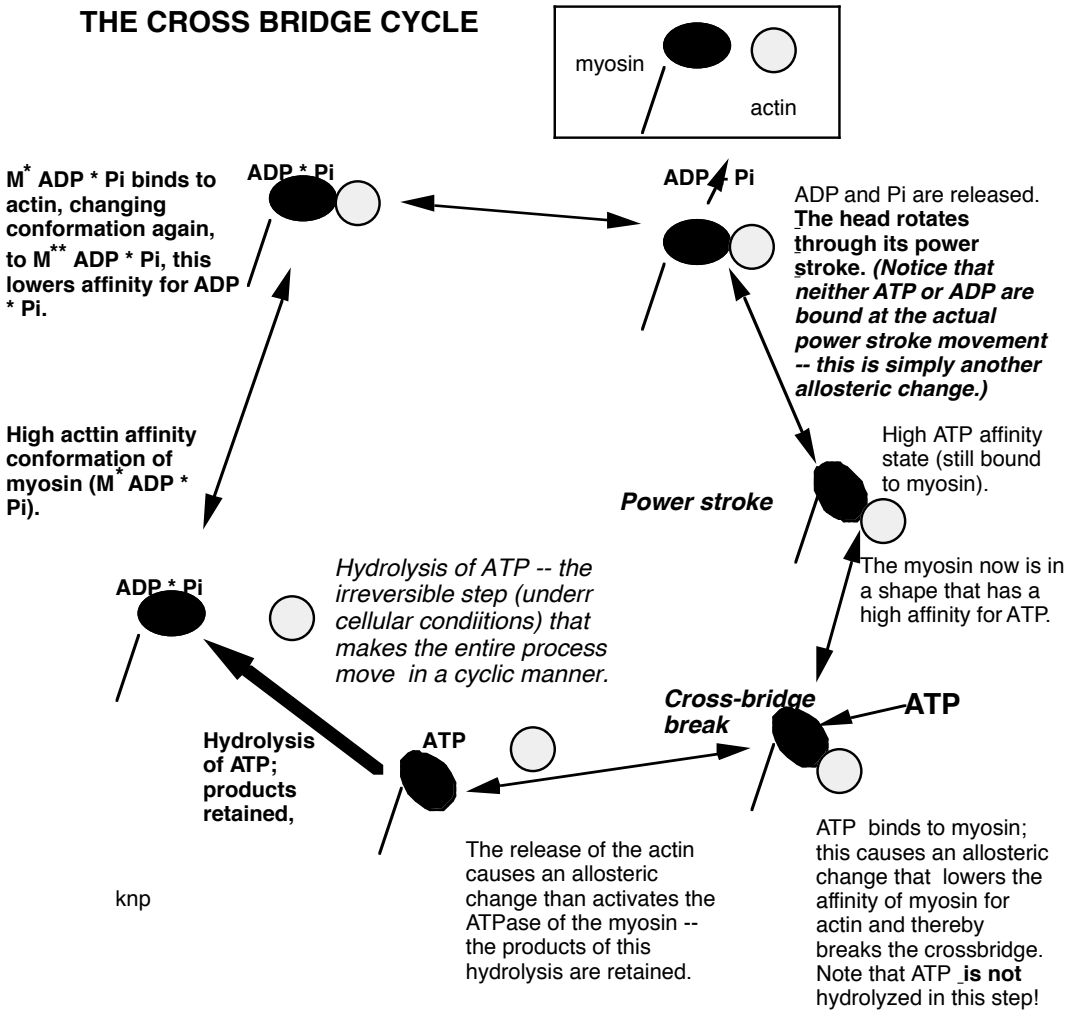
A-M^* * ADP * Pi ---> A-M^* + ADP + Pi

f. the release of the ADP and Pi triggers another allosteric change in the acto-myosin complex, this one resulting in what we call "cross-bridge rotation" where in the actin is moved a short distance by the myosin.

g. **Thus, movement of the cross-bridge is not directly fueled by the hydrolysis of ATP. ATP is used directly to break the cross-bridge and fuels the movement by participating in a chain of reactions and causing them to move in one direction.**

h. Once the cross-bridge has "rotated" (due to the myosin conformational change) the myosin-ATPase is once again in a high-affinity state for ATP. Since muscle cells normally maintain large amounts of ATP, ATP will bind to this site.

i. This binding will trigger another allosteric change that causes the cross-bridge to break.



The cross-bridge cycle. Note that most reactions are potentially reversible (as is shown by the double arrows) -- the hydrolysis of ATP after actin has been released is the key step that makes the process irreversible. Note also that movement and changes in affinity and enzyme activity (for the myosin ATPase) are all simply the result of a series of conformatinal changes. ATP does not directly fuel the movement. This point is important for the next class where we will look at the regulation of contraction in general and at the allosteric changes in the regulatory troponin in particular -- this protein undegoes large shape changes an has no direct physical interaction with ATP at all!