Muscles are biological motors which actively generate force and produce movement through the process of contraction. The molecular mechanism responsible for muscle contraction is similar in cardiac muscle, smooth muscle and skeletal muscle. However, the cellular structure and activation process differ among the three types of muscle. Here, we will consider only skeletal muscle.

We will begin by examining the structure and function of muscle at the microscopic level and building to the macroscopic level of whole muscles. This will provide an understanding of the basis for the mechanical behavior of muscle that we observe on the macroscopic level. A great leap in understanding the mechanism of muscle contraction was made when H.E. Huxley and A.F. Huxley independently proposed the sliding filament, cross-bridge model of muscle contraction in 1957. The original model has been modified over the years, but the basic principles appear to be sound. It is this model which we will use as the basis for understanding how viscoelastic properties of muscle arise.

**Microscopic Structure**

- Muscles contain two principal contractile proteins, actin and myosin, whose combined interaction with adenosine triphosphate (ATP) converts chemical energy into mechanical work.

- A myosin molecule consists of a rod (or tail) region and two elongated globular head regions. The myosin rod is about 20Å in diameter and 1500Å long while the heads are about 150Å long and up to 50Å in width. The rod is a two-chain \( \alpha \)-helical coiled-coil. Myosin rods have self-association properties, that is, they bind to each other to form longer filaments. There are about 600 myosin molecules per myosin filament. Myosin filaments are organized such that adjacent pairs of heads are 14.3 nm apart (Fig. 1.1).

- The myosin molecule can be separated into protein subunits. The myosin head is often referred to as the S1 region (subfragment 1) of the molecule. It consists of two essential, two regulatory light chains and two heavy polypeptide chains. The tails of two heavy chains are wound to form a rigid rod. The light peptide chains are located near the neck of the head region.

- Globular actin (G-actin) molecules, which are 40 to 50Å in diameter, polymerize in the form of long filaments (F-actin) arranged as two entwined \( \alpha \)-helices. Actin has approximately 13 monomers (subunits) for six turns of the helix or about 2.17 monomers per turn (Fig. 1.1).

- Myofilaments are arranged in an orderly array with six actin (thin) filaments surrounding each myosin (thick) filament. The two heads of a myosin molecule project at an angle away from the myosin rod. Three pairs of myosin heads are spaced approximately 120° apart around the circumference of the thick filament. Each pair of heads projects toward a different actin filament. Successive positions along the thick filament are occupied by pairs of myosin heads which are rotated by 60° with respect the previous group of three (Fig. 1.1).
Tens to hundreds of thousands of parallel myofilaments are contained within the cross-section of a single muscle fiber. Along the length of each muscle fiber is a repeating pattern generated by an alternation of partially overlapping arrays of actin and myosin filaments. The repeating unit is known as the sarcomere. Its length is about 2 to 3 µm at rest length in vertebrate muscle (Fig. 1.2).

In the center of each sarcomere is the A-band, which is the region in which actin and myosin filaments overlap. Between the A-band of one sarcomere and the next is the I-band, which consists of only actin filaments. In the center of the I-band is the Z-band, which is a connecting structure that mechanically links the actin filaments of one sarcomere with the next. The Z-bands consist of protein filaments that form a transverse network (Z-disc), possibly including α-actinin, whose function is to anchor the actin filaments of adjacent sarcomeres. These filaments must be sufficiently elastic to enable lateral expansion during extensive contraction and must also be responsive to strains in the longitudinal axis (Fig. 1.2).

Myosin molecules in each half of the A-band are thought to point in opposite directions, giving the myosin filament a bipolar structure. The central region of the A-band contains only myosin rods. Here the myosin filaments are cross-linked by proteins called M-line proteins (Fig. 1.2).

Other proteins, such as C, X and H proteins are involved in bundling myosin molecules together in the thick filament during development. Titin is a long protein molecule with one termination in the Z-band and the other in the M-band. It has a region in the I-band which can be stretched and likely contributes to the passive elastic force that allows a muscle fiber to return to its original length after being stretched.

Nebulin is a long protein that extends along the length of an actin filament and may act like a ruler to set the length of the actin filament.

The actin molecules are not in precise register with the myosin heads. However, if an actin molecule is sufficiently close to a myosin head, a bond can be formed linking the actin and myosin filaments. Because of their ability to form these links, myosin heads are often referred to as cross-bridges. The process of formation of a link between actin and myosin is known as cross-bridge attachment. Cross-bridge detachment is the reverse process in which the link is broken.

Myosin molecules have ATPase activity, i.e. they can bind ATP and then split (hydrolyze) the bound ATP into adenosine diphosphate (ADP) and inorganic phosphate (P_i). The myosin ATPase reaction in solution is relatively slow, but if F-actin is added the rate becomes about 500 times as fast.

In the absence of ATP, actin and myosin molecules bind spontaneously to form an actomyosin complex which has a lower free energy than either molecule separately, i.e., energy is liberated during the binding of myosin binds to actin. Unless ATP is added to the solution, actin and myosin will remain bound (Fig. 1.3).
• When ATP is present, myosin and actomyosin bind ATP. The free energy of actomyosin-ATP is similar to that of myosin-ATP, allowing myosin-ATP to detach from actin. There may be several intermediate biochemical states between those shown in Fig. 1.3. Some of these states may contribute to the passive elastic force in sarcomeres without actively generating force. Transition between different bound states may also be responsible for some of the relative motion between thick and thin filaments.

• The process of active force generation is generally considered to constitute a cycle during which a myosin cross-bridge binds to actin and then later detaches after hydrolyzing ATP. During attachment, the molecular conformation of the myosin cross-bridge changes in such a way that tension is created, pulling the actin filament towards the myosin filament (Fig. 1.3).

• There is still considerable uncertainty about how myosin bonds to actin and where in the myosin molecule motion occurs. It is generally accepted that the myosin head contains a flexible region at the light-heavy chain junction which bends, permitting the portion of the head, which is most distant from the actin attachment site, to move through a considerable angle. This bending would be transformed into linear motion of the actin filament by the lever arm action of the rigid portion of the head. However, some motion may also occur at the site of cross-bridge attachment.

• If the actin filament is held isometric so that it cannot move, the cross-bridge applies a constant tension to the actin filament. If the actin filament is free to move, the cross-bridge will pull the actin filament. Both of these processes have recently been studied in an experiment which allows the action of single cross-bridges to be measured. The force produced by a single cross-bridge is in the range of 1-7 pN (mean 4 pN) while the displacement is 7-17 nm (mean 11 nm). It is clear from this experiment that the duration of attachment increases as the concentration of ATP decreases, providing evidence that ATP is indeed necessary for detachment to take place (Fig. 1.4).

• Experiments have been conducted to investigate the relationship between the force and displacement produced by a single cross-bridge moving an elastic load. The force-displacement relation is highly linear suggesting that the development of tension and filament sliding is powered by the release of elastic energy stored in the cross-bridge.

• Because of the bipolar structure of the sarcomere, actin filaments from each half of the sarcomere are pulled towards the center, reducing distance between Z-lines (sarcomere length). The successive attachment, pulling and detachment of cross-bridges in an asynchronous manner produces sliding motion of the myofilaments with respect to each other, resulting in muscle shortening.

• This cycle can be repeated many times during sustained contraction of a muscle fiber. The cycle period is probably 100-200 ms in duration during isometric contraction, although this value will depend on factors such as the myosin ATPase reaction rate, the temperature of the muscle and the rate of change of muscle fiber length. In particular, at maximum shortening velocity, cross-bridges cannot remain attached for more than 5 ms.
Attachment and force generation, and detachment may proceed much faster than the overall cycling rate. The attachment and force generation step has been estimated to occur within as little as 7 ms while the detachment step can occur within 2.5 ms.

Myosin exists in a number of isoenzymic forms which differ in their rates of splitting ATP. These different isoenzymes are important in determining the maximum shortening velocity of a sarcomere.

**Length-tension Relation**

The force generated by a muscle fiber consists of an active component due to contraction and a passive component due to structural proteins and connective tissue throughout the fiber. In experiments done on fully activated frog semitendinosus muscle fibers, the active force was shown to correlate with the amount of overlap between actin and myosin filaments. This correlation was based on the assumptions that actin and myosin filaments normally slide past each other during sarcomere length changes without changing their lengths, and that the myosin cross-bridges form a linear array of independent force generators (Fig. 1.5).

In this particular muscle fiber, active tension begins to develop when the length of the sarcomere becomes less than 3.65 µm. This is the length at which actin and myosin filaments just begin to overlap. Between 3.65 µm and 2.25 µm the actin filaments gradually overlap more of the A-band and a linear increase in force is observed. At 2.25 µm, the ends of the thin filaments extend beyond the reach of myosin heads on the thick filament. Therefore, no additional cross-bridges are available for interaction and a plateau occurs. At sarcomere lengths less than 2.05 µm, double overlap of actin filaments from opposite ends of the sarcomere starts to occur in the middle of the A-band. This is thought to produce interference between actin filaments originating from opposite ends of the sarcomere. When an actin filament crosses the M-band, myosin heads from the opposite side of the sarcomere can bind to it. However, this will produce a force that pushes the actin filament away and will subtract from the force being generated by those cross-bridges pulling the filament. Consequently, there will be a decline in contractile force. At 1.65 µm the thick filaments contact the Z-lines and the force declines further as the sarcomere length continues to decrease (Fig. 1.5).

The form of the sarcomere length-tension relation for different muscle types and muscles from different species is similar, although specific details such as the width of the plateau region and the steepness of the ascending and descending portions of the length-tension curve may differ, due to differences in the lengths of actin and myosin filaments.
Muscle Fiber Stiffness

- Stiffness of a muscle fiber is generally measured by applying small stretches (or releases) to one end of the fiber. Stiffness is defined as the ratio of the change in force to the change in length.

\[ K = \frac{\Delta F}{\Delta x} \]

- A fundamental concept in muscle mechanics is that the stiffness of a sarcomere changes with the number of attached cross-bridges.

  - Cross-bridges behave like tiny springs. If the spring is stretched by pulling on one end, the tension in the spring increases. If the spring is shortened, the tension in the spring decreases. Tension in the spring may be modified by rotation or bending of the myosin head during the transition from a weakly bound to a strongly bound state or from one strongly bound state to another.

  - An attached cross-bridge can also be stretched or shortened by increasing or decreasing the length of the sarcomere in which it is located. Increasing the sarcomere length pulls the two sets of actin filaments apart and stretches any attached cross-bridges (Fig. 1.6C). Decreasing the sarcomere length brings the actin filaments together and shortens any attached cross-bridges (Fig. 1.6D).

  - Since all attached cross-bridges are stretched when a muscle fiber is stretched they all increase the tension being applied to the actin filaments. All of these forces are applied in parallel to the actin filaments so they sum. If there are \( N_c \) attached cross-bridges, which are each stretched by an amount \( \Delta x \) and each cross-bridge has a stiffness, \( k \), then the change in sarcomere force is given by

\[ \Delta F_s = \sum_{i}^{N_c} \Delta F = \sum_{i}^{N_c} k \Delta x = N_c k \Delta x \]

  - Recently, H.E. Huxley and others have shown that both the thin and thick filaments are extensible, i.e., they cannot be assumed to be rigid elements. Since myofilaments act in series with cross-bridges, the tension in the myofilaments must be equal to the tension in the cross-bridges. Therefore, when a muscle fiber is stretched, producing an increase in cross-bridge force, there must be an equal increase in myofilament force, resulting in stretch of the myofilaments. The amount by which the cross-bridges and myofilaments stretch is determined by their relative stiffness.

  - A muscle fiber consists of several thousand sarcomeres joined end-to-end in series. A change in the length of the muscle fiber must be equal to the sum of the length changes in the individual sarcomeres. However, the change in force must be the same for each sarcomere. This can be best illustrated by considering two neighboring sarcomeres. If
the change in force of one sarcomere were greater than in the neighboring sarcomere, it would pull on the neighboring sarcomere. This would stretch the attached cross-bridges in the neighboring sarcomere until the forces in the two sarcomeres balanced. Thus, the force generated by each sarcomere would be equal after the length change. Assuming that the forces were also equal before the length change, the changes in force would also be equal in the two sarcomeres. Consequently, the change in force produced in a single sarcomere must be the same as the change in force in the whole muscle fiber.

- Sarcomere stiffness is often estimated by applying small rapid length changes to a muscle fiber and measuring the maximum force (if the muscle fiber is stretched) or the minimum force (if the muscle fiber shortens) during the length change. Following a change in length, the force does not remain constant, but gradually approaches a value corresponding to a new point on the length-tension curve. Presumably, this occurs because cross-bridges that detach following the length change do not re-attach at the same binding sites since their length changes after detachment. Consequently, they will attach at new binding sites which allow them to return to their normal length.

- If the change in sarcomere length can be measured at the same time as the muscle force, the sarcomere stiffness can be obtained: (a) from the slope of the relation between transient force maxima or minima and length change for several different length changes; or (b) from the slope of the instantaneous force plotted against length during a single length change (Fig. 1.7).

- Suppose that the stiffness of each sarcomere in a muscle fiber is given by \( k_{si} \). When the muscle fiber length changes, the length of each sarcomere will change by an amount \( \Delta x_{si} \) resulting in a change in sarcomere force

\[
\Delta F_s = k_{si} \Delta x_{si}
\]

- The change in muscle force must be equal to the change in sarcomere force and the change in muscle length must be equal to the sum of the changes in sarcomere length.

\[
K_m \Delta x_m = \Delta F_m = \Delta F_s = k_{si} \Delta x_{si}
\]

\[
K_m = \frac{k_{si} \Delta x_{si}}{\Delta x_m} = \frac{k_{si} \Delta x_{si}}{\sum_{i}^{N_s} \Delta x_i}
\]

since

\[
\Delta x_m = \sum_{i}^{N_s} \Delta x_i
\]

- From this relation it can be seen that the greater the number of sarcomeres, \( N_s \), the lower the stiffness of the muscle fiber.
Excitation-Contraction Coupling

- Action potentials are propagated from the neuromuscular junction along the plasma membrane and reach the interior of a muscle fiber through transverse tubules (T tubules), which form a network that penetrates the muscle fiber at the junctions of the A and I bands of the sarcomere.

- The sarcoplasmic reticulum (SR), a self-contained membrane system within muscle fiber which surrounds myofibrils, expands on either side of a T tubule to form compartments called terminal cisternae. The two terminal cisternae and the T tubule are known as a triad.

- Groups of four voltage gated L-type calcium channels, located in the T tubule membrane at triads, undergo a conformational change in response to depolarization which opens these channels and induces a conformational changes in the calcium release channel (ryanodine receptor), located in the SR. Calcium moving from the extracellular fluid through L-type calcium channels also induces opening of calcium release channels by a process known as calcium–induced calcium release.

- The formation of cross-bridges is largely regulated by tropomyosin and troponin molecules associated with the actin filament. Two strands of tropomyosin molecules occur with each actin filament. The tropomyosin strands follow the actin filaments. Tropomyosin strands are composed of molecules 7 actin monomers in length which are joined at the ends by a short overlap region. One troponin complex is bound to each tropomyosin molecule. A troponin complex consists of three molecules, troponin T, I and C. Troponin T binds the complex to tropomyosin whereas troponin I binds to actin and inhibits the ATPase activity of actomyosin. Troponin C has two high affinity calcium binding sites that participate in binding of troponin to actin and two low affinity calcium binding sites that are involved in regulation of contraction (Fig. 1.1).

- In the absence of calcium binding to troponin C, the tropomyosin interferes with strong cross-bridge binding by blocking interaction of myosin with actin binding sites. Calcium binding to low affinity binding sites on troponin C causes a conformational change which moves the tropomyosin molecule away from the myosin-binding site on actin. This allows binding of myosin cross-bridges in the strong (force-generating) state. Strong cross-bridge binding apparently acts to move tropomyosin even farther away from actin binding sites to further increase the probability of strong cross-bridge binding and force generation.

- Weak cross-bridge binding can occur in the absence of calcium. Cross-bridges in the weak binding state do not exert force. However, they do contribute to the stiffness of a relaxed muscle fiber.

- After its release from the terminal cisternae, calcium quickly diffuses and rapidly binds to the troponin C, allowing strong cross-bridge binding to take place. As a consequence of cross-bridge formation, active force will be generated and muscle shortening will occur if actin and myosin filaments are free to slide past one another.
• The concentration of free calcium in the myoplasm is rapidly reduced by the action of calcium pumps in the SR, as well as by the buffering action of calcium binding proteins in the myoplasm and the mitochondria. As calcium is released from troponin molecules in the reverse reaction the contractile force quickly declines as the calcium concentration in the myoplasm is brought back to its pre-excitation level, allowing the muscle to relax. Thus, the calcium concentration within a muscle fiber regulates muscle contraction.
A thin filament contains actin, tropomyosin and troponin molecules, assembled as shown schematically here. The spherical actin molecules are arranged like a double string of beads twisted to form a helix. A tropomyosin molecule extends along seven actin molecules, and there is one troponin molecule near the end of each tropomyosin (From Murray and Weber, 1974.)

(A) A diagram of the myosin molecule with its two identical subunits. The enzyme trypsin preferentially cleaves the molecule in two between the parts labeled as heavy meromyosin (HMM) and light meromyosin (LMM) because of their relative molecular weights. Further enzymatic digestion of HMM produces subfragments S1 and S2. (B) A diagram showing the arrangement of myosin molecules in a filament. The heads of the molecules are oriented towards the two ends, while the tails of the molecules are oriented towards the center. (From Huxley, 1971.) (C) X-ray diffraction patterns indicate that the filament in part (B) is twisted into a helix with a spacing of about 43 nm. The heads of the myosin molecules, here indicated by pegs, would then be spaced just above 14 nm apart and rotated 120° from each other. (From Huxley, 1971.) (D) Cross-sectional diagram showing that over a distance of 43 nm crossbridges from each thick filament will project toward all six neighboring thin filaments in the hexagonal array. (From Huxley and Brown, 1967.)

Figure 1.1 Molecular structure and geometric arrangement of myosin and actin in thick and thin myofilaments.
Figure 1.2 Parallel and serial organization of myofilaments. Parallel bundles of myofilaments form myofibrils. Myofibrils are constructed of repeating serial units called sarcomeres. Arrangement of thick and thin filaments within a sarcomere is shown in detail in the bottom diagram.
Integration of mechanical, structural and biochemical data into an oar-like crossbridge cycle. (a) Hypothetical cycle for a single crossbridge derived from mechanical and structural studies (modified from Pringle, 1967; Huxley, 1969; Huxley, 1974). (b) Kinetic diagram of actomyosin ATPase in solution and its correlation to the various steps defined in the crossbridge cycle of (a). Binding of ATP and dissociation of myosin from actin are shown as a single step, because dissociation of myosin from actin is very fast following substrate binding (according to Lymn and Taylor, 1971).

Contraction is thought to occur by rotation of the head of the myosin molecule relative to the rest of the molecule. This rotation would produce a movement of the actin-containing thin filament with respect to the myosin-containing thick filament. (From Huxley, 1971.)

Figure 1.3 Simplified schema of the cross-bridge cycle during which myosin binds to actin and later detaches following hydrolysis of one ATP molecule. If the actin filament is held so that it cannot move tension develops due a conformational change in the molecular structure of the myosin head after cross-bridge attachment. If the actin filament is free to move the change in myosin conformation pulls the actin filament. Since myosin heads on opposite sides of the M-band pull in opposite direction this movement of the actin filaments leads to sarcomere shortening, i.e., muscle contraction.
Figure 1.4 Optical trap experiment in which an actin filament is attached to beads that are held in place by optical forces produced by a narrow laser beam. Myosin heads attached to another bead which is glued to a coverslip are brought into contact with the actin filament. The motion of the coverslip or the force applied to the coverslip by the action of the myosin on the actin can be measured. The duration of the interaction depends on ATP concentration, increasing as ATP concentration decreases.
Figure 1.5 Schema showing the dependence of sarcomere tension on sarcomere length. The force is shown to be proportional to the amount of overlap between thick and thin filaments at long sarcomere lengths, indicating that the tension is proportional to the number of cross-bridges that can be formed.
Figure 1.6 Simple spring model of cross-bridge showing that tension in the spring increases when the sarcomere lengthens ($x > 0$) and decreases when the sarcomere shortens ($x < 0$). When the cross-bridge is detached, changes in sarcomere length have no effect on the tension in the spring.
Figure 1.7 Left: Step changes in sarcomere length result in transient increases or decreases in sarcomere force. Right: A) Maximum change in tension, $T_1$, achieved during a step increase or decrease in length as shown on the left, is divided by the maximum isometric tension, $T_0$, and plotted against the change in length. The slope of this line gives an estimate of sarcomere stiffness. B) The instantaneous tension, $T$, during length changes shown on the left, is divided by the maximum isometric tension, $T_0$, and plotted against the instantaneous length. The slope of this line gives the instantaneous sarcomere stiffness.