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Chapter 10

Muscles and Animal Movement

All animal behavior—locomotion, prey capture, eating, copulation, the production of sound—is generated by three fundamentally different mechanisms: amoeboid movement, ciliary and flagellar bending, and muscle contraction. In this chapter, we focus on muscle contraction because it is responsible for most observable animal behavior and much visceral function as well. The importance of muscle contraction has been recognized from ancient times. In the second century A.D., Galen hypothesized that "animal spirits" flow from nerves into muscles, inflating them and increasing their diameter at the expense of their length. As recently as the 1950s, it was suggested that muscles contract because linear molecules of "contractile proteins" within the muscles become shorter. These molecules were thought to be helical in shape; changes in the pitch of the helix were thought to change the length of the molecules. This hypothesis was based on what was then the recent discovery of helical proteins, but it was quickly supplanted. Through evidence from electron microscopy, biochemistry, and biophysics, we have discovered in minutest detail the structure of the contractile mechanism in muscles and how it produces force and shortening. It has also become clear how contraction is initiated by electrical activity in the membrane of muscle fibers. The mechanisms of muscle contraction and relaxation are major topics in this chapter.

Muscles are classified, on both morphologic and functional grounds, into two major types, **striated muscle** and **smooth muscle.** Vertebrate striated muscle is the best understood, and in this chapter we will consider it in detail. Striated muscle can be subdivided into **skeletal muscle,** the type that underlies movement of the body, and **cardiac muscle,** the type found in the heart. Smooth muscle is found primarily in the walls of hollow organs such as the intestines and the blood vessels. Each of the three main muscle types has unique morphologic and physiological properties, but the mechanism by which all muscles contract is nearly identical. The major differences between the three types are found in how their cells are organized and how contraction is initiated. Recent research on comparative aspects of muscle function has uncovered unexpected diversity among skeletal muscles and reveals an elegant match between evolutionary "design" and function. Several examples of such varied "designs" are discussed in this chapter.

ESSENTIALS OF SKELETAL MUSCLE CONTRACTION

The general organization of skeletal muscle tissue is depicted in Figure 10-1 on the next page. Typically, a muscle is anchored at each end by a tough strap of connective tissue called a *tendon.* Each muscle consists of long, cylindrical, multinucleate cells called **muscle fibers,** which are arranged in parallel with one another. This arrangement allows all of the fibers in a muscle to pull together in the same direction. Striated muscle fibers range from 5 to 100 μm in diameter and may be many centimeters long. This extraordinary size reflects the unusual developmental pathway of muscle. Each fiber arises from many individual embryonic muscle cells, called *myoblasts,* which fuse during embryonic development to form a *myotube.* Each myotube contains many nuclei within a single plasma membrane and differentiates into an adult muscle fiber (sometimes called a *myofiber*). Each muscle fiber, in turn, is composed of numerous parallel subunits called **myofibrils,** which consist of longitudinally repeated units called **sarcomeres.** (Notice that the prefixes *myo-* and *sarco-* both signal that a word is related to muscle; for example, the cytoplasm of a muscle fiber is interchangeably called *myoplasm* or *sarcoplasm.*) The sarcomere is the functional unit of striated muscle. The myofibrils of a striated muscle fiber are lined up with the sarcomeres in register, so the fiber looks striated, or striped, when it is observed with a light microscope.

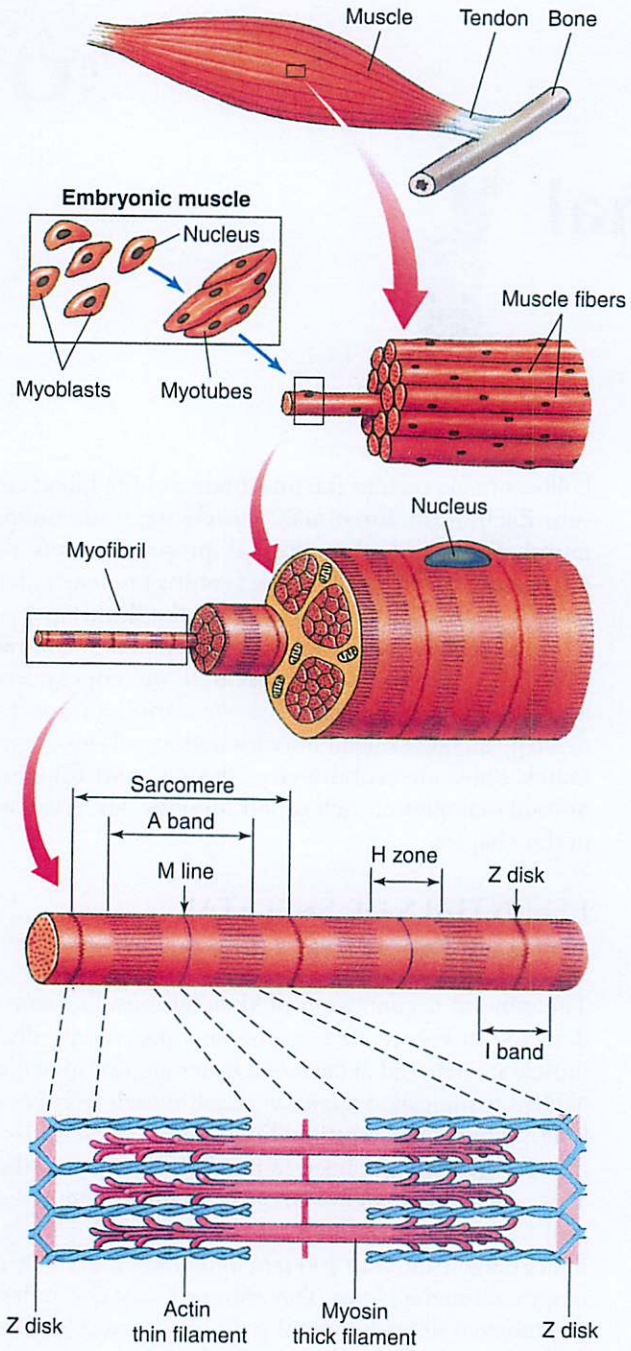


Figure 10-1 Vertebrate skeletal muscles are organized in a stereotyped hierarchy. Each muscle is made up of parallel multinucleate muscle fibers, each of which contains many myofibrils. Muscles are attached to bones or other anchor points by bands of tough connective tissue called tendons. Each muscle fiber is derived embryonically from a group of myoblasts that fuse to form a myotube. After fusion, each myotube synthesizes the proteins characteristic of muscle fibers and differentiates into its adult form. Each myofibril is made up of sarcomeres arranged end-to-end. Each sarcomere contains two kinds of protein filaments: thin filaments consisting primarily of actin, and thick filaments consisting primarily of myosin. The filaments interdigitate in a precise geometric relationship (see Figure 10-3). Thin filaments are anchored in regions called Z disks. [Adapted from Lodish et al., 1995.]

Each sarcomere contains two kinds of long, thin proteins called **myofilaments arranged** in a precise geometric pattern (Figure 10-2). Each sarcomere is bounded at either end by a **Z disk** (or *Z line*), which contains *α-actinin*, one of the proteins found in many motile cells. Extending in both directions from the Z disk are numerous **thin filaments** consisting largely of the protein **actin**. The α-actinin in the Z disk binds actin and anchors the thin filaments firmly to the disk. The thin filaments interdigitate with **thick filaments** made up primarily of the protein **myosin**. Interdigitated thick and thin filaments make up the densest portion of the sarcomere, the *A band*. The lighter portion in the center of the A band, called the *H zone*, contains only thick filaments. In the middle of the H zone is the *M line*, which has been shown to contain enzymes that are important in the energy metabolism of muscle fibers. The portion of the sarcomere between two A bands is called the *I band*.

If cross-sections are made through the various bands of a single sarcomere, the precisely arranged geometric relationship between thick and thin filaments is revealed (Figure 10-3). Only thin filaments are seen in a section through an I band, and only thick filaments are seen in a section through an H zone. At the ends of an A band in vertebrate skeletal muscle, where thick and thin

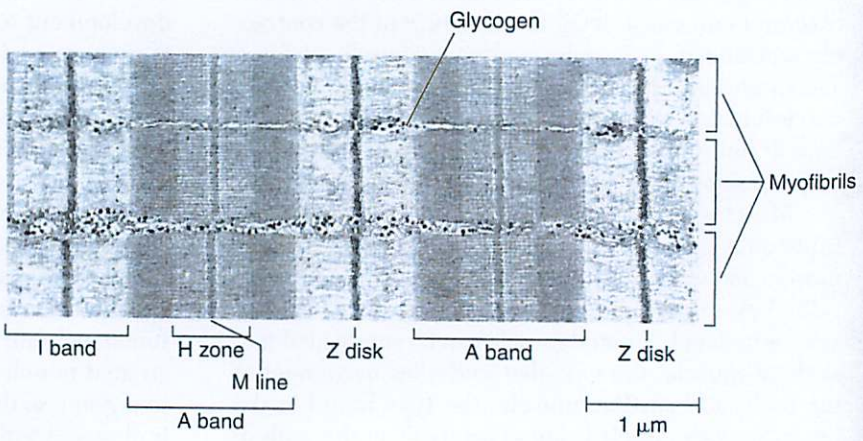


Figure 10-2 The sarcomeres within neighboring myofibrils are aligned in register, producing the characteristic banding of skeletal muscle. This electron micrograph of a longitudinal section through a frog skeletal muscle includes two complete sarcomeres of three myofibrils. The various bands and the Z disks (which appear as lines in longitudinal section, and thus have also been called Z lines) are labeled. Dark granules between fibrils are glycogen deposits. [Courtesy of L. D. Peachey.]

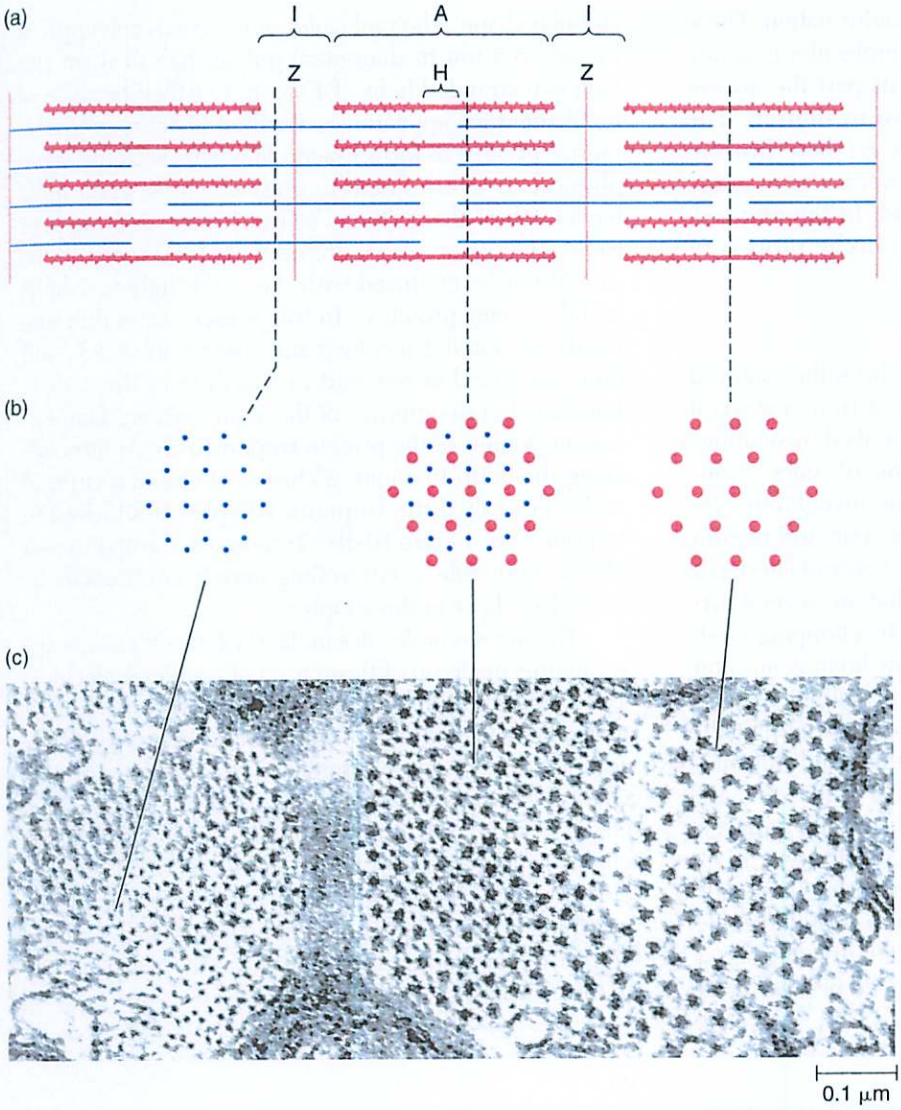


Figure 10-3 Within a myofibril, thin filaments extending from the Z disks overlap with thick filaments in a precise geometric pattern. **(a)** Diagram of three sarcomeres, showing thick and thin myofilaments forming I and A bands, H zones, and Z disks. **(b)** Diagram of the geometric relation between thick and thin filaments in cross-sections made at different locations in a sarcomere. **(c)** Electron micrograph of a cross-section through myofibrils of a spider monkey extraocular muscle, in which the sarcomeres of adjacent myofibrils are out of register so they can be matched with the profiles shown in part b. [Part c courtesy of L. D. Peachey.]

filaments overlap, each thick filament is surrounded by six thin filaments, and it shares these thin filaments with surrounding thick filaments. Each thin filament is surrounded by three thick filaments.

When a section through a sarcomere is examined at high magnification with an electron microscope, small structures, called **cross-bridges**, are visible. These projections extend outward from the thick myosin filaments and contact actin in the thin filaments during muscle contraction (Figure 10-4). Muscles contract when the cross-bridges on myosin molecules bind transiently to sites on actin molecules, causing the myosin

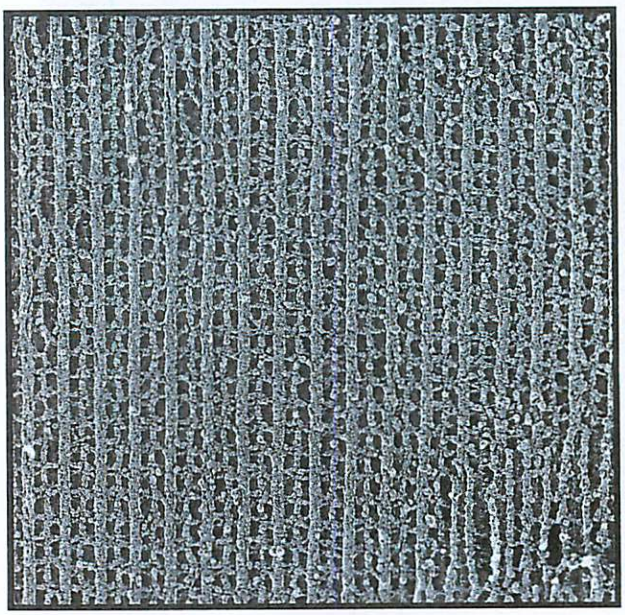


Figure 10-4 Cross-bridges extend from myosin thick filaments toward actin thin filaments. The muscle fiber shown in this figure was flash-frozen in liquid helium, etched to reveal proteins in the tissue, and stained with a heavy metal. Many cross-bridges are seen as small, horizontal projections extending from the thick filaments. [Courtesy of Professor John Heuser, M. D.]

molecules to change their physical conformation. These changes in the shape of the myosin molecules generate force, which drags the actin filaments past the myosin filaments and causes the sarcomere to shorten. The bond between actin and myosin is then broken. The cross-bridges bind and unbind over and over again, generating force each time they bind. In the next section we will consider in detail this cyclic process of cross-bridge binding and unbinding.

Myofilament Substructure

In the mid-nineteenth century, Wilhelm Kühne showed that multiple factors can be extracted from a skeletal muscle when it is minced and then soaked in solutions containing different concentrations of salts. Nonstructural soluble proteins, such as myoglobin, are extracted by distilled water, whereas actin and myosin filaments are solubilized by highly concentrated salt solutions. Fragments of myofibrils that are several sarcomeres in length can be prepared by chopping fresh muscle in a laboratory blender. If this homogenization is carried out in a "relaxing solution" containing magnesium, ATP, and a calcium-chelating agent, the myofibrils fall apart into their constituent thick and thin filaments. This result suggests that ATP and Ca^{2+} are important in regulating chemical bonds between myosin and actin, which we will soon confirm.

An actin filament resembles two strings of beads twisted around each other into a two-stranded helix (Figure 10-5a). Each "bead" in the string is a monomeric molecule of G-actin, so called because of its globular shape. The molecules of G-actin (each approximately 5.5 nm in diameter) polymerize to form the long two-stranded helix of F-actin, so called because of its filamentous appearance. Purified G-actin will polymerize *in vitro* to form F-actin filaments with the same physical structure found in muscle. The F-actin helix has a pitch of about 73 nm, so its two strands cross over each other once every 36.5 nm. (This F-actin helix should not be confused with the much tighter α helix found in many proteins.) In frog muscle, actin thin filaments are about 1 μm long and about 8 nm thick, and they are joined at one end to α-actinin in the Z disk. Positioned in the grooves of the actin helix are filamentous molecules of the protein **tropomyosin.** At intervals along the actin filament, a cluster of globular protein molecules called the **troponin complex** is attached to tropomyosin (Figure 10-5b). Troponin and tropomyosin play a major role in controlling muscle contraction, as we will see later in this chapter.

The myosin molecules in the thick myofilaments are complexes made up of three pairs of protein molecules. Two of the proteins in the complex are identical large molecules called *myosin heavy chains* (Figure 10-6). In addition, each complex contains a pair of myosin light chains called *essential light chains* and a pair of light chains called *regulatory light chains.* Each of the heavy chains is long and thin with a globular "head" region (Figure 10-6a). The long, slender portion of the complex is formed by α-helical regions of the heavy chains, which are twisted around each other to form the neck and tail of the complex.

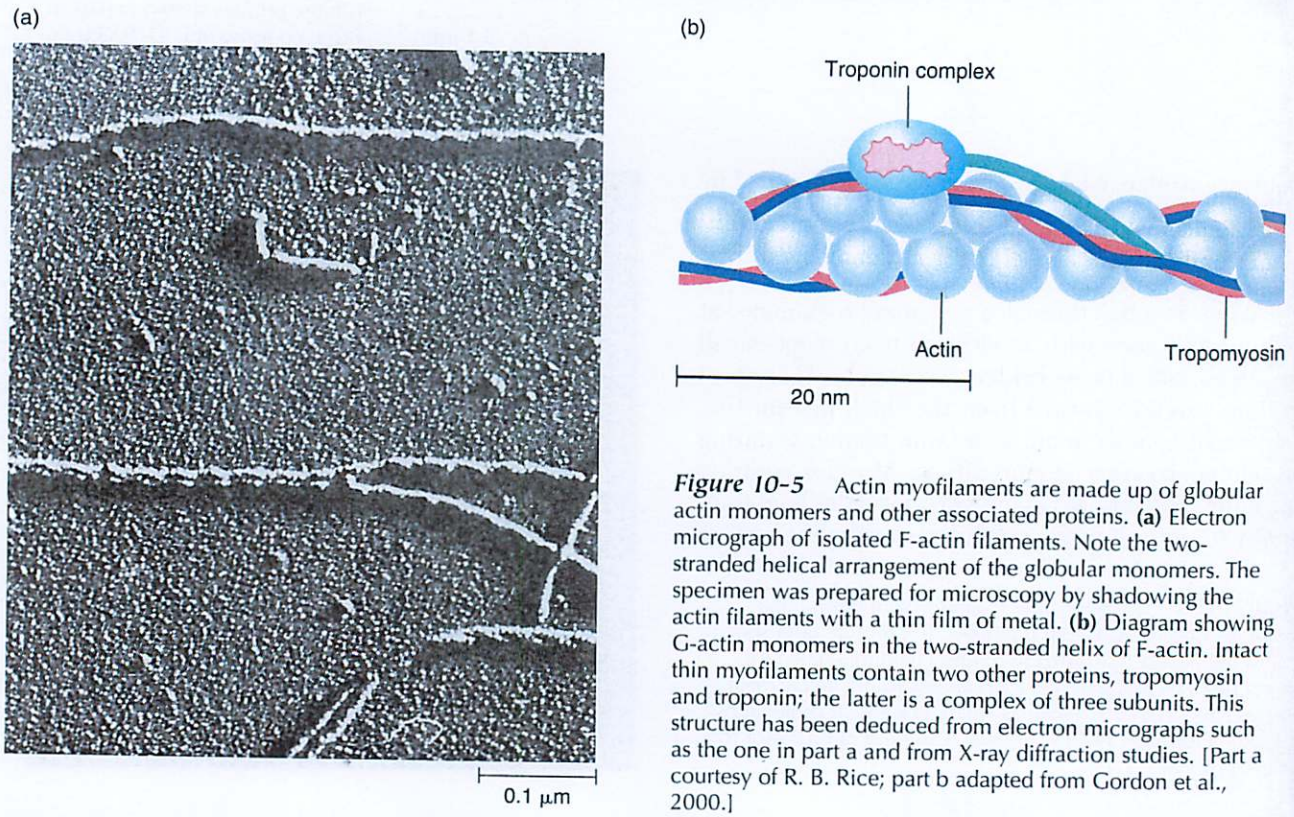


Figure 10-5 Actin myofilaments are made up of globular actin monomers and other associated proteins. **(a)** Electron micrograph of isolated F-actin filaments. Note the two-stranded helical arrangement of the globular monomers. The specimen was prepared for microscopy by shadowing the actin filaments with a thin film of metal. **(b)** Diagram showing G-actin monomers in the two-stranded helix of F-actin. Intact thin myofilaments contain two other proteins, tropomyosin and troponin; the latter is a complex of three subunits. This structure has been deduced from electron micrographs such as the one in part a and from X-ray diffraction studies. [Part a courtesy of R. B. Rice; part b adapted from Gordon et al., 2000.]

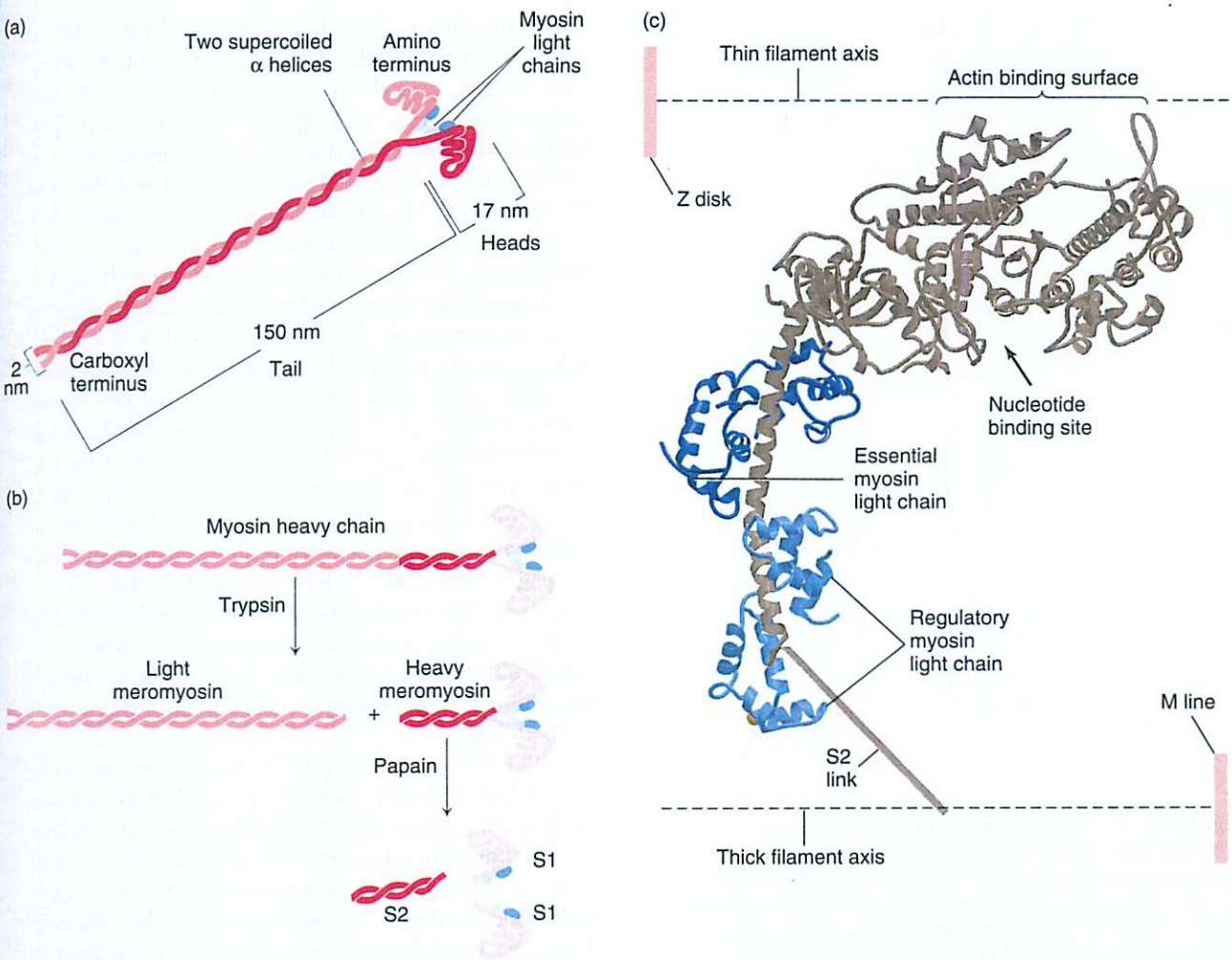


Figure 10-6 In thick myofilaments, myosin is organized into complexes consisting of six protein subunits. **(a)** Each complex has a long, thin tail made of two supercoiled α-helical segments of two myosin heavy chains. At one end are two heads, each belonging to a myosin heavy chain. In addition, two essential myosin light chains and two regulatory myosin light chains associate with the heads. **(b)** The proteolytic enzymes trypsin and papain reliably cleave the myosin complex at particular locations. Trypsin cleaves the complex into two pieces: light meromyosin, which includes most of the tail, and heavy meromyosin, which includes the head and the myosin light chains. Papain cleaves heavy meromyosin into three pieces, two called S1 and one called S2. **(c)** The three-dimensional structure of the S1 fragment has been determined, including the structure of the associated myosin light chains. In a sarcomere, the S1 fragment is connected by way of the S2 fragment to the tail, which is located in a thick myofilament. [From Nelson and Cox, 2000, with adaptations.]

When myosin complexes are treated with the proteolytic enzyme trypsin, they separate into two parts, called *light meromyosin* and *heavy meromyosin* (Figure 10-6b). Light meromyosin constitutes the major part of the tail region, and heavy meromyosin includes the globular head and the neck. Treating heavy meromyosin fragments with another proteolytic enzyme, papain, breaks them into two kinds of fragments, called S1 and S2. The three-dimensional structure of the S1 fragment has been determined, along with the structure of the myosin light chains that bind to it (Figure 10-6c). The light chains are calcium-binding proteins. Several molecular variants are known, which differ among muscle types and influence some functional properties of muscles, such as the speed of contraction.

Isolated myosin complexes in salt solution will aggregate spontaneously *in vitro* to form reconstituted thick filaments if the ionic strength of the solution is reduced. Initially, several myosin complexes aggregate with their tails overlapping and their heads pointing outward from the region of overlap and in opposite directions (Figure 10-7 on the next page). The result is a short filament in which the central region is devoid of heads. This bare zone in the middle of thick filaments has implications for muscle contraction, as we will soon see. As molecules of myosin are added to each end of the filament, all maintain this orientation, with their tails pointing toward the center of the filament and overlapping with the tails of previously added molecules. The head of each newly added myosin molecule projects laterally from the filament. Myosin molecules are added symmetrically to the two ends so that the heads on half the filament are oriented opposite to those of the other half. This reconstitution experiment

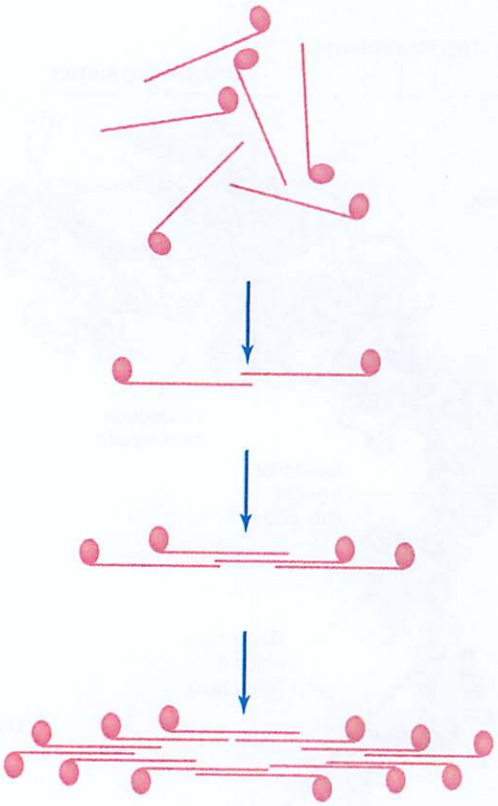


Figure 10-7 Myosin complexes will polymerize spontaneously *in vitro* to reconstitute thick filaments with an organization identical to that found in muscle. Individual myosin complexes join the nascent thick filament with their heads oriented toward either end of the filament and their tails parallel to its long axis. About equal numbers of myosin complexes make up the two ends of the filament at any given time, keeping the filament roughly symmetric in structure.

suggests that the structure of thick filaments depends directly on the physical and chemical properties of myosin molecules, and no other assembly instructions are required. Aggregation continues until, for vertebrate myosin, the filament is about 1.6 μm long and about 12 nm thick. It is as yet unclear why filaments stop growing at this particular length.

Contraction of Sarcomeres: The Sliding-Filament Theory

The striations of sarcomeres were first observed with the light microscope well over a century ago. Around the same time, it was also noted that sarcomeres change in length when a muscle contracts or is stretched, and that these changes in the sarcomeres correspond to the change in muscle length. In 1954, using a specially built light microscope that permitted accurate measurement of the sarcomeres, Andrew F. Huxley and R. Niedergerke confirmed earlier reports that the A bands, which correspond to the length of the thick filaments, maintain a constant length when a muscle shortens. In contrast, the I bands and the H zone (zones where actin and myosin filaments do not overlap in the resting muscle) become shorter (Figure 10-8a). When a muscle is stretched, the A band again maintains a constant length, and the I bands and H zone become longer. That same year, Hugh E. Huxley and Jean Hanson, working with electron micrographs, reported that neither the myosin thick filaments nor the actin thin filaments change in length when a sarcomere shortens or is stretched. Instead, it is the *extent of overlap* between actin and myosin filaments that changes.

Largely based on the observations described in the previous paragraph, H. E. Huxley and A. F. Huxley independently proposed the **sliding-filament theory** of muscle contraction. They suggested that sarcomeres shorten during muscle contraction as the thin filaments actively slide along the thick filaments. The thin filaments are pulled closer to the center of the sarcomere, and because they are firmly anchored in the Z disks, the sarcomeres become shorter (Spotlight 10-1 on pages 368–369). When a muscle relaxes or is stretched, the overlap between thin and thick filaments is reduced, and the sarcomeres elongate. We will consider how evidence was accumulated to support this theory as an example of elegant physiological methodology.

One of the strongest pieces of evidence supporting the sliding-filament theory is the **length-tension relation** for a sarcomere. A length-tension curve relates the amount of overlap between actin and myosin filaments to the tension developed by an active sarcomere under that condition. According to the sliding-filament theory, each myosin head provides a cross-bridge that interacts with an actin filament. Each cross-bridge generates force independently of all other cross-bridges and provides an increment of tension. Thus, the total tension produced by a sarcomere should be proportional to the total number of cross-bridges that can interact with actin filaments, and this number should in turn be proportional to the amount of overlap between thick and thin filaments. The sliding-filament theory thus predicts that no active tension (i.e., no tension beyond the amount due to the elasticity of the muscle fiber) will develop if a sarcomere is stretched so far that actin and myosin filaments no longer overlap.

To test the predicted relationships between filament overlap and tension generated, single frog muscle fibers were stimulated to contract at different fixed sarcomere lengths. First, the sarcomere length was adjusted with the aid of an electromechanical system. Changing the length of the sarcomeres changes the amount of overlap between the actin and myosin filaments (see Figure 10-8a). Then the fiber was electrically stimulated to contract, and the tension generated was measured and plotted as a function of sarcomere length. When the fiber was stretched so that there was no overlap at all between thick and thin filaments, stimulation produced no tension beyond what was required to stretch the fiber (Figure 10-8b). When the fiber was held so that the actin filaments overlapped completely with the parts of

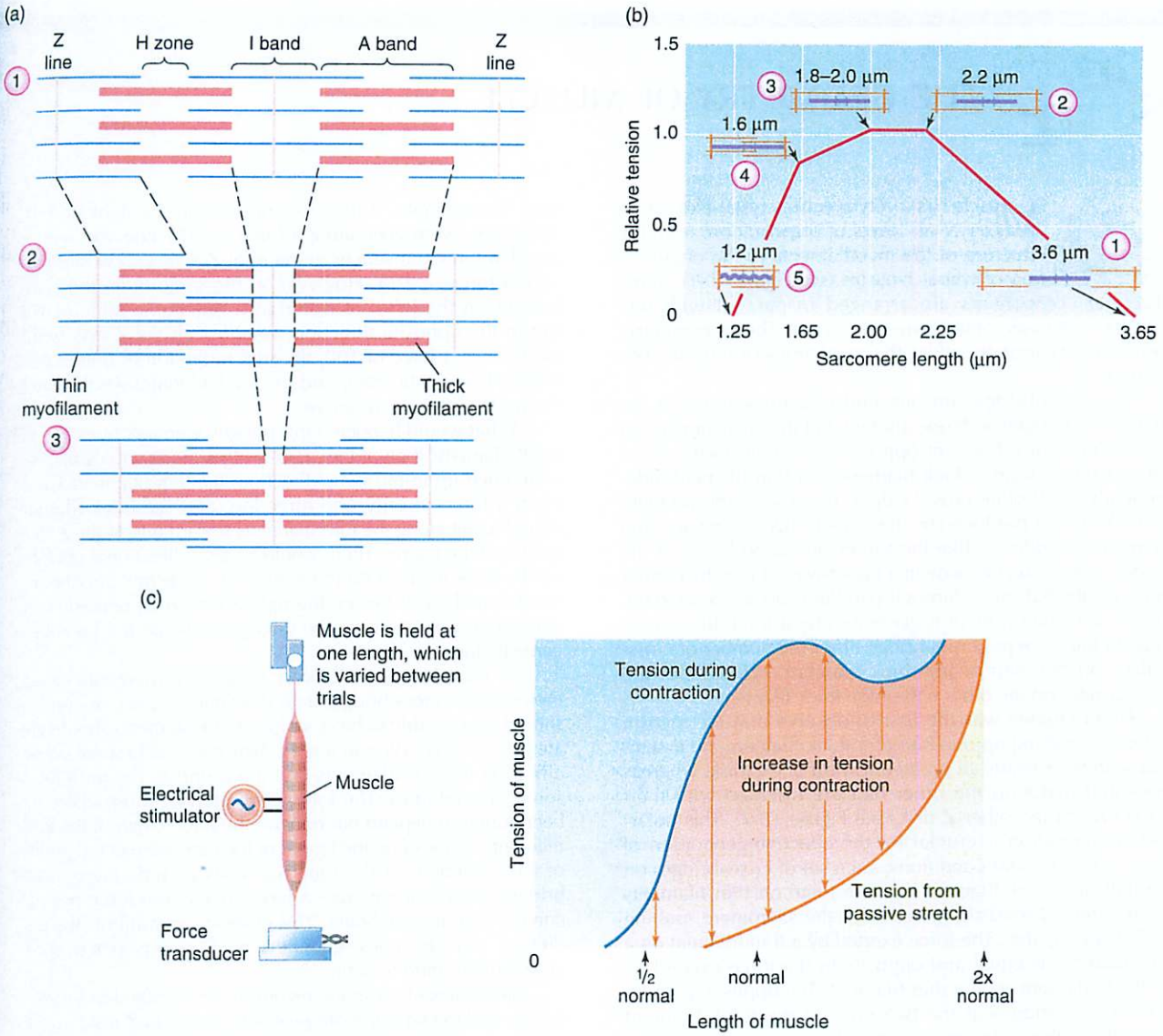


Figure 10-8 The sliding-filament theory states that sarcomeres shorten when their actin and myosin filaments move past each other. **(a)** Relations of the myofilaments when two sarcomeres shorten. Note that the lengths of the thick and thin filaments remain constant; only the amount by which they overlap changes.

Classic Work

(b) Length-tension curve for a typical vertebrate sarcomere. The length and configuration of the sarcomere are depicted schematically near the curve at critical points. (Lengths exclude the thickness of the Z disks. Adding the width of one-half of a Z disk to each end of the sarcomere would increase each length by 0.05 μm.) The tension produced by the muscle is maximal when the overlap between thick and thin filaments allows the largest number of myosin cross-bridges to bind to actin. Tension drops off with increased length, because the thick and thin filaments overlap less and fewer cross-bridges can bind. It also drops off with decreased length, because thin filaments at the two ends of the sarcomere begin to collide with each other, preventing further shortening. Skeletal muscle rarely operates over such a broad range of sarcomere lengths because the structure of the skeleton and the joints limits the range of movement. Sarcomere length normally remains within the plateau region of this curve. **(c)** The length-tension relation for whole muscle. A muscle is removed from the body and placed in an apparatus that allows the length of the muscle to be set and the tension generated by contraction to be measured (left). The red curve on the right shows the reading of the force transducer produced by manipulation of the muscle's length by the experimenter. Due to the physical properties of the muscle, the experimenter must exert more and more force to stretch the muscle to longer and longer lengths. The blue curve shows the reading of the force transducer when the muscle is stimulated to contract at each preset length. The tension produced by the muscle (called the active tension) is the difference between the red and blue curves. Like the tension produced by a single sarcomere, the active tension rises as length increases and then, at even greater lengths, it decreases.

the myosin filaments that bear the cross-bridges, the tension generated was maximal. When the fiber was so short that the actin filaments in the two halves of the sarcomere collided, the tension decreased. Tension decreased still further if the fiber was so short that the myosin filaments crumpled up against the Z disks.

Before these experiments were actually carried out, some of the properties of the length-tension curve

Spotlight 10-1

THE GEOMETRY OF MUSCLE

Muscle has a very precise, crystal-like geometry at all levels of organization, from the structure of the myofilaments to the organization of whole muscles (see Figure 10-1). Some structural components are arranged in parallel with one another, whereas others are in series. These geometric arrangements strongly affect the mechanics of muscle contraction.

The cross-bridges on one end of a thick filament are arranged in parallel with one another, but the cross-bridges on the two ends of a filament oppose each other. Every cross-bridge extends from a thick filament to a thin filament independently of all other cross-bridges. Because of this arrangement, the forces produced by the cross-bridges along one thin filament are additive, like the forces produced by all of the people lined up on one side in a tug-of-war, or like the current components that move through parallel resistors in a circuit. The force in one direction generated by a thick filament is equal to the force per cross-bridge times the number of cross-bridges on one-half of the thick filament. What about the cross-bridges on the other half of the thick filament?

Hugh Huxley was the first to observe that the myosin molecules making up one-half of a thick filament are assembled with their heads all pointed toward one Z disk, whereas those that make up the other half are oriented with their heads toward the other Z disk (see Figure 10-7). This polarized configuration is crucial for the effective generation of force. Under normal conditions, each set of cross-bridges on one-half of a thick filament exerts a force on thin filaments that is directed toward the center of the sarcomere, pulling the Z disks together. The force exerted by a thin filament on a thick filament is equal and opposite to the force exerted by the thick filament on the thin filament. The opposite polarity of the cross-bridges at the two ends of the thick filament means that a thin filament on one end of a sarcomere exerts a force onto the thick filament that is on average just balanced by the force of a matching thin filament on the other end. Hence the net force exerted on a thick filament by the surrounding thin filaments is zero, and the thick filament stays in the center of the sarcomere (part a of the accompanying figure). For example, if the cross-bridges on the right side of a sarcomere were generating a force of 100, and you were to attach a force transducer to the right Z disk, you would measure a force of 100. However, at the same time, the cross-bridges on the left side would also generate a force of 100, but in the opposite direction, so although the Z disk would experience a force of 100, the sum of the forces on the thick filament would be zero, and the thick filament would stay in the middle of the sarcomere.

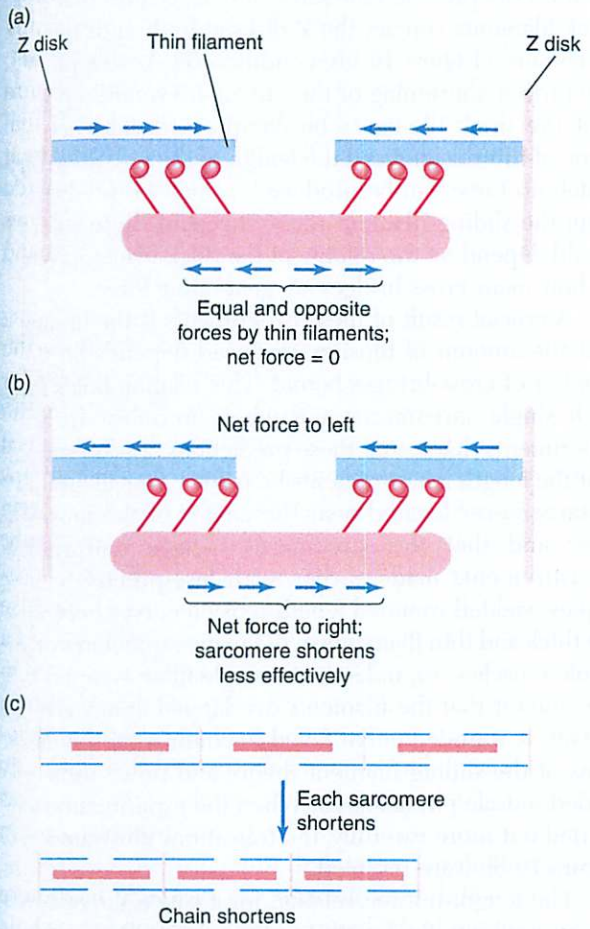
What would happen if this polarity were not built into the thick filament? If all of the cross-bridges along a thick filament were lined up in the same direction, the thin filaments would exert a force in only one direction, and the thick filament would travel along the thin filaments toward one of the Z disks (part b of the figure). There would be a unidirectional net force on the thick filament (to the right in the diagram), and the filament would travel toward the right Z disk in an unpredictable manner. In this situation, the sarcomere would not be able to generate force by shortening.

At least in principle, very long thick filaments should include more cross-bridges than short ones; more cross-bridges should thus be able to bind, so long thick filaments should create greater force. We can test this hypothesis with some unusually long myosin filaments that are found in the muscles of some invertebrates. The force generated by these fibers has been found to depend not only on the total length of the thick filaments, but also on the length of the bare zone in the middle of each filament. If the long filaments include more cross-bridges, they can generate a larger maximum force per sarcomere than in vertebrates. This observation confirms the prediction that muscles generate more force when more cross-bridges bind to actin.

Sarcomeres in a single myofibril are arranged in series—that is, end to end (or, more precisely, Z disk to Z disk), just as resistors can be placed in series in a circuit. When resistors are placed in series, the current through each resistor is the same as the current through every other resistor in the series. Similarly, the force generated by a series of sarcomeres is the same all along the chain of sarcomeres. Thus, although a myofibril might contain a huge number of cross-bridges, the

shown in Figure 10-8b could be predicted based on aspects of the sliding-filament theory. First, the theory states that the force generated by a sarcomere is proportional to the number of cross-bridges binding thick filaments to thin filaments. Second, it states that cross-bridges are evenly distributed along each thick filament, except in the central bare zone where no cross-bridges are present. (This second statement had been experimentally verified.) From these assumptions and the dimensions of the filaments, which are given in Figure 10-9a on page 370, it is possible to predict or explain the properties of the sarcomere length-tension curve. Let's consider some critical points on the length-tension curve illustrated in Figure 10-8b.

At what sarcomere length would we expect the filaments to be pulled beyond overlap and hence to generate no force? To determine the sarcomere length (excluding the Z disks) for a given amount of overlap between filaments of known lengths, imagine the path of a very tiny ant trying to crawl along the filaments from the face of one Z disk to the face of the next Z disk. In a sarcomere that has been stretched to the point where the filaments just fail to overlap, the ant would crawl along one thin filament (1.0 μm), step up to a thick filament and traverse it (1.6 μm), and step down to a thin filament on the other side of the sarcomere and traverse it (1.0 μm). The total distance traveled would be 3.6 μm (Figure 10-9b, condition 1).



The geometry of myofilaments in a sarcomere strongly affects the contractile properties of muscle. ***(a)*** *Equal numbers of myosin cross-bridges act in opposition to each other, causing Z disks to move toward the center of the sarcomere while the thick filaments remain stationary.* ***(b)*** *If the cross-bridges all worked synergistically, the thick filament would move with respect to the thin filaments, interfering with the production of force.* ***(c)*** *The amount a myofibril shortens is equal to the sum of shortening in all of its sarcomeres.*

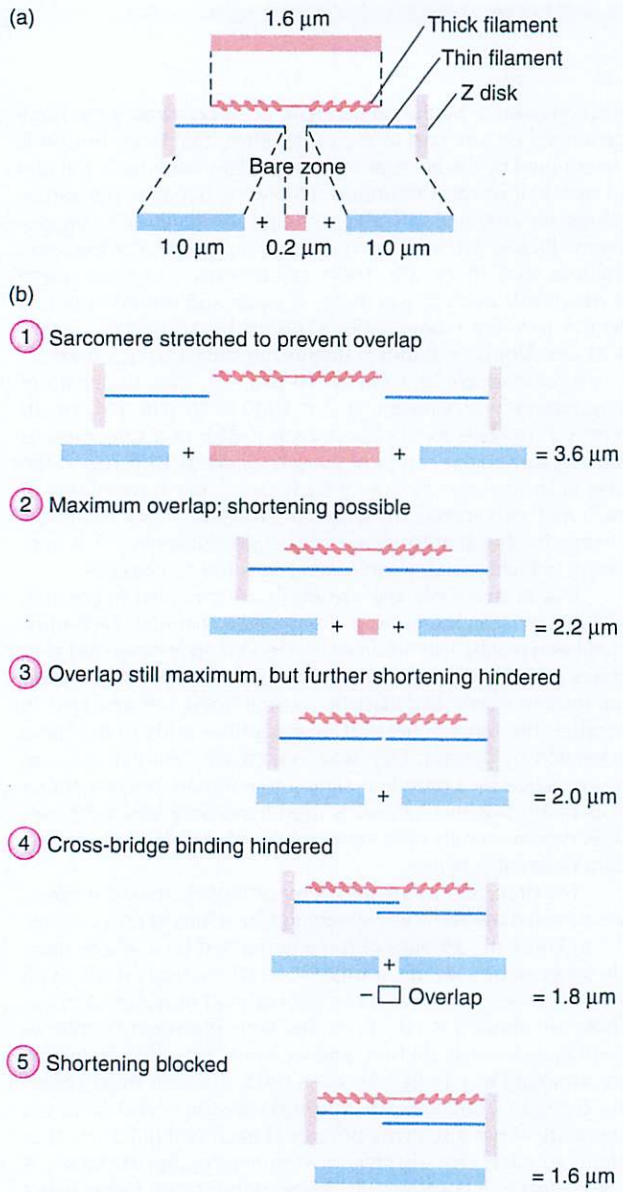
force generated by the entire chain of sarcomeres is the force generated by any one of the sarcomeres; that force in turn is determined by the number of cross-bridges working in parallel in one half of the sarcomere. However, because the sarcomeres are arranged in series, changes in length and contraction velocities are additive (part c of the figure). For instance, assume that there are 1000 sarcomeres in series along a myofibril, each 2 μm long. If each sarcomere shortens by 0.1 μm, the whole series shortens by $1000 \times 0.1\ \mu m = 100\ \mu m$. Similarly, if thin filaments move past a thick filament in each sarcomere at a rate of 10 $\mu m \cdot s^{-1}$, then the chain of sarcomeres will shorten at $2 \times 1000 \times 10\ \mu m \cdot s^{-1}$, or 20 $mm \cdot s^{-1}$. (Notice that because each Z disk in a sarcomere is moving toward the center of the sarcomere at 10 $\mu m \cdot s^{-1}$, the overall shortening velocity of each sarcomere is twice that of each half-sarcomere.) The large amplification factor for length change implies that to obtain very rapid shortening, it is necessary to have as many sarcomeres in series as possible.

In a muscle fiber, the myofibrils are arranged in parallel, and in a muscle, the fibers are arranged in parallel. Each muscle fiber typically extends from one tendon to another and generates force between the tendons independently of surrounding muscle fibers. Because the muscle fibers are arranged in parallel, the force generated by each fiber adds to the force generated by the rest. One way to increase the force that can be generated by a muscle is simply to put more fibers together in parallel. This mechanism is used transiently when the nervous system recruits different numbers of muscle fibers to perform different activities.

The precision in the geometry of muscle makes it possible to calculate the force generated by a single cross-bridge if you know the amount of force generated by a whole muscle (or even by a whole animal). Consider a frog muscle with a 1 cm^2 cross-section that can generate 30 newtons of force. There are about 5×10^{10} thick filaments per square centimeter of muscle cross-section, and we know that thick filaments are arranged in parallel. So each thick filament must generate 6×10^{-10} newtons, or 600 piconewtons (pN) of force. There are about 150 cross-bridges at each end of a thick filament, so each cross-bridge must generate approximately 4 pN of force. Experimental measurements of force using microscopic techniques yield measurements of 1.5 to 3.5 pN of force per cross-bridge—very good agreement with the calculated number.

Why is there a plateau in maximal force between 2.0 and 2.2 μm? When the sarcomere is 2.2 μm long, the ends of the thin filaments line up with the beginning of the bare zone on the thick filaments (where there are no cross-bridges). Thus all the cross-bridges on the thick filaments are optimally aligned to interact with binding sites on the thin filaments (Figure 10-9b, condition 2). As the sarcomere shortens further, no more cross-bridges are added to the number that can interact with the thin filaments, so the force generated remains the same. The end of the plateau occurs when the thin filaments meet in the center of the sarcomere (Figure 10-9b, condition 3).

Why does the force fall as the sarcomere continues to shorten? The sliding-filament theory makes no quantitative predictions about muscle force beyond the point of maximal overlap, so it has been necessary to answer this question experimentally. From one perspective, the force might remain constant because all the cross-bridges still overlap with binding sites on the thin filaments and can, at least in principle, generate force. However, two effects could reduce the force generated. First, when the thin filaments overlap at the middle of the sarcomere, binding between myosin cross-bridges and thin filaments could be sterically hindered (Figure 10-9b, condition 4). Second, some cross-bridges might bind with an inappropriate thin filament (one projecting from the



Classic Work

Figure 10-9 If the lengths of thick and thin filaments are known, the sarcomere length can be predicted for various amounts of overlap. Carefully controlled experiments have confirmed these predictions. The measured sarcomere lengths can be correlated with the known length-tension curve, providing experimental support for the sliding-filament theory. **(a)** Filament lengths measured from high-resolution electron micrographs of frog muscle fibers. The lengths of the individual components are represented by colored bars: red for thick filaments and blue for thin filaments. **(b)** Amounts of overlap between thick and thin fibers at different points in the sarcomere length-tension curve. Each condition in this figure is matched to a point on the curve in Figure 10-8b. The graphic equation accompanying each drawing calculates the length of a single sarcomere (excluding the thickness of the Z disks) for that condition of overlap. [Part b adapted from Gordon et al., 1966.]

Z disk at the other end of the same sarcomere) and thus exert a force that pushes the Z disks apart, rather than pulling them together. Such a force would be considered negative and would need to be subtracted from the force generated by the cross-bridges operating normally.

Why does force decline steeply at 1.6 µm and fall to zero at about 1.2 µm? The force generated declines steeply when the sarcomere is so short that the thick filaments contact the Z disks at both ends of the sarcomere (Figure 10-9b, condition 5). At this point, any further shortening of the sarcomere would require that the thick filaments be compressed. The actual slope of this decline, and the length of the sarcomere at which no force can be produced, cannot be predicted from the sliding-filament theory because these values would depend on the rigidity of the thick filaments and on how many cross-bridges are generating force.

A crucial result of these experiments is the finding that the amount of tension produced depends on the number of cross-bridges bound. This relation holds for both single sarcomeres and whole muscles. In the experiments that tested these predictions, it was crucial that the length measurements be made on small groups of sarcomeres located near the center of the muscle fiber and that the sarcomeres behave uniformly. Measurements made earlier, with less precise techniques, yielded rounded length-tension curves because the thick and thin filaments in the many sarcomeres of a whole muscle—or, indeed, of a single fiber—varied in the amount that the filaments overlapped at any given instant. A rounded curve failed to confirm the predictions of the sliding-filament theory and thus seriously misled muscle physiologists. When the experiment was carried out more carefully, the transitions illustrated in Figure 10-8b were revealed.

The length-tension relation for a single sarcomere is very similar to the length-tension relation for a whole muscle made up of millions of sarcomeres. In a typical length-tension experiment, a muscle is removed from the body and mounted in an apparatus that holds it at a constant length. The length of the muscle is varied by the experimenter. Once the length is set, the muscle is stimulated to contract, and the amount of tension produced is measured by a force transducer. Notice that this procedure is analogous to setting the overlap between thick and thin filaments in a single sarcomere. Figure 10-8c shows results obtained in this type of experiment. The red curve shows the reading of the force transducer produced entirely as a result of setting the length of the muscle; it is unrelated to the production of tension by the muscle. As long as the muscle is allowed to be short, the red curve has a value of zero. Once force is required to stretch the muscle to a given length, the force transducer reads out that force, but the value reflects only the activity of the experimenter to set the length of the muscle. When the muscle is stimulated to contract, the force transducer reports a higher value, shown by the blue curve. The amount of tension generated by the activity of the muscle at each length is represented by the difference between the red and blue curves at that length. Notice that subtracting the red curve from the blue produces a curve very like the one in Figure 10-8b, but without sharp transitions.

The striking similarity between the length-tension curves for whole muscle and for a single sarcomere suggests that this relation, which has been known for many decades for whole muscle, is based entirely on the behavior of the sarcomeres within the muscle.

Question

The muscle of the heart is similar to striated skeletal muscle in many ways, including its length-tension curve. If a heart attack causes the heart to stop pumping, blood returning from the circulation can cause the volume of the heart to increase beyond normal limits, stretching its walls. What effect would you expect this to have on the strength of contraction, and why?

Cross-Bridges and the Production of Force

Elucidating precisely how myosin cross-bridges and actin filaments work together to produce force continues to be one of the great challenges facing researchers who study muscle function. According to current versions of the sliding-filament theory, the force driving muscle contraction arises when several different sites on the myosin head bind sequentially to sites on the actin filament, which produces relative motion between the actin filament and the myosin filament. The bond between the head and the actin filament is then broken, freeing the head for another cycle of sequential binding at a site farther along the actin filament. We will now consider these processes in detail.

Cross-bridge chemistry

Myosin cross-bridges must attach to binding sites on actin filaments in order to generate force, but the attachment must be reversible. If the cross-bridges never detached from actin, the filaments could slide no more than a micrometer past each other. In order to shorten the sarcomere and do work, the cross-bridges must attach to the actin filament, pull on it, and then detach in a cyclic fashion.

The first hints about the chemistry of the interaction between myosin cross-bridges and actin filaments came from studies begun several decades ago on crude and purified extracts of muscle. When actin and myosin are mixed together in the absence of ATP, they form a stable complex called **actomyosin.** If ATP is added to the solution, however, it causes rapid dissociation of the complex into actin and myosin-ATP:

$$\text{Actomyosin} + \text{ATP} \rightleftharpoons \text{Actin} + \text{Myosin-ATP}$$

The observation that ATP is required for the *dissociation* of actomyosin explains a phenomenon well known to readers of detective novels. Following death, the body of a human being or other animal gradually becomes stiff and will hold the same position for hours or even days. This condition, called *rigor mortis,* differs from muscle contraction, because in rigor mortis the muscles do not shorten. Instead, they simply remain locked at the same length. This rigidity occurs in part because when all of the ATP is used up after a cell dies, myosin binds irreversibly to actin, rigidly locking the muscles in place.

When ATP binds to isolated myosin, the myosin acts as an ATPase, rapidly hydrolyzing it to ADP and P_i, but these breakdown products unbind from the myosin only slowly. As a result, the rate of this reaction is very slow, and the rate-limiting step is the release of ADP and P_i from myosin. When myosin is bound to actin, however, the release of ADP and P_i proceeds much faster due to an allosteric change in the conformation of myosin. This actin-induced effect greatly increases the rate at which myosin can hydrolyze ATP:

$$\text{Myosin-ATP} \longrightarrow \text{Myosin-ADP-P}_i \xrightarrow{\text{very slow}} \text{Myosin} + \text{ADP} + P_i$$

$$\text{Myosin-ADP-P}_i + \text{Actin} \xrightarrow{\text{fast}} \text{Actomyosin} + \text{ADP} + P_i$$

Because energy is released following the binding of actin to the myosin-ADP-P_i complex, the formation of actomyosin is kinetically favored. When the actomyosin complex forms, P_i and ADP are released and replaced by ATP, which then breaks the actomyosin complex. These reactions proceed in a cycle of binding and unbinding between myosin and actin (Figure 10-10). The net effect of one turn of the cycle is to split one molecule of ATP into ADP + P_i, liberating energy, some of which can be captured to do work.

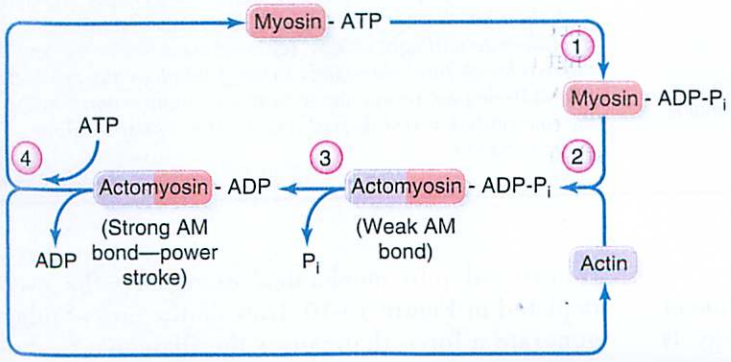


Figure 10-10 In the presence of ATP, myosin and actin filaments associate and dissociate cyclically. Myosin acts as an ATPase, hydrolyzing ATP (1), but the release of the products ADP and P_i is slow unless actin binds to the myosin (2), increasing the rate of release. Initially the actin-myosin bond is weak, but when P_i is released (3), it becomes stronger. The departure of P_i is accompanied by a release of energy, which can be used to generate force. ADP is then released and replaced by ATP (4), which breaks the actomyosin complex into actin and myosin-ATP. The cycle can then start again as long as myosin binding sites are available on actin.

Spotlight 10-2

MOTOR MOLECULES

Myosin is one of three "motor molecules" that drive movements in living organisms. Amazingly, these same three molecules drive movement at all levels of scale, from the movement of particles within a cell through the locomotion of very large animals. Myosin drives the locomotion of almost all multicellular animals, and it also participates in moving cells around, as well as in translocating organelles within a single cell. The other two motor molecules—kinesin and dynein—play important roles in the movement of organelles, and dynein also powers ciliary and flagellar movement.

The key feature of all three molecules is the ability to change chemical energy into mechanical work, a process called *chemomechanical transduction*. To accomplish this task, each molecule has ATPase activity, and each interacts with a filamentous protein to produce mechanical motion. Thus, each molecule must be able to bind ATP and catalyze the release of energy from a high-energy phosphate bond. In addition, each molecule must be able to bind to the appropriate filaments and generate mechanical force that ends in movement. Initially, it appeared that although the three motor molecules had all these features in common, they were fundamentally unrelated to one another. However, recently acquired evidence suggests that myosin and kinesin are very distantly related molecules, whereas dynein still stands apart in evolution. The function of myosin is discussed at length in the text. Here we will briefly consider kinesin and dynein.

Dynein was initially discovered in the cilia and flagella of eukaryotes. In these organelles, it binds to microtubules that are longitudinally organized along the cilium or flagellum in a conventional 9 + 2 arrangement (Figure 1a). Dynein, itself a large and complex molecule, forms one part of this active molecular complex (Figure 1b). Dynein causes flagella and cilia to bend by moving the microtubule pairs with respect to one another. It is anchored to the A tubule of one tubule pair, and its two heads transiently bind to the B tubule of the adjacent pair. The two heads "walk" along and push the B tubule by alternately binding to it, first one and then the other (Figure 1c).

Dynein also participates in intracellular traffic. In neurons, for example, it carries organelles from axon terminals

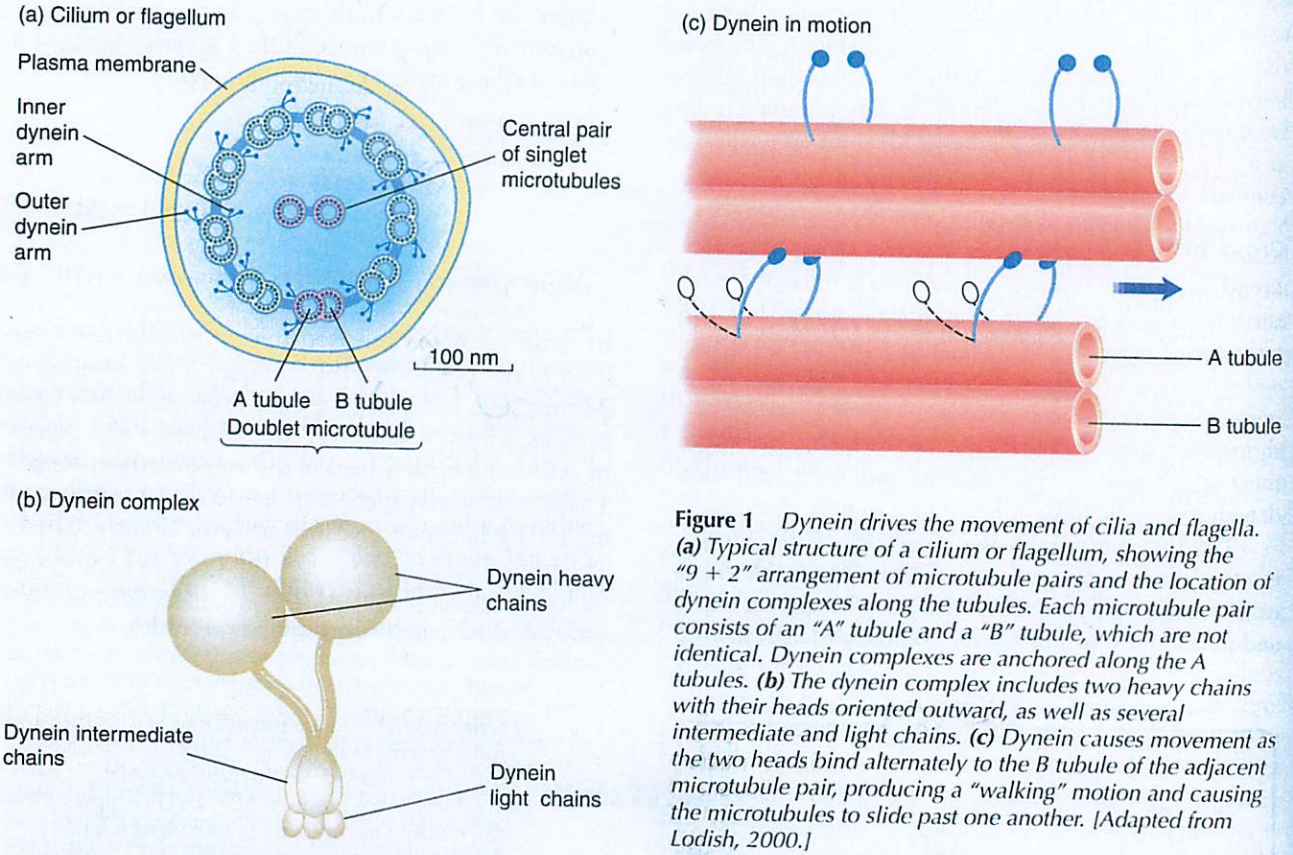
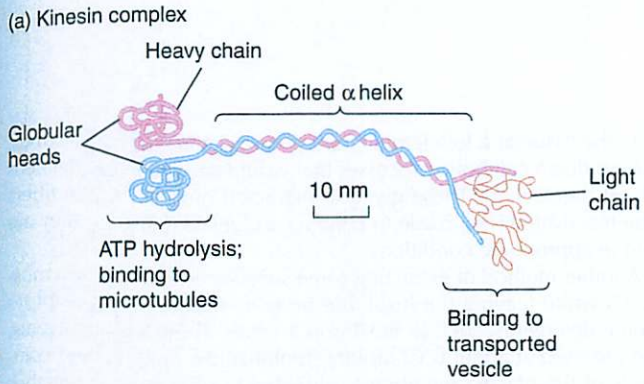


Figure 1 *Dynein drives the movement of cilia and flagella.* ***(a)*** *Typical structure of a cilium or flagellum, showing the "9 + 2" arrangement of microtubule pairs and the location of dynein complexes along the tubules. Each microtubule pair consists of an "A" tubule and a "B" tubule, which are not identical. Dynein complexes are anchored along the A tubules.* ***(b)*** *The dynein complex includes two heavy chains with their heads oriented outward, as well as several intermediate and light chains.* ***(c)*** *Dynein causes movement as the two heads bind alternately to the B tubule of the adjacent microtubule pair, producing a "walking" motion and causing the microtubules to slide past one another. [Adapted from Lodish, 2000.]*

Energy transduction by cross-bridges

One of the major questions regarding the function of myosin cross-bridges is how chemical energy is transduced into mechanical energy by the cycle depicted in Figure 10-10. How do the cross-bridges generate a force that causes the filaments to slide



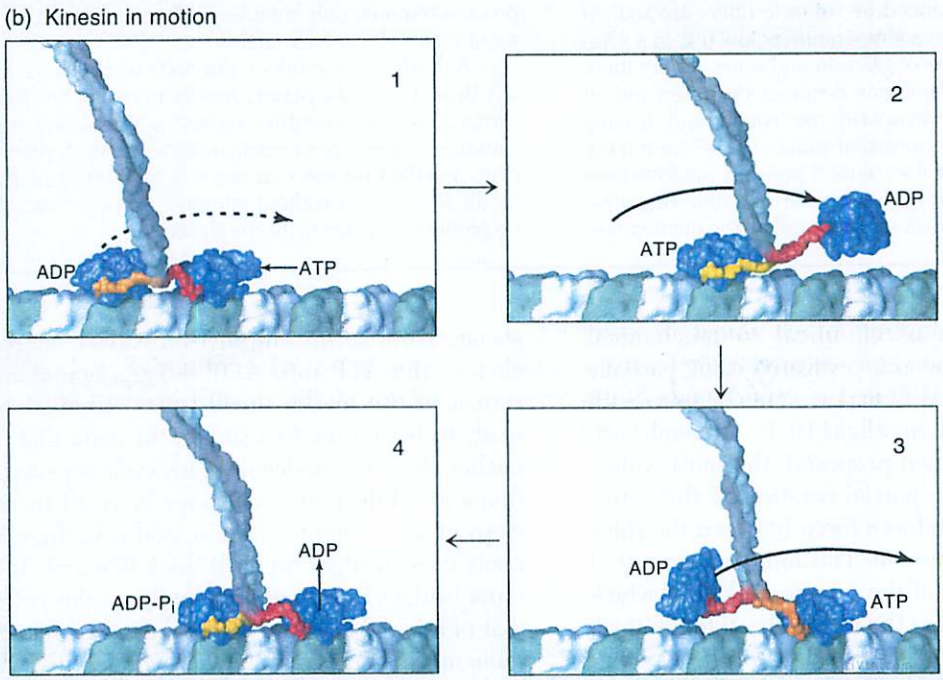


Figure 2 *Kinesin is similar to myosin in structure, but acts more like dynein.* ***(a)*** *A kinesin complex consists of two heavy chains, each of which includes a globular head and a long rod, and two light chains. The head binds to microtubules and to ATP; the opposite end of the rod binds the "cargo" to be carried.* ***(b)*** *The two heads of a kinesin complex bind alternately to a microtubule, "walking" down the structure and pulling their cargo along with them. The red linker represents the active "head." [Part b from Vale and Milligan, 2000.]*

back to the soma, a process called *retrograde axonal transport.* To perform this task, the dynein binds to microtubules arrayed along the axon and to whatever "cargo" it is transporting. In all motions based on dynein, ATP provides the energy to generate mechanical work.

Kinesin, the most recently discovered motor molecule, was identified initially for its role in *orthograde axonal transport* in neurons—that is, the movement of materials and vesicles from the soma out to the axon terminals. The structure of the kinesin complex is reminiscent of the structure of myosin—that is, it consists of paired heavy chains, each of which has a head at one end and a long rod at the other (Figure 2a). In addition, light chains associate with the end of the rod. The head end of kinesin binds to an axonal microtubule and the tail end to whatever it is transporting. The kinesin then "walks" down the microtubule from the soma toward the axon terminal by binding and then moving first one of its two heads and then the other, in a manner similar to dynein (Figure 2b).

Both dynein and kinesin produce movement by walking along microtubules, but they walk in opposite directions with respect to the orientation of the microtubules. In axons, kinesin ferries materials away from the soma; dynein, apparently moving along the same microtubules, carries materials toward the soma.

past each other? The transduction of chemical to mechanical energy in animals is accomplished by three different "molecular motors" that are ubiquitous in the animal kingdom: myosin, dynein, and kinesin (Spotlight 10-2). In muscles, it is myosin that performs this function.

Spotlight 10-3

SKINNED MUSCLE FIBERS

An early advance that contributed significantly to the study of muscle-fiber physiology was the discovery by Albert Szent-Gyorgyi of a procedure for isolating muscle fibers in which the intracellular structure remains intact, but the membrane no longer prevents free exchange of materials between the cytoplasm and the extracellular solution. This kind of preparation is called a "skinned" muscle fiber because the outer membrane of the fiber has been entirely removed or rendered so leaky that it is functionally absent. In skinned fibers, an investigator can control the composition of the intracellular fluids without any interference from regulatory mechanisms that are normally present in an intact muscle fiber.

In Szent-Gyorgyi's procedure, muscle fibers are soaked for several days or weeks at a temperature below 0°C in a solution made up of equal parts of glycerin and water. Under these conditions, the plasma membrane becomes disrupted and all soluble substances in the myoplasm are leached out, leaving intact the insoluble molecules that make up the contractile machinery. The glycerin in the solution prevents the formation of ice crystals, which could break up the structural organization of the fibers, and it also helps to solubilize the membranes. Storing the tissue at a low temperature preserves the enzymes, but slows down catabolic processes that would cause the cells to digest themselves. These glycerin-extracted muscle fibers can be reactivated (i.e., made to contract and relax) if they are placed in appropriate conditions.

Another method of extracting some substances from muscle cells while leaving the insoluble proteins intact employs nonionic detergents, such as the Triton X series. These agents, which are used at about 0°C, rapidly solubilize the lipid components of the plasma membrane, allowing soluble metabolites to diffuse out of the cell and substances in the extracellular medium to diffuse rapidly into the cell. Fibers treated in this way are called "chemically skinned muscle fibers." This process requires only minutes, rather than the days or weeks required for glycerin extraction.

A third way to produce skinned muscle fibers is to manually dissect away the plasma membrane using fine forceps. This process, which resembles removing the casing from a link sausage, requires great manual dexterity. With practice, however, a skilled person can use this method to prepare structurally intact fibers without introducing any artifacts that could be produced by detergents or glycerine.

The transduction of chemical to mechanical energy in muscle has been investigated using partially intact muscle fibers and in experiments *in vitro* with "skinned" muscle fibers (Spotlight 10-3). Although various hypotheses have been proposed, the most widely accepted view is that a partial rotation of the actin-bound myosin head produces force between the thick filament and the thin filament. This force is transmitted through the S2 portion of the myosin molecule, which forms a "neck" connecting the myosin head to the thick filament (see Figure 10-6c). According to this hypothesis, the myosin neck acts as an elastic cross-bridge, linking the myosin head and the thick filament and transmitting to the thick filament the force produced as the head rotates on the actin filament.

Evidence from a huge number of studies, including X-ray diffraction studies to determine the conformation of the myosin head under different conditions, confirms this view. The myosin head, bound to both ADP and P_i, binds to actin (Figure 10-11, step 1). Initially, this bond is relatively weak, but once it is formed, P_i is released accompanied by the release of energy, the strength of the bond between actin and myosin increases, and a major rotational movement of the myosin head with respect to the connecting link occurs (Figure 10-11, step 2). The rotation produces force on the link, which is then translated through the link to the light meromyosin rod in the thick filament. The release of ADP from the myosin head follows, and ATP replaces it in the nucleotide binding site of the head (Figure 10-11, step 3). The binding of ATP causes myosin to dissociate from actin. The myosin ATPase activity then cleaves the ATP into ADP + P_i, accompanied by a return of the myosin head to its "cocked" position, ready to bind again to a site on the actin that is a little farther along the molecule. This cycle repeats, and the filaments slide past each other in small incremental steps of attachment, rotation, and detachment of the many cross-bridges on each thick filament. Individual cross-bridges move independently, so the net effect is that of a long boat powered by many oarsmen, each of them moving in his or her own rhythm, but at least most of them rowing in the same direction.

The elasticity of the cross-bridge allows the rotational movement to occur without an abrupt change in tension. Once it is stretched, the link transmits its tension smoothly to the thick filament, generating force to push the thick filament past the firmly anchored thin filament. A major piece of evidence supporting the hypothesis that the elasticity of cross-bridge links plays a major role in mechanically coupling the myosin heads with the thick filament is the observation that the longitudinal elasticity of a muscle fiber is proportional to the amount of overlap between the thick and thin filaments. Hence it is proportional to the number of attached cross-bridges. In addition, sudden small decreases in fiber length are accompanied by very rapid recovery of tension, which presumably results from rotation of the cross-bridge heads into more stable positions of interaction with actin sites. In other words, the relative positions of the myosin head and actin adjust so that the sites that bind most strongly are favored.

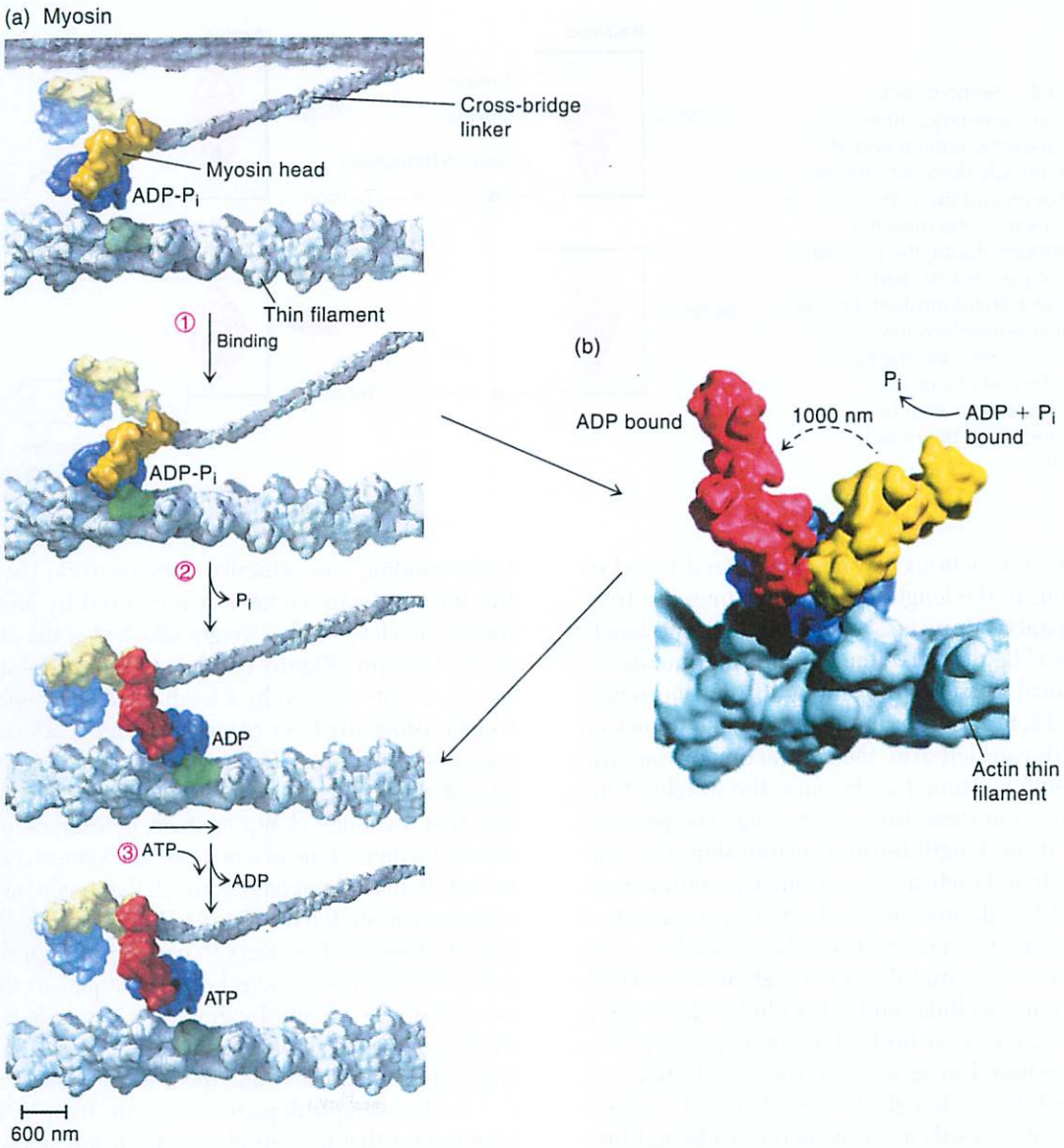


Figure 10-11 The sliding of thick and thin filaments past each other is driven by changes in the geometry of bonds between myosin cross-bridges and actin. **(a)** Space-filling models of myosin heads illustrate the sequence of events in the attachment of myosin cross-bridges to actin filaments. Although each myosin complex includes two heads, only one is active at any given time. In the relaxed state, ADP + P_i occupies the nucleotide binding site on the head (dark blue), and the myosin head is not bound to actin. One myosin head then attaches to actin (step 1) at specific binding sites (green). As a result, P_i is released (step 2), energy is liberated, and the head (now shown in red) rotates with respect to the actin filament, creating a force between the thick and thin filaments and causing them to slide past each other. The rotation is thought to accompany the sequential formation of multiple bonds between the myosin head and the actin binding site; some evidence suggests that four bonds are formed in sequence, each stronger than the last. ADP is then released from the nucleotide binding site and replaced by ATP (step 3), which weakens the bond between the myosin and actin, permitting relaxation. Hydrolysis of the ATP returns the head to its "cocked" position, ready to bind again to a site on actin. **(b)** The action of a myosin cross-bridge is similar to that of an oar pushing a boat through the water. Each swing of the myosin head "rows" the thick filament about 1000 nm past the neighboring thin filament. [Adapted from Vale and Milligan, 2000.]

Question ?

Working out at a gym causes individual skeletal muscle fibers to become larger in cross-section; no new muscle fibers are formed. How does working out make you stronger? Similarly, what allows an elephant to lift larger weights than you can?

MECHANICS OF MUSCLE CONTRACTION

Many of the mechanical properties of contracting muscle were elucidated before 1950, when the mechanism of contraction was not yet understood. It is useful to consider these classic findings and attempt to explain them in terms of our current understanding of cross-bridge behavior.

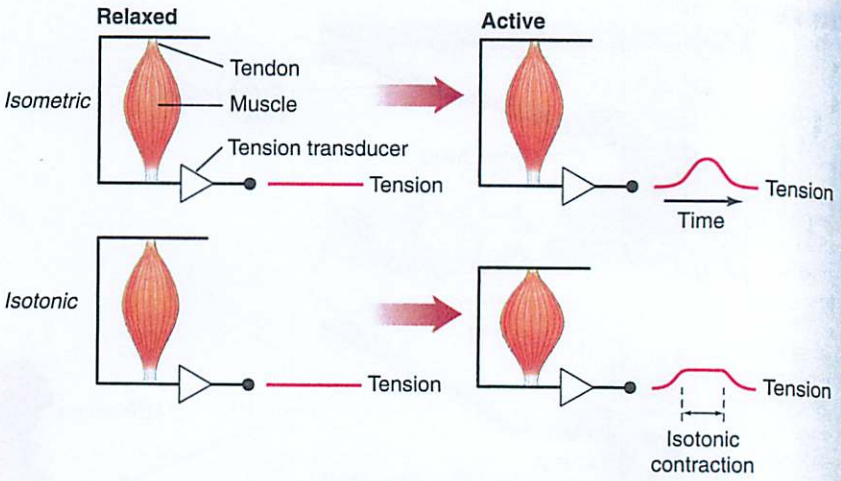


Figure 10-12 Some muscle contractions are isometric; others are isotonic. In isometric contraction, the length of the muscle does not change. Tension increases and then decreases. In isotonic contraction, the muscle is allowed to shorten during the time when tension is being generated. Strictly speaking, after a brief transitional period when the muscle develops force, the tension during isotonic contraction is constant. At the end of isotonic contraction, there is another brief dynamic period when the muscle returns to rest.

Muscle contractions can be categorized based on what happens to the length of the active muscles. In an **isometric contraction** (i.e., "same length"), the length of a muscle is held fixed, either by an experimenter or by the physical situation, preventing it from shortening (Figure 10-12, top). For example, if you tried to pick up your car with your left arm, the contraction of your arm muscles would be isometric, because the weight of the car would prevent them from shortening. The previous discussion of the length-tension relationship for a sarcomere was based exclusively on isometric contractions. Note that although no external shortening is permitted during an isometric contraction, there can be a very small amount of internal shortening (about 1%), which occurs when intracellular and extracellular elastic components—such as cross-bridge links and the connective tissue that is found in series with the muscle fibers—are stretched. Even though the muscle is not shortening, cross-bridge bonds are repeatedly made and broken, and a great deal of energy can be consumed (imagine again trying to pick up your car with one arm). In an **isotonic contraction** (i.e., "same tension"), the muscle shortens as force is generated (Figure 10-12, bottom). Although, strictly speaking, the tension generated during an isotonic contraction would be expected to remain constant, some physiologists use the term to indicate any condition in which the active muscle is allowed to shorten, whether or not the tension is indeed constant. Most natural movements are produced by muscle contractions that are neither isometric nor strictly isotonic, but many studies of muscle function are carried out in the simpler conditions of isometric or isotonic contraction, making this distinction useful.

Relation Between Force and Shortening Velocity

For animals to move, muscles must shorten and pull on parts of the skeleton. Therefore, the relation between the production of force and the rate at which a muscle shortens (the so-called *force-velocity curve*) is crucial for understanding how muscular systems work. Historically, the force-velocity curve was measured by attaching a muscle to a lever with a weight attached at the other side of the fulcrum (Figure 10-13a). In more recent experiments, a motor driven by a feedback circuit replaces the weight, providing finer control. The feedback control of such a servomotor system regulates the apparent weight against which the muscle is shortening in much the same way that a voltage clamp controls membrane potential across the membrane of a neuron. The system is arranged so that there is a limit on how much the weight, or the servomotor, can stretch the muscle. When the muscle is electrically stimulated, it starts to contract. When the force generated by the muscle becomes equal to the force exerted on the weight by gravity, the muscle begins to shorten at a constant velocity (i.e., the muscle no longer contracts isometrically), and the velocity is measured.

In the example depicted in Figure 10-13b, the maximal weight that the muscle can lift is just under 100 g; that is, if the muscle contracts against a load of 100 g or more, it cannot shorten. If the load is less than 100 g, the muscle shortens, but the rate of shortening (ΔL per unit time) depends on the size of the weight. If a 50 g weight is attached, the muscle shortens slowly; if lighter weights are attached, the muscle shortens faster. When it shortens against no weight at all, the velocity of contraction is maximal; this velocity is represented by V_{max}. This characteristic of muscles, V_{max}, will play an important role later in this chapter when we discuss the evolutionary adaptation of skeletal muscles.

Plotting the force generated by a muscle against its shortening velocity generates a hyperbolic curve whose equation was determined empirically in the 1930s by Archibald V. Hill, an important pioneer in muscle physiology:

$$V = \frac{b(P_0 - P)}{P + a} \qquad (10\text{-}1)$$

where V is the velocity of shortening; P, the force (or load); P_0, the maximal isometric tension of that muscle;

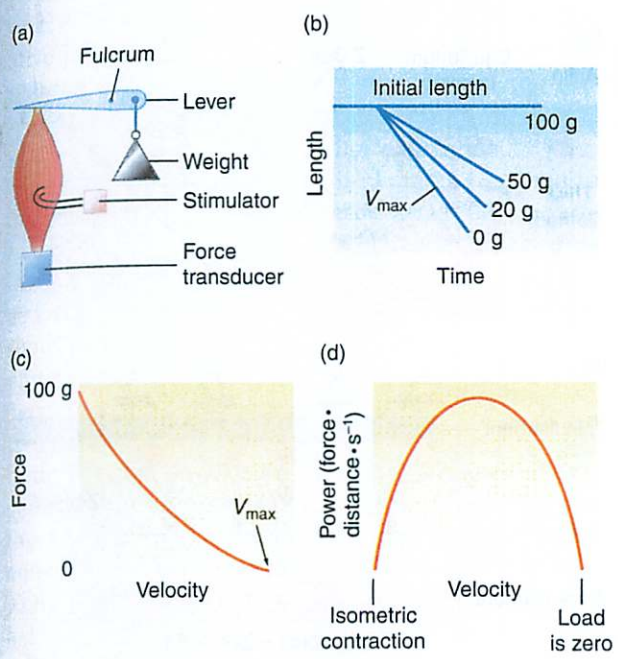


Figure 10-13 The force against which a muscle works and the velocity at which it shortens are reciprocally related. **(a)** Typical setup for measuring the relation between force and velocity for a muscle. The muscle works against a weight that is hung on the other side of the fulcrum of a lever. When the stimulated muscle generates a larger force than the weight, it shortens and pulls down on the lever. Alternatively, a servomotor system can be used to provide finer control of the initial muscle length and the load. At the start of the experiment, the length of the muscle would be set to optimize the overlap between thick and thin filaments in the sarcomeres. **(b)** Contraction of a muscle as it works against four different loads: 100 g, 50 g, 20 g, and 0 g. When the load is small, the muscle contracts more rapidly; that is, ΔL per unit time is larger. The maximal weight this muscle can lift is just under 100 g, so when it works against the 100 g weight, it cannot shorten; contraction against 100 g or more is isometric. **(c)** Force-velocity curve based on the data shown in part b. The data that produce this curve match Equation 10-1 well. At the maximal force of 100 g, the velocity of shortening is zero; that is, contraction is isometric. Shortening of the muscle is fastest when the muscle is completely unloaded. **(d)** Power-velocity curve calculated by multiplying the force and the velocity for each data point in part c. The power is zero if either the force or the velocity equals zero.

b, a constant with dimensions of velocity; and a, a constant with dimensions of force (Figure 10-13c).

Equation 10-1 implies that as the load increases, the shortening velocity decreases. You are probably familiar with this principle from personal experience; you can lift a feather from a table much more rapidly than you can lift a heavy book. Notice that the decline in force with increased velocity does not reflect a change in myofilament overlap. On the contrary, these experiments are purposely performed at the plateau of the length-tension relationship, so that the number of cross-bridges that can interact with actin remains high and constant during shortening.

The relation between power and velocity is as important to an animal's behavior as the relation between force and velocity. For a fish to swim or a frog to jump, its muscles must generate mechanical power. The mechanical work performed by a muscle is the product of force times the change in length (ΔL), and mechanical power is given by

$$\text{power} = \frac{\text{work}}{\text{time}} = \frac{(\text{force}) \times (\Delta L)}{\text{time}}$$
$$= (\text{force}) \times (\text{shortening velocity})$$

Hence, multiplying the force generated by the muscle times the velocity at which it shortens yields the power produced under each condition. As shown in Figure 10-13d, the power generated is maximal at intermediate shortening velocities. Power falls to zero if either the velocity of shortening (i.e., in isometric contraction) or the force generated is zero.

As we will see later in this chapter, it is frequently useful to describe force production or power production in terms of V/V_{max}, where V is the velocity of shortening under a particular condition and V_{max} is the maximal velocity of shortening for a particular muscle. Power production by the muscle shown in Figure 10-13 is maximal at a V/V_{max} of about 0.4; this relation has been found to hold for all muscles, no matter how fast their V_{max} is.

Effect of Cross-Bridges on the Force-Velocity Relation

From the force-velocity curve described in the previous section, we know that the force generated by a muscle drops as its shortening velocity increases. Recall that this relation is not caused by a change in the amount of overlap between thin and thick filaments; rather, it is observed at the maximal overlap. From our earlier discussion of the role played by cross-bridges in isometric contraction, we might suppose that this drop in force with increased velocity could result if fewer cross-bridges were actually attached during rapid shortening, or if each of the cross-bridges that was attached generated a smaller force, or both. This model of cross-bridge kinetics was proposed by Andrew Huxley in 1957. Although it has been superseded in some fine details, it still provides the basic principles for understanding the overall mechanics and energetics of muscle contraction.

In Huxley's model, cross-bridges are considered to be elastic structures that generate zero force when they are at equilibrium. Their behavior is similar to that of a piece of spring steel projecting out from a surface. Deforming the piece of steel by bending it creates a restoring force that can return it to its original position. Similarly, when a cross-bridge is bent toward or away from the Z disk, a restoring force is created that tends to bring it back to its original position; the magnitude of this force is proportional to the displacement of the

cross-bridge from its equilibrium position (Figure 10-14a). If a cross-bridge bent toward the Z disk were attached to a thin filament, the restoring force would pull the Z disk toward the center of the sarcomere; this force is considered to be in the "positive" direction. By contrast, if a cross-bridge bent away from the Z disk were attached to a thin filament, the restoring force would push the Z disk away from the center of the sarcomere; this force is viewed as a "negative" force.

Figure 10-14b illustrates how the forces generated by passive cross-bridge displacement cause movement of a thin filament. When the cross-bridge is at subscript the equilibrium position (0), the force generated, F_0, is zero; when the cross-bridge is bent toward the Z disk, the force is positive (F_1 and F_2); and when the cross-bridge is displaced away from the Z disk, the force is negative ($F_{1'}$ and F_3). The force generated by one thick filament is equal to $\Sigma n_i F_i$, the sum of the product of the number of attached cross-bridges at each displacement, n_i, and the force produced per cross-bridge at that displacement, F_i. As the velocity of shortening increases, the number of cross-bridges that are attached drops, and the displacement of the cross-bridges that are attached becomes smaller (Figure 10-14c). In addition, during rapid shortening, some cross-bridges become attached when they are in a position that generates negative force. As a result of all of these changes, the net force produced during rapid shortening is lower than the force produced during slow shortening.

According to Huxley's theory, unattached cross-bridges are moved away from their neutral position by random thermal motion. If cross-bridges attached randomly to thin filaments, no force would be generated as the result of this thermal motion, because the number of cross-bridges generating negative force would equal the number generating positive force. However, cross-bridges can initially attach to thin filaments only when they are in a position that would generate positive force. Thus, when a muscle is loaded maximally and is contracting isometrically, there will be an even distribution of cross-bridges that generate a positive force, and because all attached cross-bridges are generating positive force, the average force per cross-bridge will be positive and large.

If cross-bridges can attach to thin filaments only when they are displaced to a position that produces positive force, how can cross-bridges generate negative force? During shortening, the thin filaments move toward the center of the sarcomere, so any cross-bridges that are attached to them at a sharp angle toward the Z disk (e.g., cross-bridge 2 in Figure 10-14b) will be shifted closer to the equilibrium position. The amount of force they produce will thus be reduced by the movement of the thin filament. A cross-bridge that is attached at a very shallow angle (e.g., cross-bridge 1 in Figure 10-14b) can be dragged over to a position (1′) that causes it to generate a negative force ($F_{1'}$). Of course, this effect could not go on indefinitely because

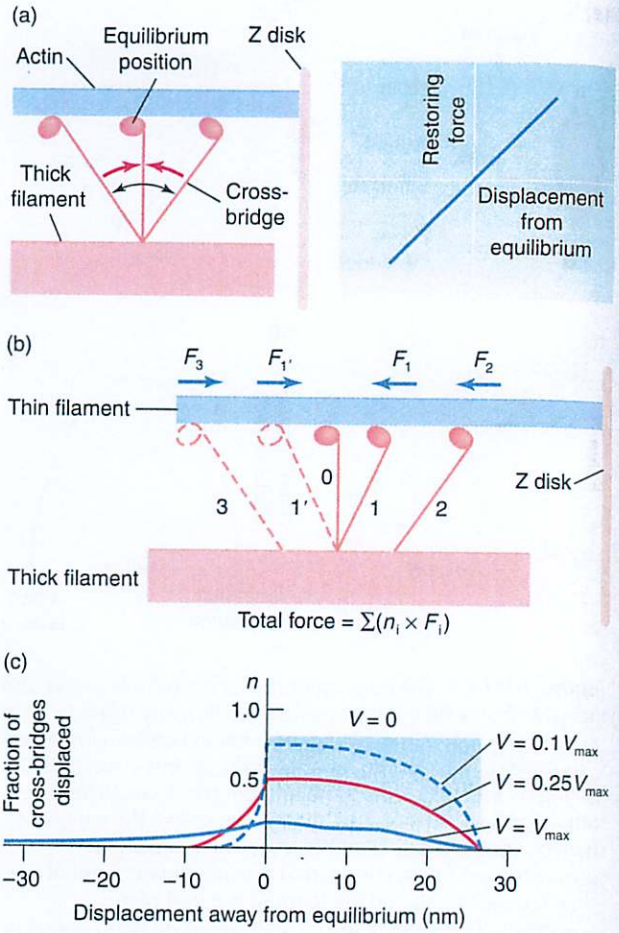


Figure 10-14 Cross-bridges generate a restoring force when they are moved away from their equilibrium position. **(a)** Relations between the position of a cross-bridge and the magnitude and direction of the force generated. Cross-bridges are assumed to behave like a strip of spring steel, fixed at one end. At the equilibrium position of the cross-bridge, no force is generated. Displacement of the cross-bridge away from equilibrium in either direction generates a restoring force that tends to bring the cross-bridge back to equilibrium. **(b)** Cross-bridges attached to a thin filament at different positions. When displaced toward the Z disk (solid lines), they generate a positive force (e.g., F_2 for cross-bridge 2); when displaced away from the Z disk (dashed lines), they generate a negative force (e.g., F_3 for cross-bridge 3). The total force at any time is the sum of the forces generated by all cross-bridges. Movement of the thin filament can change the displacement of some bound cross-bridges (e.g., position 1 to 1′ for cross-bridge 1), causing them to exert negative ($F_{1'}$) rather than positive (F_1) force. **(c)** The fraction of the total number of cross-bridges that are attached and displaced at different shortening velocities. As the velocity at which thick and thin filaments slide past each other increases, fewer cross-bridges are attached, and the average position (and hence force production) of the cross-bridges becomes more negative. At V_{max}, the net force generated by the cross-bridges equals zero, because the positive force generated by some cross-bridges equals the negative force generated by others. Conversely, when the muscle contracts isometrically ($V = 0$), the production of force is maximal because many cross-bridges are attached, and all of the attached cross-bridges are in a position that produces positive force.

such cross-bridges would produce more and more negative force, preventing further sliding of the thin filament. Each cross-bridge must detach, and the time required for a cross-bridge to detach is the key to what limits the maximal velocity of shortening.

Assuming that it takes a fixed amount of time for cross-bridges to detach, then, as the velocity at which the filaments slide past each other increases, more cross-bridges will be dragged to a position from which they can generate a negative force before they can detach. There should then be a velocity at which the negative force generated by cross-bridges that have been dragged to the negative side of the equilibrium position will just balance the positive force generated by the attached cross-bridges on the positive side. At this point, the net force generated by all attached cross-bridges is zero. Because the muscle cannot shorten any faster than this rate, this constitutes the maximal velocity of shortening, V_{max}. Thus, at V_{max}, some cross-bridges are attached, but the net force—or average force per cross-bridge—is zero. It follows that a muscle can have a fast V_{max} if its cross-bridges detach rapidly, breaking their bonds with the thin filaments before they can generate large negative forces.

Two aspects of this model explain the observed decrease in force as the velocity of shortening increases. First, the average force generated by the cross-bridges drops with increased velocity. Second, the total number of cross-bridges attached at any one time drops with increased velocity. The argument supporting this second aspect of the model is based on chemical kinetics: As cross-bridges are dragged to positions in which they generate negative force, they detach faster, which causes fewer cross-bridges to be attached at higher velocities. It is thought that at V_{max}, as few as 20% of the cross-bridges are attached; in high isometric tension, approximately 30% of cross-bridges are attached.

REGULATION OF MUSCLE CONTRACTION

Up to this point, we have considered only how cross-bridges on myosin thick filaments in an activated muscle fiber bind to and unbind from actin thin filaments, thereby generating force. Of course, if our muscles were "on," or activated, all the time, we would be in a constant state of rigidity, unable to move, talk, or breathe. Thus, to perform useful work, muscles must turn on and off at the appropriate times. The mechanisms by which contraction is regulated—that is, turned on and off—are discussed in the following sections.

The Role of Calcium in Cross-Bridge Attachment

The earliest evidence that Ca^{2+} plays a physiological role in muscle contraction came from the work of Sidney Ringer and Dudley W. Buxton in the late nineteenth century. They found that an isolated frog heart stops contracting if Ca^{2+} is omitted from the saline bath in which it is immersed. (This observation marked the origin of Ringer solution and other physiological salines.) The possibility that Ca^{2+} participates in the regulation of muscle contraction was first tested in the 1940s, when several researchers experimentally introduced a variety of cations into the interior of skinned muscle fibers. Of all the ions tested, only Ca^{2+} was found to produce contraction when it was present at concentrations similar to those normally found in living tissue. It was subsequently discovered that skeletal muscle fails to contract in response to stimulation if its internal calcium stores are depleted, although the presence of calcium in the *extracellular* fluids is not required.

The concentration of Ca^{2+} ions is normally very low in the cytosol of relaxed muscle fibers—10^{-6} M or lower. Initial attempts to study the events of contraction in solution were foiled because it was impossible to maintain the Ca^{2+} concentration of experimental solutions as low as it is in the cytosol. Even double-distilled water contains more than 10^{-6} M Ca^{2+}. The discovery of calcium-chelating agents, such as EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene-*bis*[oxyethylenenitrilo] tetraacetic acid), overcame this obstacle. The development of methods for preparing skinned muscle fibers, which lack a functional outer membrane, also facilitated research on the role of Ca^{2+} in contraction (see Spotlight 10-3).

The quantitative relation between the concentration of free cytosolic Ca^{2+} in muscle fibers and contraction has been determined by exposing skinned myofibrils to solutions containing different Ca^{2+} concentrations. Skinned myofibrils contract only if the solutions contain both Ca^{2+} and ATP, because ATP is required for muscle contraction (see Figure 10-10). If the Ca^{2+} is removed, the myofibrils relax, even if ATP is plentiful (Figure 10-15a on the next page). The amount of tension generated rises sigmoidally from zero at a Ca^{2+} concentration of about 10^{-8} M to a maximum at about 10^{-6} M (Figure 10-15b).

As we've seen already, force can be developed only when myosin cross-bridges bind to actin thin filaments, so anything that facilitates or inhibits this binding will affect contraction. The key to how Ca^{2+} induces muscle contraction lies in two proteins—tropomyosin and troponin—that are associated with actin in thin filaments (see Figure 10-5b). Tropomyosin is a filamentous protein that runs parallel to actin filaments. When a myofibril is relaxed, tropomyosin occupies a position that sterically blocks the myosin binding sites on the actin filament. Troponin is a complex of three protein subunits: troponin C, which binds Ca^{2+}; troponin T, which binds tropomyosin; and troponin I, which binds both actin

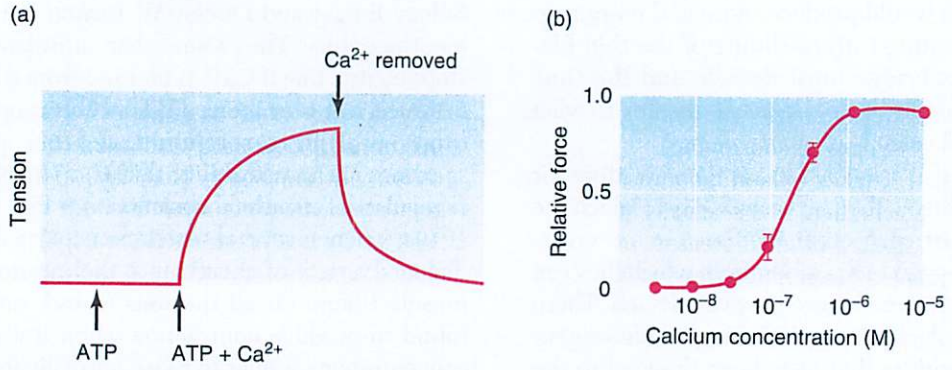


Figure 10-15 Free calcium ions in the cytosol regulate the state of muscle contraction. **(a)** Glycerin-extracted muscle fibers generate tension when they are exposed to Ca^{2+} and ATP. They relax when Ca^{2+} is removed, even if ATP is still present. **(b)** The force generated by a skinned muscle fiber varies with the concentration of Ca^{2+} in the surrounding medium. Force increases with increasing Ca^{2+} concentration, up to about 10^{-6} M. [Part b adapted from Hellam and Podolsky, 1967.]

and troponin C (Figure 10-16a). The troponin complex binds to tropomyosin about every 40 nm along the actin filament (see Figure 10-5b). Its association with troponin causes tropomyosin to change its shape and thus to change its association with the actin filaments. Troponin is the only protein in either the thin or the thick filaments of vertebrate striated muscle that has a high binding affinity for Ca^{2+}. (Myosin light chains bind Ca^{2+}, but less avidly.) When Ca^{2+} binds to troponin, the troponin molecule undergoes a change in conformation that modifies binding strengths in the complex (Figure 10-16b). The net result is that the tropomyosin moves with respect to the actin filaments, allowing myosin heads access to myosin binding sites on the actin (Figure 10-16c). Thus, when Ca^{2+} binds to troponin, it removes an otherwise constant inhibition of attachment between myosin cross-bridges and thin filaments. It is inferred from experimental results like those shown in Figure 10-15b that cross-bridges can bind to actin when the concentration of free Ca^{2+} in the cytosol reaches about 10^{-7} M.

As discussed earlier, myosin hydrolyzes ATP much more rapidly when the heads bind to actin. Ca^{2+} increases the binding of myosin heads, so adding Ca^{2+} to naked myofibrils would be expected to increase the ATPase activity of the myosin. Experiments like the one shown in Figure 10-17a on page 382 have demonstrated that this is exactly what happens. Cross-bridges cycle normally and produce tension only when both ATP and Ca^{2+} are present in the cytosol surrounding the myofibrils (Figure 10-17b). When a glycerin-extracted muscle fiber was initially exposed to Ca^{2+} in the absence of ATP, no tension was generated. When ATP was added, tension developed, and that tension was then maintained even when ATP was removed—that is, when rigor mortis set in. Once the muscle was in rigor, removing Ca^{2+} had no effect because the lack of ATP caused all the attached cross-bridges to be frozen in place. When ATP was added back to the muscle that was in rigor, but Ca^{2+} was absent, the muscle relaxed. Thus, both ATP and Ca^{2+} must be present if the thick and thin filaments are to interact effectively to produce tension, but only ATP is required for relaxation.

If this picture of how Ca^{2+} regulates contraction is correct, Ca^{2+}, troponin, and tropomyosin together would be expected to modify the ATPase activity of myosin. In the experiment shown in Figure 16-17c, S1 fragments of myosin, which are the location of myosin's ATPase activity, were mixed with ATP and other components. If only ATP and actin were present in solution with the S1 fragments, there was some ATPase activity, particularly at higher concentrations of the S1 fragments. Adding troponin and tropomyosin to the mixture reduced the ATPase activity. Troponin and tropomyosin spontaneously bind to actin, occluding potential myosin binding sites. However, adding troponin and tropomyosin along with an appropriate concentration of Ca^{2+} greatly increased the ATPase activity of the S1 fragments, indicating that just as in skinned muscle fibers, Ca^{2+} modulated the inhibitory effect of troponin and tropomyosin on cross-bridge binding.

These results showed that calcium regulates the actin-myosin interaction via troponin and tropomyosin in vertebrate striated muscles. It regulates contraction in other types of muscle as well, but by at least two other mechanisms. In most invertebrate striated muscles, calcium initiates contraction by binding to the myosin light chains of the cross-bridge heads. Contraction of vertebrate smooth muscle and of nonmuscle actomyosin depends on calcium-dependent phosphorylation of the myosin head, as described in the last section of this chapter.

Excitation-Contraction Coupling

In Chapter 6, we stated that an action potential arriving at the synapse between a motor neuron and a skeletal muscle fiber causes an action potential in the fiber, followed by a twitch. This process is summarized in Figure 10-18 on page 382. The depolarization of the muscle

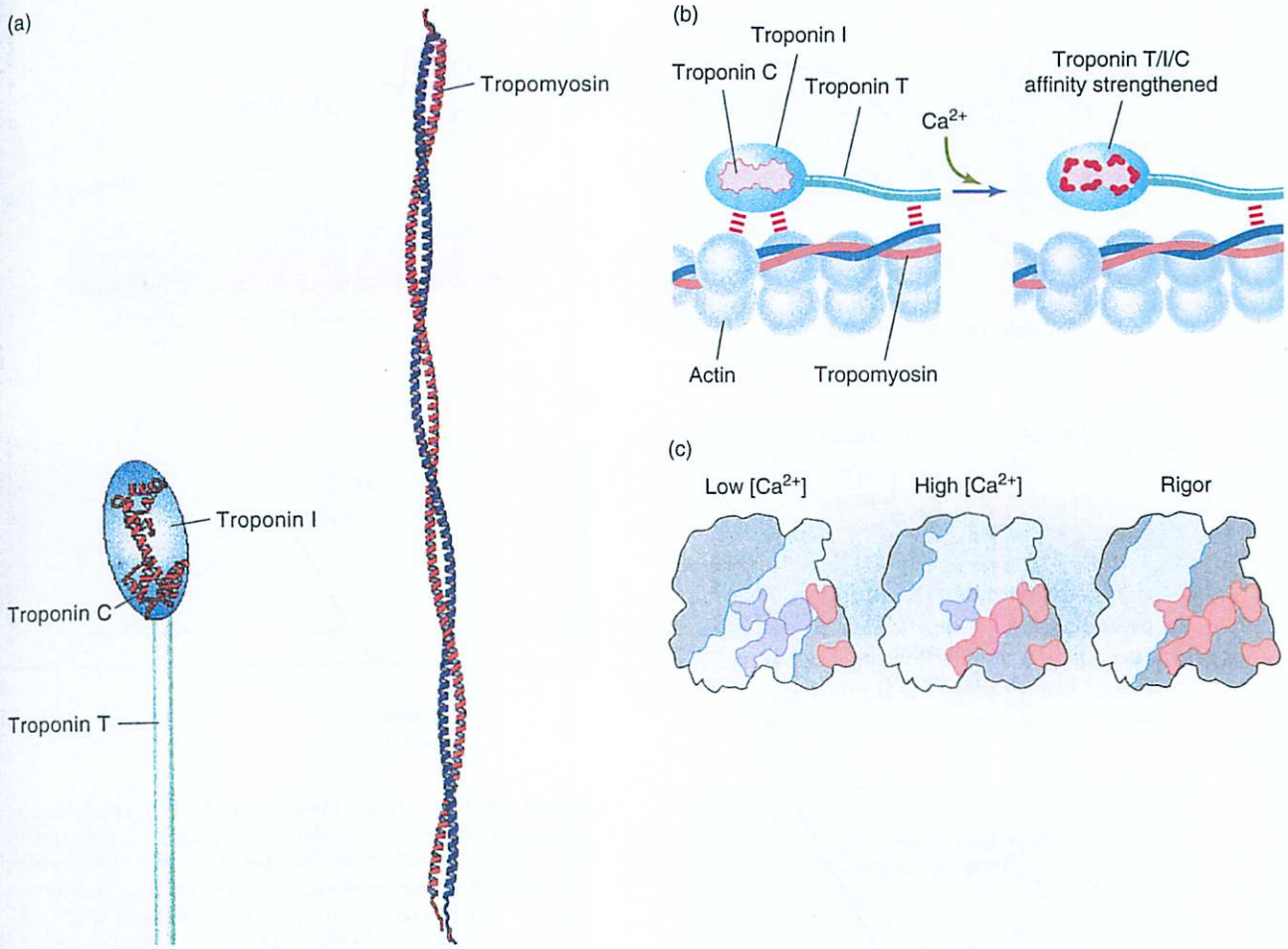


Figure 10-16 Troponin and tropomyosin regulate binding between myosin cross-bridges and actin thin filaments. **(a)** Three-dimensional models depicting the molecular structures of troponin and tropomyosin. Troponin consists of three subunits. The molecular structure of troponin C, which binds Ca^{2+}, has been worked out in detail. Troponin T, which binds to actin and blocks myosin binding sites on actin, is a long chain. The structure of troponin I, which binds to actin and to troponin C, has not yet been determined in detail, but in the complex it is located in the position shown. **(b)** Diagram illustrating bonds along thin filaments. The thickness of the red lines represents the strength of the bonds. In the absence of Ca^{2+}, troponin T binds to tropomyosin, and troponin I binds to actin. Both troponin I and troponin T bind to troponin C. When troponin C binds Ca^{2+}, however, its bond with troponin I becomes stronger, and the bond between troponin I and actin is broken. In addition, the bond between troponin C and troponin T is strengthened. In a way that is not yet understood, this shift in bond strengths exposes myosin binding sites on the actin. **(c)** Structure of a single G-actin subunit (shown in gray), including potential myosin binding sites. The part of the actin subunit covered by tropomyosin under three conditions is shown in blue. Exposed myosin binding sites are red; sites blocked by tropomyosin are purple. When Ca^{2+} is absent, tropomyosin covers most of the potential myosin binding sites. With Ca^{2+} in the cytosol, the position of tropomyosin shifts, exposing some of the myosin binding sites. In rigor, all of the binding sites become available, permitting tight binding between thick and thin filaments. When binding sites are transiently exposed in normal contraction, cross-bridges can bind cyclically until Ca^{2+} is removed from the troponin complex. [Part a adapted from Squire and Morris, 1998; parts b and c adapted from Gordon et al., 2000.]

fiber is typically large enough to elicit an action potential, which travels down its length. Following a latent period of several milliseconds, the muscle fiber then generates tension (Figure 10-18b) Given what is known about cross-bridge attachment, sliding filaments, and the crucial role played by Ca^{2+}, it seems likely that the regulation of muscle contraction includes some mechanism by which an action potential in the muscle fiber changes the concentration of free Ca^{2+} in the cytosol. This process is called **excitation-contraction coupling.** Its net effect is to link an AP in the plasma membrane of the muscle fiber to the concentration of free Ca^{2+} in the cytosol. We examine the details of this critical process in the following sections.

Membrane potential and contraction

As we saw in Figure 5-14, if some of the Na^+ ions in the normal saline bathing excitable cells are replaced by K^+ ions, the membrane potential, V_m, of the cells is depolarized. When muscle fibers are suddenly depolarized

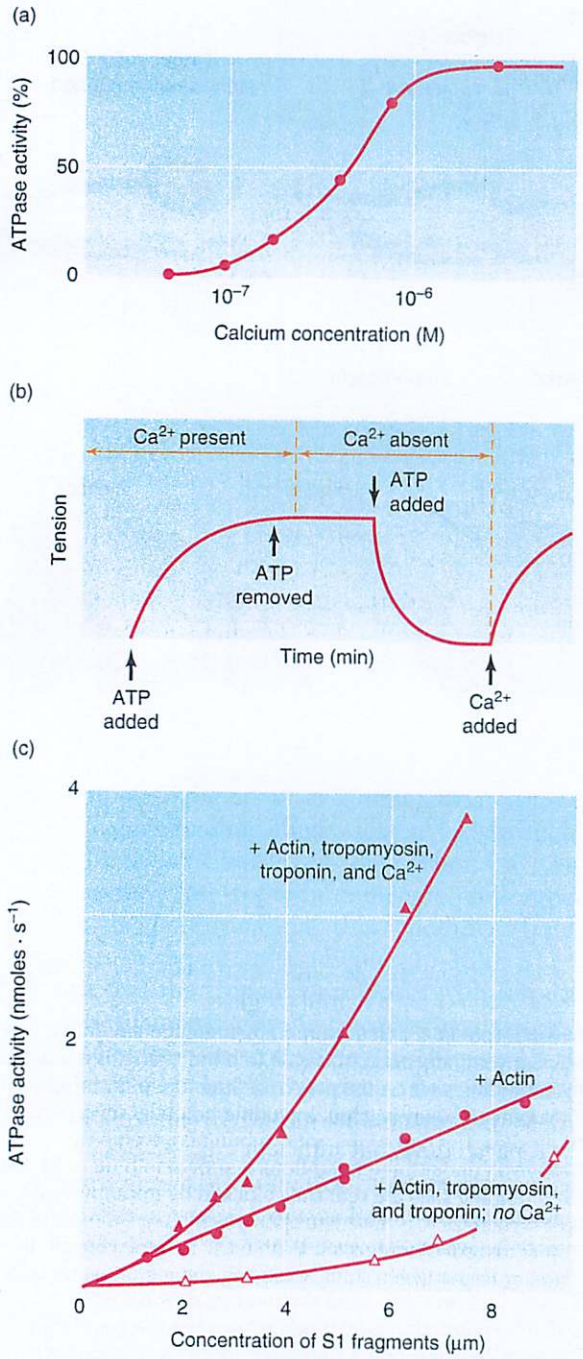


Figure 10-17 Free calcium modulates the activity of both glycerin-extracted muscle fibers and isolated myofibrils. Classic Work **(a)** The ATPase activity of myofibrils increases sigmoidally with the Ca^{2+} concentration of the surrounding solution, with a threshold somewhat lower than 10^{-7} M. **(b)** Both Ca^{2+} and ATP are required for muscles to contract, but relaxation occurs only in the presence of ATP and the absence of Ca^{2+}. If ATP is removed once tension has developed, the fiber enters rigor mortis (flat part of the curve). Rigor is relieved only by removal of Ca^{2+} *and* addition of ATP. **(c)** When actin, S1 fragments of myosin, and ATP are mixed in a solution, ATPase activity of the myosin can be detected. Adding troponin and tropomyosin to the mixture reduces the ATPase activity as troponin and tropomyosin bind to actin, blocking myosin binding sites. If Ca^{2+} is then added, the ATPase activity increases greatly due to the interactions illustrated in Figure 10-16. [Part a adapted from Bendall, 1969; part c adapted from Lehrer and Morris, 1982.]

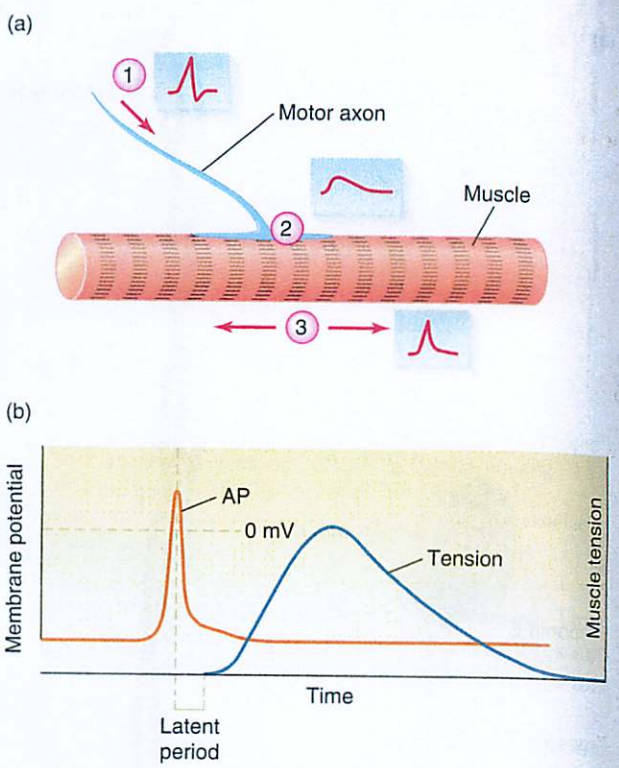


Figure 10-18 Muscle fibers contract when a postsynaptic potential at the neuromuscular junction causes a propagated action potential (AP) in the fiber. **(a)** An AP in a motor neuron (1) causes an excitatory postsynaptic potential in the muscle fiber (2), which gives rise to a propagated muscle AP (3). **(b)** The AP in the muscle fiber (red trace) is followed, after a latent period, by a transient, all-or-none contraction (blue trace) called a twitch.

in this way, they produce a transient contraction, which is called a *contracture* to differentiate it from a normal contraction. In the experiment depicted in Figure 10-19, a single frog muscle fiber was exposed to various concentrations of extracellular K^+ while the membrane potential and muscle tension were monitored. When the membrane was depolarized to about −60 mV, tension began to develop; with further depolarization, tension increased sigmoidally, reaching a maximum at about −25 mV.

This experiment demonstrates that the contractile system can produce graded contraction when the membrane is depolarized to different values. However, a single twitch in response to a single AP is typically an all-or-none event. How can these two observations be reconciled? During an AP in a muscle fiber, the membrane potential swings from a resting value of about −90 mV to an overshoot of about +50 mV. At the peak of the AP, the membrane potential is as much as 75 mV more positive than the potential required to give a maximal contracture. As a result, during an AP, the membrane potential of the muscle fiber exceeds the value at which contraction is fully activated. The twitch is all-or-none because the AP is both large and all-or-none.

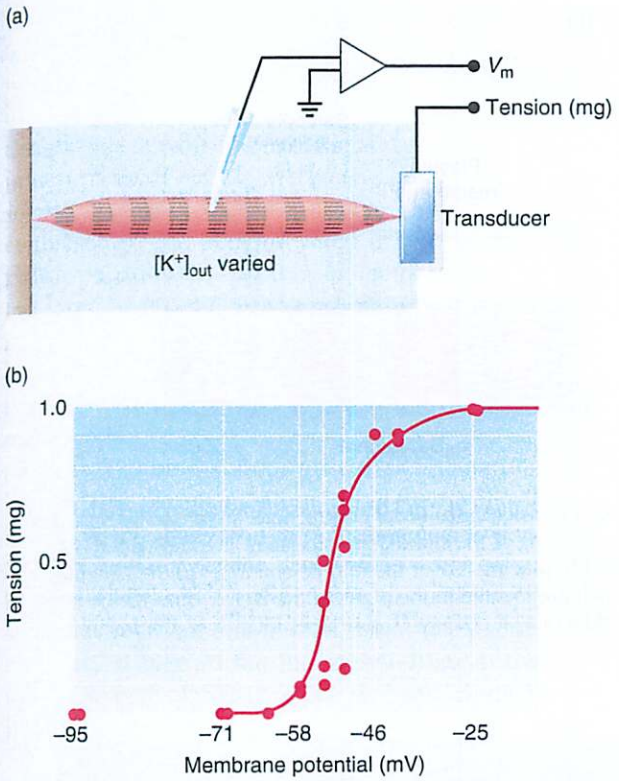


Figure 10-19 The tension developed by a muscle fiber varies with the membrane potential, V_m. **(a)** Setup for measuring the membrane potential of and tension produced by an isolated muscle fiber as the concentration of K^+ is varied in the extracellular solution. **(b)** The tension produced by the muscle fiber as a function of membrane potential. Data points are plotted, and the red curve shows the sigmoidal function that best fits the points. The threshold potential for contraction is about −60 mV. [Adapted from Hodgkin and Horowicz, 1960.]

A potential difference across the plasma membrane of a muscle fiber directly affects an intracellular region that extends at most only a fraction of a micrometer from the inner surface of the membrane. As a result, a potential change across the plasma membrane cannot directly exert any influence on the great bulk of the myofibrils in a typical skeletal muscle fiber, which is 50–100 μm in diameter. There must be something that couples depolarization of the plasma membrane to the activity of myofibrils deep within each muscle fiber. Electrotonic spread of local currents produced by a propagated AP was experimentally ruled out: when currents of physiological magnitude were passed between two microelectrodes inserted into a muscle fiber, they produced no contraction.

The hypothesis that Ca^{2+} might play a role in linking membrane potential and contraction was suggested relatively early. During the 1930s and 1940s, Lewis V. Heilbrunn argued for the importance of calcium in many cellular processes, including muscle contraction. We now know that his hypothesis that the contraction of muscle is controlled by intracellular changes in calcium concentration is essentially correct, although it was widely rejected at first because of a fundamental misunderstanding about the nature of excitation-contraction coupling. It was assumed that calcium would have to enter the cytosol of the muscle fiber (also called the myoplasm) through the plasma membrane to initiate contraction. As A. V. Hill pointed out, the rate of diffusion of an ion or a molecule from the plasma membrane to the center of a muscle fiber that is 25–50 μm in radius is several orders of magnitude too slow to account for the short observed latent period (about 2 ms) between an AP at the plasma membrane and activation of the entire cross-section of the muscle fiber. Using this logic, Hill correctly concluded that a process, rather than a substance, must couple the surface signal to myofibrils that lie deep within the muscle fiber. As we will see, it is the AP itself that is conducted deep into the cell interior, where it causes the release of intracellular Ca^{2+} from internal storage depots that surround the myofibrils. Elevation of the concentration of free Ca^{2+} in the myoplasm permits myosin cross-bridges to attach to the actin thin filaments and generate force.

T tubules

Anatomic and physiological evidence suggesting a link between the plasma membrane and the internal myofibrils came to light about 10 years after Hill's calculation. In 1958, Andrew F. Huxley and Robert E. Taylor studied the details of excitation-contraction coupling by stimulating the outside surface of single frog muscle fibers with tubular glass microelectrodes (Figure 10-20a on the next page). Their most significant findings were the following:

- Pulses of current that were too small to initiate a propagated AP, but sufficient to depolarize the membrane under the pipette opening, led to small local contractions, but only when the tip of the pipette was positioned directly over a Z disk (Figure 10-20b).
- These contractions occurred only around the perimeter of the fiber and very close to the Z disk.
- Contractions spread farther into the fiber as the intensity of the stimulating current was increased.
- Contractions were limited to the two half-sarcomeres immediately on either side of the Z disk over which the electrode was positioned. In other words, contraction occurred only in the sarcomeres lying directly under the stimulated membrane; other sarcomeres attached to the ends of the stimulated sarcomeres remained relaxed.

Electron microscopic studies of amphibian skeletal muscle performed at about the same time provided an anatomic correlate of these physiological findings. Running around the perimeter of each myofibril at the level of the Z disk is a hollow membranous tube called a

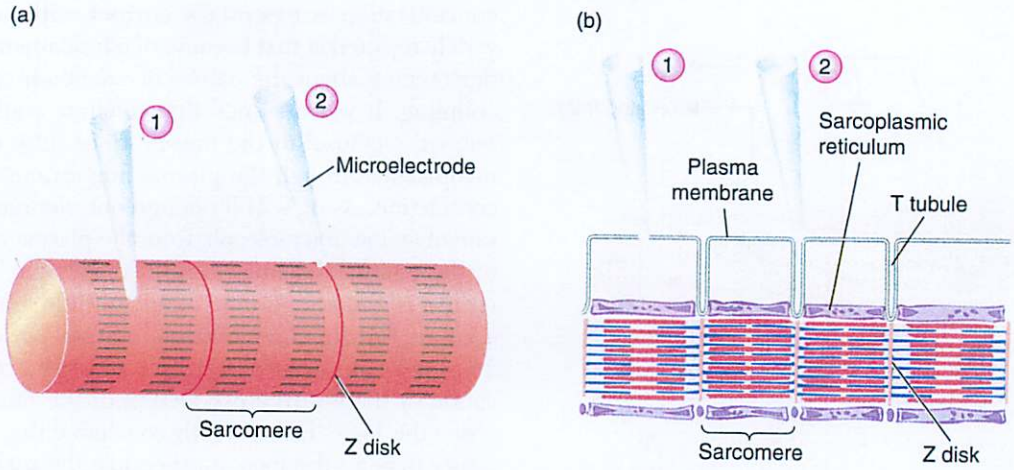


Figure 10-20 When frog muscle fibers are stimulated by an extracellular microelectrode, they can contract, but only when the pipette stimulates the fiber near a Z disk. **(a)** Experimental setup, showing the stimulating pipette positioned either in the center of a sarcomere (1) or directly over a Z disk (2). **(b)** Local contractions are observed only if the opening of the stimulating pipette is lined up with the Z disk (2), placing it over the minute entrances to the T tubules, which are located in the plane of the Z disk. Stimulation at the middle of a sarcomere (1) produces little or no contraction.

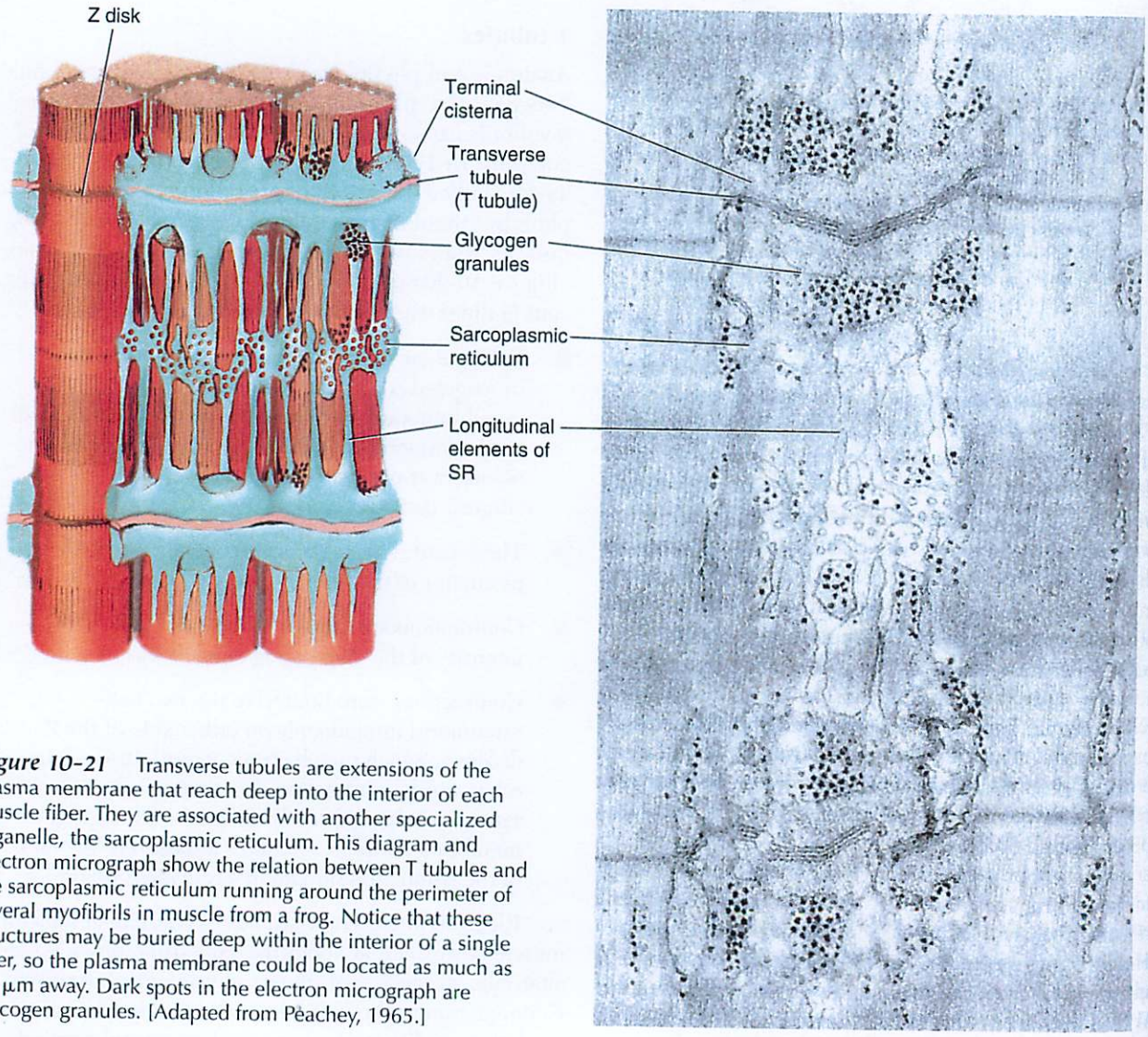


Figure 10-21 Transverse tubules are extensions of the plasma membrane that reach deep into the interior of each muscle fiber. They are associated with another specialized organelle, the sarcoplasmic reticulum. This diagram and electron micrograph show the relation between T tubules and the sarcoplasmic reticulum running around the perimeter of several myofibrils in muscle from a frog. Notice that these structures may be buried deep within the interior of a single fiber, so the plasma membrane could be located as much as 50 μm away. Dark spots in the electron micrograph are glycogen granules. [Adapted from Peachey, 1965.]

transverse tubule (or **T tubule**). T tubules are less than 0.1 μm in diameter and form a network surrounding neighboring myofibrils (Figure 10-21). The membrane of this network of tubules is connected directly to the plasma membrane of the muscle fiber, and the lumen of the T-tubule system is continuous with the solution on the outside of the fiber. This continuity was confirmed by the demonstration that ferritin or horseradish peroxidase—protein molecules that are much too large to cross plasma membranes—nevertheless appear in the lumen of the T tubules if a muscle fiber is soaked in a solution of these molecules before the tissue is fixed and then examined with an electron microscope.

The T-tubule system provides the anatomic link between the plasma membrane and the myofibrils deep inside the muscle fiber. When Huxley and Taylor placed their stimulating pipette at a Z disk, over the entrance to a T tubule (see Figure 10-20), depolarizing current spread along the membrane of the tubule and initiated contraction deep within the muscle fiber. If they produced hyperpolarizing current from their pipette instead, no contraction occurred. Comparative studies have further strengthened the conclusion that T tubules carry excitation into muscle fibers. In crabs and some lizards, the T tubules are located at the ends of the A bands, rather than at the Z disks (Figure 10-22). In these species, contraction is produced when a stimulating pipette is placed at the edge of an A band, rather than over a Z disk. From such results it has been concluded that T tubules, rather than the Z disks or any other part of the sarcomere, are most likely to transmit excitation into muscle fibers.

Further confirmation that T tubules play an important role in excitation-contraction coupling was obtained by osmotically shocking muscle fibers with a 50% glycerol solution, which disconnects the T tubules from the plasma membrane. After this treatment, membrane depolarization no longer evokes a contraction; that is, physically uncoupling the T-tubule system from the plasma membrane functionally uncouples the contractile system from action potentials propagating along the plasma membrane.

The spread of excitation to the center of a muscle fiber is reduced if tetrodotoxin is added to the bath or if the concentration of Na^+ in the extracellular fluid is reduced. Either treatment reduces or eliminates sodium-based APs, suggesting that the APs that are characteristic of the plasma membrane are actively carried deep into the muscle fiber by the membranes of the T tubules.

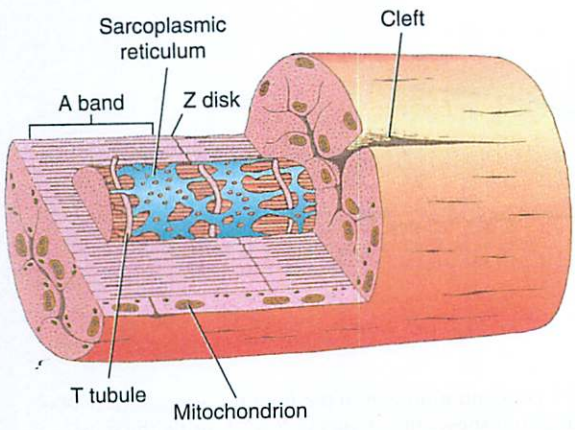


Figure 10-22 In crab muscle fibers, the T tubules are located at the A bands, rather than at the Z disks as in frog fibers. Stimulation with an extracellular micropipette produces a local contraction in crab fibers only when the tip of the pipette is placed over an A band. Compared with frog muscle fibers, crab fibers have a larger diameter and contain deep clefts. [Adapted from Ashley, 1971.]

Sarcoplasmic reticulum

In addition to the T-tubule system, striated muscle fibers contain a second intracellular membrane system, the **sarcoplasmic reticulum** (or **SR**). In frog muscle, the SR forms a hollow collar, called the terminal cisterna, around each myofibril on either side of a Z disk, and extends from one Z disk to the next as well (see Figure 10-21). The T tubules are sandwiched between the terminal cisternae of adjacent sarcomeres, but do not contact them directly.

Several different experimental approaches have demonstrated that the sarcoplasmic reticulum actively transports Ca^{2+} from the surrounding medium and concentrates it. When an AP is conducted along the membrane of a T tubule, it causes the neighboring SR to release stored Ca^{2+} ions into the cytoplasm by a mechanism that will be discussed shortly. The released Ca^{2+} ions are then available to bind to troponin, beginning the contraction process that was described in the previous section.

The calcium-sequestering activity of the sarcoplasmic reticulum is sufficiently powerful to keep the concentration of free Ca^{2+} in the myoplasm of resting muscle fibers below 10^{-7} M—low enough to remove essentially all Ca^{2+} from troponin in the cytoplasm. In other words, the SR is capable of driving the concentration of intracellular free Ca^{2+} so low that contraction is prevented. This ability of the SR to remove Ca^{2+} from the myoplasm depends on the activity of Ca^{2+}/Mg^{2+} ATPases, or calcium pumps, proteins in the SR membrane that bind and transport Ca^{2+} ions. In freeze-fracture electron micrographs, many densely packed inclusions can be seen in the membrane of the longitudinal elements of the SR; these inclusions have been associated with calcium pump molecules. The calcium pump, like other active transport molecule, requires ATP as its energy source—another key role for ATP in muscle contraction.

Under resting conditions in the sarcoplasmic reticulum, Ca^{2+} is bound to a protein called **calsequestrin**.

As a result, even though the total amount of Ca^{2+} within the SR is high, the concentration of *free* Ca^{2+} remains relatively low, reducing the gradient against which the pumps must work.

Combining observations of Ca^{2+} uptake and release by the sarcoplasmic reticulum with the role Ca^{2+} was known to play in the interaction between thin and thick filaments strongly suggested to physiologists that muscle contraction is initiated when the SR releases Ca^{2+} into the myoplasm. The first direct evidence that the concentration of free Ca^{2+} in muscle fibers rises in response to electrical stimulation came from a photometric method using the calcium-sensitive bioluminescent protein aequorin, isolated from a species of jellyfish (see Figure 6-27). The light-emitting reaction of aequorin is complex, however, and its response to changes in the concentration of free Ca^{2+} is rather slow. In more recent experiments, dyes have been used that respond to changes in Ca^{2+} concentration with rapid changes in their fluorescent properties. The dye furaptra, for example, fluoresces in the absence of Ca^{2+}; that is, it emits light of a particular wavelength when it is illuminated with exciting light of a different wavelength. The intensity of furaptra's fluorescence decreases as the Ca^{2+} concentration increases, so furaptra can be used to monitor changes in Ca^{2+} concentration within muscle fibers. When a furaptra-loaded muscle fiber is stimulated with a brief electric shock, the fluorescence of the dye first declines and then returns to its initial value (Figure 10-23). This result indicates that when the muscle is electrically stimulated, the amount of free Ca^{2+} in the myoplasm transiently increases. A very small fraction of the newly released Ca^{2+} binds to the furaptra, and the fluorescence of the dye declines. Most of the Ca^{2+} released is free to bind to troponin. As the released Ca^{2+} is resequestered, Ca^{2+} dissociates from the dye, and the dye's fluorescence rises again.

All of this evidence indicates that contraction is activated when Ca^{2+} ions are released from the sarcoplasmic reticulum, and that this release is linked to APs that are initiated at the plasma membrane and transmitted into the depths of the muscle fiber along the T tubules. Notice that in vertebrate skeletal muscle, essentially

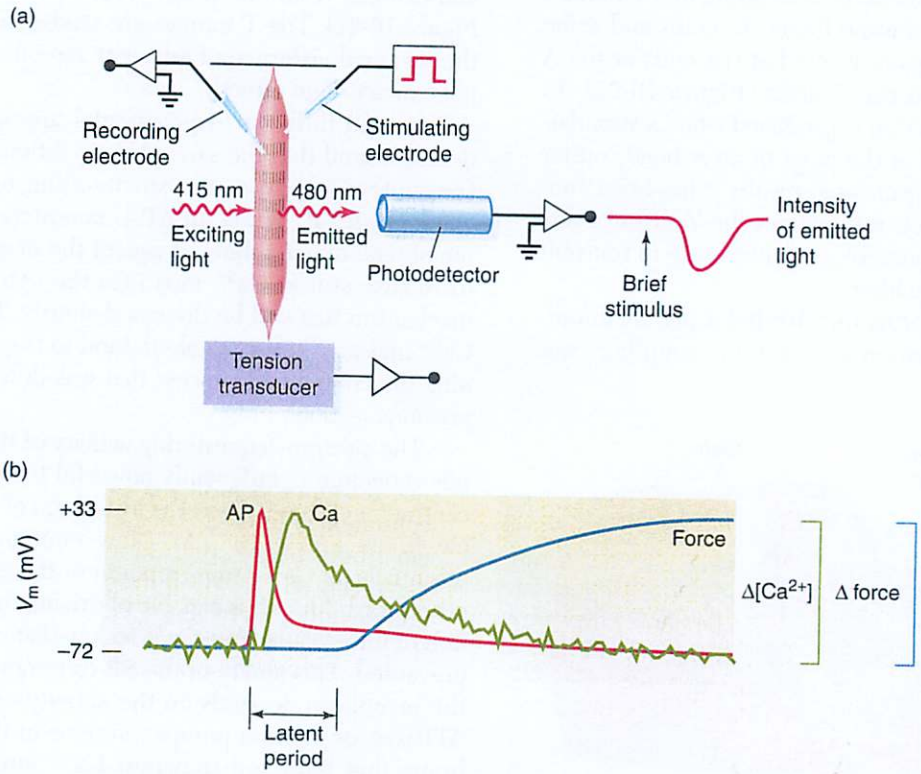


Figure 10-23 The concentration of free Ca^{2+} in a muscle fiber can be measured using a calcium-sensitive fluorescent dye such as furaptra. **(a)** In this experimental setup, a muscle fiber injected with furaptra is electrically stimulated and the subsequent changes in fluorescence, the membrane potential, and the production of tension by the fiber are recorded. **(b)** When the muscle fiber is stimulated, an AP propagates along the plasma membrane and is recorded by the recording microelectrode. A short time later, the fluorescence signal from the calcium-sensitive dye inside the fiber indicates that the Ca^{2+} concentration within the fiber has increased. (Notice that this graph shows the changing $[Ca^{2+}]$ inside the muscle fiber, rather than the fluorescence of the furaptra, although the concentration was inferred from the dye's fluorescence. The fluorescence of furaptra varies inversely with $[Ca^{2+}]$.) Even later, the tension transducer measures the production of tension by the fiber. Notice that the tension begins to rise only after the AP is over and the intracellular Ca^{2+} concentration has already begun to decline. [Part b courtesy of S. M. Baylor.]

none of the regulatory Ca^{2+} enters the cell across the plasma membrane; *the only source of the regulatory calcium is the SR.* The anatomy of the T tubules and SR suggests how this coupling happens. As noted above, each T tubule is located in close apposition to the terminal cisternae of the SR (see Figure 10-21). In fact, histologists have for decades called this portion of a muscle fiber the *triad* because sections through this region consistently revealed three associated tubes or sacks. Two of the sacks are always relatively large, and they are located on either side of a much smaller central tube or sack. We now know that the two large sacks are terminal cisternae of the SR and the smaller central sack is a T tubule. As we will see, the triads play a crucial role in linking a muscle AP to a change in myoplasmic free Ca^{2+}.

Receptor molecules in triads

In 1970, electron microscopy experiments by Clara Franzini-Armstrong revealed electron-dense particles in the part of the SR membrane that lies adjacent to the T tubule (Figure 10-24a on the next page). She called these structures "feet." More recently, these feet have been identified as the cytoplasmic portion of membrane-associated protein complexes, called **ryanodine receptors** because they bind the drug ryanodine. Ryanodine receptors are tetrameric proteins that span the SR membrane (Figure 10-24b). Half of them are lined up with proteins in the T-tubule membrane, called **dihydropyridine receptors** for their ability to bind dihydropyridine drugs (Figure 10-24c). The dihydropyridine receptors have been more precisely identified as a type of voltage-gated calcium channel, called an L-type channel, which explains how they can respond to APs traveling along the membrane of T tubules. It is noteworthy that in skeletal muscle, little or no Ca^{2+} passes from the T-tubule lumen to the myoplasm through these channels, but in vertebrate contractile cardiac muscle, Ca^{2+} entering activated fibers through dihydropyridine receptor channels plays an important role, as we will see. In skeletal muscle it is a *mechanical* interaction between activated dihydropyridine receptors and ryanodine receptors that permits Ca^{2+} to move from the lumen of the sarcoplasmic reticulum into the myoplasm. Figure 10-24d summarizes the important molecular players in the Ca^{2+} economy of the SR.

Shortly after Franzini-Armstrong described the feet located between the T tubule and SR membranes, Knox Chandler and his colleagues suggested that these proteins are Ca^{2+} channels and incorporated them into a "plunger model" for release of Ca^{2+} from the sarcoplasmic reticulum (Figure 10-25 on page 389). They suggested that depolarization of the T tubule causes a plug to be removed from Ca^{2+} channels in the SR membrane, allowing Ca^{2+} to escape into the myoplasm, driven down its steep electrochemical gradient. When the T-tubule membrane repolarizes, the plug is replaced, preventing further Ca^{2+} release. The current version of the plunger model proposes that when the T tubule depolarizes, a change in the conformation of a voltage-sensitive dihydropyridine receptor forces the calcium channel of an associated ryanodine receptor to open, allowing Ca^{2+} to rush into the myoplasm from the lumen of the SR.

Interestingly, only about half of the ryanodine receptors in the sarcoplasmic reticulum are associated directly with dihydropyridine receptors in the T-tubule membrane. The other, independent ryanodine receptors are activated by the increase in myoplasmic free Ca^{2+} following opening of the mechanically linked channels, a process called *calcium-induced calcium release.* Activation of these unlinked ryanodine receptors in turn opens more Ca^{2+} channels in the membrane of the sarcoplasmic reticulum. Calcium-induced calcium release has been found in many cell types, and it plays an important role in excitation-contraction coupling in cardiac muscle.

Time course of calcium release and reuptake

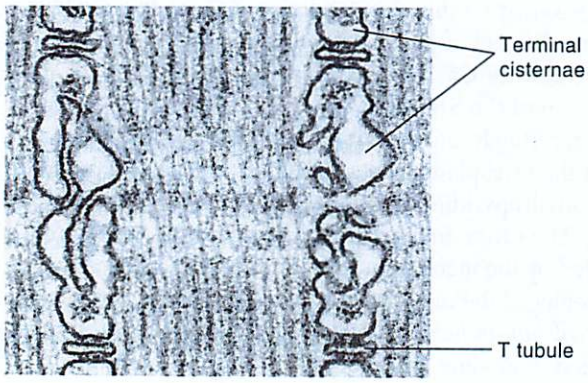
The ability to measure rapid changes in the myoplasmic Ca^{2+} concentration, combined with knowledge of calcium binding by troponin and the kinetics of the calcium pumps in the SR membrane, has permitted the Ca^{2+} fluxes during muscle contraction and relaxation to be modeled. The results suggest that when T tubules become depolarized, Ca^{2+} flows out of the sarcoplasmic reticulum for several milliseconds, then the Ca^{2+} channels close. Most of the Ca^{2+} that leaves the sarcoplasmic reticulum binds very quickly to troponin. The concentration of troponin in muscle fibers is about 240 μM, which represents a large buffer for Ca^{2+} ions. Thus, only a very small amount of the released Ca^{2+} remains free in the myoplasm. During and after the release of Ca^{2+} from the SR, the free Ca^{2+} in the myoplasm is pumped back into the SR lumen, lowering the myoplasmic level of free Ca^{2+}. As the concentration of free Ca^{2+} in the myoplasm becomes very low, Ca^{2+} bound to troponin is released back into the myoplasm and is subsequently pumped back into the SR, where it binds to calsequestrin.

The Contraction-Relaxation Cycle

Starting with a relaxed skeletal muscle fiber, let's summarize the sequence of events that leads to contraction and then relaxation.

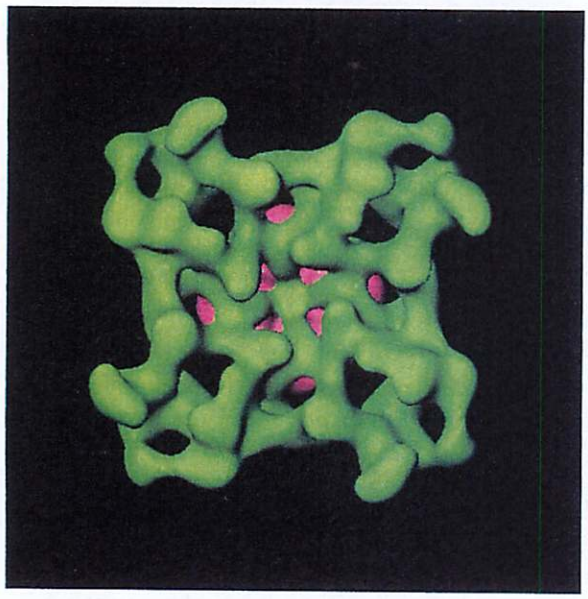
1. The plasma membrane of the fiber is depolarized by an AP or, in some muscles, by electrotonically conducted postsynaptic potentials. In the body of an animal, APs in skeletal muscle fibers are generated by postsynaptic potentials, so neuronal input is required for initiating contraction in skeletal muscle.
2. The AP is conducted deep into the muscle fiber along the T tubules.
3. In response to depolarization of the T-tubule membrane, dihydropyridine receptors in the membrane

(a)

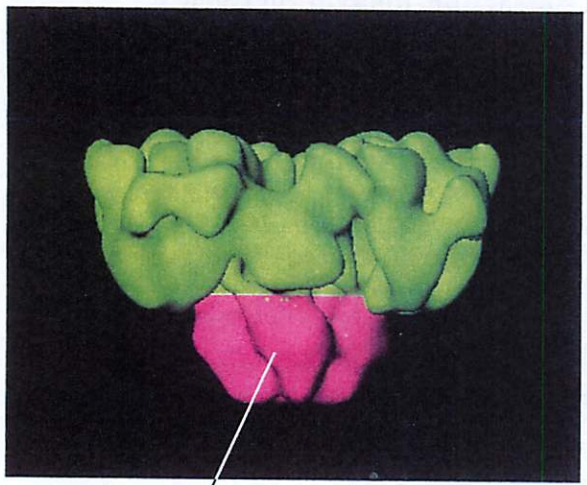


(b) Ryanodine receptor

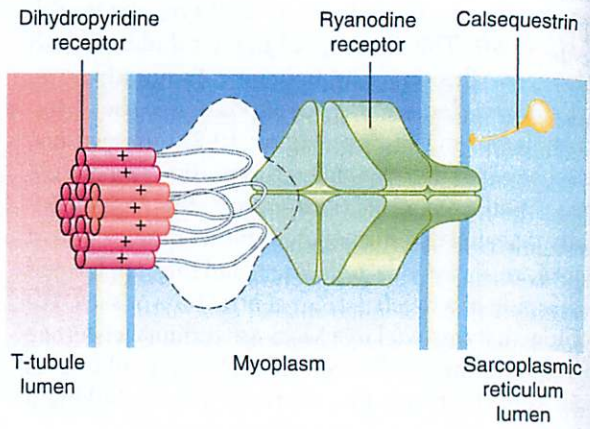
Viewed from outside SR



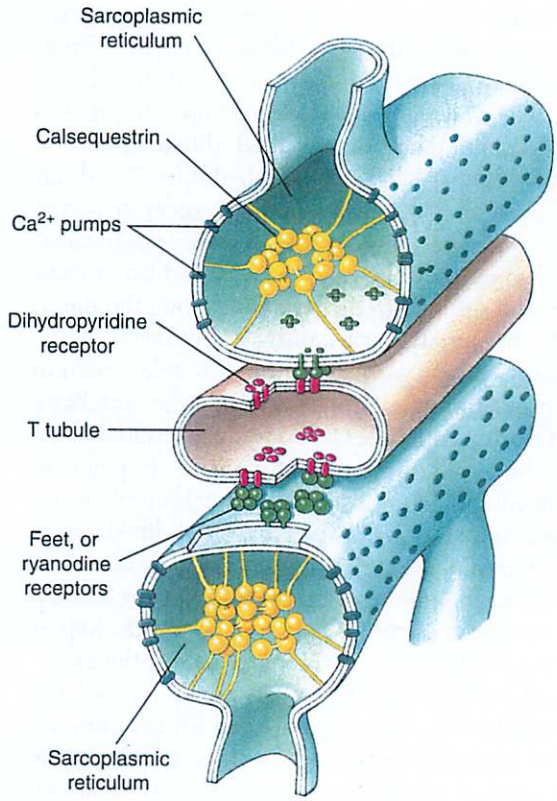
Viewed from side



(c)



(d)



undergo a conformational change that—through direct mechanical linkage to ryanodine receptors in the SR membrane—causes the opening of Ca^{2+} channels in the SR membrane (see Figure 10-25, step 2).

4. As Ca^{2+} flows from the lumen of the sarcoplasmic reticulum into the myoplasm, the free Ca^{2+} concentration of the myoplasm increases within a few milliseconds from a resting value of below 10^{-7} M to an active level of about 10^{-6} M or higher. The Ca^{2+} channels in the SR membrane then close because V_m of the T tubules has returned to V_{rest}.

5. Most of the Ca^{2+} that enters the myoplasm binds rapidly to troponin, inducing a conformational change in the troponin molecules. This conformational change causes a change in the position of the associated tropomyosin molecule, allowing myosin cross-bridges to bind to actin thin filaments (see Figure 10-16c).

6. Myosin cross-bridges attach to the actin filaments and go through a series of binding steps that cause the myosin head to rotate against the actin filaments, pulling on the cross-bridge link (see Figure 10-11). This pulling produces force on, and in some cases sliding of, the thin filaments that is directed toward the center of the sarcomere, causing the sarcomere to shorten by a small amount (see Figure 10-8a).

7. ATP binds to the ATPase site on the myosin head, causing the myosin head to detach from the thin filament. ATP is then hydrolyzed, and the energy of hydrolysis is stored as a "recocking" of the myosin head, which then attaches to the next site along the actin filament as long as binding sites are still available, and the cycle of binding and unbinding is repeated (see Figure 10-10). During a single contraction, each cross-bridge attaches, pulls, and detaches many times as it "rows" itself along the actin filament toward the Z disk.

8. Finally, calcium pumps in the SR membrane actively transport Ca^{2+} from the myoplasm back into the SR lumen (see Figure 10-25, step 3). As the concentration of free Ca^{2+} in the myoplasm drops, Ca^{2+} bound to troponin is released, allowing tropomyosin again to prevent cross-bridge attachment, and as long as ATP is present, the muscle relaxes. The muscle remains relaxed until the next depolarization.

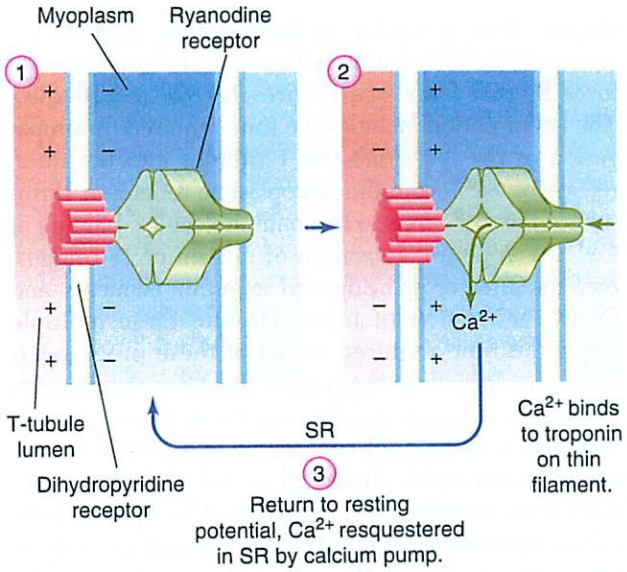


Figure 10-25 Depolarization of the T-tubule membrane indirectly causes calcium channels in the sarcoplasmic reticulum to open. When the membrane of the T tubule is at rest (1), the calcium-conducting channels of ryanodine receptors are closed. When the T-tubule membrane depolarizes (2), dihydropyridine receptors convey the signal to the ryanodine receptors, and their calcium channels open, allowing Ca^{2+} to flow out of the SR lumen into the myoplasm. The free Ca^{2+} binds to troponin, exposing cross-bridge binding sites on actin molecules. When the membrane potential returns to its resting value (3), the ryanodine receptor calcium channels close. Calcium pumps in the SR resequester Ca^{2+}, shifting the equilibrium of Ca^{2+} binding to troponin and causing the cross-bridge binding sites on actin to be concealed. [Adapted from Berridge, 1993.]

Figure 10-24 (*on facing page*) Dihydropyridine receptors in the T-tubule membrane and ryanodine receptors in the SR membrane interact at triads. **(a)** Near the Z disks of mammalian and amphibian skeletal muscle, T tubules and the terminal cisternae of two sarcomeres form triads. Darkly stained "feet" connect the terminal cisternae with the T tubule in each triad. **(b)** The molecular structure of a ryanodine receptor reveals fourfold symmetry, suggesting that it is a tetramer. The boundaries of the monomers have not yet been resolved. In this diagram, the feet would be some part of the green region of the complex, and the Ca^{2+}-conducting pore is colored pink. **(c)** Dihydropyridine receptors span the T-tubule membrane and are organized in clusters of four. Each dihydropyridine receptor associates closely with the extracellular "foot" of a ryanodine receptor, which extends from the SR membrane. The molecular structure of each unit of the dihydropyridine receptor is similar to that of the voltage-gated Ca^{2+} channel illustrated in Figure 5-27. Calsequestrin inside the SR binds Ca^{2+}. **(d)** In an intact triad, several molecules contribute to the control of the myoplasmic Ca^{2+} concentration. The voltage-sensitive dihydropyridine receptors and the ryanodine receptors work together, linking depolarization of the T tubule to the opening of calcium channels in the SR membrane. A calcium ATPase (calcium pump) in the SR membrane resequesters Ca^{2+} from the myoplasm, and calsequestrin inside the SR binds Ca^{2+}, reducing the concentration of ionic free Ca^{2+} inside the SR. [Part a courtesy of Clara Franzini-Armstrong; part b adapted from Samsó and Wagenknecht, 1998; part c adapted from Lamb, 2000; part d adapted from Block et al., 1988.]

THE TRANSIENT PRODUCTION OF FORCE

Up to now, we have considered the mechanics of muscle fibers during a single twitch. As we have seen

already, there is a delay, or *latent period,* between the action potential in a muscle fiber and the generation of force by that fiber (see Figures 10-18b and 10-23b). The latent period includes the time required for propagation of the AP along the T tubules into the fiber, release of Ca^{2+} from the sarcoplasmic reticulum, diffusion of the Ca^{2+} ions to troponin molecules, binding of Ca^{2+} to troponin, activation of myosin cross-bridges, binding of the cross-bridges to actin thin filaments, and finally, generation of force. Despite these multiple steps, the time required for all of these processes is short: the delay from the peak of the AP to the first sign of tension in some muscles can be as short as 2 ms. Although a single brief contraction in a muscle fiber is complex and rapid, almost all useful muscle contractions in an animal's body are produced by repeated individual contractions that take place simultaneously in many muscle fibers. We now will consider how the activities of individual fibers and individual twitches are coordinated to do useful work.

Series Elastic Components of Muscle

A muscle can be modeled as a contractile element that is arranged in parallel with one elastic component and in series with another elastic component, as depicted in Figure 10-26a. The *parallel elastic component* in this model represents the properties of the plasma membrane of the muscle fibers and the connective tissues that run in parallel with them. The *series elastic component,* also called the *series elastic elements,* represents tendons, connective tissues that link muscle fibers to the tendons, and perhaps the Z disks of the sarcomeres. An additional important constituent of the series elastic elements appears to be the myosin cross-bridge links themselves, which undergo some stretch when tension is generated (see Figure 10-11). Representing all of the elastic components of a muscle with only these two components greatly simplifies the model, making it easier to manipulate mathematically while maintaining sufficient accuracy to increase our understanding of the mechanics of muscle contraction.

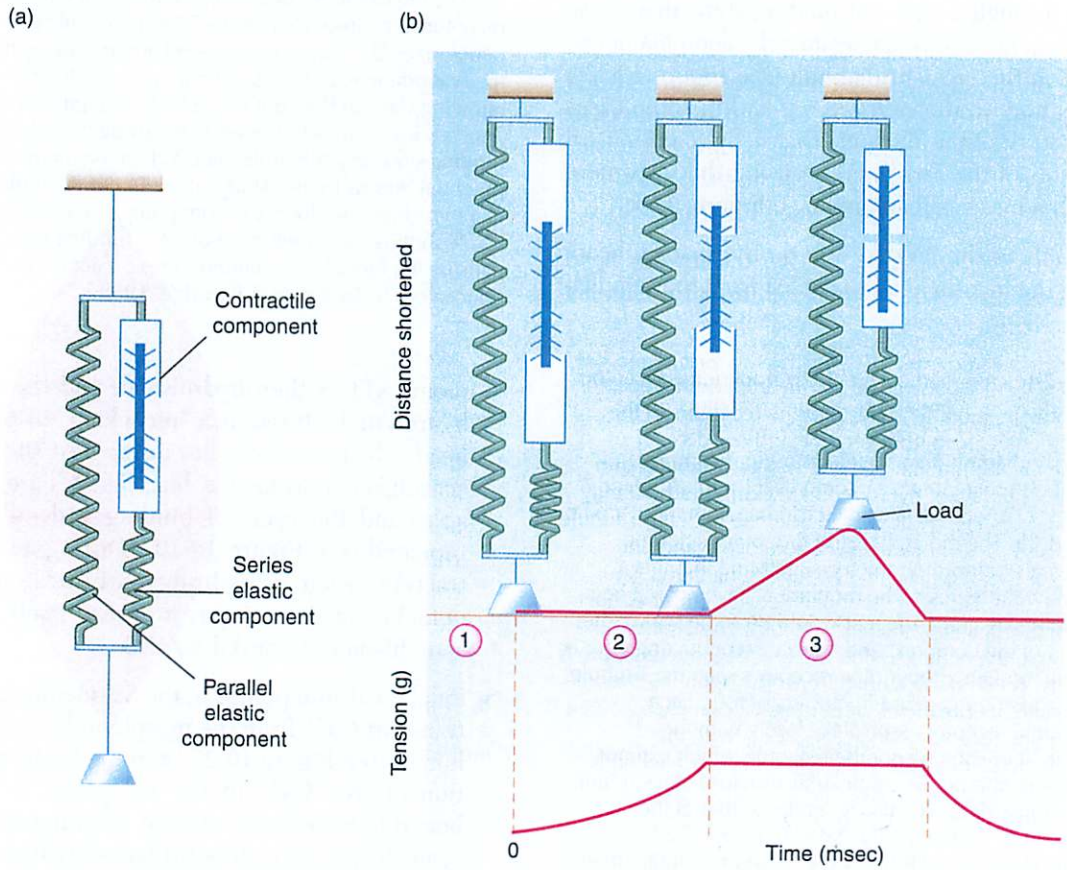


Figure 10-26 A muscle fiber, or an entire muscle, can be represented by a mechanical model that includes a contractile component and elastic components. **(a)** Mechanical model of a muscle consisting of a contractile component (the sarcomeres), in series with one elastic component (e.g., tendons) and in parallel with another elastic component (e.g., connective tissue layers within the muscle). **(b)** Effect of series elastic components on muscle contraction. At the beginning of contraction in this model muscle, the load rests on a surface (1). As the thick and thin filaments begin to slide past each other and tension increases, the series elastic components are stretched (1 → 2), but the length of the muscle has not yet changed; contraction up to this point (2) is isometric. Once the muscle generates tension that is equal to or greater than the weight of the load, the load is lifted and the contraction becomes isotonic (3). Note that as contraction progresses, the thick and thin filaments overlap increasingly and more cross-bridges can bind. [Adapted from Vander et al., 1975.]

As a muscle becomes activated and the contractile component begins to shorten, the series elastic component must be stretched before full tension can be transmitted to the external load (steps 1 and 2 in Figure 10-26b). When the tension developed equals the weight of the load, the muscle begins to lift the load off of the surface (step 3). In steps 1 and 2, the contraction is essentially isometric, whereas in going from step 2 to 3 it becomes more isotonic as the load is finally lifted. If the load were sufficiently heavy that the muscle never produced tension equal to its weight, the contraction would remain isometric throughout. (At maximal tension during an "isometric" contraction, a small amount of shortening in the contractile component stretches the series elastic component by an amount equivalent to about 1% of the muscle length, even though the external length of the muscle does not change.)

Time is required for the thin and thick filaments to slide past each other and stretch the series elastic component as tension builds up. Thus the series elastic component slows the development of tension in the muscle and smooths out abrupt changes in tension.

The Active State

During contraction, external shortening of the muscle fiber and production of tension reach a maximum within 10 to 500 milliseconds, depending on the kind of muscle, the temperature, and the load. At first glance, this statement might suggest that the contractile mechanism is activated with a similar slowly rising time course. It is important, however, not to confuse the time it takes a muscle to develop tension with the time course of cross-bridge activity. Cross-bridges become activated and attach to thin filaments before the filaments begin to slide past each other. Then once the filaments begin to slide, they must take up the slack in the series elastic component before tension can be fully developed.

The state of the cross-bridges after activation, but before the muscle has had a chance to develop full tension, can be determined by the application of quick stretches with a special apparatus. These stretches can be applied at various times after stimulation, both before and during contraction. The rationale for quick-stretch experiments is that the series elastic elements are stretched by the apparatus when it applies force, eliminating the time that is normally required for the contractile mechanism to take up this slack. This maneuver thus improves the time resolution when measuring the state of cross-bridge activity. The "internal" tension recorded by the sensing device during a quick stretch represents the tensile strength of the bonds between the thick and thin filaments, which depends on the holding strength of the cross-bridges at the instant of the stretch. If the stretch applied is stronger than the holding strength of the cross-bridge bonds, the cross-bridges will slip, and the filaments will slide past each other in a negative direction. Thus, loading during a quick stretch that is just sufficient to make the thick and thin filaments slide apart approximates the load-carrying capacity of the muscle at the time of stretch. This tension should be proportional to the average number of active cross-bridges per sarcomere.

In the relaxed state, a muscle has very little resistance to stretch aside from the contribution made by connective tissue, the plasma membranes, and other elastic components. Quick-stretch experiments revealed that a muscle's resistance to stretch rises steeply very soon after stimulation and reaches a maximum at about the time when external shortening or tension in an unstretched muscle is just getting under way. After a brief plateau, the resistance to stretch returns to the low level characteristic of the relaxed muscle.

The term **active state** is used to describe this state of increased stretch resistance, or internal tension, in a muscle following a brief stimulation (Figure 10-27a on the next page). The active state corresponds to the formation of bonds between myosin cross-bridges and actin thin filaments and to the subsequent slight internal shortening generated by the cross-bridges. Because cross-bridge activity is controlled by the concentration of free Ca^{2+} in the myoplasm, the time course of the active state is believed to approximately parallel changes in myoplasmic Ca^{2+} concentration following stimulation. The brief increase in tension due to cross-bridge activity is called a **twitch.**

If stimulation of a muscle is prolonged, the active state persists. A prolonged active state produced by a barrage of high-frequency APs is called **tetanus.** In this state, the measurable external isometric tension can increase until it reaches the value of internal tension during the active state as measured by quick-stretch experiments (Figure 10-27b).

Contractile States: Twitches and Tetanus

The graphs in Figure 10-27 raise a question: Why is the maximal external isometric tension produced by the muscle during a twitch so much lower than the internal tension associated with the active state, or than the tension produced during tetanus? In other words, during a brief contraction, why does the muscle produce so much less tension than it is actually capable of producing?

During a single twitch, the active state is rapidly terminated by the calcium-sequestering activity of the sarcoplasmic reticulum, which efficiently removes Ca^{2+} from the myoplasm soon after it is released. Thus, the active state begins to decay even before the filaments have had time to slide far enough to stretch the series elastic component to a fully developed tension. For this reason, the tension of which the contractile system is capable cannot be realized in a single twitch.

Before the peak of the twitch tension, the contractile elements store potential energy in the series elastic component by progressively stretching it. If a second

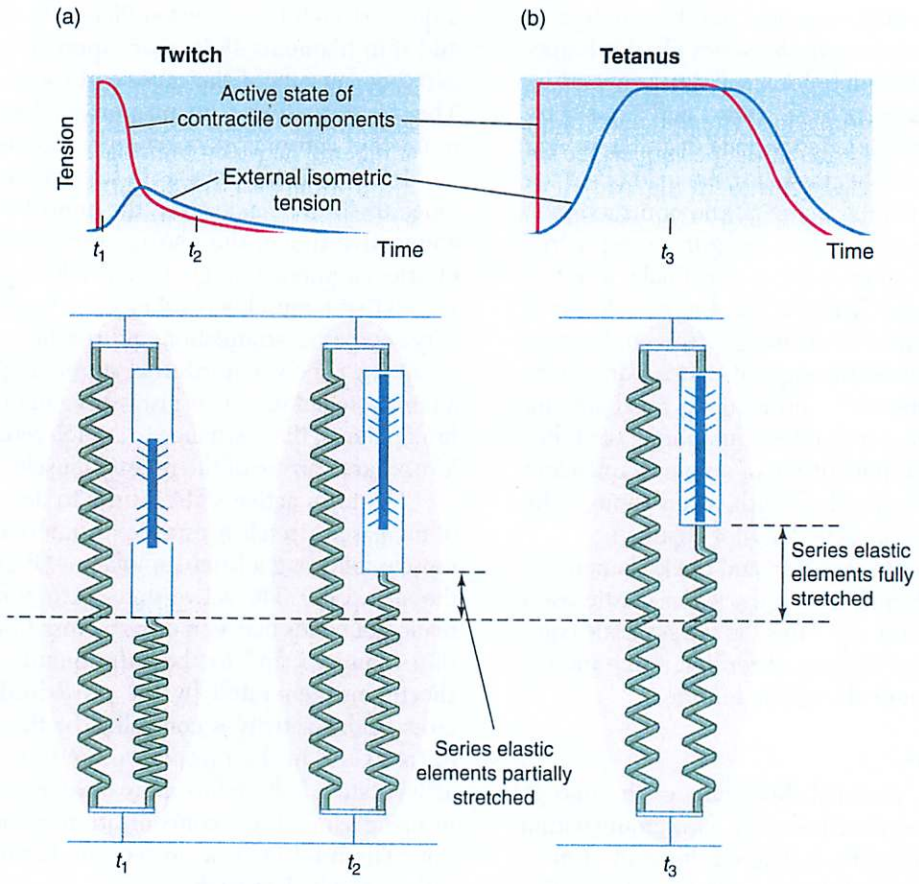


Figure 10-27 The time course of the active state differs from the time course of tension production. **(a)** The active state—as measured in quick-stretch experiments—develops rapidly in response to a short stimulus. This brief response is called a twitch. Measurable external isometric tension develops considerably more slowly, and it fails to reach the same tension that can be measured during a quick stretch because without the quick stretch, the muscle must first do work to take up slack in the series elastic elements. **(b)** In response to a prolonged stimulus, a tetanic contraction develops. In this case, the external isometric tension has time to reach the same value as the internal tension that is measured during quick-stretch experiments. The time period shown in part a is much shorter than the time period shown in part b. [Adapted from Vander et al., 1975.]

AP follows the first before the sarcoplasmic reticulum can entirely remove the previously released Ca^{2+} from the myoplasm, the concentration of Ca^{2+} remains high in the myoplasm, and the active state is prolonged. If the active state continues long enough, the isometric tension increases over time until the tension produced by the internal shortening of the contractile components and the stretching of the series elastic component is just sufficient to cause cross-bridges to slip and prevent further shortening of the contractile components. The muscle has then reached full tetanus.

Depending on the repetition rate of muscle APs, the amount by which individual twitches fuse can vary, reaching a maximal value in tetanus (Figure 10-28). The addition of tension due to repeated rapid stimulation is called **summation of contraction.** Notice that summation depends partly on the release of free Ca^{2+} into the cytoplasm faster than the pumps in the SR membrane can remove it and partly on the inability of the series elastic component to relax back to its slack condition between stimuli.

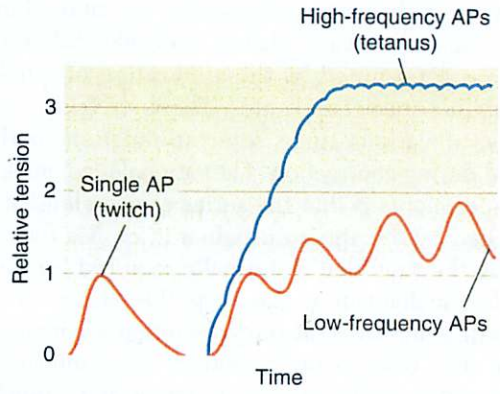


Figure 10-28 Twitches fuse when stimulating APs arrive in rapid succession, generating summed tension. A single AP produces a single twitch. If a set of relatively low-frequency APs is conducted along the muscle fiber, each successive twitch begins before the muscle has time to return to its fully relaxed condition following the previous twitch, so tension builds up moderately. At a maximum frequency of APs, the twitches fuse with one another, producing a long, strong contraction called tetanus.

ENERGETICS OF MUSCLE CONTRACTION

In muscle contraction, two major processes require the expenditure of energy. The most obvious is the hydrolysis of ATP by myosin cross-bridges as they cyclically attach to and detach from actin thin filaments (see Figure 10-10). The other process that consumes ATP is the pumping of Ca^{2+} back into the sarcoplasmic reticulum against a Ca^{2+} concentration gradient (see Figure 10-25, step 3).

Biochemical studies have determined that two molecules of ATP are required to pump each Ca^{2+} ion into the sarcoplasmic reticulum. During a twitch, some amount of Ca^{2+} is released during the few milliseconds following the AP, and exactly that amount of Ca^{2+} must eventually be pumped back into the sarcoplasmic reticulum if the muscle fiber is to relax. During summation (just as in a twitch), the calcium pumps immediately start to resequester the Ca^{2+} released during the first AP, but they do not have time to remove all of it from the myoplasm before the next AP occurs, and each successive AP causes more Ca^{2+} to be released. The buildup of Ca^{2+} in the myoplasm keeps troponin saturated with Ca^{2+} until the APs cease; at that point, the calcium pumps eventually can return all of the released Ca^{2+} to the SR. To maintain the condition of tetanus, ATP is steadily hydrolyzed by both the myosin ATPase and the calcium pumps. Then further ATP is used by the pumps to return the muscle to its relaxed state.

ATP Consumption by the Myosin ATPase and Calcium Pumps

The relative consumption of ATP by myosin ATPase and the calcium pumps was determined in experiments with tetanized frog muscle. In these experiments, muscles were stretched to different degrees, producing different amounts of overlap between thick and thin filaments. As the muscle is progressively stretched, fewer cross-bridges can interact with actin, reducing both the amount of force that can be produced and the amount of ATP hydrolyzed by the myosin ATPase. (Remember that myosin can hydrolyze ATP on its own, but when myosin is not bound to actin, the hydrolysis products ADP and P_i are released very slowly. Therefore, if myosin cross-bridges have few available sites where they can bind to actin, their ATPase activity will be low.) By contrast, stretching the muscle should have little or no effect on the rate at which Ca^{2+} is released from and resequestered by the sarcoplasmic reticulum, because these processes are mediated by membrane proteins whose activity is unrelated to the amount of myofilament overlap. Thus, as the muscle is increasingly stretched, the total ATPase activity declines. At the length where the myofilaments no longer overlap, any ATPase activity can be attributed entirely to the calcium pumps.

Using this experimental approach, researchers determined that the calcium pumps accounted for about 25%–30% of total ATPase activity during muscle contraction. It is generally assumed that this percentage is the same for all muscles; that is, we believe that muscles with a higher maximal contraction velocity, and hence higher myosin ATPase activity, also have faster calcium pumps in their sarcoplasmic reticulum. It is possible, however, that in the very fast sound-producing muscles discussed later in this chapter, pumping of Ca^{2+} may account for a larger fraction of overall energy usage.

Regeneration of ATP During Muscle Activity

As the previous discussion indicates, muscles use exclusively ATP to power their contraction. Yet early measurements of overall ATP usage during muscle contraction produced a surprising result: the ATP concentrations in stimulated and unstimulated muscles (matched as closely as possible for other variables) were nearly identical. For many years, this finding caused some muscle physiologists to hypothesize that muscles use some molecule other than ATP to power their contractions. However, an alternative explanation turned out to be correct. In addition to ATP, muscle fibers contain a second high-energy molecule: creatine phosphate, also known as phosphocreatine (see Figure 3-36). Within muscle fibers, the enzyme creatine phosphokinase transfers a high-energy phosphate from creatine phosphate to ADP, regenerating ATP so quickly that the ATP concentration remains constant, even when the muscle is using energy at a high rate. Because of this reaction, accurately measuring the amount of ATP hydrolyzed by the muscle is best done by measuring either the drop in the concentration of creatine phosphate or the rise in the concentration of P_i.

Beyond the technical issue of accurately measuring the rate of ATP hydrolysis, the creatine phosphokinase reaction is extremely important for effective muscle function. If a muscle runs out of ATP, it goes into rigor (see Figure 10-17b). It is therefore essential that the concentration of ATP in muscles be buffered. Under most circumstances, oxidative or anaerobic metabolism can generate ATP fast enough to power muscle contraction. However, during high-intensity, short-duration activity (e.g., when an animal sprints to run down prey or to avoid becoming prey), ATP may be used up too fast to be replenished by these mechanisms. Under these circumstances, continuous rephosphorylation of ADP by the creatine phosphokinase reaction can keep the muscle supplied with ATP for a short time (Figure 10-29 on the next page). The concentration of creatine phosphate in muscle fibers (20–40 mM) is several times greater than the reserve of ATP (about 5 mM in muscle fibers), so an animal can use this reserve of high-energy phosphate to power muscle contraction under sudden load conditions until anaerobic and oxidative metabolism can catch up to the increased need. An animal's life may depend on this short-lived extra source of energy.

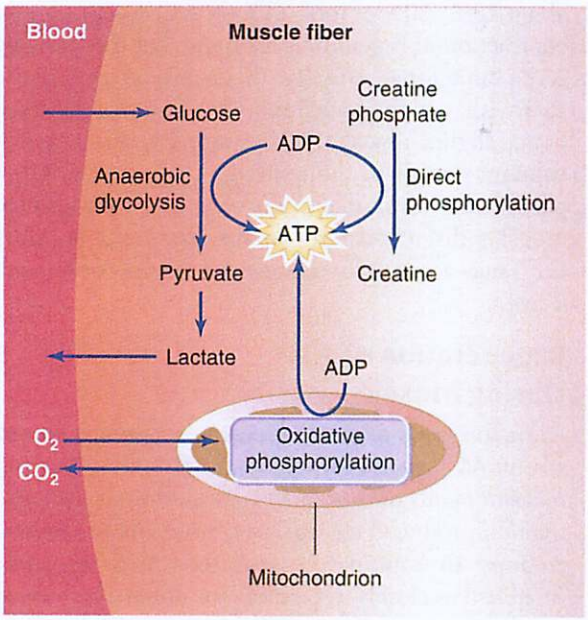


Figure 10-29 The ATP that provides energy for muscle contraction comes from several different sources. In direct phosphorylation, high-energy phosphates are transferred from creatine phosphate to ADP, regenerating ATP. The concentration of creatine phosphate in muscle fibers is higher than the concentration of ATP, so creatine phosphate effectively buffers the ATP concentration during short periods of intense demand. Anaerobic glycolysis metabolizes glucose, rephosphorylating ADP in the process. Lactate accumulates as a by-product and leaks into the blood. Oxidative phosphorylation of ADP regenerates ATP, but produces it more slowly than the other two processes and requires O_2 to proceed (see Chapter 3).

Moreover, the ATP concentration is stabilized because the creatine phosphokinase reaction greatly favors phosphorylation of ADP by creatine phosphate. Under most conditions, only the concentration of creatine phosphate falls in a working muscle, while the concentration of ATP remains nearly constant.

Recently a dietary supplement called creatine monohydrate has been advertised to improve athletic training and performance. Can you propose a physiological mechanism that might support this claim? Would this supplement be equally effective at improving performance in all kinds of activities? Why or why not?

FIBER TYPES IN VERTEBRATE SKELETAL MUSCLE

The muscular systems of animals perform a great variety of motor tasks, ranging from the high-speed movements of sound production, which occur at a frequency of several hundred contractions per second, to sustained locomotion during long-distance migrations, in which an individual may cover thousands of miles. Even a casual observer notices the diversity in the external attributes of muscular systems, such as lobster claws and human legs. There is an equally impressive diversity in the characteristics of the muscles themselves. To produce such a broad range of activities, different muscles must be organized to perform very differently. Recent experiments have shown that the properties of a muscle are in many cases well matched to the other components in a system, optimizing the system for its biological function. To appreciate how well muscles are adapted to their biological roles, we now examine the properties of a variety of muscles in light of their primary functions.

Classification of Fiber Types

The components of an individual muscle fiber confer on it several important characteristics that differ among fibers. The skeletal muscles of vertebrates typically contain muscle fibers of more than one type. Among the biochemical, metabolic, and histochemical properties that distinguish the various fiber types are the following:

- The electrical properties of the plasma membrane determine whether a fiber will respond with an all-or-none twitch or with a graded contraction. If the membrane produces APs, the fiber will contract with all-or-none twitches.
- The rate at which cross-bridges detach from actin thin filaments determines the maximal rate of contraction, V_{max}. The chemical nature of the myosin heavy chains determines the rate of cross-bridge detachment.
- The density of calcium pump molecules in the SR membrane determines how long the myoplasmic free Ca^{2+} remains elevated following an AP.
- The number of mitochondria and the density of a fiber's blood supply determine its maximum rate of sustained oxidative ATP production and hence its resistance to fatigue.

Based on these and other properties, four major groups of vertebrate skeletal muscle are recognized—**tonic fibers** and three types of **twitch** (or *phasic*) fibers (Table 10-1).

Tonic muscle fibers contract very slowly and do not produce twitches. They are found in the postural muscles of amphibians, reptiles, and birds, as well as in the muscle spindles that house muscle stretch receptors and in the extraocular muscles (the muscles that move the eyeball in its socket) of mammals. Tonic fibers normally produce no APs, and APs are not required to spread excitation because the innervating motor neuron runs the length of the muscle fiber, making repeated synapses all along it. In tonic muscle fibers, the myosin cross-bridges attach and detach very slowly, accounting for the

Table 10-1 Properties of twitch (phasic) fibers in mammalian skeletal muscles

Property	Slow oxidative (type I)	Fast oxidative (type IIa)	Fast glycolytic (type IIb)
Fiber diameter	↓	→	↑
Force per cross-sectional area	↓	→	↑
Rate of contraction (V_{max})	↓	↑	↑
Myosin ATPase activity	↓	↑	↑
Resistance to fatigue	↑	→	↓
Number of mitochondria	↑	↑	↓
Capacity for oxidative phosphorylation	↑	↑	↓
Enzymes for anaerobic glycolysis	↓	→	↑

Source: Adapted from Sherwood, 2001. Key = ↓ Low → Intermediate ↑ High

fibers' extremely slow shortening velocity and their ability to generate isometric tension very efficiently.

Slow-twitch (or *type I*) *fibers* contract slowly and fatigue slowly; they are found, for example, in mammalian postural muscles. They are characterized by a slow to moderate V_{max} and slow Ca^{2+} kinetics. They generate all-or-none APs, so they respond to motor-neuron input with all-or-none twitches. Like other twitch fibers, type I fibers typically have one or at most a few motor endplates; in most vertebrates, all synapses on a single fiber are made by a single motor neuron. Slow-twitch fibers are used both for maintaining posture and for moderately fast repetitive movements. They fatigue very slowly for two reasons. First, they contain a large number of mitochondria and have a rich blood supply bringing plenty of oxygen, which supports sustained oxidative phosphorylation. Second, they use ATP at a relatively slow rate. They are characterized by a reddish color (examples are the dark-colored meat of fish and fowl) conferred by a high concentration of the oxygen-storage protein myoglobin (see Chapter 13). Muscles that contain a high proportion of type I fibers are often called red muscle.

Fast-twitch oxidative (or *type IIa*) *fibers* have a high V_{max} and activate quickly. They are specialized for rapid repetitive movements, such as sustained, strenuous locomotion—the flight muscles of migratory birds are a striking example. With their many mitochondria, they produce enough ATP by oxidative phosphorylation to support work over long periods. They are thus relatively resistant to fatigue, although they are not as tireless as type I fibers.

Fast-twitch glycolytic (or *type IIb*) *fibers* contract very rapidly and fatigue quickly. They have a high V_{max}, and they activate and relax quickly because of their rapid Ca^{2+} kinetics. These fibers contain few mitochondria, depending instead on anaerobic glycolysis to generate ATP. A familiar example of this type of fiber is found in the white breast muscles of domestic fowl, which are never used for flying and cannot produce sustained activity. (The breast muscles of migratory birds feature type IIa fibers, consistent with their locomotory function.) Ectothermic vertebrates, such as amphibians and reptiles, also make extensive use of glycolytic muscle fibers.

These categories are somewhat arbitrary because some muscle fibers combine properties of different types. In addition, the numerical values for many of the parameters vary among species. The slow-twitch fibers of a mouse, for instance, have a faster V_{max} than the fast-twitch oxidative fibers of a horse. Within a given muscle, however, the fiber types can be distinguished by their histological properties. For example, histochemical staining reveals differences in the properties of the myosin ATPase in different fiber types (Figure 10-30). Another useful histochemical method for distinguishing fiber types is based on the abundance of the protein complexes that carry out oxidative phosphorylation.

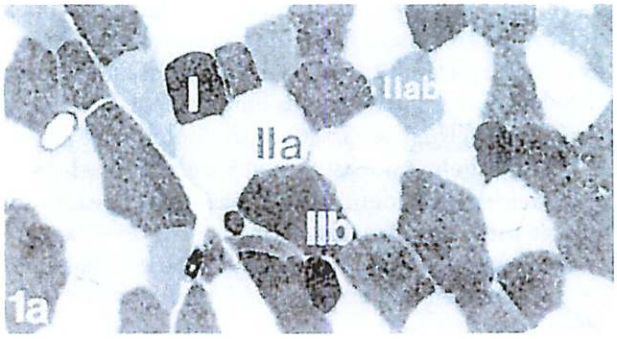


Figure 10-30 Histochemical staining for myosin ATPase activity reveals different types of fibers within a single muscle. This section through a muscle from a horse contains slow oxidative (type I), fast oxidative (type IIa), and fast glycolytic (type IIb) fibers. The fiber labeled IIab has intermediate properties. [Courtesy of L. Rome.]

Question

All mammalian muscles contain a variety of fiber types, but the percentages of the different types vary from muscle to muscle and from animal to animal. Can you predict which type of muscle fibers would predominate in the large leg muscles of the antelope on the cover of this book? Compare this pattern with what you would expect to find in a sprinter, such as a cheetah.

Functional Rationale for Different Fiber Types

What do animals gain by having different types of muscle fibers? Fast-twitch fibers obviously are necessary if an animal is to move its limbs or fins very rapidly, but why, then, have slow-twitch fibers? A basic principle in muscle physiology is that there is always a trade-off between speed and energetic cost. Very fast muscles require a large amount of ATP. Slow muscles perform less rapidly, but they also use relatively little energy. To better understand this trade-off, it is useful to compare the energetic costs and mechanical abilities of fiber types with different values of V_{max}.

The technique that has the best time resolution for measuring energy utilization by muscle and the one on which many conclusions about muscle energetics have been based is measurement of heat production. The hydrolysis of ATP by muscles is exothermic—heat is released by the reaction. This heat production can be used by homeotherms to warm the entire body—for example, in shivering (see Chapter 16). During a typical single contraction, the temperature of a muscle increases by a very tiny amount, about 0.001–0.01°C. Very fast and very sensitive thermometers called *thermopiles* can be used to measure the heat generated. In theory, the amount of ATP hydrolyzed by a muscle could be calculated by measuring the work done during contraction and dividing that value by the energy liberated as heat when a given amount of ATP is hydrolyzed. However, other heat-absorbing and heat-producing processes unrelated to the hydrolysis of ATP interfere with this measurement, so it is impossible to relate heat production during contraction precisely to the use of ATP. Nonetheless, measurements of heat production have yielded considerable insight into how different types of muscle use energy during contractions.

A muscle fiber's mechanical properties (that is, its force generation and power production) and energetic properties (that is, its rate of ATP use and efficiency) depend on both its velocity of shortening, V, and V/V_{max}. For a given rate of shortening, V, the force and mechanical power produced per cross-sectional area can be considerably higher in a fiber with a high V_{max} than in a slower fiber (Figure 10-31a,b). Furthermore, the generation of power is maximal at intermediate values of V/V_{max}. It therefore takes fewer high-V_{max} fibers than low-V_{max} fibers to generate a given amount of power.

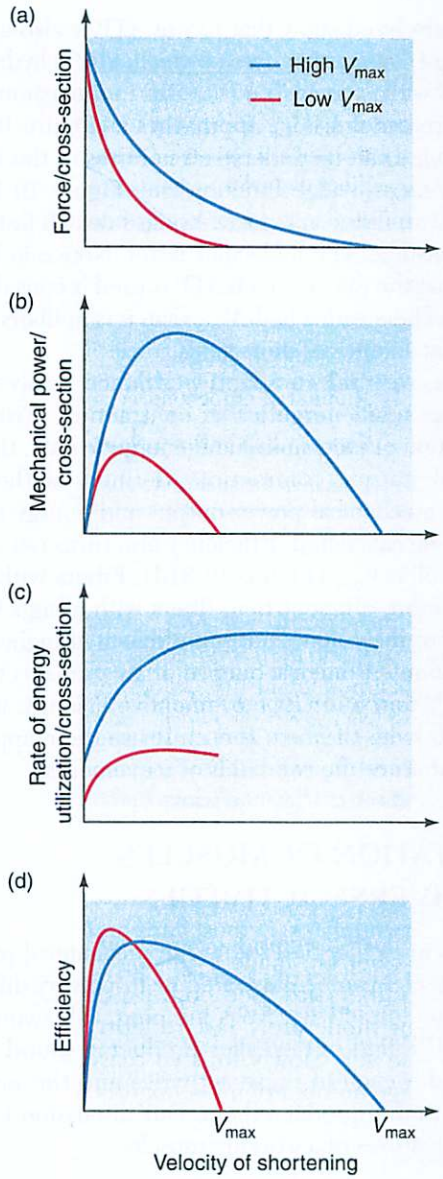


Figure 10-31 Force, power, rate of energy utilization, and efficiency vary as a function of shortening velocity. Fibers with a high V_{max} can generate more force **(a)** and mechanical power **(b)** than those with a low V_{max}, but they also use more energy at all shortening velocities **(c)**. The efficiency of contraction is calculated as the power output divided by the energy used **(d)**. Note that low-V_{max} fibers are more efficient at low shortening velocities, whereas high-V_{max} fibers are more efficient at higher rates of shortening. These curves were derived from heat-production, oxygen-usage, and mechanical measurements of frog muscle contraction. [Adapted from Hill, 1964; Hill, 1938; and Rome and Kushmerick, 1983.]

It might, then, seem advantageous to have only muscle fibers with high values of V_{max}. There is, however, an energetic price to be paid for a high V_{max}. Measurements of heat liberated and high-energy phos-

phate hydrolyzed show that use of ATP is also a direct function of V/V_{max}. The rate at which ATP is hydrolyzed increases with increasing V/V_{max} up to a maximum and then decreases as V/V_{max} approaches 1 (Figure 10-31c). This increase can be understood in terms of the Huxley model of cross-bridge function (see Figure 10-10). In muscles with faster V_{max}, cross-bridges detach faster and hence consume ATP molecules faster. Notice in Figure 10-31c that the rate at which ATP is used is considerably higher in fibers with a high V_{max} than it is in fibers with a low V_{max} at all rates of shortening.

Thus, we find an adaptive balance between the mechanics and energetics of contraction. From the combination of mechanical and energetic data, the efficiency of muscle contraction (defined as the ratio between mechanical power output and energy utilization) can be calculated. Efficiency also turns out to be a function of V/V_{max} (Figure 10-31d). Fibers with a low V_{max} are more efficient than fibers with a high V_{max} at low rates of shortening, but less efficient at higher rates of shortening. As a consequence, if an animal is to produce both slow and fast movements efficiently, it must have both kinds of fibers and must use them appropriately to produce the two kinds of movements.

ADAPTATION OF MUSCLES FOR DIVERSE ACTIVITIES

The principles that determine the mechanical properties of muscles can be illustrated by three very different kinds of motor activity: frogs jumping, fish swimming, and toadfish and rattlesnakes producing sound. Here we consider each of these activities and the muscles that are used to produce them. Our discussion focuses on three features of a working muscle:

- the amount of overlap between thick and thin filaments (that is, where on its length-tension curve the muscle is working)
- the relative velocity of shortening, V/V_{max}, during the activity, which determines the power and efficiency of the muscle
- the timing and duration of the muscle's active state

In this section we will draw heavily from the work of Lawrence Rome and his colleagues, who have contributed much to our understanding of comparative muscle physiology.

Adaptation for Power: Jumping Frogs

When a frog jumps, it moves from a crouched to an extended position in just 50 to 100 ms. The work performed per unit time is high, so the muscles that produce the jump must generate considerable power. The distance covered in a single jump depends directly on how much power the muscles produce. From our earlier discussion, we would expect a muscle that generates high power to exhibit three properties: (1) it should operate within the plateau of the sarcomere length-tension curve, where maximal force is generated (see Figure 10-8b); (2) it should shorten at a rate at which maximal power is generated (see Figure 10-13d); and (3) it should become maximally activated (that is, every fiber should be in the active state) before shortening begins. To determine whether a frog's jumping muscles actually have these properties, G. Lutz and L. C. Rome, using *Rana pipiens* as their model system, observed both jumping frogs and frog muscles in isolation, integrating the results of the two kinds of experiments.

Length-tension relation

To examine the length-tension relation of muscles during a jump, Lutz and Rome measured the length and changes in length of the semimembranosus muscle, a hip extensor. They took measurements from videotapes of frogs jumping and from isolated limbs whose positions were manipulated to match the shape of a jumping frog's legs (Figure 10-32 on the next page). By plotting the changes in muscle length against the pelvic angle, they determined the *moment arm* of the muscle. (The moment arm is the distance separating a fixed fulcrum from a point at which a force is exerted that will tend to rotate a mass around the fixed point, as illustrated in the inset in Figure 10-32.) The length of the moment arm is crucial because it determines both the leverage of the muscle (that is, the angular acceleration around the fulcrum point that will be produced by contraction of the muscle) and how much the muscle must shorten to produce any given change in the angle of the hip joint.

The length of sarcomeres in the hip extensor was determined when the hip was in the crouched position and when it was in the extended, jumping position. During a jump, the sarcomere length changes from 2.34 μm in the crouched position to 1.82 μm at the point of take-off. To determine where these lengths fall in the sarcomere length-tension relation, they were compared with a length-tension curve measured in a closely related species of frog *(Rana temporaria)*, as shown in Figure 10-33a on the next page. The measured lengths of the hip extensor sarcomeres fell along the plateau of the sarcomere length-tension curve; thus, as expected for a power muscle, the fibers of the hip extensor operate very near their optimum during a jump. It has been calculated that this muscle generates at least 90% of its maximal tension throughout the jump. In fact, if the initial sarcomere length were either longer or shorter than the measured length, the muscle would produce less power.

A number of factors must be matched to produce this optimal behavior. The lengths of the myofilaments and the number of sarcomeres per muscle fiber must

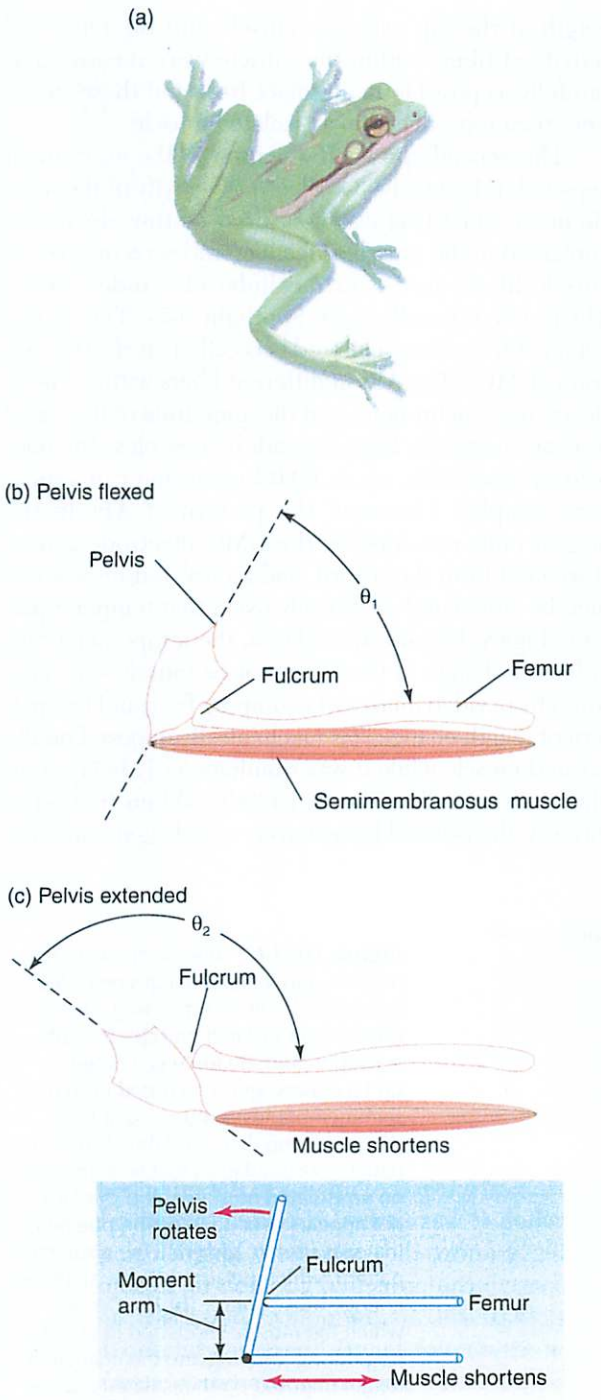


Figure 10-32 When the hip extensor muscle of a frog contracts, the hip joint rotates around the point at which the femur attaches to the pelvis, pushing the frog off the substrate. **(a)** In a sitting frog, the thigh is held at a small angle with respect to the longitudinal axis of the body, as diagrammed in part b. **(b)** When the frog is in a crouched position, the angle of the hip joint (θ_1) is small, and the hip extensor muscle (the semimembranosus muscle) is relaxed. **(c)** When the semimembranosus muscle contracts, the angle of the hip joint increases (θ_2) as contraction of the muscle acts through a moment arm to pull on the pelvis. As a result, the frog's legs push against the substrate, propelling the body upward and forward. Inset: Schematic diagram illustrating geometric relations among the mechanical components contributing to jumping. [Part a adapted from Pough et al., 1996; part b adapted from Lutz and Rome, 1996a, b.]

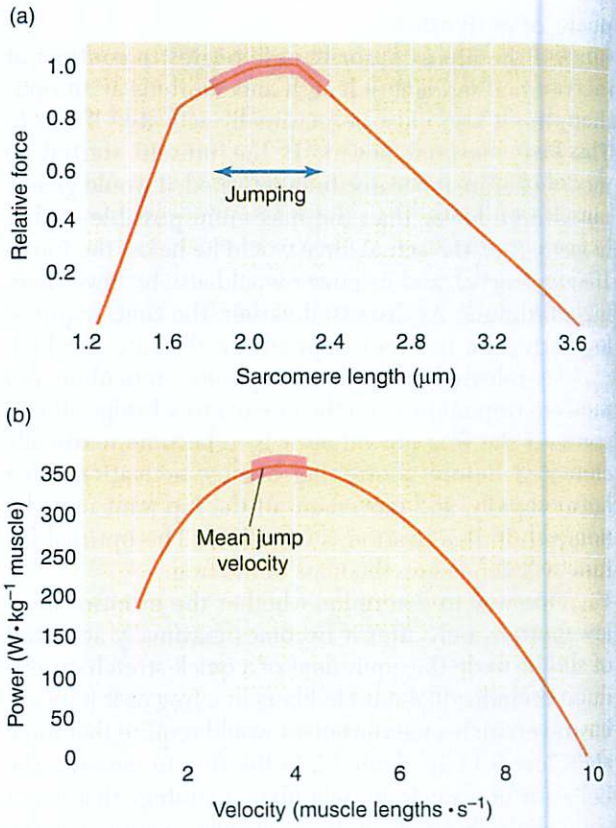


Figure 10-33 The mechanics of the frog's hip extensor muscle operate optimally during a jump. **(a)** At the beginning of a jump, the sarcomeres in the semimembranosus muscle are 2.34 μm long; they shorten to 1.82 μm during the jump (highlighted portion of curve). Even at the shortest sarcomere length, the muscle still generates over 90% of its maximal tension. **(b)** At the shortening velocity used during jumping, the muscle operates in the highlighted portion of the power curve in which at least 99% of maximal power is generated. The velocity of shortening is expressed in terms of muscle lengths per second to take into account the difference in length of muscles taken from different-sized frogs. [Adapted from Lutz and Rome, 1994.]

combine to produce optimal overlap of thick and thin filaments when the frog is in the crouched position. In addition, given the change in the angle of the hip joint observed during jumping, the moment arm of the hip joint must allow the muscle and its sarcomeres to undergo appropriate changes in length while maintaining optimum overlap.

Value of V/V_{max}

The V_{max} of the hip extensor muscle is about 10 muscle lengths per second, and it generates maximal power at 3.44 muscle lengths per second (Figure 10-33b). The mean rate at which the muscle shortens during a jump is 3.43 muscle lengths per second; that is, at a V/V_{max} of 0.33, almost exactly the rate at which the muscle produces maximal power. Thus, the frog's muscles, joint configuration, and mass are all matched to allow the hip extensor muscle to shorten at a V/V_{max} appropriate for maximal generation of power.

State of activation

Even if the hip extensor muscle begins to contract at the optimal sarcomere length and shortens at an optimal rate, it also must be maximally activated if it is to generate maximal power. If the muscle started to shorten before it became fully activated, it would generate a force lower than the maximum possible at that velocity (i.e., the actual force would lie below the force-velocity curve), and its power would also be lower than the maximum. As discussed earlier, the time required for activation to occur depends on the rate at which Ca^{2+} is released from the sarcoplasmic reticulum and binds to troponin and on the rate of cross-bridge attachment. If the frog hip extensor is to become maximally activated before shortening begins, activation must occur rapidly, and movement of the hip joint must be delayed until activation is complete. The optimal lag time will depend on the mass of the frog.

One way to determine whether the extensor muscle shortens only after it becomes maximally activated would be to do the equivalent of a quick-stretch experiment on individual muscle fibers in a frog as it jumped. However, such a measurement would require that force transducers be implanted into the frog to measure the behavior of a single muscle fiber, a strategy that is not yet technically practical. In an alternative approach, the length of the hip extensor muscle and the electrical activity of fibers within the muscle were measured as carefully as possible in an intact frog, and these values were then reproduced in an isolated muscle.

This second approach was used in the experiment depicted in Figure 10-34. Electrical activity of the muscle in an intact frog was measured by tiny electrodes implanted in the muscle; such electrodes record APs in muscle fibers, just as extracellular electrodes record APs in nerve bundles (see Spotlight 6-2). The record obtained from these electrodes is called an *electromyogram* (EMG). The APs in different fibers within a muscle are not synchronous, and the amplitude of the signal from any particular fiber depends on how close the fiber is to the electrode, so an EMG recording can appear very complex. However, the pattern of APs in the biggest units recorded by the EMG electrode can be abstracted from the record, and an isolated muscle can then be stimulated electrically using that temporal pattern (Figure 10-34a). In addition, the temporal pattern of length change in the hip extensor muscle was measured from video images of a jumping frog, and this pattern of length change was mechanically imposed on the isolated muscle while it was simultaneously being stimulated electrically (Figure 10-34b). When treated in this way, the isolated hip extensor muscle generated the

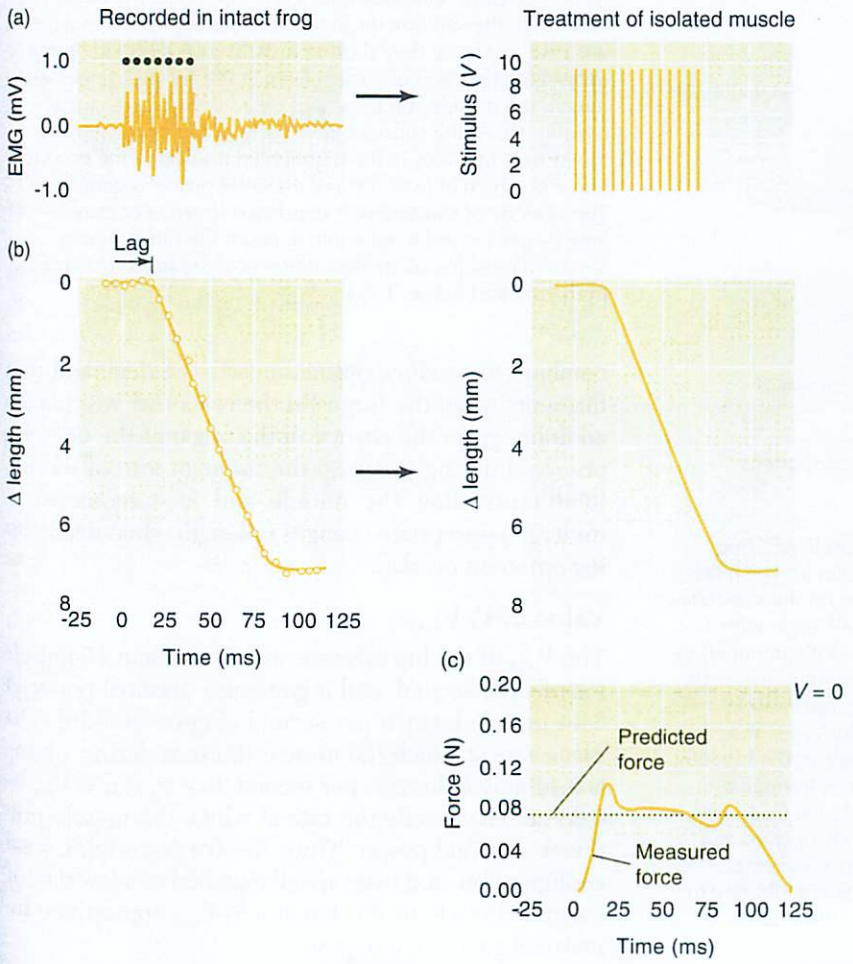


Figure 10-34 The electrical activity patterns and length changes recorded from muscles in an intact frog can be imposed on isolated muscles to study activation and the force generated. **(a)** Electromyogram recorded from the semimembranosus muscle of a frog during a jump (left), and the abstracted pattern of stimulation that was imposed on an isolated muscle (right). A black dot appears over each action potential that was used to generate the abstracted pattern, which persisted longer than the endogenous pattern. **(b)** The rate at which the muscle shortened in an intact frog during jumping and the change in length imposed on the isolated muscle while it was being electrically stimulated as shown in part a. **(c)** Force produced by the isolated muscle (red line) during the experimental manipulation. The dashed line shows the isometric force produced by this muscle when $V = 0$, and the dotted line shows the force that would be expected in the same muscle contracting at the imposed shortening velocity during a force-velocity experiment similar to the one shown in Figure 10-31a. The time axis is identical in all panels. Time equals zero at the start of stimulation. Notice that there is a lag between the start of stimulation and the time when muscle length begins to change in part b. [Adapted from Lutz and Rome, 1994.]

maximal force expected at the imposed shortening velocity (Figure 10-34c), strongly suggesting that it is maximally activated during jumping. The implication of this result is that the molecular components of activation in this muscle are strikingly matched to the biomechanics of jumping.

Adaptation for Contrasting Functions: Swimming Fish

The study of muscles in fish has for two reasons been particularly useful in elucidating how muscular systems are organized. First, fish make many different kinds of movements that can be elicited readily and analyzed quantitatively. Second, different movements are powered by different muscle fiber types, which in fish are anatomically separated, permitting the activity of individual fiber types to be monitored by electromyogram electrodes (Figure 10-35). (This arrangement contrasts with that of the muscles in most other vertebrates, which contain more than one type of fiber, making electrical monitoring of activity in one particular fiber type difficult or impossible.)

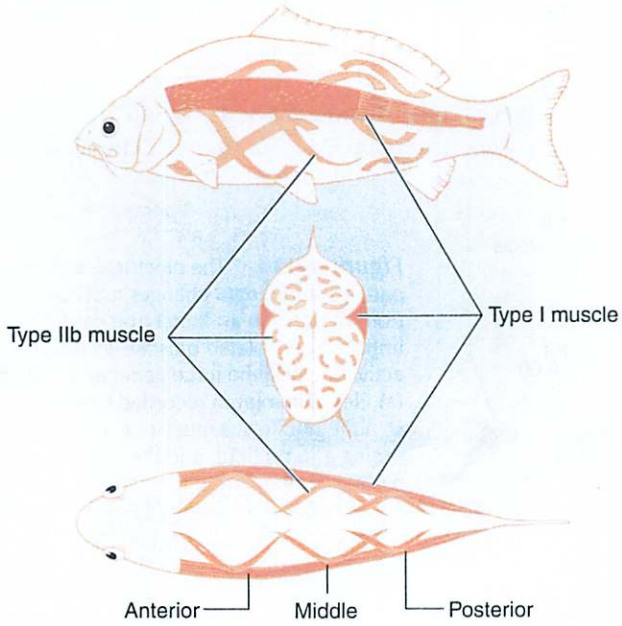


Figure 10-35 In fish, the muscle fiber types are anatomically separate from one another, facilitating electromyographic monitoring of activity in specific fiber types. These diagrams show the arrangement of two muscle fiber types in a carp. Type I (slow-twitch oxidative) muscle fibers (dark red) are found in red muscles, which lie in a thin layer just under the skin; the thickness of this layer has been enlarged in this drawing. These muscles run parallel to the body axis, so the change in sarcomere length during shortening is directly related to the curvature of the spine and to the distance separating the muscle layer from the spine. Notice that details have been omitted from the anterior end of the muscle band. Type IIb (fast-twitch glycolytic) fibers (light red) compose the white muscles, which are located deeper in the body. These muscles run helically, rather than parallel to the long axis of the body. Because of their anatomic arrangement, white muscles need to shorten only about 25% as much as red muscles to produce a given change in the curvature of the body. [Adapted from Rome et al., 1988.]

During the many movements of which fish are capable, the change in sarcomere length is roughly proportional to the curvature of the spine. When a carp is swimming steadily at a velocity of 25 $cm \cdot s^{-1}$, the curvature changes very little along most of its spine (Figure 10-36a), indicating that the lengths of sarcomeres along its body change very little. In contrast, when the fish is startled—for example, by a loud sound—and produces an escape response, its spine curves markedly, indicating that sarcomeres have shortened on one side of the body and lengthened on the other (Figure 10-36b). Notice the difference in time scale between steady swimming and the escape response. During steady swimming, one tailbeat takes about 400 ms, whereas in the escape response, the body of the fish changes from straight to highly curved in only 25 ms.

The muscles of a fish must, then, be able to generate both slow, low-amplitude movements and fast, high-amplitude movements. Earlier in this chapter, it was argued that muscles must be finely tuned to a particular activity in order to perform optimally, but these two behaviors seem to require very different properties. Can muscle fibers perform such different tasks while still operating optimally? If so, how do they do it?

Electromyograms recorded from fish swimming normally and responding to a loud sound revealed that different muscles, containing different fibers types, are active during the two behaviors. When a fish is swimming steadily, only red muscles are active; these are composed of slow-twitch oxidative (type I) fibers. In contrast, white muscles, composed of fast-twitch glycolytic (type IIb) fibers, are recruited to produce fast swimming or large, rapid movements such as the escape response. A fish is able to produce these very different kinds of movements effectively because for each movement, it uses muscles that are specialized to match the demands of the particular task. Let's examine the same three properties of these fish muscles that we considered for the hip extensor muscles of a frog.

Length-tension relation

Measurements of sarcomeres in fish frozen into the shapes assumed by living fish as they perform different behaviors reveal that sarcomere lengths in the red muscles of slowly swimming fish vary rhythmically between 1.89 μm and 2.25 μm, centered around 2.07 μm (see Figure 10-36a). These values then need to be compared with the length-tension curve for fish sarcomeres to determine whether the thick and thin filaments maintain optimal overlap in this range. Although the length-tension curve for a single fish sarcomere has yet to be determined, electron microscopic examination of red and white muscles from carp reveals that the lengths of myofilaments in fish muscles are nearly identical to the

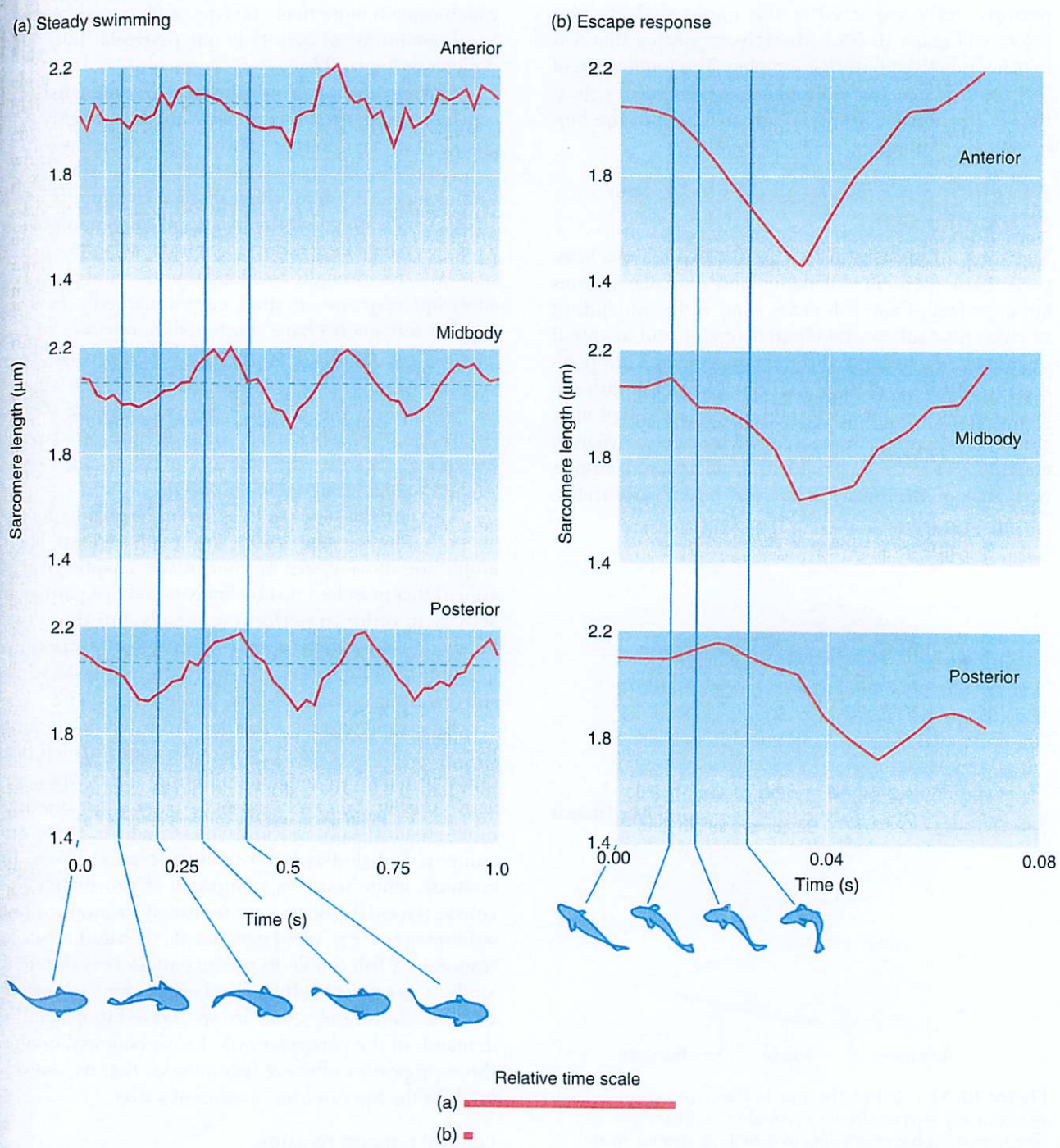


Figure 10-36 Steady swimming movements and the escape response in fish differ greatly in magnitude and time course. This figure shows inferred changes in sarcomere length within the red muscles located on one side of the anterior, midbody, and posterior of a carp engaged in two activities: **(a)** swimming steadily at 25 $cm \cdot s^{-1}$ or **(b)** making an abrupt escape response. The changes in sarcomere length were calculated from the shape of the fish at each time point in a high-speed movie of the behavior. The shape of the body at selected time points is indicated in figures below each graph. Type I (slow-twitch oxidative) muscles are active during steady swimming. In contrast, type IIb (fast-twitch glycolytic) muscles produce the escape response (see Figure 10-37). [Part a adapted from Rome et al., 1990; part b adapted from Rome et al., 1988.]

lengths of these filaments in frog muscles. Therefore, the sarcomere length-tension curve for frog muscle is likely to provide a good approximation of the same relation in carp. Comparison of the sarcomere lengths measured in swimming carp with the frog length-tension curve shows that in swimming fish, the red muscles at peak tension generate at least 96% of their maximal force (Figure 10-37a on the next page).

In the escape response, the fish moves rapidly, and its body curves dramatically. As Figure 10-35 shows, the

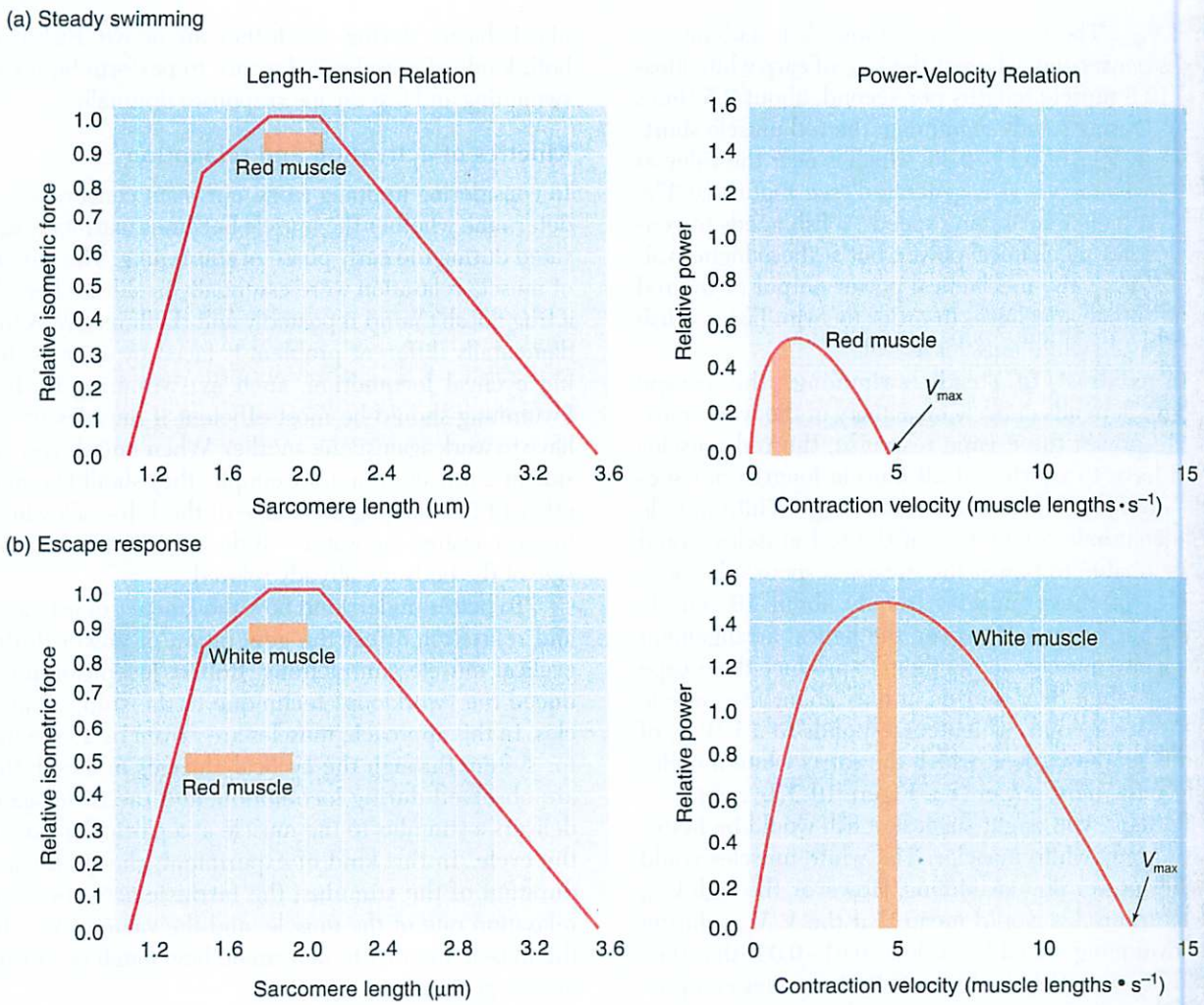


Figure 10-37 The properties of the red and white muscles of fish make the two kinds of muscle optimally suited for different kinds of activities. **(a)** The changes in the sarcomere length of red muscles during steady swimming coincide with the plateau of the sarcomere length-tension curve (left). The orange bar indicates the lengths of red muscle sarcomeres during slow, steady swimming. In addition, the contraction velocities of red muscles during swimming correspond to values of V/V_{max} between 0.17 and 0.36, near the value at which red fibers produce maximal power (right; orange region under curve). **(b)** Because of their anatomic arrangement, white fibers can produce the escape response at a more favorable region of the sarcomere length-tension curve than can red fibers (left). In addition, the high V_{max} of white fibers allows them to generate power when they are shortening very rapidly (right). Indeed, during the escape response, the V/V_{max} for white muscles (orange bar) is 0.38, which is the peak of their power curve. [Adapted from Rome and Sosnicki, 1991.]

red muscles in carp run parallel to the long axis of the fish, whereas the white muscles run helically. To produce the escape response, the sarcomeres of the red muscle would have to shorten to 1.4 μm, a length at which their force production would be low (Figure 10-37). In contrast, the sarcomeres in the helically arranged white muscles need shorten only to about 1.75 μm during this behavior. In other words, the mechanical advantage conferred by the anatomic arrangement of the white muscles allows them to produce any given change in the curvature of the spine with much less sarcomere shortening than would be required by the red muscles. Thus, white muscles are much better suited to produce the escape response, and they generate about 85% of their maximal force during this behavior (see Figure 10-37b). When white muscle is used in less extreme movements (e.g., when a fish is swimming rapidly) and the curvature of the spine is not nearly as extreme, the sarcomeres shorten less, and the muscles generate nearly maximal force.

Because the fish uses different muscles to produce different movements, the myofilament overlap (sarcomere length) is never far from its optimal level, even in the most extreme movements. The lengths of the thick and thin filaments and the anatomic arrangement of the muscle fiber types combine to allow this optimization.

Value of V/V_{max}

In addition to their different anatomic arrangements, the red and white muscles of a carp have different val-

ues of V_{max}. The V_{max} of carp red muscle is 4.65 muscle lengths per second, whereas the V_{max} of carp white muscle is 12.8 muscle lengths per second, about 2.5 times higher. During steady swimming, the red muscle shortens at a V/V_{max} of 0.17–0.36, which is near the value at which maximal power is generated (see Figure 10-37a, right). At higher swimming speeds, a fish needs to generate greater mechanical power, but at these higher values of V/V_{max}, the mechanical power output of the red muscle actually declines. In order to swim faster, a fish must activate white muscles as well.

In contrast to steady swimming, the escape response depends entirely on activity in the white muscles. To power the escape response, the red muscles would have to shorten at 20 muscle lengths per second—four times faster than their V_{max}. White muscle in the anatomic orientation of the red muscles would also be unable to power the escape response, because the V_{max} of these muscles is only about 13 muscle lengths per second. However, the helical arrangement of the white muscles allows them to produce the escape response when they shorten at only about five muscle lengths per second, which corresponds to a V/V_{max} of about 0.38, the value at which the carp's white muscles produce the most power (see Figure 10-37b, right).

Perhaps, you might suggest, a fish would be better off with only white muscles. The white muscles could certainly power slow swimming. However, the high V_{max} of white muscles would mean that the V/V_{max} during slow swimming would be so low (0.01–0.03) that they would be extremely inefficient. Red muscles can produce adequate power to generate slow swimming, and they do it much more efficiently than white muscles could. Thus, the anatomic arrangement and the V_{max} of the two kinds of muscles suit each of them to the particular behavior during which they are active. Fish need both kinds of muscles if they are to perform both slow swimming and fast escape responses optimally.

Kinetics of activation and relaxation

In considering jumping frogs, our main concern was to determine whether the muscle becomes maximally activated during the early phase of shortening. The kinetics of muscle relaxation were essentially irrelevant because a frog doesn't jump repeatedly and rhythmically. A fundamentally different problem is faced by animals during cyclical locomotion, such as swimming by fish. Swimming should be most efficient if muscles do not have to work against one another. When muscles on one side of a fish shorten, for example, they should be most efficient in changing the shape of the fish—allowing it to push against the water—if the muscles on the other side of the body are already relaxed.

To better understand how the kinetics of activation and relaxation affect the generation of power during cyclical muscle contractions, Robert Josephson introduced the "workloop" technique to the study of muscles. In this approach, muscles are driven by a servomotor system through the cyclical changes in length that are observed during locomotion, and the investigator delivers a stimulus to the muscle at a particular time in the cycle. In this kind of experiment, the timing and duration of the stimulus, the intrinsic activation and relaxation rate of the muscle, and the value of V_{max} for the muscle interact to determine how much power the muscle generates.

A useful way to quantify these potentially complex interactions is to measure the amount of net work (force × change in length) a muscle generates during one cycle of shortening and lengthening (Figure 10-38a).

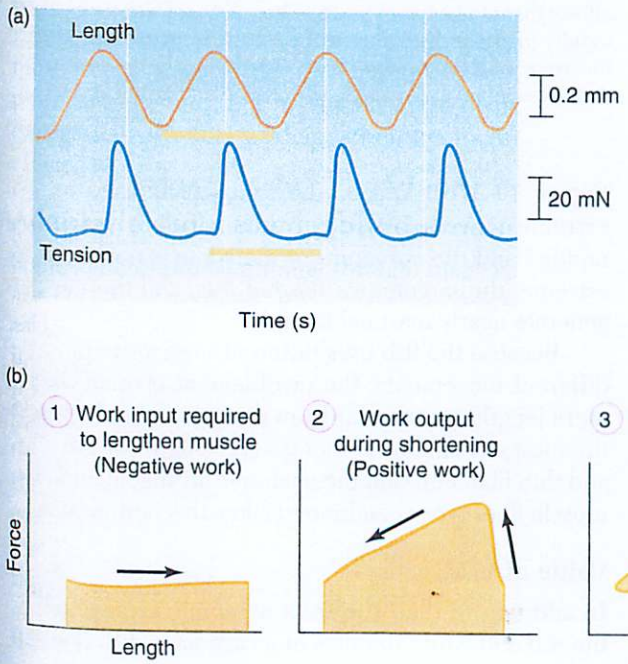


Figure 10-38 Workloops graphically depict the net work done during cyclical muscle contractions. **(a)** The length (red upper record) and tension (blue lower record) of a katydid flight muscle was recorded as it was being driven to shorten and lengthen cyclically. The orange bar indicates the duration of a single cycle. **(b)** The force-length relation during one complete cycle. In (1) the muscle is becoming longer because it is being stretched by an outside force; the shaded area under the curve represents the "negative work" done during this phase. In (2) the muscle shortens; the shaded area represents the positive work done during this phase. The net work (3) is the difference between the negative work and the positive work and equals the area encompassed by the force-length curve or workloop. [Adapted from Josephson, 1985.]

Net work is graphically equivalent to the area contained within a force-length loop (Figure 10-38b). A muscle does positive work only when it is shortening; thus positive work is equal to the area under the force-length curve during the shortening phase of a cycle. A muscle generates "negative work" when it is forcibly lengthened by an antagonist muscle (or a servomotor system); thus negative work is equal to the area under the force-length curve during the lengthening phase of each cycle. The net work—the difference between the positive and negative work done during one cycle—is equal to the area between the positive and negative legs of the force-length curve for one cycle. In other words, the net work is equal to the area inside the force-length loop, or work-loop. For a muscle to generate net positive work, it must generate a greater force during shortening than was required to stretch it to its initial length. The net power generated by a cyclical contraction is expressed by the equation

$$\text{net power} = (\text{positive work} - \text{negative work})_{\text{cycle}} \times \text{frequency of cycles}$$

It would seem that muscles might operate optimally if their fibers were fully activated during shortening (as in the frog) and could fully relax before they were forced to elongate by the activity of other muscles. If a muscle could be fully activated instantaneously and then relax instantaneously, the generation of force during shortening would be given by the force-velocity curve. There is, however, a problem. A muscle that was maximally activated throughout shortening and that then relaxed instantly at the end of shortening would be very energetically expensive, for two reasons. First, such a muscle would have to pump Ca^{2+} back into its sarcoplasmic reticulum very rapidly, requiring a huge number of calcium pumps to be continuously active—an unrealistically large expenditure of ATP. Second, instantaneous relaxation would require that cross-bridges detach very rapidly, but rapidly cycling cross-bridges use ATP much faster than cross-bridges that cycle more slowly. A muscle with more modest rates of calcium pumping and cross-bridge cycling will be energetically less expensive, allowing it to work more efficiently. Efficiency of operation is important in muscles that are used almost continuously, such as the swim muscles of an active fish.

If a muscle has a slow relaxation time, allowing it to be metabolically efficient, the timing of stimulation becomes important. Remember that there is a lag between the muscle AP and the onset of tension. For a slowly relaxing muscle to be appropriately relaxed before contralateral muscles pull on it, electrical stimulation activating the muscle must start *during* the lengthening phase and continue into only the very earliest part of the shortening phase. Otherwise the stimulation will continue too long to allow the muscle to relax soon enough. However, this pattern of stimulation reduces the amount of work the muscle can do. Once again, there is a trade-off between two desirable features—in this case, the ability of the muscle to do work versus its metabolic efficiency.

Workloop experiments have been performed on swimming fish to determine whether swim muscles emphasize rapid relaxation, which is metabolically costly, or lower work output, which is less metabolically expensive. The basic experimental approach used in these studies was similar to that described for the frog hip extensor muscle. The electrical activity of muscles

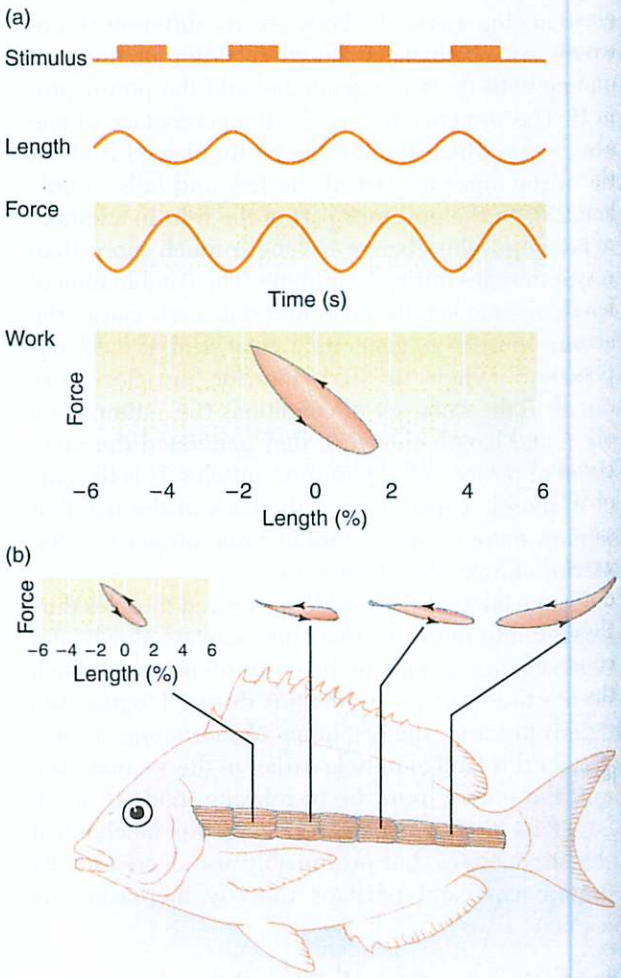


Figure 10-39 During slow, steady swimming, the posterior muscles do more net work than the anterior muscles. **(a)** Typical stimulus, length, and force records during "swimming" by isolated muscles, and a workloop calculated from these records. **(b)** The workloops determined for muscles at several locations along the body of a scup indicate the net work performed by the various muscles. The scale of the workloops for each position along the body is the same, so the relative area within the workloop reflects the relative amount of work done by muscles at the different locations. The length and force axes are shown for the most anterior workloop; only the shape and relative size of the workloops are shown for other locations. [Adapted from Rome et al., 1993.]

and the changes in muscle length were determined in swimming fish. Then, using the type of setup illustrated in Figure 10-34, isolated muscles were stimulated in a temporal pattern identical to that of the electromyogram, and the length of the muscles was controlled to match the changes measured during swimming. The force and power generated by the muscles under these conditions were determined, and the net work done by the muscles was calculated by plotting the workloop. These experiments revealed that during slow, steady swimming, the posterior muscles do more net work than the anterior muscles (Figure 10-39).

During cyclic swimming, muscles at different locations along the axis of the body receive different stimulus patterns and change in length by different amounts, affecting both the force generated and the power produced. The *stimulus duty cycle* (the percentage of one cycle during which the muscle is stimulated) is about 50% in the anterior part of the fish and falls to only about 25% in the posterior part of the fish. In addition, posterior muscles change in length much more than anterior muscles during swimming. The combination of large changes in length and a short duty cycle causes the posterior muscles to generate a great deal of mechanical power. When isolated anterior muscles were exposed to the same set of conditions (i.e., stimulation pattern and length changes), they generated the same amount of power as the posterior muscles. It is the *pattern* of muscle contraction at the back of the fish that generates more power, not an intrinsic property of the posterior muscle fibers themselves.

Examination of the workloops for red muscles during swimming indicates that they activate slowly and relax slowly. Stimulation to the posterior muscles, which generate the most power, begins during lengthening and ends just after the beginning of shortening, as predicted for this kind of muscle earlier in this section. As a result, the muscle must be in relaxing mode through most of its power stroke, reducing the mechanical power it generates, but presumably also decreasing its energetic cost and perhaps thereby increasing its efficiency.

Adaptation for Speed: Sound Production

Some animals produce sound through mechanisms that are not directly coupled to muscle contraction—for example, the movement of a column of air past a vibrating membrane or past the vocal cords. In other animals, however, muscles directly cause structures, such as the swimbladder of a toadfish or the rattle on the tail of a rattlesnake, to vibrate, generating sound. In these animals, the sound-producing (or sonic) muscles must undergo contraction-relaxation cycles at the frequency at which the sound is made, which can be 10 to 100 times faster than most locomotory muscles operate.

In the last section, we saw that the swim muscles of fish have relatively slow rates of relaxation, allowing them to avoid the high energetic cost of excessive calcium pumping. When these swim muscles are experimentally stimulated at the high frequencies needed for sound production, they are unable to relax between stimuli, and they go into tetanus (see Figure 10-28). If sound muscles became tetanic in the same way, the animal would be rendered silent. Sonic muscles must, therefore, have unique properties that allow them to operate at the high frequencies associated with sound production.

Toadfish swimbladder

The male toadfish *(Opsanus tau)* produces a "boatwhistle" mating call ten to twelve times per minute for hours on end to attract females to its nest. This call is generated by rapid oscillatory contractions of the muscles encircling the fish's gas-filled swimbladder. The sonic muscles of the swimbladder must contract and relax at frequencies of several hundred hertz. In contrast, the steady swimming movements of toadfish are made at about 1–2 Hz, and the escape response of these fish operates at 5–10 Hz. To understand the differences among muscles that allow them to function with such different kinetics, the properties of three kinds of muscles in the toadfish—swim, escape, and sonic—have been studied.

The time course of many biological events is characterized by their **half-width**, which is the width of the event on the temporal axis when the measured variable is equal to half of its peak value (Figure 10-40a on the next page). The half-width of a single muscle twitch in the toadfish is 500 ms in red (swim) muscle, 200 ms in white (escape) muscle, and only 10 ms in swimbladder (sonic) muscle (Figure 10-40b).

If a muscle is to activate and relax rapidly, two conditions must be met. First, Ca^{2+}, the trigger for muscle contraction, must enter the myoplasm rapidly and be removed rapidly. Second, myosin cross-bridges must attach to actin and generate force soon after the myoplasmic Ca^{2+} concentration rises, then detach and stop generating force soon after the Ca^{2+} concentration falls. In the red and white muscles of the toadfish, myoplasmic free Ca^{2+} rises and falls with typical kinetics, but in the sonic muscles, these Ca^{2+} transients are the fastest ever measured for any fiber type from any animal. Similarly, force measurements indicate that the sonic muscles both contract and relax about 50 times faster than the red muscles (see Figure 10-40b).

The effect of these very fast Ca^{2+} transients is most obvious during repeated stimulation. When red muscle is stimulated at 3.5 stimuli per second (3.5 Hz), Ca^{2+} lingers in the myoplasm so long that the concentration of myoplasmic free Ca^{2+} cannot return to its resting value between stimuli. Indeed, between stimuli delivered at this rate, the myoplasmic Ca^{2+} concentration remains constantly above the threshold level required for contraction, so a partially fused tetanus is

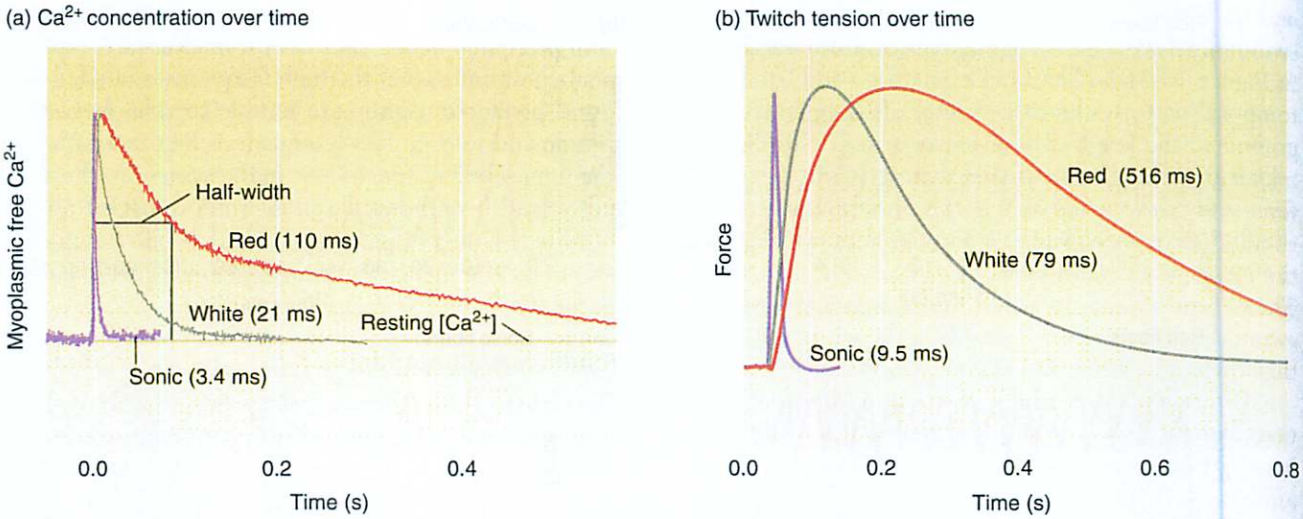


Figure 10-40 Because the sonic muscles of the toadfish activate and relax much more rapidly than its red (swim) and white (escape) locomotory muscles, they can operate at higher stimulation frequencies without becoming tetanic. **(a)** Time course of myoplasmic Ca^{2+} concentration following stimulation at 16°C in three types of muscle fibers isolated from toadfish. The time course of many biological events is characterized by the half-width of the event, illustrated for the red muscle. The half-width is the duration separating the rising and falling phases of the event when the value of the variable is one-half of its peak value. The vertical black line intersects the record at one-half of its peak value; the horizontal black line shows the time between the rising and falling phases. The average half-width of the Ca^{2+} transients in these muscles ranges from 3.4 to 110 ms, as indicated on the graph. **(b)** Time course of twitch tension in the three fiber types measured under the same conditions as in part a. Sonic muscles both contract and relax much faster than do the red or white fibers. The average half-width of twitch tension ranges from 9.5 to 516 ms. Thus sonic muscle operates more than 50 times faster than red muscle. The Ca^{2+} concentration and tension records are normalized to their maximal values for all fiber types. [Adapted from Rome et al., 1996.]

produced (Figure 10-41a). By contrast, the swimbladder sonic muscle has such fast Ca^{2+} transients that even at 67 Hz, the myoplasmic Ca^{2+} concentration returns to its baseline value between stimuli. Because the myoplasmic free Ca^{2+} concentration is below the threshold for the generation of force for much of the time between stimuli, only the first two twitches in the series are fused (Figure 10-41b). The production of an individual twitch in response to each stimulus is required for generating the oscillation of the swimbladder that produces sound.

The ability of a muscle to relax rapidly requires not only a very short Ca^{2+} transient, but also the rapid release of bound Ca^{2+} from troponin. Comparing the time course of force generation and of myoplasmic Ca^{2+} transients in frog white muscle fibers and in toadfish sonic muscles suggests that Ca^{2+} must be released from troponin in the sonic muscles three times faster than in the frog fibers.

Finally, for force to drop quickly following the dissociation of Ca^{2+} from troponin, myosin cross-bridges must detach rapidly from actin filaments. The Huxley model discussed earlier in this chapter suggests that the maximal velocity of shortening of a muscle, V_{max}, must be proportional to the rate at which cross-bridges detach from actin. Indeed, the V_{max} of toadfish sonic muscle (about 12 muscle lengths per second) is exceptionally fast—five times higher than that of toadfish red muscle and two and a half times higher than that of toadfish white muscle. (Notice that toadfish white muscle is slower than the white muscles of carp discussed earlier.)

Ultrastructural and biochemical studies of toadfish sonic fibers have revealed adaptations that allow these muscles to contract at such high frequencies. It appears that the short Ca^{2+} transient depends on an unusually high density of Ca^{2+} channels and calcium pumps in the SR membrane, an increased concentration of calcium-binding proteins (e.g., troponin), and a fiber morphology in which the distance between the SR membrane and the myofilaments is particularly short, reducing the time required for diffusion. The rapid release of Ca^{2+} from troponin probably reflects an unusually low affinity of the troponin for Ca^{2+}. Finally, the rapid detachment of cross-bridges implies that the myosin in sonic fibers also has special molecular properties, although this aspect of the fibers remains to be tested.

To emit continuous sound, sonic muscle must do work to overcome frictional energy losses in the sound-producing system and to produce sound energy. Workloop experiments, similar to those described previously for the swim muscles of fish, show that swimbladder fibers can perform positive work at frequencies above 200 Hz at 25°C, the highest frequency for work production ever recorded in vertebrate muscle. By comparison, the highest frequency known for vertebrate locomotory muscles is 25–30 Hz, measured in mouse and lizard fast-twitch muscles at 35°C.

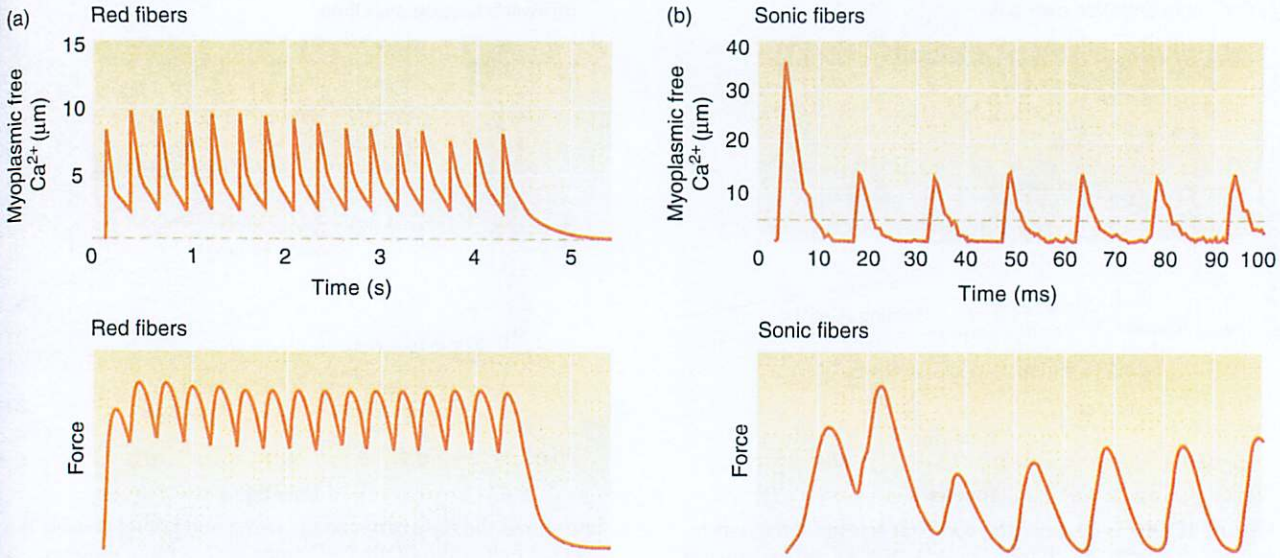


Figure 10-41 Red muscle from a toadfish contracts tetanically in response to relatively low-frequency stimulation, whereas sonic muscle produces individual twitches, even when stimulated at a much higher frequency. **(a)** Myoplasmic free Ca^{2+} in and force generated by a red fiber from a toadfish stimulated at 3.5 Hz. The threshold concentration of myoplasmic free Ca^{2+} necessary for the generation of force is shown by the dashed line on the Ca^{2+} trace. **(b)** Myoplasmic free Ca^{2+} in and force generated by a sonic fiber from a toadfish swimbladder stimulated at 67 Hz. The threshold Ca^{2+} concentration for force production (dashed line) is much higher in the swimbladder sonic fiber than in the red fiber, and the Ca^{2+} transient is fast enough that the concentration falls below the threshold value between each pair of stimuli. Notice the difference in time scale between parts a and b. [Adapted from Rome et al., 1996.]

Rattlesnakes

Rattlesnakes in the genus *Crotalus* also use special noise-making muscles, but as a warning to members of other species rather than to attract conspecifics for mating. Rattling is a loud and effective warning that renders these snakes, like many venomous animals, very conspicuous; the sound is produced when muscle fibers ("shaker fibers") rapidly shake the rattle on the snake's tail. Unlike the periodic toadfish boatwhistle, rattling can go on continuously for as long as 3 hours.

Comparing shaker muscles and sonic muscles illustrates how important individual aspects of fiber function can be. However, because muscle contraction, like most biochemical reactions, is very sensitive to temperature, experimental conditions must be carefully controlled. This comparison is complicated because, whereas toadfish normally live in cool seawater, rattlesnakes are typically desert dwellers. Hence, to match temperature conditions, one muscle type is forced to operate away from its usual temperature. At a typical temperature for a toadfish, 16°C, rattlesnake shaker fibers have a very rapid calcium transient, with a half-width of 4–5 ms, only 1–2 ms slower than that of swimbladder sonic fibers at the same temperature (Figure 10-42a on the next page). In contrast, at 16°C, the half-width of the shaker muscle *twitch* is considerably longer than that of swimbladder muscle (Figure 10-42b). It is likely that the shaker muscle twitch is slower than the sonic muscle twitch, even though the calcium transient is almost the same length, because its cross-bridges detach more slowly. This hypothesis is based on measurements showing that the V_{max} of the shaker muscle at 16°C is about 7 muscle lengths per second, only about half the V_{max} of the swimbladder muscle. In addition, Ca^{2+} may detach from troponin more slowly, although this feature has not been conclusively demonstrated. The properties of rattlesnake shaker muscle demonstrate that a rapid calcium transient is not sufficient for the production of very rapid contractions. The release of Ca^{2+} from troponin and the detachment of cross-bridges from actin filaments must be unusually fast as well. For example, at 16°C, shaker fibers can be stimulated up to only about 20 Hz before summation of contraction begins, with tetanic fusion occurring at about 50 Hz. In contrast, sonic fibers produce individual twitches at 67 Hz at 16°C.

However, we must consider the function of shaker fibers in their usual environment. Many rattlesnakes are active at temperatures above 30°C, and at 35°C *Crotalus* snakes rattle at 90 Hz. At this temperature, the calcium transient and the twitch speed are even faster in the shaker muscle than the frequencies measured in the swimbladder sonic muscle at a toadfish's typical ambient temperature (16°C) (see Figure 10-42). Most likely, both the V_{max} of shaker fibers and the rate at which Ca^{2+} is released from troponin are higher at 35°C than at 16°C. At 35°C, shaker fibers can be stimulated at 100 Hz without going into complete tetanus, and they can perform work at 90 Hz. The similarities in the properties of toadfish swimbladder sonic muscles

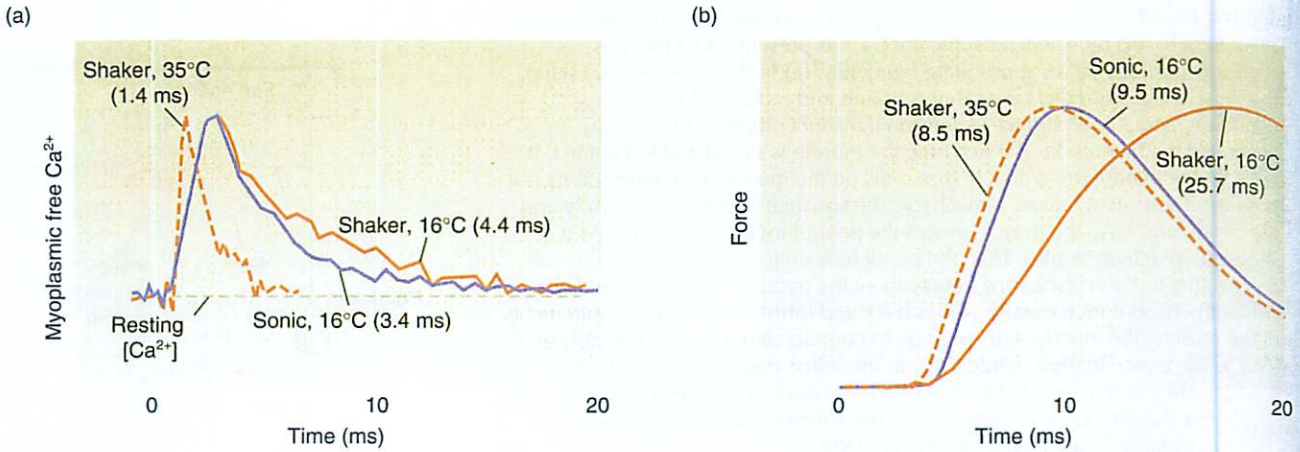


Figure 10-42 Comparing shaker muscle from a rattlesnake with sonic muscle from a toadfish reveals a complex relation between the calcium transient and the length of a twitch. **(a)** Myoplasmic free Ca^{2+} following stimulation of toadfish sonic fibers and rattlesnake shaker fibers at the indicated temperatures. The half-widths of the calcium transient (indicated in parentheses) are quite similar in sonic and shaker fibers at 16°C. At 35°C, a typical ambient temperature for rattlesnakes, the calcium transient in shaker fibers becomes shorter. **(b)** Time course of twitch tension in toadfish sonic fibers and rattlesnake shaker fibers measured under the same conditions as in part a. At 16°C, the half-width of twitch tension is nearly three times longer in shaker fibers than in sonic fibers, even though the calcium transients have about the same half-width. At 35°C, the shaker fibers contract and relax considerably faster than at the lower temperature. The Ca^{2+} concentration and force records are normalized to their maximal values. [Adapted from Rome et al., 1996.]

and rattlesnake shaker muscles suggest that in these species, convergent evolution has arrived at similar solutions to the challenges posed by high-frequency oscillatory contraction.

What difference would you expect to hear in a rattlesnake's rattle between early morning and high noon?

High Power and High Frequency: Insect Asynchronous Flight Muscles

In muscle fibers that contract and relax very rapidly, extremely large calcium fluxes must be supported by many SR Ca^{2+} channels and by large numbers of calcium pumps, which must be powered by large amounts of ATP. To meet the requirement for rapid Ca^{2+} flux across the membrane of the sarcoplasmic reticulum, we would expect the surface area and volume of the sarcoplasmic reticulum to be relatively high. Toadfish sonic fibers provide one such example. In these fibers, Ca^{2+} is removed from the myoplasm 50 times faster than in the red fibers, and about 30% of the entire volume of each fiber is occupied by SR. Furthermore, in fast muscles operating continuously, such as the rattlesnake shaker muscle, only aerobic metabolism can generate enough ATP to fuel the high rate of calcium pumping; thus each fiber might be expected to contain many mitochondria. An extensive SR and numerous mitochondria both take up space within the cell at the expense of space for myofilaments—the structures that generate force. In other words, once again we find a trade-off. If too much space is occupied by the calcium regulatory machinery, there is not enough space left for the contractile apparatus. The energetics of such a fiber might be impressive, but there might be too few myofilaments to generate enough power to do the required work.

Fortunately, producing sounds does not require a lot of force, and in many species, the effort lasts for only short periods of time. The situation is quite different for flight in some insects, however, which depends on muscles that can operate at high frequency and produce considerable power at the same time. We have just made the argument that a muscle *cannot* produce both high power and high frequency. Then how do these insects fly? The answer lies in the cellular properties and anatomic arrangement of their highly specialized flight muscles.

Many species among the Hymenoptera (bees and wasps), Diptera (flies), Coleoptera (beetles), and Hemiptera (true bugs) have special flight muscles that are a notable exception to the rule that only one contraction is evoked by a single AP. Although contraction of the fibers in these muscles is **neurogenic**—that is, it is initiated and maintained by activity in motor neurons—unlike more typical striated fibers, they produce many contractions for each arriving nerve impulse. Consequently, these flight muscles are called **asynchronous muscles** (or sometimes *fibrillar muscles*) to distinguish them from other skeletal muscles that contract in synchrony with APs from their motor neuron.

The first clues that asynchronous flight muscles are special came from observing insect flight. In many small insects, the wingbeat frequency (and hence, you might

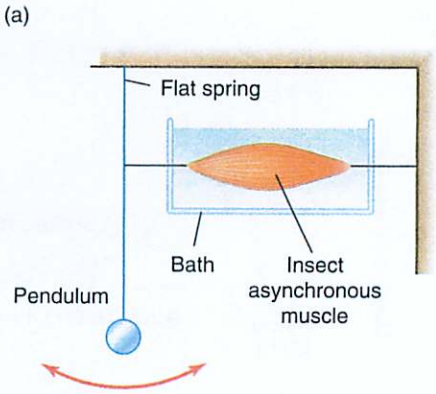


Figure 10-43 A glycerin-extracted asynchronous muscle will contract and relax rapidly and repeatedly if sufficient Ca^{2+} is present *and* it receives mechanical stretch at an appropriate frequency. **(a)** In this experimental setup, the muscle is surrounded by a saline solution and mounted between a pendulum and a fixed surface. A tension transducer monitors the force generated by the muscle. The first time the muscle is stimulated to contract, it pulls on the pendulum, which in turn pulls on the muscle as it returns to its rest position. The system creates a mechanical resonance between the muscle and the pendulum. First, the muscle moves the pendulum by contracting. As it shortens, its activity wanes. Then the pendulum stretches the muscle, reactivating it. If the resonance frequency of the pendulum (that is, the rate at which the pendulum naturally swings back and forth) matches the requirements of the muscle, the muscle will continue to contract and relax rhythmically as long as the concentration of free Ca^{2+} in the saline is sufficiently high. **(b)** Contraction of the asynchronous muscle in the setup shown in part a depends on sufficient Ca^{2+} in the solution bathing the muscle. [Adapted from Jewell and Ruegg, 1966.]

Classic Work

conclude, the frequency of wing muscle contractions) far exceeds the maximal maintained discharge rates of which axons are capable. Wingbeat frequency has been found to vary inversely with wing size. A tiny midge or mosquito, for example, beats its wings at a frequency of 1000 Hz or more, producing the annoying high-pitched sound that causes campers and picnickers to reach for their insect repellent.

Three different aspects of the insect's body combine to produce these rapid oscillations of the wings: first, the physiology of asynchronous flight muscles; second, the anatomic arrangement of the muscles in the thorax; and third, the mechanical properties of the thorax and wing joints. In some respects, asynchronous muscles are very similar to the synchronous flight muscles found in most flying insects. As in all other muscles, the level of myoplasmic free Ca^{2+} must rise above some threshold before the muscle is activated. In addition, neuronal input is required to initiate contraction in both synchronous and asynchronous muscle fibers, and neuronal input regulates the myoplasmic Ca^{2+} concentration in both. However, even if myoplasmic free Ca^{2+} is elevated, the active state is not initiated in asynchronous muscles until the muscle is given a quick stretch. The active state is terminated if tension on the muscle is released. In the presence of a constant concentration of Ca^{2+} higher than 10^{-7} M, a skinned asynchronous muscle actively develops tension following a quick tug, and it will oscillate repeatedly between contraction and relaxation if it is coupled to a mechanical system that oscillates at an appropriate frequency (Figure 10-43). Each asynchronous muscle has a contraction frequency at which it generates the most power, although what tunes the muscle is not yet understood.

In addition to these cellular differences, the mechanics of flight differ considerably between insects with asynchronous flight muscles and most other insects, which fly with conventional skeletal muscles. In insects with synchronous flight muscles (e.g., the damselfly), the wings are elevated and depressed by simple lever mechanics (Figure 10-44a on the next page). One end of the flight muscle is attached to the wing and the other end is attached to the floor of the thorax. Contraction of the elevator muscles causes the wings to move up; contraction of the depressors causes them to move down. The wingbeats of these insects are limited largely to frequencies below 100–200 Hz. (For comparison, the frequency of a hummingbird's wingbeat, which is also driven by synchronous muscles, is in the range of 70 Hz.)

Insects with asynchronous flight muscles have a more complex arrangement. In these insects, contractions of two sets of flight muscles arranged perpendicularly to one another change the shape of the thorax to elevate and depress the wings (Figure 10-44b). Neither set of muscles directly contacts the wings or the wing hinges. Instead, changes in the shape of the thorax act through a complex hinge structure to move each wing. The elevator muscles are attached dorsal to ventral; the depressor muscles run from the anterior to the posterior. Contraction of the elevator muscles flattens the thorax dorsoventrally and lengthens it, and this change in the shape of the thorax acts through the hinges to move the wings up. At the same time, the lengthening

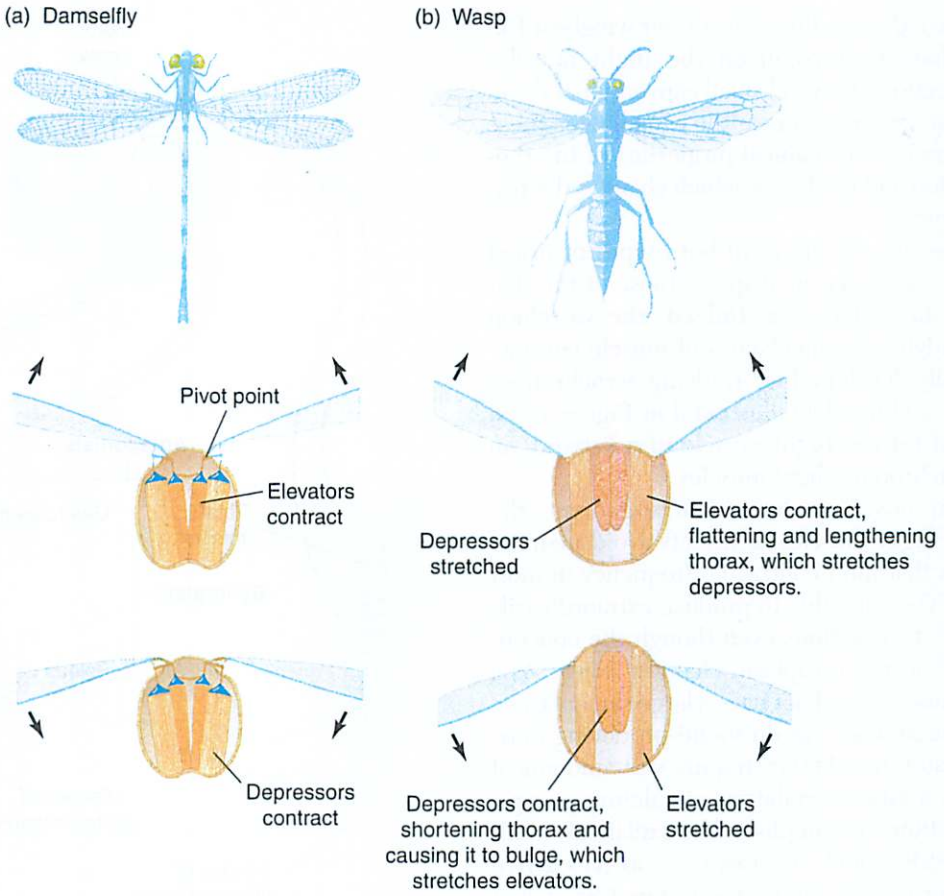


Figure 10-44 The mechanics of insect flight powered by synchronous flight muscles differ from the mechanics of flight powered by asynchronous muscles. **(a)** The wings of a damselfly act like a simple lever, pivoting about a fixed point. The synchronous flight muscles of the wings are arranged so that elevator muscles (contracted in the middle image) and depressor muscles (contracted in the bottom image) work vertically to raise and lower the wings. Both elevator and depressor muscle fibers run dorsal to ventral. **(b)** In contrast, the asynchronous flight muscles of a wasp are arranged roughly perpendicular to each other. In this transverse section through the thorax, elevators have been cut in longitudinal section and depressors cut in cross-section. The elevators run dorsal to ventral, and contraction of these muscles flattens and lengthens the thorax. This change in the shape of the thorax works through a complex hinge to raise the wings. Contraction of the depressors (bottom), which are attached at the anterior and posterior ends of the thorax, causes the roof of the thorax to bulge upward. This change in the shape of the thorax works through the hinge to lower the wings. These alternating changes in the shape of the thorax alternately stretch, and thus activate, the asynchronous elevators and depressors.

of the thorax stretches and activates the depressor muscles, while the reduced length of the elevator muscles reduces the tension on the elevators, causing them to relax. As the depressor muscles contract, the thorax is pulled shorter and becomes thicker from dorsal to ventral; this change in shape acts through the hinges to move the wings downward. In the shortened thorax the elevator muscles are stretched, while the stretch on the depressors is reduced. This alternation between contraction in the elevator and depressor muscles continues as long as conditions are appropriate for the thick and thin filaments of the fibers to slide past each other and generate force. For decades it was believed that the thorax had only two stable conformations, wing-up and wing-down, and that the instability of any state between these two contributed to the mechanical wingbeat. More recent evidence has called this "click" hypothesis into question, and the controversy remains unresolved, but there is agreement that both stretch-based activation of the muscle fibers and the mechanical properties of the thorax contribute to moving the wings.

When electrical stimulation of an asynchronous flight muscle ceases, the muscle membrane repolarizes, the myoplasmic free Ca^{2+} concentration drops, and myosin cross-bridges can no longer bind to actin filaments. If stretch is applied when myoplasmic Ca^{2+} is too low, the muscle fibers cannot be activated, and flight movements cease. Thus the motor neurons innervating asynchronous muscles act largely as an on-off switch, rather than regulating the frequency of contraction. The frequency of the wingbeat depends instead on the mechanical properties of the muscle and the mechanical resonance of the flight apparatus (thorax, muscles, and wings). If a fly's wings are clipped short, for example, its wingbeat frequency increases, even though the frequency of incoming APs remains unchanged. Under

more normal conditions, flies adjust their wingbeat frequency by changing tension on the flight muscles through contraction of several small conventional skeletal muscles that attach on or near the wing hinge. These muscles modify the mechanical properties of the thoracic exoskeleton and the hinge, which changes the resonance frequency.

The force-velocity curves of both types of insect flight muscles are similar in shape to those of the striated muscles in vertebrates. Indeed, the workloop method of studying the mechanics of muscle contraction was initially developed for studying asynchronous flight muscles, and the data illustrated in Figure 10-38 were recorded for the flight muscle of a katydid, an insect with synchronous flight muscles.

With their novel mechanical arrangement, the asynchronous flight muscles of insects avoid many of the constraints that limit contractile frequency in most muscle fibers. They are able to produce extraordinarily high-frequency contractions even though the concentration of Ca^{2+} in the myoplasm changes slowly. As a result, these muscles need not have a large sarcoplasmic reticulum to pump Ca^{2+}, as do sound-producing muscles in vertebrates, nor do they require vast amounts of ATP to power a large population of calcium pumps. Calcium regulation is accomplished by a relatively modest amount of SR, which releases Ca^{2+} as APs arrive along the motor neurons and resequesters Ca^{2+} when APs cease. Asynchronous fibers instead devote much of their volume to force-generating myofilaments, containing only enough mitochondria to supply ATP for the myosin ATPase and the modest amount of SR.

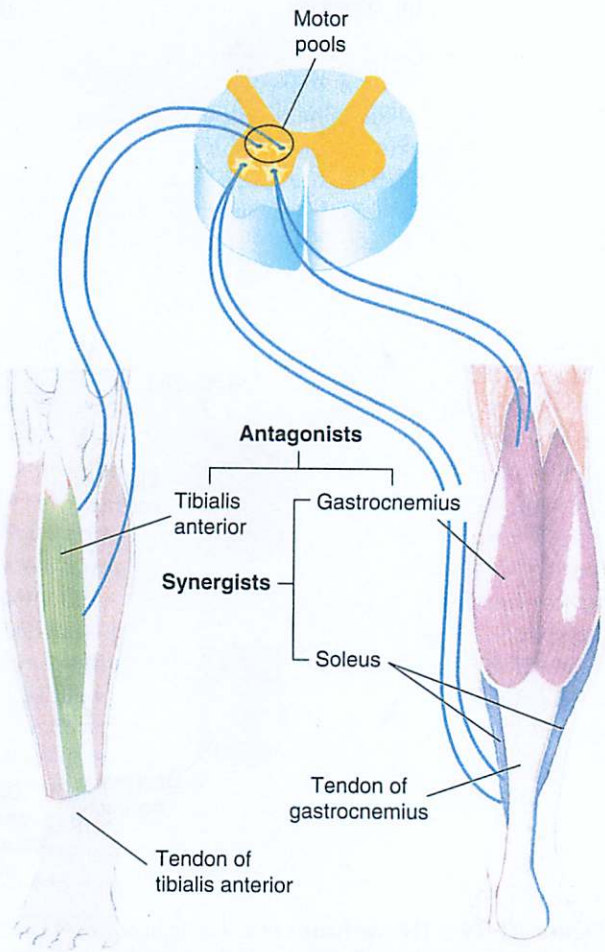


Figure 10-45 Vertebrate muscles are typically arranged in antagonistic pairs; each muscle is innervated by a separate group of motor neurons, called its motor pool. Here the pattern is illustrated for the human calf and foot. The tibialis anterior muscle flexes (closes) the ankle joint when it contracts. Its action is opposed by two other muscles, the gastrocnemius and soleus, which extend (open) the ankle joint. These two muscles are antagonists to the tibialis anterior, and they are synergists to each other because they both produce the same effect on the ankle joint. The motor pools of synergistic muscles tend to be active at the same time. In contrast, if the motor pool of a flexor is active, the motor pool of the antagonistic extensor is likely to be less active.

NEURONAL CONTROL OF MUSCLE CONTRACTION

Effective animal movement requires that the contractions of many fibers within a muscle—and of many muscles within the body—be correctly timed with respect to one another. This coordination is generated within the nervous system, as most muscles contract only when APs arrive at the neuromuscular junction. In addition to controlling the timing of contractions, the nervous system regulates their strength by selecting among different fiber types and by determining how many fibers will be active simultaneously. Several means for achieving fine control of muscle contraction have arisen during the course of evolution. We will use the neuromuscular mechanisms of vertebrates and arthropods as particularly well studied examples of these contrasting mechanisms.

Motor Control in Vertebrates

Vertebrate muscles are typically arranged in antagonistic pairs (Figure 10-45). That is, if a muscle pulls on a joint and causes that joint to close (which would make the muscle a **flexor**), its action is opposed by that of a muscle that causes the joint to open (an **extensor**). Each vertebrate skeletal muscle is innervated by motor neurons whose somata are located in the ventral horn of the gray matter of the spinal cord or in particular parts of the brain. The axon of a spinal motor neuron leaves the spinal cord through a ventral root, continues to the muscle by way of a peripheral nerve, and finally branches repeatedly to innervate skeletal muscle fibers. A single motor neuron may innervate only a few fibers or a thousand or more. However, in vertebrates, each muscle fiber receives input from only one motor neuron.

The collection of motor neurons that innervate a particular muscle is called its **motor pool**. The somata of each motor pool are clustered together in the ventral

horn of a spinal cord segment that is relatively near the location of the muscle. For example, the motor pool of the biceps muscle, which flexes the elbow joint, is located in the cervical spinal cord, whereas the motor pool of the hamstring muscle, which flexes the knee joint, is located in the lumbar spinal cord.

A motor neuron and the muscle fibers that it innervates form a **motor unit.** Motor units in vertebrates typically consist of about 100 muscle fibers, although this number can be much smaller or reach more than 2000 in some locations. The size of motor units in a muscle determines the precision of movement possible for that muscle. The motor units in primate fingers and in the human tongue are extremely small, permitting very finely modulated movements, whereas the motor units in the big muscles of the trunk are very large.

All vertebrate motor neurons are excitatory. They receive an enormous number of synaptic inputs from sensory neurons and from interneurons. In vertebrates, the spinal motor neurons are the only means available for controlling contraction of the muscles, so they have been called "the final common pathway" of neuronal output. When an AP is initiated in a motor neuron as a consequence of its synaptic inputs, the membrane excitation spreads into all of its terminal branches, activating all of its endplates (see Figure 6-12). As we saw in Chapter 6, all vertebrate spinal α-motor neurons produce the neurotransmitter acetylcholine (ACh). When the endplates of a motor neuron are activated, acetylcholine is released onto all of the fibers in the neuron's motor unit. In twitch muscle fibers, the postsynaptic membrane of the neuromuscular junction is typically sufficiently depolarized by a single incoming AP to bring the fiber above threshold (see Figure 10-18), and produce an AP and a twitch in all of the muscle fibers of its motor unit (Figure 10-46a). Whether the resulting contractions are single twitches or sustained contractions depends on the frequency of the APs in the motor neuron. Higher-frequency APs in the muscle fiber produce summation of contraction and hence stronger contractions.

Because APs in a twitch muscle fiber are so tightly correlated with the APs in its motor neuron, there is no gradation in a motor unit between total inactivity and a twitch. If many APs are carried in succession along the motor axon, the result is either partial or full tetanus (see Figure 10-28). In the vertebrates, the problem of how to increase muscle tension in a graded fashion is solved by activating increasing numbers of motor units, as well as by varying the average frequency at which neurons in the motor pool fire. For example, if a small number of motor units in a muscle are maximally active, the muscle will contract with a small fraction of its total maximal tension. On the other hand, if all the motor neurons innervating the muscle are recruited to fire at a high rate, all the motor units are brought into a state of full tetanus, producing the maximal contraction of which the muscle is capable. In addition, many vertebrate muscles contain different types of fibers (see Figure 10-30), which vary in their ability to produce

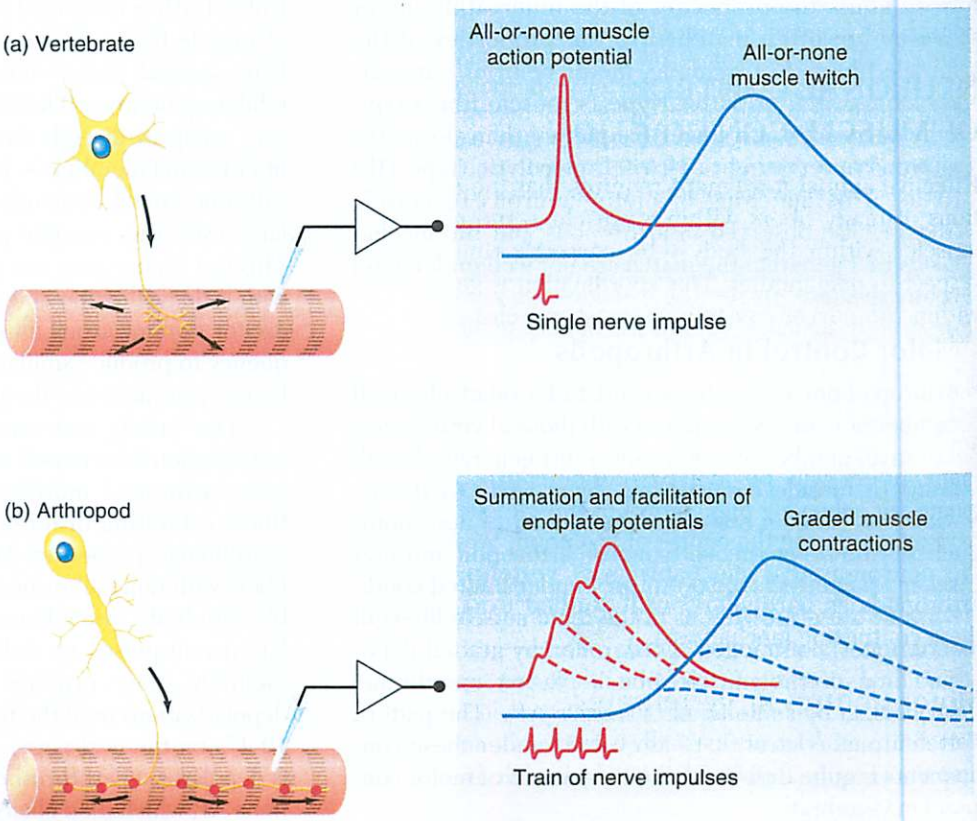


Figure 10-46 Most vertebrate muscles are made up of twitch fibers, whereas fibers in many invertebrate muscles produce graded contractions. **(a)** Vertebrate twitch muscle fibers produce an all-or-none twitch in response to each all-or-none AP traveling along the membrane of the fiber. **(b)** Many arthropod muscle fibers, as well as vertebrate tonic muscle fibers, produce graded contractions in response to the overlapping postsynaptic potentials at multiple motor synapses distributed along the fiber length.

force, so the nervous system can modulate which fibers are active, as well as how many are active. By differentially activating motor neurons, the central nervous system determines the strength and duration of muscle contractions.

The pattern of muscle contraction around a joint depends on the patterns of activity in the motor pools of different muscles. If the motor pool of a flexor muscle at a joint is active, neurons in the motor pool controlling the antagonistic extensor receive inhibitory input. If both motor pools are active simultaneously, the position of the joint will be locked as both muscles pull on the joint. Thus, the orchestration of body movements is rooted in the activity patterns of motor pools within the central nervous system.

The tonic muscle fibers of vertebrates (found primarily in amphibians and lizards) are unusual in that they receive *multiterminal innervation*—that is, a motor neuron makes many synapses along the length of each fiber. (Notice that they do not receive *multineuronal* input.) In these fibers, which lack all-or-none APs, the synaptic potentials produced by the broadly distributed neuromuscular junctions are sufficient to generate graded contractions (Figure 10-46b). The tension produced by these muscles depends strongly on the frequency of incoming motor neuron APs. As we have seen, these tonic muscle fibers generally are found where slow, sustained contractions are required.

As noted already, most vertebrate skeletal muscles contain several types of twitch fibers. Typically, all the fibers within a single motor unit are of the same type. In addition, the properties of the innervating motor neuron are often matched to the properties of the muscle fibers. For example, motor neurons innervating slow-twitch oxidative (type I) muscle fibers typically carry APs at a lower frequency than do motor neurons innervating fast-twitch glycolytic (type IIb) fibers. This matching of motor neurons to muscle fibers is also observed in arthropods, but the mechanisms that generate the match are not well understood in any species.

Motor Control in Arthropods

Arthropod nervous systems consist of a relatively small number of neurons compared with those of vertebrates, so a small number of motor units must generate the full range of muscle contractions, from weak to strong, without relying on extensive recruitment of new motor units. Moreover, many types of arthropod muscles never produce APs, or do so only under limited conditions. In these muscles, as in the tonic muscle fibers of vertebrates, contraction is controlled by graded depolarization of the muscle fiber's plasma membrane, rather than by summation of muscle APs. The pattern of neuronal control that has evolved under these constraints is quite different from the pattern of motor control in vertebrates.

Each vertebrate twitch muscle fiber is typically innervated at only one endplate, at which APs are initiated. The APs then propagate along the muscle fiber. In contrast, crustacean skeletal muscle fibers, like vertebrate tonic fibers, receive many synaptic terminals located along the entire length of the muscle fiber, so no propagated AP is required to spread the signal in the muscle fiber (see Figure 10-46b). Postsynaptic potentials arising along the distributed neuromuscular junctions are summed; the closer together excitatory postsynaptic potentials fall in time and space, the greater the depolarization of the muscle membrane. In vertebrate twitch muscles, Ca^{2+} is released from the sarcoplasmic reticulum in an all-or-none fashion in response to all-or-none APs, whereas in tonic muscles, Ca^{2+} is released from the SR in a graded fashion, because the electrical signals conducted along the membrane are graded rather than all-or-none. Because the coupling between membrane potential and tension is graded, each muscle fiber can produce tension within a wide range, instead of being limited to either all-or-none twitches or tetanus, with few possibilities in between. For this reason, even with very few motor units, arthropod muscles function well over a large range of tensions. The variation in the tension produced by single fibers replaces the recruitment of multiple fibers that is seen in most vertebrate muscles. In some arthropod muscles, one motor neuron may innervate all—or at least most—of the fibers in a muscle, so the entire muscle is one motor unit, yet contraction of the muscle can be finely graded.

In many invertebrates, the flexibility of motor control is further enhanced by multineuronal innervation of muscle fibers. Each muscle fiber receives synapses from several motor neurons, including one or two inhibitory neurons. The effects of inhibitory and excitatory synapses directly sum at the level of the muscle fiber plasma membrane. In these systems, there is typically one excitatory neuron that produces exceptionally large excitatory synaptic potentials in the muscle fiber. This fast exciter axon can generate a strong contraction with little facilitation and summation, whereas the slow exciter axon or axons must fire repeatedly at high frequency to produce similar levels of depolarization and, hence, contraction in the muscle fiber.

The variety and complexity of peripheral motor organization is increased still further by the presence in many arthropod muscles of several types of muscle fibers exhibiting different electrical, contractile, and morphologic properties. At one end of the spectrum are fibers with rapid all-or-none contractions, which resemble vertebrate twitch fibers. When a series of intracellular current pulses are delivered to these fibers experimentally, they produce a series of subthreshold depolarizations until the firing level is exceeded (Figure 10-47a on the next page). Once the plasma membrane is depolarized past threshold, it responds with an all-or-none AP, which then elicits an all-or-none fast twitch. At

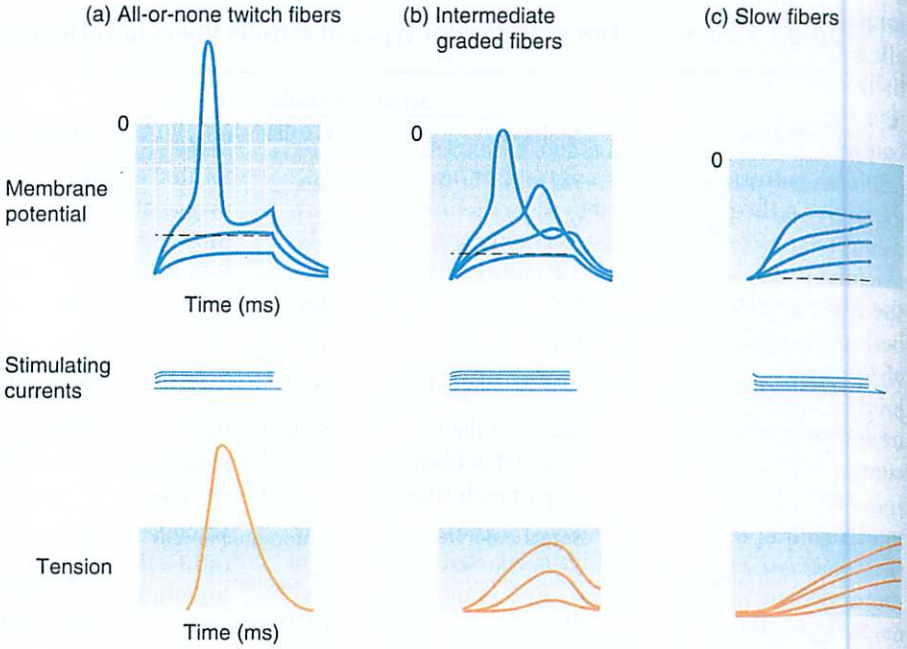


Figure 10-47 Many crustacean muscles contain fibers with highly diverse properties. Shown here are the membrane potentials (top) and tension generated (bottom) in three types of crustacean muscle fibers following intracellular stimulation (middle). **(a)** All-or-none twitch fibers produce APs and fast twitches. **(b)** Intermediate graded fibers produce nonpropagating graded potentials and graded contractions. **(c)** Slow fibers produce only small and slow depolarizations, and they contract very slowly. [Adapted from G. Hoyle, 1967, in *Invertebrate Nervous Systems,* C. A. G. Wiersma, ed. © 1967 by University of Chicago Press.]

the opposite end of the spectrum in crustacean muscle are fibers in which the electrical responses show little sign of regenerative depolarization, and the contractions are fully graded with the amount of depolarization (Figure 10-47c). Between these two extremes is a continuum of intermediate muscle fiber types (Figure 10-47b). The differences in the contractile behavior of these fiber types are correlated with morphologic differences. The slowly contracting fibers have relatively fewer T tubules and less sarcoplasmic reticulum than their more rapidly contracting counterparts. Even more than in the vertebrates, the properties of the motor neurons innervating each type of muscle fiber are at least approximately matched to the properties of the fibers themselves.

Question

What are some potential advantages and disadvantages of the vertebrate pattern of motor control? What are some potential advantages and disadvantages of the arthropod pattern of motor control? Most vertebrates are larger than most arthropods. Might this difference be related to the two patterns?

CARDIAC MUSCLE

The vertebrate heart consists primarily of muscle fibers. Unlike the large and multinucleate skeletal muscle fibers that we have been describing, each cardiac muscle fiber is a small and elongated cell, tapered at both ends, containing a single nucleus. Individual fibers are electrically connected to neighboring fibers by gap junctions, particularly at structures called intercalated disks. In addition, the fibers are tightly bound together by anchoring structures called *desmosomes* (see Figure 4-34). Histologists recognized intercalated disks as a unique structural feature of cardiac muscle long before the role of these junctions was determined. Just like other gap junctions, those of the intercalated disks allow electric current to pass unimpeded between cardiac muscle fibers, an important feature of cardiac physiology.

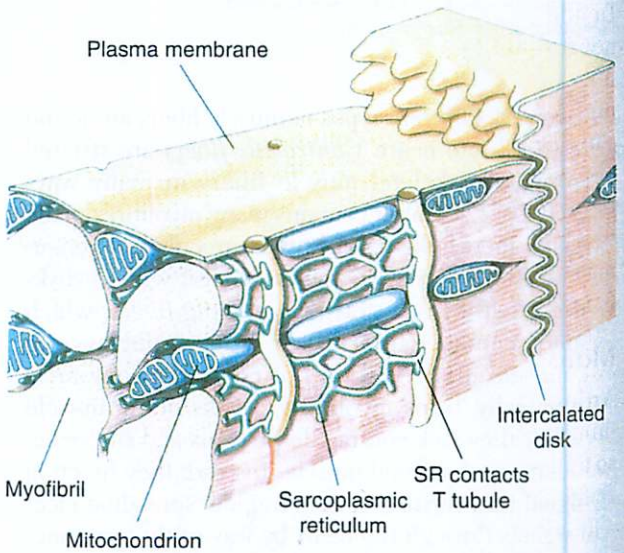


Figure 10-48 Adult mammalian contractile cardiac muscle has an extensive sarcoplasmic reticulum. As in skeletal muscle, T tubules are associated with Z disks and come into close apposition with the SR. Unlike skeletal muscle fibers, cardiac contractile fibers are connected to one another electrically through intercalated disks, at which the extensive membranes of neighboring cells are coupled by numerous gap junctions and held tightly together by desmosomes. [Adapted from Fawcett, 1986.]

Table 10-2 Characteristics of the major types of muscle fibers in vertebrates

	Striated muscle		Smooth (nonstriated) muscle	
Property/component	Skeletal	Cardiac	Multi-unit	Single-unit
Visible banding pattern	Yes	Yes	No	No
Myosin thick filaments and actin thin filaments	Yes	Yes	Yes	Yes
Tropomyosin and troponin	Yes	Yes	No	No
Transverse tubules	Yes	Yes	No	No
Sarcoplasmic reticulum	Well developed	Well developed	Very little	Very little
Mechanism of contraction	Sliding of thick and thin filaments past each other	Sliding of thick and thin filaments past each other	Sliding of thick and thin filaments past each other	Sliding of thick and thin filaments past each other
Innervation	Somatic nerves	Autonomic nerves	Autonomic nerves	Autonomic nerves
Initiation of contraction*	Neurogenic	Myogenic	Neurogenic	Myogenic
Source of Ca^{2+} for activation†	SR	ECF and SR	ECF and SR	ECF and SR
Gap junctions between fibers?	No	Yes	No	Yes
Speed of contraction	Fast or slow depending on fiber type	Slow	Very slow	Very slow
Clear-cut relationship between length and tension	Yes	Yes	No	No

*Neurogenic muscles contract only when stimulated by synaptic input from a neuron. Myogenic muscles endogenously produce depolarizing membrane potentials, allowing them to contract independently of any neuronal input.
†SR, sarcoplasmic reticulum; ECF, extracellular fluid.
Source: Adapted from Sherwood, 2001.

Two very different types of muscle fibers are found in the vertebrate heart. *Contractile fibers* are striated and similar to skeletal muscle fibers in many ways (Table 10-2). Each fiber contains many myofibrils made up of sarcomeres plus an elaborate sarcoplasmic reticulum and T tubules that are associated with Z disks (Figure 10-48). In contrast, *conducting fibers,* which include **pacemaker fibers,** bear little resemblance to most muscle fibers and do not contract. They arise embryonically from myoblasts, as do other muscle fibers, but they lack contractile proteins and the structural features of striated muscle. Instead, they function as a signal-transmission system, rapidly spreading electrical signals through the heart by way of the gap junctions they form among themselves and with contractile fibers.

Heart function will be considered in detail in Chapter 12, but let's look briefly here at the properties of the cardiac muscles. The contraction of heart muscle is **myogenic;** that is, it is initiated in the muscle fibers themselves. An electrical signal arises endogenously in the pacemaker fibers and spreads as APs through the heart by way of gap junctions. Unlike all of the types of skeletal muscle we have been discussing, cardiac muscle does not depend on neuronal input to initiate or sustain contraction. Cardiac muscle fibers do, however, receive input from neurons of the sympathetic and parasympathetic divisions of the autonomic system (see Chapter 8 for more discussion of the autonomic nervous system). This innervation of the contractile fibers produces no discrete postsynaptic potentials; instead, it serves a modulatory role. The strength and rate of cardiac muscle contractions are increased by input from sympathetic neurons and decreased by parasympathetic input.

Although the contractile mechanism of vertebrate cardiac muscle resembles that of skeletal twitch muscle, their membrane APs differ. In contrast to the very brief AP in skeletal muscles, the AP in cardiac contractile muscle fibers has a plateau phase that is hundreds of milliseconds long following the upstroke (Figure 10-49a on the next page; also see Figure 12-7). The long duration of the cardiac-muscle AP, combined with a long refractory period of several hundred milliseconds, prevents tetanic contraction and permits the muscle to relax. As a result of regularly paced, prolonged APs, the

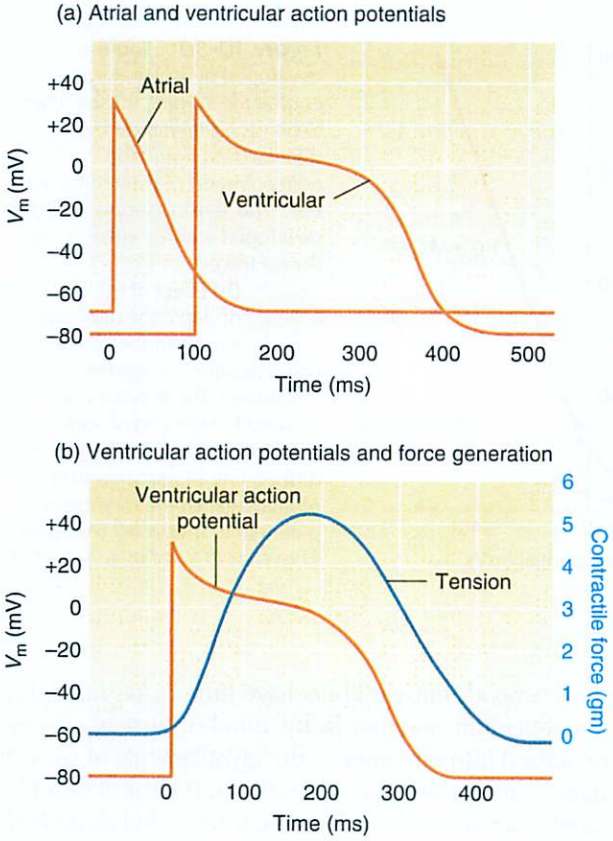


Figure 10-49 Mammalian cardiac action potentials last much longer than skeletal muscle action potentials. **(a)** This graph shows action potentials from muscle fibers in the atria (top two chambers) and ventricles (bottom two chambers) of the heart. Atrial action potentials are shorter than ventricular action potentials and precede them in time by about 100 ms, but the duration of both types of APs greatly exceeds the duration of skeletal muscle APs. **(b)** Action potential and force generation in a ventricular fiber. Notice that the force curve overlaps the AP significantly, which is quite different from skeletal muscle (compare with Figure 10-18). [Adapted from Rhoades and Pflanzer, 1996.]

heart contracts and relaxes at a rate suitable for its function as a pump.

As in skeletal twitch muscle, contraction of cardiac muscle is activated when the cytosolic Ca^{2+} concentration rises. In cardiac fibers, however, the rise in cytosolic Ca^{2+} depends on an influx of Ca^{2+} across the plasma membrane as well as on its release from the sarcoplasmic reticulum. Mammalian cardiac muscle fibers possess an elaborate SR and system of T tubules (see Figure 10-48). Membrane depolarization activates dihydropyridine receptors in the T tubules, allowing an inward flow of Ca^{2+} from the extracellular space. In skeletal muscle, the flux of Ca^{2+} through dihydropyridine receptors is negligible, but in cardiac muscle, the influx of Ca^{2+} triggers the release of a much larger amount of Ca^{2+} from the SR via ryanodine receptors, leading to contraction. Interestingly, the dihydropyridine receptors expressed in cardiac fibers appear to be unable to mechanically affect the function of ryanodine receptors. Instead, release of Ca^{2+} from the SR depends on this calcium-induced calcium release. Calcium is removed rapidly from the cytosol by calcium pumps in the SR membrane and by Na^+/Ca^{2+} exchange proteins in the plasma membrane.

In cardiac contractile fibers, the long plateau of the AP depends on the influx of Ca^{2+} through voltage-gated Ca^{2+} channels. Just as in other types of muscles, the amount of tension that can be developed by a cardiac muscle depends on the amount of Ca^{2+} in the myoplasm, and contraction persists in these fibers as long as Ca^{2+} continues to cross the plasma membrane and be released from the sarcoplasmic reticulum, making the timing of contraction quite unlike that in striated skeletal muscle fibers (Figure 10-49b; compare with Figure 10-18b).

The relative importance of the sarcoplasmic reticulum and the plasma membrane for Ca^{2+} regulation of cardiac muscle varies among species. In frogs, cardiac muscle fibers have only a rudimentary SR and T-tubule system. Most of the Ca^{2+} that regulates contraction in amphibian heart cells enters across the plasma membrane when depolarization opens voltage-gated ion channels. The fibers of the frog heart are much smaller than adult mammalian cardiac muscle fibers, and their resulting large surface-to-volume ratio appears to reduce the need for an elaborate intracellular SR to store, release, and resequester Ca^{2+}. When muscle cells from a frog heart are depolarized experimentally, Ca^{2+} flows into the cell through open Ca^{2+} channels in the depolarized membrane. Because the influx of Ca^{2+} is voltage-dependent, the amount of tension developed depends on the amount of depolarization, with greater induced depolarization producing greater tension (Figure 10-50a). Reducing the extracellular Ca^{2+} concentration causes a weaker contraction for a given amount of depolarization, because the reduced pool of available Ca^{2+} reduces the driving force on Ca^{2+}, and fewer ions enter the cell (Figure 10-50b). In contrast, reducing the amount of extracellular Ca^{2+} has little or no effect on the contractile force of a mammalian heart, because most of the rise in intracellular Ca^{2+} in these fibers is caused by release from the SR.

The intracellular Ca^{2+} concentration in cardiac muscle is determined not only by depolarization but also by a number of other factors, including the action of catecholamines on the heart. The catecholamines epinephrine and norepinephrine, which circulate in the blood or are released from neuron terminals, activate α- and β-adrenoreceptors on the surface of cardiac muscle cells and augment cardiac contractile force. Stimulation of α-adrenoreceptors activates the inositol phospholipid second-messenger system (see Figures 9-23 and 9-24), resulting in increased Ca^{2+} release from the sarcoplasmic reticulum. β-adrenoreceptor stimulation activates the adenylate cyclase second-messenger system (see

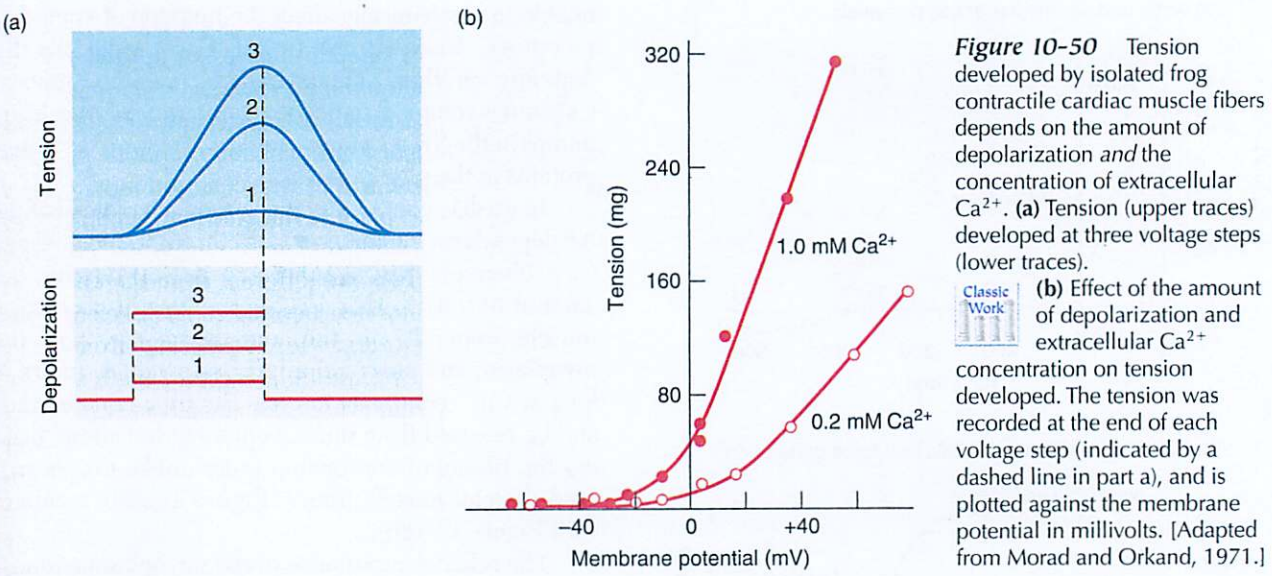


Figure 10-50 Tension developed by isolated frog contractile cardiac muscle fibers depends on the amount of depolarization *and* the concentration of extracellular Ca^{2+}. **(a)** Tension (upper traces) developed at three voltage steps (lower traces). Classic Work **(b)** Effect of the amount of depolarization and extracellular Ca^{2+} concentration on tension developed. The tension was recorded at the end of each voltage step (indicated by a dashed line in part a), and is plotted against the membrane potential in millivolts. [Adapted from Morad and Orkand, 1971.]

Figures 9-20 and 9-21), resulting in increased calcium flux across the plasma membrane.

SMOOTH MUSCLE

A large class of muscles are called *smooth muscles* because they lack sarcomeres and do not appear striated when viewed with a microscope. Many smooth muscles are found in the walls of hollow organs—for example, in the alimentary canal, the urinary bladder, the uterus, and many blood vessels. Smooth muscle primarily supports visceral functions, rather than locomotion and other behavior. It has some properties in common with skeletal striated muscle, others in common with cardiac striated muscle, and some features that are unique to smooth muscle. A major impediment to a brief description of smooth muscles is their heterogeneity. Unlike striated muscles, all of which function pretty much alike, smooth muscles can be divided into subclasses that are quite different from one another. Smooth muscle is less well understood than striated muscle, but some generalizations can be made. Many smooth muscles function more independently of the nervous system than striated skeletal muscles do, and all are innervated by neurons of the autonomic nervous system, rather than the voluntary nervous system. Many smooth muscles can produce more force per cross-sectional area than striated muscles, and some can generate prolonged contractions that require much less energy per unit time than would be required by striated muscles.

Like striated muscle, smooth muscle contracts when actin and myosin filaments slide past each other, pulled by myosin cross-bridges. As in cardiac muscle, each fiber is an individual small cell containing a single nucleus. Unlike either class of striated muscle fibers, many smooth muscle fibers have little or no sarcoplasmic reticulum, and they lack T tubules. Instead of being organized into sarcomeres, the myofilaments of smooth muscle are gathered into bundles of thick and thin filaments that are anchored in structures called *dense bodies,* or they are connected to the inside surface of the plasma membrane at sites called *attachment plaques* (Figure 10-51 on the next page). Attachment plaques contain high concentrations of α-actinin, which is also found in the Z disks of skeletal muscle, and the protein vinculin, which is not. In smooth muscle fibers, vinculin anchors actin filaments to the plasma membrane by binding α-actinin.

The myofilaments of many smooth muscles are arranged helically within the fibers. Compare this pattern with the musculature of a fish to consider whether this geometric arrangement produces a mechanical advantage for these fibers.

Vertebrate Single-Unit and Multi-Unit Smooth Muscles

Vertebrate smooth muscles can be divided into two categories, single-unit and multi-unit, depending on how contraction arises and is controlled (see Table 10-2). In **single-unit smooth muscles**, the individual muscle cells, like cardiac fibers, are typically small, elongated, and tapered at both ends. They are coupled with one another through electrically conducting gap junctions. If one or a few of the cells spontaneously depolarize, the rest of the cells depolarize soon afterward as excitation is passed from fiber to fiber through the gap

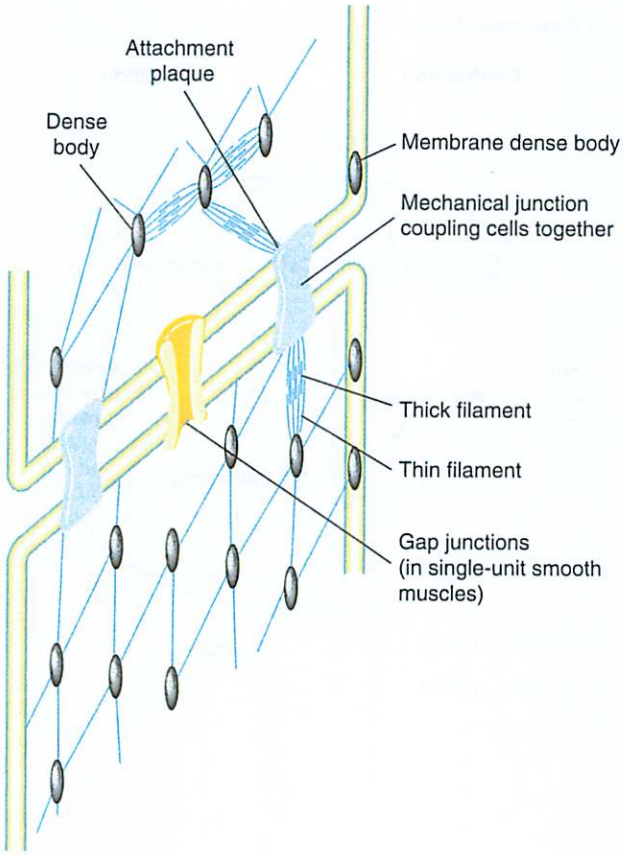


Figure 10-51 In smooth muscle, thin and thick filaments interdigitate, but they are not organized into sarcomeres. Parts of two fibers are shown in this diagram. Thin filaments are anchored to dense bodies and to dense areas abutting the membrane (attachment plaques); thick filaments lie between thin filaments. The two fibers illustrated are coupled by gap junctions, indicating that these fibers are electrically coupled. In addition, fibers are held together by strong mechanical junctions. [Adapted from Berne and Levy, 1998.]

junctions. As in cardiac muscle, the contraction of these muscles is myogenic, and the activation of a few fibers can generate contraction that moves throughout the entire organ in a wave. One example is the wavelike peristaltic contraction of the intestinal tract that propels food along the intestine. These muscles are called "single-unit" because the entire set of fibers behaves as a unit, rather than as a set of independently controlled fibers. Autonomic neurons synapse onto single-unit muscle fibers and can modulate the rate, strength, and frequency of contraction, but neuronal input is not required to initiate contraction. Single-unit smooth muscle forms the walls of vertebrate visceral organs (e.g., the alimentary canal, urinary bladder, ureters, and uterus).

In contrast, the cells in **multi-unit smooth muscles** act independently and contract only when stimulated by neurons, or in some cases hormones; in other words, their contraction is neurogenic. These fibers are not coupled to one another by gap junctions. The muscles that regulate the diameter of the pupil in the iris of the eye are multi-unit smooth muscles, as are the smooth muscles in the walls of blood vessels.

The smooth muscle of vertebrates is generally under autonomic or hormonal control and is not controlled "voluntarily" in the way contraction of skeletal muscle is (one exception to this rule may be the urinary bladder). The synapses of autonomic neurons with smooth muscle fibers are different from the endplates formed by the motor neurons that control skeletal muscle fibers. Neurotransmitter is released from many swellings, called *varicosities,* along the length of autonomic axons running within the smooth muscle tissue. Transmitter released from a varicosity diffuses over some distance, encountering a number of smooth muscle cells along the way. Receptor molecules on the smooth muscle cells appear to be distributed diffusely over the cell surface. Notice that this pattern is somewhat similar to that of neuromodulatory synapses, described in Chapter 6.

Regulation of Smooth-Muscle Contraction

As in striated muscle fibers, the cyclic binding and unbinding of myosin and actin myofilaments in smooth muscle depends on the presence of free Ca^{2+} in the cytoplasm. Most smooth muscle cells contract and relax far more slowly than striated muscle fibers and are capable of more sustained contraction. This difference in contraction kinetics is reflected in the duration and amplitude of the cytosolic Ca^{2+} pulse. Excitation-contraction coupling operates more slowly, and by different mechanisms, in smooth muscle than it does in striated muscle. The slow release and uptake of Ca^{2+} in smooth-muscle cells is associated with a relatively undeveloped sarcoplasmic reticulum, which is composed only of smooth, flat vesicles located close to the inner surface of the plasma membrane. Because the cells are small and slender, no point in the cytoplasm is more than a few micrometers away from the plasma membrane. As a result, diffusion of Ca^{2+} between the membrane and the myofilaments seems sufficient for regulating the slow contraction of smooth muscles, and the plasma membrane of smooth muscle cells performs calcium-regulating functions similar to those of SR membranes in striated muscle.

In smooth muscle cells—as in all cells—Ca^{2+} is actively and continuously pumped outward across the plasma membrane, keeping the cytosolic Ca^{2+} concentration very low. When the membrane is depolarized, voltage-gated Ca^{2+} channels open, permitting an influx of Ca^{2+}, which activates contraction. Relaxation occurs when the Ca^{2+} permeability returns to its low resting level and pumps in the plasma membrane push Ca^{2+} back out of the cell. In some smooth muscles, although not all, depolarization of the plasma membrane generates APs in which Ca^{2+} carries the inward current, causing the cytosolic Ca^{2+} to rise sharply. As in other muscle

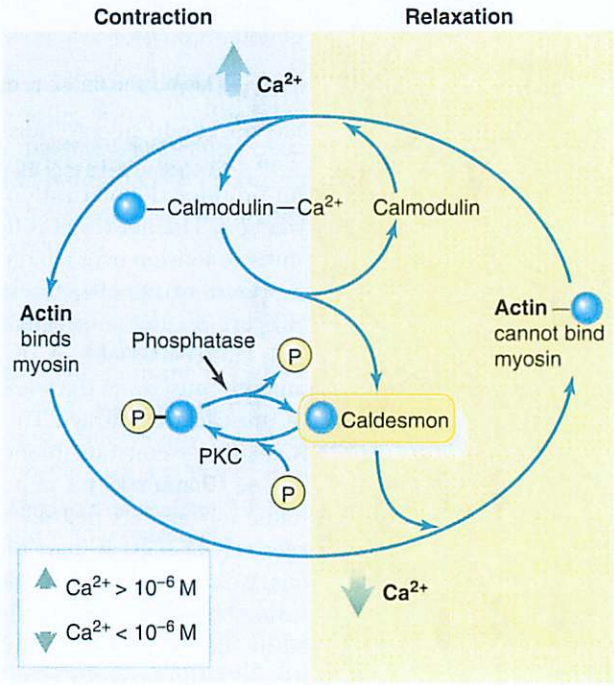
types, the tension generated is proportional to the intracellular level of Ca^{2+}.

The regulation of contraction in smooth muscle has been found to depend on a variety of mechanisms, unlike that in striated muscle, in which troponin and tropomyosin control access to myosin binding sites. Smooth muscles in general lack troponin. Instead, another filamentous protein, **caldesmon**, binds to thin filaments in smooth muscle, preventing binding between myosin and actin. Caldesmon is removed from the thin filaments by either of two events. One depends on yet another protein, **calmodulin**, which is a calcium-binding protein that has been found in a large number of cell types and is implicated in the control of a number of metabolic pathways and other cellular functions regulated by calcium. When Ca^{2+} binds to calmodulin and the Ca^{2+}/calmodulin complex binds to caldesmon, myosin cross-bridges are permitted to bind to the thin filaments (Figure 10-52a). In addition, caldesmon may be phosphorylated by an enzyme called *protein kinase C*. Phosphorylated caldesmon cannot bind to thin filaments and therefore does not inhibit myosin-actin interactions.

Three other mechanisms for regulating smooth-muscle contraction depend on the regulatory light chains of myosin (Figure 10-52b). In vertebrate smooth muscle and in some invertebrate muscles, binding of Ca^{2+} directly to the myosin regulatory light chains induces a conformational change in the myosin head that allows it to bind to actin, and the muscle contracts. Phosphorylation of myosin light chains by *myosin light-chain (LC) kinase* is another pathway to contraction in vertebrate smooth muscle. Myosin LC kinase is activated by Ca^{2+}/calmodulin, making the rate at which the myosin light chains are phosphorylated calcium-dependent. In a third mechanism, phosphorylation of another site on the myosin regulatory light chain by protein kinase C induces a conformational change that prevents actin-myosin interactions, resulting in relaxation (see Figure 10-52b, bottom). The slow actions of the protein kinases, along with the slow changes in cytosolic Ca^{2+} levels, contribute to the slow rate of contraction seen in many smooth muscles.

Smooth-muscle contraction is modulated by a wide variety of factors, both neuronal and humoral, which can inhibit or activate contraction. All of these factors operate to influence the cytosolic Ca^{2+} concentration or the activity of protein kinase C, myosin LC kinase, and myosin phosphatases. The diverse mechanisms that

(a) Regulation of actin



(b) Regulation of myosin light chains

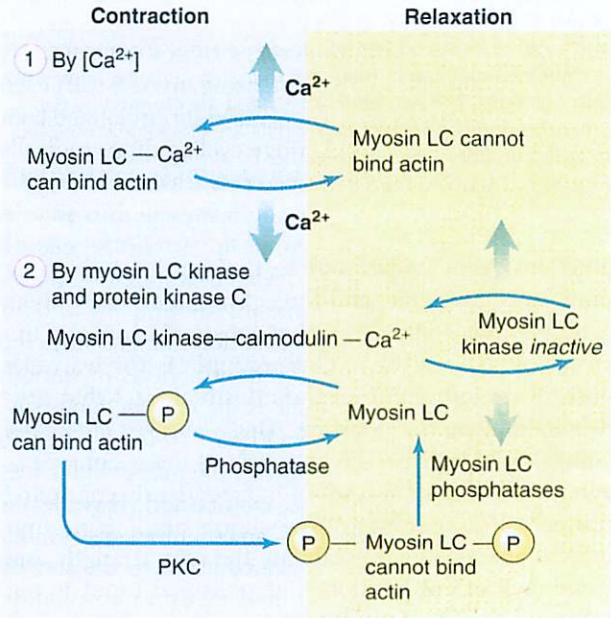


Figure 10-52 Both actin-dependent and myosin-dependent mechanisms control smooth-muscle contraction and relaxation. **(a)** Binding of caldesmon to the actin of thin filaments prevents contraction. At cytosolic Ca^{2+} levels above 10^{-6} M, Ca^{2+}/calmodulin complexes form. When this complex binds to caldesmon, myosin binding sites are revealed on actin, allowing the muscle to contract. Phosphorylation of caldesmon by protein kinase C (PKC) also prevents it from binding to thin filaments and promotes contraction. **(b)** Binding of Ca^{2+} to myosin regulatory light chains (1) allows actin-myosin binding and promotes contraction. Phosphorylation of myosin regulatory light chains (2) by myosin LC kinase, which is activated by Ca^{2+}/calmodulin, also promotes contraction. Phosphorylation of myosin regulatory light chains by PKC at a different site inhibits myosin-actin interactions and causes smooth-muscle relaxation. [Adapted from Lodish et al., 1995.]

control smooth muscle result in the complex patterns of contraction observed in this type of muscle.

Unusual Features of Smooth-Muscle Contraction

An interesting feature of some vertebrate smooth muscle is its sensitivity to mechanical stimulation. Stretching these muscles can produce depolarization, which in turn produces contraction. The net result is to maintain relatively constant muscle tension over a large range of fiber lengths. The response of smooth muscle to stretch accounts, at least in part, for the autoregulation seen in small blood vessels called arterioles. A rise in blood pressure stretches smooth muscles in the walls of arterioles, which leads the muscles to contract. This response helps to maintain relatively constant blood flow in the peripheral tissues (see Chapter 12 for more details). Likewise, the peristaltic movements that push food along the intestinal tract rely at least in part on stretch-induced contractions of single-unit smooth muscles. This "stretch activation" might seem to indicate physiological similarities between vertebrate smooth muscle and the striated asynchronous flight muscles of insects, but we do not yet understand enough about either kind of contraction to know whether the similarity is more than superficial.

In both vertebrates and invertebrates, some smooth muscles are specialized to maintain the contracted state for long periods of time while expending a minimum amount of energy. In striated muscle, tension can be maintained only if cross-bridges continuously bind to and unbind from actin, using up ATP with each cycle. In smooth muscles specialized for prolonged contraction, the rate of cross-bridge cycling drops radically when contraction is prolonged, drastically reducing the energy cost. In vertebrate smooth muscle, this state is called "latch," whereas in some invertebrate smooth muscle, it is called "catch." The two states may well arise by different mechanisms.

The initial phase of a contraction that goes into the latch state appears similar to contractions in smooth muscles that do not latch, but as the contraction is sustained, the picture changes (Figure 10-53a). After an initial surge in cytoplasmic Ca^{2+}, the intracellular free Ca^{2+} drops, yet the force is maintained. How is this accomplished? As yet there is no complete answer, but one hypothesis is that the balance between the activity levels of myosin LC kinase and myosin phosphatase determines the rate at which cross-bridges cycle. According to this hypothesis, as a fiber enters into latch, the activation of both enzymes drops, causing cross-bridges to cycle more slowly. Most cross-bridges enter the bound state and stay there. In this state, the number of bound cross-bridges remains high, so the static force produced by the muscle is large, but the amount of ATP hydrolyzed goes down because few cross-bridges detach. However, evidence is accumulating that components of the thin filaments in smooth muscle—particularly caldesmon and calmodulin—play a role in establishing latch, so a full explanation awaits further experimentation. Whatever the explanation, latch allows very thrifty generation of tension. In this state, a smooth muscle may use only 0.3% of the

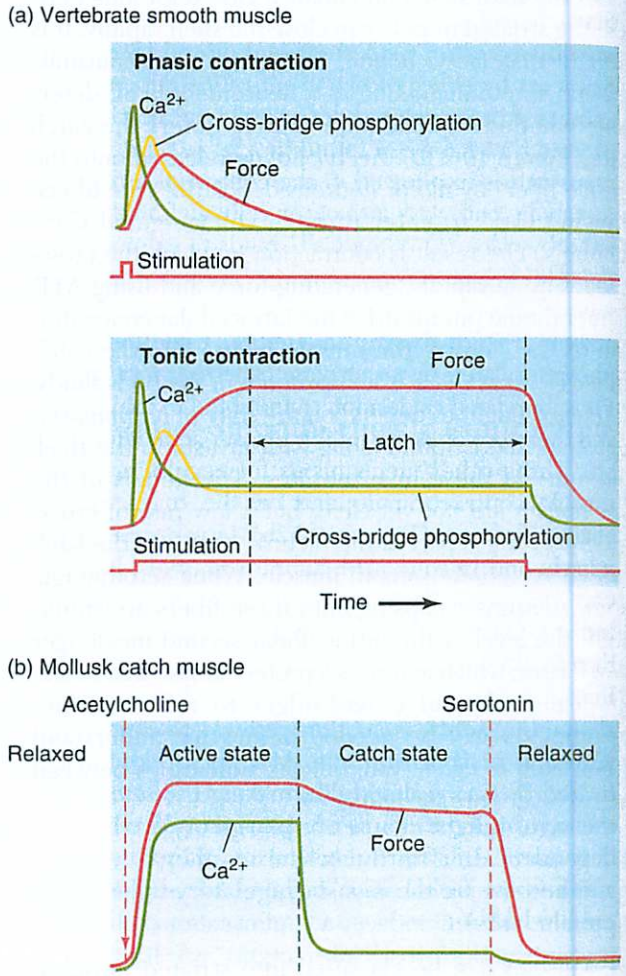


Figure 10-53 Some smooth muscles are able to generate force very economically over long periods of time. **(a)** Events in a vertebrate smooth muscle during a typical short-term (phasic) contraction and during a long-term (tonic), or "latch," contraction. In both cases, contraction begins with a pulse of intracellular Ca^{2+}. In the phasic contraction, cross-bridges are phosphorylated for a short time, and force is generated, but both phosphorylation and force dissipate quickly. When the muscle goes into the latch state, although intracellular Ca^{2+} drops to a level, cross-bridges remain phosphorylated and the generation of force continues for much longer. **(b)** Control of the contractile state in a mollusk catch muscle. Contraction is initiated by cholinergic synaptic input, which causes an increase in intracellular Ca^{2+}, permitting cross-bridges to cycle. When intracellular Ca^{2+} drops, the muscle enters the catch state, in which cross-bridges remain bound but cycle very slowly. Input from a serotonergic motor neuron releases the muscle from catch through a process that depends on an increase in intracellular cAMP. [Part a adapted from Berne and Levy, 1998; part b adapted from Withers, 1992.]

energy that a striated muscle would require to do the same job.

The classic catch muscles are the smooth muscle components of the adductor muscles that hold together the two shells of bivalve mollusks. Typically, the adductor muscle also includes a section of striated skeletal muscle. The smooth-muscle in the adductor closes the shell slowly and holds it closed for long periods; the striated muscle can close the shell rapidly. It is thought that acetylcholine, acting as a neurotransmitter, and serotonin, acting as a neuromodulator, determine whether the adductor muscle enters the catch state (Figure 10-53b). Acetylcholine released onto the muscle fibers by motor neurons depolarizes the fibers, allowing Ca^{2+} to enter through voltage-gated Ca^{2+} channels. The result is contraction in which the cross-bridges cycle rapidly, generating force and using ATP. As membrane pumps drive the intracellular concentration of Ca^{2+} down, the muscle moves into the catch state as long as there is no serotonin in the body fluids. Catch is thought to depend on the state of *paramyosin,* a protein that is found along with myosin in the thick filaments of these invertebrates. The details of the mechanism are not yet clear, but a low rate of cross-bridge cycling is part of the picture, as it is in the latch state of vertebrate smooth muscle. When serotonergic motor neurons synapsing onto these fibers are stimulated, the level of the intracellular second messenger cAMP rises, which activates a protein kinase that somehow causes bound cross-bridges to release. More experiments will be needed before we understand either latch or catch well, but the similarities between the two states are intriguing.

SUMMARY

■ Muscles can be classified into striated muscles, which appear striped when viewed with a light microscope, and smooth muscles, which lack this striated appearance.

■ Muscles produce movement of the body or within the body by generating force and in many cases becoming shorter, a process that is based universally on two proteins: actin and myosin. This process is called contraction.

Essentials of skeletal muscle contraction

■ Each skeletal muscle fiber is a large multinucleated cell containing many subunits called myofibrils. Each myofibril is made up of sarcomeres arranged end-to-end.

■ The sarcomere is the functional unit of vertebrate skeletal muscle. Within each sarcomere, thick filaments composed principally of myosin interdigitate with thin filaments composed primarily of actin. Thin filaments are anchored at the ends of each sarcomere in Z disks.

■ Muscle fibers generate force when the sarcomeres shorten. Sarcomeres shorten as thin and thick filaments slide past each other, pulling the Z disks closer together.

■ Thick and thin filaments slide past each other, generating force, as the "heads" of myosin molecules repeatedly make and break bonds with actin in the thin filaments; in effect, the thick filaments "row" themselves along the thin filaments by means of these cross-bridges.

■ The amount of force a muscle generates depends directly on the number of bound cross-bridges.

■ The amount by which thick and thin filaments overlap, which determines how many cross-bridges can bind, determines the maximum amount of tension a muscle can generate. This geometry produces the length-tension relation of both individual sarcomeres and whole muscles.

■ The energy to do the work of muscle contraction is derived from the hydrolysis of ATP by myosin.

Mechanics of muscle contraction

■ In isometric contraction, a muscle generates force but does not shorten. In isotonic contraction, a muscle generates constant force and becomes shorter.

■ A muscle shortens fastest when it is unloaded. As the load increases, the velocity of shortening decreases, until at high loads the muscle cannot shorten at all (isometric contraction).

■ The force-velocity relation of muscle can be explained in terms of cross-bridge binding properties.

Regulation of muscle contraction

■ Myosin cross-bridges can bind to actin only when binding sites are available. In resting muscle, the myosin binding sites on the actin thin filaments are covered by the protein tropomyosin.

■ Tropomyosin moves away from the myosin binding sites when Ca^{2+} binds to troponin, another protein associated with thin filaments.

■ A striated muscle fiber contracts when an AP travels along its plasma membrane. Through the mechanism of excitation-contraction coupling, the AP is linked to a rise in intracellular free Ca^{2+}.

■ During muscle activation, the concentration of intracellular free Ca^{2+} increases as much as a hundredfold, Ca^{2+} binds to troponin, tropomyosin shifts on the thin filaments, myosin binding sites are revealed on actin, and the cyclic binding of myosin cross-bridges pulls the thick and thin filaments past each other.

■ In vertebrate striated muscle, the rise in intracellular free Ca^{2+} depends on release of Ca^{2+} from intracellular stores in the sarcoplasmic reticulum.

- Calcium is released from the SR when an AP travels along the plasma membrane and into the muscle fiber through a network of T tubules.

- An AP activates dihydropyridine receptors in the T-tubule membrane. Dihydropyridine receptors are themselves voltage-gated Ca^{2+} channels, but in vertebrate skeletal muscle fibers, little or no Ca^{2+} enters the cell through these channels. Instead, the activated dihydropyridine receptors mechanically open ryanodine receptor Ca^{2+} channels in the SR membrane, allowing Ca^{2+} to escape. In contrast, in cardiac muscle fibers, Ca^{2+} enters through the dihydropyridine receptors and contributes to calcium-induced calcium release from the SR.

- Following an AP, myoplasmic free Ca^{2+} returns to its resting level when ryanodine receptor channels close and calcium pumps in the SR membrane resequester Ca^{2+} in the SR.

Energetics of muscle contraction

- ATP is required for two crucial processes in muscle contraction: breaking the myosin-actin bond and powering the calcium pumps that resequester Ca^{2+} in the sarcoplasmic reticulum.

- ATP in muscle fibers is generated by way of oxidative metabolism, glycolysis, and the direct phosphorylation of ADP by creatine phosphate.

Fiber types in vertebrate skeletal muscle

- Vertebrate skeletal muscle fibers can be classified based on how fast they contract and how fast they fatigue.

- Type I slow-twitch fibers contract slowly, fatigue very slowly, and depend largely on oxidative metabolism for ATP production. Type IIb fast-twitch fibers contract rapidly, fatigue rapidly, and depend largely on glycolysis for ATP production. Type IIa fast-twitch fibers contract relatively rapidly, fatigue relatively slowly, and can usually meet their energy needs through oxidative metabolism.

- There typically are trade-offs between strength of contraction, speed of contraction, and efficiency in using ATP. No one muscle type is best for all tasks. Evolution has produced elegant matching of task and fiber type in many animals.

Neuronal control of muscle contraction

- Each vertebrate skeletal muscle fiber is controlled by a single motor neuron, although one motor neuron can innervate a few or a large number of muscle fibers.

- All vertebrate motor neurons are excitatory. Vertebrate skeletal muscles contract only when they receive synaptic input from their innervating motor neuron, making the motor neurons the final common pathway of behavioral control.

- The pattern of muscle contraction—its strength, duration, and speed—is thus determined by the pattern of activity in motor neurons controlling contraction of the many fibers in a muscle.

- In contrast, invertebrate muscle fibers are typically innervated by multiple motor neurons; some of these neurons are excitatory and some inhibitory. The pattern of muscle contraction is determined by summation of the many synaptic inputs to the muscle fiber.

Cardiac muscle

- Cardiac contractile muscle fibers are striated and have many features in common with vertebrate skeletal muscle. However, they have some major differences: they are small, contain a single nucleus, and are coupled to other fibers by way of gap junctions.

- Cardiac conducting fibers lack contractile machinery. Instead, they serve as a conducting system, spreading depolarization throughout the heart by way of gap junctions that link the conducting fibers to one another and to contractile fibers.

- The signal initiating contraction in the heart is myogenic; it arises in pacemaker fibers and spreads to the rest of the heart through gap junctions. Autonomic neurons modulate contraction of cardiac muscle, but are not required to initiate it.

- The APs of cardiac contractile fibers are prolonged and include a significant plateau phase based on a Ca^{2+} current through the plasma membrane. The direct role played by this Ca^{2+} in regulating contraction varies from relatively minor in the mammalian heart to enormously significant in the frog heart.

Smooth muscle

- Smooth muscles form a large and varied group. They are typically found in the walls of hollow organs.

- Smooth muscles lack sarcomeres, although their contraction is based on myosin thick and actin thin filaments that slide past each other.

- Contraction of some smooth muscles (called single-unit muscles) is myogenic and consists of fibers that are electrically coupled to one another. Contraction of other smooth muscles (called multi-unit muscles) is neurogenic; fibers of these muscles are not coupled electrically.

- Contraction of smooth muscles depends on increased intracellular free Ca^{2+}, but the means by which Ca^{2+} regulates binding between thick and thin filaments are varied and complex.

- Some smooth muscles are specialized to hold tension for very long periods of time while using much less ATP than a skeletal or cardiac muscle would.

REVIEW QUESTIONS

1. Describe the organization and components of each of these structures: myofilaments, myofibrils, muscle fibers, and muscles.
2. What kinds of evidence led A. F. Huxley and H. E. Huxley to propose the sliding-filament hypothesis?
3. Draw a sarcomere and label its components. Briefly state the function of each component.
4. Discuss the contributions of myosin, actin, troponin, and tropomyosin to contraction of striated muscle.
5. Predict the shape of the sarcomere length-tension graph of a muscle with the following filament dimensions: thick filament, 1.6 μm; bare zone, 0.4 μm; thin filament, 1.1 μm.
6. Why do muscles become rigid several hours after an animal dies (rigor mortis)?
7. How do myosin cross-bridges produce the force that causes the thick and thin filaments to slide past each other?
8. When a muscle is shortening at V_{max}, what is the net force generated by its cross-bridges? What is the power produced? (Hint: See Figure 10-14.)
9. Why does the velocity of shortening decrease as heavier loads are placed on a muscle?
10. Explain how intracellular free Ca^{2+} is regulated in striated muscle fibers and how it controls their contraction.
11. List the steps of muscle activation and relaxation in striated muscle. List the same steps for smooth muscle.
12. How does depolarization of the plasma membrane of a striated muscle fiber cause the release of Ca^{2+} from the sarcoplasmic reticulum? (There are two mechanisms.) What molecules play a role in each mechanism?
13. What are the major processes in muscle function that require ATP?
14. What limits the tension that can be produced by a myofibril? By a muscle fiber? By a muscle?
15. What allows a muscle fiber to produce greater tension during tetanic contraction than during a single twitch?
16. Define *mechanical power*. Define *efficiency*. Why are mechanical power and efficiency equal to zero during isometric contractions and when a muscle shortens at V_{max}?
17. During locomotion, what is the disadvantage of using a muscle that is too slow (V_{max} is too low) to power a movement requiring a given shortening velocity? What is the disadvantage of using a muscle that is too fast (V_{max} is too high)? What is an optimal value of V_{max}?
18. Describe the features of the fish muscular system that enable a fish to produce both relatively slow movements with little backbone curvature and very fast movements with large backbone curvature.
19. Why must the muscles that produce sound relax very rapidly? What are some adaptations that permit a muscle to relax quickly?
20. Why is a large energetic cost associated with rapidly relaxing muscles? How do insect asynchronous muscles avoid some of this cost?
21. What factors determine frequency of contraction in insect asynchronous muscle?
22. Compare and contrast the neuronal control of vertebrate twitch muscle fibers and arthropod muscle fibers.
23. Compare and contrast skeletal, smooth, and the two types of cardiac muscle fibers with respect to structure, excitation-contraction coupling, speed of contraction, relaxation, and energetics.
24. What are the advantages of latch and catch in smooth muscle?

SUGGESTED READINGS

Alexander, R. M., and G. Goldspink. 1977. *Mechanics and Energetics of Animal Locomotion.* London: Chapman and Hall. (A discussion of how a very wide variety of animals move around and what it costs them to do so.)

Huxley, H. E. 1969. The mechanism of muscular contraction. *Science* 164:1356–1365. (One of the landmark papers in the history of muscle physiology.)

Josephson, R. E. 1993. Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* 55:527–546. (A thorough review of muscle function by the physiologist who first applied work-loop methods to contracting muscles.)

Lamb, G. D. 2000. Excitation-contraction coupling in skeletal muscle: Comparisons with cardiac muscle. *Clin. Exp. Pharmacol. Physiol.* 27:216–224. (A concise review of this complex process in two important types of striated muscle.)

Lutz, G., and L. C. Rome. 1994. Built for jumping: The design of frog muscular system. *Science* 263:370–372. (A brief introduction to studies described in this chapter.)

Marden, J. H. 2000. Variability in the size, composition, and function of insect flight muscles. *Annu. Rev. Physiol.* 62:157–178. (A review of the ways in which insects have solved the puzzle of flight.)

Maughm, D. W., and J. O. Vigoreaux. 1999. An integrated view of insect flight muscle: Genes, motor molecules, and motion. *News Physiol. Sci.* 14: 87–92. (A molecular approach to insect flight.)

Reggiani, C., R. Bottinelli, and G. H. M. Stienen. 2000. Sarcomeric myosin isoforms: Fine tuning of a molecular motor. *News Physiol. Sci.* 15:26–33. (A molecular approach to understanding how different types of striated muscle can behave differently.)

Rome, L. C., R. P. Funke, et al. 1988. Why animals have different muscle fibre types. *Nature* 355:824–827. (More detail on one theme of this chapter.)

Squire, J. M., and E. P. Morris. 1998. A new look at thin filament regulation in vertebrate skeletal muscle. *FASEB J.* 12:761–771. (A summary of interactions among troponin, tropomyosin, and actin, including some great three-dimensional figures presented as stereo pairs.)

Taylor, C. R., E. Weibel, and L. Bolis, eds. 1985. *Design and Performance of Muscular Systems. J. Exp. Biol.*, Vol. 115. Cambridge: The Company of Biologists, Ltd. (A collection of papers on muscle physiology and motor systems.)

Vale, R. D., and R. A. Milligan. 2000. The way things move: Looking under the hood of molecular motor proteins. *Science* 288:88–95. (An enlightening exposition comparing myosin-based and kinesin-based motion.)