Cellular Physiology of Skeletal, Cardiac, and Smooth Muscle

The primary function of each of the three fundamental types of muscle—skeletal, cardiac, and smooth muscle—is to generate force or movement in response to a physiological stimulus. All muscles transduce a chemical or electrical command into a mechanical response. However, the unique physiological role of each of the three basic muscle types dictates inherent differences in the rate and duration of contraction, metabolism, fatigueability, and the ability to regulate contractile strength. For example, both skeletal and cardiac muscle must be capable of rapid force development and shortening. However, skeletal muscle must be able to maintain contractile force for relatively long periods, whereas cardiac muscle need only contract briefly with each heartbeat, although it must sustain this periodic activity for an entire lifetime. Smooth muscle, like skeletal muscle, must be able to regulate contraction over a wide range of force. However, in some tissues (e.g., sphincters), smooth muscle must be able to sustain contraction without fatigue for very long periods. In spite of these differences, the trigger for muscle contraction is the same for all three types of muscle: a rise in the free cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)].

In muscle of all types, certain components of the muscle cell are highly specialized to accomplish the muscle’s unique function. Given the disparity in their physiological roles, it is not surprising that each muscle type has evolved unique anatomic structures and functional mechanisms. Thus, the various types of muscle cells have specialized plasma membranes, cytoskeletons, endoplasmic reticulum, and metabolic pathways for energy generation and utilization.

**EXCITATION OF MUSCLE CELLS**

The plasma membrane—or sarcolemma—of each of the types of muscle cells has regions that are specialized to facilitate communication between cells. In skeletal muscle, this specialization takes the form of the postsynaptic portion of the neuromuscular junction. Neuromuscular junctions (i.e., chemical synapses) are also present in cardiac and smooth muscle. In cardiac muscle, neuromuscular transmission does not initiate contraction; it only modulates it. On the other hand, neuromuscular transmission may initiate contraction in smooth muscle cells, or it may modulate contraction that has been initiated by another mechanism. In both smooth and cardiac muscle cells, gap junctions (i.e., electrical synapses) couple the sarcolemma of neighboring cells; these also play an important role in intercellular communication.
Skeletal Muscle Contracts in Response to Neuromuscular Synaptic Transmission

The mature skeletal muscle cell has a single neuromuscular junction where acetylcholine (ACh) receptors are concentrated (p. 209). A single muscle cell responds to only a single neuron. However, a single neuronal axon may bifurcate to innervate several individual muscle cells. The group of muscle cells innervated by a single neuron is referred to as a motor unit.

The neuromuscular junction is the focus of Chapter 8. Briefly, the ACh released by the presynaptic nerve terminal binds to inotropic (nicotinic) ACh receptors at the neuromuscular junction. These receptors are nonselective cation channels that open when ACh binds to a specific site on the channel, and a depolarization known as the end-plate potential is produced. If this end-plate potential exceeds the threshold for activating Na⁺ channels, an action potential results. Generation of an action potential initiates the sequence of processes leading to contraction. The ACh is rapidly inactivated by acetylcholinesterase, an enzyme that is manufactured by the muscle cell, and muscle contraction stops a few milliseconds after neuronal activity ceases.

Cardiac Muscle Contracts in Response to the Propagation of Electrical Signals from One Cardiac Cell to Another Across Gap Junctions

Cardiac muscle cells also have chemical synapses, but the sympathetic and parasympathetic branches of the autonomic nervous system (see Chapter 15) use these synapses to modulate, rather than to initiate, cardiac muscle function. In contrast to skeletal muscle, cardiac muscle contraction is triggered by electrical signals from neighboring cardiac muscle cells. These electrical impulses originate in the pacemaker region of the heart, the sinoatrial node (p. 489), which spontaneously and periodically generates action potentials. To facilitate direct electrical communication between cardiac muscle cells, the sarcolemma of cardiac muscle is specialized to contain gap junctions (p. 164), electrical synapses that couple neighboring cells (Fig. 9–1). When an action potential is initiated in one cell, current flows through the gap junctions and depolarizes neighboring cells. If depolarization causes the membrane potential (V_m) to be more positive than threshold, self-propagating action potentials occur in the neighboring cells as well. Thus, the generation of an action potential is just as critical for initiating contraction in cardiac muscle as it is in skeletal muscle.

Smooth Muscles May Contract in Response to Either Neuromuscular Synaptic Transmission or Electrical Coupling

Like skeletal muscle, smooth muscle receives synaptic input from the nervous system. However, the synaptic input to smooth muscle differs from that to skeletal muscle in two ways. First, the neurons are part of the autonomic nervous system rather than the somatic nervous system (see Chapter 15). Second, the neuron makes multiple contacts with a smooth muscle cell. At each contact point, the axon diameter expands to form a varicosity that contains the presynaptic machinery. The varicosity is in close proximity to the postsynaptic membrane of the smooth muscle cell, but there is relatively little specialization of the postsynaptic membrane. Rather than the neurotransmitter receptors being closely clustered at the neuromuscular junction, as in skeletal muscle, in smooth muscle the receptors are spread more widely across the postsynaptic membrane.

The mechanisms of intercellular communication among smooth muscle cells are more diverse than are those of skeletal or cardiac muscle. In some organs, smooth muscle is innervated in a manner similar to skeletal muscle in that each smooth muscle cell receives synaptic input. However, a difference is that a smooth muscle cell may receive input from more than one neuron. Moreover, there is little electrical coupling among these smooth muscle cells (i.e., few gap junctions). As a result, each smooth muscle cell may contract independently of its neighbor. Because this type of smooth muscle behaves as multiple, independent cells or groups of cells, it is called multiunit smooth muscle (Fig. 9–2A). Note that the "multi" in "multiunit" refers to the muscle fibers acting independently of one another as multiple units. Multiunit smooth muscles are capable of finer control. Indeed, multiunit smooth muscle is found in the iris and ciliary body of the eye, the piloerector muscles of the skin, and some blood vessels.

In contrast to multiunit smooth muscle, the smooth muscle cells of most organs have extensive intercellular communication in the manner of cardiac muscle cells. In
this type of smooth muscle, gap junctions permit electrical communication between neighboring cells. This communication allows coordinated contraction of many cells. Because these cells contract as a single unit, this type of smooth muscle is called unitary smooth muscle (Fig. 9–2B). Unitary smooth muscle is the predominant smooth muscle type within the walls of visceral organs such as the gastrointestinal tract, the uterus, and many blood vessels. For this reason, unitary smooth muscle is often referred to as visceral smooth muscle. Among unitary smooth muscles, variation in the strength of intercellular coupling from organ to organ leads to variation in the spatial extent of a single unit. For example, in the bladder, extensive coupling among cells defines large functional units, which allows the muscular wall of the bladder to contract in synchrony. On the other hand, the smooth muscle cells of blood vessels couple to form smaller, independently functioning units that are more akin to multiunit smooth muscle. In fact, electrical coupling of smooth muscle units exhibits a tissue-specific continuum from multiunit to unitary coupling.

Action Potentials of Smooth Muscles May Be Brief or Prolonged

Whereas both skeletal muscle and cardiac muscle produce action potentials that initiate contraction, smooth muscle cells produce a wide range of $V_m$ variations that can either initiate or modulate contraction. Action potentials that are similar to those seen in skeletal muscle are observed in unitary smooth muscle and in some multiunit muscle. Like cardiac muscle cells, some smooth muscle cells exhibit prolonged action potentials that are characterized by a prominent plateau. Still other smooth muscle cells cannot generate action potentials at all. In these cells, $V_m$ changes in a graded fashion (p. 173) rather than in the all-or-none manner of action potentials. The stimuli that produce a graded response of $V_m$ include many circulating and local humoral factors, as well as mechanical stimuli such as stretching the cell. These graded $V_m$ changes may be either hyperpolarizing or depolarizing; they sum temporally as well as spatially. If the summation of graded depolarizations brings $V_m$ above threshold—in a smooth muscle cell capable of producing an action potential—an action potential will then ensue.

Action potentials are usually seen in unitary (visceral) smooth muscle. These action potentials typically have a slower upstroke and longer duration (up to ~100 ms) than do skeletal muscle action potentials (~2 ms). The action potential in a smooth muscle cell can be a simple spike, a spike followed by a plateau, or a series of spikes on top of slow waves of $V_m$ (Fig. 9–3A). In any case, the upstroke or depolarizing phase of the action potential reflects opening of voltage-gated Ca$^{2+}$ channels. The inward Ca$^{2+}$ current further depolarizes the cell and thereby causes still more voltage-gated Ca$^{2+}$ channels to open. Thus, some smooth muscle cells can undergo the same type of regenerative depolarization that is seen in skeletal muscle. However, the rate of rise of the action potential in smooth muscle is lower because Ca$^{2+}$ channels open more slowly than do Na$^+$ channels in skeletal and cardiac muscle (p. 189). Repolarization of the smooth muscle cell is also relatively slow. Two explanations may be offered for this slower repolarization. First, voltage-gated Ca$^{2+}$ channels, which are responsible for the depolarization phase of the action potential, inactivate slowly. Second, the repolarization phase of the action potential reflects the delayed activation of voltage-gated K$^+$ channels and, in some cases, Ca$^{2+}$-activated K$^+$ channels.

Some smooth muscle cells have fast, voltage-gated Na$^+$ channels. However, even when these channels are present, they do not appear to be necessary for generating an action potential. Their main role may be to allow more rapid activation of voltage-gated Ca$^{2+}$ channels and thus contribute to a faster rate of depolarization.

In some unitary smooth muscle, repolarization is so delayed that the action potential contour displays a prominent plateau. These plateau potentials may be several hundred milliseconds in duration, as in cardiac muscle. Plateau action potentials occur in smooth muscle of the genitourinary tract, including the ureters, bladder, and uterus. The long $V_m$ plateau allows the entry of Ca$^{2+}$ to continue for a longer period and thus allows [Ca$^{2+}$], to remain high for a longer period, thereby prolonging the contraction.
FIGURE 9-3. Action potentials and slow waves in smooth muscle.
Some Smooth Muscle Cells Can Initiate Spontaneous Electrical Activity

Although smooth muscle cells undergo changes in $V_m$ in response to neural, hormonal, or mechanical stimulation, many smooth muscle cells are capable of initiating spontaneous electrical activity. In some cells, this spontaneous activity results from pacemaker currents. These currents result from time- and voltage-dependent properties of ion currents that produce either a spontaneous increase in inward, or depolarizing, currents (e.g., voltage-gated $Ca^{2+}$ currents) or a spontaneous decrease in outward, or hyperpolarizing, currents (e.g., voltage-gated $K^+$ currents). The pacemaker currents cause the cell to depolarize until $V_m$ reaches threshold, triggering an action potential.

In other smooth muscle cells, this spontaneous electrical activity occurs in regular, repetitive oscillations in $V_m$. These $V_m$ oscillations occur at a frequency of several oscillations per minute and are referred to as slow waves (Fig. 9-38). One hypothesis for the origin of slow-wave potentials suggests that voltage-gated $Ca^{2+}$ channels—active at the resting $V_m$—depolarize the cell enough to activate more voltage-gated $Ca^{2+}$ channels. This activation results in progressive depolarization and $Ca^{2+}$ influx. The increase in $[Ca^{2+}]$ activates $Ca^{2+}$-dependent $K^+$ channels, which leads to progressive hyperpolarization and termination of the depolarization phase of the wave. These periodic depolarizations and $[Ca^{2+}]$ increases cause periodic, tonic contractions of the smooth muscle. When the amplitude of the slow $V_m$ waves is sufficient to depolarize the cell to threshold, the ensuing action potentials lead to further $Ca^{2+}$ influx and phasic contractions.

Other hypotheses to explain spontaneous electrical and mechanical activity in smooth muscle cells are based on oscillatory changes in other intracellular ions or molecules. For example, increased $[Ca^{2+}]$, during an action potential might stimulate Na-Ca exchange and lead to a cyclic increase in $[Na^+]$, and thus an increase in the rate of Na$^+$ extrusion by the electrogenic Na-K pump. Alternatively, the inositol 1,4,5-triphosphate (IP$_3$) receptor channel (p. 100) might spontaneously open and release $Ca^{2+}$. The effect on $[Ca^{2+}]$ would be self-reinforcing because of $Ca^{2+}$-activated inward and outward currents.

Some smooth muscle cells contract without any change in $V_m$. For example, a neurotransmitter can bind to a receptor, activate a G protein, and lead to the generation of IP$_3$, which in turn leads to the release of $Ca^{2+}$ from the SR. The eventual depletion of $Ca^{2+}$ in the SR may in turn stimulate $Ca^{2+}$ influx across the cell membrane via so-called store-operated $Ca^{2+}$ channels.

**MUSCLE CONTRACTION**

**Striated Muscle Cells Are Densely Packed with Myofibrils That Contain Ordered Arrays of Thick and Thin Filaments**

As summarized in Figure 9-4A, each individual skeletal muscle cell (or myocyte or fiber) contains a dense parallel array of smaller, cylindrical elements called myofibrils that have the diameter of a Z disk (p. 45). Each of these myofibrils comprises repeating units, or sarcomeres, that consist of smaller interdigitating filaments called myofilaments. These myofilaments come in two types (see Fig. 9-4B), thick filaments composed primarily of myosin and thin filaments composed primarily of actin (p. 27). The sarcomere extends from one Z disk to another. Sarcomeres stacked end to end make up a myofibril. The repeating sarcomeres are most highly organized within skeletal and cardiac muscle and impart a striped appearance. Thus, both skeletal and cardiac muscle are referred to as striated muscle.

In smooth muscle, striations are not visible. Although actin and myosin are present in smooth muscle cells, the relationship between actin and myosin (thin and thick filaments) is less highly organized. The actin filaments are oriented mainly parallel or oblique to the long axis of the cell. Multiple actin filaments appear to join at electron-dense regions called dense bodies, which are found immediately beneath the cell membrane, as well as within the interior of the myocyte. The thick filaments are interspersed among the thin filaments in smooth muscle, and are far less abundant than in skeletal or cardiac muscle. Thin filaments are 5 to 8 nm in diameter and, in striated muscle, 1 μm in length. In striated muscle, the thin filaments are tethered together at one end, where they project from a dense disk known as the Z disk. The Z disk is oriented perpendicular to the axis of the muscle fiber; thin filaments project from both its faces. Not only do Z disks tether the thin filaments of a single myofibril together, but connections between the Z disks also tether
FROM MUSCLE TO MYOFILAMENTS

Thick and thin filaments (myofilaments)

FIGURE 9-4. Structure of the sarcomere, "each myofibril to its neighbors. These interconnections align the sarcomeres and give skeletal and, to a lesser extent, cardiac muscle its striated appearance.

The thick filaments are 10 nm in diameter and, in striated muscle, 1.6 μm in length. They lie between and partially interdigitate with the thin filaments. This partial interdigitation results in alternating light and dark bands along the axis of the myofibril (see Fig. 9-4C). The light bands, which represent regions of the thin filament that do not lie alongside thick filaments, are known as I bands because they are isotropic to polarized light. The Z disk is visible as a dark perpendicular line at the center of the I band. The dark bands, which represent the myosin filaments, are known as A bands because they are anisotropic to polarized light. During contraction, the A bands are unchanged in length whereas the I bands shorten.

Within the A bands, the pivoting heads of the thick myosin filaments, the molecular motors, establish cross-bridges to the thin actin filaments. As discussed later, the adenosine triphosphate (ATP)-dependent cycle of making and breaking cross-bridges causes the actin filament to be drawn over the myosin filament and thereby results in muscle contraction.

The Thin and Thick Filaments Are Supramolecular Assemblies of Protein Subunits

THIN FILAMENTS. Thin filaments (Fig. 9-5A) consist of actin, tropomyosin, and troponin. The backbone of the filament is a double-stranded α-helical polymer of actin molecules. Each helical turn of a single strand of filamentous or F-actin consists of 13 individual actin monomers and is approximately 70 nm long. F-actin is associated with two important regulatory, actin-binding proteins: tropomyosin and troponin.

Individual tropomyosin molecules consist of two identical α helices that coil around each other and sit near the two grooves that are formed by the two helical actin strands. Head-to-tail contact between neighboring tropomyosin molecules results in two nearly continuous helical
filaments that shadow the actin double helix. The length of a single tropomyosin molecule corresponds to about seven actin monomers (i.e., a half turn of the actin helix). As we shall see later, the role of tropomyosin is to interfere with the binding of myosin to actin.

**Troponin** is a heterotrimer consisting of tropinin T (which binds to a single molecule of tropomyosin), tropinin C (which binds Ca\(^{2+}\)), and troponin I (which binds to actin and inhibits contraction). Tropinin C is closely related to another Ca\(^{2+}\)-binding protein, calmodulin (CaM; p. 102). Thus, each tropinin heterotrimer interacts with a single tropomyosin molecule, which in turn interacts with seven actin monomers. The tropinin complex also interacts directly with the actin filaments. The coordinated interaction among tropinin, tropomyosin, and actin allows actin-myosin interactions to be regulated by changes in [Ca\(^{2+}\)].

**THICK FILAMENTS.** Like thin actin filaments, thick filaments are polymers of proteins (see Fig. 9–5B). Thick filaments are bipolar assemblies composed of multiple myosin-II molecules. Each myosin-II molecule is a hexamer (actually a double trimer) composed of two intertwined heavy chains, two regulatory light chains, and two alkali (or essential) light chains. The two heavy chains have three regions: a rod, a hinge, and a head region. The rod portions are \(\alpha\) helices that wrap around each other. At the hinge regions, the molecule flares open to form two globular heads, which are the cross-bridges between the thick and thin filaments of the sarcomere. The heads of the heavy chains—also called S1 fragments—each possess a site for binding actin as well as a site for binding and hydrolyzing ATP. The head portion of each myosin forms a complex with two light chains, one regulatory and one alkali. The **alkali light chain** plays an essential role in stabilizing the myosin head region. The **regulatory light chain**, as its name implies, regulates the ATPase activity of myosin. The activity of the myosin regulatory light chain is in turn regulated via phosphorylation by Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent kinases.

Figure 9–5C summarizes the interaction between a thin filament and a single pair of head groups from the myosin of a thick filament.

**In All Three Muscle Types, An Increase in [Ca\(^{2+}\)], Triggers Contraction By Removing the Inhibition of Cross-Bridge Cycling**

Underlying muscle contraction is a cycle in which myosin-II heads bind to actin, these cross-bridges become distorted, and finally the myosin heads detach from actin. Energy for this cycling comes from the hydrolysis of ATP. However, if unregulated, the cycling would continue until the myocyte was depleted of ATP. It is not surprising, then, that skeletal, cardiac, and smooth muscle each have mechanisms for regulating cross-bridge cycling. In all three cell types, an increase in [Ca\(^{2+}\)], initiates and allows cross-bridges to cycle to continue. During this excitatory increase, [Ca\(^{2+}\)], may rise from its resting level of less than 10\(^{-7}\) M to greater than 10\(^{-5}\) M. The subsequent decrease in [Ca\(^{2+}\)], is the signal to cease cross-bridge cycling and relax.

Regardless of the muscle type, Ca\(^{2+}\) modulates contraction through regulatory proteins rather than interacting directly with the contractile proteins. In the absence of Ca\(^{2+}\), these regulatory proteins act in concert to inhibit actin-myosin interactions, thus inhibiting the contractile process. When Ca\(^{2+}\) binds to one or more of these proteins, a conformational change takes place in the regulatory complex that releases the inhibition of contraction.

**SKELETAL MUSCLE.** The heterotrimeric tropinin molecule contains the key Ca\(^{2+}\)-sensitive regulator troponin C (Fig. 9–6A). Each **troponin C** molecule in skeletal muscle has two high-affinity Ca\(^{2+}\)-binding sites that participate in binding of troponin C to the thin filament. Ca\(^{2+}\)-binding to these high-affinity sites does not change during muscle activation. Each troponin C molecule in skeletal muscle also has two **additional**, low-affinity Ca\(^{2+}\)-binding sites. Binding of Ca\(^{2+}\) to these low-affinity sites induces a conformational change in the troponin complex that has two effects. The first effect is that the C terminus of the
**A INITIATION OF CROSS-BRIDGE CYCLING IN SKELETAL AND CARDIAC MUSCLE**

Actin  
Tropomyosin  
Troponin complex

The movement of the tropomyosin deeper into the actin groove unmask the myosin binding sites.

**B INITIATION OF CROSS-BRIDGE CYCLING IN SMOOTH MUSCLE**

Calmodulin

**FIGURE 9-6.** The role of Ca$^{2+}$ in triggering muscle contraction. MLCK, myosin light chain kinase.

Inhibitory troponin I moves away from the actin/tropomyosin filament, thereby permitting the tropomyosin molecule to move. According to one hypothesis, the other effect, transmitted through troponin T, is to push tropomyosin away from the myosin-binding site on the actin and into the actin groove. With the steric hindrance removed, the myosin head is able to interact with actin and engage in cross-bridge cycling.

**CARDIAC MUSCLE.** The regulatory mechanism within cardiac muscle is similar to that of skeletal muscle, although troponin C from cardiac muscle has just a single, active low-affinity Ca$^{2+}$-binding site.

**SMOOTH MUSCLE.** An entirely different mechanism controls cross-bridge turnover in smooth muscle. Here, an increase in [Ca$^{2+}$] initiates a slow chain of events that...
ultimately increases the ATPase activity of the myosin (see Fig. 9–68). The first step is the binding of four Ca\(^{2+}\) ions to calmodulin, which, as we noted earlier, is closely related to troponin C. Next, the Ca\(^{2+}\)-CaM complex activates an enzyme known as myosin light chain kinase (MLCK), which in turn phosphorylates the regulatory light chain that is associated with the myosin-II molecule. Phosphorylation of the light chain alters the conformation of the myosin head, which greatly increases its ATPase activity and allows it to interact with actin and act as a molecular motor. Thus, in smooth muscle, CaM rather than troponin C is the Ca\(^{2+}\)-binding protein responsible for transducing the contraction-triggering increases in [Ca\(^{2+}\)].

Note that in smooth muscle, contraction cannot begin until MLCK increases the ATPase activity of myosin, which is a time-consuming process. In skeletal and cardiac muscle, on the other hand, the ATPase activity of the myosin head is constitutively high, and cross-bridge cycling can begin as soon as the tropomyosin is moved out of the way.

The mechanism just outlined activates the thick filaments in smooth muscle. Other mechanisms act on the thin filaments of smooth muscle to remove the tonic inhibition in actin-myosin interactions that are caused by steric hindrance. Two proteins—caldesmon and calponin—tonically inhibit the interaction between actin and myosin. Both are Ca\(^{2+}\)-CaM–binding proteins, and both bind to actin and tropomysin. Calponin, which is found in a fixed stoichiometry with tropomysin and actin (one calponin—one tropomysin—seven actin monomers), tonically inhibits the ATPase activity of myosin. As we saw earlier, the increase in [Ca\(^{2+}\)], that triggers smooth muscle contraction activates Ca\(^{2+}\)-CaM. Besides activating MLCK, this Ca\(^{2+}\)-CaM complex has two effects on calponin. First, Ca\(^{2+}\)-CaM binds to calponin. Second, Ca\(^{2+}\)-CaM activates Ca\(^{2+}\)-CaM–dependent protein kinase, which phosphorylates calponin. Both effects reduce calponin’s inhibition of myosin’s ATPase activity. Like calponin, caldesmon also tonically inhibits the ATPase activity of myosin in smooth muscle.

### During the Cross-Bridge Cycle, Contractile Proteins Convert the Energy of ATP Hydrolysis Into Mechanical Energy

The cross-bridge cycle that we introduced in the previous section occurs in five steps (Fig. 9–7). Initially, the myosin head is attached to an actin filament after the “power stroke” from the previous cycle and after the actomyosin complex has released adenosine diphosphate (ADP). In the absence of ATP, the system could remain in this rigid state for an indefinitely long period, as is indeed the case in rigor mortis. In this rigid state, the myosin head is at a 45-degree angle with respect to the actin and myosin filaments.

**Step 1: ATP binding.** ATP binding to the head of the myosin heavy chain (MHC) reduces the affinity of myosin for actin, causing the myosin head to release from the actin filament. If all cross-bridges in a muscle were in this state, the muscle would be fully relaxed.

**Step 2: ATP hydrolysis.** The breakdown of ATP to ADP and inorganic phosphate (P\(_i\)) occurs on the myosin head; the products of hydrolysis are retained on the myosin. As a result of hydrolysis, the myosin head pivots around the hinge into a “cocked” position (perpendicular or at a 90-degree angle to the thick and thin filaments). This rotation causes the tip of the myosin to move about 11 nm along the actin filament so that it now lines up with a new actin monomer. Two monomers further along the actin filament (see the box titled Measuring the Force of a Single Cross-Bridge Cycle). Once again, if all cross-bridges in a muscle were in this state, the muscle would be fully relaxed.

**Step 3: Cross-bridge formation.** The cocked myosin head now binds to its new position on the actin filament. This binding reflects the increased affinity of the myosin-ADP-P, complex for actin.

**Step 4: Release of P, from the myosin.** Dissociation of P\(_i\) from the myosin head triggers the power stroke, a conformational change in which the myosin head bends approximately 45 degrees about the hinge and pulls the actin filament about 11 nm toward the tail of the myosin molecule. This conformational change causes the actin filament to be drawn along the myosin filament, thereby generating force and motion.

**Step 5: ADP release.** Dissociation of ADP from myosin completes the cycle, and the actomyosin complex is left in a rigid state. The myosin head remains in the same position and at a 45-degree angle with respect to the thick and thin filaments. The ADP–free myosin complex remains bound to actin until another ATP binds and initiates another cycle.

The ADP–free myosin complex (“attached state” in Fig. 9–7) would quickly bind ATP at the concentrations of ATP normally found within cells. If unrestrained, this cross-bridge cycling would continue until depleting the cytoplasm of ATP. At that time, the muscle would remain in the stiff “attached state” because release of the cross-bridges from actin requires binding of ATP to myosin.

As discussed earlier, muscle cells do not regulate cross-bridge cycling by modifying ATP. Instead, skeletal muscle and cardiac muscle control this cycle at the third step by preventing cross-bridge formation until the tropomyosin moves out of the way in response to an increase in [Ca\(^{2+}\)]. Smooth muscle controls the cycle at the second step by preventing ATP hydrolysis until the ATPase activity of the myosin head increases in response to an increase in [Ca\(^{2+}\)].

Although this general schema of cross-bridge cycling occurs in smooth muscle as well as skeletal and cardiac muscle, the frequency of cross-bridge cycling in smooth muscle is less than one tenth the frequency encountered in skeletal muscle. This variation reflects differences in the properties of the various myosin isoforms that are expressed in various cell types. Even though cross-bridge cycling occurs less frequently in smooth muscle, force generation may be as great or greater, perhaps because the cross-bridges remain intact for a longer period with each cycle. It is likely that this longer period during which the cross-bridges are intact reflects a lower rate of ADP release from the smooth muscle isoform of myosin.
IS
POWER-STROKE STATE

ADP is released.

RELEASED STATE

ATP is hydrolyzed, causing myosin heads to return to their resting conformation.

CROSS-BRIDGE STATE

A cross-bridge forms and the myosin head binds to a new position on actin.

Because ATP Stores Are Small, the Cell Must Regenerate the ATP Needed for Muscle Contraction

Each cross-bridge cycle consumes one molecule of ATP. In skeletal muscle, the entire cellular store of ATP is sufficient to allow only a few seconds of continuous maximal contraction. Therefore, the muscle cell must resynthesize ATP from ADP at a rate comparable to the rate of ATP consumption. Skeletal muscle has specialized energy stores that permit rapid regeneration of ATP. The most readily available pool of this energy is the high-energy phosphate bond of phosphocreatine. The enzyme creatine phosphotransferase transfers the high-energy phosphate of phosphocreatine to ADP, thereby rephosphorylating ADP to ATP. The phosphocreatine content of skeletal muscle is adequate to replenish the ATP pool several times, but it is still inadequate for sustaining the energy needs of contracting muscle for more than 10 seconds.

In comparison with the energy stored as phosphocreatine, glycogen is a far more abundant energy source within skeletal muscle. Glycogen that has been previously stored by muscle can be enzymatically degraded to pyruvic acid. Degradation of glycogen to pyruvate is rapid and liberates energy that the cell invests in phosphorylating ADP to yield ATP. Pyruvate can be further degraded along with other foodstuffs by oxidative metabolism, which over the long term is the primary mechanism for the regeneration of ATP (p. 1220). The rate of ATP generation by oxidative metabolism is limited by the rate of oxygen delivery to the muscle. However, glycolytic formation of pyruvate occurs independently of oxygen, as does the conversion of pyruvate to lactate. This anaerobic metabolism of muscle glycogen ensures that energy stores are sufficient to sustain muscle activity for nearly a minute even when oxygen is unavailable. In Chapter 59 we will discuss the aerobic and anaerobic metabolism of exercising muscle in more depth.
FIGURE 9–8. Plasma-membrane invaginations. A, The transverse tubules (T tubules) are extensions of the plasma membrane, penetrating the muscle cell at two points in each sarcomere: the junctions of the A and I bands. B, Smooth-muscle cells have rudimentary invaginations of the plasma membrane, called caveoli, contacting with the sarcoplasmic reticulum.

**EXCITATION-CONTRACTION COUPLING**

In our discussion of the mechanism of muscle contraction, we saw that regardless of whether the muscle is skeletal, cardiac, or smooth, it is an increase in $[Ca^{2+}]$, that triggers muscle contraction. The time during which $[Ca^{2+}]$ remains elevated determines the duration of muscle contraction. The process by which "excitation" triggers the increase in $[Ca^{2+}]$ is known as excitation-contraction coupling. Different kinds of myocytes have specialized mechanisms that regulate the entry of $Ca^{2+}$ into the cytoplasm, as well as remove $Ca^{2+}$ from the cytoplasm once the stimulus for muscle contraction subsides. $Ca^{2+}$ can enter the cytoplasm from the extracellular space through voltage-gated or ligand-gated ion channels, or alternatively, $Ca^{2+}$ can be released into the cytoplasm from the SR. Thus, both extracellular and intracellular sources contribute to the increase in $[Ca^{2+}]$. However, the relative importance of these two sources varies among the different muscle types.

Invaginations of the Sarcolemma Facilitate Communication Between the Surface of the Cell and Its Interior

The plasma membranes of muscle cells display invaginations that extend the surface membrane into the muscle cell. In skeletal and cardiac muscle, these invaginations take the form of radially projecting tubes called transverse tubules or T tubules (Fig. 9–8A). T tubules are highly organized and penetrate the muscle at two points in each sarcomere: at the junctions of the A and I bands. A cross section through the A-I junction would show a complex, branching array of T tubules penetrating to the center of the muscle cell and surrounding the individual myofibrils. Along its length the tubule associates with two cisternae, which are specialized regions of the SR. The sarcoplasmic reticulum is the muscle equivalent of the endoplasmic reticulum, and it serves as a storage organelle for intracellular $Ca^{2+}$. The combination of the T-tubule membrane and its two neighboring cisternae is called a triad, this structure plays a crucial role in the...
coupling of excitation to contraction in skeletal and cardiac muscle. Smooth muscle, in contrast, has more rudimentary and shallow invaginations called caveoli (see Fig. 9–8B).

In Skeletal Muscle, Depolarization of the T-Tubule Membrane Leads to Ca²⁺ Release from the Sarcoplasmic Reticulum at the Triad

Action potentials originating from depolarizations at the motor end plate propagate along the skeletal muscle membrane and down the T tubules. Depolarization of the triad region of the T tubules activates L-type Ca²⁺ channels (p. 190), which are clustered in groups of four called “tetrads” (Fig. 9–9). These voltage-gated channels play a pivotal role in coupling electrical excitation to contraction because they function as the voltage sensor in EC coupling. Electron microscopy reveals a checkerboard pattern of projections arising from the T-tubule membrane and extending toward the cisternae of the SR; these projections probably represent the cytoplasmic face of these L-type Ca²⁺ channels. Each of the four voltage-gated Ca²⁺ channels in a tetrad is in fact a heteropentameric protein (see Fig. 7–12B). Each of the four Ca²⁺ channels is also called a DHP receptor, because it is inhibited by a class of antihypertensive drugs known as dihydropyridines. Depolarization of the T-tubule membrane evokes conformational changes in each of the four L-type Ca²⁺ channels and has two effects. First, the conformational changes allow Ca²⁺ to enter through the four channel pores. Second, and much more important, the conformational changes in the four L-type Ca²⁺ channels induce a conformational change in each of the four subunits of another channel—the Ca²⁺-release channel—that is located in the SR membrane.

The Ca²⁺-release channel (Table 6–2, #18) has a homotetrameric structure quite different from that of the L-type Ca²⁺ channel that constitutes the voltage sensor. The Ca²⁺-release channel in the SR is also known as the ryanodine receptor because it is inhibited by a class of drugs that include the plant alkaloids ryanodine and caffeine. Ca²⁺-release channels cluster in the portion of the SR membrane that faces the T tubules. Each of the four subunits of these channels has a large extension—also known as a “foot.” These feet project as a regular array into the cytosol. The foot of each of the four Ca²⁺-release
In Cardiac Muscle, Ca²⁺ Entry Through L-Type Ca²⁺ Channels Is Amplified By Ca²⁺-Induced Ca²⁺ Release from the Sarcoplasmic Reticulum

Whereas EC coupling in skeletal muscle does not require Ca²⁺ influx through L-type Ca²⁺ channels, cardiac contraction has an absolute requirement for Ca²⁺ influx through these channels during the action potential. Because the T-tubule lumen is an extension of the extracellular space, it facilitates the diffusion of Ca²⁺ from bulk extracellular fluid to the site of the L-type Ca²⁺ channels on the T-tubule membrane. Thus, the Ca²⁺ can simultaneously reach superficial and deep regions of the muscle. The increase in [Ca²⁺], resulting from Ca²⁺ influx alone is not, however, sufficient to initiate contraction. Rather, the increase in [Ca²⁺], that is produced by the L-type Ca²⁺ channels is greatly amplified by CICR from the SR via the Ca²⁺-release channels. Indeed, because the Ca²⁺-release channels remain open for a longer period than do L-type Ca²⁺ channels, the contribution of CICR to the rise in [Ca²⁺], is greater than the flux contributed by the L-type Ca²⁺ channels of the T tubules.

It appears that each L-type Ca²⁺ channel controls only one SR Ca²⁺-release channel. The close physical proximity of L-type Ca²⁺ channels of the T-tubule membrane and the Ca²⁺-release channel in the SR at the triad junctions allows for this tight local control. Although Ca²⁺ diffuses in the cytosol away from its SR release site, Ca²⁺ release at one site does not appear to be able to induce Ca²⁺ release from a neighboring SR Ca²⁺-release channel. Thus, Ca²⁺ release events are not propagated along the myocyte. In fact, the SR Ca²⁺-release channel does not appear to respond to generalized increases in cytoplasmic [Ca²⁺]. Generalized cardiac muscle contractions occur as a result of the spatial and temporal summation of individual CICR events.

In Smooth Muscle, Both Extracellular and Intracellular Ca²⁺ Activate Contraction

In smooth muscle cells, three major pathways—which are not mutually exclusive—can lead to the increase in [Ca²⁺], that triggers contraction: (1) Ca²⁺ entry through voltage-gated channels in response to cell depolarization, (2) Ca²⁺ release from the SR, and (3) Ca²⁺ entry through voltage-independent channels.

Ca²⁺ Entry Through Voltage-Gated Channels. As noted earlier, smooth muscle cells respond to stimulation with graded depolarizations or action potentials. In either case, depolarization may produce an influx of Ca²⁺ through voltage-gated l-type Ca²⁺ channels (Fig. 9–10).

Ca²⁺ Release FROM the SR. This Ca²⁺ release may occur by either of two mechanisms: CICR or IP₃-mediated Ca²⁺ release. As we have already seen, CICR plays a key role in EC coupling in cardiac muscle, where the L-type Ca²⁺ channels are highly ordered and in close proximity to the Ca²⁺-release channels in the SR. Thus, Ca²⁺ influx through L-type Ca²⁺ channels can trigger CICR. In smooth muscle, the relationship between the plasma membrane and the SR is not as regularly organized as it is in striated muscle. Nevertheless, electron-dense couplings have been observed bridging the 8- to 10-nm gap between the cell membranes and elements of the SR in smooth muscle. Although CICR occurs in smooth muscle cells under some conditions, it requires [Ca²⁺] levels that are higher than those that typically occur under physiological conditions, and its role remains unclear.

A more important mechanism for Ca²⁺ release from the SR of smooth muscle is the IP₃ pathway. The existence of this pathway is supported by the observation that some extracellular agonists can elicit smooth muscle contraction with minimal depolarization and negligible Ca²⁺ influx. Furthermore, even for agonists such as serotonin or norepinephrine, which activate a Ca²⁺-influx pathway, the observed increase in [Ca²⁺], is out of proportion to that expected from Ca²⁺ influx alone. Thus, another pathway must exist for increasing [Ca²⁺]. Some agonists cause smooth muscle contraction by triggering the production of IP₃ (p. 100), which binds to a specific receptor on the...
MALIGNANT HYPERTHERMIA

Malignant hyperthermia (MH) is a genetic disorder affecting between 1 in 10,000 and 1 in 50,000 individuals. Affected individuals are at risk for a potentially life-threatening syndrome when exposed to any of the various inhalation anesthetic agents, particularly halothane. Administration of succinylcholine can also trigger or exaggerate MH. This drug is a short-acting inotropic (nicotinic) acetylcholine-receptor antagonist that acts by first opening the acetylcholine receptor channel and then blocking it, thereby resulting in a burst of muscle activity, followed by paralysis. Onset of the syndrome in the setting of the operating room is typified by the development of tachypnea (rapid breathing), low plasma \([O_2]\), high plasma \([CO_2]\), tachycardia (rapid heart rate), and hyperthermia (rising body temperature), as well as rigidity, sweating, and dramatic swings in blood pressure. The patient's temperature may rise as rapidly as 1°C every 5 minutes. The onset of MH is usually during anesthesia, but it can occur up to several hours later. If untreated, the patient will develop respiratory and lactic acidosis, muscular rigidity, and a breakdown of muscle tissue that leads to the release of \(K^+\) and thus profound hyperkalemia. These episodes reflect a progressively severe hypermetabolic state in the muscle tissues. Fortunately, our evolving understanding of the physiology of MH has led to the development of a therapeutic regimen that has greatly improved the once-dismal prognosis.

The major features of the syndrome—hyperthermia, muscular rigidity, and an increased metabolic rate—led early investigators to suggest that MH was a disease of abnormal regulation of muscle contraction. Accurate to this hypothesis, uncontrolled muscle contraction— somehow triggered by the administration of halothane and succinylcholine—causes excessive adenosine triphosphate (ATP) hydrolysis to provide energy for contraction. The increased rate of ATP hydrolysis leads to an increased metabolic rate as muscle tries to replenish and sustain its ATP stores. Hyperthermia develops because of the heat liberated by the hydrolysis of ATP.

Further support for this hypothesis came from the observation that more tension developed in muscle fibers obtained by biopsy from susceptible individuals than in fibers from normal individuals when the fibers were exposed to halothane. In muscle fibers from both normal subjects and a strain of swine susceptible to MH, \(Ca^{2+}\)-induced \(Ca^{2+}\) release from the sarcoplasmic reticulum (SR) is enhanced when compared with fibers from unaffected subjects. Furthermore, caffeine, which causes the \(Ca^{2+}\)-release channels to open, induced greater contractions in fibers from susceptible subjects. Taken together, these observations suggested the possibility that MH results from an abnormality in the \(Ca^{2+}\)-release channel in the SR membrane.

In both humans and animals, inheritance of MH follows a mendelian autosomal-dominant pattern. Cloning of the gene (\(RyR1\)) encoding the \(Ca^{2+}\)-release channel (ryanodine receptor) allowed genetic linkage analysis to demonstrate that human MH is closely linked in some families to the \(RyR1\) gene on chromosome 19. In swine, MH results from a single amino-acid substitution in \(RyR1\) (Cys for Arg at position 614). An analogous substitution is present in some human kindreds as well. This substitution increases the probability that the \(Ca^{2+}\)-release channel will be open. In other families, MH has been associated with other genetic abnormalities in the \(RyR1\) gene.

In still others, MH does not appear to be genetically linked to the \(RyR1\) gene. It is possible that defects in other steps along the excitation-contraction cascade can result in abnormal regulation of muscle contraction and the MH phenotype. For example, when under anesthesia, patients with some forms of muscular dystrophy may have metabolic crises that resemble MH.

MH also occurs in domestic livestock. The incidence of MH is particularly high in swine, where episodes are triggered by a variety of physical and environmental stresses (porcine stress syndrome). MH in animals has significant economic importance in view of the potential loss from fatal episodes and the devaluation of meat as a result of muscle destruction during non-fatal episodes.

In humans, a condition similar to MH may occur in patients treated with neuroleptic agents such as the phenothiazines or haloperidol. It is called the neuroleptic malignant syndrome and appears to result from abnormally high neuronal input to the muscle cells.

Therapy for MH now involves administration of the drug dantrolene, cessation of anesthesia, and aggressive efforts aimed at cooling the body. Dantrolene is an effective therapeutic agent because it blocks excitation-contraction coupling between T tubules and the SR, thus interrupting the otherwise uncontrolled progression of muscular contractions. The drug can be given acutely in an effort to abort an ongoing attack or, in a person known to be at risk, it can be given before the initiation of anesthesia in order to prevent onset of the syndrome. Therapy also includes intravenous hydration and the judicious use of diuretics to keep the urine flowing. The latter lessens damage to the kidneys from the release of breakdown products, such as myoglobin from the damaged muscles. Sodium bicarbonate is given to counter the lactic acidosis, and patients may be mechanically hyperventilated to blow off the excess \(CO_2\).

Despite the intensive protocol just outlined, MH is still associated with high mortality. The relatives of a patient with a documented history of one episode of MH should be carefully screened to see whether they, too, carry the inherited trait; many of the affected relatives may demonstrate baseline elevations in muscle enzyme levels in their blood (e.g., an increase in creatine kinase levels).
transmitters that lead to the discharge—and thus the depletion—of SR Ca\(^{2+}\) stores (i.e., second pathway) somehow lead to the activation of store-operated Ca\(^{2+}\) channels in the plasma membrane. The Ca\(^{2+}\) entering through these channels allows [Ca\(^{2+}\)] to remain elevated even after SR depletion and also appears to replenish SR Ca\(^{2+}\) stores.

Together, the second pathway for increasing [Ca\(^{2+}\)] (Ca\(^{2+}\) release from the SR) and the third pathway (Ca\(^{2+}\) influx through store-operated channels) have been called pharmacomechanical coupling because these excitatory neurotransmitters and hormones can induce smooth muscle contraction that is independent of action-potential generation. Regardless of the source of the Ca\(^{2+}\), the resultant increase in [Ca\(^{2+}\)] leads to contraction via a Ca\(^{2+}\)-CaM-dependent increase in MLCK activity and myosin phosphorylation.

**Smooth Muscle Contraction May Also Occur Independently of Increases in [Ca\(^{2+}\)]**

Whereas many excitatory stimuli rely on increases in [Ca\(^{2+}\)] to evoke contraction, some stimuli appear to cause contraction without a measurable increase in [Ca\(^{2+}\)]. One mechanism by which excitatory stimuli might induce Ca\(^{2+}\)-independent contractions is by modulating the activity of contractile or regulatory proteins directly. Thus, the amount of force developed at any given [Ca\(^{2+}\)] may vary. This force/[Ca\(^{2+}\)] ratio may be increased or decreased and is generally higher during pharmacomechanical activation than during depolarization-activated contractions. Because phosphorylation of the myosin light chain (MLC) is a major determinant of contractile force in smooth muscle, Ca\(^{2+}\)-independent contractions may result either from an increase in the rate of MLC phosphorylation by MLCK or from a decrease in the rate of MLC dephosphorylation by MLC phosphatase. One second-messenger system that can decrease the activity of phosphatases is protein kinase C (PKC) (p. 102). Some excitatory stimuli are therefore capable of initiating smooth muscle contraction by inducing IP\(_3\)-mediated release of Ca\(^{2+}\) from intracellular stores, as well as by producing PKC-mediated decreases in MLC phosphatase activity. These pathways are further examples of pharmacomechanical coupling.

**TERMINATING CONTRACTION**

**In Skeletal, Cardiac, and Smooth Muscle, Terminating Contraction Requires Re-Uptake of Ca\(^{2+}\) Into the Sarcoplasmic Reticulum**

After the contraction-activating stimulus has subsided, Ca\(^{2+}\) must be removed from the cytoplasm for contrac-
tion to cease and for relaxation to occur. Ca²⁺ may be extruded across the cell membrane or sequestered within intracellular compartments (Fig. 9–11).

The cell may extrude Ca²⁺ using either an Na-Ca exchanger (NCX. p. 68) or a Ca²⁺ pump at the plasma membrane (PMCA. p. 64). Extrusion across the cell membrane, however, would eventually deplete the cell of Ca²⁺ and is therefore a minor mechanism for Ca²⁺ removal from the cytoplasm. Instead, Ca²⁺ re-uptake into the SR is the most important mechanism by which the cell returns [Ca²⁺]₁ to resting levels. Ca²⁺ re-uptake by the SR is mediated by a SERCA-type Ca²⁺ pump (p. 64).

It is possible that the rate of Ca²⁺ re-uptake into the SR may be regulated by modulating the activity of the SR Ca²⁺ pump. For example, in cardiac muscle, SR Ca²⁺ pump activity is inhibited by the regulatory protein phospholamban. When phospholamban is phosphorylated by cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA), its ability to inhibit the SR Ca²⁺ pump is lost. Thus, activators of PKA, such as the neurotransmitter epinephrine, may enhance the rate of cardiac myocyte relaxation (p. 525).

REGULATING MUSCLE CONTRACTION

Muscle Contractions Produce Force and/or Shortening and, in the Extreme, Can Be Studied Under Either Isometric or Isotonic Conditions

The total force generated by a muscle is the sum of the forces generated by many independently cycling actin–myosin cross-bridges. The number of simultaneously cycling cross-bridges depends substantially on the initial length of the muscle fiber and on the pattern or frequency of muscle-cell stimulation. When muscle is stimulated to contract, it exerts a force tending to pull the attachment points at either end toward each other. This force is referred to as the tension developed by the muscle.

Two mechanical—and artificial—arrangements can be used to study muscle contraction. In one, the attachment points are immobile, thereby fixing the muscle length. Here, stimulation causes an increase in tension, but no shortening. Because these contractions occur at constant length, they are referred to as isometric contractions (Fig. 9–12A). In the second arrangement, one of the two attachment points is mobile, and a force—or load—

In Smooth Muscle, Terminating Contraction Also Requires Dephosphorylation of the Myosin Light Chain

Because Ca²⁺ triggers smooth muscle contraction by inducing phosphorylation of the myosin regulatory light chain, merely restoring [Ca²⁺], to its low resting value may not allow muscle relaxation. Rather, relaxation of smooth muscle requires MLC dephosphorylation, which is accomplished by myosin light chain phosphatase. This phosphatase is a heterotrimer consisting of subunits with molecular masses of 130, 20, and 37 kDa. The 130-kDa subunit confers specificity by binding to myosin, whereas the 37-kDa protein is the catalytic subunit responsible for the dephosphorylating activity.
FIGURE 9-12. Isometric and isotonic contraction. A. Experimental preparation for studying muscle contraction under isometric conditions. B. Experimental preparation for studying muscle contraction under isotonic conditions. C. The "passive" curve represents the tension that is measured at various muscle lengths prior to muscle contraction. The "total" curve represents the tension that is measured at various muscle lengths during muscle contraction. D. The "active" tension is the difference between the "total" and the "passive" tensions in C. E. Each of the three blue curves shows that the velocity of muscle shortening is faster if the muscle lifts a lighter weight—it is easier to lift a feather (left side of each curve/low load) than to lift a barbell (right side of each curve/high load). The three blue curves also show that, for any given velocity of shortening, a longer muscle can develop a greater tension than can a shorter muscle.
The force of a single cross-bridge cycle has been measured directly. Finer, Simmons, and Spudich used optical tweezers to manipulate a single actin filament and place it in proximity to a myosin molecule immobilized on a bead (Fig. 9–13). With the use of video-enhanced microscopy these investigators were able to detect movements of the actin filament as small as 1 nm. The optical tweezers could also exert an adjustable force opposing movement of the actin filament. When the tweezers applied only a small opposing force and the experiment was conducted in the presence of adenosine triphosphate (ATP), they observed that the actin moved over the myosin bead in step-like displacements of 11 nm. This observation, made under “microscopically isotonic” conditions, suggests that the quantal displacement of a single cross-bridge cycle is approximately 11 nm. When the tweezers applied a force sufficiently large to immobilize the actin filament, the investigators observed step-like impulses of force that averaged around 5 pN. This observation, made under “microscopically isotonic” conditions, suggests that the quantal force developed during a single cross-bridge cycle is about 5 pN. Interestingly, these isotonic force impulses lasted longer when the ATP concentration was lower. This last finding is consistent with the notion that ATP binding to myosin must occur to allow detachment of the cross-bridges (step 7 in the cycle in Fig. 9–7).

### Measuring the Force of a Single Cross-Bridge Cycle

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This length-tension relationship is a direct result of the anatomy of the thick and thin filaments within individual sarcomeres (see Fig. 9–12D). As muscle length increases, the ends of the actin filaments arising from neighboring Z disks are pulled away from each other. When length is increased beyond 150% of its resting sarcomere length, the ends of the actin filaments are pulled beyond the ends of the myosin filaments. Under this condition, no interaction occurs between actin and myosin filaments and hence no development of active tension. As muscle length shortens from this point, actin and myosin filaments begin to overlap and tension can develop; the amount of tension developed corresponds to the degree of overlap between the actin and the myosin filaments. As the muscle shortens further, opposing actin filaments slide over one another and the ends of the myosin filaments and—with extreme degrees of shortening—eventually butt up against the opposing Z disks. Under these conditions, the spatial relationship between actin and myosin is distorted and active tension falls. The maximal degree of overlap between actin and myosin filaments, and hence maximal active tension, corresponds to a sarcomere length that is near its normal resting length.

### Muscle Length Influences Tension Development By Determining the Degree of Overlap Between Actin and Myosin Filaments

The isometric force of contractions depends on the initial length of the muscle fiber. Unstimulated muscle may be elongated somewhat by applying tension and stretching it. The tension measured before muscle contraction is referred to as passive tension (see Fig. 9–12C). Because muscle gets stiffer as it is distended, it takes increasing amounts of “passive” tension to progressively elongate the muscle cell. If at any fixed length (i.e., isometric conditions) the muscle is stimulated to contract, an additional active tension develops because of cross-bridge cycling. The total measured tension is thus the sum of the passive and active tension. This incremental or active tension—the difference between total tension and passive tension—is quite small when the muscle is less than approximately 70% of its normal resting length (see Fig. 9–12D). As muscle length increases toward its normal length, active tension increases. Active tension is maximal at a length—usually called L₀—that is near the normal muscle length. Active tension decreases with further lengthening; thus, active tension is again small when the muscle is stretched beyond 150% of its normal resting length. Although the relationship between muscle length and tension has been best characterized for skeletal muscle, the tension of cardiac and smooth muscle also appears to depend on length in a similar manner.

At Higher Loads, the Velocity of Shortening Is Lower Because More Cross-Bridges Are Simultaneously Active

Under isotonic conditions, the velocity of shortening decreases as the applied load opposing contraction of the muscle fiber increases. This point is obvious; anyone can lift a single French fry much faster than a sack of potatoes. As shown for any of the three blue curves in Figure 9–12E—each of which represents a different initial length of muscle—the relationship between velocity and load is hyperbolic. Thus, the smaller the load applied to the muscle, the greater its velocity of shortening. Conversely, the greater the load, the lower the velocity of shortening.
The load (or tension)-velocity relationship is perhaps best understood by considering the situation at maximum load for a resting muscle length (i.e., isometric conditions). This situation is represented by the upper blue curve in Figure 9-12E. At any time, all the available cross-bridges are engaged in resisting the opposing force. None are left over to make the muscle shorten. At a slightly smaller load but at the same isotonic muscle length, fewer cross-bridges need to be engaged to resist the opposing load. Thus, extra cross-bridges are available to ratchet the thick myosin filaments over the thin actin filaments, but at a very low velocity. At a still lower load, even more cross-bridges are available for ratcheting the myosin over the actin, and the velocity increases further. At very low loads, it is reasonable to expect that as the myosin filament slides along the thin filament, only a tiny fraction of the actin monomers need to interact with myosin heads to overcome the load. Under these conditions of vanishingly small loads, the speed with which the thick and thin filaments slide over each other is limited only by the time that it takes for the ATP-consuming cross-bridge cycle to occur. With increasing velocity, the probability of actin-myosin interactions decreases. Thus, fewer cross-bridges are simultaneously active at higher shortening velocities, and less tension develops.

Note that the upper blue curve in Figure 9-12E applies to a particular initial length of the muscle, that is, the resting length. We already saw in Figure 9-12C that the total isometric tension (i.e., the maximal load that the muscle can sustain at zero velocity) increases with initial muscle length. This principle is confirmed in Figure 9-12E: the longer the initial length, the larger the maximal load under zero-velocity conditions (i.e., the three different intercepts with the abscissa). In contrast to these
In a Single Skeletal Muscle Fiber, the Force Developed May Be Increased By Summing Multiple Twitches In Time

At sufficiently low stimulation frequencies, the tension developed falls to the resting level between individual twitches (Fig. 9-14A). Single skeletal-muscle twitches last between 25 and 200 msec, depending on the type of muscle. Although each twitch is elicited by a single muscle action potential, the duration of contraction is long when compared with the duration of the exciting action potential, which lasts only several milliseconds. Because the muscle twitch far exceeds the duration of the action potential, it is possible to initiate a second action potential before a first contraction has fully subsided. When this situation occurs, the second action potential stimulates a twitch that is superimposed on the residual tension of the first twitch, thereby achieving greater isometric tension than the first (compare Fig. 9-14A and B). This effect is known as summation.

If multiple action potentials occur closely enough in time, the multiple twitches can summate and thus greatly increase the tension developed. Summation is more effective at increasing tension when the action potentials are grouped more closely in time, as in Figure 9-14C. In other words, tension is higher when action potentials are evoked at higher frequency. Because this type of tension enhancement depends on the frequency of muscle stimulation, it is referred to as frequency summation.

When the stimulation frequency is increased sufficiently, the individual twitches occur so closely together in time that they fuse (see Fig. 9-14D) and cause the muscle tension to remain at a steady plateau. The state in which the individual twitches are no longer distinguishable from each other is referred to as tetanus. Tetanus arises when the time between successive action potentials is insufficient to return enough Ca^{2+} back to the SR to lower [Ca^{2+}] below a level that initiates relaxation. In fact, a sustained increase in [Ca^{2+}] persists until the tetanic stimulus ceases. At stimulation frequencies above the fusion frequency that causes tetanus, muscle fiber tension increases very little.

In a Whole Skeletal Muscle, the Force Developed May Be Increased By Summing the Contractions of Multiple Fibers

In addition to determining the frequency with which it stimulates a single muscle fiber, the central nervous system (CNS) can control muscle force by determining the number of individual muscle fibers that it stimulates at a given time. As each additional motor-neuron cell body within the spinal cord is excited, those muscle fibers that are part of the motor unit of that motor neuron are added to the contracting pool of fibers (Fig. 9-15). This effect is known as multiple-fiber summation. Generally, smaller motor neurons serve motor units consisting of fewer individual muscle fibers. Because a given excitatory stimulus will generate a larger excitatory postsynaptic potential (p
A MOTOR UNIT

A motor neuron innervates one set of muscle fibers.

B MOTOR NEURON POOL

A pool consists of many motor neurons, each of which innervates a motor unit with the muscle.

FIGURE 9-15. The motor unit and the motor-neuron pool.

In Cardiac Muscle, Increasing the Entry of Ca\(^{2+}\) Enhances the Contractile Force

Whereas frequency summation and multiple-fiber summation are important mechanisms for regulating the strength of skeletal-muscle contractions, these mechanisms would not be consistent with the physiological demands of cardiac muscle. Because cardiac muscle must contract only once with each heartbeat and must fully relax between each contraction, frequency summation is precluded. Furthermore, the extensive electrical coupling between cardiac myocytes, as well as the requirement that cardiac muscle contract homogeneously, eliminates the potential for multiple-fiber summation. Therefore, the strength of cardiac muscle contraction must be regulated by modulating the contractile force generated during each individual muscle twitch. This type of regulation is an important part of the adaptive response to exercise and is mediated by norepinephrine, a neurotransmitter released by the sympathetic nervous system.

Because an increase in \([\text{Ca}^{2+}]\) \textsuperscript{+}, activates contraction by removing the inhibitory influence of the regulatory proteins, it is reasonable to consider that contractile function may be regulated either by modulating the magnitude of the rise in \([\text{Ca}^{2+}]\), or by altering the \(\text{Ca}^{2+}\) sensitivity of the regulatory proteins. In fact, both these mechanisms are important in controlling the force of cardiac muscle contraction.

In cardiac muscle, a significant proportion of the activator \(\text{Ca}^{2+}\) enters the cell via voltage-gated \(\text{Ca}^{2+}\) channels that open during the cardiac action potential. Most of this \(\text{Ca}^{2+}\) influx occurs via \(\text{L-type Ca}^{2+}\) channels. How does norepinephrine increase the contractile force of the heart? This hormone acts through the \(\beta\)-type adrenergic receptor to increase the generation of cAMP, activate PKA (p. 97), and in turn phosphorylate the \(\text{L-type Ca}^{2+}\) channels, thereby increasing the passive influx of \(\text{Ca}^{2+}\). An increased \([\text{Ca}^{2+}]\), leads to an increase in contractile force. The cAMP pathway also appears to increase the \(\text{Ca}^{2+}\) sensitivity of the contractile apparatus by phosphorylating one or more of the regulatory proteins. Thus, cAMP causes an increase in the force generated for any given \([\text{Ca}^{2+}]\).

Reciprocal control over \(\text{Ca}^{2+}\) entry is provided by cyclic guanosine monophosphate (cGMP)-dependent phosphorylation of the \(\text{L-type Ca}^{2+}\) channels. Acetylcholine, acting through muscarinic ACh receptors, raises intracellular cGMP concentrations. In turn, the cGMP-dependent phosphorylation of \(\text{L-type Ca}^{2+}\) channels, at sites distinct from those phosphorylated by the cAMP-dependent kinase, causes a decrease in \(\text{Ca}^{2+}\) influx during the cardiac action potential and thus a decrease in the force of contraction.

\(\text{Ca}^{2+}\) entry may also be regulated indirectly by modulating other ion channels so that they either change their \(\text{Ca}^{2+}\) permeability or alter the duration of the action potential. Norepinephrine, for example, may increase the \(\text{Ca}^{2+}\) permeability of voltage-gated \(\text{Na}^{+}\) channels. Receptor transduction mechanisms that inhibit voltage-gated \(\text{K}^{+}\) currents may prolong the cardiac action potential and thereby increase net \(\text{Ca}^{2+}\) influx through \(\text{L-type Ca}^{2+}\) channels without modulating the \(\text{Ca}^{2+}\) channels themselves.

In Smooth Muscle, Contractile Force Is Enhanced By Increasing the Entry of \(\text{Ca}^{2+}\), As Well As By Increasing the \(\text{Ca}^{2+}\) Sensitivity of the Contractile Apparatus

Unlike skeletal muscle, in which force development results from the summation of individual muscle twitches,
individual smooth muscle cells can maintain a sustained contraction that can be graded in strength over a wide range. Contractile force in smooth muscle largely depends on the relative balance between the phosphorylation and dephosphorylation of MLCs. The rate of MLC phosphorylation is regulated by the Ca^{2+}-CaM complex, which in turn depends on levels of intracellular Ca^{2+}. Smooth muscle cells can regulate [Ca^{2+}], over a wider range than skeletal and cardiac muscle can for several reasons. First, some smooth muscle cells do not generate action potentials. Rather, their membrane potential varies slowly in response to neurotransmitters or hormones. This graded response of V_m allows finer regulation of Ca^{2+} influx through voltage-gated channels. Second, release of Ca^{2+} from intracellular stores may be modulated through neurotransmitter-induced generation of intracellular second messengers such as IP_3. This modulation allows finer control of Ca^{2+} release than occurs in the SR Ca^{2+} release channel by L-type Ca^{2+} channels in skeletal and cardiac muscle.

A second level of control over contractile force occurs by regulating the Ca^{2+} sensitivity of proteins that regulate contraction. For example, inhibiting myosin light chain phosphatase alters the balance between phosphorylation and dephosphorylation, in effect allowing a greater contraction at a lower [Ca^{2+}]. Some neurotransmitters act by inhibiting the phosphatase, which appears to occur through activation of G-protein-coupled receptors. Another mechanism for governing the Ca^{2+} sensitivity of proteins that regulate contraction is alteration of the Ca^{2+} sensitivity of the myosin light chain kinase. For example, MLCK itself is phosphorylated at specific sites by several protein kinases, including PKA, protein kinase C, and Ca^{2+}-CaM-dependent kinases. Phosphorylation by any of these kinases decreases the sensitivity of MLCK to activation by the Ca^{2+}-CaM complex.

**Smooth Muscle Maintains High Force at Low Energy Consumption**

Smooth muscle is often called upon to maintain high force for long periods. If smooth muscle consumed ATP at rates similar to striated muscle, metabolic demands would be considerable and the muscle would be prone to fatigue. Unlike striated muscle, however, smooth muscle is able to maintain high force at a low rate of ATP hydrolysis. This low-energy consumption/high-tension state is referred to as the latch state. The latch state in smooth muscle is unique because high tension can be maintained despite a decrease in the degree of muscle activation by excitatory stimuli. As a result, force is maintained at a lower level of MLCK phosphorylation.

The mechanism underlying the latch state is not entirely known, although it appears to be due in large part to changes in the kinetics of actin-myosin cross-bridge formation and detachment. These changes may be a direct result of a decrease in the rate at which dephosphorylated cross-bridges detach. Tension is directly related to the number of attached cross-bridges. Furthermore, the proportion of myosin heads cross-bridged to actin is related to the ratio of attachment rates to detachment rates. Therefore, it is reasonable to expect that a decrease in the detachment rate would allow a greater number of cross-bridges to be maintained and would result in a lower rate of cross-bridge cycling and ATP hydrolysis. Thus, smooth muscle appears to be able to slow down cross-bridge cycling just before detachment, a feat that can be accomplished in skeletal muscle (see Fig. 9-7) only at low ATP levels (as in rigor mortis).

**DIVERSITY AMONG MUSCLES**

Each muscle type (i.e., skeletal, cardiac, and smooth) is distinguishable on the basis of its unique histology, ECCcoupling mechanisms, and regulation of contractile function. However, even within each of the three categories, muscle in different locations must serve markedly different purposes, with different demands for strength, speed, and fatigability. This diversity is possible because of differences in the expression of specific isoforms for various contractile and regulatory proteins (Table 9-1).

**Skeletal Muscle Is Composed of Slow-Twitch and Fast-Twitch Fibers**

Some skeletal muscles must be resistant to fatigue and be able to maintain tension for relatively long periods, although they need not contract rapidly. Examples are muscles that maintain body posture, such as the soleus muscle of the lower part of the leg. In contrast, some muscles need to contract rapidly, yet infrequently. Examples are the extraocular muscles, which must contract rapidly to redirect the eye as an object of visual interest moves about.

Individual muscle fibers are classified as slow twitch (type I) or fast twitch (type II), depending on their rate of force development. These fiber types are also distinguished by their histologic appearance and their ability to resist fatigue.

**Slow-twitch fibers** (Table 9-2) are generally thinner and have a more dense capillary network surrounding them. Slow-twitch fibers also appear red because of a large amount of the oxygen-binding protein myoglobin (p. 656) within the cytoplasm. This rich capillary network together with myoglobin facilitates oxygen transport to the slow-twitch fibers, which mostly rely on oxidative metabolism for energy. The metabolic machinery of the slow-twitch fiber also favors oxidative metabolism because it has low glycogen content and glycolytic-enzyme activity but a rich mitochondrial and oxidative-enzyme content.

**Fast-twitch fibers** differ among themselves with respect to fatigability. Some fast-twitch fibers are fatigue resistant; they rely on oxidative metabolism (type IIa) and are quite similar to slow-twitch fibers with respect to myoglobin content (indeed, they are red) and metabolic machinery. One important difference is that fast-twitch oxidative fibers contain abundant glycogen and have a greater number of mitochondria than slow-twitch fibers do. These features ensure adequate ATP generation to compensate for the increased rate of ATP hydrolysis in fast-twitch fibers.
Other fast-twitch fibers are not capable of sufficient oxidative metabolism to sustain contraction. Because these fibers must rely on the energy that is stored within glycogen (and phosphocreatine), they are more easily fatigable. Fatigable fast-twitch fibers (type IIb) have fewer mitochondria and lower concentrations of myoglobin and oxidative enzymes. Because of their low myoglobin content, type IIb muscle fibers are white. They are, however, richer in glycolytic enzyme activity than other fiber types are.

In reality, slow- and fast-twitch fibers represent the extremes of a continuum of muscle fiber characteristics. Moreover, each whole muscle is composed of fibers of each twitch type, although in any given muscle one of the fiber types predominates. The differences between fiber types derive in large part from differences in isoform expression of the various contractile and regulatory proteins (see Table 9–1).

Differences in the rate of contraction, for example, may be directly correlated with the maximal rate of myosin ATPase activity. As many as 10 different myosin heavy chain isoforms have been identified. Individual isoform expression varies among muscle types and is developmentally regulated. At least four isoforms of the MHC protein are expressed in skeletal muscle (MHC-I, MHC-IIa, MHC-IIb, MHC-IIx/d). For the most part, a muscle fiber type expresses a single MHC isoform, the ATPase activity of which appears to correspond to the rate of contraction in that fiber type. Whereas most fibers express one of these isoforms, some fibers express a combination of two different isoforms. These hybrid cells have rates of contraction that are intermediate between the two pure fiber types.

Differences in the rates and strength of contraction may also result from differences in myosin light chain isoform expression or from isoform differences among other components of the EC coupling process. Three skeletal muscle isoforms have been identified. MLC-1a and MLC-1b are expressed in slow-twitch fibers, whereas MLC-1f and MLC-3f are expressed in fast-twitch fibers.

Isoform differences also exist for the SR Ca2+ pump (i.e., the SERCA), calsequestrin, the Ca2+ release channel,
and troponin C. Furthermore, some proteins, such as phospholamban, are expressed in one fiber type (slow twitch) and not the other.

One particularly interesting feature of muscle differentiation is that fiber-type determination is not static. Through exercise training or changes in patterns of neuronal stimulation, alterations in contractile and regulatory protein isoforms may occur. For example, it is possible for a greater proportion of fast-twitch fibers to develop in a specific muscle with repetitive training. It is even possible to induce cardiac-specific isoforms in skeletal muscle, given appropriate stimulation patterns.

The Properties of Cardiac Cells Vary with Location in the Heart

Just as skeletal muscle consists of multiple fiber types, so too does heart muscle. The electrophysiological and metabolic properties of cardiac muscle vary with their location within the heart (epicardial cells versus conducting system versus ventricle). In among cells within one anatomic location, differences may exist between muscle cells near the surface of the heart versus those lining the interior or the interior of the heart versus the exterior. Differences in isoform expression of the various contractile and regulatory proteins. Although some of the protein isoforms expressed in cardiac tissue are identical to those expressed in skeletal muscle, many of the proteins have cardiac-specific isoforms (see Table 9-1). The MHC in heart, for example, exists in two isoforms, α and β, which may be expressed alone or in combination.

<table>
<thead>
<tr>
<th>Table 9-3: Summary of Comparisons Between Muscle Types</th>
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<tbody>
<tr>
<td><strong>SKELETAL</strong></td>
</tr>
<tr>
<td>Mechanism of excitation</td>
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<tr>
<td>Electrical activity of muscle cell</td>
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<tr>
<td>Ca²⁺ sensor</td>
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<tr>
<td>Excitation-contraction coupling</td>
</tr>
<tr>
<td>Terminates contraction</td>
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<tr>
<td>Twitch duration</td>
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<td>Regulation of force</td>
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<td>Metabolism</td>
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ACh, acetylcholine; DHP, dihydropyridine; IP₃, inositol 1,4,5-trisphosphate receptor; MLCK, myosin light chain kinase; SR, sarcoplasmic reticulum.
Smooth muscle—the myometrium—that undergoes remarkable transformation during gestation as it prepares for parturition (see Chapter 55). In addition to hypertrophy, greater coupling develops between smooth muscle cells through the increased formation of gap junctions. The cells also undergo changes in their expression of contractile protein isoforms. Changes in the expression of ion channels and hormone receptors facilitate rhythmic electrical activity. This activity is coordinated across the myometrium by propagation of action potentials and increases in $[\text{Ca}^{2+}]_i$, through the gap junctions. These rhythmic, coordinated contractions develop spontaneously, but they are strongly influenced by the hormone oxytocin, levels of which increase just before and during labor and just after parturition.

These differences in smooth muscle function among various tissues or even over the lifetime of a single cell probably reflect differences in protein composition. Indeed, in comparison to striated muscle, smooth muscle cells express a wider variety of isoforms of contractile and regulatory proteins (see Table 9-1). This variety is a result of both multiple genes and alternative splicing (p. 139). This richness in diversity is likely to have important consequences for smooth muscle cell function, although the precise relationship between the structure and function of these protein isoforms is not yet clear.

Smooth Muscle Cells Express a Wide Variety of Neurotransmitter and Hormone Receptors

Perhaps one of the most impressive sources of diversity among smooth muscle relates to differences in response to neurotransmitters, environmental factors, and circulating hormones. Smooth muscle cells differ widely with respect to the types of cell-surface receptors that mediate the effects of these various mediators. In general, smooth muscle cells each express a variety of such receptors, and receptor stimulation may lead to either contraction or relaxation. Many substances act through different receptor subtypes in different cells, and these receptor subtypes may act through different mechanisms. For example, whereas some neurotransmitter/hormone receptors may be ligand-gated ion channels, others act through heterotrimeric G proteins that either act directly on targets or act through intracellular second messengers such as cAMP, cGMP, or IP, and DAG.

The list of neurotransmitters, hormones, and environmental factors regulating the function of vascular smooth muscle cells alone is vast (see Chapter 22). A few of these vasoactive substances include epinephrine, norepinephrine, serotonin, angiotensin, vasopressin, neuropeptide Y, nitric oxide, endothelin, and oxygen. Identical stimuli, however, may result in remarkably different physiologic responses by smooth muscle in different locations. For example, systemic arterial smooth muscle cells relax when the oxygen concentration around them decreases, whereas pulmonary arterial smooth muscle contracts when local oxygen decreases (p. 699).

See Table 9-3 for a comparison of muscle types.

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