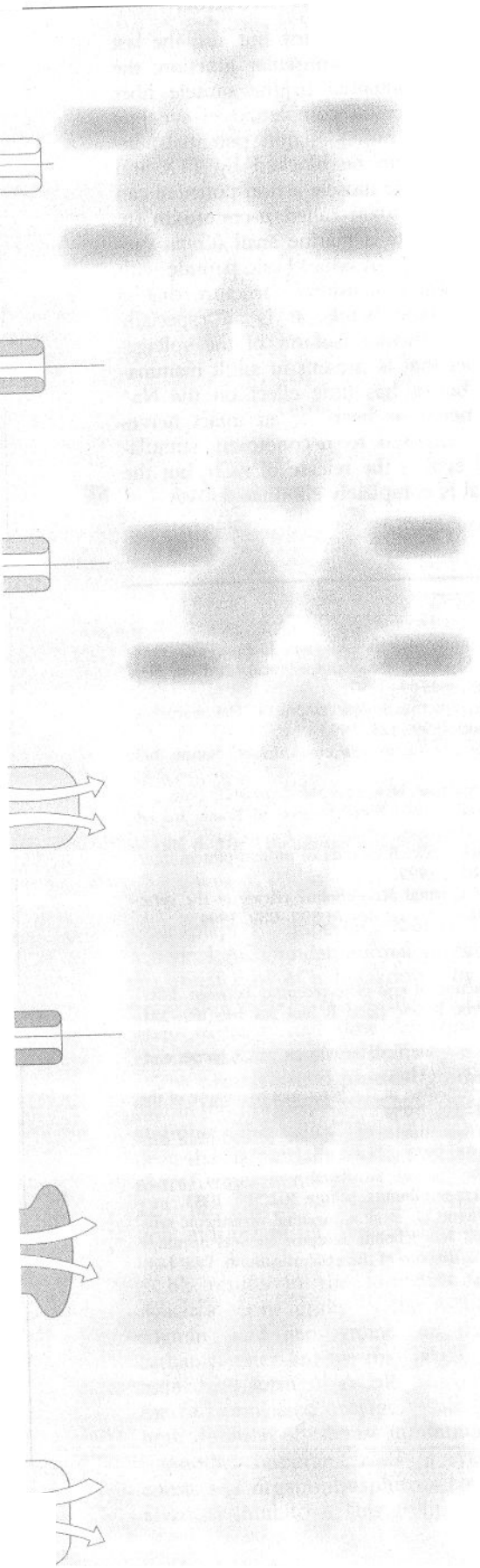

Cellular Physiology of Skeletal, Cardiac, and Smooth Muscle

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, fatigability, 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 ($[Ca^{2+}]_i$).

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.

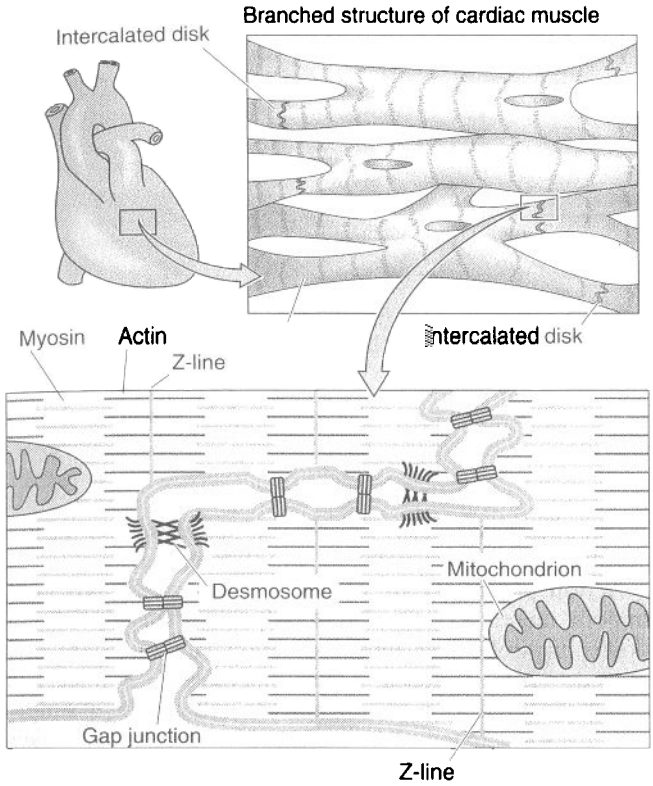

FIGURE 9–1. Electrical coupling of cardiac myocytes.

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. 9–2*A*). 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

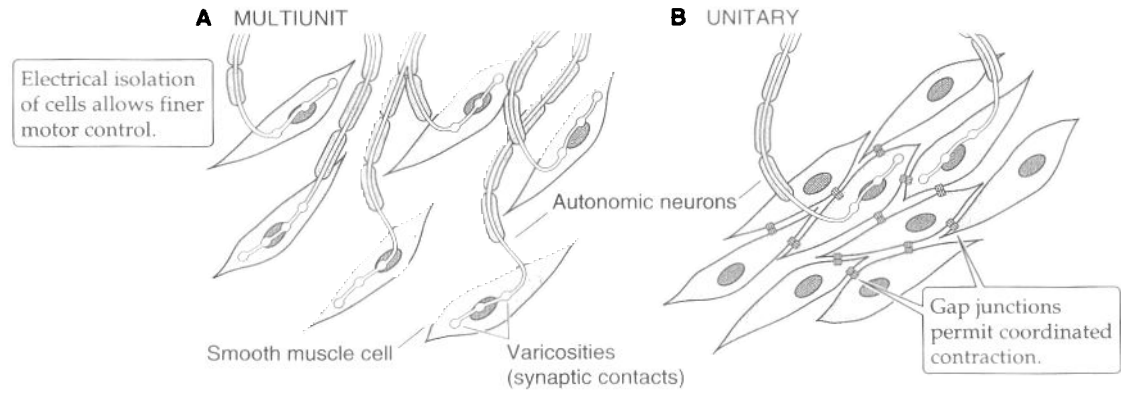

FIGURE 9–2. Smooth-muscle organization. *A*, Each smooth muscle cell receives its own synaptic input. *B*, Only a few of the smooth muscle cells receive direct synaptic input.

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. 9–2*B*). 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. 9–3*A*). 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 $[Ca^{2+}]_i$ to remain high for a longer period, thereby prolonging the contraction.

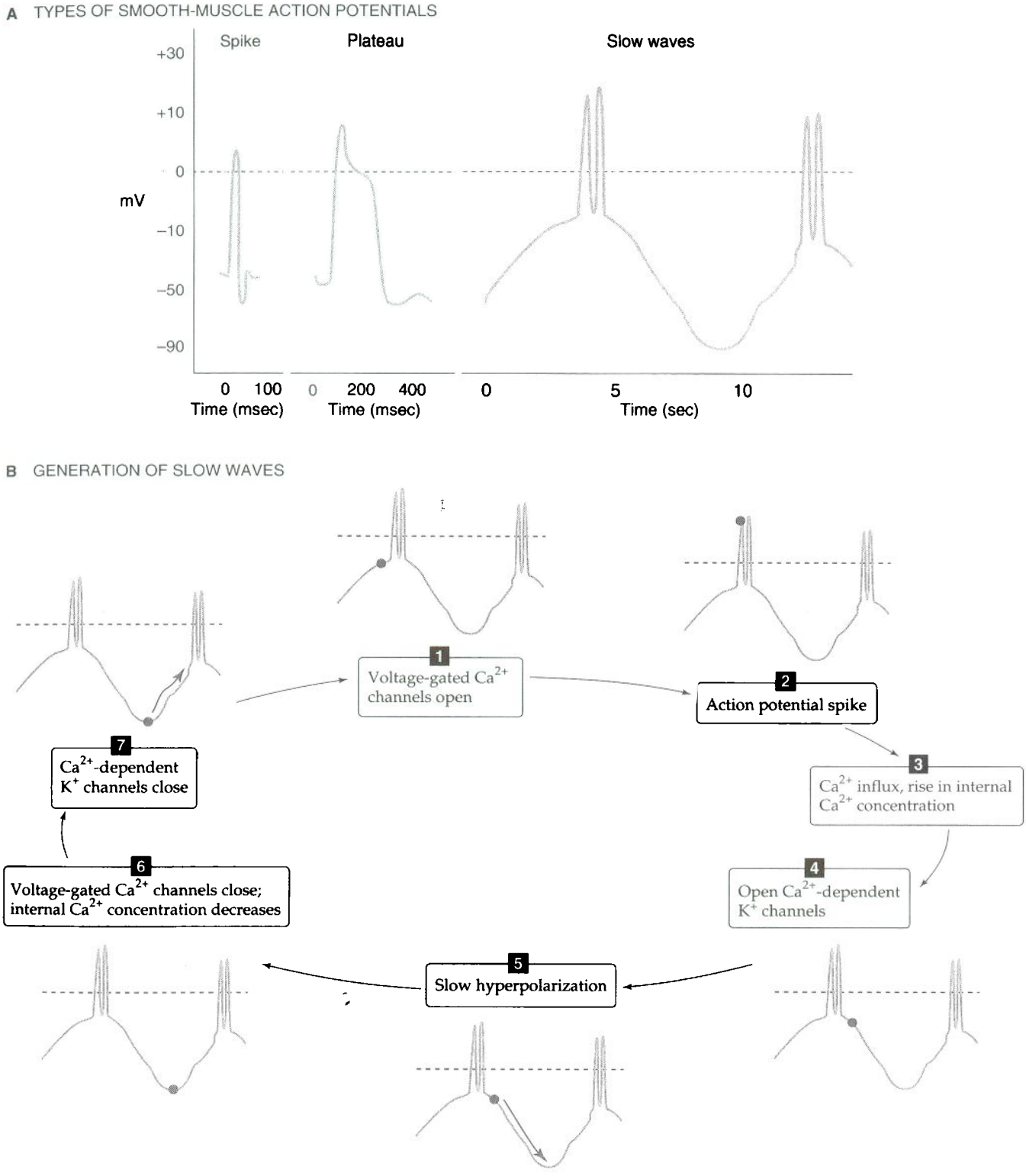

FIGURE 9–3. Action potentials and slow waves in smooth muscle.

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 results 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. 9–3*B*). 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 $[Ca^{2+}]_i$ activates Ca^{2+}-dependent K^+ channels, which leads to progressive hyperpolarization and termination of the depolarization phase of the wave. These periodic depolarizations and $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ during an action potential might stimulate Na-Ca exchange and lead to a cyclic increase in $[Na^+]_i$ 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 $[Ca^{2+}]_i$ would be self-reinforcing because of Ca^{2+}-activated Ca^{2+} release via the IP_3 receptor. At high $[Ca^{2+}]_i$, this channel is inhibited and the Ca^{2+} release event is terminated, followed by re-uptake of Ca^{2+} into the sarcoplasmic reticulum (SR). The $[Ca^{2+}]_i$ increases may themselves lead to periodic electrical activity by stimulating Ca^{2+}-activated inward and outward currents.

Some Smooth Muscles Contract Without Action Potentials

Whereas action-potential generation is essential for initiating contraction of skeletal and cardiac muscle, many smooth muscle cells contract despite being unable to generate an action potential. As discussed previously, V_m oscillations can lead to tonic contractions in the absence of action potentials. Action potentials usually do not occur in **multiunit** smooth muscle. For example, in the smooth muscle that regulates the iris, excitatory neurotransmitters such as norepinephrine or ACh cause a local depolarization, the **junctional potential**, which is similar to the end-plate potential in skeletal muscle. Junctional potentials spread electrotonically (i.e., in a graded fashion) throughout the muscle fiber, thereby altering V_m and affecting the entry of Ca^{2+} through voltage-gated slow (L-type) Ca^{2+} channels. Changes in V_m—by an unknown mechanism—may also modulate the activity of the enzyme phospholipase C, which cleaves phosphoinositides to release the intracellular second messengers diacylglycerol (DAG) and IP_3 (p. 100). Both these second messengers are modulators of contractile force. In the absence of action potentials, some unitary smooth muscle, including some vascular smooth muscle, also contracts as a result of graded V_m changes.

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+} stores 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 9–4*A*, 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. 9–4*B*), 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

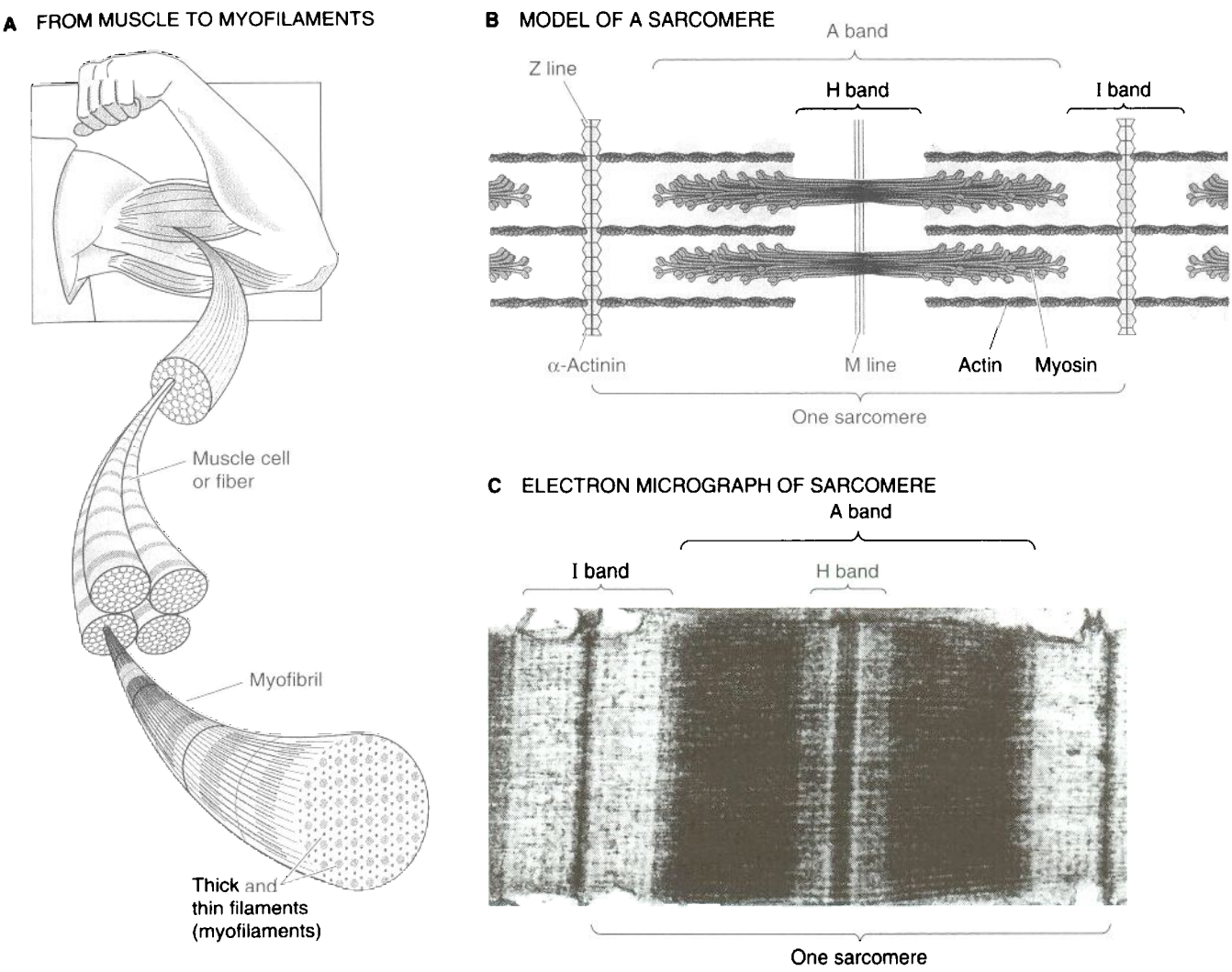

FIGURE 9–4. Structure of the sarcomere.

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. 9–4*C*). 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. 9–5*A*) 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

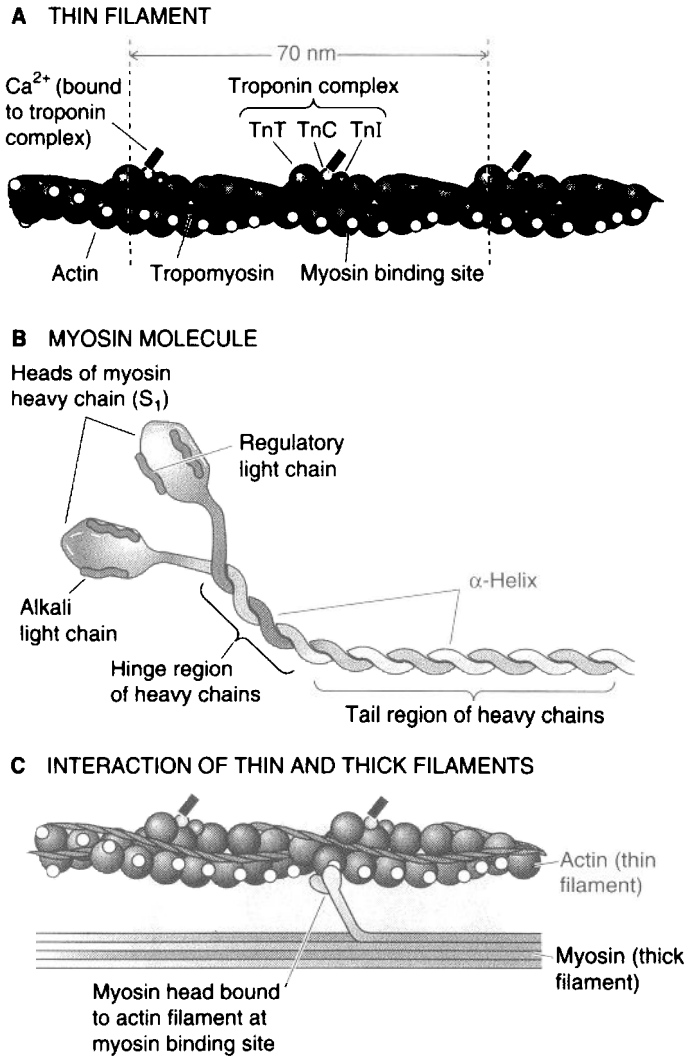

FIGURE 9–5. Structure of thin and thick filaments.

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

THICK FILAMENTS. Like thin actin filaments, thick filaments are polymers of proteins (see Fig. 9–5*B*). 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 α 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 9–5*C* 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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ initiates and allows cross-bridge cycling to continue. During this excitatory increase, $[Ca^{2+}]_i$ may rise from its resting level of less than 10^{-7} M to greater than 10^{-5} M. The subsequent decrease in $[Ca^{2+}]_i$ 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 troponin molecule contains the key Ca^{2+}-sensitive regulator troponin C (Fig. 9–6*A*). 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

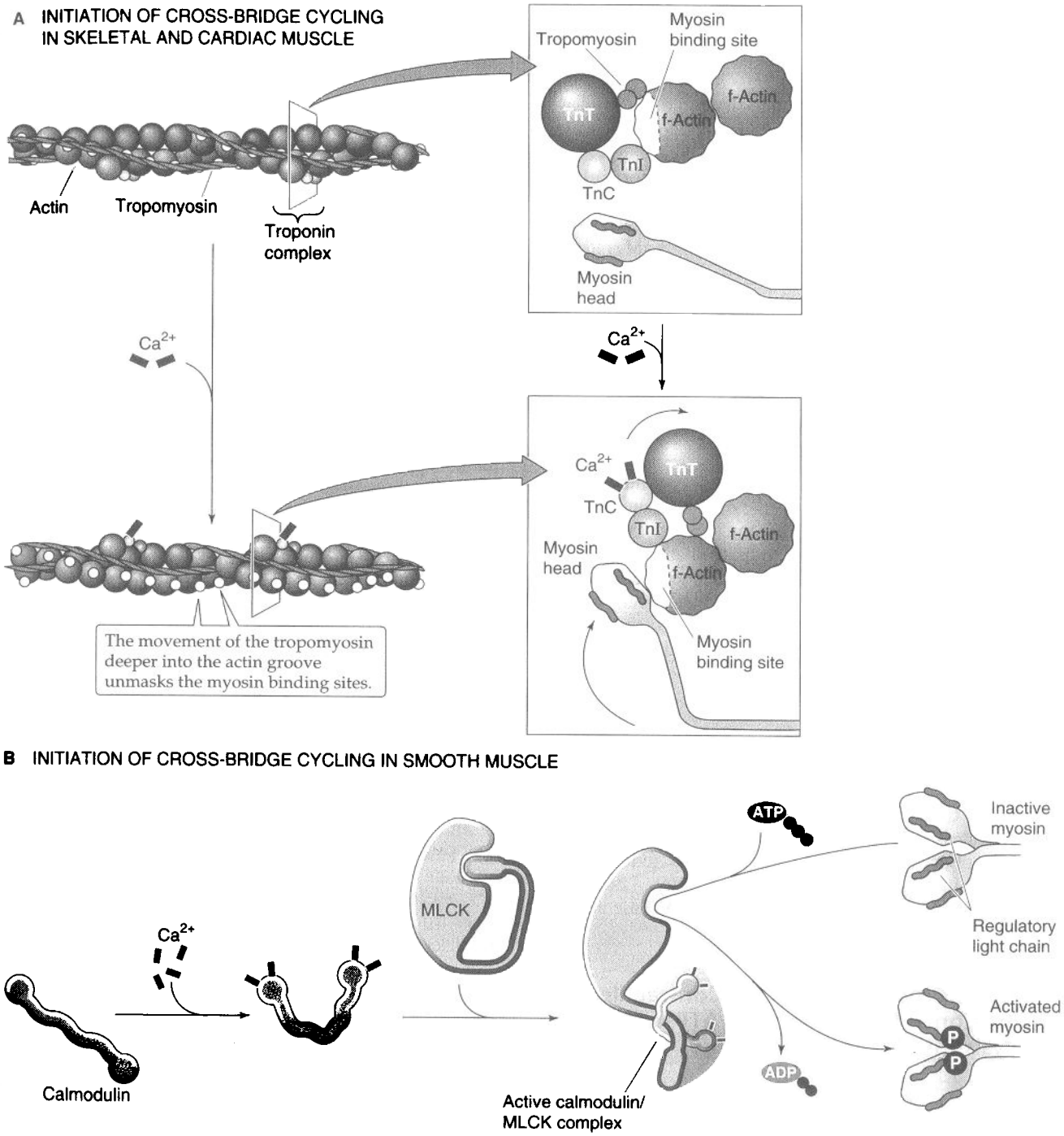

FIGURE 9–6. The role of Ca^{2+} in triggering muscle contraction. MLCK, myosin light chain kinase.

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 $[Ca^{2+}]_i$ initiates a slow chain of events that

ultimately increases the ATPase activity of the myosin (see Fig. 9–6*B*). 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 $[Ca^{2+}]_i$. 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 to 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 tropomyosin. **Calponin**, which is found in a fixed stoichiometry with tropomyosin and actin (one calponin—one tropomyosin—seven actin monomers), tonically inhibits the ATPase activity of myosin. As we saw earlier, the increase in $[Ca^{2+}]_i$ 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_i complex for actin.

Step 4: **Release of P_i 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 $[ATP]_i$. 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 $[Ca^{2+}]_i$. 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 $[Ca^{2+}]_i$.

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.

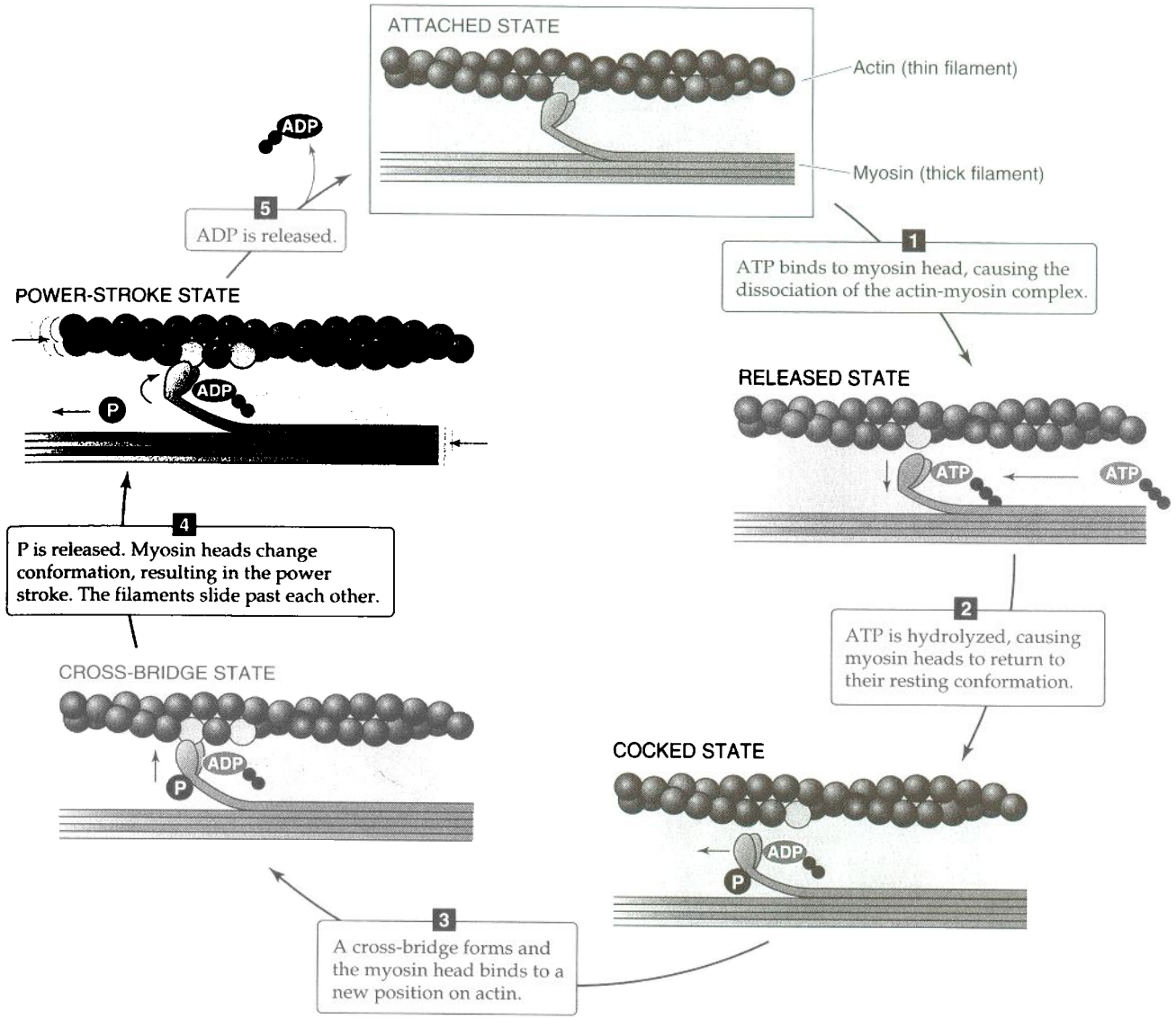

FIGURE 9–7. The cross-bridge cycle in skeletal and cardiac muscle. Each cycle advances the myosin head by two actin monomers, or approximately 11 nm.

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.

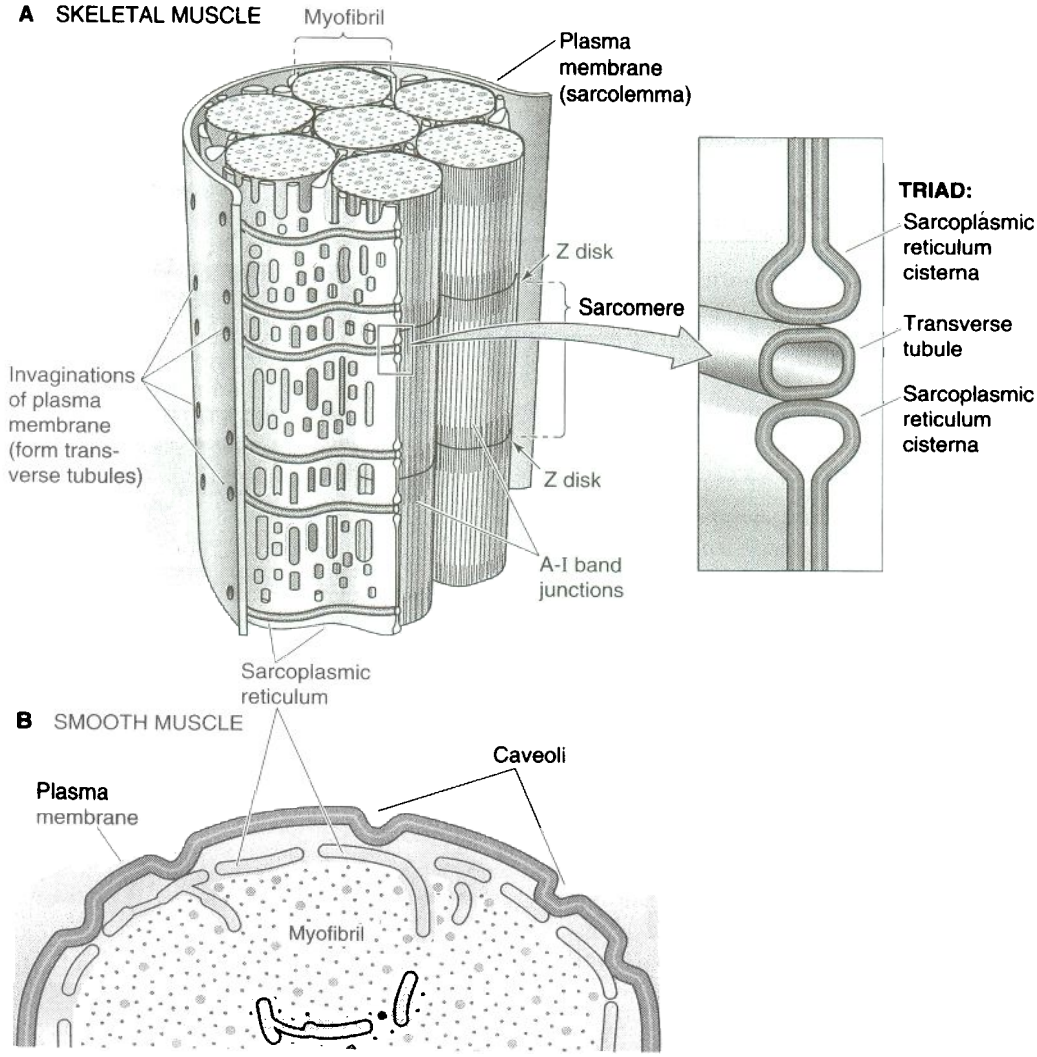

FIGURE 9–8. Plasma-membrane invaginations. *A*, The transverse tubules (T tubules) are extensions of the plasma membrane, penetrating the muscle cell at two points in each sarcomere: the junctions of the A and I bands. *B*, Smooth-muscle cells have rudimentary invaginations of the plasma membrane, called 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 $[Ca^{2+}]_i$ that triggers muscle contraction. The time during which $[Ca^{2+}]_i$ remains elevated determines the duration of muscle contraction. The process by which "excitation" triggers the increase in $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$. 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. 9–8*A*). 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

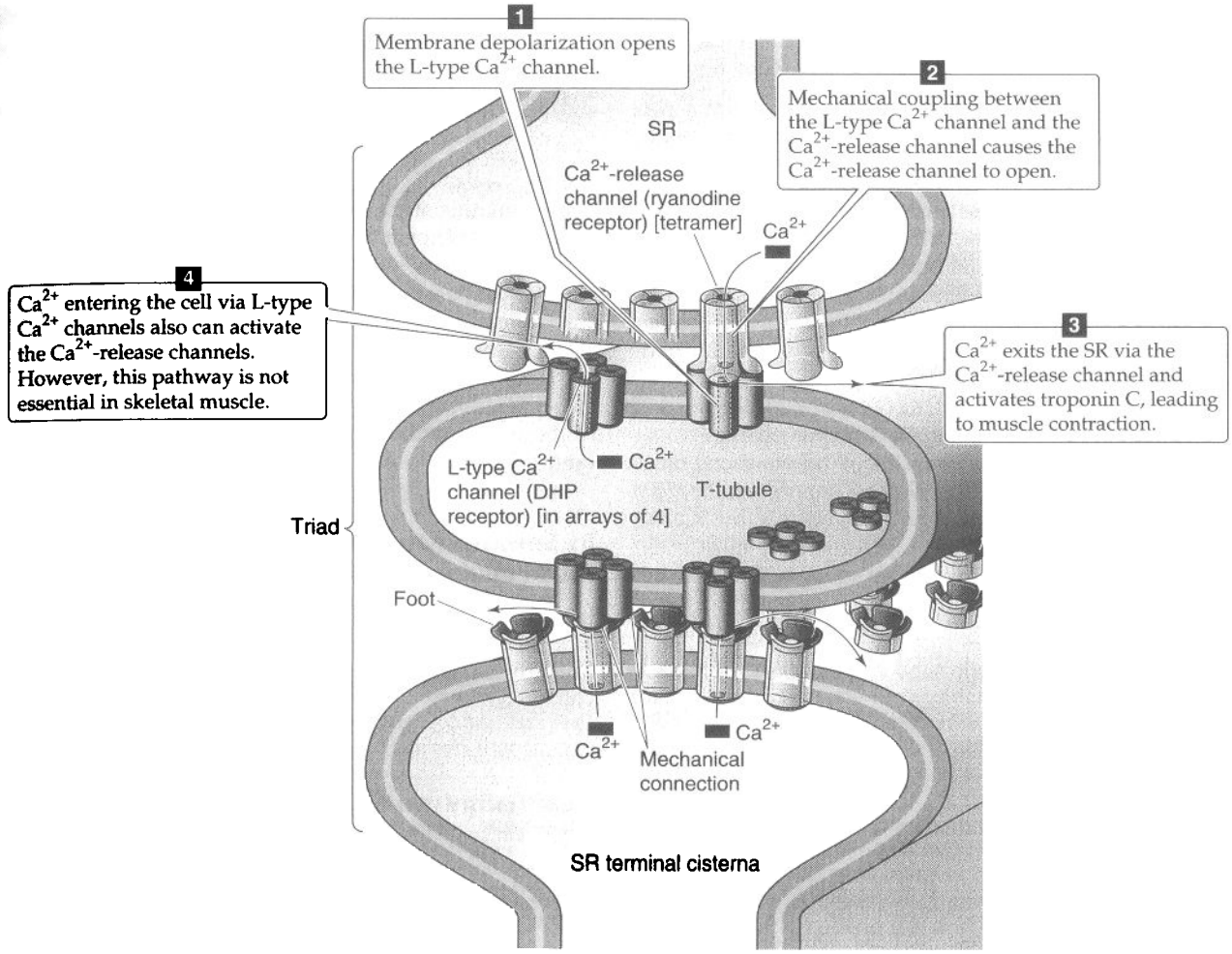

FIGURE 9–9. Excitation-contraction coupling in skeletal muscle. A tetrad of four L-type Ca^{2+} channels on the T tubules faces a single Ca^{2+}-release channel of the SR, so that each L-type Ca^{2+} channel interacts with the foot of one of the four subunits of the Ca^{2+}-release channel. Note that half of the Ca^{2+}-release channels lack associations with L-type Ca^{2+} channels. DHP, dihydropyridine; SR, sarcoplasmic reticulum.

coupling of excitation to contraction in skeletal and cardiac muscle. *Smooth* muscle, in contrast, has more rudimentary and shallow invaginations called **caveoli** (see Fig. 9–8*B*).

In Skeletal Muscle, Depolarization of the T-Tubule Membrane Leads to Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} channels. Each of the four voltage-gated Ca^{2+} channels in a tetrad is in fact a heteropentameric protein (see Fig. 7–12*B*). Each of the four Ca^{2+} 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 Ca^{2+} channels and has two effects. First, the conformational changes allow Ca^{2+} to enter through the four channel pores. Second, and much more important, the conformational changes in the four L-type Ca^{2+} channels induce a conformational change in each of the four subunits of another channel—the Ca^{2+}-release channel—that is located in the SR membrane.

The **Ca^{2+}-release channel** (Table 6–2, #18) has a homotetrameric structure quite different from that of the L-type Ca^{2+} channel that constitutes the voltage sensor. The Ca^{2+}-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*. Ca^{2+}-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 Ca^{2+}-release

channel subunits is complementary to the cytoplasmic projection of one of the four L-type Ca^{2+} channels in a tetrad on the T tubule (see Fig. 9–9). The close physical proximity of these two proteins, as well as the ability of both DHP and ryanodine to block muscle contraction, suggests that interaction between these two different Ca^{2+} channels underlies EC coupling. The precise mechanism of interaction between these proteins is not yet fully understood, although we know that it is not electrical inasmuch as ion conductance of the Ca^{2+}-release channel is not strongly voltage dependent. A large cytoplasmic projection on the α_1 subunit of the L-type Ca^{2+} channel appears to be necessary for interaction between the two Ca^{2+} channels on opposing T-tubule and SR membranes. Thus, it is possible that direct *mechanical* coupling exists between this projection and the Ca^{2+}-release channel.

As the L-type Ca^{2+} channel on the T-tubule membrane mechanically opens the Ca^{2+}-release channel in the SR, Ca^{2+} sequestered in the SR rapidly leaves via the Ca^{2+}-release channel. The resultant rapid increase in $[Ca^{2+}]_i$ activates troponin C, thus initiating cross-bridge cycling as described earlier. The entire process, extending from depolarization of the T-tubule membrane to the initiation of cross-bridge cycling, is termed **excitation-contraction coupling**.

Although we have stressed activation of the Ca^{2+}-release channel in the SR by *mechanical* coupling between it and the L-type Ca^{2+} channel in the T-tubule membrane, local elevations in $[Ca^{2+}]_i$ can also activate the Ca^{2+}-release channel in skeletal muscle. When the L-type Ca^{2+} channel opens during depolarization, it allows an influx of Ca^{2+} that locally increases $[Ca^{2+}]_i$. This mechanism of activating the Ca^{2+}-release channel in the SR is known as **Ca^{2+}-induced Ca^{2+} release** (CICR). Although L-type Ca^{2+} channels allow $[Ca^{2+}]_i$ to rise during action potentials in skeletal muscle, CICR is not necessary for contraction. Indeed, skeletal muscle contraction persists even when Ca^{2+} is absent from the extracellular fluid. However, as we will see later, CICR plays a critical role in EC coupling in *cardiac* muscle.

In Cardiac Muscle, Ca^{2+} Entry Through L-Type Ca^{2+} Channels Is Amplified By Ca^{2+}-Induced Ca^{2+} Release from the Sarcoplasmic Reticulum

Whereas EC coupling in skeletal muscle does not require Ca^{2+} influx through L-type Ca^{2+} channels, cardiac contraction has an *absolute requirement* for Ca^{2+} 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 Ca^{2+} from bulk extracellular fluid to the site of the L-type Ca^{2+} channels on the T-tubule membrane. Thus, the Ca^{2+} can simultaneously reach superficial and deep regions of the muscle. The increase in $[Ca^{2+}]_i$ resulting from Ca^{2+} influx alone is not, however, sufficient to initiate contraction. Rather, the increase in $[Ca^{2+}]_i$ that is produced by the L-type Ca^{2+} channels is greatly amplified by CICR from the SR via the Ca^{2+}-release channels. Indeed, because the Ca^{2+}-release channels remain open for a longer period than do L-type Ca^{2+} channels, the contribution of CICR to the rise in $[Ca^{2+}]_i$ is greater than the flux contributed by the L-type Ca^{2+} channels of the T tubules.

It appears that each L-type Ca^{2+} channel controls only one SR Ca^{2+}-release channel. The close physical proximity of L-type Ca^{2+} channels of the T-tubule membrane and the Ca^{2+}-release channel in the SR at the triad junctions allows for this tight local control. Although Ca^{2+} diffuses in the cytosol away from its SR release site, Ca^{2+} release at one site does not appear to be able to induce Ca^{2+} release from a neighboring SR Ca^{2+}-release channel. Thus, Ca^{2+} release events are not propagated along the myocyte. In fact, the SR Ca^{2+}-release channel does not appear to respond to generalized increases in cytoplasmic $[Ca^{2+}]_i$. 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 Ca^{2+} Activate Contraction

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

Ca^{2+} 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 Ca^{2+} through voltage-gated L-type Ca^{2+} channels (Fig. 9–10).

Ca^{2+} RELEASE FROM THE SR. This Ca^{2+} release may occur by either of two mechanisms: CICR or IP_3-mediated Ca^{2+} release. As we have already seen, CICR plays a key role in EC coupling in cardiac muscle, where the L-type Ca^{2+} channels are highly ordered and in close proximity to the Ca^{2+}-release channels in the SR. Thus, Ca^{2+} influx through L-type Ca^{2+} 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 $[Ca^{2+}]_i$ levels that are higher than those that typically occur under physiological conditions, and its role remains unclear.

A more important mechanism for Ca^{2+} release from the SR of smooth muscle is the IP_3 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 Ca^{2+} influx. Furthermore, even for agonists such as serotonin or norepinephrine, which activate a Ca^{2+}-influx pathway, the observed increase in $[Ca^{2+}]_i$ is out of proportion to that expected from Ca^{2+} influx alone. Thus, another pathway must exist for increasing $[Ca^{2+}]_i$. Some agonists cause smooth muscle contraction by triggering the production of IP_3 (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. According 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 humans 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).

membrane of the smooth muscle SR (p. 100). This IP_3 receptor is itself a ligand-gated Ca^{2+} channel. Thus, a receptor in the plasma membrane can indirectly induce Ca^{2+} release from the SR and hence contraction.

Ca^{2+} ENTRY THROUGH VOLTAGE-*INDEPENDENT* CHANNELS. In smooth muscle it is possible that extracellular ligands may trigger the influx of Ca^{2+} through either ligand-gated channels (p. 170) or channels that are activated via G-protein–coupled receptors. Nevertheless, it is not clear to what extent, if any, these types of Ca^{2+} channels contribute to $[Ca^{2+}]_i$ increases in smooth muscle. However, another class of channels—**store-operated Ca^{2+} channels**—appears to play an important role. Neu-

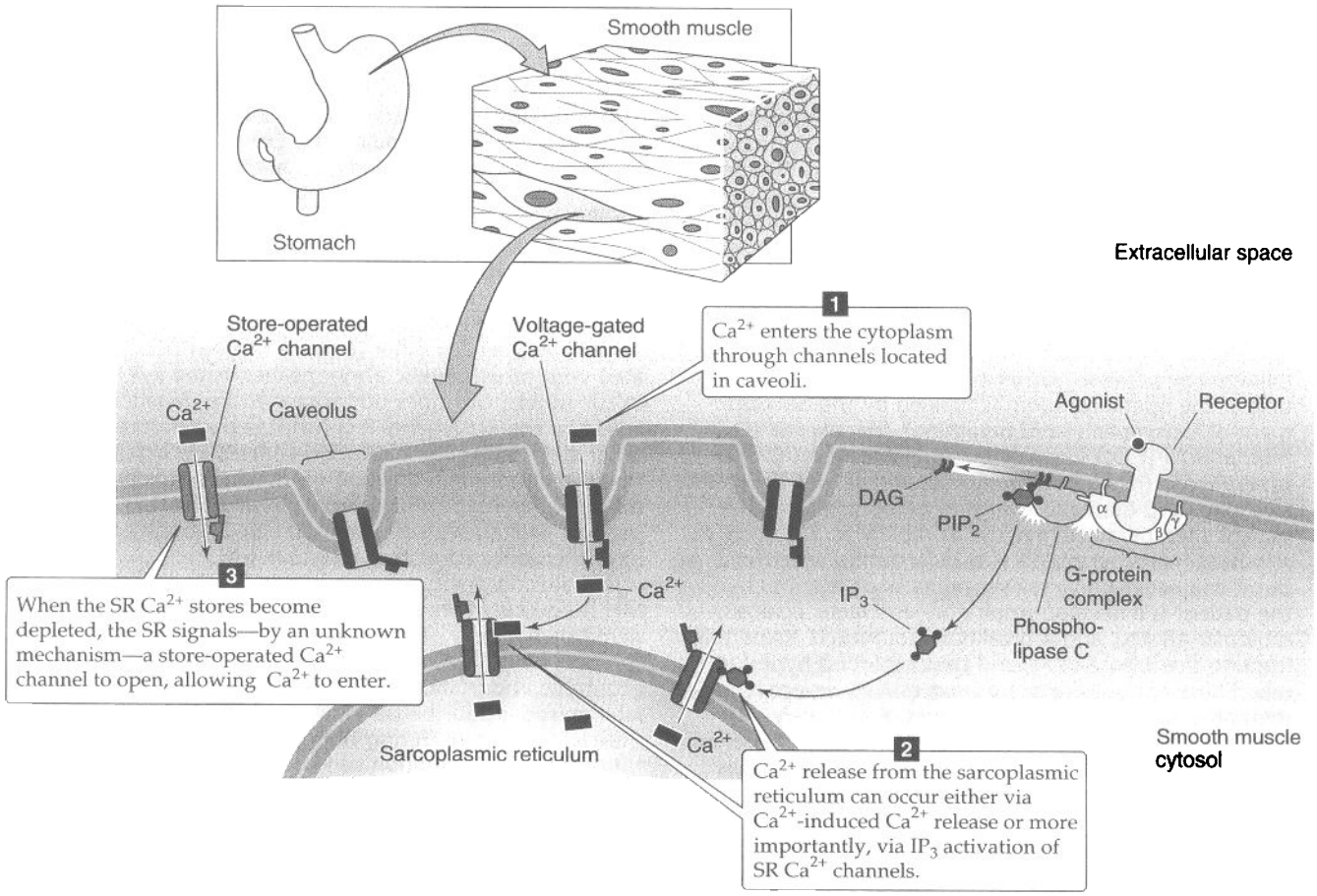

FIGURE 9–10. Excitation-contraction (E-C) coupling in smooth muscle. DAG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate; PIP_2, phosphatidyl inositol 4,5-biphosphate; SR, sarcoplasmic reticulum.

rotransmitters 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 $[Ca^{2+}]_i$ to remain elevated even after SR depletion and also appears to replenish SR Ca^{2+} stores.

Together, the second pathway for increasing $[Ca^{2+}]_i$ (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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$

Whereas many excitatory stimuli rely on increases in $[Ca^{2+}]_i$ to evoke contraction, some stimuli appear to cause contraction without a measurable increase in $[Ca^{2+}]_i$. 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 $[Ca^{2+}]_i$ may vary. This force/$[Ca^{2+}]_i$ 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-

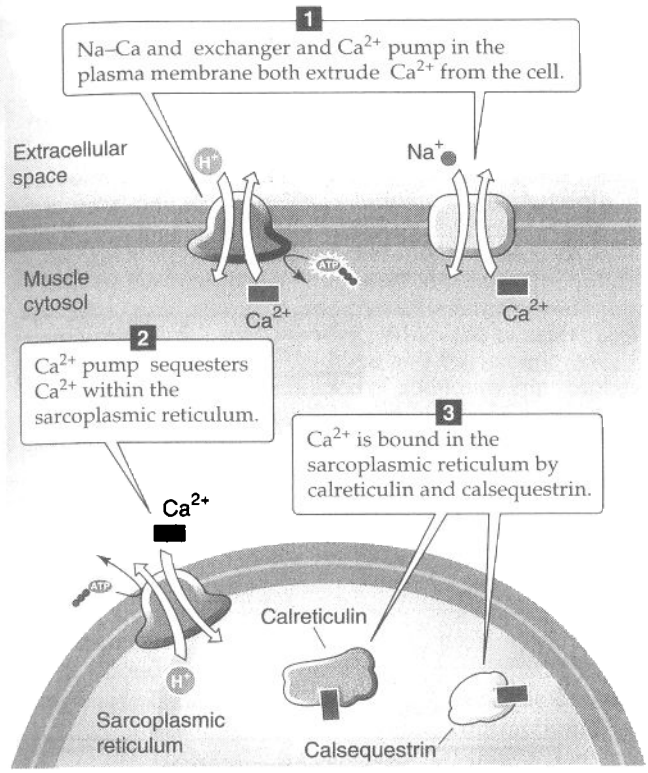

FIGURE 9–11. Mechanisms of Ca^{2+} removal from the cytoplasm.

tion to cease and for relaxation to occur. Ca^{2+} may be extruded across the cell membrane or sequestered within intracellular compartments (Fig. 9–11).

The cell may extrude Ca^{2+} using either an Na-Ca exchanger (NCX, p. 68) or a Ca^{2+} pump at the plasma membrane (PMCA, p. 64). Extrusion across the cell membrane, however, would eventually deplete the cell of Ca^{2+} and is therefore a minor mechanism for Ca^{2+} removal from the cytoplasm. Instead, Ca^{2+} re-uptake into the SR is the most important mechanism by which the cell returns $[Ca^{2+}]_i$ to resting levels. Ca^{2+} re-uptake by the SR is mediated by a SERCA-type Ca^{2+} pump (p. 64).

It is possible that the rate of Ca^{2+} re-uptake into the SR may be regulated by modulating the activity of the SR Ca^{2+} pump. For example, in cardiac muscle, SR Ca^{2+}-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 Ca^{2+} pump is lost. Thus, activators of PKA, such as the neurotransmitter epinephrine, may enhance the rate of cardiac myocyte relaxation (p. 525).

SR Ca^{2+}-pump activity is also inhibited by high $[Ca^{2+}]$ within the SR lumen. This inhibition of SR Ca^{2+}-pump activity is delayed by Ca^{2+}-binding proteins within the SR lumen. These Ca^{2+}-binding proteins buffer the $[Ca^{2+}]$ increase in the SR during Ca^{2+} re-uptake and thus markedly increase the Ca^{2+} capacity of the SR. The principal Ca^{2+}-binding protein in skeletal muscle, **calsequestrin**, is also present in cardiac and some smooth muscle. **Calreticulin** is a ubiquitous Ca^{2+}-binding protein that is found in particularly high concentrations within the SR of smooth muscle. These proteins have a tremendous capacity to bind Ca^{2+}, with up to 50 binding sites per protein molecule.

Ca^{2+}-binding proteins are not located diffusely within the SR. Rather, calsequestrin is highly localized to the region of the SR immediately beneath the triad junction. Calsequestrin appears to bind directly at the triad, where it forms a complex with the Ca^{2+}-release channel and with two other triad proteins, junctin and triadin. Here, calsequestrin is poised not only to aid muscle relaxation by buffering Ca^{2+} within the SR lumen but also to unload its Ca^{2+} in the vicinity of the Ca^{2+}-release channel and thus facilitate EC coupling. It has been hypothesized that EC coupling promotes Ca^{2+} release from calsequestrin, making Ca^{2+} available for exit from the SR.

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

Because Ca^{2+} triggers smooth muscle contraction by inducing phosphorylation of the myosin regulatory light chain, merely restoring $[Ca^{2+}]_i$ 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.

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. 9–12*A*). In the second arrangement, one of the two attachment points is mobile, and a force—or load—

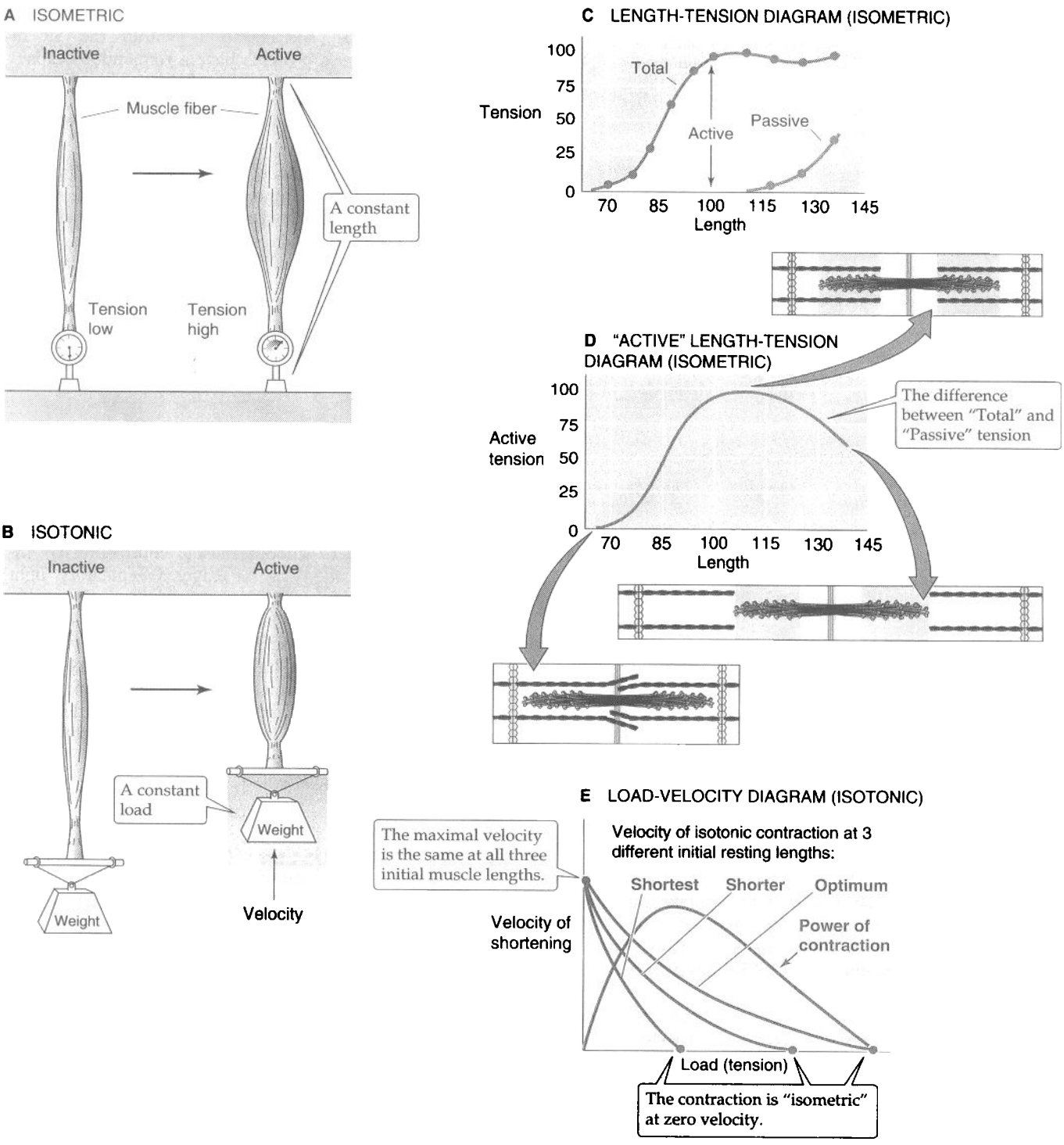

FIGURE 9–12. Isometric and isotonic contraction. *A*, Experimental preparation for studying muscle contraction under *isometric* conditions. *B*, Experimental preparation for studying muscle contraction under *isotonic* conditions. *C*, The *"passive" curve* represents the tension that is measured at 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.

MEASURING THE FORCE OF A SINGLE CROSS-BRIDGE CYCLE

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 isometric" conditions, suggests that the quantal force developed during a single cross-bridge cycle is about 5 pN. Interestingly, these isometric 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 1* in the cycle in Fig. 9–7).

tends to pull this mobile point away from the fixed one. Here, stimulation causes shortening, provided that the tension developed by the muscle is greater than the opposing load. Because these shortenings occur at constant load, they are referred to as **isotonic contractions** (see Fig. 9–12*B*). Both isometric and isotonic contractions can be examined at different initial muscle lengths. Moreover, they can be measured during individual muscle twitches that are evoked by single muscle action potentials, as well as during other patterns of stimulation.

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

The iso*metric* 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. 9–12*C*). 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. 9–12*D*). As muscle length increases toward its normal length, active tension increases. Active tension is maximal at a length—usually called L_0—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.

This length-tension relationship is a direct result of the anatomy of the thick and thin filaments within individual sarcomeres (see Fig. 9–12*D*). 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.

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

Under iso*tonic* 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 9–12*E*—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.

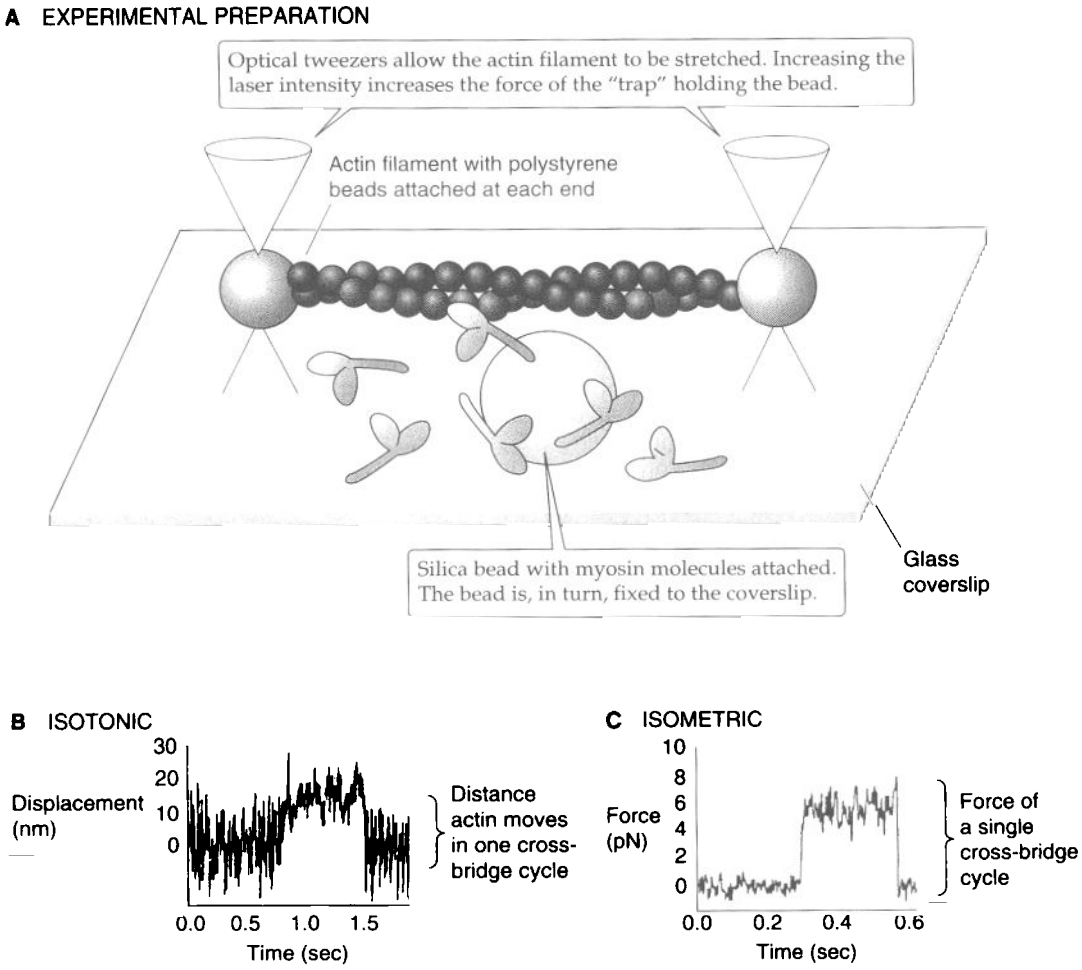

FIGURE 9–13. Microscopic measurements of cross-bridge force and displacement. *A*, An actin filament is attached at each end to a polystyrene bead. The "optical tweezers," a finely focused beam of laser light, can "trap" the bead at its focal point and physically move it. By adjusting the laser intensity, the experimenter can alter the strength of the trap (i.e., the force with which the bead is held). In this experiment, two optical tweezers were used to suspend the actin filament above a coverglass. Attached to this coverslip is a silica bead, and myosin molecules are bound to the bead. *B*, In an "isotonic" experiment, the force between the actin filament and the fixed myosin/silica bead is kept constant by using a stable laser intensity. The experimenter measures, as a function of time, the displacement of the polystyrene bead (*blue*) away from the center of the trap. Thus, in one cross-bridge cycle, the myosin-actin interaction pulls the polystyrene bead approximately 11 nm away from the center of the trap. *C*, In an "isometric" experiment, the experimenter measures, as a function of time, the extra force that needs to be applied (i.e., increase in laser intensity) to keep the polystyrene bead (*blue*) at a fixed position near the center of the trap. Thus, in one cross-bridge cycle, the myosin-actin interaction exerts a force of approximately 5 pN. (Data from Finer JT, Mehta AD, Spudich JA: Characterization of single actin-myosin interactions. Biophys J 68:291s–296s, 1995.)

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 9–12*E*. At any time, all the available cross-bridges are engaged in resisting the opposing force. None are left over to make the muscle shorten. If the number of engaged cross-bridges were decreased, the muscle would lengthen. 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 actin 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 9–12*E* applies to a particular initial length of the muscle, that is, the *resting* length. We already saw in Figure 9–12*C* 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 9–12*E*: 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 this maxi-

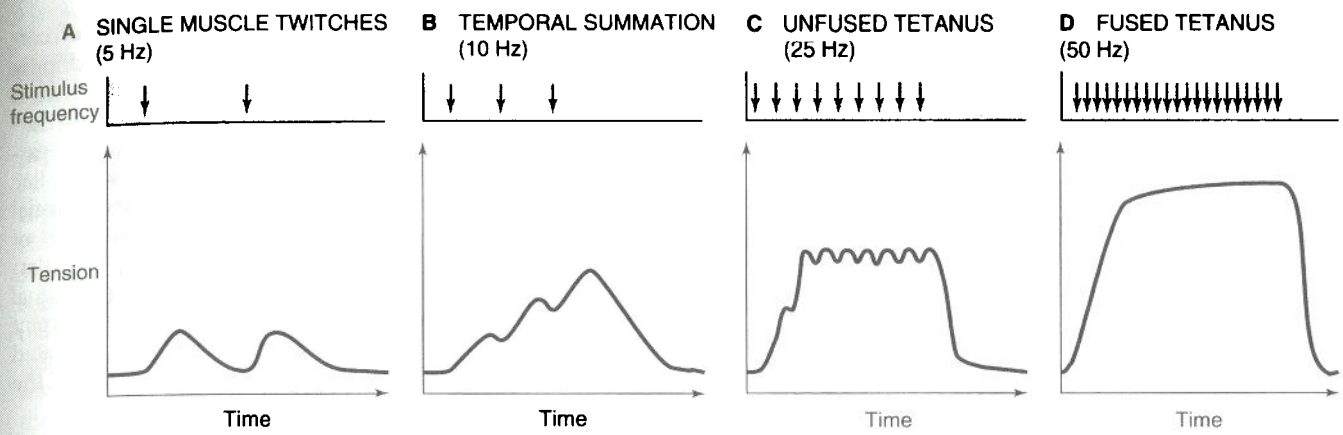

FIGURE 9–14. Frequency summation of skeletal muscle twitches.

mal *load*, which depends very much on length, maximal *velocity* is independent of length, as shown by the common intercept of the family of curves with the ordinate. The explanation for this effect—as we have already noted—is that maximal velocity (at no load) depends on the maximal rate of cross-bridge turnover, not on the initial overlap of the thin and thick filaments.

The velocity-tension curve reveals an interesting relationship between muscle power and applied load. Muscle does measurable mechanical work only when it displaces a load. This mechanical **work** (W) is the product of load (F) and displacement (Δx). **Power** (P) is the rate at which work is performed, or work per unit time (Δt):

Equation 9–1

$$P = W/\Delta t = F \times \Delta x/\Delta t$$

Because velocity (v) is $\Delta x/\Delta t$, it follows that

Equation 9–2

$$P = F \times v$$

For a given load (F), we can calculate the power by reading the velocity (v) from the uppermost of the three blue load-velocity relationships in Figure 9–12*E*. Power is maximal at intermediate loads (where both F and v are moderate) and falls to zero at maximum load (where $v = 0$) and at zero load (where $F = 0$).

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. 9–14*A*). 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. 9–14*A* 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 9–14*C*. 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. 9–14*D*) 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 $[Ca^{2+}]_i$ below a level that initiates relaxation. In fact, a sustained increase in $[Ca^{2+}]_i$ 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.

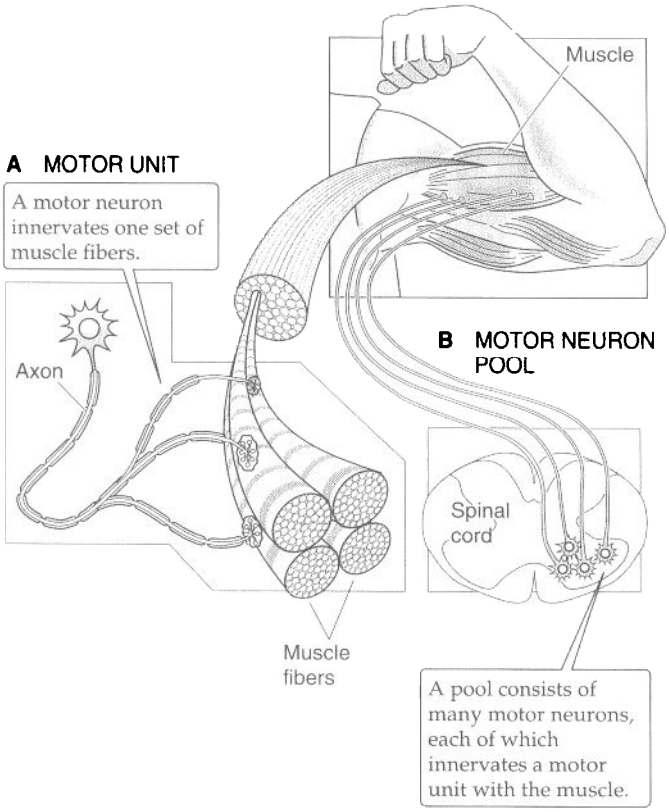

FIGURE 9–15. The motor unit and the motor-neuron pool.

212) in motor neurons with smaller cell bodies, the small motor units are recruited even with minimal neuronal stimulation. As neuronal stimulation intensifies, larger motor neurons innervating larger motor units are also recruited. The progressive recruitment of first small and then larger and larger motor units is referred to as the **size principle**. The group of all motor neurons innervating a single muscle is called a **motor-neuron pool**.

Multiple-fiber summation, sometimes referred to as **spatial summation**, is an important mechanism that allows the force developed by a whole muscle to be relatively constant in time. It is true that the CNS could direct the force to be relatively constant over time merely by driving a fixed number of motor units within the muscle to tetanus, where the force fluctuations are very small (see Fig. 9–14*D*). However, adding tetanic motor units would increase total muscle force by rather large individual increments. Instead, the CNS can activate individual motor units asynchronously so that some units are developing tension while others are relaxing. Thus, whole-muscle force can be relatively constant with time, even when individual fibers are not stimulated to tetanus. Smooth, nontetanic contraction is essential for fine motor control.

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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ or by altering the 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 Ca^{2+} enters the cell via voltage-gated Ca^{2+} channels that open during the cardiac action potential. Most of this Ca^{2+} influx occurs via L-type Ca^{2+} channels. How does norepinephrine increase the contractile force of the heart? This hormone acts through the β-type adrenergic receptor to increase the generation of cAMP, activate PKA (p. 97), and in turn phosphorylate the L-type Ca^{2+} channels, thereby increasing the passive influx of Ca^{2+}. An increased $[Ca^{2+}]_i$ leads to an increase in contractile force. The cAMP pathway also appears to increase the 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 $[Ca^{2+}]_i$.

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

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

In Smooth Muscle, Contractile Force Is Enhanced By Increasing the Entry of Ca^{2+}, As Well As By Increasing the 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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$. 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, EC-coupling 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.

TABLE 9–1

ISOFORM EXPRESSION OF CONTRACTILE AND REGULATORY PROTEINS

	SKELETAL SLOW (I)	SKELETAL FAST OXIDATIVE (IIa)	SKELETAL FAST FATIGABLE (IIb)	CARDIAC	SMOOTH
Myosin heavy chain	MHC-I	MHC-IIa	MHC-IIb, -IIx	MHC-α and -β	MHC-SM1, 2 (multiple isoforms)
Myosin light chain	MLC-1aS, -1bS	MLC-1f, -3f	MLC-1f, -3f	MLC-1v, -1a	MLC-17a, -17b
SR Ca ATPase	SERCA2a	SERCA1	SERCA1	SERCA2a	SERCA2a, 2b (b > > > a)
Phospholamban	Present	Absent	Absent	Present	Present
Calsequestrin	"Fast" and "cardiac"	"Fast"	"Fast"	"Cardiac"	? "Cardiac" ? "Fast"
Ca^{2+} release mechanisms	RyR1 (Ca^{2+} release channel or "ryanodine" receptor)	RyR1	RyR1	RyR2	IP_3R (3 isoforms) RyR3
Ca^{2+} sensor	Troponin C_1	Troponin C_2	Troponin C_2	Troponin C_1	Calmodulin (multiple isoforms)

IP_3R, inositol 1,4,5-triphosphate receptor; SR, sarcoplasmic reticulum.

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-1as and MLC-1bs 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 Ca^{2+} pump** (i.e., the SERCA), calsequestrin, the Ca^{2+}-release channel,

TABLE 9–2

PROPERTIES OF FAST- AND SLOW-TWITCH MUSCLE FIBERS

	SLOW TWITCH	FAST TWITCH	FAST TWITCH
Synonym	Type I	Type IIa	Type IIb
Fatigue	Resistant	Resistant	Fatigable
Color	Red (myoglobin)	Red (myoglobin)	White (low myoglobin)
Metabolism	Oxidative	Oxidative	Glycolytic
Mitochondria	High	Higher	Fewer
Glycogen	Low	Abundant	High

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 isoform expression 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 mechanical properties of cardiac muscle vary with their location (i.e., atria versus conducting system versus ventricle). Moreover, even among cells within one anatomic location, functional differences may exist between muscle cells near the surface of the heart (*epicardial cells*) and those lining the interior of the same chambers (*endocardial cells*). As in skeletal muscle, many of these differences reflect 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.

Smooth Muscle Cells May Differ Markedly Among Tissues and May Adapt Their Properties with Time Even in a Single Tissue

When one considers that smooth muscle has a broad range of functions, including regulating the diameter of blood vessels, propelling food through the gastrointestinal tract, regulating the diameter of airways, and delivering a newborn infant from the uterus, it is not surprising that smooth muscle is a particularly diverse type of muscle. In addition to being distinguished as unitary or multiunit muscle (p. 231), smooth muscle in different organs diverges with respect to nerve and hormonal control, electrical activity, and characteristics of contraction.

Even among smooth muscle cells within the same sort of tissue, important functional differences may exist. For example, vascular smooth muscle cells within the walls of two arterioles that perfuse different organs may vary in their contractile response to various stimuli. Differences may even exist between vascular smooth muscle cells at two different points along one arterial pathway.

The phenotype of smooth muscle within a given organ may change with shifting demands. The uterus, for exam-

TABLE 9–3

SUMMARY OF COMPARISONS BETWEEN MUSCLE TYPES

	SKELETAL	CARDIAC	SMOOTH
Mechanism of excitation	Neuromuscular transmission	Pacemaker potentials Electrotonic depolarization via gap junctions	Synaptic transmission Hormone-activated receptors Electrical coupling Pacemaker potentials
Electrical activity of muscle cell	Action potential spikes	Action potential plateaus	Action potential spikes, plateaus Graded membrane potential changes Slow waves
Ca^{2+} sensor	Troponin	Troponin	Calmodulin
Excitation-contraction coupling	L-type Ca^{2+} channel (DHP receptor) in T-tubule membrane coupling to Ca^{2+} release channel (ryanodine receptor) in SR	Ca^{2+} entry via L-type Ca^{2+} channel (DHP receptor) triggers Ca^{2+}-induced Ca^{2+} release from SR	Ca^{2+} entry via voltage-gated Ca^{2+} channels Ca^{2+}- and IP_3-mediated Ca^{2+} release from SR Ca^{2+} entry through store-operated Ca^{2+} channels
Terminates contraction	Breakdown of ACh by acetylcholinesterase	Action potential repolarization	Myosin light chain phosphatase
Twitch duration	20–200 msec	200–400 msec	200 msec—sustained
Regulation of force	Frequency and multifiber summation	Regulation of calcium entry	Balance between MLCK phosphorylation and dephosphorylation Latch state
Metabolism	Oxidative, glycolytic	Oxidative	Oxidative

ACh, acetylcholine; DHP, dihydropyridine; IP_3, inositol 1,4,5-triphosphate receptor; MLCK, myosin light chain kinase; SR, sarcoplasmic reticulum.

ple, is composed of 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 $[Ca^{2+}]_i$ 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_3 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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