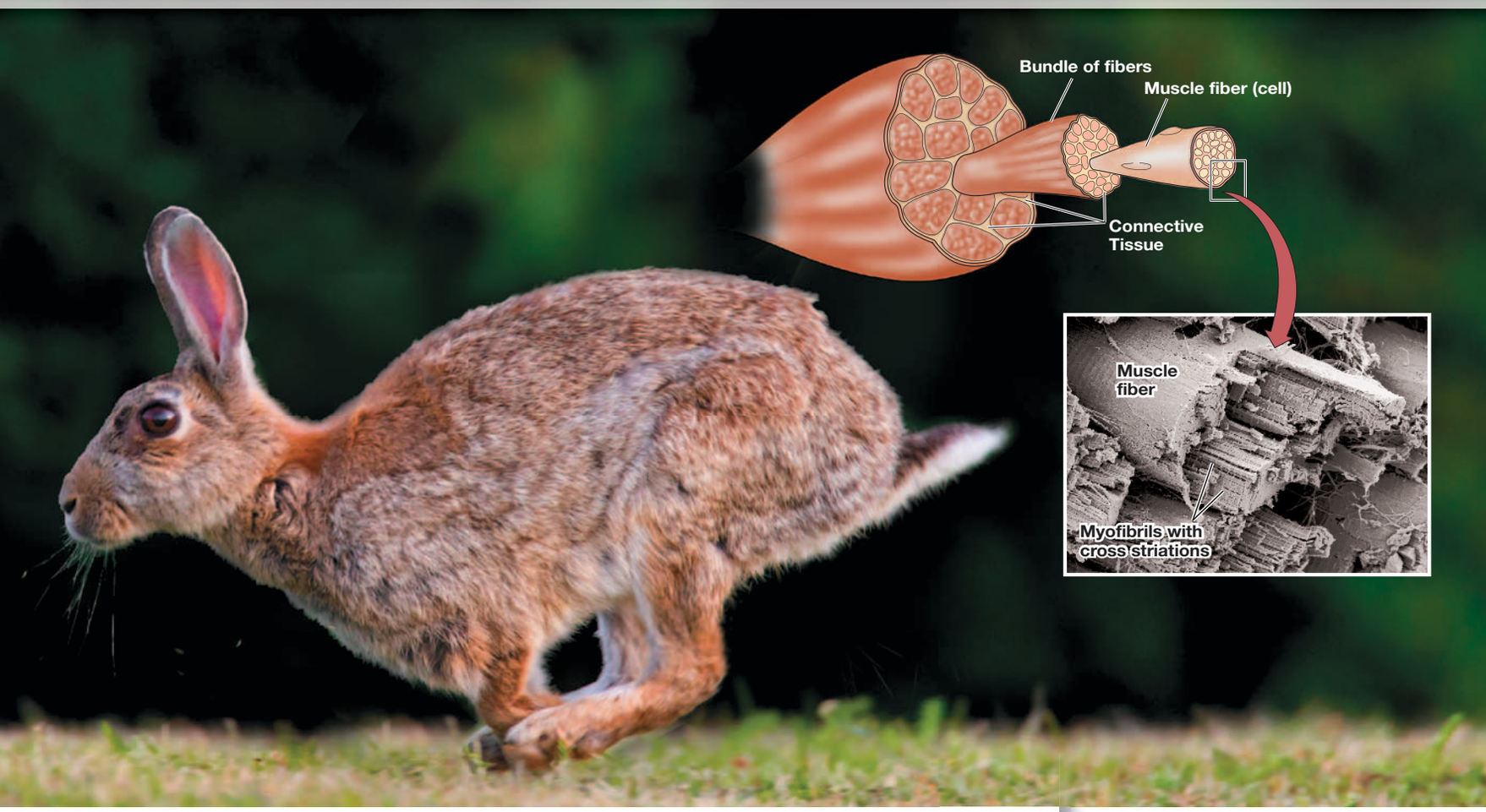
20 Muscle



Muscles are specialized for movement. All animals use muscles to generate movements that accomplish behaviors, such as bees collecting nectar from flowers or a rabbit dashing away from a predator. Animals also use muscles to accomplish physiological functions, such as generating heartbeats or mixing and propelling foods for digestion.

Muscle is a tissue that consists of contractile cells. To produce movements, muscle cells use the *molecular motor* **myosin**[1] to capture and convert the chemical energy of ATP into the mechanical energy of movement. Myosin is a large protein that interacts with another protein, **actin**, to generate force. Myosin and actin are referred to as *contractile proteins*.

All animal phyla have two categories of muscle cells: striated and smooth (or unstriated). **Striated muscle cells** have transverse bands, giving them a striped appearance. The pattern of bands reflects the organization of myosin and actin into regularly repeating units, called *sarcomeres*. In vertebrates, **skeletal** muscles (attached to bones) and **cardiac** (heart) muscles are both striated muscles. The figure shown here of a whole skeletal muscle illustrates the

[1] The myosin molecules that produce contractile force in smooth and striated muscles are classified as myosin II. They are part of the myosin superfamily, which consists of at least 18 different classes of myosins found in protozoans, fungi, plants, and animals.

Skeletal muscles consist of bundles of longitudinally arranged muscle fibers (cells) Connective tissue wraps around individual muscle fibers and bundles of fibers, and it weaves into the tendon which attaches the muscle to the skeleton. The scanning electron micrograph reveals individual fibers of the rabbit psoas muscle, a skeletal muscle that rotates and flexes the thigh. Each muscle fiber contains longitudinally arranged *myofibrils* with cross striations that delineate *sarcomeres* arranged end to end. (Micrograph courtesy of Richard Briggs, Smith College.)

longitudinal arrangement of muscle cells in this type of muscle, and the scanning electron micrograph reveals the internal components of a single striated muscle cell. Each cell (fiber) contains *myofibrils* arranged in parallel, and each myofibril has cross-striations, which delineate sarcomeres.

Smooth (**unstriated**) **muscle cells** also use actin and myosin to generate contractions, but these proteins are not organized into sarcomeres. Smooth muscles of vertebrates are found primarily in hollow or tubular organs such as the intestine, uterus, and blood vessels. Invertebrates also have striated and smooth muscles, but they are not always found in the same distribution as in vertebrates. In arthropods, for example, the skeletal (attached to the exoskeleton) and cardiac muscles are both striated, but so are muscles of the alimentary (digestive) tract.

In this chapter we consider muscles in light of the major themes of this book: mechanism and adaptation. We examine first the physiological and biochemical mechanisms that underlie muscle contraction, and then the adaptations of certain muscles specialized to perform different functions. Because vertebrate muscles have been the focus of in-depth experimental studies, we examine them in detail, and we complement our observations with examples of some well-studied invertebrate muscles.

Vertebrate Skeletal Muscle Cells

Skeletal muscles (see chapter opener figure) are composed of bundles of long, cylindrical **muscle fibers**, or **muscle cells** (the two terms are used interchangeably). Connective tissue surrounds individual muscle fibers, bundles of fibers, and the muscle itself. The connective tissue holds fibers together, provides a matrix for nerve fibers and blood vessels to gain access to the muscle cells, contributes elasticity to the whole muscle, and weaves itself into **tendons**. The tendons attach the muscles to bones and thereby transmit force generated by the muscle fibers to the skeleton. Whereas small muscles may contain only a few hundred muscle fibers, large limb muscles of mammals contain thousands of fibers. Single muscle fibers can be as long as 0.3 m (1 ft). Single fibers are typically 10 to 100 micrometers (μm) in diameter, although some (such as those in certain Antarctic fish) can reach several hundred μm in diameter. Skeletal muscle fibers are multinucleate (contain many nuclei) because they form developmentally by the fusion of individual uninucleate cells called **myoblasts**. A muscle fiber is surrounded by a cell membrane sometimes referred to as the **sarcolemma** (**FIGURE 20.1A**) (The prefixes *myo-* and *sarco-* both denote "muscle.")

Each muscle fiber contains hundreds of parallel, cylindrical **myofibrils** (**FIGURE 20.1B,C** and the opening scanning electron micrograph). The myofibrils are 1 to 2 μm in diameter and as long as the muscle fiber. Each myofibril has regularly repeating, transverse bands. The major bands are the dark **A bands** and the lighter **I bands**.[2] In the middle of each I band is a narrow, dense **Z disc**, or **Z line**. The portion of a myofibril between two Z discs is called a **sarcomere** (see Figure 20.1B,C). Thus one myofibril consists of a longitudinal series of repeating sarcomeres. The Z discs of adjacent myofibrils are lined up in register with each other, so the pattern of alternating A bands and I bands appears continuous for all the myofibrils of a muscle fiber. This alignment of banding within a muscle fiber gives the fiber its striated appearance.

Higher-magnification electron micrographs show that the myofibrils contain two kinds of **myofilaments** (**FIGURE 20.2**). The **thick filaments** (see Figure 20.2C) are composed primarily of the protein *myosin* and are confined to the A band of each sarcomere. A single thick filament consists of 200 to 400 myosin molecules. The **thin filaments** (see Figure 20.2B) are composed primarily of *actin*. A single thin

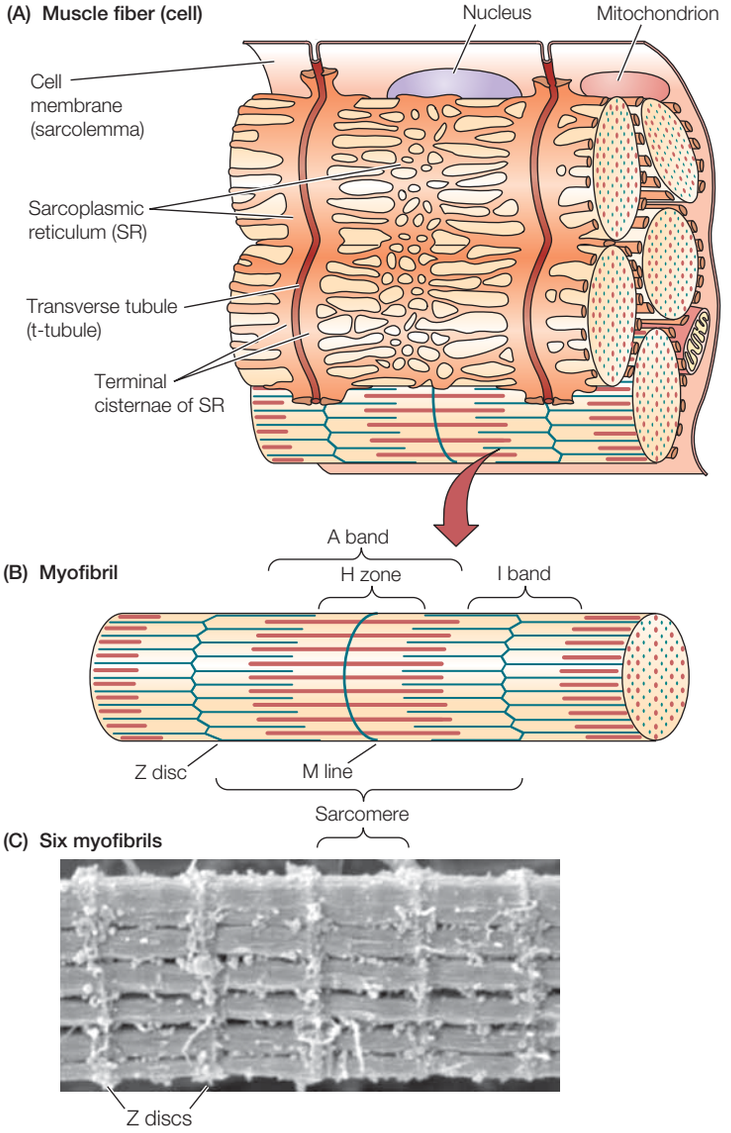


FIGURE 20.1 Each muscle cell contains many myofibrils
(A) The myofibrils are enclosed by the sarcoplasmic reticulum, which forms intimate associations with transverse tubules that extend from the cell membrane (sarcolemma). The transverse tubules and sarcoplasmic reticulum couple excitation of the muscle fiber membrane with contraction. (B) Contraction is accomplished by interaction of the myosin and actin filaments of the sarcomeres, which are arranged end to end to form the myofibrils. (C) This enlarged segment of the scanning electron micrograph in the chapter opener figure illustrates a series of sarcomeres in six myofibrils. Their alignment in register gives a striated appearance.

[2] Microscopists gave the A band its name because they observed that it is *anisotropic* (strongly polarizes visible light). They named the I band *isotropic* because it does not polarize visible light. The names of other components of the sarcomere come from the German language. The Z disc that separates sarcomeres comes from *Zwischenscheibe* ("between line"); the bright H zone at the center of the A band from *hell* ("clear or bright"); and the M line down the middle of the H zone from *Mittelscheibe* ("middle line").

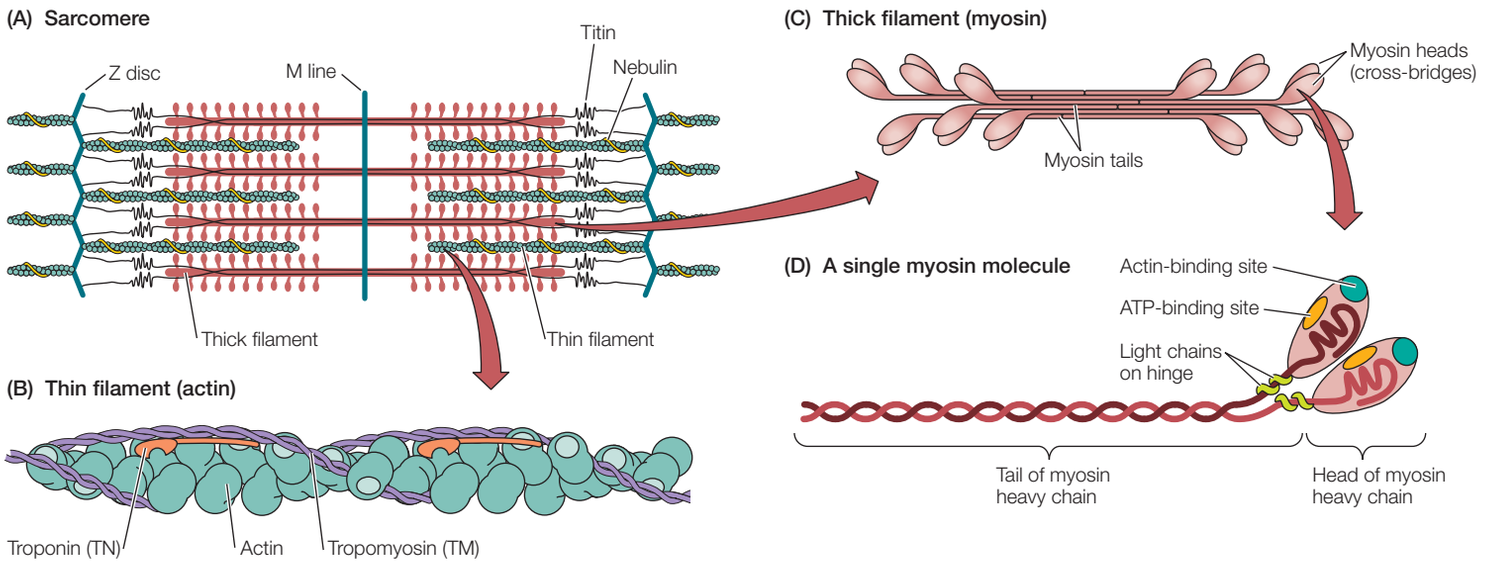


FIGURE 20.2 The sarcomere is the functional unit of striated muscle (A) Thick and thin myofilaments overlap and slide by each other to generate contractions. (B) Each thin filament is made of two chains of globular actin molecules arranged in a loose helix. The regulatory proteins tropomyosin (TM) and troponin (TN) are also components of the thin filament. (C) Myosin molecules form the thick filament. (D) Each myosin molecule contains two heavy chains of amino acids. The two chains coiled around each other form the tail. The amino-terminal end of each heavy chain forms one of the heads. The head region has a site for binding actin and a different site for binding and hydrolyzing ATP. A hinge region connects the head to the tail. The myosin molecule also includes two smaller light chains associated with each head. Thus each complete myosin molecule contains six polypeptide chains: two heavy and four light. The molecular composition of the heavy and light chains varies in different types of muscles. The different myosin isoforms of heavy chains and light chains confer variations of functional properties, such as the rate at which the myosin ATPase hydrolyzes ATP.

filament consists of two chains of globular actin molecules wrapped around each other in a loose helix. Thin filaments are anchored to proteins in the Z discs (see Figure 20.2A). They extend from the Z discs partway into the A bands of each flanking sarcomere, where they interdigitate with thick filaments. The central region of the A band, which contains only thick filaments and appears lighter than the rest of the A band, is called the **H zone**. A narrow dense region called the **M line** bisects the H zone.

In the M line, the thick filaments of the myofibril are webbed together with accessory proteins to maintain their regular spacing. The Z disc and M line ensure that neither the thin filaments nor the thick filaments float free. In vertebrates, the thick filaments are about 1.6 μm long and 12 to 15 nanometers (nm) across. The thin filaments are about 1.0 μm long and 7 to 8 nm across. Muscle fibers also contain *intermediate filaments*, so named because their diameters are about 10 nm, intermediate between those of thick and thin filaments. Intermediate filaments contribute to the architectural integrity of the muscle fiber. The protein *desmin*, for example, forms a scaffold around Z discs of adjacent myofibrils to hold them together. This scaffold extends to the cytoskeleton that lies beneath the cell membrane as well as to the nucleus and mitochondria. These connections help maintain the structural organization of the cell during contractile activity.

Cross sections of a myofibril show the relationship of thick and thin filaments in a sarcomere (**FIGURE 20.3**). A cross section through the I band shows only thin filaments. A section through the part of the A band in which the thick and thin filaments overlap shows each thick filament surrounded by six thin filaments. A section through the H zone shows only thick filaments.

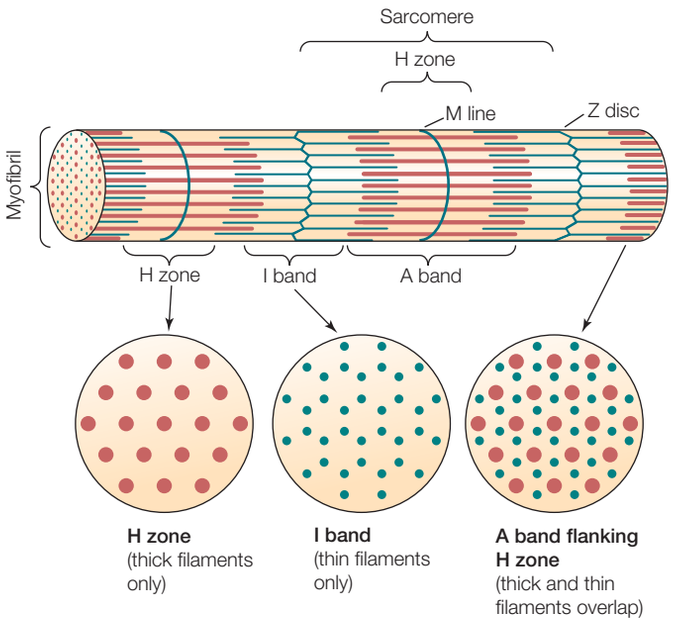


FIGURE 20.3 Thick (myosin) and thin (actin) myofilaments are arranged in parallel in a sarcomere Cross sections of a single myofibril illustrate the regions of overlap of the thick and thin myofilaments. Both the M line and the Z disc contain accessory proteins that anchor the thick and thin filaments.

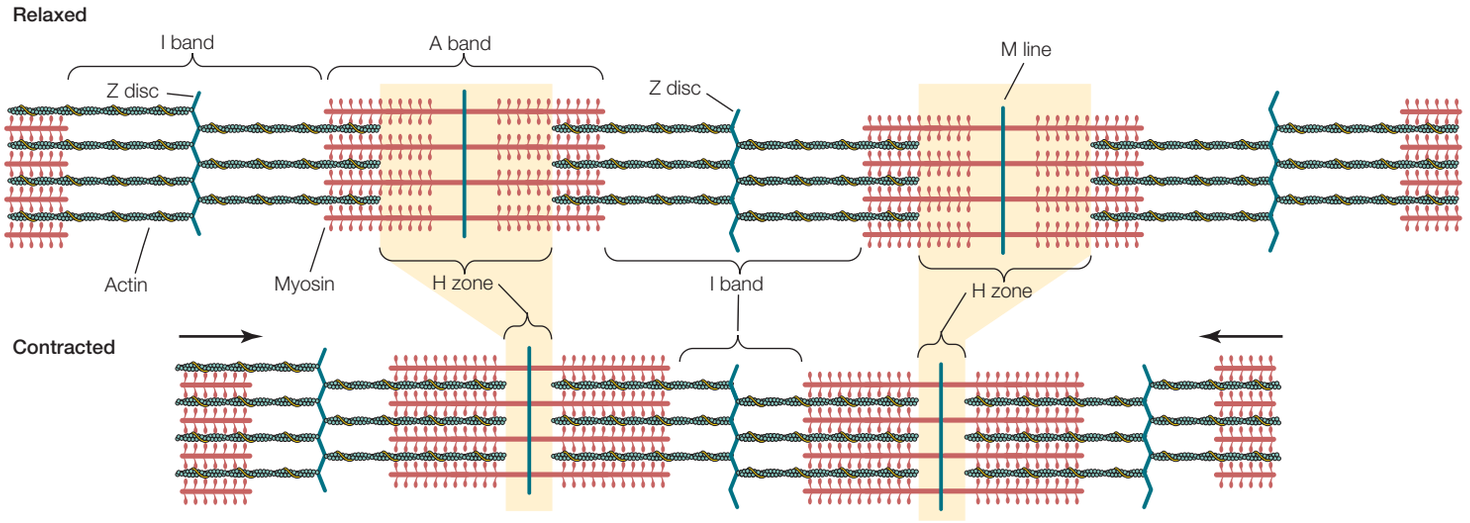


FIGURE 20.4 Thick and thin myofilaments slide by one another to produce contractions Cross-bridges of the thick filaments draw the thin filaments toward the center of each sarcomere. The myofilaments do not shorten, but the I band and H band of each sarcomere do. Because the sarcomeres are arranged in series in a myofibril, the entire myofibril shortens. Arrows indicate shortening of two adjacent sarcomeres during contraction.

The myosin molecules have radial projections on them called heads or **cross-bridges** (see Figure 20.2C,D). When the muscle cell is stimulated to contract, the myosin cross-bridges interact transiently with the overlapping actin thin filaments. The interactions of the myosin cross-bridges with actin molecules generate the force for muscle contraction. These contractile proteins function together with additional proteins in the sarcomere. For example, **titin** and **nebulin** (see Figure 20.2A) are giant proteins that help align actin and myosin. A single molecule of titin attaches to both the Z disc and the M line and spans the distance between them.[3] Titin's properties vary along its length: The region along the I band is highly folded and elastic; the region along the A band is integrated into the lattice of the thick filaments and is inelastic. This big titin molecule maintains the thick filament at the center of the sarcomere, and its elasticity over the region of the I band confers the ability of the sarcomere to spring back after the muscle fiber is stretched.

Nebulin is inelastic; it runs the length of a thin filament and stabilizes it. The nebulin molecule also specifies the length of the thin filament to optimize the overlap between thick and thin filaments. **Obscurin** is a third giant protein, which binds with proteins in the M line and links to the sarcoplasmic reticulum. Like titin and nebulin, obscurin plays an important role in maintaining the structural organization of the sarcomere. **Troponin** (**TN**) and **tropomyosin** (**TM**) are protein molecules associated with the actin chains of the thin filaments (see Figure 20.2B). They regulate the process of contraction by controlling whether or not the myosin cross-bridges can interact with the thin filaments.[4]

Thick and thin filaments are polarized polymers of individual protein molecules

Individual myosin molecules are large proteins of about 500 kilodaltons (kDa), each consisting of two globular heads joined to a long rod, or tail. The heads are the cross-bridges, and the tail contributes to the backbone of the thick filament (see Figure 20.2C,D). During polymerization the myosin molecules orient themselves with their tails pointing toward the center of the thick filament and their heads toward the ends. As a result, the two halves of the thick filament become mirror images of each other with a short bare zone of only tails in the middle of the filament. The cross-bridges on either side of the bare zone point in opposite directions.

Each actin molecule is a globular protein (42 kDa) called *G-actin*. G-actin monomers form chains of *F-actin* (filamentous actin). The two chains of F-actin wind around each other in a loose helix (see Figure 20.2B). Like the myosin molecules in thick filaments, G-actin molecules in thin filaments are arranged so that those on one side of the Z disc have one orientation, and those on the other side have the opposite orientation. The consequence of the polarized organization of the thick and thin filaments is that the cross-bridges in contact with the thin filament act like oars to pull the thin filaments toward the center of the sarcomere.

Thus, when a muscle fiber contracts, the thick and thin filaments do not shorten but instead slide by one another. In 1954 two independent teams—A. F. Huxley and R. Niedergerke, and H. E. Huxley and J. Hanson—formulated the *sliding-filament theory* of muscle contraction, which has since been amply confirmed. It states that the force of contraction is generated by the cross-bridges of the thick filaments attaching to the thin filaments and actively pulling them toward the center of the sarcomere (**FIGURE 20.4**).

[3] Titin (also known as connectin) is the largest known protein. Composed of nearly 27,000 amino acids, it has a molecular weight of 3 million Daltons (Da). Whereas the thick and thin myofilaments of similar length are polymers made up of hundreds of myosin or actin molecules, a single molecule of titin extends from the Z disc to the center of the sarcomere!

[4] The characteristics of many proteins in striated muscle fibers continue to be studied and refined. Currently, investigators are using proteomics to characterize the profiles of many proteins in different muscles and to follow changes that occur within muscles over time, such as those in holometabolous insects that undergo complete metamorphosis (see Chapter 16) or those in disease states.

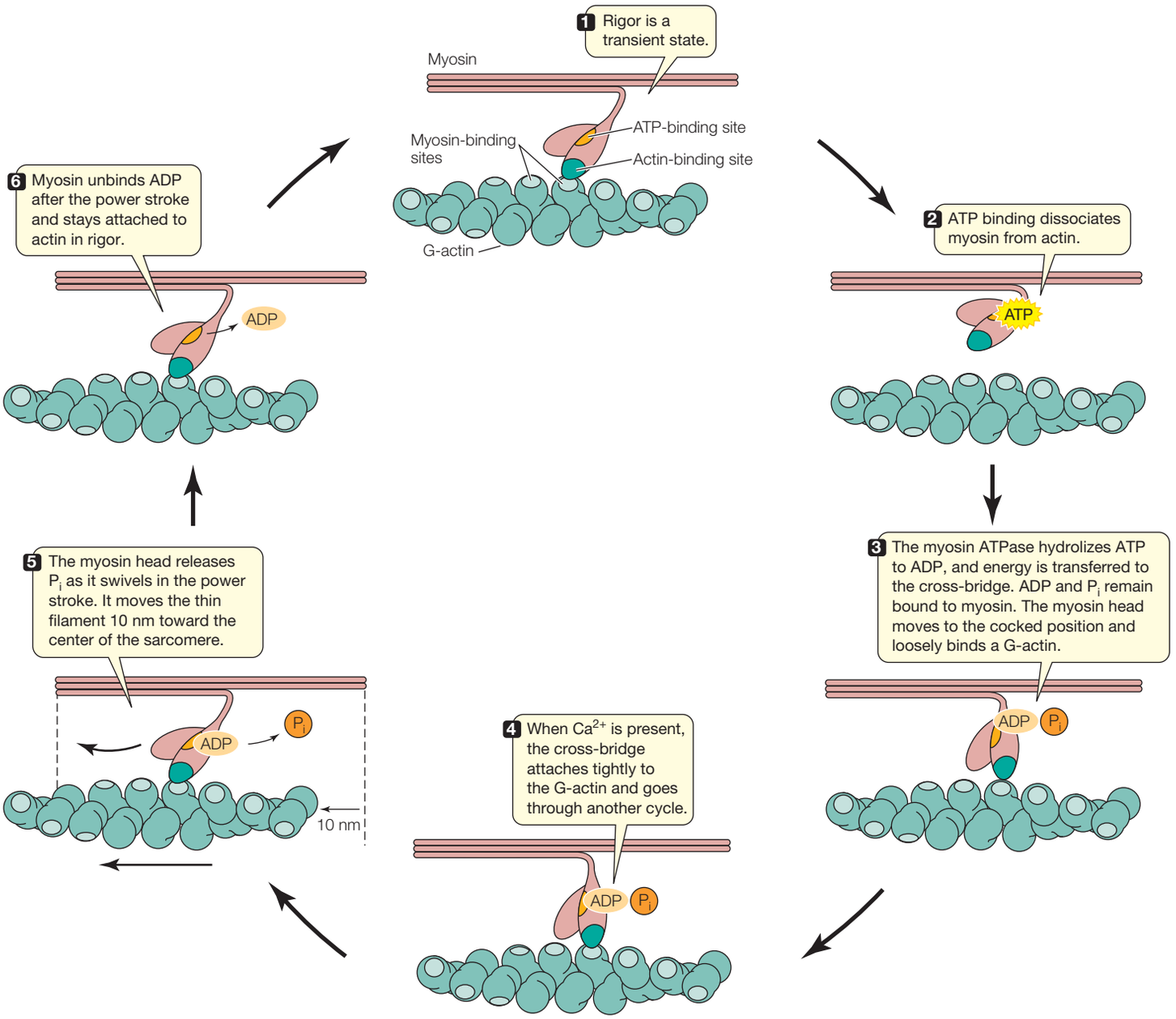


FIGURE 20.5 A single cross-bridge cycle uses one molecule of ATP and moves the actin filament about 10 nm Each cross-bridge goes through several cycles during a single contraction. The two myosin heads function independently, and only one binds to actin at a time. Structural studies suggest that no more than four myosin heads can attach over a span of seven G-actin monomers.

Muscles require ATP to contract

Myosin heads cyclically attach to actin molecules and then swivel to pull on the actin filament. Each myosin head has two binding sites: one for actin and the other for ATP. The binding site for ATP is an ATPase with enzymatic activity that splits inorganic phosphate from the ATP molecule and captures the released energy. The energy is used to power cross-bridge action.

The cycle of molecular interactions underlying contraction is shown in **FIGURE 20.5**. In step ❶ the myosin head is bound to actin at the conclusion of a power stroke. This is the *rigor* conformation, as in *rigor mortis*, in which muscles become stiff after death because of the absence of ATP. In rigor, the actin and myosin filaments are rigidly fixed in place until a new molecule of ATP binds to the myosin head and triggers its *unbinding* from actin (step ❷). In life, the rigor stage of each cross-bridge cycle is brief because the globular myosin head readily binds ATP and detaches from actin. It is important to understand that the detachment of myosin from actin requires the *binding* of ATP to change the conformation of myosin's actin-binding site, but it does not require the energy derived from the ATP.

Once released from actin, the myosin head hydrolyzes the ATP to ADP and inorganic phosphate (P_i) and the released energy is transferred to the cross-bridge and stored there. The ADP and

P_i remain attached to the head. A change in angle of the myosin head (termed *cocking*) accompanies hydrolysis. The cocked head interacts loosely with a G-actin monomer to form an actin–myosin–ADP–P_i complex (step ❸). To make another power stroke, the complex must bind tightly to the actin monomer (step ❹). We will see in the next paragraphs and Figure 20.6 that this tight binding requires the presence of Ca^{2+} ions. When the complex binds tightly to actin it triggers the power stroke and release of P_i (step ❺). The myosin head swivels, pulling the attached actin toward the middle of the myosin filament. At the end of the power stroke, the ADP is released and the myosin remains tightly bound to the actin (step ❻). A new molecule of ATP then binds to the myosin head, triggering its release from actin.

With each cycle, one ATP is consumed, and the myosin molecule moves the actin filament a short distance (about 10 nm). During a single contractile event (stimulated by a muscle fiber action potential), each cross-bridge repeats several binding/unbinding cycles. The cross-bridges work independently and asynchronously, so that at any instant during a contraction some of the cross-bridges are bound to actin while the rest are in other phases of the cycle. At no time during contraction do all cross-bridges simultaneously detach from actin. The summed effect of all the repeated cross-bridge cycles is to pull the thin filaments toward the middle of the sarcomere. In living, relaxed muscle, all cross-bridges have stored energy and bound ADP and P_i (as in Figure 20.5 step ❸), but most are temporarily unable to bind tightly to actin to generate a power stroke. As we will see next, cross-bridge cycling requires the presence of calcium.

Calcium and the regulatory proteins tropomyosin and troponin control contractions

In a resting muscle, each myosin head has detached from actin, hydrolyzed the ATP, and stored the energy obtained from hydrolysis. It is "primed" for another cycle. However, the regulatory proteins tropomyosin (TM) and troponin (TN) prevent contraction by inhibiting the myosin heads from tightly engaging with actin. TM is a protein dimer of two polypeptides that form an α-helical coiled-coil, which lies along the groove between the two actin chains of the thin filament (**FIGURE 20.6**).

A single TM molecule extends the length of seven globular actin molecules. Each TM molecule is associated with one TN molecule. In the resting state (see Figure 20.6A), the TM molecule lies over the myosin-binding sites of the adjacent actin molecules and prevents myosin cross-bridges from interacting with actin. TN is a golf club–shaped complex of three subunits. The "handle" is troponin T (TN-T), which binds to tropomyosin. The "club" includes troponin I (TN-I), which binds to actin, and troponin C (TN-C), which binds Ca^{2+} ions. For contraction to occur, Ca^{2+} must bind to TN.

The key physiological regulator of muscle contraction is calcium. When Ca^{2+} ions bind to TN-C, they trigger conformational changes that remove TM's steric blocking of the myosin-binding sites on actin (see Figure 20.6B). Once interaction between actin and myosin is possible, the primed myosin cross-bridges are permitted to go through repeated cross-bridge cycles as long as Ca^{2+} is present in the cytoplasm. The muscle will therefore contract only when Ca^{2+} ions are available to bind TN. In relaxed skeletal muscle fibers, the intracellular concentration of calcium is extremely low: less than 1×10^{-7} *M*, which is below the concentration that will induce (by mass action) calcium association with troponin.

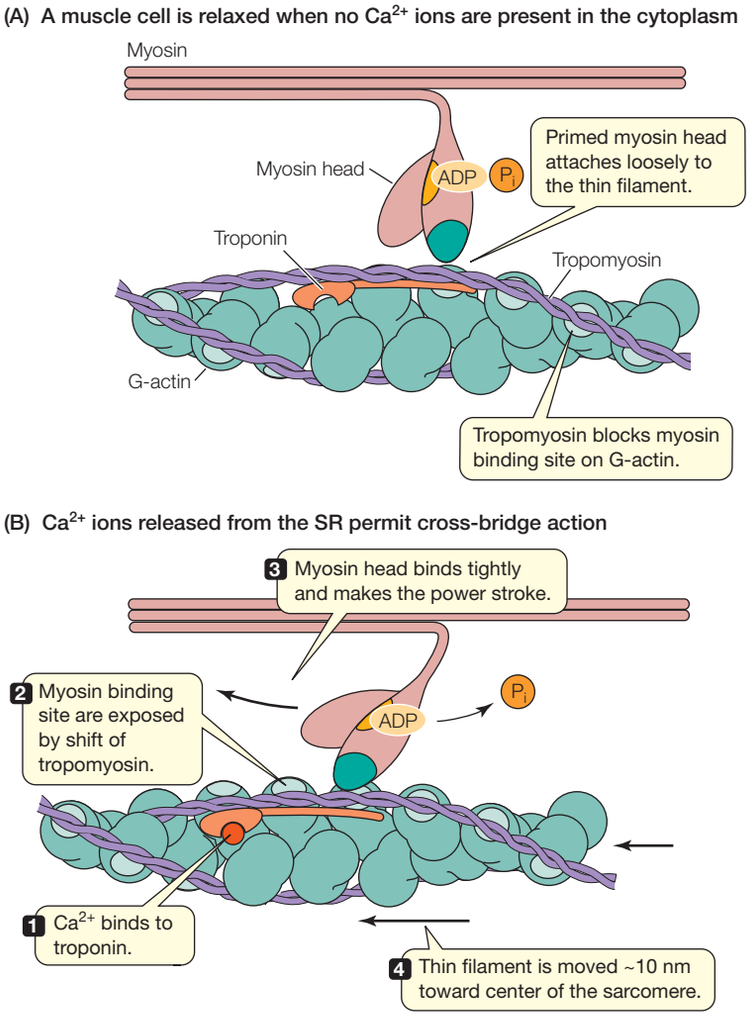


FIGURE 20.6 Ca^{2+} ions, troponin (TN), and tropomyosin (TM) regulate contraction (A) When Ca^{2+} ions are scarce in the cytoplasm, the TN-I subunit binds to two adjacent actin monomers, and the TN-T subunit binds to the tropomyosin molecule. These connections hold TM in a position that covers the myosin-binding sites on actin and inhibits cross-bridge action. (B) The TN-C subunit binds to Ca^{2+} ions when they are released from the sarcoplasmic reticulum. This binding causes conformational changes that detach TN-I from actin and allow TM to roll over the actin surface. The changed position of TM, as well as allosteric changes, permits cross-bridge action.

SUMMARY

Vertebrate Skeletal Muscle Cells

- Each whole muscle consists of bundles of longitudinally arrayed muscle fibers, which in turn consist of longitudinally arranged myofibrils made up of thick (myosin) and thin (actin) myofilaments organized into sarcomeres.

- A single myofibril consists of a longitudinal series of sarcomeres. The myofibrils are aligned in register in a muscle fiber so that they give the fiber a striped, or striated, appearance. Titin, nebulin, and obscurin are giant proteins that maintain the internal structural organization of the muscle fiber.
- The contractile proteins actin and myosin polymerize in a polarized fashion to form the thin and thick filaments. During contraction, the heads of individual myosin molecules bind to sites on individual actin molecules and draw the thin filaments toward the center of each sarcomere.
- Each myosin head also functions as an ATPase to provide the energy required to power cross-bridge motion. In relaxed muscle, each cross-bridge contains ADP, P_i, and stored energy obtained from the hydrolysis of ATP. The cross-bridge is oriented in the cocked position, loosely attached to actin, and is primed for its next power stroke.
- The regulatory proteins troponin (TN) and tropomyosin (TM), located on the thin filament, inhibit myosin cross-bridges from interacting tightly with actin, except when cytoplasmic Ca^{2+} is elevated. When Ca^{2+} binds to TN-C, it triggers conformational changes that allow myosin cross-bridges to bind tightly to myosin-binding sites on actin molecules and produce a power stroke.

Excitation–Contraction Coupling

Neural excitation triggers skeletal muscle contraction. Each skeletal muscle fiber is innervated by a motor neuron at the motor end-plate (**FIGURE 20.7**; see also Figure 13.9). An action potential conducted to the axon terminal of the motor neuron releases acetylcholine (ACh), which binds to postsynaptic ACh receptors in the end-plate and causes permeability changes that depolarize the muscle fiber membrane (sarcolemma) and produce an action potential. Depolarization of the muscle fiber is referred to as *excitation*. This excitation leads to rapid activation of the contractile machinery of the muscle fiber. The relationship between depolarization and contraction is called **excitation–contraction coupling**.

Excitation and contraction are coupled by two separate but intimately associated membrane systems (see Figures 20.1 and 20.7). The first of these is a system of tubules that is continuous with the sarcolemma: the **transverse tubules**, or **t-tubules**. Each t-tubule dips into the muscle fiber at an angle perpendicular to the sarcolemma, transverse to the long axis of the muscle fiber. The t-tubule invaginations occur at regular intervals along the length of the sarcolemma. The position of invagination varies among phyletic groups, usually at the level of the Z discs (e.g., amphibian muscle) or at the junction of the A and I bands (e.g., mammalian and reptilian muscles). Because the t-tubule membrane is a continuation of the outer sarcolemma, the tubule lumen is continuous with extracellular space. When the sarcolemma is depolarized, the t-tubules conduct this excitation deep into the interior of the muscle fiber. The t-tubules come into close association with the second membrane system required for excitation–contraction coupling, the **sarcoplasmic reticulum** (**SR**).

The SR is a branching lacework of tubules contained entirely within the muscle fiber. Each myofibril is enveloped in SR. The SR membrane has Ca^{2+}-ATPase active-transport pumps that maintain a low concentration of Ca^{2+} ions in the cytoplasm and a high concentration (~1×10^{-3} *M*) of Ca^{2+} ions within the SR.[5]

The SR between two t-tubules is called an SR *compartment*. Each compartment of the SR forms a sleeve of branching tubules around each myofibril (see Figure 20.1A). Enlarged sacs called *terminal cisternae* (singular *cisterna*) lie next to the t-tubules. In resting muscle, Ca^{2+} is largely confined to the terminal cisternae of the SR. Once an action potential conducted along the sarcolemma depolarizes the t-tubule, Ca^{2+} ions are released from the SR into the cytoplasm. How does depolarization of the t-tubule membrane produce Ca^{2+} release from the separate membrane system of the sarcoplasmic reticulum? In skeletal muscle, the two membrane systems are linked by two kinds of membrane proteins (see Figure 20.7): the *dihydropyridine receptors* (DHPRs) of the t-tubules and the *ryanodine receptors* (RyRs) of the SR. Both of these proteins were named for the drugs that bind to them specifically. In skeletal muscle, the t-tubular DHPRs and the SR RyR Ca^{2+} channels interact directly with each other in a one-on-one fashion.

The DHPRs are voltage-sensitive calcium channels in some tissues. However, in skeletal muscle, the DHPRs do not open to permit calcium flux from the extracellular fluid into the cytoplasm. Instead, their sensitivity to voltage changes plays a significant role in excitation–contraction coupling. When the t-tubule is depolarized, the DHPRs change conformation. Because the DHPRs are intimately associated with the RyRs, their altered form causes the RyRs to open. When the RyRs open, Ca^{2+} diffuses out of the SR into the cytoplasm.[6]

Figure 20.7 illustrates a motor neuron action potential triggering excitation (top panel) and contraction (middle panel) of a muscle fiber. When the RyR channels of the SR open, Ca^{2+} ions rapidly diffuse the short distance to the adjacent myofilaments, bind to troponin, and initiate processes that allow cross-bridge action. In vertebrate skeletal muscle, sufficient Ca^{2+} diffuses to the myofibrils so that every TN–TM complex moves to allow all cross-bridges to function. Indeed, the cytoplasmic concentration of Ca^{2+} increases from $<10^{-7}$ *M* to $>10^{-6}$ *M*. When the muscle fiber action potential ends (bottom panel), the t-tubules repolarize and the RyRs close. Ca^{2+} ions no longer leave the SR, and those that left when the channels were open are returned by the action of Ca^{2+}-ATPase pumps. As the cytoplasmic Ca^{2+} concentration decreases, Ca^{2+} ions unbind from TN, TM again blocks the myosin-binding sites on actin to prevent cross-bridge action, and relaxation occurs. In some fast-contracting muscles, *parvalbumin* (a low-molecular-weight protein in the cytoplasm) binds Ca^{2+}. The action of parvalbumin in concert with the SR Ca^{2+}-ATPase pumps enhances the rate of removal of Ca^{2+} from TN and hastens

[5] The sarcoplasmic reticulum is homologous to the smooth endoplasmic reticulum of other cells. Its Ca^{2+}-ATPase pumps are often referred to as SERCA (SarcoEndoplasmic Reticulum Calcium transport ATPase). Ca^{2+} is stored in the SR both free and bound to the protein calsequestrin.

[6] Cardiac muscle also has both DHPR and RyR proteins (in different isoforms), but their interaction is entirely indirect. The DHPR in cardiac muscle functions as a Ca^{2+} channel. It opens in response to depolarization and lets in extracellular Ca^{2+} from the t-tubular lumen. This Ca^{2+} from outside the cell opens the RyR calcium channel of the SR by a process called Ca^{2+}-induced Ca^{2+} release. The indirect coupling seen in cardiac muscle is probably more primitive and was replaced in skeletal muscle by evolution of the faster direct coupling of the two proteins.

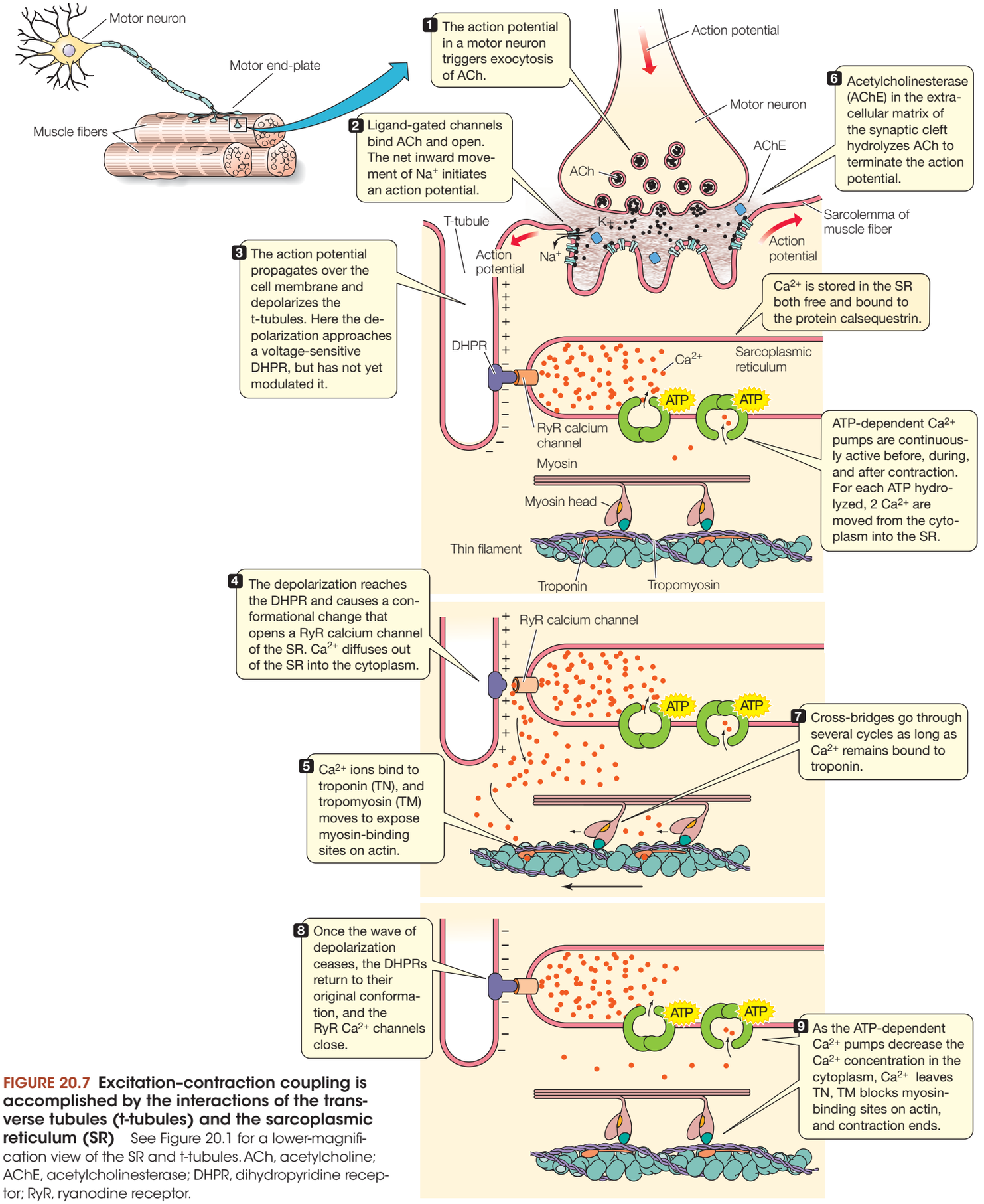


FIGURE 20.7 Excitation–contraction coupling is accomplished by the interactions of the transverse tubules (t-tubules) and the sarcoplasmic reticulum (SR) See Figure 20.1 for a lower-magnification view of the SR and t-tubules. ACh, acetylcholine; AChE, acetylcholinesterase; DHPR, dihydropyridine receptor; RyR, ryanodine receptor.

relaxation. Quicker relaxation ensures sooner readiness for the next contraction. The Ca^{2+} ions bound to parvalbumin later unbind and are transported back into the SR.

SUMMARY

Excitation–Contraction Coupling

- The sarcoplasmic reticulum (SR) sequesters Ca^{2+} ions to keep the cytoplasmic concentration of Ca^{2+} low. The terminal cisternae of the SR possess RyR calcium channels. Transverse tubules include voltage-sensitive DHPRs that come into intimate contact with the RyRs of the SR.
- Each skeletal muscle contraction is initiated by an action potential in a motor neuron that releases acetylcholine, which in turn gives rise to a muscle fiber action potential.
- The action potential propagates over the cell membrane of the muscle fiber and depolarizes the DHPRs in the t-tubules. The DHPRs cause the RyR calcium channels to open and allow Ca^{2+} ions to diffuse out of the terminal cisternae of the SR into the cytoplasm.
- Ca^{2+} ions bind to TN and cause conformational changes of TN and TM that expose the myosin-binding sites of adjacent actin molecules. Previously primed myosin heads bind to the actin sites. Repeated cross-bridge cycles continue as long as sufficient Ca^{2+} is present. The cross-bridges move the thick and thin filaments relative to each other, pulling the thin filaments toward the center of the sarcomere.
- Once the muscle fiber action potential is over, the RyR channels close. The Ca^{2+}-ATPase pumps of the SR sequester Ca^{2+} back into the SR. As the Ca^{2+} concentration in the cytoplasm decreases, Ca^{2+} dissociates from TN, and the TN–TM complex again prevents actin–myosin interactions. The muscle relaxes. Parvalbumin (prevalent in fast muscles) also binds cytoplasmic Ca^{2+} and thereby enhances the rate of relaxation.

Whole Skeletal Muscles

Many skeletal muscles in vertebrates work in **antagonistic pairs** arranged around joints. When one muscle shortens, its antagonist lengthens; and vice versa. Muscles generate force only by contraction; they lengthen passively. The antagonistic arrangement ensures relengthening of the member of a pair that shortened during contraction. For example, the quadriceps muscles on the front of the thigh and the hamstring muscles on the back of the thigh work together as an antagonistic pair of muscles. The hamstring muscles shorten to bend the knee joint. The quadriceps muscles shorten to straighten the knee joint. Often muscles work in combination with connective tissues that store elastic energy. For example, grazing animals such as camels and cows use muscles to pull their heads down to feed. Lowering the head stretches a ligament that attaches to the back of the head at one end and to the vertebral column at the other end. The stretched ligament stores energy like that in a stretched spring. This energy is expended as the ligament springs back to its original length, helping the muscles that lift the animal's head.

Muscle contraction is the force generated by a muscle during cross-bridge activity

Although the term *contraction* suggests that the muscle shortens during cross-bridge activity, this is not always the case. For example, you can "tighten up" your biceps without allowing your elbow joint to flex. Even though cross-bridge cycling occurs, the bones do not move, and the whole muscle stays the same length. This type of contraction is called **isometric** ("same length") **contraction**. The sarcomeres in the muscles shorten slightly during isometric contraction (the biceps "bulges") because they pull on elastic elements within the muscle. Elastic structures include not only the connective tissue surrounding the muscle fibers, which continue into tendons, but also components of the myofibrils such as titin and the cross-bridge links themselves. **FIGURE 20.8** illustrates the relationship of contractile and elastic components in a muscle.

Whole muscles can indeed shorten. For example, when your hand brings a heavy book toward your face, the biceps muscle shortens to decrease the angle at your elbow. This type of contraction, in which the whole muscle shortens, is called **concentric contraction** because the muscular action brings the hand closer to the center of the body. Cross-bridges can also be active when the muscle is lengthening. For example, if you hold a 10-pound weight in your hand with your elbow bent, and slowly extend your arm, the sarcomeres of your biceps are lengthening at the same time that the cross-bridges are generating force. Similarly, when you go through the motion of sitting down, or hike down an incline, the quadriceps muscles on the top of your thighs are actively contracting, but the muscles are actually longer than they are when the knee is not bent. The contractile activity in these cases is resisting stretch imposed by an external force. These contractions are called **lengthening**, or **eccentric**, **contractions**. Lengthening contractions are thought to produce minor damage to muscle fibers that lead to delayed soreness following exercise.

Concentric and eccentric contractions are both examples of **isotonic** ("same tension") **contractions** (see Figure 20.8B), which we explain below. Muscles hardly ever produce pure isotonic contractions. Most muscle activity involves dynamic combinations of both isometric and isotonic contractions. Physiologists separate these types of contractions experimentally in order to study particular properties of muscles.

A muscle exerts its force on a **load**. For example, when you lift an object with your hand, the load on which the biceps muscle exerts force includes the mass of the lower arm plus the mass of the object. The force of the muscle is opposed by the force of the load. If the force developed by a muscle is greater than the force exerted on it by a load, the muscle will change length.

Once the muscle begins to change length, the force it produces is constant and equal to the force of the load. Experimenters recording *isotonic* contractions measure changes in *length* of the muscle. If the force exerted by the load is greater than the muscle force (e.g., an extremely heavy weight), the muscle will produce an isometric contraction. Experimenters recording *isometric* contractions measure the *tension* developed by the muscle. **Tension** is the force exerted on a load by a unit of cross-sectional area of muscle.[7]

[7] Physiologists often use the terms *muscle tension* and *muscle force* interchangeably. Tension has the units of force/cross-sectional area. We know that cross-bridge action at the level of the sarcomere underlies the action produced by a whole muscle. The tension generated by a muscle fiber is directly proportional to the number of attached cross-bridges between the thick and thin filaments.

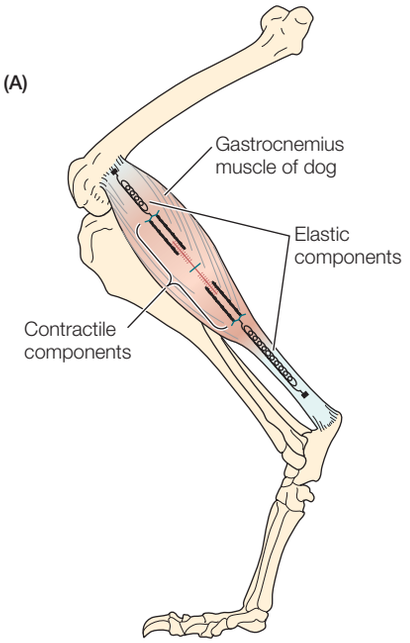
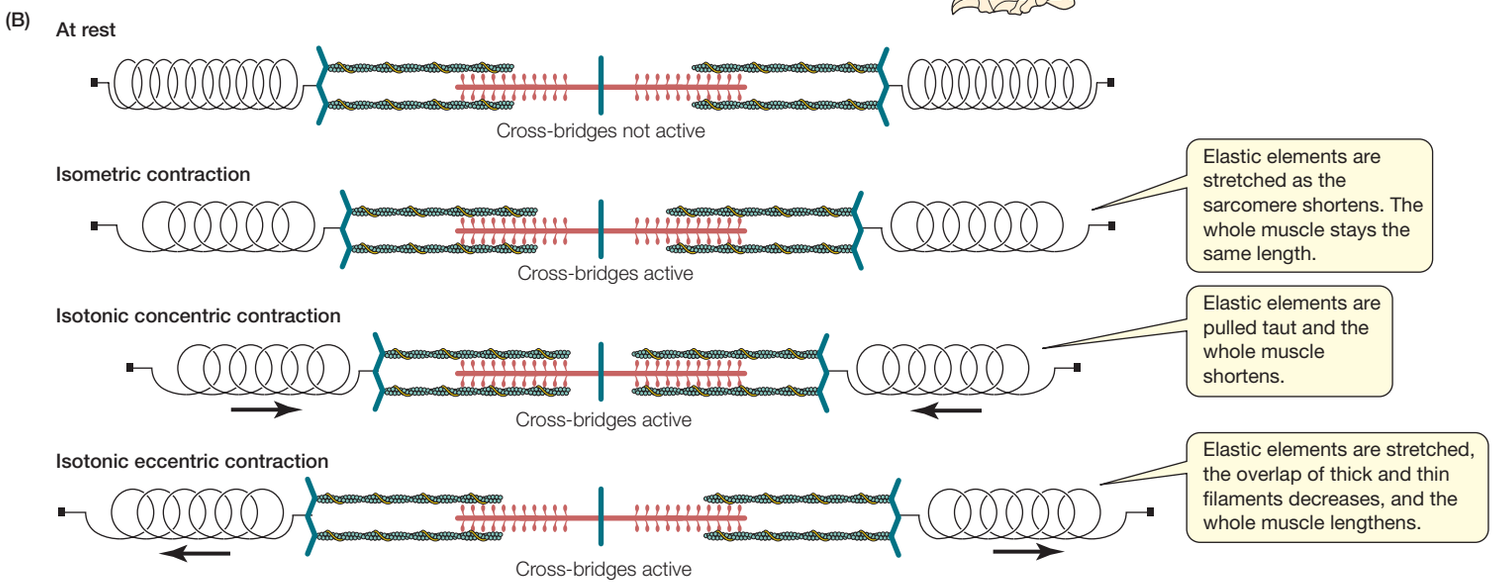


FIGURE 20.8 Contractile and elastic components interact during contraction The cross-bridge action within sarcomeres pulls on immediately adjacent sarcomeres and also on elastic elements within the muscle. (A) A single schematic sarcomere represents contractile components that are associated with intracellular and extracellular elastic elements. (B) Schematic representations show contractile and elastic elements at rest (when cross-bridges are not active) and during contractions (when cross-bridges cycle).



To record isometric contractions, experimenters usually attach the muscle to a very stiff force transducer that measures tension (force/cross-sectional area) while permitting only minuscule changes in length.

A twitch is the mechanical response of a muscle to a single stimulus

FIGURE 20.9 shows the twitch response of the same mammalian muscle recorded under isometric and isotonic conditions. Both twitches have three phases: a latent period, a contraction phase, and a relaxation phase. The isometric twitch has a brief latent period before any contractile tension is recorded (see Figure 20.9A); this latent period largely reflects the time required for excitation–contraction coupling to occur. The isotonic twitch has a longer latent period (see Figure 20.9B). Before the muscle can lift the load and shorten, excitation–contraction coupling must occur, and the cross-bridges must develop enough tension isometrically to overcome the force exerted by the load. If the load were greater, the latency would be longer because additional time would be required to develop tension to equal the heavier load.

The velocity of shortening decreases as the load increases

Isotonic recordings are ideal for revealing that the load directly influences the velocity (speed) at which a muscle shortens. You know from experience that you can lift a pencil faster than an unabridged dictionary, and you cannot lift your car at all. The fact that velocity of shortening decreases progressively with increasing loads is referred to as the **load–velocity relationship**. Current models suggest that greater loads somehow decrease the rate at which the myosin heads detach from actin, and therefore slow the speed of shortening. This relationship is also referred to as the **force–velocity relationship**. The words *load* and *force* can be used interchangeably because—in isotonic contractions—the force produced by the muscle equals the force of the load. Indeed, the force generated by the muscle decreases with velocity of shortening because there is decreased probability of cross-bridge action as a sarcomere's speed starts from zero to reach a finite value. The load–velocity (force–velocity) relationship is considered further in Chapter 21 (see page 551xx) and is illustrated in Figure 21.1, which also shows muscle *power* (power = force × velocity).

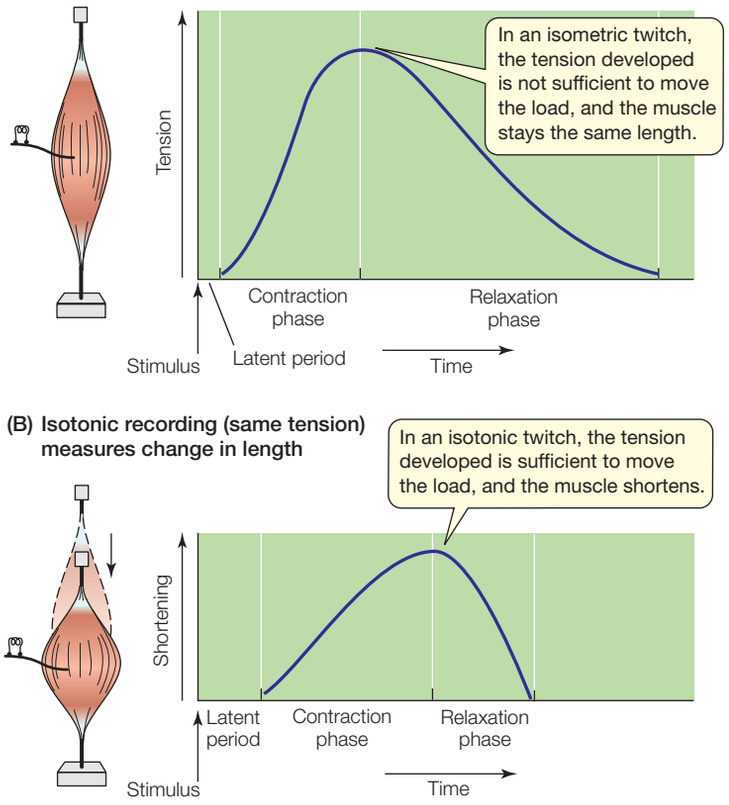


FIGURE 20.9 Recordings reveal differences between isometric and isotonic contractions (twitches) (A) In the isometric experimental arrangement, the muscle contracts but is not allowed to move a load. (B) In the isotonic experimental arrangement, the muscle shortens and moves the load, once its contractile activity generates tension (force) that equals the force of the load.

The frequency of action potentials determines the tension developed by a muscle

Depending on the muscle, twitches can vary from tens to hundreds of milliseconds (ms) in duration—much longer than the duration of the skeletal muscle action potential, which is about 2 ms. When a muscle is stimulated more than once within a brief period, the successive twitches produced add to each other, so the overall response is greater than the twitch response to a single stimulus. Such addition is called **summation**. Summation can be recorded either isometrically or isotonically. **FIGURE 20.10** shows isometric records of summation. The electrical events triggering the contractions (the action potentials of the motor neurons and muscle fibers) are all-or-none and do not sum. However, because the action potentials last only 1 to 2 ms, and a muscle twitch lasts many milliseconds, the muscle can generate a second (or even multiple) action potential(s) before the end of the twitch produced by the first action potential.

The amplitude of the summed contractions depends on the interval of time between stimuli. Low frequencies of stimulation produce contractions that sum but are not fused. Higher-frequency stimulation produces a fused contraction called **tetanus**—the maximum contractile response the muscle can achieve. In mammalian muscle, the amplitude of the tetanus is usually three or four times the amplitude of a single twitch. In amphibian muscle, the tetanic response can exceed ten times the amplitude of a single twitch.

A sustained high calcium concentration in the cytoplasm permits summation and tetanus

As we noted earlier, each action potential triggers the release of a sufficient number of Ca^{2+} ions from the SR to allow every TN–TM complex to move away from the myosin-binding sites on the actin thin filaments. Thus every cross-bridge in every stimulated skeletal muscle fiber is capable of interacting with actin and pulling the thin

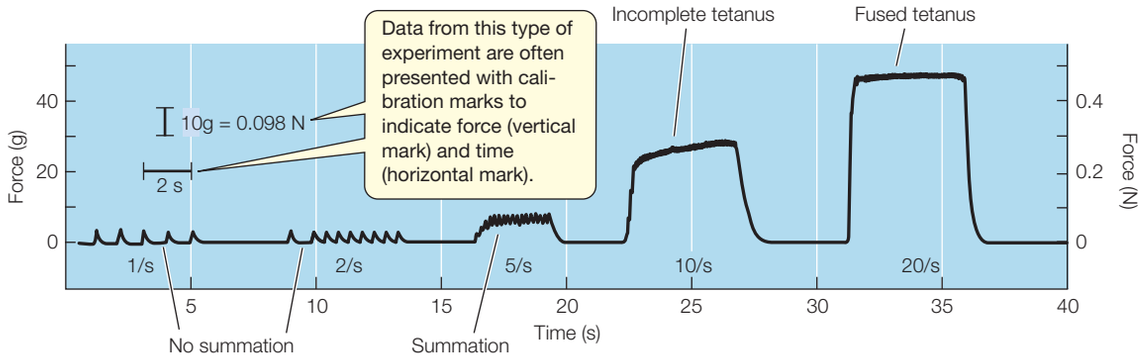


FIGURE 20.10 Summation and tetanus Increasing the frequency of stimulation produces summation of twitches up to a maximum contractile response called *fused tetanus*. In this example, short trains of stimuli were applied to the sciatic nerve that innervates the gastrocnemius muscle of a frog. The muscle was allowed to rest briefly between trains of stimuli that were applied at different frequencies. Experimenters use known weights to calibrate the recording apparatus. Because weight is the magnitude of the force of gravity on an object, it is expressed as the product of the mass of the object (in kilograms, kg) times the strength of the gravitational field (9.8 N/kg). Therefore tension produced by the muscle is accurately expressed in units of newtons (N). (Published values of muscle tension are often expressed in units of grams instead of newtons.) *Inset*: Tetanus toxin prevents the exocytosis of neurotransmitter from inhibitory interneurons onto α motor neurons in the spinal cord. The motor neurons lack inhibitory synaptic input to balance excitatory inputs, and they become hyperexcitable. In severe cases, essentially all motor neurons generate action potentials and cause tetanic contractions of the skeletal muscles. (Painting by Sir Charles Bell, 1809.)

filaments toward the center of the sarcomere. If all cross-bridges are fully engaged, how is it possible to produce a tetanic force several times greater than the response to a single stimulus?

The answer is that the contractile apparatus requires *time* to pull on the various elastic components of the muscle. The elastic components include the connective tissue associated with the muscle fibers and the elastic components of the myofibrillar apparatus. These all lie in series with each other. Thus the elastic structures are referred to as *series elastic elements*. For maximum tension to be recorded at the ends of the muscle fiber, the elastic elements must be stretched taut.

During a single twitch produced by a single action potential, the Ca^{2+} released into the cytoplasm is pumped back into the SR before the cross-bridges can fully stretch out the elastic elements.[8] Successive action potentials, however, open the RyR channels with sufficient frequency to keep the cytoplasmic concentration of Ca^{2+} high enough so that the actin-binding sites for myosin remain exposed over time. Thus cross-bridges can cycle repeatedly until the elastic elements are stretched taut and the full contractile potential of the muscle fiber is realized.

[8] The condition of the muscle fiber during the time Ca^{2+} ions are available to permit cross-bridge action is often referred to as the *active state*.

The amount of tension developed by a muscle depends on the length of the muscle at the time it is stimulated

Whole skeletal muscles, because of their attachments to bones (or to exoskeleton in invertebrates), do not change greatly in length. Nevertheless, muscles develop the most tension if they start contracting at an ideal initial length. Isometric recordings from isolated whole muscles illustrate this idea. **FIGURE 20.11A** shows the tension produced by a muscle when it was set at several different lengths prior to stimulation. Maximum tension was achieved when the muscle was set at lengths near its normal relaxed length in the animal. When the muscle was set at shorter lengths or stretched to longer lengths, the development of tension dropped off.

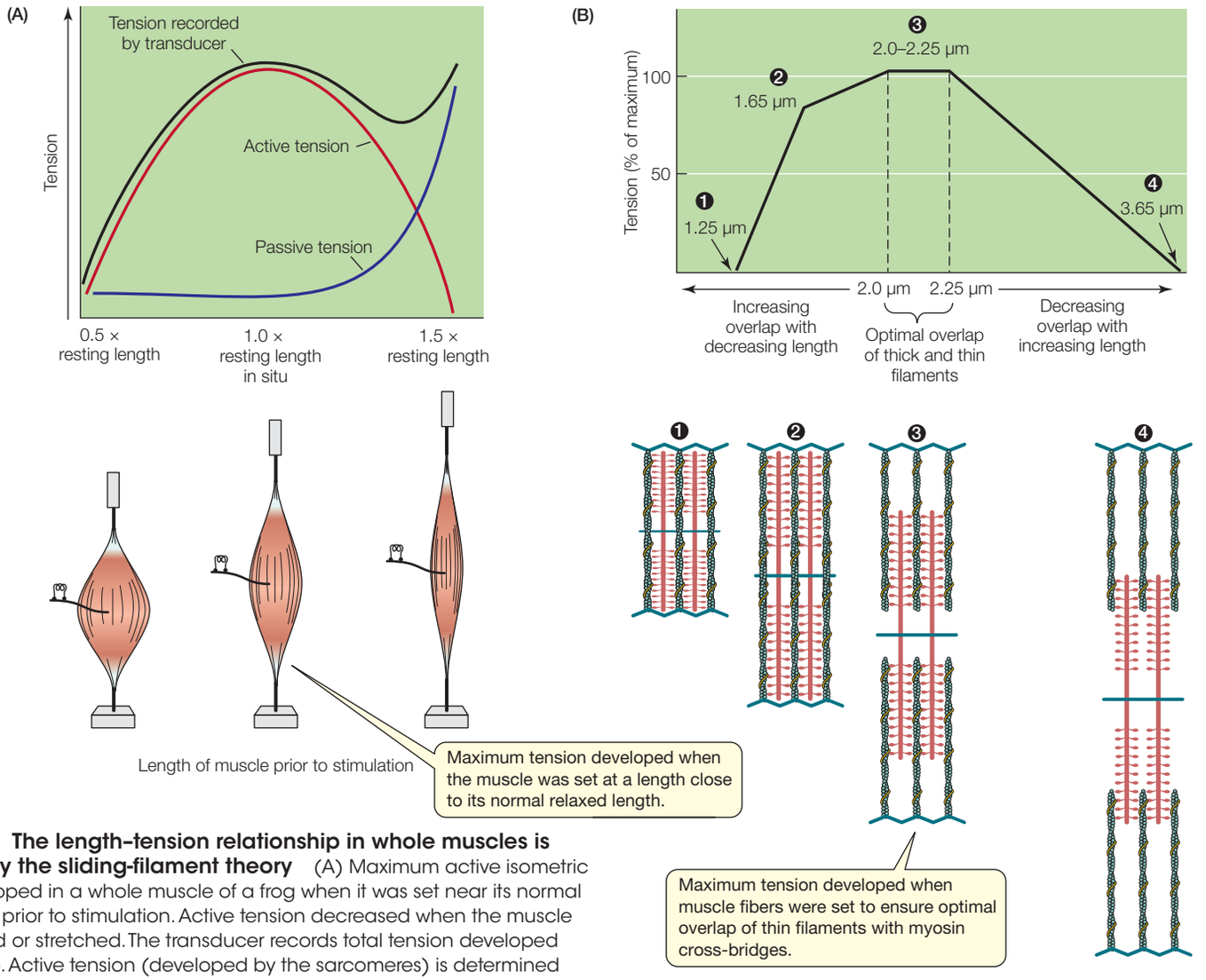


FIGURE 20.11 The length–tension relationship in whole muscles is explained by the sliding-filament theory (A) Maximum active isometric tension developed in a whole muscle of a frog when it was set near its normal resting length prior to stimulation. Active tension decreased when the muscle was shortened or stretched. The transducer records total tension developed by the muscle. Active tension (developed by the sarcomeres) is determined by subtracting the passive tension (produced by the experimenter pulling on the muscle to stretch it) from the total tension recorded during contraction. (B) Isometric tension recorded from single fibers is related to the sarcomere length set prior to stimulation. It is at its maximum when the actin filaments overlap the greatest number of myosin cross-bridges. (B from Gordon et al. 1966.)

This **length–tension relationship** is entirely explained by the sliding-filament model of muscle contraction. Elegant isometric recordings of tension developed by *single* frog skeletal muscle fibers unambiguously demonstrated the length–tension relationship at the level of the sarcomeres. **FIGURE 20.11B** shows the tension developed by single muscle fibers set at different lengths prior to stimulation. The set length of the muscle fiber affected the length of the sarcomeres within it and therefore the degree of overlap of the thick and thin filaments within each sarcomere. The experimenters plotted the amount of tension developed upon stimulation as a function of sarcomere length. Maximum tensions were recorded when the sarcomere lengths were set near those found in the intact animal.

The diagrams in Figure 20.11B illustrate that, at the lengths that yielded maximum tension, the overlap of thick and thin filaments permits optimal cross-bridge binding with actin (❸). Stretching or compressing the sarcomeres leads to less tension developed in response to stimulation. Sarcomeres set at longer-than-ideal lengths have less overlap of thick and thin filaments and therefore fewer available sites for myosin cross-bridges to bind (❹). At sarcomere lengths that are shorter than ideal, the thin filaments overlap each other, probably interfering with myosin cross-bridge action (❷), and ultimately push up against the Z disc (❶).

The striking agreement of the length–tension curve of single muscle fibers with the observed regions of filament overlap strongly implies that each cross-bridge contributes an independent and equal increment of tension, and provides compelling support for the sliding-filament theory of muscle contraction. Below we will see the powerful effects of sarcomeres arranged in series within myofibrils, and of myofibrils arranged in parallel.

In general, the amount of work a muscle can do depends on its volume

Work performed by a muscle can best be understood by the use of isotonic recording. **Work** is the product of force produced by the muscle and the distance that the muscle shortens. **FIGURE 20.12** shows the distance a muscle shortened when it was given increasingly greater loads to lift. The muscle shortened the greatest distance when it lifted no load. It did not shorten at all when the force of the load exceeded the maximum force it could develop. When the muscle lifted no load, although it shortened, it performed little work because it exerted negligible force. When the muscle attempted to lift a very heavy load, it exerted isometric force but performed no work because it did not move the load. At intermediate loads, the muscle did increasing amounts of work, up to about 40% of the maximum load, and then it did progressively decreasing amounts of work while lifting loads of increasing mass.

The *force* exerted by a muscle is proportional to the cross-sectional area (CSA) of its contractile elements. In all muscles examined that use actin and myosin as contractile proteins, the diameters of the thick and thin filaments are essentially the same. This means that a cross section through the contractile components of any muscle would reveal the same number of cross-bridges per unit of area. Because of this constant number of cross-bridges, most vertebrate skeletal muscle fibers (and many invertebrate muscle fibers) exert about the same amount of force per unit of area. A muscle that has a greater total CSA would therefore be able to produce a greater total force than a thinner muscle, because of the additive effect of myofibrils in parallel. Investigators refine this concept by taking into account

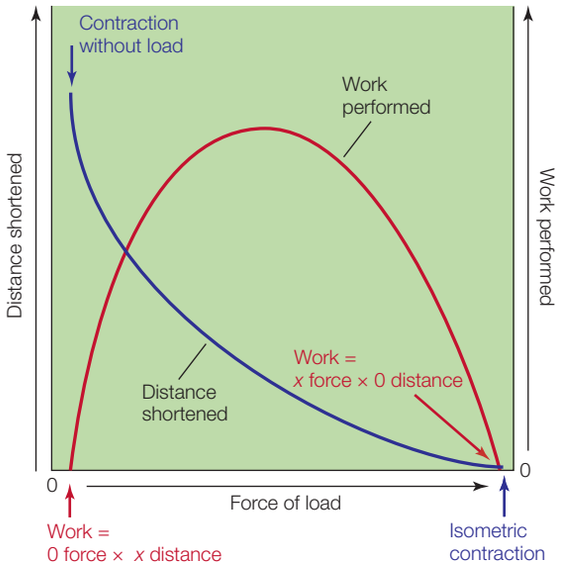


FIGURE 20.12 Work of contraction Isotonic recordings show that the muscle shortens the greatest distance when it lifts no load, and shortens progressively shorter distances with increasing loads. Multiplying the force developed (equal to the force of the load, in newtons, N) by the distance shortened for each load (in meters, m) gives a curve that represents work performed by the muscle (in joules, J). (After Schmidt-Nielsen 1997.)

the orientation of the muscle fibers within a given muscle. In some muscles, the individual muscle fibers are arranged in parallel and extend the full length of the muscle. However, other muscles may include shorter muscle fibers arranged at an angle to the long axis of the muscle. This arrangement adds complexity to understanding the amount of force generated by a particular type of muscle.

The *length* of a muscle fiber does not contribute to the force it generates. However, the length is important in determining how much work the muscle can do. The sarcomeres in most vertebrates are about 2.5 μm long. Thus, if each sarcomere contracts, for example, 10% of its length during a twitch, a muscle that has myofibrils consisting of 100 sarcomeres in series will shorten 25 μm. A muscle that has myofibrils consisting of 300 sarcomeres in series would shorten three times that distance. Assuming that both muscles had the same CSA, they would both exert the same force. However, the longer muscle would perform more work because work is the product of force times the distance shortened. A muscle that was both greater in CSA and longer would produce even more work because it would both exert greater force and shorten a greater distance. Thus muscles that have a greater volume of contractile machinery are generally capable of doing greater work.

The length of a muscle fiber also affects the velocity of shortening. Because the sarcomeres are arranged in series, velocities—like length changes—are also additive. For example, if each sarcomere shortened at a speed of 20 μm/s, then a 100-sarcomere myofibril would shorten at (20 μm/s) × 100 = 2 mm/s. A 300-sarcomere myofibril would shorten at a speed of 6 mm/s. Therefore, assuming sarcomeres of equivalent lengths, the longer a muscle fiber, the greater its velocity of shortening.

Interestingly, the muscles of some animals have been so drastically modified that they possess hardly any contractile machinery at all. For example, the electric organs of some fish do not do contractile work but instead produce electric shocks (**BOX 20.1**).

BOX 20.1 Electric Fish Exploit Modified Skeletal Muscles to Generate Electric Shocks

In addition to using skeletal muscles for locomotion, electric fish have incorporated highly modified skeletal muscle cells—called *electrocytes*—into **electric organs** (**EOs**), which they use for stunning prey, exploring the environment, and communicating. Researchers recently investigated the specific effects on prey of electric discharges produced by the electric eel *Electrophorus electricus* from freshwater rivers of South America (Figure A). They first showed that high-voltage electric discharges produced by the eel's EO specifically stimulated the prey's efferent motor neurons, causing them to release acetylcholine (ACh) at skeletal muscle end-plates. The researchers then demonstrated how the electric eels time the discharges so that they either immobilize free-swimming fish or reveal the locations of hidden fish. When an eel targets free-swimming prey, it emits high-frequency discharges (~400 Hz) that remotely stimulate the prey's motor neurons at such a high frequency that they cause the skeletal muscles to undergo tetanus and temporarily immobilize the prey. During this short period of immobility, the electric eel captures the prey. To flush out hidden prey, the electric eel emits two or three high-voltage discharges that stimulate the prey's motor neurons to produce full-body twitches that give away the animal's hiding spot. Within 20 to 40 ms of having revealed the prey's location—before the prey can escape—the eel attacks it with a high-frequency discharge and immobilizes it for capture.

Some strongly electric fish were known in ancient times. Aristotle described how the Mediterranean torpedo, which can deliver shocks of 50 to 60 V and several amperes of current, hides in wait to stun and then consume its prey. Francesco Redi (in 1671) and Stephano Lorenzini (in 1678) dissected torpedoes and con-

FIGURE A **The electric eel *Electrophorus electricus***

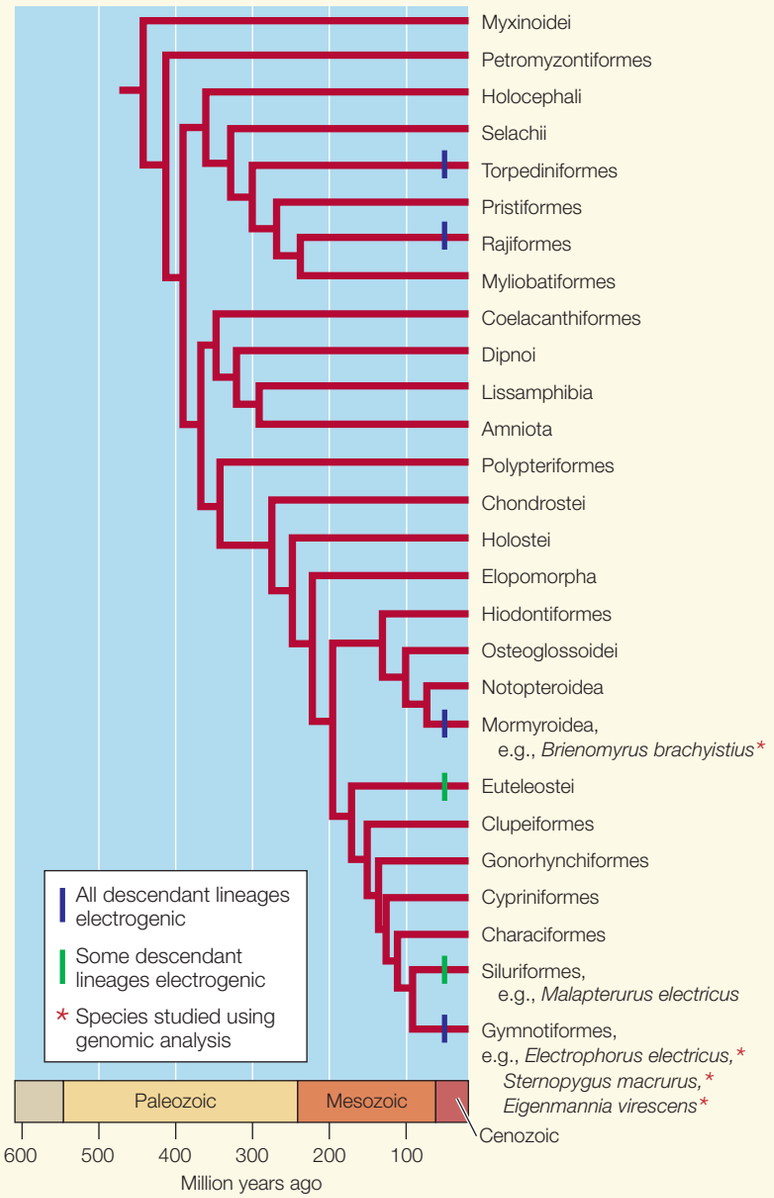


FIGURE B **Phylogeny of electric fishes** This phylogenetic tree shows that electric organs evolved independently in six different orders of vertebrates. (After Gallant et al. 2014.)

cluded that the EO was derived from muscle. Nearly 300 years later, in the 1950s, fish with EOs that generate only weak electrical pulses were discovered in Africa and South America.

Combined observations of EOs in many unrelated fish indicate that EOs have evolved independently at least six different times. Recently, researchers have studied the genomic basis of the convergent evolution of EOs. Figure B illustrates the phylogeny of known groups of electric fish and identifies those used in a study of gene expression in EO tissues. The cellular morphology of EOs varied greatly among the animals studied (marked with asterisks), yet these divergent animals showed striking convergence in patterns of upregulation and downregulation of genes and transcription factors expressed to produce the physiological features of EOs.

Whereas genes involved in differentiation of muscle structures and functions are downregulated in all EOs, those involved in EO function are upregulated. For example, the genes for certain ion channels and transporters are highly expressed, which would contribute to increased excitability of the electrocytes. Certain collagen genes are also upregulated, presumably to produce the insulating connective tissue that prevents dissipation of the current produced by the electrocytes. Finally, the electric fish from divergent taxa all upregulated genes for insulin-like growth factors (IGFs), which turn on cellular mechanisms that contribute to large cell size. Thus, despite the widely varied morphologies of EO tissues in divergent fish, selection pressures appear to have exploited convergent use of gene expression patterns that yield the features of functional EOs.

In the process of studying the physiology of EOs and learning how electric fish use them in their native habitats, investigators have also made discoveries about convergent evolution, animal behavior, and cellular differentiation. Furthermore, because EOs yield abundant amounts of nearly pure excitable membranes, they provide tissues for ever-more-sophisticated studies of channels and membrane receptors. Finally, the large quantities of AChE in many EOs provide a plentiful source of enzyme for studies of anticholinesterases, which are of interest both to environmentalists (who find synthetic anticholinesterases in toxic wastes) and investigators seeking ways to prolong ACh signals at specific synapses. The structure and diverse functions of EOs are explored in **Box Extension 20.1**.

SUMMARY

Whole Skeletal Muscles

- Cross-bridge activity within individual muscle fibers accounts for the force generated by a muscle. Force exerted by a muscle is proportional to the cross-sectional area of its contractile elements.
- The tension (force per cross-sectional area) generated by a whole muscle is directly related to the number of actively contracting muscle fibers.
- The amount of tension developed by each contracting fiber in a muscle is determined by the frequency of action potentials from its motor neuron (to produce summation of twitches and tetanus) and the length of the muscle fiber at the time it is stimulated (the length–tension relationship).
- The speed with which a muscle shortens decreases as the load it lifts increases (the load–velocity relationship).
- Work performed by a muscle is the product of force produced by the muscle and the distance it shortens.

Muscle Energetics

In this section we examine the sources of energy available to muscle fibers and the ways in which energy is used by different types of muscle fibers.

ATP is the immediate source of energy for powering muscle contraction

ATP performs at least three functions in the contraction–relaxation cycle:

1. ATP binding to the cross-bridge (but not hydrolysis) is necessary for detachment of myosin from actin.
2. Hydrolysis of ATP primes (cocks) the myosin cross-bridge in preparation for binding to actin and undergoing a power stroke.
3. Energy from the hydrolysis of ATP drives the calcium pump that transports Ca^{2+} ions into the sarcoplasmic reticulum.

Interestingly, muscle contains only enough ATP (2–4 m*M*) to sustain contraction for a few seconds. Thus nearly all forms of muscular work require the production of ATP while the muscle is active. The rate of work produced depends on the rate at which ATP is provided to the contractile apparatus.

In broad outline, vertebrate muscle fibers possess three biochemical mechanisms that produce ATP (see Chapter 7 for a detailed discussion of ATP resupply):

❶ *Use of the phosphagen creatine phosphate.* Phosphagens temporarily store high-energy phosphate bonds. The high-energy phosphate of creatine phosphate can be donated to ADP to produce ATP, as shown in Figure 8.7. Creatine phosphate is produced in resting muscle from creatine and ATP. The formation of ATP from creatine phosphate is driven by simple mass action. Whereas creatine phosphate is the phosphagen found in muscles of vertebrates, it and other phosphagens, such as arginine phosphate, are found in invertebrates (see Chapter 8, page 192xx).

❷ *Anaerobic glycolysis.* This form of catabolism requires no oxygen (see Figure 8.5). It must have glucose or glycogen

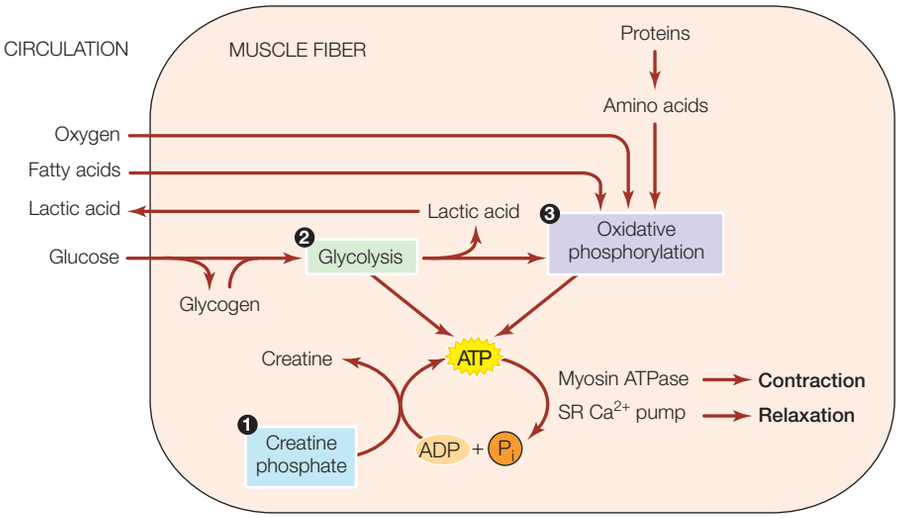


FIGURE 20.13 Three metabolic pathways supply the ATP for muscle contractile activity: ❶ Transfer of high-energy phosphate from creatine phosphate to ADP; ❷ anaerobic glycolysis; and ❸ aerobic catabolism involving oxidative phosphorylation.

as fuel. In addition to ATP, it produces *lactic acid*, which in vertebrates is always retained in the body and disposed of metabolically.

❸ *Aerobic catabolism.* This form of catabolism requires oxygen and can use all three major classes of foodstuff as fuel (see Figures 8.1–8.3). It produces ATP principally by *oxidative phosphorylation*. Its other major products are CO_2 and H_2O.

FIGURE 20.13 illustrates major elements in the production and use of ATP in a vertebrate muscle fiber. The three mechanisms of ATP production differ greatly in how fast they can make ATP when operating at peak output, how much ATP they can make, and how quickly they can accelerate their rate of ATP production. **TABLE 20.1** summarizes these attributes (see Table 8.1 for more details).

If a resting muscle is called upon suddenly to engage in all-out effort, creatine phosphate supplies much of the ATP in the first seconds because phosphagen-based ATP synthesis can be accelerated very rapidly. The rate of ATP supply is exceedingly high because of the intrinsic properties of the phosphagen mechanism. But because the muscle soon runs out of creatine phosphate, this high rate of ATP supply is short-lived.

With continued contractile activity, anaerobic glycolysis takes over as the principal mechanism of ATP synthesis. Because the peak rate of ATP synthesis by anaerobic glycolysis is lower than that using creatine phosphate, the rate of ATP supply to the contractile apparatus decreases (although it is still very high). Anaerobic glycolysis can make somewhat more total ATP than phosphagen, but it, too, exhausts its ability to make ATP if a muscle stays in a state of all-out exertion.

At that point, aerobic catabolism becomes the sole source of ATP. The rate of ATP supply falls still further because aerobic catabolism exhibits the lowest rate of ATP synthesis. But the aerobic mechanism can make ATP on a sustained basis. These transitions in the biochemistry of ATP synthesis are the reason that the rate of work by a muscle declines with time during all-out exercise. Figure 8.12 illustrates this concept.

Vertebrate muscle fibers vary in their use of ATP

Tonic muscle fibers are relatively rare. They are found mainly in postural muscles of lower vertebrates.[9] Tonic muscle fibers do not generate action potentials, but they do undergo changes in membrane potential. They contract more slowly than any other types of vertebrate muscle fibers, and their slow cross-bridge cycling permits long-lasting contractions with low energetic costs. The most common types of muscle fibers are **twitch fibers**. These fibers generate action potentials, and each action potential gives rise to a muscle twitch (see Figure 20.9). The biochemical and metabolic features of twitch fibers vary, which gives them different contractile capabilities.

Twitch fibers are generally classified into three main categories based on differences in isoforms of the myosin ATPase and metabolic features of the cells: *slow oxidative* (*SO*), *fast oxidative glycolytic* (*FOG*), and *fast glycolytic* (*FG*) *fibers* (**TABLE 20.2**). In mammals, the myosin ATPases in FG and FOG fibers hydrolyze about 600 ATP molecules per second, whereas those in SO fibers split ATP about half as rapidly. Because the rate of ATP hydrolysis governs the rate of cross-bridge cycling, higher ATPase activity allows faster contraction.

It is important to remember that the amount of tension developed *per cross-bridge cycle* is the same in both fast and slow types of muscle, but the number of cycles accomplished per unit of time differs. Earlier we saw that the velocity of contraction of a muscle fiber depends on the load being moved (the *load–velocity*, or *force–velocity*, *relationship*).

TABLE 20.1 Characteristics of the three principal mechanisms of ATP regeneration in vertebrate muscle

	Use of phosphagen	Anaerobic glycolysis	Aerobic catabolism
Peak rate of ATP synthesis	Very high	High	Moderate
Total possible yield of ATP in one episode of use	Small	Moderate	High (maintained indefinitely)
Rate of acceleration of ATP production	Fast	Fast	Slow

Note: See Table 8.1 for more detail.

[9] In mammals, tonic fibers occur only as intrafusal fibers of muscle spindles and in extraocular muscles. In many mammals, the extraocular muscles, which control complex motions of the eyes, also contain extremely fast-contracting muscle fibers.

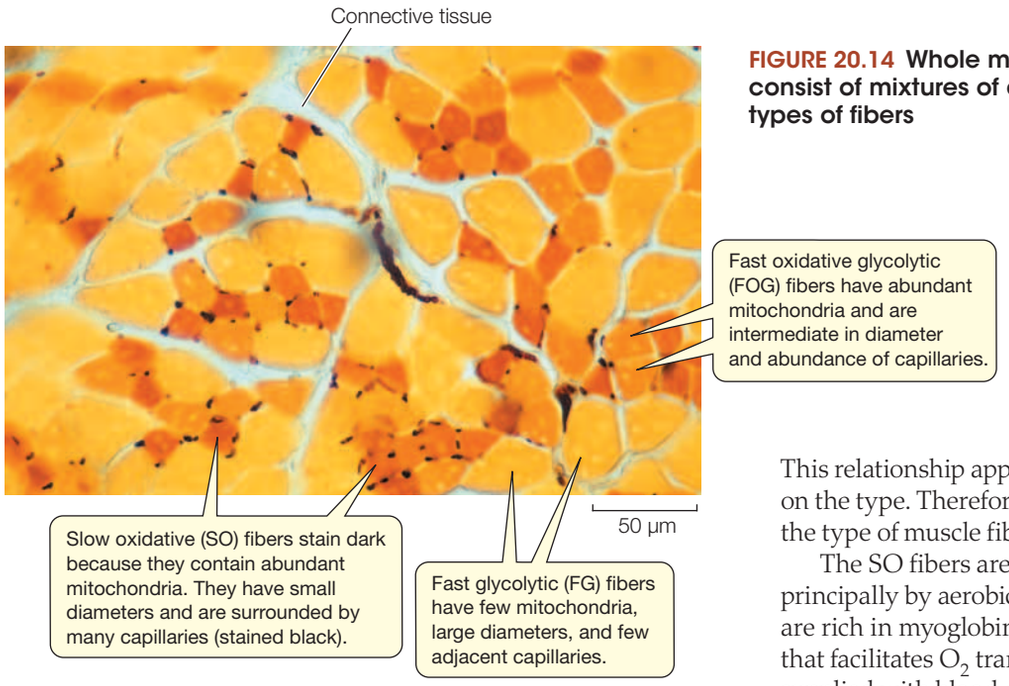


FIGURE 20.14 Whole muscles often consist of mixtures of different types of fibers

This relationship applies to all muscle fibers but varies depending on the type. Therefore the velocity of contraction depends on *both* the type of muscle fiber and the load against which it exerts force.

The SO fibers are mitochondria-rich and poised to make ATP principally by aerobic catabolism; they have small diameters and are rich in myoglobin (an intracellular hemoglobin-like molecule that facilitates O_2 transport), red (because of the myoglobin), well supplied with blood capillaries, and slow to fatigue. The FG fibers have large diameters, are invested with fewer capillaries, have little myoglobin, and are *white*. They have few mitochondria and make ATP mainly by anaerobic glycolysis; they are rich in glycogen (the principal fuel of anaerobic glycolysis), quickly accumulate lactic acid, and fatigue rapidly. The FOG fibers are intermediate. Although the FOG isoform of myosin is different from that of FG fibers, the activity of myosin ATPase in FOG fibers is high, and they are therefore capable of rapid tension development. Unlike FG fibers, however, FOG fibers are relatively rich in mitochondria, and because they make ATP aerobically, they are relatively resistant to fatigue.

Whole muscles often include mixtures of different types of muscle fibers, and their performance reflects their composition. The light micrograph in **FIGURE 20.14** shows an example of SO, FOG and FG fibers intermingled within a muscle. Table 20.2 provides a comparative guide to the three fiber types. The distinctions, although useful, should not be viewed too rigidly, however, because the fiber types vary considerably in other characteristics. For example, fibers of a given type may differ from each other because they have different isoforms of troponin, tropomyosin, or other proteins. Furthermore, varying

TABLE 20.2 Characteristics of mammalian skeletal twitch muscle fibers

	Slow oxidative (SO)	Fast oxidative glycolytic (FOG)	Fast glycolytic (FG)
Myosin ATPase activity	Slow	Fast	Fast
Speed to reach peak tension	Slow	Intermediate to fast	Fast
Duration of twitches	Long	Short	Short
Rate of Ca^{2+} uptake by sarcoplasmic reticulum	Slow to intermediate	High	High
Resistance to fatigue	High	Intermediate	Low
Number of mitochondria	Many	Many	Few
Myoglobin content	High	High	Low
Color	Red	Red	White
Diameter of fiber	Small	Intermediate	Large
Number of surrounding capillaries	Many	Many	Few
Levels of glycolytic enzymes	Low	Intermediate	High
Ability to produce ATP using oxidative phosphorylation	High	High	Low
Force developed per cross-sectional area of entire fiber	Low	Intermediate	High
Function in animal	Posture	Standing, walking, rapid repetitive movements	Jumping, bursts of high-speed locomotion
Frequency of use by animal	High	Intermediate to high	Low

Note: The names of different types of skeletal muscle fibers vary in the literature. Slow oxidative fibers are also called Type I; and fast oxidative fibers, Type IIa. Different types of fast glycolytic fibers are found in mammals, IIb in small mammals and IIx in large mammals.

conditions of use can cause one type of fiber to be converted into another (see Chapter 21).

Different fiber types are specifically adapted to serve different functions, which gives muscles a broad repertoire of contractile abilities. SO fibers (and tonic fibers when present) do not generate much tension, but they operate efficiently and without fatigue. They are adapted for isometric postural functions and for small, slow movements. The myosin isoform in SO fibers has low ATPase activity and therefore can produce tension economically. FOG fibers generate more tension and faster contractions, yet they are fatigue-resistant. They are adapted for repeated movements such as locomotion. FG fibers generate rapid contractions and large increments of tension, but they lack endurance. They are used for occasional, forceful, fast movements such as leaps or bursts of speed in escape or prey capture.

The ankle extensors in the cat hindlimb illustrate the functional roles of different fiber types. Three muscles—the *soleus*, *medial gastrocnemius*, and *lateral gastrocnemius*—compose the ankle extensors. They all insert on the Achilles tendon at the heel. The soleus contracts slowly and consists entirely of SO fibers. It is most active in postural standing. The medial and lateral gastrocnemii are faster muscles of mixed fiber composition. For example, the medial gastrocnemius contains approximately 45% FG, 25% FOG, and 25% SO fibers. (The remaining 5% of fibers are intermediate in their properties between those of FG and FOG fibers.)

Because the FG fibers have relatively greater diameters (see Table 20.2), the 45% that they contribute to the muscle fibers contributes 75% of the maximum total tension of the medial gastrocnemius. However, walking and most running use only about 25% of the maximum tension of the medial gastrocnemius. This is the amount of tension produced by the FOG and SO fibers without any contribution from the FG fibers. Thus these fatigue-resistant fibers are sufficient for most locomotion. The large force contributed by FG fibers is believed to be reserved for short bursts of contraction required in motions such as jumping.

Different animals employ different types of muscles that contribute to their achieving success

In fish, the trunk muscles of the body are divided into separate regions of red slow muscle and white fast muscle. The muscle fibers in the two regions bear many histological, biochemical, and physiological similarities to mammalian fiber types. SO-like fibers are found in the red muscle and FG-like fibers in the white muscle. The slow red muscle makes up less than 10% of the total trunk muscle in most fish species, and it never exceeds 25%. Yet only the slow red muscle is used at all speeds of steady cruising. The white fast muscle that constitutes the great bulk of the muscle mass is used only for bursts of high-speed swimming, and it fatigues rapidly. The shear size of the white muscle is a testimony to the extreme importance to the fish of being able to accelerate rapidly through its dense water environment when necessary to capture food or escape a predator.

Several animals possess exceptional muscles that are adapted for very rapid contractions. Certain vertebrates possess rapidly contracting muscles that consist of fibers that are oxidative and fatigue-resistant. Hummingbird flight muscle, for example, can contract and relax at frequencies approaching 80 times/s (hertz, Hz), so the contraction–relaxation cycle is completed in less than 15 ms. Sound-producing muscles of insects, fish, birds, and bats can be even faster.[10] In all of these cases there are extreme adaptations for rapid generation of tension, and also rapid relaxation.

Experimenters have shown that three main factors contribute to increased speeds of contraction: (1) myosin isoforms capable of rapid cross-bridge cycling, (2) troponin isoforms that have a low affinity for Ca^{2+} so that Ca^{2+} unbinds rapidly, and (3) increased density of Ca^{2+}-ATPase pumps in the SR and parvalbumin in the cytoplasm for rapid relaxation. Large amounts of ATP are required to support rapid cross-bridge cycling and pump functions, and not surprisingly, these muscles require a well-developed SR, many mitochondria, and a rich supply of capillaries to deliver O_2 and nutrients.

The benefit of rapid contraction brings with it a cost of limited ability to generate tension, because space in cells is limited. In most muscles used for locomotion, about 90% of the space is filled by myofibrils; mitochondria, glycogen, and SR fill the remaining 10% of the space. Consider the tail-shaker muscle of the rattlesnake, which can produce contractions at a frequency of up to 90 Hz (at optimum temperatures). Rattlesnakes make themselves conspicuous by rattling their tails continuously, sometimes for hours. The tail-shaker muscle fibers have high metabolic demands and require reserves of fuel, abundant mitochondria, and also extensive SR.

The space required for these "supporting" components necessarily limits the space available for contractile proteins (the tension-generating components). Indeed, in rattlesnake shaker muscle fibers, only about 30% of the space is occupied by myofibrils. The remaining space is filled by SR (26%), glycogen (17%), and mitochondria (26%). The diminished contractile machinery results in less ability to generate tension. These muscle fibers illustrate a general point: that space in cells can be at a premium, and thus trade-offs may be required among various cell components. In contrast, the asynchronous flight muscles of some insects produce extremely high frequencies of robust contractions, and their fibers contain a large volume of contractile elements with relatively few mitochondria and little SR. In a dramatic departure from all other known skeletal muscles, the asynchronous flight muscles of insects have evolved an excitation–contraction mechanism in which one action potential triggers several contraction–relaxation cycles. In *Drosophila melanogaster*, for example, the motor neurons to the asynchronous flight muscles generate action potentials at a rate of about 5 Hz, but the muscles' contraction frequency is approximately 200 Hz. The wing beats of these insects result from changes in the shape of the thorax produced by opposing sets of muscles that are alternately activated by stretch. The action potentials ensure that sufficient Ca^{2+} is present in the cytoplasm to permit actin–myosin cross-bridge action. In **BOX 20.2** we compare asynchronous and synchronous flight muscles of insects.

[10] The sound-producing muscles of the male toadfish swim bladder are the fastest known vertebrate muscles, contracting at frequencies of up to 200 Hz. In insects, the sound-producing muscle of the shrill-chirping male cicada can contract and relax at a frequency of 550 Hz!

BOX 20.2 Insect Flight

Honeybee (*Apis mellifera*)

Humans have long admired and envied the ability of other animals to fly. Insects—from lazily looping butterflies to dive-bombing mosquitoes—have captivated our attention. Insect flight muscles possess the familiar features of striated skeletal muscle fibers found in other animals. The myofibrils are organized into sarcomeres; t-tubules and sarcoplasmic reticulum are present; and Ca^{2+} ions bind to the TN–TM complex to permit cross-bridge cycling that produces tension. **Box Extension 20.2** describes the special features of insect flight muscles that underlie insects' aerial feats.

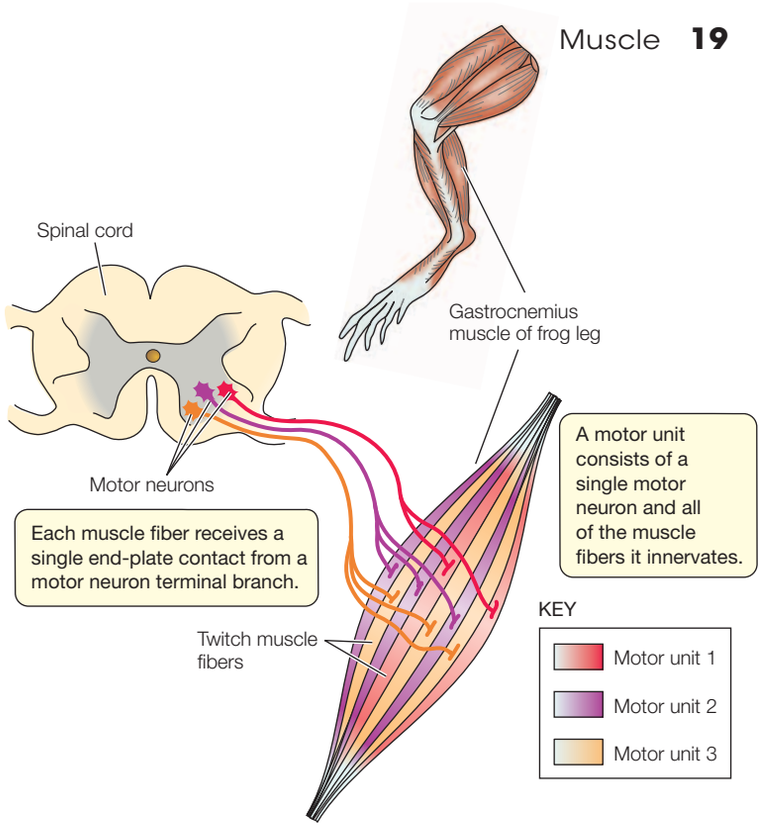


FIGURE 20.15 Vertebrate skeletal muscles consist of many different, independent motor units An action potential in a motor neuron stimulates an action potential and contraction in all of the muscle fibers it innervates. Varying the number of active motor units varies the amount of tension produced by the whole muscle.

SUMMARY

Muscle Energetics

- Contractile activity requires the hydrolysis of ATP to provide energy for cross-bridge power strokes and to support the Ca^{2+}-ATPase pumps of the sarcoplasmic reticulum.
- ATP is produced by three principal means: (1) transfer of the high-energy phosphate from creatine phosphate to ADP, (2) glycolysis, and (3) oxidative phosphorylation.
- Vertebrate muscle fibers are classified into different types on the basis of their biochemical and metabolic features, and each type is adapted to serve different functions. Muscles usually contain a mixture of different fiber types.
- Muscles adapted for extremely rapid contractions typically produce less tension than muscles that contract at slower rates. The presence of large numbers of mitochondria and abundant SR reduces the cross-sectional area of contractile machinery, and therefore the ability to generate tension.

Neural Control of Skeletal Muscle

Whole skeletal muscles in both vertebrates and invertebrates produce smooth, fluid movements that are generated by continuous and finely controlled neural input. Unlike smooth and cardiac muscles (which may generate contractions endogenously and may respond to hormonal as well as neural control), skeletal muscles contract only when stimulated by motor neurons. Two contrasting evolutionary approaches are known to provide gradation of tension in a muscle, one exemplified by vertebrates (the *vertebrate plan*) and the other by arthropods (the *arthropod plan*). In most of the well-studied invertebrate groups other than arthropods, muscle tension is controlled by variations on the arthropod plan.

The vertebrate plan is based on muscles organized into motor units

A vertebrate skeletal muscle is innervated typically by about 100 to 1000 motor neurons. The axon of each motor neuron branches to innervate several muscle fibers, and each muscle fiber receives synaptic input from only one motor neuron. A motor neuron and all the muscle fibers it innervates are collectively termed a **motor unit** (**FIGURE 20.15**). When the motor neuron generates an action potential, all of the muscle fibers in the motor unit generate action potentials and contract to produce a twitch. Trains of action potentials of increasing frequencies can produce summation of twitches up to fused tetanic contraction.[11] Thus, as in whole muscles, the amount of tension produced by a single motor unit can be varied by varying the frequency of action potentials generated by the motor neuron. Although the amount of tetanic tension varies in different animals, in many vertebrate muscles it is about two to five times greater than the twitch tension.

A more dramatic effect on the amount of tension developed by a whole muscle can be accomplished by varying the number

[11] In mammals, fused tetanus occurs at about 300 action potentials/s in slow-twitch, oxidative muscle fibers, and at about 100 action potentials/s in fast-twitch, glycolytic fibers.

of active motor units. Increasing the number of active motor units is called **recruitment of motor units**. Recruitment requires stimulating increasing numbers of motor neurons that innervate the muscle. For example, the tension in a muscle innervated by 100 motor neurons could be graded in 100 steps by recruitment. The amount of tension developed by the whole muscle increases as more motor units are activated (recruited). Recruitment is the dominant means used to control the amount of tension produced in vertebrate twitch muscles. Varying the number of active motor units, as well as the timing of their activation, ensures precise and smooth movements. The elastic properties of the muscle also contribute to the smoothness of movement.

The innervation of vertebrate tonic muscle is intermediate between the vertebrate and arthropod plans

Like twitch muscles, tonic muscles also are made up of motor units that consist of a single motor neuron and the muscle fibers it innervates. However, whereas each fiber of a twitch muscle has a single end-plate contact near the middle of the fiber, each muscle fiber of a tonic muscle receives many synaptic contacts distributed over its length. This pattern, shown in **FIGURE 20.16A**, is termed **multiterminal innervation**. An action potential generated by a motor neuron produces an excitatory postsynaptic potential (EPSP) at each of the distributed junctions on all fibers in the motor unit. Tonic muscle fibers have little or no ability to generate action potentials. Instead, each depolarizing EPSP spreads passively over a region of membrane and down the t-tubules in that area. Because an EPSP is produced at each of the many terminals along the entire length of the fiber, the depolarized t-tubules trigger opening of RyRs at many points, and contractile elements are activated along the entire fiber. The amount of tension generated depends directly on the amount of depolarization produced by the EPSPs.

The arthropod plan employs multiterminal and polyneuronal innervation

Although the fibers of arthropod skeletal muscles share many features of vertebrate skeletal muscle, including the organization of thick and thin filaments into sarcomeres and excitation–contraction coupling by way of t-tubules and SR, they show interesting differences in their patterns of innervation. A typical arthropod whole muscle is innervated by one to ten motor neurons, in contrast to the hundreds or thousands of motor neurons that innervate a whole vertebrate muscle. Most individual arthropod muscle *fibers* are innervated by more than one motor neuron, a pattern termed **polyneuronal innervation** (**FIGURE 20.16B**).

As in vertebrate tonic muscle, each neuron in arthropod skeletal muscle branches to provide multiterminal innervation to several muscle fibers. Arthropod muscle fibers typically do not generate all-or-none action potentials. (Insect flight muscles, which do generate action potentials, are an exception.) Because arthropod muscle fibers are innervated polyneuronally, the motor units of arthropods overlap; each muscle fiber is part of several motor units. Thus arthropods have only a few overlapping motor units per muscle, whereas vertebrates have many, nonoverlapping motor units per muscle.

Some arthropod muscles are innervated by both excitatory and inhibitory motor neurons. This feature—distinctly different from vertebrate muscles, which are innervated solely by excitatory neurons—allows *peripheral inhibition*. In arthropods, the excitatory transmitter is typically glutamate (not acetylcholine) and the inhibitory transmitter is gamma-aminobutyric acid (GABA). The algebraic summation of graded inhibitory postsynaptic potentials (IPSPs) and EPSPs *in the muscle fiber* determines the amount of tension developed. The greater the depolarization, the greater the amount of Ca^{2+} released from the SR, and the greater the tension developed. Thus the dominant mechanism for controlling tension in arthropod muscles is *controlling the degree of depolarization of muscle*

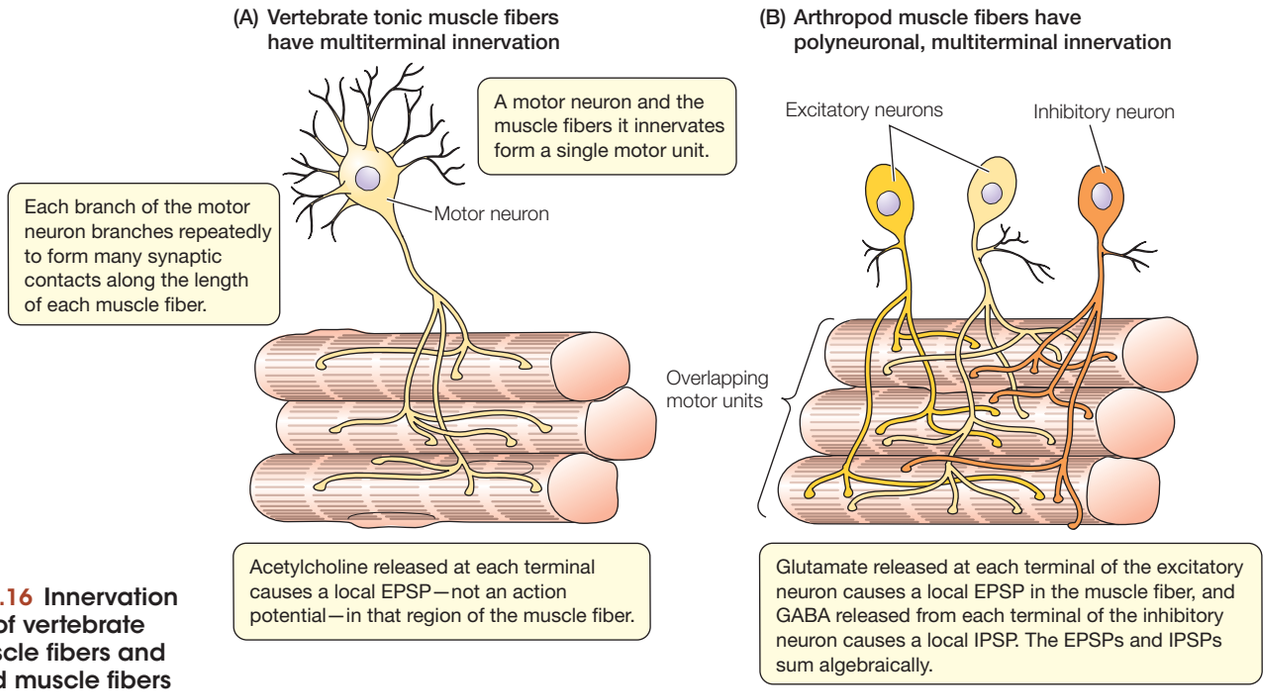


FIGURE 20.16 Innervation patterns of vertebrate tonic muscle fibers and arthropod muscle fibers

fibers, which in turn depends on the frequency of action potentials in the excitatory and inhibitory motor neurons.

Arthropod muscle fibers have a range of speeds of contraction, but unlike in vertebrate fibers, the velocity of contraction of arthropod muscle fibers is associated with different sarcomere lengths: Short-sarcomere fibers contract quickly, and long-sarcomere fibers contract slowly. Most arthropod muscles contain a variety of fibers with different sarcomere lengths and contraction speeds. Some muscles, however, are composed of all long-sarcomere slow fibers or all short-sarcomere fast fibers. For example, the muscles of crayfish and lobsters that control flexion and extension of the abdomen are made up of either all fast or all slow fibers. Thus there are fast and slow flexor muscles and fast and slow extensor muscles. The slow flexor and extensor muscles each receive up to five excitatory motor neurons and one inhibitory neuron. Many of the fast muscles receive three excitatory axons and one inhibitory axon.

An additional pattern of innervation is found only in insects. The skeletal muscles in insects receive synaptic input not only from excitatory and inhibitory neurons but also from a third type of neuron that releases octopamine or tyramine. The octopamine/tyramine transmitters do not directly trigger or inhibit muscle contraction, but instead perform two different functions that affect muscle activity. First, they *modulate* neuromuscular activity elicited by input from glutamatergic excitatory motor neurons and GABA-ergic inhibitory neurons. For example, octopamine accelerates the relaxation rate of muscles by influencing the functions of chloride and potassium channels in the muscle membrane. Second, the octopamine/tyramine neurons to skeletal muscle fibers also promote glycolysis, and therefore ATP production from carbohydrates, during contractions. This direct neural control of metabolism plays an adaptive role in adjusting muscle ATP production to the energy demands of motor behaviors. Sometimes, however, it is not adaptive to use carbohydrates as metabolic fuel. Indeed, the flight muscles of certain insects that possess synchronous flight muscles (see Box Extension 20.2) switch from carbohydrate to lipid metabolism during long flights. For example, locusts have synchronous flight muscles and are well known for their ability to fly across oceans. In these animals, the octopamine neurons to the flight muscles stimulate glycolysis of carbohydrate stores during rest, but these neurons are inhibited during flight. In the absence of octopamine input, the flight muscles metabolize lipids instead of carbohydrates.

SUMMARY

Neural Control of Skeletal Muscle

- The neuromuscular organization of vertebrates is characterized by many nonoverlapping motor units, each controlled by a single motor neuron. Each muscle fiber within a motor unit generates an action potential that spreads rapidly over the entire cell membrane and triggers contraction.
- Vertebrate tonic fibers are organized into nonoverlapping motor units. They usually do not generate action potentials. Each fiber is innervated by a branch of a motor neuron that makes multiple synaptic contacts along its length. Excitatory postsynaptic potentials (EPSPs) produce local contractions near each synaptic contact.
- The neuromuscular organization of arthropods is characterized by few motor neurons, overlapping motor units, and in some cases, by peripheral inhibition. Each muscle fiber is typically innervated by more than one motor neuron, and each neuron makes multiple synaptic contacts on the fiber. Arthropod muscle fibers typically do not generate action potentials. Instead, the postsynaptic potentials produced at several points along the length of the fiber provide graded electrical signals that each trigger the contractile machinery in a small section of the fiber and control the degree of tension developed. Insect muscles are innervated not only by excitatory and inhibitory neurons but also by neurons that release octopamine or tyramine at synaptic contacts. These transmitters modulate neuromuscular activity and regulate energy metabolism.

Vertebrate Smooth (Unstriated) Muscle

Whereas the main function of skeletal muscle in vertebrates is to accomplish locomotion, and that of cardiac muscle is to pump blood through the heart, smooth muscles are important in the homeostatic functions of many different systems within vertebrate animals. Smooth muscles are found in the gastrointestinal, respiratory, reproductive, and urinary tracts and in the blood vessels. In addition, smooth muscles are in the eye (they control the size of the pupil and shape of the lens) and at the base of hairs or feathers (see Chapter 10, page 256xx). In hollow and tubular organs, smooth muscles permit a variety of functions, including changing size and volume (such as the bladder or stomach), propelling materials along a tube (such as chyme through the intestine or urine through the ureter), and maintaining tension for long periods of time (as in the walls of arterioles or sphincter muscles).

Compared with vertebrate skeletal muscles, which are relatively uniform in their structure and function, smooth muscles are richly varied in their architectural arrangement within organs, the types of stimuli that trigger their contraction, and the types of electrical activities they generate. Smooth muscles use the contractile proteins actin and myosin, but these proteins are not organized into sarcomeres, and so smooth muscle cells do not appear striated. Interdigitating myosin and actin filaments are organized into bundles around the periphery of the cell, and cross-bridge action causes them to slide by one another to accomplish contraction (**FIGURE 20.17A**). Smooth muscle cells have a greater proportion of actin relative to myosin than do striated muscles—a difference that is reflected in the larger ratio of thin to thick filaments in smooth muscles (about 12–15 thin filaments per thick filament) relative to striated muscles (about 2–4 thin filaments per thick filament). The actin filaments attach to **dense bodies** in the cytoplasm and on the inner surface of the cell membrane. Intermediate filaments also attach to the dense bodies to help form a stable cytoskeleton. The thick myosin filaments have cross-bridges along their entire length (unlike skeletal and cardiac thick filaments, which are "bald" in the middle). The myosin structure increases the probability that actin-binding sites will overlap cross-bridges even when the muscle is stretched.

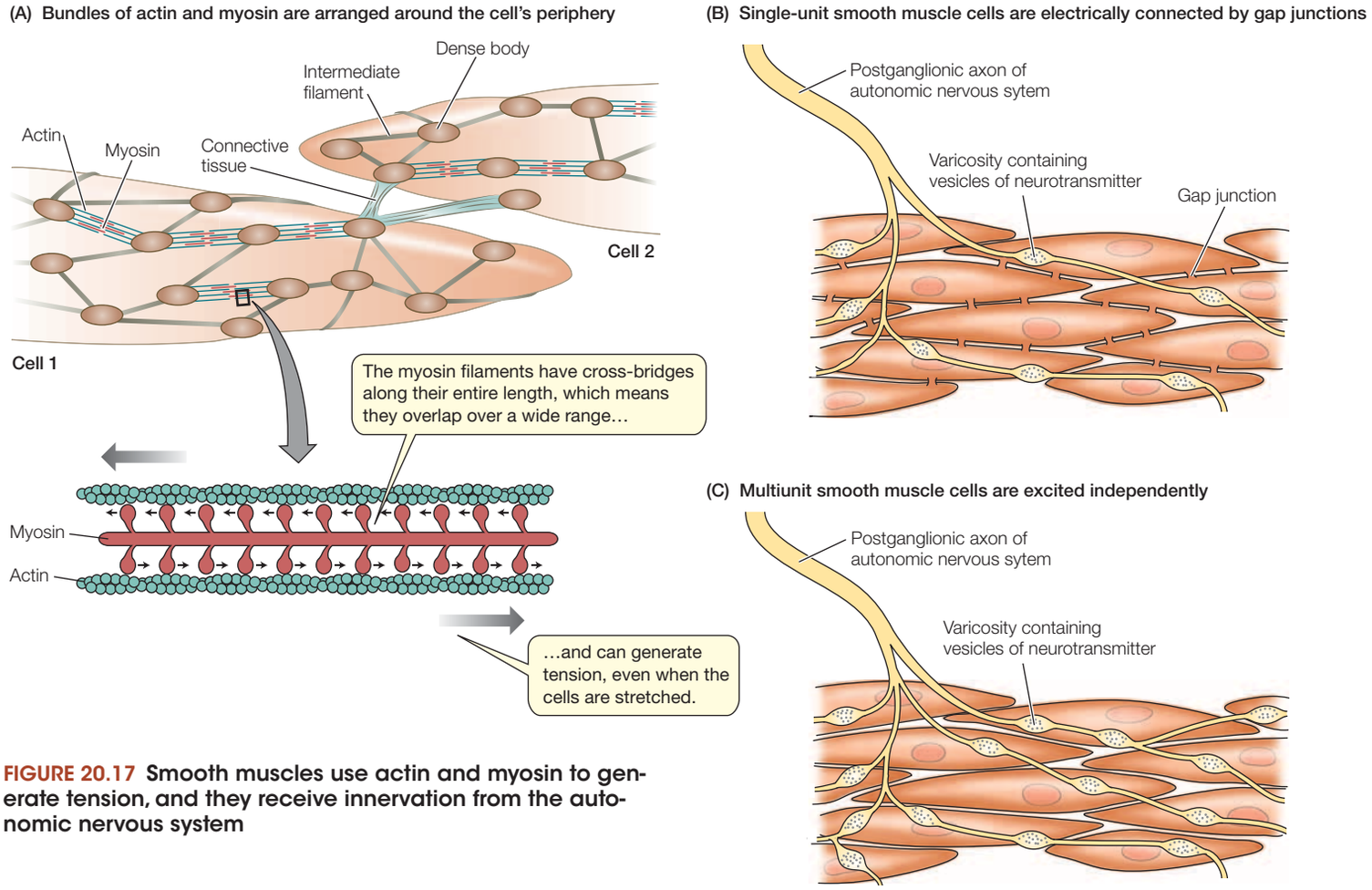


FIGURE 20.17 Smooth muscles use actin and myosin to generate tension, and they receive innervation from the autonomic nervous system

Smooth muscle cells range from 40 to 600 μm in length (shorter than most skeletal muscle fibers). They are widest in diameter around the single nucleus (2–10 μm, only somewhat wider than the 1–2 μm diameter of a single myofibril of a skeletal muscle fiber) and taper toward the ends. This cell shape is referred to as *spindle-shaped*. When a smooth muscle cell is stimulated to contract, the cross-bridge action of the peripherally arranged myofilaments causes the cell to shorten and plump up in the center. Smooth muscle cells are linked by connective tissue to each other and to surrounding connective tissue to ensure transmission of contractile force throughout a tissue or organ.

In addition to their small dimensions and single nucleus, smooth muscle cells are characterized by the absence of transverse tubules, troponin, and nebulin. They have a reduced sarcoplasmic reticulum (SR) but typically have *caveolae*, invaginations of the cell membrane that are thought to contribute to the rise of Ca^{2+} in the cytoplasm when the cell is activated. As in cardiac and skeletal muscles, the myosin ATPase of smooth muscle hydrolyzes ATP to power cross-bridge motions. However, the smooth muscle myosin ATPase hydrolyses ATP much more slowly than do the ATPases of different types of skeletal and cardiac myosins. Because of the slow rate of ATP hydrolysis, myosin cross-bridges cycle at a slower rate in smooth muscle, resulting in slower speed of contraction and longer contraction time. Many smooth muscles can maintain contractions for long periods using only a small portion of available cross-bridges and small expenditures of energy. The smooth muscle of the esophageal sphincter that guards the opening of the stomach is a good example. Except when food is swallowed, this muscle stays contracted continuously and prevents stomach acid and enzymes from entering the esophagus.

Smooth muscle cells are broadly classified

One useful classification scheme differentiates vertebrate smooth muscle into two main types: *single-unit* and *multiunit* smooth muscles. In **single-unit smooth muscle**, the muscle cells are electrically coupled by gap junctions (**FIGURE 20.17B**). Because of this coupling, groups of muscle cells are depolarized and contract together, functioning as a single unit. The smooth muscles of the gastrointestinal tract and small-diameter blood vessels are examples of the single-unit type. Single-unit smooth muscle is often spontaneously active, with electrical activity propagating from cell to cell via the gap junctions. This type of muscle can also be activated by stretch. Neural and hormonal controls may modulate the endogenous activity to varying degrees.

Multiunit smooth muscles have few if any gap junctions, so the muscle cells function as independent units (**FIGURE 20.17C**). They are innervated by autonomic nerves, and individual cells are under more direct neural control than are cells of single-unit smooth muscles. Multiunit smooth muscles may or may not generate action potentials, and they may be activated hormonally or by local chemical stimuli as well as neurally. They are not stretch-sensitive. Smooth muscles of the hair and feather erectors, eye, large arteries, and respiratory airways are examples of multiunit smooth muscles.

The smooth muscle of the mammalian uterus changes between multiunit and single-unit depending on circulating levels of reproductive steroid hormones. For example, during late pregnancy, when estrogen is present in the blood at high levels, the uterine smooth muscle cells form gap junctions that electrically couple adjacent cells. Thus the uterus is able to function as a single-unit smooth muscle to produce coordinated contractions during the birthing process.

A second classification used to distinguish different types of smooth muscle is based on contractile and electrophysiological properties. **Tonic smooth muscles**, such as those in the airways and certain sphincter muscles, maintain contractile force ("tone") for long periods and do not generate spontaneous contractions or action potentials (although they do undergo changes in membrane potential). **Phasic smooth muscles**, such as those in the stomach and small intestine, produce rhythmic or intermittent activity. They contract rapidly, produce spontaneous contractions, and generate action potentials that propagate through gap junctions from cell to cell. These gastrointestinal muscles can also be classified as single-unit smooth muscles. It is important to keep in mind that although classification schemes are useful when considering smooth muscles broadly, smooth muscles are hugely diverse, so not all of them fit neatly into specific categories.

Some smooth muscle cells undergo slow-wave changes in membrane potential in the absence of external stimulation. Slow waves recorded in these muscle cells may trigger action potentials if they exceed the voltage threshold of the fiber. In smooth muscle, inward Ca^{2+} current produces the rising phase of the action potential, so action potentials lead to a direct increase in intracellular Ca^{2+} concentration. However, because action potentials are not required to open voltage-gated Ca^{2+} channels, even subthreshold depolarizations will allow an influx of Ca^{2+} ions that may produce measurable tension in the muscle.

Ca^{2+} availability controls smooth muscle contraction by myosin-linked regulation

Like skeletal and cardiac muscles, smooth muscles maintain a low resting internal Ca^{2+} concentration (using pumps in both the SR and the cell membrane), and a rise in cytoplasmic Ca^{2+} initiates contraction. As we have seen, different smooth muscle cells respond to different types of stimuli, some respond to more than one type of stimulus, and some undergo spontaneous changes in membrane potential. The sum of inputs and membrane functions determines the moment-to-moment level of Ca^{2+} in the cytoplasm. Because the amount of available cytoplasmic Ca^{2+} determines the degree of force generated by the contractile proteins, smooth muscle cells produce *graded contractions*.

When a smooth muscle cell is stimulated to contract, Ca^{2+} enters the cytoplasm down its concentration gradient from both the SR and the extracellular space. The cells are small, so diffusion distance for Ca^{2+} is short, whether it enters from the SR or across the cell membrane. Unlike skeletal and cardiac muscles, smooth muscles do not use the thin filament regulatory proteins troponin (TN) and tropomyosin (TM) to regulate contraction. Instead, the proteins that regulate smooth muscle contraction are on the thick filament. **FIGURE 20.18** shows that Ca^{2+} activates smooth muscle predominantly by regulating the phosphorylation of myosin light chains (MLCs) (see Figure 20.2D). In this *myosin-linked regulation*,[12] entering Ca^{2+} ions combine with the Ca^{2+}-binding protein *calmodulin*, which is present in the cytoplasm. The Ca^{2+}–calmodulin complex activates the enzyme *myosin light-chain kinase* (*MLCK*), which phosphorylates one of each pair of MLCs of individual myosin molecules. Phosphorylation of the MLCs enhances the ATPase activity of the myosin heads and triggers them to bind to actin filaments and generate cross-bridge motions. As in skeletal muscle, hydrolysis of one ATP molecule powers one cross-bridge cycle. As long as Ca^{2+} is present and the MLC remains phosphorylated, repeated cross-bridge cycles occur. The degree of force generated by the contractile proteins reflects the number of active cross-bridges. The number of active cross-bridges increases with increasing Ca^{2+} in the cytoplasm. This property of smooth muscle, in which the number of active cross-bridges is variable, is similar to cardiac muscle. However it is distinctly different from skeletal muscle, in which a muscle action potential triggers the release of sufficient Ca^{2+} from the SR to allow every cross-bridge to be active.

Relaxation is accomplished by the pumping of Ca^{2+} from the cytoplasm into the SR or out of the cell. As free Ca^{2+} decreases, Ca^{2+} unbinds from calmodulin, and the MLCK becomes inactive. Another enzyme, *myosin light-chain phosphatase* (*MLCP*), dephosphorylates the light chains.

The processes shown in Figure 20.18 are often modulated by additional signaling molecules that influence the functions of MLCK and MLCP. For example, a smooth muscle cell activated by one type of signal may also receive an additional signal to turn on the G protein RhoA and its effector Rho kinase (ROK). The RhoA/ROK system inhibits MLCP. By preventing the dephosphorylation of MLCs, more myosin heads retain ATPase activity, so that—at any level of Ca^{2+} in the cytoplasm—more cross-bridges are active and generating force. This effect is referred to as *Ca^{2+} sensitization*. Interestingly, in tonic smooth muscles that produce especially prolonged contractions, such as the lower esophageal sphincter, the cross-bridges remain attached to actin in a **latch state** long after cytoplasmic Ca^{2+} is reduced. In this condition, ATP replaces the bound ADP of the myosin head ATPase extremely slowly, so the majority of cross-bridges "latched" to actin maintain tension without using ATP. The mechanisms responsible for maintaining the energy-saving latch state, and terminating it, are not fully clarified. It is possible, for example, that the relative activities of

[12] Additional mechanisms, including those that involve proteins on the thin filament, also play a role in regulating contraction in smooth muscle. Caldesmon, calponin, and tropomyosin are three such proteins thought to influence cross-bridge action.

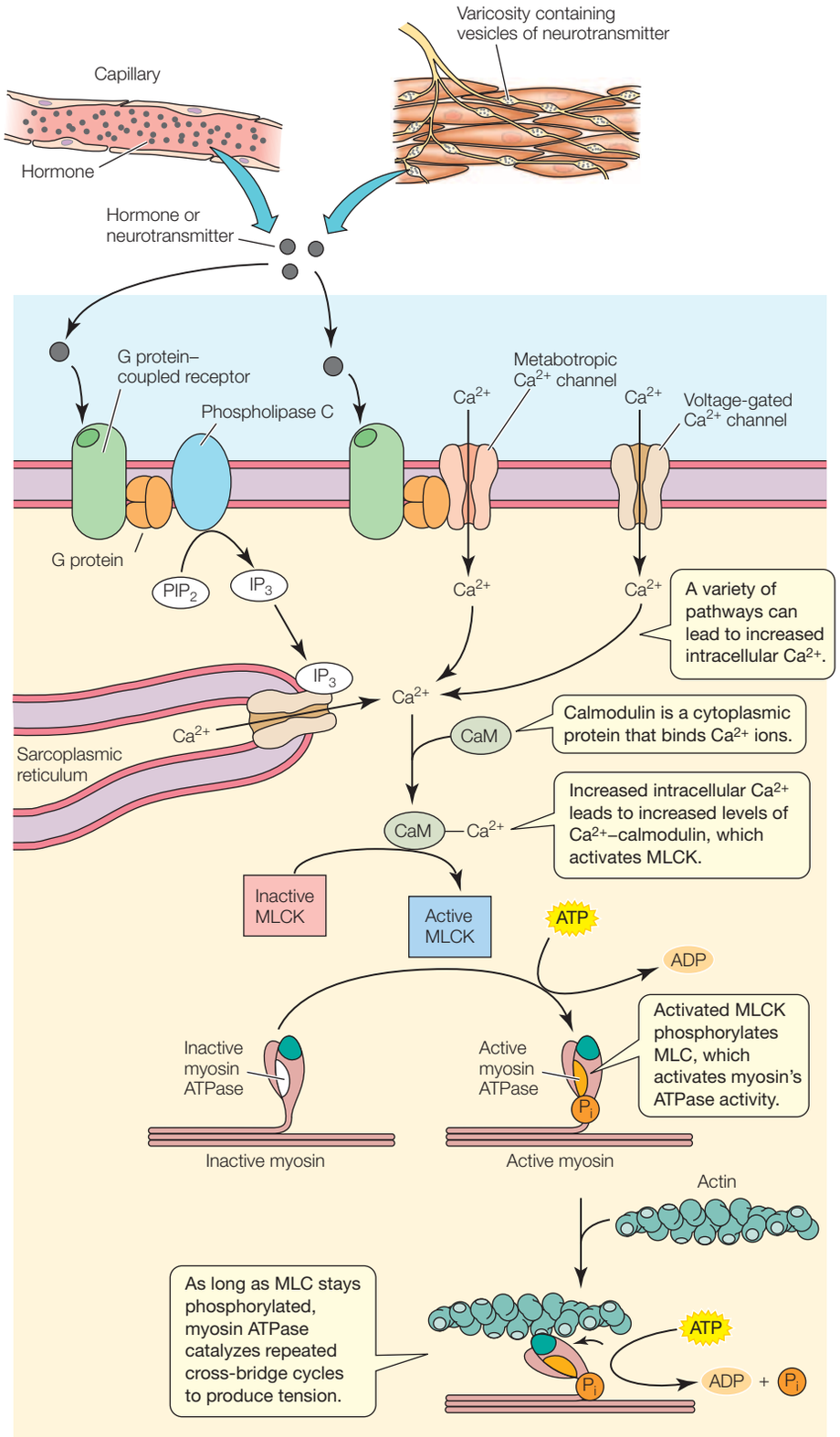


FIGURE 20.18 Myosin-linked regulation of smooth muscle contraction requires Ca^{2+} ions, calmodulin, and myosin light-chain kinase MLC, myosin light chain; MLCK, myosin-light chain kinase.

MLCK and MLCP are involved, or that proteins of the thin filament play a role, or that second messengers, such as those influenced by the paracrine nitric oxide (NO), have an effect.

Myosin-linked regulation of contraction (instead of troponin–tropomyosin–actin-linked regulation) also occurs in the muscles of molluscs and several other invertebrate groups. The muscles that hold shut the shells of bivalve molluscs (such as scallops) are known to remain contracted for hours or even days with very little O_2 consumption. These muscles actively contract, but relax extremely slowly, a condition termed the *catch state*. In this state, the muscles are stiff and resistant to stretch. Some investigators have suggested that an intermediate state of actin–myosin–ADP similar to the latch state of vertebrate smooth muscle could account for the economical maintenance of tension in molluscan muscle. An alternative idea is that the catch state is produced by the formation of a rigid network of connections between the myofilaments, a condition not dependent on myosin cross-bridges. These ideas are currently under investigation.

The autonomic nervous system (ANS) innervates smooth muscles

Postganglionic sympathetic and parasympathetic axons branch and ramify among the muscle cells (see Figure 20.17). The autonomic axons have repeated swellings, or varicosities, near their terminations, giving them a beaded appearance. The varicosities of the postganglionic axons function in a similar way to the presynaptic axon terminals of the somatic nervous system. Neurotransmitters are synthesized in the varicosities, stored in vesicles, and released by exocytosis. Neural activity triggers transmitter release, and the transmitter molecules diffuse over the surface of the muscle cells until they encounter receptor molecules. Unlike skeletal muscle fibers, smooth muscle cells lack distinct postsynaptic regions such as end-plates.

We conclude this section with two comparisons that illustrate the versatility of function that can be attained by interactions of the ANS and smooth muscles. In the first case, the smooth muscle of the urinary bladder wall is innervated by both divisions of the ANS. The parasympathetic transmitter acetylcholine stimulates the smooth muscle cells to contract and cause voiding of urine. By contrast, the sympathetic transmitter norepinephrine inhibits the smooth muscle cells from contracting so that urine is retained. Thus different behaviors of the same muscle can be achieved by activating different divisions of the ANS. A second comparison points out that the

same neurotransmitter from the same autonomic division can cause either relaxation or contraction depending on the type of receptor expressed by the smooth muscles. Thus whereas norepinephrine causes relaxation of smooth muscle cells of the bladder wall, it causes contraction of the smooth muscle cells in most blood vessels. In sum, combining different presynaptic and postsynaptic elements permits rich versatility in controlling functions to achieve homeostasis. Chapter 15 provides a detailed description of the autonomic nervous system.

SUMMARY

Vertebrate Smooth (Unstriated) Muscle

- Smooth muscles make up the walls of tubular and hollow organs, and are found in the eye and at the base of hairs and feathers. Smooth muscles contract slowly because their myosin ATPase isomers hydrolyze ATP very slowly. Some types of smooth muscles maintain contractions for protracted lengths of time using very little energy.
- Smooth muscle cells are small, spindle-shaped, and uninucleate. They contain thin actin filaments and thick myosin filaments arranged around the periphery of the cell. Although the thick and thin filaments overlap with each other, they do not form sarcomeres, which accounts for the muscles' "smooth" appearance.
- Smooth muscles receive innervation from the autonomic nervous system, and may be influenced by hormones, paracrines, and even stretch. Smooth muscles vary in the number of gap junctions present and in their contractile and electrophysiological properties. Cells in *single-unit* smooth muscles are connected by numerous gap junctions so that excitation spreads from cell to cell. Cells in *multiunit* smooth muscles have few gap junctions and function independently of each other. *Tonic* smooth muscles contract for long periods of time and typically generate only graded membrane depolarizations. *Phasic* smooth muscles produce rhythmic or intermittent contractions and generate action potentials.
- Smooth muscle contraction is controlled by Ca^{2+}, which enters the cytoplasm from the extracellular space or SR and binds to calmodulin. The Ca^{2+}–calmodulin complex activates MLCK, which phosphorylates myosin light chains and thereby increases the ATPase activity of the myosin head. Because the number of active cross-bridges in a smooth muscle varies depending on the amount of Ca^{2+} present at any given time, smooth muscle cells are capable of producing graded contractions. Relaxation occurs when cytoplasmic Ca^{2+} decreases, Ca^{2+} unbinds from calmodulin, and MLCK is no longer activated. MLCP dephosphorylates the light chains. Other signaling pathways can influence MLCK and MLCP activity and thus modulate the Ca^{2+} sensitivity of smooth muscle cells.

Vertebrate Cardiac Muscle

Vertebrate cardiac muscle, the muscle that forms the walls of the heart and functions to propel blood through the vascular system,

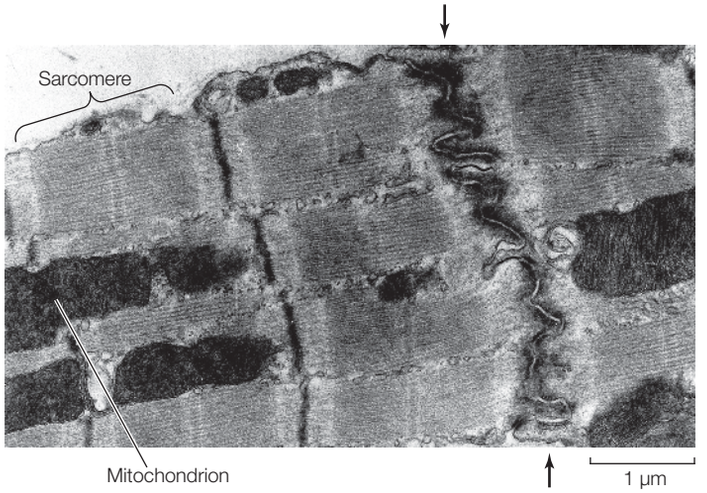


FIGURE 20.19 Cardiac muscle Striated cardiac muscle fibers are connected by intercalated discs (arrows), which include electrical gap junctions and two types of mechanical connections called desmosomes and fasciae adherentes.

is discussed in Chapters 12 and 25. We note its main features here to provide a comparison with smooth and skeletal muscle. Cardiac muscle is classified as striated because its myofibrils are organized into sarcomeres, which possess the same structural and regulatory proteins that skeletal muscle sarcomeres have (see pages 524–525xxx) (**FIGURE 20.19**). The cells are typically branched instead of straight like skeletal muscle fibers or spindle-shaped like smooth muscle cells. They are usually uninucleate. In mammals, the SR and t-tubules are well developed, but they are variable in other vertebrate animals.

Cardiac muscle fibers have functional properties that contribute to their effectiveness in pumping blood. First, they are characterized by the presence of **intercalated discs** between adjacent cells. Intercalated discs include gap junctions and localized mechanical adhesions called *desmosomes* and *fasciae adherentes* (singular *fascia adherens*). The adhesions provide mechanical strength so that the force of contraction generated by one cell can be transmitted to the next to ensure coordinated pumping. The electrical coupling at gap junctions ensures that all cells connected by gap junctions contract (beat) nearly synchronously. Gap junctions and desmosomes are illustrated in Figure 2.7.

A second property of cardiac muscle cells is that they are capable of generating endogenous action potentials at periodic intervals. Typically, specialized pacemaker cells with the fastest endogenous rate impose their rhythm on the contractile activity of the rest of the heart. Finally, the action potentials of vertebrate cardiac fibers have very long durations, typically 100 to 500 ms (see Figure 12.23). Their long durations ensure a prolonged contraction rather than a brief twitch. Indeed, the action potentials last as long as the contractions. Because the cardiac cells are refractory during the prolonged action potentials, contractions cannot sum; thus the coordinated pumping of blood is ensured.

TABLE 20.3 summarizes the properties of vertebrate skeletal, smooth, and cardiac muscles.

TABLE 20.3 Characteristics of the three major types of muscles in vertebrates

	Skeletal	Multiunit smooth	Single-unit smooth	Cardiac
Structure	Large, cylindrical, multinucleate fibers	Small, spindle-shaped, uninucleate cells	Small, spindle-shaped, uninucleate cells	Branched uninucleate fibers, shorter than skeletal muscle fibers
Visible striations	Yes	No	No	Yes
Mechanism of contraction	Thick myosin and thin actin filaments slide by each other	Thick myosin and thin actin filaments slide by each other	Thick myosin and thin actin filaments slide by each other	Thick myosin and thin actin filaments slide by each other
Cross-bridge action regulated by Ca^{2+} ions	Yes	Yes	Yes	Yes
Innervation	Somatic nervous system initiates contractions	Autonomic nervous system initiates contractions	Autonomic nervous system modulates contractions	Autonomic nervous system modulates contractions
Spontaneous production of action potentials by pacemakers	No	No	Yes	Yes
Hormones influence function	No	Yes	Yes	Yes
Gap junctions present	No	No (few)	Yes	Yes
Transverse tubules	Yes	No	No	Yes
Sarcoplasmic reticulum	Abundant	Sparse	Sparse	Moderate
Source of Ca^{2+} ions for regulation	Sarcoplasmic reticulum	Extracellular fluid and sarcoplasmic reticulum	Extracellular fluid and sarcoplasmic reticulum	Extracellular fluid and sarcoplasmic reticulum
Troponin and tropomyosin	Both present	Tropomyosin only	Tropomyosin only	Both present
Ca^{2+} regulation	Ca^{2+} and troponin; tropomyosin–troponin complex moves to expose myosin-binding sites on actin	Ca^{2+} and calmodulin; phosphorylation of myosin light chains	Ca^{2+} and calmodulin; phosphorylation of myosin light chains	Ca^{2+} and troponin; tropomyosin–troponin complex moves to expose myosin-binding sites on actin
Speed of contraction (reflecting myosin ATPase activity)	Varies from fast to slow depending on fiber type	Very slow	Very slow	Slow

STUDY QUESTIONS

1. Knowing the dimensions of a vertebrate skeletal muscle and the relationship between the SR and the myofilaments, estimate the approximate distance that a single Ca^{2+} ion would travel from a terminal cisterna of the SR to a TN-binding site.
2. Experimenters can separate F-actin thin myofilaments from myosin thick myofilaments. First they homogenize muscle cells in a blender (to break cell membranes); then they place the homogenate in a Ca^{2+}-free "relaxing solution" that contains ATP. Explain why ATP must be present and Ca^{2+} ions must not be present in order to isolate thick and thin myofilaments from each other.
3. List and describe the events that take place (and the structures involved) between excitation of the skeletal muscle cell membrane by an action potential and the initiation of cross-bridge action.
4. Combining your knowledge of rates of diffusion with your knowledge of muscle physiology, explain why it is advantageous for oxidative muscle fibers (which depend on aerobic metabolism to generate ATP) to have smaller diameters than glycolytic muscle fibers have.
5. What is the difference between a single cross-bridge power stroke and a single twitch of a skeletal muscle fiber?
6. (a) In skeletal muscle, if all cross-bridges are activated when a single action potential triggers Ca^{2+} release from the SR, why is the amount of tension produced by a train of action potentials greater than the amount of tension of a single twitch? (b) The maximum contractile response of a muscle stimulated by a train of stimuli is called tetanus. Tetanus toxin prevents inhibitory control of motor neurons, which as a consequence release ACh from their terminals unremittingly. Describe the effects of tetanus toxin on contractile activity of skeletal muscles in an individual infected by *Clostridium tetanii*, the bacterium that produces tetanus toxin.
7. Arthropod muscle fibers typically do not generate action potentials. Using your knowledge of their innervation, explain how their contractile elements are activated in a rapid and coordinated fashion.

8. Describe the organization of a motor unit in vertebrate skeletal muscle, and explain how recruitment of motor units influences the amount of tension produced by a whole muscle.

9. Two muscles have the same diameter, but one is twice as long as the other. Which muscle produces more work? Explain your answer.

10. Contraction in both skeletal and smooth muscles requires the influx of Ca^{2+} into the cytoplasm. Compare and contrast the locations and functions of the molecules to which Ca^{2+} binds in skeletal and smooth muscles, and explain the steps that lead to cross-bridge cycling in each type of muscle.

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See also **Additional References** *and Figure and Table Citations.*