12

Skeletal Muscle Physiology

LEARNING OBJECTIVES

Upon completion of this chapter, the student should be able to answer the following questions:

1. What structures within skeletal muscle fibers participate in the longitudinal generation of force to the tendon, and which structures participate in the lateral transmission of force to the extracellular matrix?
2. How does a mutation/deficiency in dystrophin affect skeletal muscle?
3. What is the sequence of events and molecular interactions by which an action potential in the sarcolemma of a skeletal muscle fiber results in muscle contraction?
4. By what mechanisms can the force of contraction of skeletal muscle be increased within a minute?
5. What is the basis for the classification of Type 1 and Type 2 skeletal muscle fibers, and their recruitment pattern?
6. Under what circumstances can Type 1 skeletal muscle fibers be converted to Type 2 skeletal muscle fibers, or vice versa?
7. What changes typically occur in skeletal muscle as a consequence of endurance exercise training and resistance exercise training, and what signaling mechanisms participate in each of these training effects.
8. What factors contribute to the development of fatigue?
9. Mutations in which proteins in skeletal muscle are often associated with dysregulation of intracellular Ca^{++}?
10. By what mechanisms can skeletal muscle fibers be repaired?

Skeletal Muscle Physiology

Muscle cells are highly specialized for the conversion of chemical energy to mechanical energy. Specifically, muscle cells use the energy in adenosine triphosphate (ATP) to generate force or do work. Because work can take many forms (such as locomotion, pumping blood, or peristalsis), several types of muscle have evolved. The three basic types of muscle are **skeletal muscle, cardiac muscle,** and **smooth muscle.**

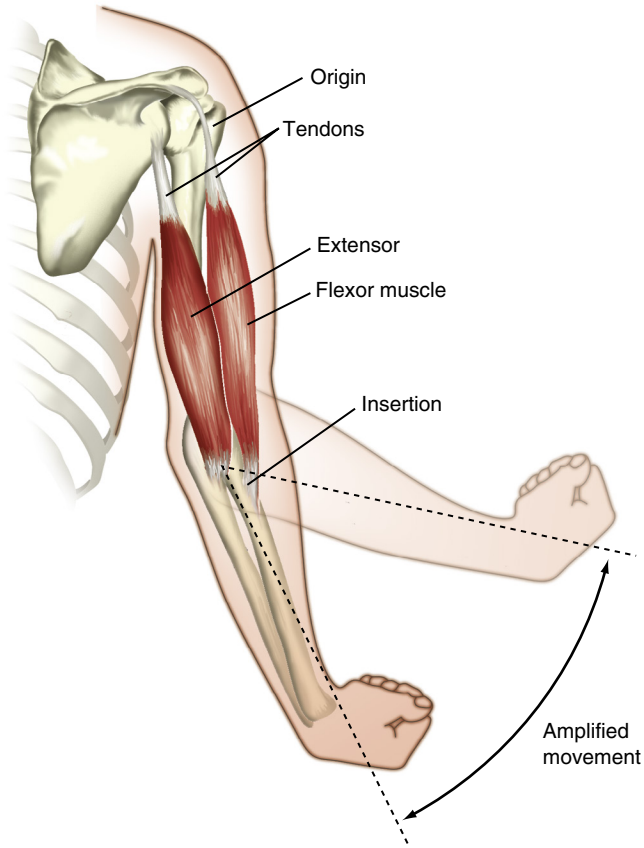
Skeletal muscle acts on the skeleton. In limbs, for example, skeletal muscle spans a joint, thereby allowing a lever action. Skeletal muscle is under voluntary control (i.e., controlled by the central nervous system) and plays a key role in numerous activities such as maintenance of posture, locomotion, speech, and respiration. When viewed under the microscope, skeletal muscle exhibits transverse striations (at intervals of 2–3 μm) that result from the highly organized arrangement of actin and myosin molecules within the skeletal muscle cells. Thus skeletal muscle is classified as a **striated muscle.** The heart is composed of cardiac muscle, and although it is also a striated muscle, it is an involuntary muscle (i.e., controlled by an intrinsic pacemaker and modulated by the autonomic nervous system). Smooth muscle (which lacks the striations evident in skeletal and cardiac muscle) is an involuntary muscle typically found lining hollow organs such as the intestine and blood vessels. In all three muscle types, force is generated by the interaction of actin and myosin molecules, a process that requires transient elevation of intracellular $[Ca^{++}]$.

In this chapter, attention is directed at the molecular mechanisms underlying contraction of skeletal muscle. Mechanisms for regulating the force of contraction are also addressed. To put this information into perspective, it is important to first examine the basic organization of skeletal muscle.

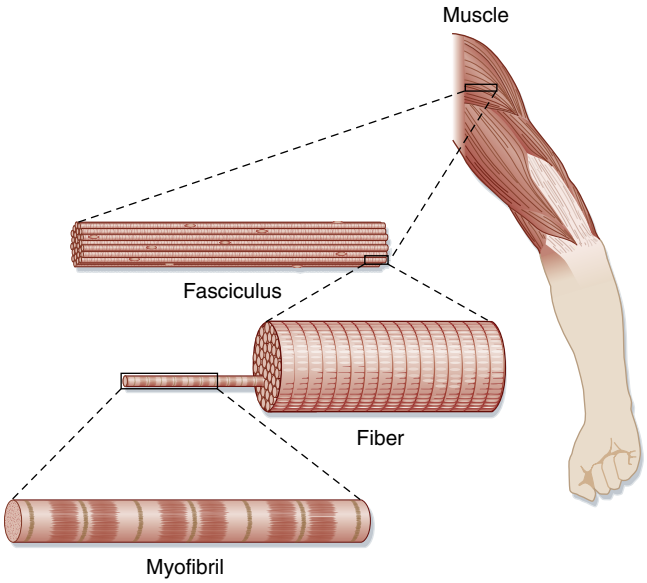
Organization of Skeletal Muscle

Fig. 12.1 illustrates skeletal muscles spanning the elbow joint. The muscles are attached to bone on either side of the joint. The point of attachment closest to the spine (proximal) is called the **origin,** whereas the point of attachment on the far side of the joint (distal) is called the **insertion.** These points of attachment occur through **tendons** (connective tissue) at the end of the muscle. Note that the point of insertion is close to the elbow joint, which enables a broad range of motion. Also note that the joint is spanned by a **flexor** muscle on one side and an **extensor** muscle on the opposite side of the joint. Thus contraction of the flexor muscle (see the biceps muscle in Fig. 12.1) results in a decrease in the angle of the elbow joint (bringing the forearm closer to the shoulder), whereas contraction of the extensor muscle (see the triceps muscle in Fig. 12.1) results in the reverse motion (extending the arm).

The basic structure of skeletal muscle is shown in Fig. 12.2. Each muscle is composed of numerous cells called **muscle fibers.** A connective tissue layer called the **endomysium** surrounds each of these fibers. Individual muscle fibers are then grouped together into **fascicles,** which are surrounded by another connective tissue layer called the



• **Fig. 12.1** Skeletal muscle attaches to the skeleton by way of tendons and typically spans a joint. The proximal and distal points of attachment of the tendon are termed *origin* and *insertion,* respectively. Note that the insertion is close to the joint, which allows a broad range of motion. Also note that skeletal muscles span both sides of the joint, which allows both flexion and extension of the forearm.



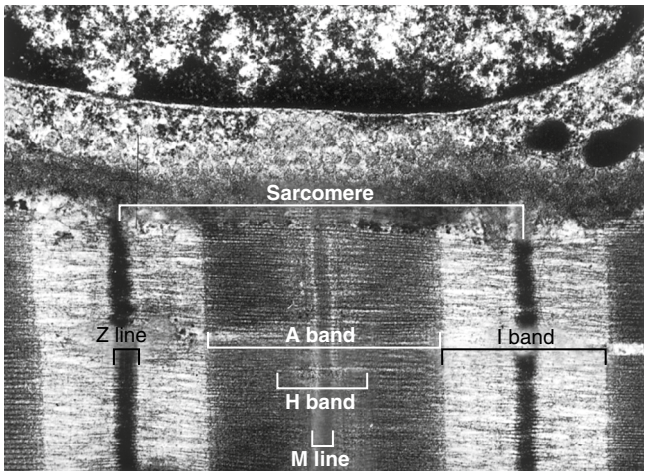
• **Fig. 12.2** Skeletal muscle is composed of bundles of muscle fibers; each such bundle is called a *fasciculus.* A muscle fiber represents an individual muscle cell and contains bundles of myofibrils. The striations are due to the arrangement of thick and thin filaments. See text for details. (Redrawn from Bloom W, Fawcett DW. *A Textbook of Histology.* 10th ed. Philadelphia: Saunders; 1975.)

perimysium. Within the perimysium are the blood vessels and nerves that supply the individual muscle fibers. The fascicles are joined together to form the muscle. The connective tissue sheath that surrounds the muscle is called the **epimysium.** At the ends of the muscle, the connective tissue layers come together to form a tendon, which attaches the muscle to the skeleton. The **myotendinous junction** is a specialized region of the tendon where the ends of the muscle fibers interdigitate with the tendon for the transmission of the force of contraction of the muscle to the tendon to effect movement of the skeleton (discussed later in this section). The tendon and the connective tissue layers are composed mainly of elastin and collagen fibers, and thus they also contribute to passive tension of muscle and prevent damage to the muscle fibers as a result of overstretching or contraction.

Individual skeletal muscle cells are narrow (≈10–80 μm in diameter), but they can be extremely long (up to 25 cm in length). Each skeletal muscle fiber contains bundles of filaments, called **myofibrils,** running along the axis of the cell. The gross striation pattern of the cell results from a repeating pattern in the myofibrils. Specifically, it is the regular arrangement of the thick and thin filaments within these myofibrils, coupled with the highly organized alignment of adjacent myofibrils, that gives rise to the striated appearance of skeletal muscle. Striations can be observed in intact muscle fibers and in the underlying myofibrils.

A myofibril can be subdivided longitudinally into **sarcomeres** (Fig. 12.3). The sarcomere is demarcated by two dark lines called **Z lines** and represents a repeating contractile unit in skeletal muscle. The average length of a sarcomere is 2 μm. On either side of the Z line is a light band **(I band)** that contains thin filaments composed primarily of the protein **actin.** The area between two I bands within a sarcomere is the **A band,** which contains thick filaments composed primarily of the protein **myosin.** The thin actin filaments extend from the Z line toward the center of the sarcomere and overlap a portion of the thick filaments. The dark area at each end of the A band represents this region of overlap between thick and thin filaments. A light area in the center of the sarcomere is called the **H band.** This area represents the portion of the A band that contains myosin thick filaments but no thin actin filaments. Thus thin actin filaments extend from the Z line to the edge of the H band and overlap a portion of the thick filament in the A band. A dark line called the **M line** is evident in the center of the sarcomere and includes proteins that appear to be critical for organization and alignment of the thick filaments in the sarcomere.

As illustrated in Fig. 12.3*B*, each myofibril in a muscle fiber is surrounded by **sarcoplasmic reticulum (SR).** The SR is an intracellular membrane network that plays a critical role in the regulation of intracellular [Ca^{++}]. Invaginations of the sarcolemma, called **T tubules,** pass into the muscle fiber near the ends of the A band (i.e., close to the SR). The SR and the T tubules, however, are distinct membrane systems: The SR is an intracellular network, whereas the T tubules are in contact with the extracellular space. A gap (≈15 nm



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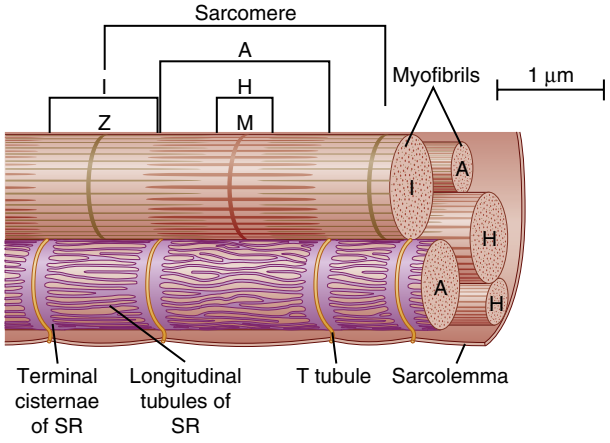

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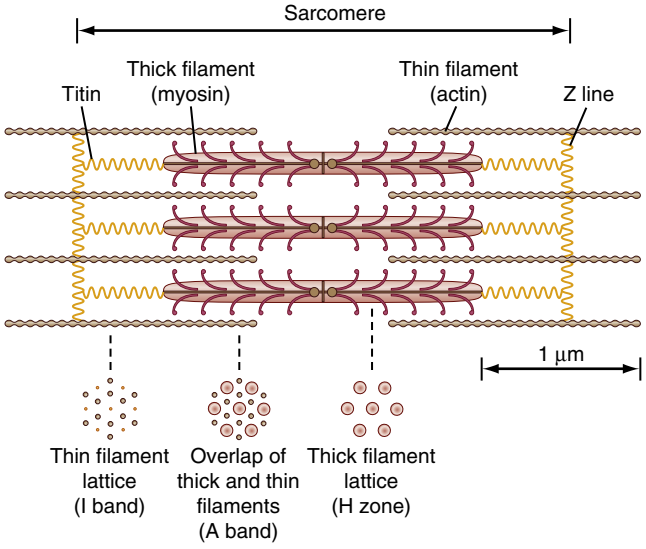


C

• **Fig. 12.3 A,** Myofibrils are arranged in parallel within a muscle fiber. **B,** Each fibril is surrounded by sarcoplasmic reticulum *(SR)*. Terminal cisternae of the SR are closely associated with T tubules and form a triad at the junction of the I and A bands. The Z lines define the boundary of the sarcomere. The striations are formed by overlap of the contractile proteins. Three bands can be observed: the A band, I band, and H band. An M line is visible in the middle of the H band. **C,** Organization of the proteins within a single sarcomere. The cross-sectional arrangement of the proteins is also illustrated.

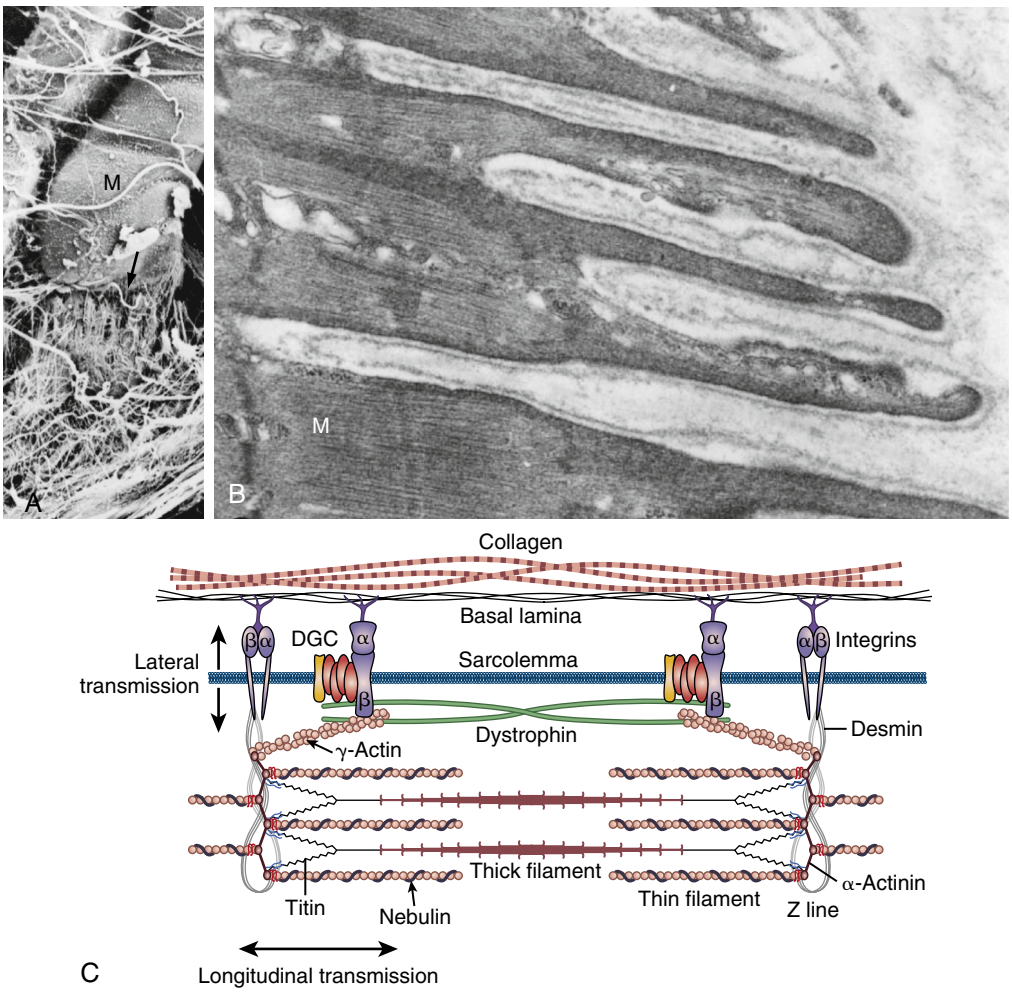
in width) separates the T tubules from the SR. The portion of the SR nearest the T tubules is called the **terminal cisternae,** and it is the site of Ca^{++} release, which is critical for contraction of skeletal muscle (see the section "Excitation-Contraction Coupling"). At the myofibrils, the T tubule is positioned between two terminal cisternae (see Fig. 12.3*B*). The term "triad" refers to this region of the T tubule where it is coupled to two adjacent terminal cisternae, and hence is the site where excitation-contraction coupling is initiated.

The longitudinal portions of the SR are continuous with the terminal cisternae and extend along the length of the sarcomere. This portion of the SR contains a high density of Ca^{++} pump protein (i.e., **SERCA**: **S**arcoplasmic **E**ndoplasmic **R**eticulum **C**a^{++}-**A**TPase), which is critical for reaccumulation of Ca^{++} in the SR and hence for relaxation of the muscle.

The thick and thin filaments are highly organized in the sarcomere of myofibrils. The thin actin filaments extend from the Z line toward the center of the sarcomere, whereas thick myosin filaments are centrally located and overlap a portion of the opposing thin actin filaments. The thick and thin filaments are oriented in such a way that in the region of overlap within the sarcomere, each thick myosin filament is surrounded by a hexagonal array of thin actin filaments (see Fig. 12.3*C*). The Ca^{++}-dependent interaction of the thick myosin filaments and the thin actin filaments generate the force of contraction after stimulation of the muscle (see the section "Actin-Myosin Interaction: Cross-Bridge Formation").

The thin filament is formed by aggregation of actin molecules (termed **globular actin** or **G-actin**) into a two-stranded helical filament called **filamentous actin** or **F-actin**. The elongated cytoskeletal protein **nebulin** extends along the length of the thin filament and may participate in regulation of the length of the thin filament. Dimers of the protein **tropomyosin** extend over the entire actin filament and cover myosin-binding sites on the actin molecules. Each tropomyosin dimer extends across seven actin molecules, with sequential tropomyosin dimers arranged in a head-to-tail configuration. A **troponin complex** consisting of three subunits **(troponin T, troponin I,** and **troponin C)** is present on each tropomyosin dimer and influences the position of the tropomyosin molecule on the actin filament and hence the ability of tropomyosin to inhibit binding of myosin to the actin filament at low cytosolic Ca^{++} concentrations (see the section "Actin-Myosin Interaction: Cross-Bridge Formation"). Additional proteins associated with the thin filament include **tropomodulin, α-actinin,** and **CapZ protein.** Tropomodulin is located at the end of the thin filament, toward the center of the sarcomere, and may participate in setting the length of the thin filament. CapZ protein and α-actinin serve to anchor the thin filament to the Z line.

The thick myosin filaments are tethered to the Z lines by the cytoskeletal protein **titin**. Titin is a very large protein (molecular weight > 3000 kDa) that extends from the Z line to the center of the sarcomere (see Fig. 12.3*C*), and appears to



• **Fig. 12.4** The force of contraction of the muscle fiber is transmitted both longitudinally to the tendon (at the myotendinous junction) and laterally to adjacent extracellular connective tissue (at costameres). The force of contraction is transmitted from the end of the muscle fiber *(M)* to the tendon by connections with numerous collagen fibers (**A,** tip of *arrow*). Folds in the sarcolemma at the end of the muscle fiber **(B)** result in an interdigitation of the muscle fiber with the tendon, and represents the myotendinous junction. Costameres are located on the sides of the muscle fibers, and represent the bridges between the Z lines in the subsarcolemmal myofibrils and the extracellular connective tissue **(C).** Costameres facilitate the lateral transmission of force of contraction, which helps stabilize the sarcolemma. *DGC*, Dystrophin-associated glycoprotein complex. (**A** and **B,** From Tidball JG. Myotendinous junction: morphological changes and mechanical failure associated with muscle cell atrophy. *Exp Molec Pathol* 1984;40:1-12. **C,** From Hughes D, Wallace M, Baar K. Effects of aging, exercise, and disease on force transfer in skeletal muscle. *Am J Physiol Endocrin Metab* 2015;309:E1-E10.)

be important for the organization and alignment of the thick filaments in the sarcomere. Some forms of muscular dystrophy have been attributed to defects in titin (i.e., titinopathies). Additional proteins found in the thick filaments (e.g., **myomesin** and **C protein**) may also participate in the bipolar organization or packing of the thick filament (or both).

The cytoskeleton (including the intermediate filament protein desmin) participates in the highly organized alignment of sarcomeres. Desmin extends from the Z lines of adjacent sarcomeres to the integrin protein complexes on the sarcolemma and thus participates in both the alignment of sarcomeres across muscles and the lateral transmission of force (described later in this section). Defects in desmin have been associated with myofibrillar myopathies.

The force of contraction is transmitted both longitudinally to the tendon (via myotendinous junctions) and laterally to connective tissue adjacent to the muscle fibers (via costameres). The myotendinous junction represents a specialized region where the muscle fiber connects to the tendon (Fig. 12.4*A,B*). Folding of the sarcolemma at the myotendinous junction results in an interdigitation of the tendon with the end of the muscle fiber, which increases the contact area between the muscle fiber and the connective tissue and hence reduces the force per unit area at the end of the muscle fiber.

Lateral transmission of the force of contraction involves costameres, which link the Z lines of subsarcolemmal sarcomeres to extracellular matrix through a series of proteins (see

Fig. 12.4*C*). The lateral transmission of force is thought to stabilize the sarcolemma and to protect it from damage during contraction. Defects in the myotendinous junction and/or costameres (which includes the dystrophin-glycoprotein complex) have been associated with some forms of muscular dystrophy. The myotendinous junction and costameres also contain signaling molecules.

AT THE CELLULAR LEVEL

The muscular dystrophies constitute a group of genetically determined degenerative disorders. **Duchenne's muscular dystrophy** (described by G.B. Duchenne in 1861) is the most common of the muscular dystrophies and affects 1 per 3500 boys (3–5 years of age). Severe muscle wasting occurs, and most affected patients are wheelchair bound by the age of 12; many die of respiratory failure in adulthood (30–40 years of age). Duchenne's muscular dystrophy is an X-linked recessive disease that has been linked to a defect in the dystrophin gene that leads to a deficiency of the dystrophin protein in skeletal muscle, brain, retina, and smooth muscle. **Dystrophin** is a large (427-kDa) protein that is present at low levels (0.025%) in skeletal muscle. It is localized on the intracellular surface of the sarcolemma in association with several integral membrane glycoproteins (forming a dystrophin-glycoprotein complex; Figs. 12.4*C* and 12.5*A*). This dystrophin-glycoprotein complex provides a structural link between the subsarcolemmal cytoskeleton of the muscle cell and the extracellular matrix and appears to stabilize the sarcolemma and hence prevent contraction-induced injury (rupture). The dystrophin-glycoprotein complex may also serve as a scaffold for cell signaling cascades. The enzyme nitric oxide synthase is present in the dystrophin-glycoprotein complex.

Although defects in the dystrophin-glycoprotein complex are involved in many forms of muscular dystrophy, some forms of muscular dystrophy that involve other mechanisms have been identified. Specifically, a defect in sarcolemma repair (attributed to loss/mutation of the protein dysferlin) appears to underlie at least one form of muscular dystrophy (**limb-girdle muscular dystrophy 2B,** associated with muscle wasting in the pelvic region). Defects in the protein titin (titinopathies) have been implicated in other forms of muscular dystrophy (e.g., **limb-girdle muscular dystrophy 2J** and **tibial muscular dystrophy**). Mutations in the protease **calpain 3** (resulting in loss of protease activity) have also been implicated in some types of muscular dystrophy (e.g., limb-girdle muscular dystrophy 2A), apparently secondary to apoptosis.

Organization of the thick filament is shown in Fig. 12.6. Each myosin molecule (≈480 kDa) consists of two heavy chains (≈200 kDa) and four light chains (≈20 kDa). The heavy chains are wound together in an α-helical configuration to form a long rod-like segment (which forms the backbone of the thick filament), and an N-terminal globular head (which extends from each myosin heavy chain toward the actin filament).

The globular head of each myosin molecule contains an *essential light chain* (which is crucial for the ATPase activity of myosin), and a *regulatory light chain.* The regulatory light chain can be phosphorylated by Ca^{++}/calmodulin-dependent myosin light chain protein kinase, which can influence the interaction of myosin with actin (see the section "Skeletal Muscle Types"). Thus myosin ATPase activity occurs in the two globular heads of myosin and requires the presence of the "essential" light chain in each globular head.

Myosin filaments form by a tail-to-tail association of myosin molecules, which results in a bipolar arrangement of the thick filament (see Fig. 12.6*A*). The thick filament then extends on either side of the central bare zone by a head-to-tail association of myosin molecules, thus maintaining the filament's bipolar organization centered on the M line. Such a bipolar arrangement is critical for drawing the Z lines together (i.e., shortening the length of the sarcomere) during contraction.

Control of Skeletal Muscle Activity

Motor Nerves and Motor Units

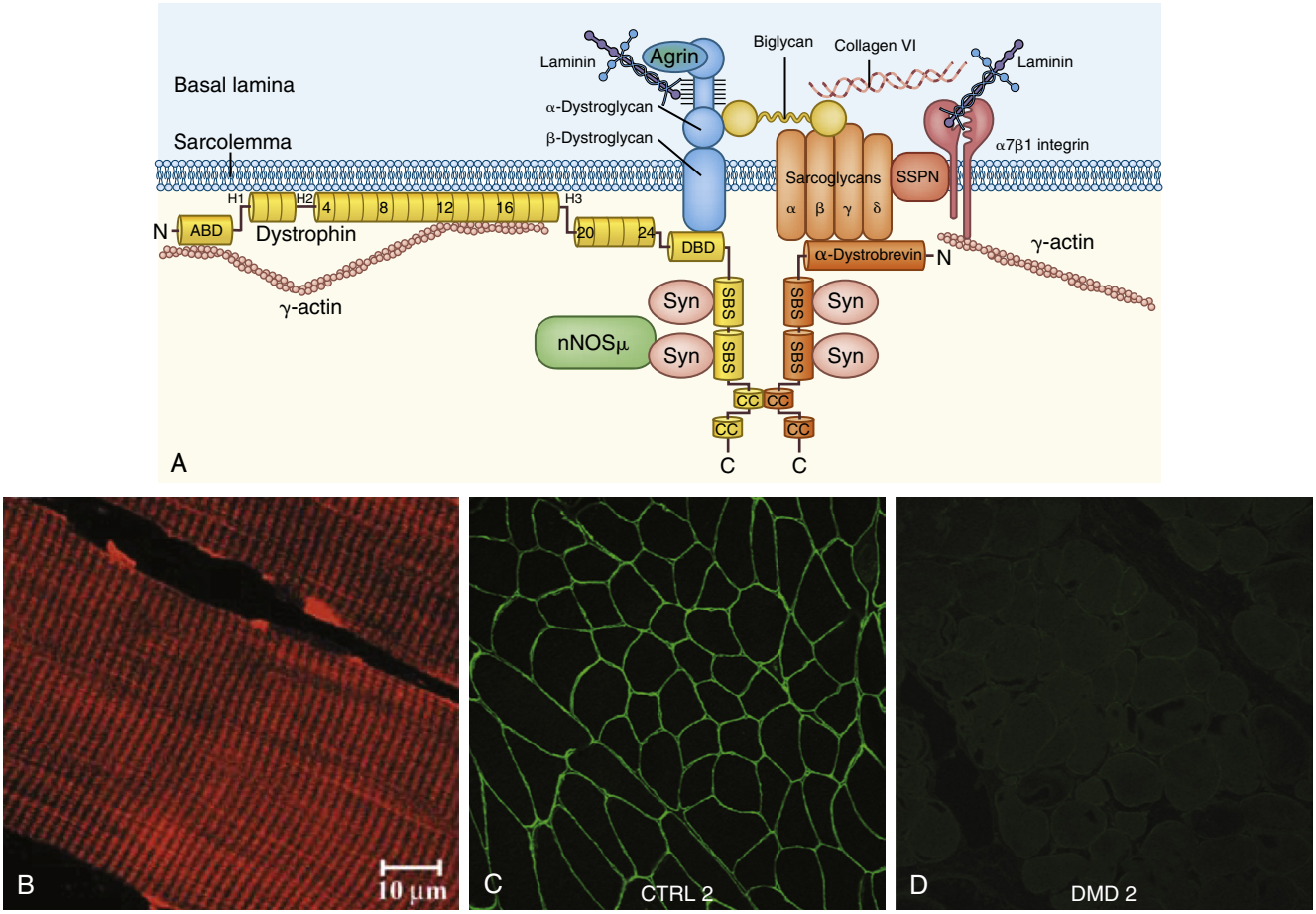
Skeletal muscle is controlled by the central nervous system. Specifically, each skeletal muscle is innervated by an α **motor neuron.** The cell bodies of α motor neurons are in the ventral horn of the spinal cord (Fig. 12.7; see also Chapter 9). The motor axons exit via the ventral roots and reach the muscle through mixed peripheral nerves. The α motor nerves branch in the muscle, and each branch innervates a single muscle fiber. The specialized cholinergic synapse that forms the **neuromuscular junction** and the neuromuscular transmission process that generates an action potential in the muscle fiber are described in Chapter 6.

A **motor unit** consists of the α motor nerve and all the muscle fibers innervated by the nerve. The motor unit is the functional contractile unit because all the muscle cells within a motor unit contract synchronously when the motor nerve fires. The size of motor units within a muscle varies, depending on the function of the muscle. Activation of motor units with a small number of fibers facilitates fine motor control. Activation of varying numbers of motor units within a muscle is one way in which the tension developed by a muscle can be controlled (see "Recruitment" in the section "Modulation of the Force of Contraction").

The neuromuscular junction formed by the α motor neuron is called an **end plate** (see Chapter 6 for details). Acetylcholine released from the α motor neuron at the neuromuscular junction initiates an action potential in the muscle fiber that rapidly spreads along its length. The duration of the action potential in skeletal muscle is less than 5 milliseconds. The short duration of the skeletal muscle action potential allows very rapid contractions of the fiber and provides yet another mechanism by which the force of contraction can be increased. Increasing tension by repetitive stimulation of the muscle is called *tetany* (see the section "Modulation of the Force of Contraction").

Excitation-Contraction Coupling

When an action potential is transmitted along the sarcolemma of the muscle fiber and then down the T tubules,

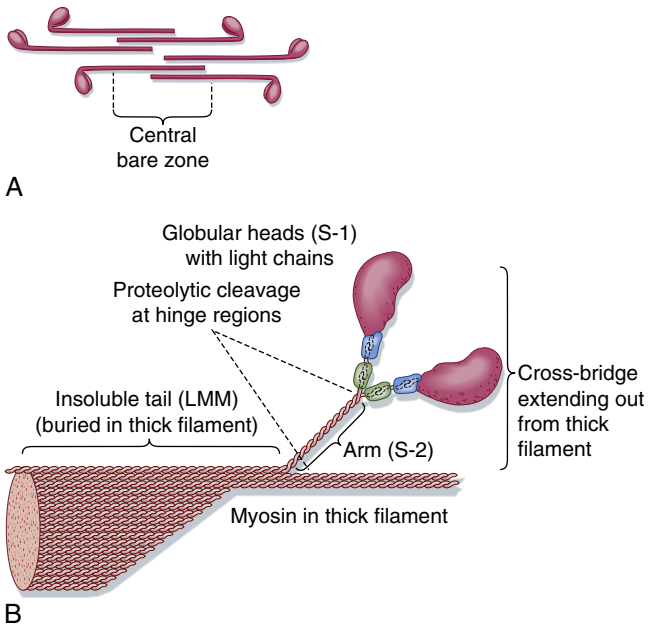


• **Fig. 12.5** **A,** Organization of the dystrophin-glycoprotein complex in skeletal muscle. The dystrophin-glycoprotein complex provides a structural link between the cytoskeleton of the muscle cell and the extracellular matrix, which appears to stabilize the sarcolemma and hence prevents contraction-induced injury (rupture). Duchenne's muscular dystrophy is associated with loss of dystrophin. *Numbers* in dystrophin indicate hinge regions (e.g., H1, H2) and spectrin-like repeat domains (e.g., 4, 8, 12). *ABD*, Actin-binding domain; *C*, carboxy terminus; *CC*, coiled-coil domain; *DBD*, dystroglycan-binding domain; *N*, amino terminus; *nNOSμ*, neuronal nitric oxide synthase μ; *SBS*, syntrophin-binding site; *SSPN*, sarcospan; *Syn*, syntrophin. Electron micrographs of a longitudinal view **(B)** and a cross-sectional view **(C)** show the distribution of dystrophin in skeletal muscle of a normal patient *(CTRL)*. Another cross-sectional view **(D)** shows the loss of dystrophin from skeletal muscle in a patient with Duchenne's muscular dystrophy *(DMD)*. (**A,** From Allen D, Whitehead N, Froehner S. Absence of dystrophin disrupts skeletal muscle signaling: roles of Ca^{2+}, reactive oxygen species, and nitric oxide in the development of muscular dystrophy. *Physiol Rev* 2016;96:253-305. **B,** From Anastasi G, Cutroneo G, Santoro G, et al. Costameric proteins in human skeletal muscle during muscular inactivity. *J Anat* 2008;213:284-295. **C** and **D,** From Beekman C, Sipkens J, Testerink J, et al. A sensitive, reproducible and objective immunofluorescence analysis method of dystrophin in individual fibers in samples from patients with Duchenne muscular dystrophy. *PLoS One* 2014;9[9]:e107494.)

Ca^{++} is released from the terminal cisternae of the SR into the myoplasm (Fig. 12.8*A*). This Ca^{++} release causes intracellular [Ca^{++}] to rise, which in turn promotes actin-myosin interaction and hence contraction (see Fig. 12.8*B*). The action potential is extremely short-lived (<5 milliseconds). The elevation in intracellular [Ca^{++}] begins slightly after the action potential and peaks at approximately 20 milliseconds. This increase in intracellular [Ca^{++}] initiates a contraction called a *twitch.*

The mechanism by which an action potential in a skeletal muscle fiber can induce Ca^{++} release from the SR involves an interaction of voltage-gated Ca^{++} channels ($Ca_V1.1$) in the T tubules with nearby Ca^{++} release channels (RYR1) in the terminal cisternae of the SR (see Fig. 12.8*A*). Calcium flux through the voltage-gated Ca^{++} channels in the T tubule is not needed to induce Ca^{++} release from the nearby SR. Instead, a depolarization-induced conformational change in the voltage-gated Ca^{++} channel in the T tubule appears to promote a protein-protein interaction with the nearby SR Ca^{++} release channel, resulting in Ca^{++} release from the SR into the cytoplasm. The rise in cytosolic Ca^{++} then promotes actin-myosin interaction, and hence contraction

The proposed organization of the voltage-gated Ca^{++} channel and SR Ca^{++} release channel is shown in Fig. 12.9*A*. The voltage-gated Ca^{++} channel contains five subunits (α_{1s}, α_2, δ, β_{1a}, γ), with the α_{1s} subunit acting as a voltage sensor



• **Fig. 12.6** Organization of a thick filament. A thick filament is formed by the polymerization of myosin molecules in a tail-to-tail configuration extending from the center of the sarcomere **(A).** An individual myosin molecule has a tail region and a cross-bridge region. The cross-bridge region is composed of an arm and globular heads **(B).** The globular heads contain light chains that are important for the function of myosin ATPase activity. *LMM*, Light meromyosin; *S-1 and S-2*, myosin subfragments 1 and 2.

and Ca^{++} channel. The α_{1s} subunit is also called $Ca_V1.1$. Historically, this voltage-gated Ca^{++} channel was isolated using the dihydropyridine class of L-type voltage-gated Ca^{++} channel blockers, so the α_{1s} subunit ($Ca_V1.1$) is also called the dihydropyridine receptor (DHPR).

The SR Ca^{++} release channel is called the ryanodine receptor (RYR), as it was isolated using the compound ryanodine. The isoform of the ryanodine receptor in skeletal muscle is RYR1. The ryanodine receptor (RYR1) is a large protein (~480 kDa), that forms a homotetrameric Ca^{++} channel in the terminal cisternae. Much of the RYR1 extends from the terminal cisternae, across the ~15 nm gap, to approach the T tubule. Structural analyses confirm a close association of the voltage-gated Ca^{++} channel in the T tubule and the cytosolic portion of the RYR1 extending from the terminal cisternae (see Fig. 12.9*A*).

Recent studies have shown that the depolarization-induced Ca^{++} release from the SR characteristic of the excitation-contraction-coupling in skeletal muscle can be reconstituted in an expression system using the following 5 proteins: (1) $Ca_V1.1$, (2) the β_{1a} auxiliary subunit of voltage-gated Ca channel, (3) the adapter protein STAC3, (4) RYR1, and (5) junctiphilin. The proposed interactions among these proteins are depicted in Fig. 12.9*B*. Junctiphilin is not shown in Fig. 12.9, but serves a critical function of promoting formation/maintenance of junctions between the SR and T tubule membrane. Junctiphilin may also participate in localizing this calcium release complex.

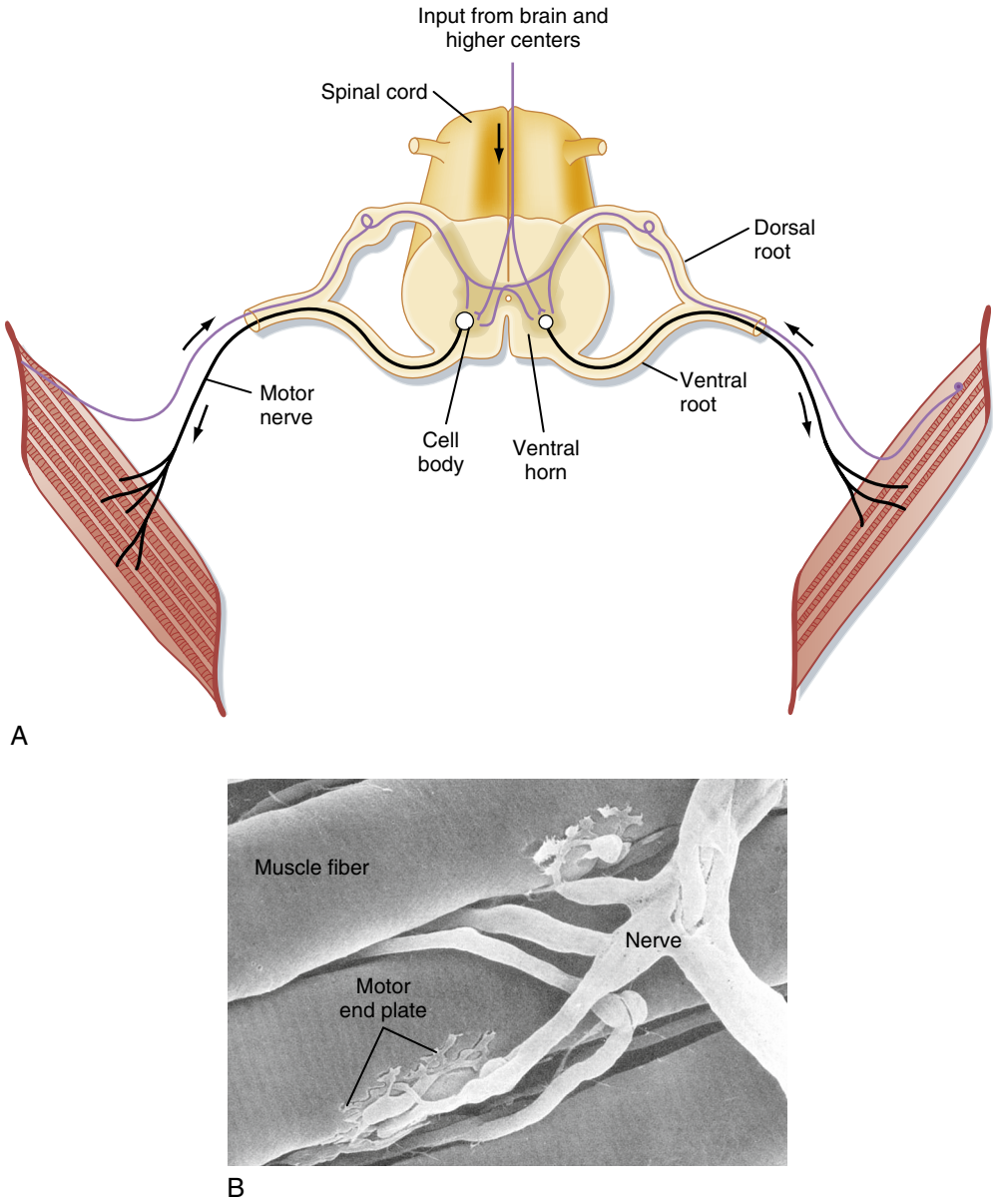
The lumen of the terminal cisternae contains the low-affinity Ca^{++}-binding protein calsequestrin, that allows Ca^{++} to be "stored" at high concentration and thereby establishes a favorable concentration gradient that facilitates the efflux of Ca^{++} from the SR into the cytoplasm when the RYR1 opens. The proteins triadin and junctin are also in the terminal cisternae membrane and bind both RYR and calsequestrin; they could anchor calsequestrin near RYR1 and thereby increase Ca^{++} buffering capacity at the site of Ca^{++} release. Histidine-rich calcium-binding protein is another low-affinity Ca^{++}-binding protein in the SR lumen, although it is less abundant than calsequestrin.

There is also evidence for the presence of **s**tore-**o**perated **Ca** **e**ntry (**SOCE**) in skeletal muscle (e.g., via the Orai/Stim1 complex) during tetany. Inhibition of Ca^{++} influx did not affect excitation-contraction coupling but did reduce maximal tetanic tension at high rates of electrical stimulation, which suggests that there may be some extrusion of intracellular Ca^{++} during tetany, which is compensated by Ca^{++} influx to maintain maximal tetanic tension.

AT THE CELLULAR LEVEL

Recent studies indicate that the electromechanical coupling between $Ca_V1.1$ and the RYR1 can be accomplished with the following 5 proteins: (1) $Ca_V1.1$, (2) β_{1a} auxiliary subunit of $Ca_V1.1$, (3) Stac3, (4) RYR1, and (5) junctiphilin (see Fig. 12.9*B*). It is hypothesized that as the wave of depolarization from an action potential spreads down the T tubule, the $Ca_V1.1$ responds to the voltage through a conformational change that opens the underlying RYR1, resulting in Ca^{++} release from the terminal cisternae of the SR into the muscle cytoplasm, which promotes actin-myosin interaction and hence contraction. The voltage-sensing region of the $Ca_V1.1$ involved in intramembranous charge movement is thought to reside in the S_4 transmembrane segments of $Ca_V1.1$, whereas the myoplasmic loop between transmembrane domains II and III in $Ca_V1.1$ appears to be important for the interaction between $Ca_V1.1$, Stac3, and RYR1. Mutations in $Ca_V1.1$, RYR1, and/or Stac3 have been linked to pathologies characterized by altered regulation of intracellular $[Ca^{++}]$. Specifically, mutations in $Ca_V1.1$ have been associated with hypokalemic periodic paralysis, and myotonic dystrophy type 1. Malignant hyperthermia susceptibility has been linked to mutations in either $Ca_V1.1$ or RYR1. Central core disease involves a defect in the RYR1, discussed later. A mutation in Stac3 is present in the rare congenital disorder Native American myopathy.

Relaxation of skeletal muscle occurs as intracellular Ca^{++} is resequestered by the SR. Uptake of Ca^{++} into the SR is due to the action of a Ca^{++} pump (i.e., Ca^{++}-ATPase). This pump is not unique to skeletal muscle; it is found in all cells in association with the endoplasmic reticulum. Accordingly, it is named **SERCA,** which stands for **s**arcoplasmic **e**ndoplasmic **r**eticulum **c**alcium **A**TPase. SERCA is the most abundant protein in the SR of skeletal muscle, and it is distributed throughout the longitudinal tubules and the terminal cisternae. It

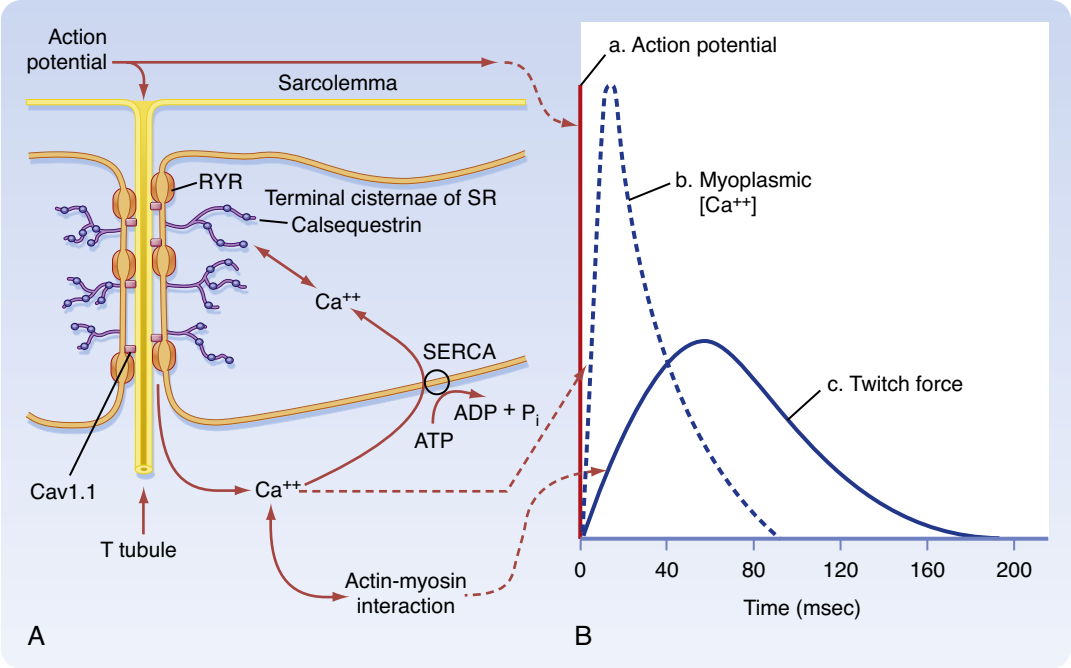


• **Fig. 12.7** Skeletal muscle is voluntary muscle controlled by the central nervous system, with efferent signals (i.e., action potentials) passing through an α motor neuron to muscle fibers. Each motor neuron may innervate many muscle fibers within a muscle, although each muscle fiber is innervated by only one motor neuron **(A).** A scanning electron micrograph **(B)** shows innervation of several muscle fibers by a single motor neuron. (**B,** From Bloom W, Fawcett DW. *A Textbook of Physiology.* 12th ed. New York: Chapman & Hall; 1994.)

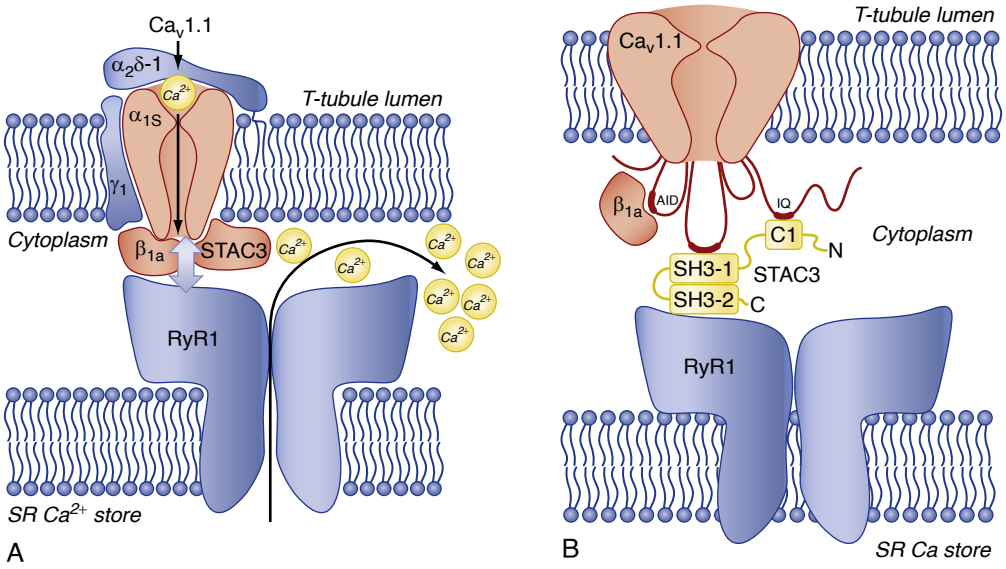
transports two molecules of Ca^{++} into its lumen for each molecule of ATP hydrolyzed.[a] Thus the Ca^{++} transient seen during a twitch contraction (see Fig. 12.8*B*) reflects release of Ca^{++} from the terminal cisternae via RYR1 and reuptake primarily into the longitudinal portion of the SR by SERCA. The low-affinity Ca^{++}-binding protein **sarcalumenin** is present throughout the longitudinal tubules of the SR and nonjunctional regions of the terminal cisternae and is thought to be involved in the transfer of Ca^{++} from sites of Ca^{++} uptake in the longitudinal tubules to sites of Ca^{++} release in the terminal cisternae. Results of studies suggest that sarcalumenin increases Ca^{++} uptake by SERCA, at least in part by buffering luminal Ca^{++} near the pump.

The endogenous micropeptides phospholamban, sarcolipin, and myoregulin have been shown to regulate the activity of SERCA by decreasing the Ca^{++} sensitivity of Ca^{++} uptake. Protein kinase A–dependent phosphorylation of phospholamban in slow-twitch skeletal muscle has been reported to increase Ca^{++} transport in the SR, similar to the effect of phospholamban phosphorylation in the heart. Phospholamban and sarcolipin are present in slow-twitch muscle, whereas myoregulin is present in both fast- and slow-twitch muscle.

[a]During the transport of Ca^{++}, SERCA exchanges two Ca^{++} ions for two H^+ ions (i.e., H^+ is pumped out of the SR).



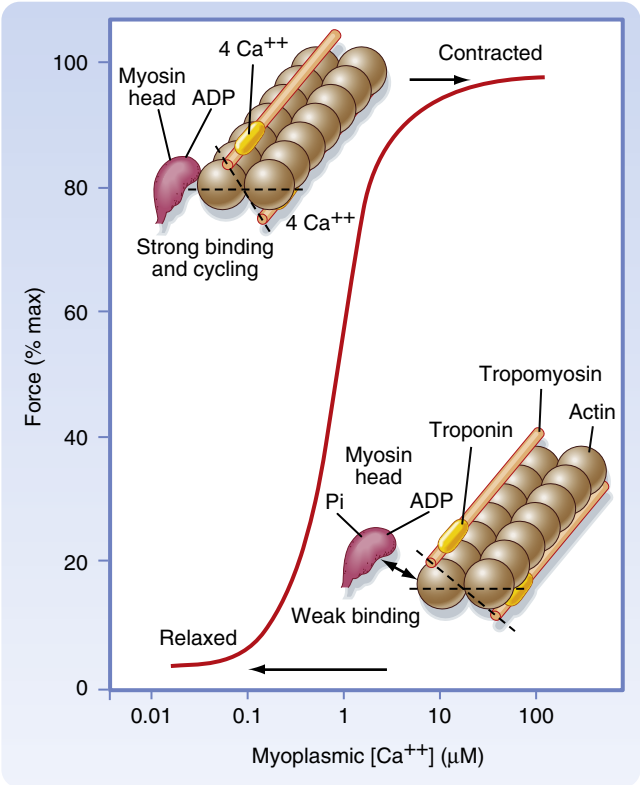
• **Fig. 12.8** **A,** Stimulation of a skeletal muscle fiber initiates an action potential in the muscle that travels down the T tubule and induces release of Ca^{++} from the terminal cisternae of the sarcoplasmic reticulum (SR). The rise in intracellular [Ca^{++}] causes a contraction. As Ca^{++} is pumped back into the SR by sarcoplasmic endoplasmic reticulum Ca^{++}-ATPase *(SERCA)*, relaxation occurs. *$Ca_V1.1$*, α_{1s} subunit of voltage-gated Ca channel; *P_i*, inorganic phosphate; *RYR*, ryanodine receptor. **B,** Time courses of the action potential, myoplasmic Ca^{++} transient, and force of the twitch contraction.



• **Fig. 12.9** Organization of Ca^{++} release complex at the junction of the T-tubule membrane and the terminal cisternae of the SR in skeletal muscle. **A,** The voltage-gated Ca^{++} channel in the T-tubule is close to the Ca^{++} release channel in the terminal cisternae. **B,** The proposed interactions between proteins needed for an action potential in the T-tubule to induce Ca^{++} release from the terminal cisternae of the SR. The protein junctiphilin is not depicted, though it is needed for forming and maintaining this junction between the T-tubule and the terminal cisternae of the SR. The subunits of the voltage-gated Ca channel are shown (α_{1s}, β_{1a}, $\alpha_2\delta$-1, γ_1), along with the adaptor protein STAC3 and the ryanodine receptor *(RYR1)*. *$Ca_V1.1$*, α_{1s} subunit of the voltage-gated Ca channel. See text for details. (From Fluher B, Campiglio M. STAC proteins: the missing link in skeletal muscle EC coupling and new regulators of calcium channel function. *Biochim Biophys Acta - Mol Cell Res* 2019;1866:1101.)

Actin-Myosin Interaction: Cross-Bridge Formation

As noted, contraction of skeletal muscle requires an increase in intracellular [Ca^{++}]. Moreover, the process of contraction is regulated by the thin filament. As shown in Fig. 12.10, contractile force (i.e., tension) increases in a sigmoidal manner as intracellular [Ca^{++}] is elevated above 0.1 μM, with half-maximal force occurring at less than 1 μM Ca^{++}. The mechanism by which Ca^{++} promotes this increase in tension is as follows: Ca^{++} released from the SR binds to troponin C. Once bound with Ca^{++}, troponin C facilitates movement of the associated tropomyosin molecule toward the cleft of the actin filament. This movement of tropomyosin exposes myosin-binding sites on the actin filament and allows a cross-bridge to form and thereby generate tension (see section "Cross-Bridge Cycling: Sarcomere Shortening"). Troponin C has four Ca^{++}-binding sites. Two of these sites have high affinity for Ca^{++} but also bind Mg^{++} at rest. These sites seem to be involved in controlling and enhancing the interaction between the troponin I and troponin T subunits. The other two binding sites have lower affinity and bind Ca^{++} as its concentration rises after release from the SR. Binding of myosin to the actin filaments appears to cause a further shift in tropomyosin. Although a given tropomyosin molecule extends over seven actin molecules, it is hypothesized that the strong binding of myosin to actin results in movement of an adjacent tropomyosin molecule, perhaps exposing myosin-binding sites on as many as 14 actin molecules. This ability of one tropomyosin molecule to influence the movement of another may be a consequence of the close proximity of adjacent tropomyosin molecules.



• **Fig. 12.10** The contractile force of skeletal muscle increases in a Ca^{++}-dependent manner as a result of binding of Ca^{++} to troponin C and the subsequent movement of tropomyosin away from myosin-binding sites on the underlying actin molecules. See text for details. (Redrawn from Hartshorne DJ. In: Lapedes DN, ed. *Yearbook of Science and Technology.* New York: McGraw-Hill; 1976.)

Cross-Bridge Cycling: Sarcomere Shortening

Once myosin and actin are bound, ATP-dependent conformational changes in the myosin molecule result in movement of the actin filaments toward the center of the sarcomere. Such movement shortens the length of the sarcomere and thereby contracts the muscle fiber. The mechanism by which myosin produces force and shortens the sarcomere is thought to involve four basic steps that are collectively termed the *cross-bridge cycle* (labeled *a* to *d* in Fig. 12.11). In the resting state, myosin is thought to have partially hydrolyzed ATP (state *a*). When Ca^{++} is released from the terminal cisternae of the SR, it binds to troponin C, which in turn promotes movement of tropomyosin on the actin filament in such a way that myosin-binding sites on actin are exposed. This then allows the "energized" myosin head to bind to the underlying actin (state *b*). Myosin next undergoes a conformational change termed "ratchet action" that pulls the actin filament toward the center of the sarcomere (state *c*). Myosin releases adenosine diphosphate (ADP) and inorganic phosphate during the transition to state *c*. Binding of ATP to myosin decreases the affinity of myosin for actin, thereby resulting in the release of myosin from the actin filament (state *d*). Myosin then partially hydrolyzes the ATP, and part of the energy in the ATP is used to recock the head and return to the resting state.

If intracellular [Ca^{++}] is still elevated, myosin undergoes another cross-bridge cycle and produces further contraction of the muscle. The ratchet action of the cross-bridge is capable of moving the thin filament approximately 10 nm. The cycle continues until the SERCA pumps Ca^{++} back into the SR. As [Ca^{++}] falls, Ca^{++} dissociates from troponin C, and the troponin-tropomyosin complex moves and blocks the myosin-binding sites on the actin filament. If the supply of ATP is exhausted, as occurs with death, the cycle stops in state *c* with the formation of permanent actin-myosin complexes (i.e., the rigor state). In this state, the muscle is rigid, and the condition is termed **rigor mortis.**

As already noted, formation of the thick filaments involves the association of myosin molecules in a tail-to-tail configuration to produce a bipolar orientation (see Fig. 12.6). Such a bipolar orientation allows myosin to pull the actin filaments toward the center of the sarcomere during the cross-bridge cycle. The myosin molecules are also oriented in a helical array in the thick filament in such a way that cross-bridges extend toward each of the six thin filaments surrounding the thick filament (see Fig. 12.3*C*). These myosin projections/cross-bridges can be seen on electron micrographs of skeletal muscle and appear to extend

IN THE CLINIC

Genetic diseases that cause disturbances in Ca^{++} homeostasis in skeletal muscle include **malignant hyperthermia, central core disease,** and **Brody's disease.** Malignant hyperthermia is an autosomal dominant trait that has life-threatening consequences in certain surgical instances. Anesthetics such as halothane or ether and the muscle relaxant succinylcholine can produce uncontrolled release of Ca^{++} from the SR, thereby resulting in skeletal muscle rigidity, tachycardia, hyperventilation, and hyperthermia. This condition is lethal if not treated immediately (typically by administering dantrolene to block this uncontrolled Ca^{++} release from the SR). The incidence of malignant hyperthermia susceptibility is approximately 1 per 15,000 children and 1 per 50,000 adults treated with anesthetics. Malignant hyperthermia is the result of a defect in the SR Ca^{++} release channel (RYR1), which becomes activated in the presence of the aforementioned anesthetics, causes the release of Ca^{++} into the cytoplasm, and hence prolongs muscle contraction (rigidity). The defect in the RYR1 is not restricted to a single locus. In some cases, malignant hyperthermia has been linked to a defect in the $Ca_V1.1$ of the T tubule.

Central core disease is a rare autosomal dominant trait that results in muscle weakness, loss of mitochondria in the core of skeletal muscle fibers, and some disintegration of contractile filaments. It is often closely associated with malignant hyperthermia, and so patients with central core disease are treated as though they are susceptible to malignant hyperthermia in surgical situations. It is hypothesized that central cores devoid of mitochondria represent areas of elevated intracellular Ca^{++} secondary to a mutation in the RYR. The loss of mitochondria is thought to occur when they take up the elevated Ca^{++}, which leads to mitochondrial Ca^{++} overload.

Brody's disease is characterized by painless muscle cramping and impaired muscle relaxation during exercise. While an affected person runs upstairs, for example, muscles may stiffen and temporarily cannot be used. This relaxation abnormality is seen in muscles of the legs, arms, and eyelid, and the response is worsened in cold weather. Brody's disease can be either autosomal recessive or autosomal dominant and may involve mutations in up to three genes; however, it is rare (affecting 1 per 10,000,000 births). It appears to result from decreased activity of the SERCA1 Ca^{++} pump found in fast-twitch skeletal muscle (see the section "Skeletal Muscle Types"). The decreased activity of SERCA1 has been associated with mutations in the gene that encodes SERCA1, although another accessory factor may contribute to the decreased SR Ca^{++} uptake in the fast-twitch skeletal muscle of individuals with Brody's disease.

Myotonia congenita is also associated with prolonged muscle contractions (painless cramping) after voluntary contractions, as a result of mutations in the *CLCN1* gene, which encodes the chloride voltage-gated channel 1 in skeletal muscle sarcolemma and T tubules. Chloride conductance in the skeletal muscle is important for repolarization and stabilization of the membrane potential, and so the reduced chloride conductance in skeletal muscles of individuals with myotonia congenita results in hyperexcitability of the muscle fiber. Voluntary contraction may therefore be followed by a series of action potentials (afterdepolarizations) in the muscle that result in prolonged contractions (i.e., cramping). Epinephrine (e.g., during stressful situations) often worsens the condition, as shown in myotonic ("fainting") goats. Muscle stiffness can be relieved by repeated contractions (i.e., the warm-up phenomenon), although the mechanism underlying the warm-up phenomenon is not known. Mutations in the *CLCN1* gene in myotonia congenita may be transmitted in either an autosomal recessive manner (as in Becker's disease, one type of myotonia congenita) or an autosomal dominant manner (as in Thomsen's disease, the other type of myotonia congenita). The prevalence of myotonia congenita is approximately 1 per 100,000 worldwide; the incidence is higher (≈1 per 10,000) in northern Scandinavia.

perpendicular from the thick filaments at rest. In the contracted state, the myosin cross-bridges slant toward the center of the sarcomere, which is consistent with the ratchet action of the myosin head.

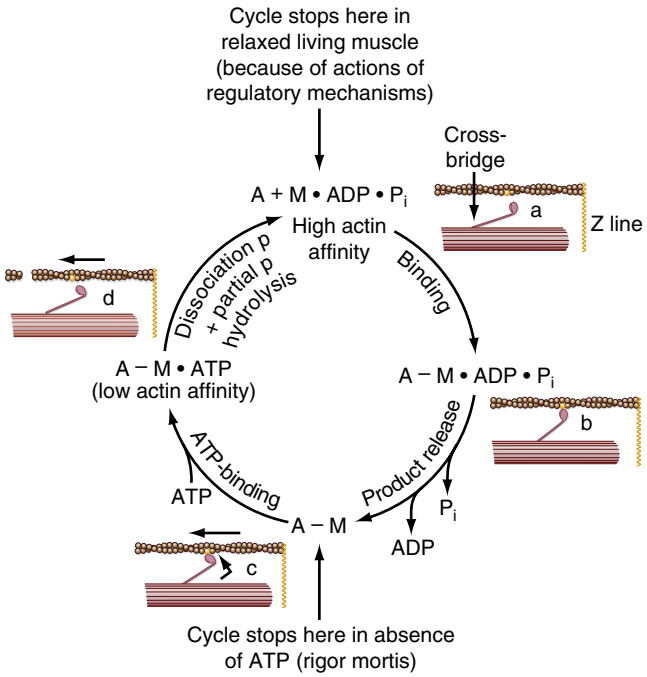
The cross-bridge cycling mechanism just described is called the **sliding filament theory** because the myosin cross-bridge is pulling the actin thin filament toward the center of the sarcomere, which results in an apparent "sliding" of the thin filament past the thick filament. There is, however, uncertainty about how many myosin molecules contribute to the generation of force and whether both myosin heads in a given myosin molecule are involved. It has been calculated that there may be 600 myosin heads per thick filament, with a stoichiometry of 1 myosin head per 1.8 actin molecules. As a result of steric considerations, it is unlikely that all myosin heads can interact with actin, and calculations suggest that even during maximal force generation, only 20% to 40% of the myosin heads bind to actin.

The conversion of chemical energy (i.e., ATP) to mechanical energy by muscle is highly efficient. In isolated muscle preparations, maximum mechanical efficiency (≈65% efficiency) is obtained at a submaximal force of 30% maximal tension. In humans performing steady-state ergometer exercise, mechanical efficiencies range from 40% to 57%.

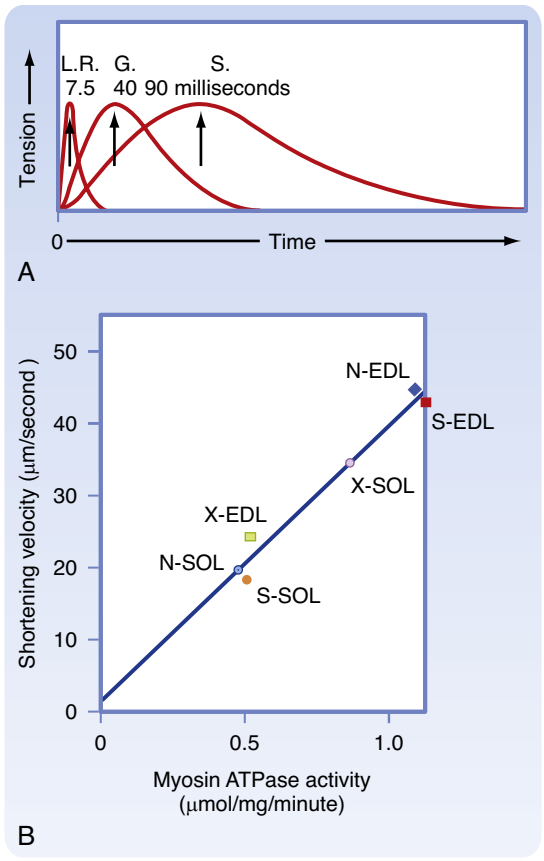
Skeletal Muscle Types

Skeletal muscle fibers can be classified into two main groups according to the speed of contraction: fast-twitch and slow-twitch muscle fibers. As shown in Fig. 12.12*A*, the lateral rectus of the eye contracts very quickly in response to an action potential, reaching peak tension within 8 milliseconds, and then relaxes quickly, which results in a short duration of contraction. The soleus muscle of the leg, in contrast, requires 90 milliseconds to reach peak tension in response to an action potential, and then it relaxes slowly. The gastrocnemius muscle requires an intermediate time to reach peak tension (40 milliseconds) because of the presence of both fast-twitch and slow-twitch muscle fibers in this muscle.

The difference in speed of contraction between fast-twitch and slow-twitch muscles is correlated with myosin ATPase activity (see Fig. 12.12*B*), which in turn reflects the type of myosin present in the muscle fiber. Thus fast-twitch muscle fibers contain myosin isoforms that hydrolyze ATP



• **Fig. 12.11** Cross-bridge cycle. In the relaxed state (state *a*), ATP is partially hydrolyzed (M • ADP • P_i). In the presence of elevated myoplasmic Ca^{++} (state *b*), myosin *(M)* binds to actin *(A)*. Hydrolysis of ATP is completed (state *c*) and causes a conformational change in the myosin molecule that pulls the actin filament toward the center of the sarcomere. A new ATP molecule binds to myosin and causes release of the cross-bridge (state *d*). Partial hydrolysis of the newly bound ATP recocks the myosin head, which is now ready to bind again and again. If myoplasmic [Ca^{++}] is still elevated, the cycle repeats. If myoplasmic [Ca^{++}] is low, relaxation results.

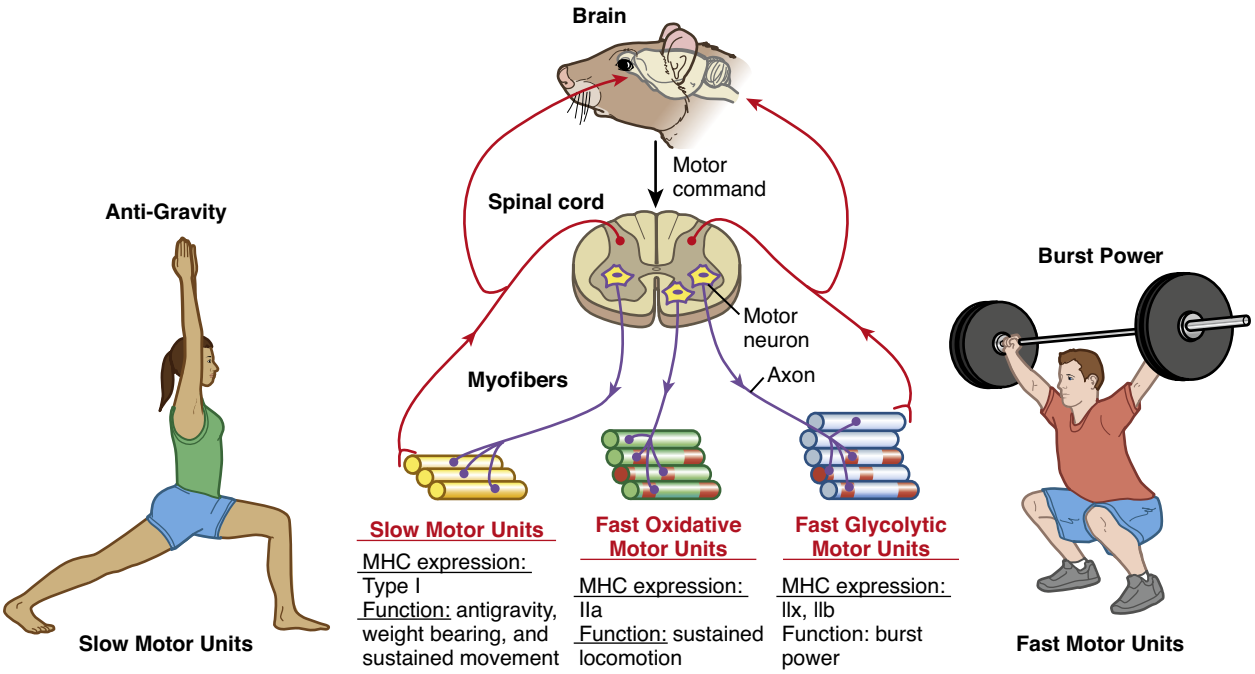


• **Fig. 12.12** **A,** Muscles vary in terms of the speed of contraction. *G*, Gastrocnemius muscle of the leg; *LR*, lateral rectus muscle of the eye; *S*, soleus muscle of the leg. **B,** The speed of shortening is correlated with myosin ATPase activity. *N-SOL*, Normal soleus muscle (slow twitch); *N-EDL*, normal extensor digitorum longus muscle (fast twitch); *S-EDL*, self-innervated extensor digitorum longus muscle (EDL motor nerve transected and resutured); *S-SOL*, self-innervated soleus muscle (soleus motor nerve transected and resutured); *X-EDL*, cross-innervated extensor digitorum longus muscle (EDL innervated by soleus motor nerve); *X-SOL*, cross-innervated SOL muscle (soleus innervated by EDL motor nerve). (**A,** From Mountcastle V. *Medical Physiology.* 12th ed. St. Louis: Mosby; 1974. **B,** From Bárány M, Close RI. *J Physiol* 1971;213:455.)

quickly, whereas slow-twitch muscle fibers contain myosin isoforms that hydrolyze ATP slowly. These two types of myosin isoforms have the same basic structure described previously, with two heavy chains and two pairs of light chains, although they differ in amino acid composition.

It is very difficult to convert a slow-twitch muscle fiber into a fast-twitch fiber, although it can be accomplished by cross-innervation, which involves surgically interconnecting two motor neurons. As shown in Fig. 12.12*B,* when the soleus muscle and extensor digitorum longus muscle underwent cross-innervation, so that contraction of the soleus muscle was controlled by the extensor digitorum motor neuron (and vice versa), the speed of the contraction and the myosin ATPase activity of the soleus muscle increased (labeled *X-SOL* in Fig. 12.12*B*), whereas the extensor digitorum longus exhibited a decrease in shortening velocity and myosin ATPase activity (labeled *X-EDL*). Thus the motor innervation of the muscle fiber plays an important role in determining which type of myosin isoform is expressed in the muscle fiber. Further study showed that the intracellular Ca^{++} concentration in the muscle (secondary to differences in the activity pattern of the motor neuron) was an important determinant of whether the muscle fiber expressed the slow myosin isoform or the fast myosin isoform (see the section "Growth and Development").

The myosin isoforms expressed in skeletal muscle can be distinguished on the basis of myosin heavy chain composition. Slow-twitch muscle fibers express Type I myosin heavy chain, whereas fast-twitch skeletal muscle fibers could contain Type IIa, Type IIx, or Type IIb myosin heavy chains (Fig. 12.13). The Type IIb myosin isoform is not present in human skeletal muscle, so human skeletal muscle fiber types are classified as Type 1, Type IIa, or Type IIx. The distribution of Type I, Type IIa, and Type IIx myosins in a biopsy of human vastus lateralis muscle is shown in Fig. 12.14*A*. Note that a few muscle fibers (denoted with an asterix) contain two types of myosin heavy chain. Endurance training or chronic stimulation promotes the expression of the Type 1 myosin isoform, whereas strength training promotes the expression of the Type II myosin isoform (as depicted in Fig. 12.13). Typically, changes in the expression of myosin isoforms follow a progression, wherein



• **Fig. 12.13** Comparison of three basic motor unit phenotypes in skeletal muscle of extremities and trunk. *MHC*, Myosin heavy chain. (Redrawn from Baldwin K, Haddad F, Pandorf C, et al. Alterations in muscle mass and contractile phenotype in response to unloading models: role of transcriptional/pretranslational mechanisms. *Front Physiol* 2013;4:284.)

Type IIx myosin < - > Type IIa myosin < - > Type I myosin.

The maximal contraction speeds of human and mouse skeletal muscle fibers expressing Type 1, Type IIa, Type IIx, or Type IIb myosins are shown in Fig. 12.14*B*. The human maximal contraction velocities were determined from the Y-intercepts of the force-velocity relationships shown in Fig. 12.14*C*, using permeabilized single muscle fibers from biopsies of human vastus lateralis muscle. In both humans and mice, the contraction speeds were consistent with the myosin isoform expressed in the fiber in that:

Type I contraction speed < Type IIa contraction speed

< Type IIx contraction speed

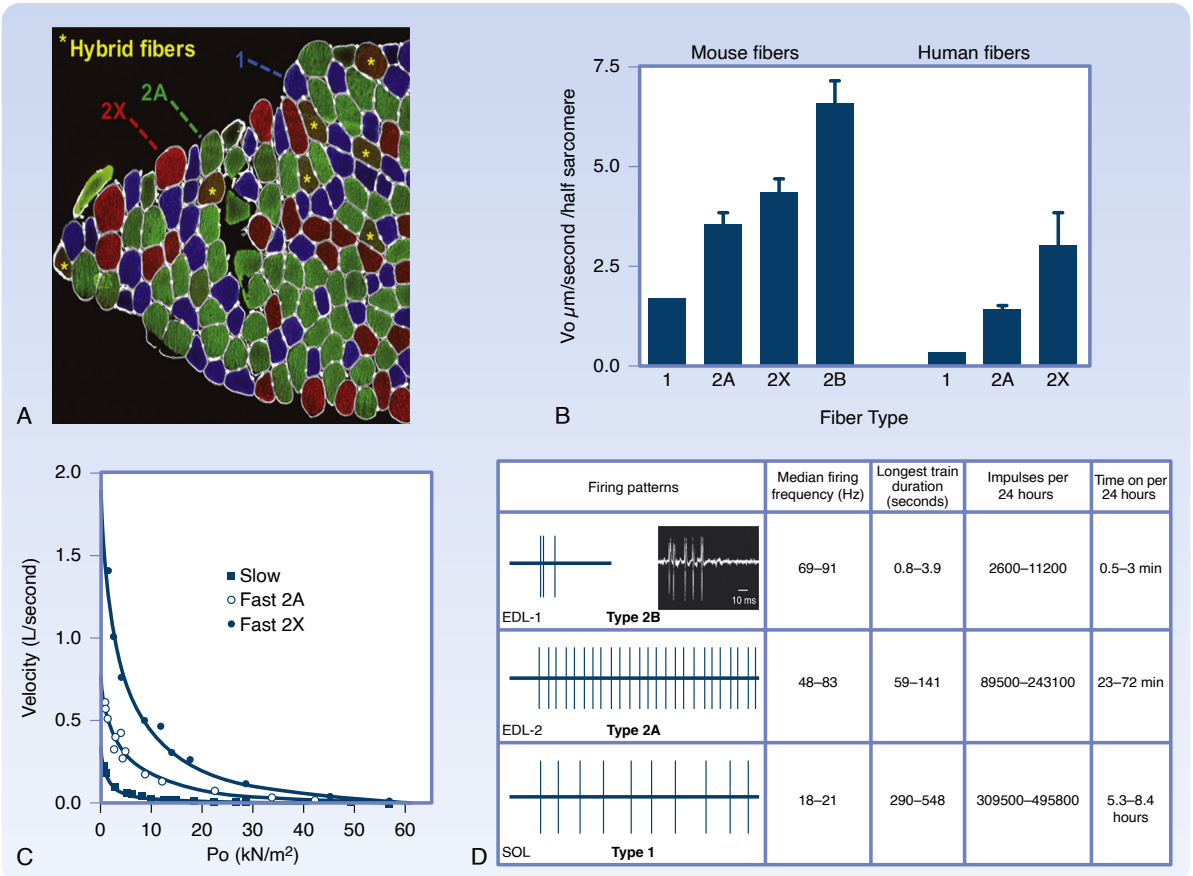
Type IIb myosin is rarely expressed in humans but is expressed in rodents. The contraction speed of mouse muscle fibers expressing Type 1Ib myosins was the fastest of the four myosin isoforms. Additional characteristics of the Type 1, Type IIa, Type IIx, and Type IIb muscle fibers are shown in Table 12.1.

All of the muscle fibers innervated by a given α motor neuron typically express the same myosin isoform, so there are slow motor units and two or three types of fast motor units (for human and rodents, respectively) (see Fig. 12.13). The α motor neurons innervating Type I muscle fibers have small cell bodies, and are easily excited (Table 12.2). The α motor neurons innervating Type II muscle fibers are larger, with a higher threshold for activation. The data in Fig. 12.14*D* are consistent with this recruitment pattern, in that over a 24-hour period the cumulative activation time of rat slow-twitch motor units greatly exceeded that for rat fast-twitch motor units. Type IIb motor units in the rat were rarely recruited. This pattern of recruitment is consistent with the size principle for motor unit recruitment (see Chapter 9), wherein motor units with small motor axons are more easily activated than large motor neurons,

Slow-twitch skeletal muscles are also characterized by a high oxidative capacity (see Table 12.1), which in combination with the low myosin ATPase activity contribute to the fatigue resistance of slow-twitch muscle fibers. The oxidative capacity of the fast-twitch muscle fiber ranges from relatively high (in muscle fibers expressing Type IIa myosin heavy chain) to low (in muscle fibers expressing Type IIb myosin heavy chains). Muscle fibers expressing a Type IIx myosin heavy chain have a speed of contraction and an oxidative capacity that is intermediate between fiber types IIa and IIb, so in human skeletal muscle (which lacks Type IIb fibers), the Type IIx muscle fibers have a slightly higher contraction speed but lower oxidative capacity than Type I muscle fibers (see Table 12.1)

Although motor units are generally composed of only one type of muscle fiber (see Fig. 12.13), there are conditions that may trigger a change in the type of myosin expressed in a muscle fiber. Chronic conditions such as microgravity (in space flight), denervation, spinal cord injury, and chronic unloading, for example, are associated with severe atrophy and promote the gradual transition from the expression of slow muscle myosin (Type I) in the fiber to the expression of fast muscle myosin (Types IIa and IIx).

An important function of slow motor units is in the maintenance of posture (see Fig. 12.13). The low ATPase



• **Fig. 12.14** Type I muscle fibers exhibit a slower contraction and lower isometric force of contraction than Type II muscle fibers. **A**, Immunostaining of human vastus lateralis muscle with antibodies to Type I, Type IIa, and Type IIx myosin isoforms. Note the presence of a few hybrid fibers that express more than one type of myosin. **B,** Maximum unloaded contraction velocities of Type I and Type II muscle fibers in mouse and human. **C**, Force-velocity relationship of Type I and Type II muscle fibers from human vastus lateralis muscle. **D**, 24-hour continuous monitoring of Type I, Type IIa, and Type IIb motor units in a rat hindlimb. (**A,** From Schiafino S. Muscle fiber type diversity revealed by anti-myosin heavy chain antibodies. *FEBS J* 2018;285:3688. **B–D,** From Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev* 2011;91:1447.

TABLE 12.1 Basic Classification of Skeletal Muscle Fiber Types

Classification Parameters	Type I Slow-Oxidative	Type IIa Fast-Oxidative	Type IIx Fast-Intermediate	Type IIb[a] Fast-Glycolytic
Myosin isoenzyme Myosin gene	Type I MYH7	Type IIa MYH2	Type IIx MYH1	Type IIb MYH4
Myosin ATPase activity	Slow	Fast	Faster	Fastest
Maximum shortening velocity	Slow	Fast	Faster	Fastest
SR Ca^{++}-pumping rate	Moderate	High	High	High
Capillary density	Moderate	Moderate	Lower	Lowest
Oxidative capacity: Mitochondrial content	High	High	Low	Lowest
Glycolytic capacity	Moderate	High	High	High

[a]Human skeletal muscle fibers rarely express the Type IIb myosin isoenzyme.

Type IIx muscle fibers express metabolic properties intermediate between those of Type IIa and Type IIb.

SR, Sarcoplasmic reticulum.

TABLE 12.2 Properties of Motor Units

Characteristics	Motor Unit Classification	
	Type I	Type II
Properties of Nerve		
Cell diameter	Small	Large
Conduction velocity	Fast	Very fast
Excitability	High	Low
Properties of Muscle Cells		
Number of fibers	Few	Many
Fiber diameter	Moderate	Large
Force of unit	Low	High
Metabolic profile	Oxidative	Glycolytic
Contraction velocity	Moderate	Fast
Fatigability	Low	High

activity of myosin in slow motor units, coupled with their high oxidative capacity, facilitates the ability of these slow motor units to maintain posture at low energy cost and thus resist fatigue. The smaller diameter of slow muscle fibers, and the higher capillary density in slow muscle, also helps slow muscle resist fatigue.

Fast muscle, in contrast, is recruited for activities that require faster movements, more force, or both (see Fig. 12.13). Weightlifting, for example, can require a lot of power for short duration. To meet the demands for more force, additional motor units are recruited. In comparison with slow motor units, the fast motor units typically contain more muscle fibers (see Table 12.2). Fast muscle fibers also have a larger diameter than do slow muscle fibers. Thus recruitment of fast motor units can help meet the increased demands of burst activities such as weightlifting. The high myosin ATPase activity in fast muscle fibers and the increase in diffusion distance (resulting from the large diameter of the fast muscle fibers), however, increase the susceptibility of fast muscle fibers to fatigue.

Additional differences between fast and slow muscles include the following:

1. The neuromuscular junction of fast muscle differs from that in slow muscle in terms of acetylcholine vesicle content, the amount of acetylcholine released, the density of nicotinic acetylcholine receptors, the acetylcholine esterase activity, and Na^+ channel density, all of which endow the fast muscle with a higher safety factor for initiation of an action potential. During repetitive stimulation, however, the safety factor in fast muscle drops quickly (faster than that seen in slow muscle).
2. The SR is more highly developed in fast muscle than in slow muscle, with higher levels of RYR1, SERCA, luminal Ca^{++}, and a higher $Ca_V1.1$/RYR1 ratio, all of which promote the development of a larger, faster intracellular Ca^{++} transient in fast muscle, which is important for quick, forceful contraction.

In addition to the differences between fast and slow fibers just noted, other muscle proteins are also expressed in a fiber type–specific manner. Such proteins include the three troponin subunits, tropomyosin, and C protein. The differential expression of troponin and tropomyosin isoforms influences the dependency of contraction on Ca^{++}. Slow fibers begin to develop tension at lower [Ca^{++}] than fast fibers do. This difference in sensitivity to Ca^{++} is related in part to the fact that the troponin C isoform in slow fibers has only a single low-affinity Ca^{++}-binding site, whereas the troponin C of fast fibers has two low-affinity binding sites. Changes in the dependence of contraction on Ca^{++}, however, are not restricted to differences in the troponin C isoforms. Differences in troponin T and tropomyosin isoforms are also found. Thus regulation of the dependence of contraction on Ca^{++} is complex and involves contributions from multiple proteins on the thin filament. Phosphorylation of the regulatory light chain of myosin by Ca^{++}/calmodulin-dependent myosin light chain kinase, however, can increase Ca^{++} sensitivity of contraction, particularly in fast muscle fibers (partly because of the reported higher activity of myosin light chain kinase in fast muscle fibers).

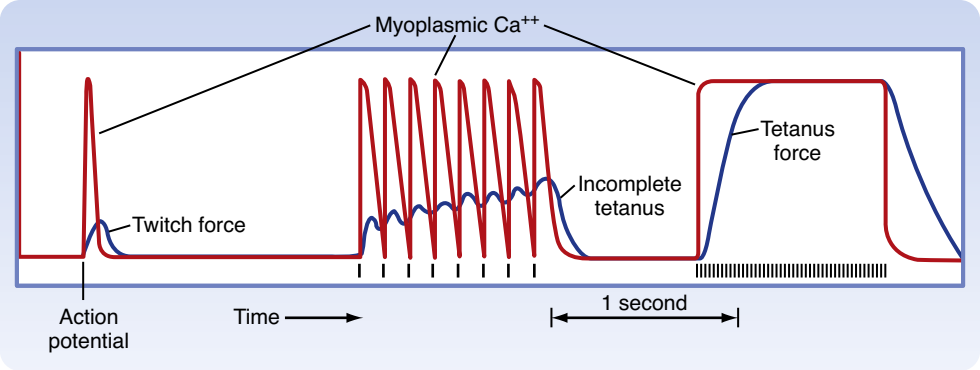
Modulation of the Force of Contraction

Recruitment

A simple means of increasing the force of contraction of a muscle is to recruit more muscle fibers. Because all the muscle fibers within a motor unit are activated simultaneously, a muscle recruits more muscle fibers by recruiting more motor units. As already noted, muscle fibers can be classified as fast-twitch or slow-twitch. The type of fiber is determined by its innervation. Because all fibers in a motor unit are innervated by a single α motor neuron, all fibers within a motor unit are of the same type. Slow-twitch motor units tend to be small (100–500 muscle fibers) and are innervated by an α motor neuron that is easily excited (see Table 12.2). Fast-twitch motor units, in contrast, tend to be large (containing 1000–2000 muscle fibers) and are innervated by α motor neurons that are more difficult to excite. Thus slow-twitch motor units tend to be recruited first. As more and more force is needed, fast-twitch motor units are recruited. The advantage of such a recruitment strategy is that the first muscle fibers recruited are those that have high resistance to fatigue. Moreover, the small size of slow-twitch motor units allows fine motor control at low levels of force. The process of increasing the force of contraction by recruiting additional motor units is termed **spatial summation** because forces from muscle fibers are being "summed" within a larger area of the muscle. This contrasts with **temporal summation (tetany),** discussed below.

Tetany

Action potentials in skeletal muscles are quite uniform and lead to the release of a reproducible pulse of Ca^{++} from the SR (Fig. 12.15). A single action potential releases sufficient

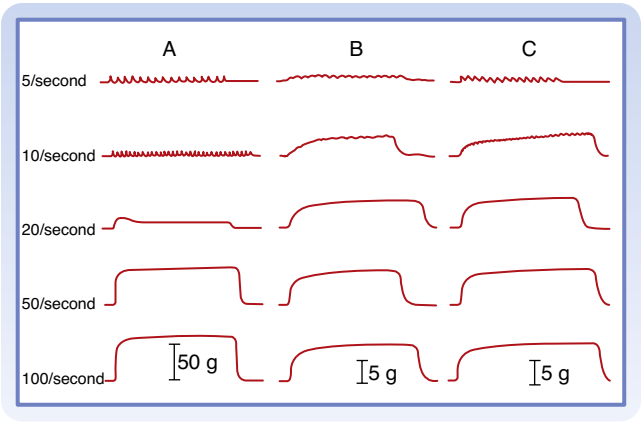


• **Fig. 12.15** Increasing the frequency of electrical stimulation of skeletal muscle results in an increase in the force of contraction. This is attributable to prolongation of the intracellular Ca^{++} transient and is termed *tetany.* Incomplete tetany results from initiation of another intracellular Ca^{++} transient before the muscle has completely relaxed. Thus there is a summation of twitch forces. See text for details.

Ca^{++} to cause a twitch contraction. However, the duration of this contraction is very short because Ca^{++} is very rapidly pumped back into the SR. If the muscle is stimulated a second time before it is fully relaxed, the force of contraction increases (see Fig. 12.15, *middle*). Thus twitch forces are amplified as stimulus frequency increases. At a high level of stimulation, intracellular [Ca^{++}] increases and is maintained throughout the period of stimulation (see Fig. 12.15, *right*), and the amount of force developed greatly exceeds that observed during a twitch. The response is termed *tetany.* At intermediate stimulus frequency, intracellular [Ca^{++}] returns to baseline just before the next stimulus. However, there is a gradual rise in force (see Fig. 12.15, *middle*). This phenomenon is termed *incomplete tetany.* In both cases, the increased frequency of stimulation is said to produce a fusion of twitches.

The low force generation during a twitch, in comparison with that during tetany, may be due to the presence of a series elastic component in the muscle. Specifically, when the muscle is stretched a small amount shortly after initiation of the action potential, the muscle generates a twitch force that approximates the maximal tetanic force. This result, coupled with the observation that the size of the intracellular Ca^{++} transient during a twitch contraction is comparable with that during tetany, suggests that enough Ca^{++} is released into the cytoplasm during a twitch to allow the actin-myosin interactions to produce maximal tension. However, the duration of the intracellular Ca^{++} transient during a twitch is sufficiently short that the contractile elements may not have enough time to fully stretch the series elastic components in the fiber and muscle. As a result, the measured tension is submaximal.

An increase in the duration of the intracellular Ca^{++} transient, as occurs with tetany, provides the muscle with sufficient time to completely stretch the series elastic component and thereby results in expression of the full contractile force of the actin-myosin interactions (i.e., maximal tension). Partial stretching of the series elastic component (as might be expected during a single twitch), followed by restimulation of the muscle before complete relaxation, however,



• **Fig. 12.16** Slow-twitch muscles exhibit tetany at a lower stimulation frequency than do fast-twitch muscles. **A,** Fast-twitch motor unit in the gastrocnemius muscle. **B,** Slow-twitch motor unit in the gastrocnemius muscle. **C,** Slow-twitch muscle unit in the soleus muscle. The motor units were stimulated at the frequencies indicated on the *left*. The calibration bar for tension (in grams) generated during concentration is indicated by the *vertical brackets* under the curves. Note the large force generated by the fast-twitch motor unit **(A)**. (From Montcastle V. *Medical Physiology.* 12th ed. St. Louis: Mosby: 1974.)

would be expected to yield an intermediate level of tension, similar to that seen with incomplete tetany. The location of the series elastic component in skeletal muscle is not known. One potential source is the myosin molecule itself. In addition, it is likely that there are other sources of the series elastic component, such as the connective tissue and titin.

The stimulus frequency needed to produce tetany depends on whether the motor unit consists of slow or fast fibers (Fig. 12.16). Slow fibers can be tetanized at lower frequencies than can fast fibers. The ability of slow-twitch muscle to tetanize at lower stimulation frequencies reflects, at least in part, the longer duration of contraction seen in slow fibers. As also illustrated in Fig. 12.16, fast fibers develop a larger maximal force than slow fibers do because fast fibers are larger in diameter than slow fibers and there are more fibers in fast motor units than in slow motor units. Even when normalized for the cross-sectional area of the

Type I muscle fibers, however, the maximal isometric tension of the human Type I muscle fibers was less than that for the human Type II muscle fibers. Specifically, the maximal isometric tension of the human Type I muscle fiber, normalized for cross-sectional area (determined from the X-intercept of Fig. 12.14*C*), was ~20% lower than that seen in the Type II muscle fibers. Thus, Type II motor units are well suited for bursts of high levels of force.

Modulation of Force by Reflex Arcs

Stretch Reflex

Skeletal muscles contain sensory fibers (**muscle spindles;** also called **intrafusal fibers**) that run parallel to the skeletal muscle fibers. The muscle spindles assess the degree of stretch of the muscle, as well as the speed of contraction. In the stretch reflex, rapid stretching of the muscle (e.g., tapping the tendon) lengthens the spindles in the muscle and results in an increased frequency of action potentials in the afferent sensory neurons of the spindle. These afferent fibers in turn excite the α motor neurons in the spinal cord that innervate the stretched muscle. The result is that the reflex arc is a stretch-induced contraction of the muscle that does not require input from high centers in the brain. As the muscle shortens, efferent output is also sent to the spindle, which thereby takes the slack out of the spindle and ensures its ability to respond to stretch at all muscle lengths. By their action, muscle spindles provide feedback to the muscle in terms of its length and thus help maintain a joint at a given angle.

Golgi Tendon Organ

Golgi tendon organs are in the tendons of muscles and provide feedback regarding contraction of the muscle. The main component of the tendon organ is an elongated fascicle of collagen bundles that is in series with the muscle fibers and can respond to contractions of individual muscle fibers. A given tendon organ may attach to several fast-twitch or slow-twitch muscle fibers (or both) and sends impulses through Type Ib afferent nerve fibers in response to muscle contraction. The Type Ib afferent impulses enter the spinal cord, which can promote inhibition of α motor neurons to the contracting (and synergistic) muscles while promoting excitation of α motor neurons to antagonistic muscles. The inhibitory actions are mediated through interneurons in the cord that release an inhibitory transmitter to the α motor neuron and create an inhibitory postsynaptic potential. The Type Ib afferent impulses are also sent to higher centers of the brain (including the motor cortex and cerebellum). It is hypothesized that feedback from the tendon organs in response to muscle contraction may smooth the progression of muscle contraction by limiting the recruitment of additional motor units. Of interest is that the response of the tendon organ is not linearly related to force; rather, it drops off at higher levels of force, which may facilitate the recruitment of motor units at higher levels of effort.

Skeletal Muscle Tone

The skeletal system supports the body in an erect posture with the expenditure of relatively little energy. Nonetheless, even at rest, muscles normally exhibit some level of contractile activity. Isolated (i.e., denervated) unstimulated muscles are in a relaxed state and are said to be *flaccid.* However, relaxed muscles in the body are comparatively firm. This firmness, or tone, is caused by low levels of contractile activity in some of the motor units and is driven by reflex arcs from the muscle spindles. Interruption of the reflex arc by sectioning of the sensory afferent fibers abolishes this resting muscle tone. The tone in skeletal muscle is distinct from the "tone" in smooth muscle (see Chapter 14).

Energy Sources During Contraction

Adenosine Triphosphate

Muscle cells convert chemical energy to mechanical energy. ATP is the energy source used for this conversion. The ATP pool in skeletal muscle is small and capable of supporting only a few contractions if not replenished. This pool, however, is continually replenished during contraction, as described later, so that even when the muscle fatigues, ATP stores are only modestly decreased.

Creatine Phosphate

Muscle cells contain creatine phosphate, which is used to convert ADP to ATP and thus replenish the ATP store during muscle contraction. The creatine phosphate store represents the immediate high-energy source for replenishing the ATP supply in skeletal muscle, especially during intense exercise. The enzyme **creatine phosphokinase** catalyzes the reaction:

$$\text{ADP} + \text{creatinephosphate} \rightarrow \text{ATP} + \text{creatine}$$

Although much of the creatine phosphokinase is present in the myoplasm, a small amount is in the thick filament (near the M line). The creatine phosphokinase in the thick filament may participate in the rapid resynthesis of ATP near the myosin heads during muscle contraction. The creatine phosphate store, however, is only about five times the size of the ATP store and thus cannot support prolonged periods of contraction (less than a minute of maximal muscle activity). Skeletal muscle fatigue during intense exercise is associated with depletion of the creatine phosphate store, although as described subsequently, this does not necessarily imply that the fatigue is caused by depletion of the creatine phosphate store. Because the creatine phosphokinase–catalyzed reaction is reversible, the muscle cell replenishes the creatine phosphate pool during recovery from fatigue by using ATP synthesized through oxidative phosphorylation.

Carbohydrates

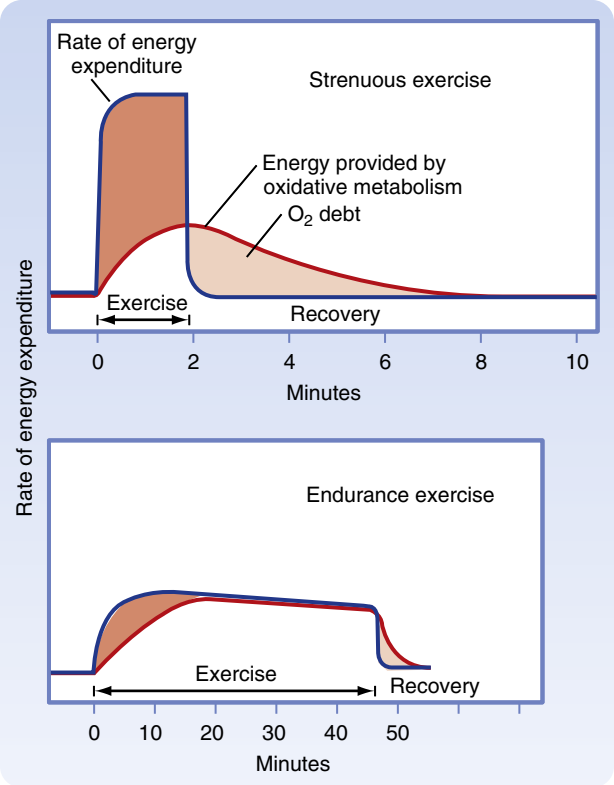
Muscle cells contain glycogen, which can be metabolized during muscle contraction to provide glucose for oxidative phosphorylation and glycolysis, both of which generate ATP to replenish the ATP store. Muscle cells can also take up glucose from blood, a process that is stimulated by insulin (see Chapter 39). The cytosolic enzyme phosphorylase releases glucose 1-phosphate residues from glycogen, which are then metabolized by a combination of glycolysis (in the cytosol) and oxidative phosphorylation (in the mitochondria) to yield the equivalent of 37 mol of ATP per mole of glucose 1-phosphate. Blood glucose yields 36 mol of ATP per mole of glucose because 1 ATP is used to phosphorylate glucose at the start of glycolysis. These ATP yields, however, are dependent on an adequate oxygen supply. Under anaerobic conditions, in contrast, metabolism of glycogen and glucose yields only 3 and 2 mol of ATP per mole of glucose 1-phosphate and glucose, respectively (along with 2 mol of lactate). As discussed later, muscle fatigue during prolonged exercise is associated with depletion of glycogen stores in the muscle.

Fatty Acids and Triglycerides

Fatty acids represent an important source of energy for muscle cells during prolonged exercise. Muscle cells contain fatty acids but can also take up fatty acids from blood. In addition, muscle cells can store triglycerides, which can be hydrolyzed when needed to produce fatty acids. The fatty acids are subjected to β oxidation within the mitochondria. For fatty acids to enter the mitochondria, however, they are converted to acylcarnitine in the cytosol and then transported into the mitochondria, where they are converted to acyl coenzyme A (CoA). Within the mitochondria, the acyl CoA is subjected to β oxidation and yields acetyl CoA, which then enters the citric acid cycle and ultimately produces ATP.

Oxygen Debt

If the energy demands of exercise cannot be met by oxidative phosphorylation, an **oxygen debt** is incurred. After completion of exercise, respiration remains above the resting level to "repay" this oxygen debt. The extra oxygen consumption during this recovery phase is used to restore metabolite levels (such as creatine phosphate and ATP) and to metabolize the lactate generated by glycolysis. The increased cardiac and respiratory work during recovery also contributes to the increased oxygen consumption seen at this time and explains why more oxygen has to be "repaid" than was "borrowed." Some oxygen debt occurs even with low levels of exercise because slow oxidative motor units consume considerable ATP, derived from creatine phosphate or glycolysis, before oxidative metabolism can increase ATP production to meet steady-state requirements. The oxygen debt is much greater with strenuous exercise, when fast glycolytic motor units are used (Fig. 12.17). The oxygen debt is approximately equal to the energy consumed during exercise minus that supplied by oxidative metabolism (i.e., the dark- and light-colored areas in Fig. 12.17 are approximately equal). As indicated earlier, the additional oxygen used during recovery from exercise represents the energy requirements for restoring normal cellular metabolite levels.

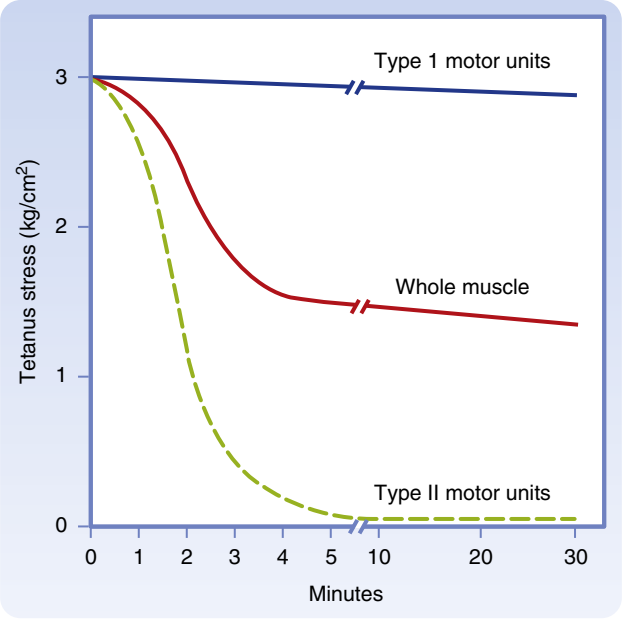


• **Fig. 12.17** An oxygen debt is incurred by the exercising of muscle when the rate of energy expenditure exceeds the rate of energy production by oxidative metabolism. *Upper panel*, Energy expenditure during strenuous exercise. *Lower panel*, Energy expenditure during endurance exercise. See text for details.

Fatigue

The ability of muscle to meet energy needs is a major determinant of the duration of the exercise. However, fatigue is not the result of depletion of energy stores. Instead, metabolic by-products seem to be important factors in the onset of fatigue. Fatigue may potentially occur at any of the points involved in muscle contraction, from the brain to the muscle cells, as well as in the cardiovascular and respiratory systems that maintain energy supplies (i.e., fatty acids and glucose) and oxygen delivery to the exercising muscle.

Several factors have been implicated in **muscle fatigue.** During brief periods of tetany, the oxygen supply to the muscle is adequate as long as the circulation is intact. However, the force/stress generated during these brief tetanic periods decays rapidly to a level that can be maintained for long periods (Fig. 12.18). This decay represents the rapid and almost total failure of the fast motor units. The decline



• **Fig. 12.18** A series of brief tetanic stimulations of skeletal muscle result in a rapid decrease in force (tetanic stress, exemplified by the "Whole muscle" line in plot) that is attributable to fatigue of fast-twitch (Type II) motor units in the muscle. Under these conditions, however, slow-twitch (Type I) motor units are resistant to fatigue.

in force/stress is paralleled by depletion of glycogen and creatine phosphate stores and the accumulation of lactic acid. Of importance is that the decline in force/stress occurs when the ATP pool is not greatly reduced, so that the muscle fibers do not go into rigor. In contrast, the slow motor units can meet the energy demands of fibers under this condition, and they do not exhibit significant fatigue, even after many hours. Evidently, some factor associated with energy metabolism can inhibit contraction (e.g., in the fast fibers), but this factor has not been clearly identified.

During intense exercise, accumulation of inorganic phosphate (P_i) and lactic acid in the myoplasm contributes to muscle fatigue. The accumulation of lactic acid, to levels as high as 15 to 26 mmol/L, decreases myoplasmic pH (from ≈7 to ≈6.2) and inhibits actin-myosin interactions. This decrease in pH reduces the sensitivity of the actin-myosin interaction to Ca^{++} by altering Ca^{++} binding to troponin C and by decreasing the maximum number of actin-myosin interactions. P_i has also been implicated as an important factor in the development of fatigue during intense exercise. Phosphate concentrations can increase from approximately 2 mmol/L at rest to nearly 40 mmol/L in working muscle. Such an elevation in [P_i] can reduce tension by at least the following three different mechanisms: (1) inhibition of Ca^{++} release from the SR, (2) decrease in the sensitivity of contraction to Ca^{++}, and (3) alteration in actin-myosin binding. Several other factors, including glycogen depletion from a specialized compartment, a localized increase in [ADP], extracellular elevation of [K^+], and generation of oxygen free radicals, have also been implicated in various forms of exercise-induced muscle fatigue. Finally, the central nervous system contributes to fatigue, especially in how fatigue is perceived by the individual.

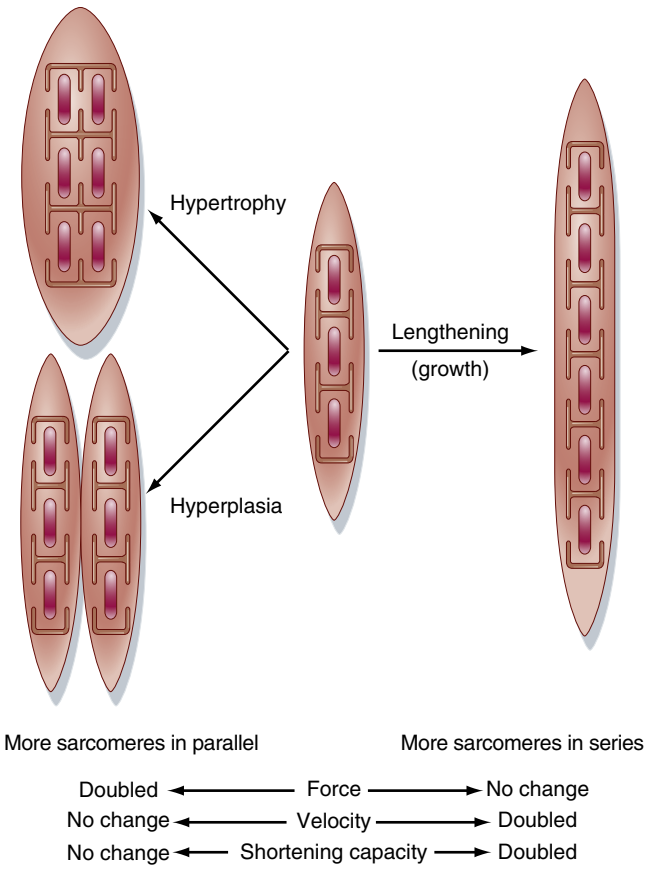
Regardless of whether the muscle is fatigued during high-intensity exercise or prolonged exercise, the myoplasmic ATP level does not decrease substantially. In view of the reliance of all cells on the availability of ATP to maintain viability, fatigue has been described as a protective mechanism to minimize the risk of muscle cell injury or death. Consequently, it is likely that skeletal muscle cells have developed redundant systems to ensure that ATP levels do not drop to dangerously low levels and hence risk the viability of the cell.

Most persons tire and cease exercise long before the motor unit fatigues. General physical fatigue may be defined as a homeostatic disturbance produced by work. The basis for the perceived discomfort (or even pain) probably involves many factors. These factors may include a decrease in plasma glucose levels and accumulation of metabolites. Motor system function in the central nervous system is not impaired. Highly motivated and trained athletes can withstand the discomfort of fatigue and may exercise to the point at which some motor unit fatigue occurs. Part of the enhanced performance observed after training involves motivational factors.

Growth and Development

Skeletal muscle fibers differentiate before they are innervated, and some neuromuscular junctions are formed well after birth. Before innervation, the muscle fibers physiologically resemble slow (Type I) cells. **Acetylcholine receptors** are distributed throughout the sarcolemma of these noninnervated cells and are supersensitive to that neurotransmitter. An end plate is formed when the first growing nerve terminal establishes contact with a muscle cell. The cell forms no further association with nerves, and receptors to acetylcholine become concentrated in the end plate membranes. Cells innervated by a small motor neuron form slow (Type I) oxidative motor units. Fibers innervated by large motor nerves develop all the characteristics of fast (Type II) motor units. Innervation produces major cellular changes, including synthesis of the fast and slow myosin isoforms, which replace embryonic or neonatal variants. Thus muscle fiber type is determined by the nerves that innervate the fiber.

An increase in muscle strength and size occurs during maturation. As the skeleton grows, the muscle cells lengthen. Lengthening is accomplished by the formation of additional sarcomeres at the ends of the muscle cells (Fig. 12.19), a process that is reversible. For example, the length of a cell decreases when terminal sarcomeres are eliminated, which can occur when a limb is immobilized with the muscle in a shortened position or when improper setting of a fracture causes shortening of the limb segment. Changes in muscle length affect the velocity and extent of shortening but do not influence the amount of force that can be generated by the muscle. The gradual increase in strength and diameter of a muscle during growth is achieved mainly by hypertrophy.



• **Fig. 12.19** Effects of growth on the mechanical output of a muscle cell. Typically, skeletal muscle cell growth involves either lengthening (adding more sarcomeres to the ends of the muscle fibers) or increasing muscle fiber diameter (hypertrophy as a result of the addition of more myofilaments/myofibrils in parallel within the muscle fiber). The formation of new muscle fibers is called *muscle hyperplasia,* and it is infrequent in skeletal muscle.

Doubling the myofibrillar diameter by adding more sarcomeres in parallel (**hypertrophy,** for example) may double the amount of force generated but has no effect on the maximal velocity of shortening. Resistance exercise can promote hypertrophy by activation of the Akt-mTOR signaling pathway (Fig. 12.20*A*) and simultaneous inhibition of the forkhead box O protein (FoxO)–atrogene pathway, which results in an increase in net protein synthesis.

The signaling pathways contributing to skeletal muscle hypertrophy and atrophy are complex, with hypertrophy resulting when the rate of contractile protein synthesis exceeds the rate of contractile protein degradation. During development, and in response to resistance exercise, elevated levels of insulin-like growth factor 1 (IGF-1) typically promote the development of skeletal muscle hypertrophy through the Akt-mTOR pathway (see Fig. 12.20*A*). Many other stimuli, however, have also been identified or proposed, including the transduction of the mechanical force of contraction (particularly in resistance exercise training) through adhesion complexes between the skeletal muscle cytoskeleton and the extracellular matrix/tendon (e.g., via the dystrophin-associated glycoprotein complex [DGC)]). Signaling pathways that have been identified and/or proposed for the response of skeletal muscle to endurance exercise are shown in Fig. 12.20*B*, with muscular contraction resulting in an elevated intracellular [Ca^{++}] that stimulates the expression of contractile protein genes through calcineurin and Ca-calmodulin–dependent protein kinase pathways. Endurance exercise also promotes increases in oxidative capacity (including mitochondrial biogenesis) and perfusion (via angiogenesis) in the exercising muscles through signaling pathways that appear to involve stimulation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α; see Fig. 12.20*B*)

Atrophy of skeletal muscles occurs when the rate of contractile protein degradation exceeds the rate of contractile protein synthesis. This occurs in many situations, such as (1) immobilization of an extremity in a cast, (2) prolonged bedrest, (3) spinal cord injury, and (4) spaceflight (microgravity). Aging and serious illnesses (such as late stages of cancer) can also promote atrophy of skeletal muscles (termed sarcopenia and cachexia, respectively). In addition, space flight exposes astronauts to a microgravity environment that mechanically unloads their muscles. Such unloading leads to rapid loss of muscle mass (i.e., **atrophy**) and weakness. Disuse atrophy appears to involve both inhibition of protein synthesis and stimulation of protein degradation (with net activation of the FoxO-atrogene pathway).

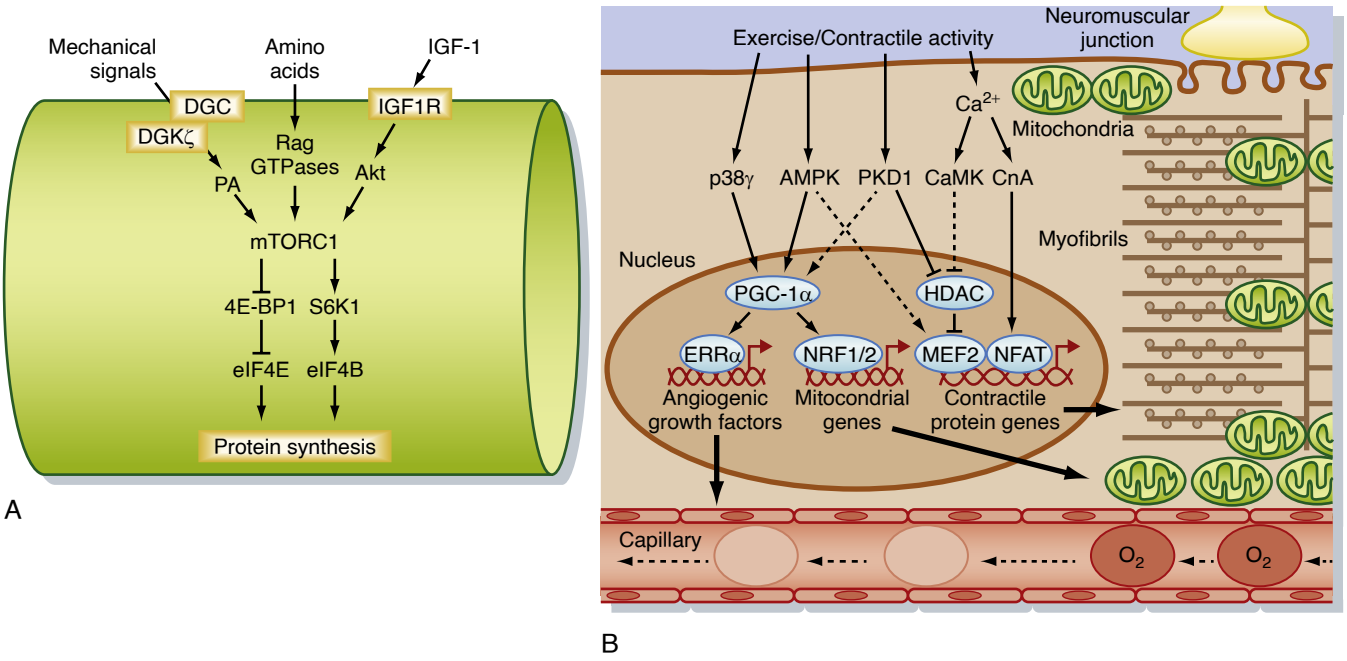
Muscles that frequently contract to support the body typically have a high number of slow (Type I) oxidative motor units. These slow motor units atrophy more rapidly than the fast (Type II) motor units during prolonged periods of unloading. This atrophy of slow motor units is associated with a decrease in maximal tetanic force but also an increase in maximal shortening velocity. The increase in velocity is correlated with expression of the fast myosin isoform in these fibers. An important aspect of space medicine is the design of exercise programs that minimize such phenotypic changes during prolonged space flight.

Testosterone is a major factor responsible for the greater muscle mass in men because it has both myotrophic action and androgenic (masculinization) effects (see Chapter 44).

Skeletal muscles have a limited ability to form new fibers **(hyperplasia).** These new fibers result from activation/differentiation of satellite cells that are present under the basal lamina of the muscle fibers (discussed later).

Denervation, Reinnervation, and Cross-Innervation

As already noted, innervation is crucial for the skeletal muscle phenotype. If the motor nerve is cut, muscle fasciculation occurs. **Fasciculation** is characterized by small, irregular contractions caused by release of acetylcholine from the terminals of the degenerating distal portion of the axon. Several days after denervation, muscle fibrillation begins. **Fibrillation** is characterized by spontaneous, repetitive contractions. At this time, the cholinergic receptors have



• **Fig. 12.20** Basic molecular signaling pathways involved in contributing to net protein synthesis **(A)**, mitochondrial biogenesis and angiogenesis **(B)** in skeletal muscle in response to exercise. See text for details. *AKT*, A serine/threonine-specific protein kinase (protein kinase B); *AMPK*, 5′ adenosine monophosphate–activated protein kinase; *CaMK*, Ca^{++}/calmodulin-dependent protein kinase; *CnA*, calcineurin A; *DGC*, dystrophin-glycoprotein complex; *DGKζ*, diacylglycerol kinase-zeta; *ERRα*, estrogen-related receptor α; *HDAC*, histone deacetylase; *IGF-1*, insulin-like growth factor; *IGF1R*, IGF-1 receptor; *MEF2*, myocyte enhancer factor 2; *mTORC1*, mammalian target of rapamycin complex 1; *NFAT*, nuclear factor of activated T cells; *NRF1/2*, nuclear respiratory factor 1/2; *PA*, phosphatidic acid; *PGC-1α*, peroxisome proliferator-activated receptor γ coactivator 1α; *PKD1*, protein kinase D1. (**A,** Reprinted from Schiaffino S, Reggiani C, Akimotod T, Blaauw B. Molecular mechanisms of skeletal muscle hypertrophy. *J Neuromuscul Dis* 2021;8(2):169, with permission from IOS Press. The publication is available at IOS Press through http://dx.doi.org/10.3233/JND-200568. **B,** From Yan Z, Okutsu M, Akhtar Y, Lira V. Regulation of exercise-induced fiber type transformation, mitochondrial biogenesis, and angiogenesis in skeletal muscle. *J Appl Physiol* 2011;110(1):264.)

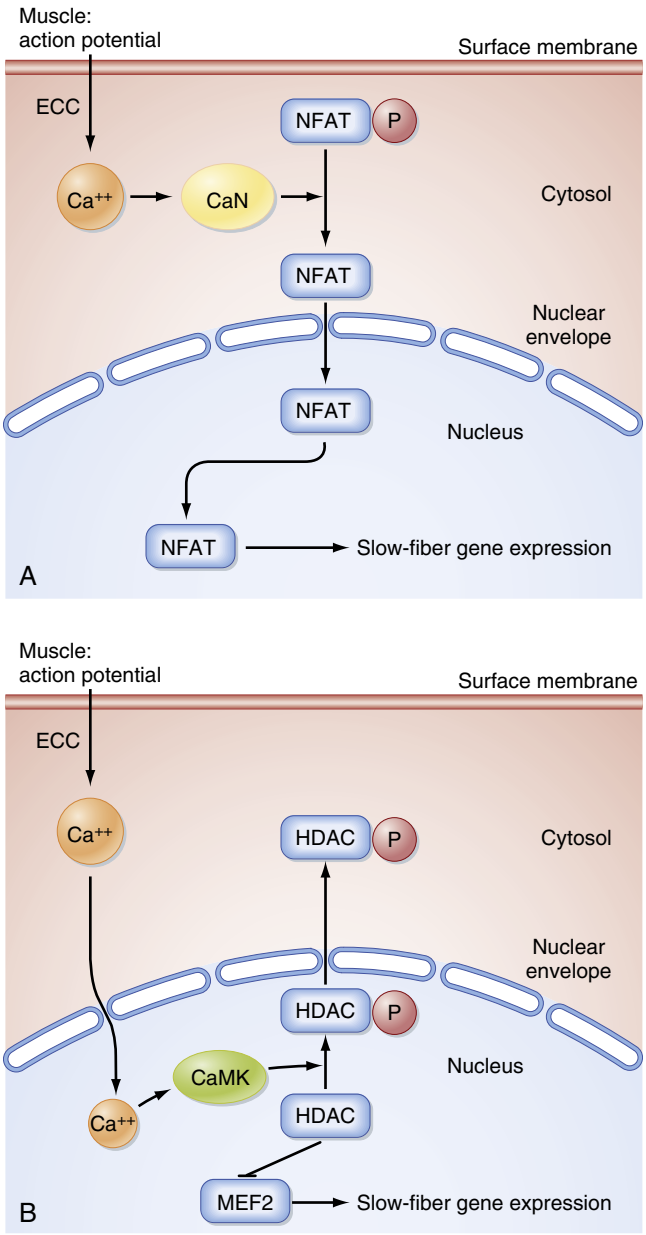
spread out over the entire cell membrane, in effect reverting to their preinnervation embryonic arrangement. The muscle fibrillations reflect supersensitivity to acetylcholine. Affected muscles also atrophy, with a decrease in the size of the muscle and its cells. Atrophy is progressive in humans, with degeneration of some cells 3 or 4 months after denervation. Most of the muscle fibers are replaced by fat and connective tissue after 1 to 2 years. These changes can be reversed if reinnervation occurs within a few months. Reinnervation is normally achieved by growth of the peripheral stump of motor nerve axons along the old nerve sheath.

Reinnervation of formerly fast (Type II) fibers by a small motor axon causes that cell to redifferentiate into a slow (Type I) fiber, and vice versa. This suggests that large and small motor nerves differ qualitatively and that the nerves have a specific "trophic" effect on the muscle fibers. This "trophic" effect reflects the rate of fiber stimulation. For example, stimulation via electrodes implanted in the muscle can lessen denervation atrophy. More strikingly, chronic low-frequency stimulation of fast motor units causes these units to be converted to slow motor units.

The frequency of contraction determines fiber development and phenotype through changes in gene expression and protein synthesis. Fibers that undergo frequent contractile activity form many mitochondria and synthesize the slow myosin isoform. Fibers innervated by large, less excitable axons contract infrequently. Such relatively inactive fibers typically form few mitochondria, have large concentrations of glycolytic enzymes, and synthesize the fast myosin isoform.

AT THE CELLULAR LEVEL

The transcription factor **nuclear factor of activated T cells (NFAT)** has been implicated in this transition from fast-twitch to slow-twitch muscle (Fig. 12.21*A*). Specifically, it appears that stimulation of adult fast-twitch muscle cells at a frequency consistent with slow-twitch muscle cells can activate the Ca^{++}-dependent phosphatase calcineurin, which in turn can dephosphorylate NFAT and result in translocation of NFAT from the myoplasm to the nucleus, followed by the transcription of slow-twitch muscle genes (and inhibition of fast-twitch muscle genes). In accordance with this mechanism, expression of constitutively active NFAT in fast-twitch muscle promotes the expression of slow-twitch myosin while inhibiting the expression of fast-twitch myosin. The transcription factor **myocyte enhancing factor 2 (MEF2)** has also been implicated in this transition from fast-twitch to slow-twitch muscle (see Fig. 12.21*B*). Activation of MEF2 is thought to result from Ca^{++}/calmodulin-dependent phosphorylation of an inhibitor of MEF2: namely, histone deacetylase (HDAC).



• **Fig. 12.21** Molecular signaling pathways contributing to the transition from fast-twitch muscle to slow-twitch muscle. Chronic electrical stimulation of a fast-twitch muscle in a pattern consistent with a slow-twitch muscle results in development of the slow-twitch muscle phenotype because of dephosphorylation of the transcription factor nuclear factor of activated T cells *(NFAT)* by the Ca^{++}/calmodulin-dependent protein phosphatase calcineurin *(CaN)*; this in turn results in nuclear translocation of NFAT and expression of slow-twitch muscle fiber genes **(A).** Activation of the transcription factor myocyte enhancer factor 2 *(MEF2)* also appears to contribute to this fiber type transition **(B),** in which activation of MEF2 involves Ca^{++}/calmodulin-dependent phosphorylation of an inhibitor, histone deacetylase *(HDAC)*. *CaMK*, Ca^{++}/calmodulin-dependent protein kinase; *ECC*, excitation-contraction coupling; *P*, phosphorylation of HDAC. (From Liu Y, Shen T, Randall W, Schneider M. Signaling pathways in activity-dependent fiber type plasticity in adult skeletal muscle. *J Muscle Res Cell Motil* 2005;26:13-21.)

Intracellular $[Ca^{++}]$ appears to play an important role in expression of the slow myosin isoform. Slow-twitch muscle fibers have a higher resting level of intracellular Ca^{++} than do fast-twitch muscle fibers. In addition, chronic electrical stimulation of fast-twitch muscle is accompanied by a 2.5-fold increase in resting myoplasmic $[Ca^{++}]$ that precedes the increased expression of slow-twitch myosin and decreased expression of fast-twitch myosin. Similarly, chronic elevation of intracellular Ca^{++} (approximately fivefold) in muscle cells expressing fast-twitch myosin induces a change in gene expression from the fast muscle myosin isoform to the slow myosin isoform within 8 days. An increase in citrate synthetase activity (an indicator of oxidative capacity) and a decrease in lactate dehydrogenase activity (an indicator of glycolytic capacity) accompany this Ca^{++}-dependent transition from fast-twitch to slow-twitch myosin. These Ca^{++}-dependent changes are reversible by a reduction of intracellular $[Ca^{++}]$.

Response to Exercise

Exercise physiologists identify three categories of training regimens and responses: **learning, endurance,** and **strength training** (Table 12.3). Typically, most athletic endeavors involve elements of all three. The learning aspect of training involves motivational factors, as well as neuromuscular coordination. This aspect of training does not involve adaptive changes in the muscle fibers per se. However, motor skills can persist for years without regular training, unlike the responses of muscle cells to exercise.

Muscle strength can be increased by regular massive efforts that involve most motor units. Such efforts recruit fast glycolytic motor units, as well as slow oxidative motor units. During these efforts, blood supply to the working muscles may be interrupted as tissue pressures rise above intravascular pressure. The reduced blood flow limits the duration of the contraction. Regular maximal-strength exercise, such as weightlifting, induces the synthesis of more myofibrils and hence hypertrophy of the active muscle cells. The increased stress also induces the growth of tendons and bones.

Mechanisms by which resistance exercise stimulates hypertrophy are complex, but typically involve a stimulation of the Akt-mTOR signaling pathway (see Fig. 12.20*A*). Exercise-induced production of IGF-1 can originate from multiple sources including the skeletal muscle, thereby constituting an autocrine or paracrine effect. The force of contraction transduced through adhesion complexes between the muscle cytoskeleton and the extracellular matrix or tendon has also been reported to contribute to hypertrophy of the exercising muscle through stimulation of the Akt-mTOR pathway (see Fig. 12.20*A*). Micro-RNA and long-noncoding RNA produced during exercise may also influence the signaling pathways contributing to the exercise-induced hypertrophy of skeletal muscle.

Endurance exercise has been shown to promote increased oxidative capacity and increased perfusion (angiogenesis) of the exercising muscle fibers. The increased oxidative capacity was associated with increases in the levels of oxidative enzymes as well as mitochondrial biogenesis in both Type I and Type II muscle fibers. The endurance

TABLE 12.3 Effects of Exercise

Type of Training	Example	Major Adaptive Response
Learning/coordination skills	Typing	Increased rate and accuracy of motor units (central nervous system)
Endurance (submaximal, sustained efforts)	Marathon running	Increased oxidative capacity in all involved motor units, with limited cellular hypertrophy
Strength (brief, maximal efforts)	Weightlifting	Hypertrophy and enhanced glycolytic capacity of the motor units used

exercise-induced increase in capillary density can occur in both Type I and Type II muscle fibers. The signaling mechanism(s) underlying these changes in oxidative capacity and angiogenesis are complex, but seem to involve activation of the transcription coactivator PGC-1a (see Fig. 12.20*B*). The angiogenesis appears to involve VEGF, downstream from the stimulation of PGC-1a. Micro-RNA and/or long noncoding RNA produced during endurance exercise may also influence signaling pathways leading to the above changes in oxidative capacity, mitochondrial biogenesis, and angiogenesis.

Hypertrophy could accompany these endurance exercise-induced changes in oxidative capacity and perfusion through the calcineurin and calcium-calmodulin–dependent protein kinase signaling pathways (see Fig. 12.20*B*). An important point to appreciate is that exercise routines are unlikely to convert a Type II muscle fiber to a Type I muscle fiber. There may be changes in the expression of Type IIx vs Type IIa in a muscle fiber (as evidenced by an occasional hybrid fiber), but exercise routines have not shown conversion of a muscle fiber from Type II to Type I. Instead, dramatic changes in the stimulation frequency were required to make a change in the expression pattern from Type II myosin to Type I myosin (as evidenced by chronic electrical stimulation studies and cross-innervation studies).

Muscle fibers can be injured during exercise. This is particularly true for eccentric exercises at high load. If the injury to the sarcolemma of the muscle is small (resulting in a small influx of Ca^{++}), dysferlin in combination with annexin II may be able to repair the sarcolemma. The injury may also initiate a cascade of repair mechanisms that involves activation of satellite cells on the outside of the sarcolemma (but beneath the basal lamina). Activated satellite cells undergo (1) proliferation, (2) maturation/differentiation phase, and (3) fusion to form myotube (with centrally located nuclei), which can then mature to become functional skeletal muscle fibers (with subsarcolemma nuclei) (Fig. 12.22*A*). The regenerating fibers can be identified by the expression of an embryonic myosin isoform, and the location of central nuclei. The presence of regenerating skeletal muscle fibers can also be observed in pathologies such as muscular dystrophy and polymyositis (see Fig. 12.22*B*). An important point to appreciate is that this regeneration also involves the participation of the immune system, with (1) M1 macrophages participating in the removal of debris and promoting proliferation of the satellite cells, and (2) M2 macrophages promoting the maturation of the satellite cells and the fusion.

Delayed-Onset Muscle Soreness

Activities such as hiking or, in particular, downhill running, in which contracting muscles are stretched and lengthened too vigorously, are followed by more pain and stiffness than after comparable exercise that does not involve vigorous muscle stretching and lengthening (e.g., cycling). The resultant dull, aching pain develops slowly and reaches its peak within 24 to 48 hours. The pain is associated with reduced range of motion, stiffness, and weakness of the affected muscles. The prime factors that cause the pain are swelling and inflammation from injury to muscle cells, most commonly near the myotendinous junction. Type II motor units are affected more than Type I motor units because the maximal force is highest in large cells, in which the loads imposed are approximately 60% greater than the maximal force that the cells can develop. Recovery is slow and depends on regeneration of the injured sarcomeres.

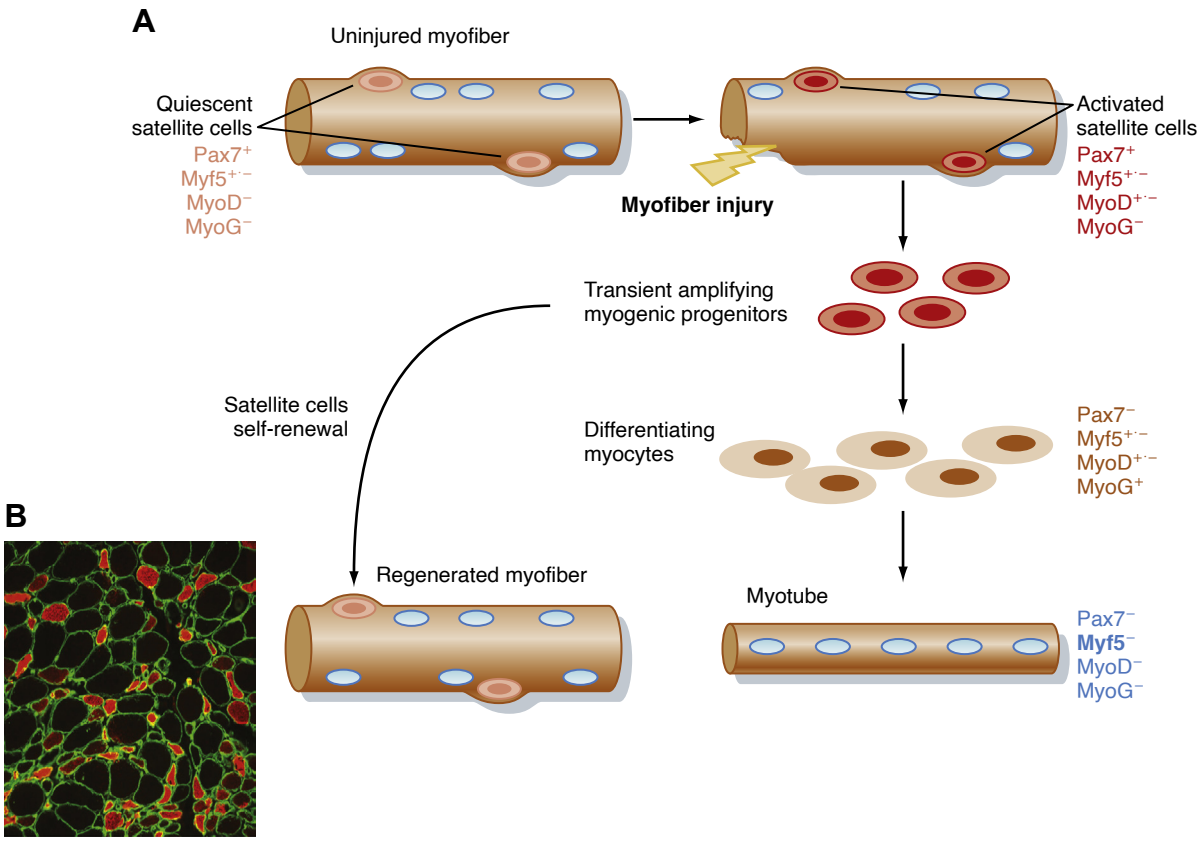
Biophysical Properties of Skeletal Muscle

The molecular mechanisms of muscle contraction described earlier underlie and are responsible for the biophysical properties of muscle. Historically, these biophysical properties were well described before elucidation of the molecular mechanisms of contraction. They remain important ways of describing muscle function.

Length-Tension Relationship

When muscles contract, they generate force (often measured as tension or stress) and decrease in length. In examination of the biophysical properties of muscle, one of these parameters is usually held constant, and the other is measured after an experimental maneuver. Accordingly, an **isometric contraction** is one in which muscle length is held constant, and the force generated during the contraction is then measured. An **isotonic contraction** is one in which the force (or tone) is held constant, and the change in length of the muscle is then measured.

When a muscle at rest is stretched, it resists stretch by a force that increases slowly at first and then more rapidly as the extent of stretch increases (Fig. 12.23). This purely passive property is due to the elasticity of the muscle tissue. If

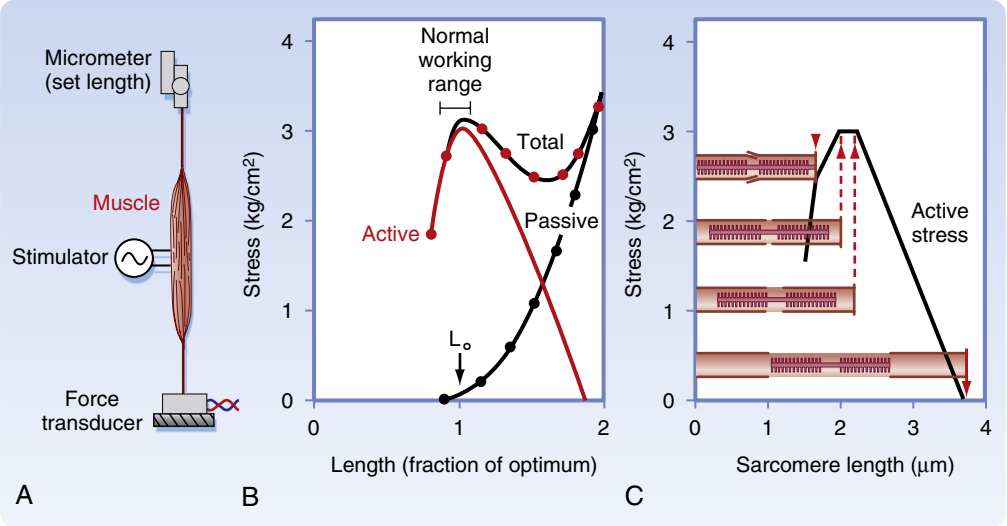


• **Fig. 12.22** Regeneration of injured skeletal muscle. **A,** Injury of a skeletal muscle results in the activation of satellite cells under the basal lamina of the injured skeletal muscle fiber. The satellite cells progress through a series of steps to the regenerate the muscle fiber. **B,** Regenerating muscle fibers express an embryonic myosin isoform. This biopsy sample is from a patient with polymyositis, stained with antibodies to embryonic myosin heavy chain *(red)* and laminin *(green)* (see text for details). (**A,** From Giordani L, Paris A, Le Grand F. Satellite cell self-renewal. *Curr Top Dev Biol* 2018;126:177. **B,** From Schiafino S. Muscle fiber type diversity revealed by anti-myosin heavy chain antibodies. *FEBS J* 2018;285:3688.)

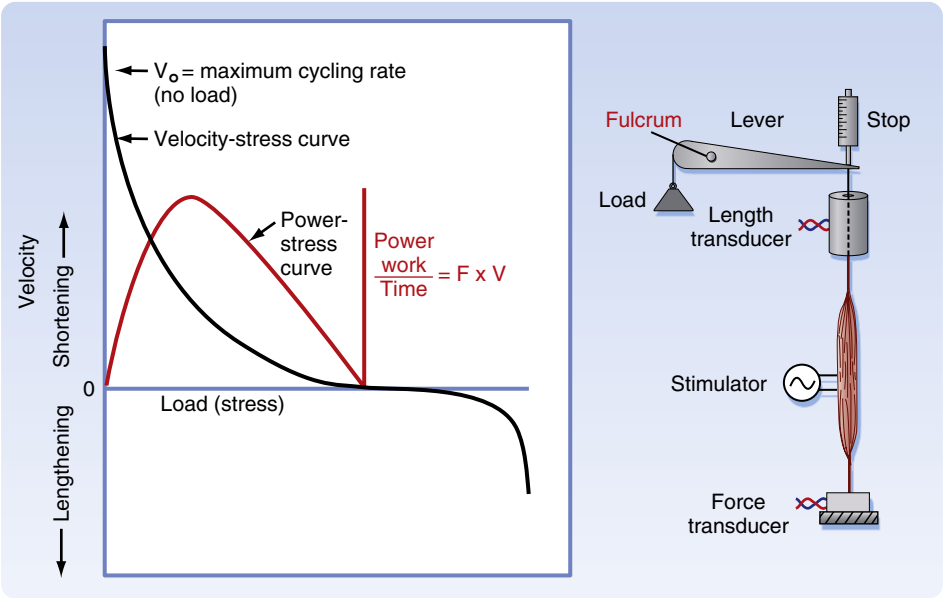
the muscle is stimulated to contract at these various lengths, a different relationship is obtained. Specifically, contractile force increases as muscle length is increased up to a point (designated L_O to indicate optimal length). As the muscle is stretched beyond L_O, contractile force decreases. This length-tension curve is consistent with the sliding filament theory, described previously. At a very long sarcomere length (3.7 µm), actin filaments no longer overlap with myosin filaments, and so there is no contraction. As muscle length is decreased toward L_O, the amount of overlap increases, and contractile force progressively increases. As sarcomere length decreases below 2 µm, the thin filaments collide in the middle of the sarcomere, the actin-myosin interaction is disturbed, and hence contractile force decreases. For construction of the length-tension curves, muscles were maintained at a given length, and then contractile force was measured (i.e., isometric contraction). Thus the length-tension relationship supports the sliding filament theory of muscle contraction.

Force-Velocity Relationship

The velocity at which a muscle shortens is strongly dependent on the amount of force that the muscle must develop (Fig. 12.24). In the absence of any load, the shortening velocity of the muscle is maximal (denoted as V_0). V_0 corresponds to the maximal cycling rate of the cross-bridges (i.e., it is proportional to the maximal rate of energy turnover [ATPase activity] by myosin). Thus V_0 for fast-twitch muscle is higher than that for slow-twitch muscle (see Fig. 12.14*B,C*). Increasing the load decreases the velocity of muscle shortening until, at maximal load, the muscle cannot lift the load and hence cannot shorten (zero velocity). Further increases in load result in stretching the muscle (negative velocity). The maximal isometric tension (i.e., force at which shortening velocity is zero) is proportional to the number of active cross-bridges between actin and myosin, and it is usually greater for fast-twitch motor units (because of the larger diameter of fast-twitch muscle fibers and greater number of muscle fibers in a typical fast-twitch motor unit). In Fig. 12.24, the power-stress curve reflects the rate of work done at each load and shows that the maximal rate of work was done at a submaximal load (namely, when the force of contraction was approximately 30% of the maximal tetanic tension). To calculate the latter curve, the x- and y-coordinates were simply multiplied, and then the product was plotted as a function of the x-coordinate.



• **Fig. 12.23** Length-tension relationship in skeletal muscle. **A,** Experimental setup in which maximal isometric tetanic tension is measured at various muscle lengths. **B,** How active tension was calculated at various muscle lengths (i.e., by subtracting passive tension from total tension at each muscle length). **C,** Plot of active tension as a function of muscle length, with the predicted overlap of thick and thin filaments at selected points.



• **Fig. 12.24** Force-velocity relationship of skeletal muscle. The experimental setup is shown on the *right*. The initial muscle length was kept constant, but the amount of weight that the muscle had to lift during tetanic stimulation varied. While these various amounts of weight were lifted, muscle-shortening velocity was measured. See text for details. *F*, Force; *V*, velocity.

Key Points

1. Skeletal muscle is composed of numerous muscle cells (muscle fibers) that are typically 10 to 80 μm in diameter and up to 25 cm in length. The appearance of striations in skeletal muscle is due to the highly organized arrangement of thick and thin filaments in the myofibrils of skeletal muscle fibers. The sarcomere is a contractile unit in skeletal muscle. Each sarcomere is approximately 2 μm in length at rest and is bounded by two Z lines. Sarcomeres are arranged in series along the length of the myofibril. Thin filaments, containing actin, extend from the Z line toward the center of the sarcomere. Thick filaments, containing myosin, are positioned in the center of the sarcomere and overlap the actin thin filaments. Muscle contraction results

from the Ca^{++}-dependent interaction of myosin and actin, in which myosin pulls the thin filaments toward the center of the sarcomere.

2. Contraction of skeletal muscle is under control of the central nervous system (i.e., voluntary). Motor centers in the brain control the activity of α motor neurons in the ventral horns of the spinal cord. These α motor neurons, in turn, synapse on skeletal muscle fibers. Whereas each skeletal muscle fiber is innervated by only one motor neuron, a motor neuron innervates several muscle fibers within the muscle. A *motor unit* refers to all the muscle fibers innervated by a single motor neuron.
3. The motor neuron initiates contraction of skeletal muscle by producing an action potential in the muscle fiber. As the action potential passes down the T tubules of the muscle fiber, voltage-gated Ca^{++} channels ($Ca_V1.1$) in the T tubules undergo conformational changes that result in the opening of neighboring SR Ca^{++} channels called ryanodine receptors (RYR1), which then release Ca^{++} to the myoplasm from the SR. Ca^{++} influx through $Ca_V1.1$ is not needed for the action potential in the T tubule to induce Ca^{++} release from RYR1. Instead, the voltage-induced conformational change in the $Ca_V1.1$ protein in the T tubule promotes a protein-protein interaction with RYR1, stimulating Ca^{++} release from the SR. The increase in myoplasmic Ca^{++} promotes muscle contraction by exposing myosin-binding sites on the actin thin filaments (a process that involves binding of Ca^{++} to troponin C, followed by movement of tropomyosin toward the groove in the thin filament). Myosin cross-bridges then appear to undergo a ratchet action, with the thin filaments pulled toward the center of the sarcomere and contracting the skeletal muscle fiber. Relaxation of the muscle follows as myoplasmic Ca^{++} is resequestered by Ca^{++}-ATPase (SERCA) in the SR.
4. The force of contraction can be increased by the activation of more motor neurons (i.e., recruiting of more muscle fibers) or by an increase in the frequency of action potentials in the muscle fiber, which produces tetany. The increase in force during tetanic contractions is due to prolonged elevation of intracellular $[Ca^{++}]$.
5. The two basic types of skeletal muscle fibers are distinguished on the basis of their speed of contraction (i.e., fast-twitch versus slow-twitch). The difference in speed of contraction is attributed to the expression of different myosin isoforms that differ in myosin ATPase activity. In addition to the difference in myosin ATPase activity, fast-twitch and slow-twitch muscles also differ in metabolic activity, fiber diameter, motor unit size, sensitivity to tetany, and recruitment pattern.
6. Typically, slow-twitch muscles are recruited before fast-twitch muscle fibers because of the greater excitability of motor neurons innervating slow-twitch muscles. The high oxidative capacity of slow-twitch muscle fibers supports sustained contractile activity. Motor units composed of fast-twitch glycolytic fibers (Type IIx) have a higher threshold for activation, larger number of fibers, and lower oxidative capacity, and so are best suited for short periods of activity when high levels of force are required.
7. Fast-twitch muscle fibers can be converted to slow-twitch muscle fibers (and vice versa), depending on the stimulation pattern. Chronic electrical stimulation of a fast-twitch muscle results in the expression of slow-twitch myosin and decreased expression of fast-twitch myosin, along with an increase in oxidative capacity. The mechanism or mechanisms underlying this change in gene expression are unknown, but the change appears to be secondary to an elevation in resting intracellular $[Ca^{++}]$. The Ca^{++}-dependent phosphatase calcineurin and the transcription factor NFAT have been implicated in this transition from the fast-twitch to the slow-twitch phenotype. Ca^{++}/calmodulin-dependent kinase and the transcription factor MEF2 may also participate in the phenotype transition.
8. Skeletal muscle fibers atrophy after denervation. Muscle fibers depend on the activity of their motor nerves for maintenance of the differentiated phenotype. Reinnervation by axon growth along the original nerve sheath can reverse these changes. Skeletal muscle has a limited capacity to replace cells lost as a result of trauma or disease. Inhibition of the PI3K/Akt signaling pathways and activation of the FoxO pathway appears to contribute to the decreased rate of protein synthesis and the increased rate of protein degradation (respectively) observed during disuse atrophy. The increased protein degradation during atrophy is attributed to increases in both protease activity (e.g., activation of caspase 3) and ubiquitination (through elevated levels of ubiquitin ligases).
9. Skeletal muscle exhibits considerable phenotypic plasticity. Normal growth is associated with cellular hypertrophy, caused by the addition of more myofibrils and more sarcomeres at the ends of the cell to match skeletal growth. Strength training induces cellular hypertrophy (typically through a signaling pathway that involves activation of the Akt-mTOR pathway). Endurance training increases the oxidative capacity and capillary density of all involved motor units, typically through a signaling pathway that involves stimulation of the transcription coactivator PGC-1a. Training regimens cannot convert Type II muscle fibers to Type I muscle fibers
10. Muscle fatigue during exercise is not due to depletion of ATP. The mechanism or mechanisms underlying exercise-induced fatigue are not known, although the accumulation of various metabolic products (lactate, P_i, ADP) has been implicated. In view of the importance of preventing depletion of myoplasmic ATP, which would affect the viability of the cell, it is likely that multiple mechanisms may have been developed to

induce fatigue and hence lower the rate of ATP hydrolysis before the individual risks injury or death of the skeletal muscle cell.

11. When the energy demands of an exercising muscle cannot be met by oxidative metabolism, an oxygen debt is incurred. Increased breathing during the recovery period after exercise reflects this oxygen debt. The greater the reliance on anaerobic metabolism to meet the energy requirements of muscle contraction, the greater the oxygen debt.
12. Minor injuries to the sarcolemma of skeletal muscle can be repaired by the annexin/dysferlin, whereas more extensive damage may lead to regeneration of muscle fibers. Regeneration of the muscle fibers involves activation of satellite cells under the basal lamina of the muscle. The activated satellite cells proliferate and differentiate to become myocytes, which fuse to yield myotubes. The myotubes mature to become regenerated muscle fibers with centrally located nuclei and embryonic myosin heavy chain. Depending on the α motor nerve innervation, the regenerated muscle fibers will express either Type I or Type II myosin.