Contractile Mechanisms in Skeletal Muscle 3.5

Learning Objectives

- Explain why muscle fibers are multinucleated
- Distinguish among muscle fiber, myofibrils, and myofilaments
- Identify the various cross-striations as evidenced by electron microscopy including the A-band, I-band, Z-line, M-line, and H-zone
- Define the sarcomere
- Describe which filaments are found in which microscopic zone of the sarcomere
- Be able to show the relationship among the sarcoplasmic reticulum, T-tubules, and myofibrils
- Draw the length—tension curve using sarcomere length and explain the origin of its major points
- Describe the structure of the thick filament and explain the origin of the bare zone and its width
- Name the constituents of the thin filament and each of their functions
- Describe the polarity of thin filaments at the Z-disk
- Explain the function of the cross-bridge cycle and write a simplified reaction mechanism
- Describe how myosin isoforms generate muscle heterogeneity
- Define myosin turnover number and explain why it correlates with muscle speed
- Describe costameres and how force is thought to be distributed through the sarcolemma to the extracellular matrix

INTRODUCTION

Chapter 3.4 has shown us how the overall muscle behaves: muscles are heterogeneous with respect to contractile properties. They can be classified according to their twitch times, velocity of shortening, and resistance to fatigue: fibers can be slow, fast fatigue resistant, fast intermediate, and fast fatigable. Muscle force can be graded by the recruitment of motor units, by varying the frequency of motor neuron firing, and by varying the length of the muscle. Of these, recruitment offers control of the greatest range of force, frequency the next greatest, and length the least. There is an inverse relationship between velocity and force of shortening. The power of the muscle peaks at about one-third maximal force and at about one-third maximal velocity. What we seek now is some explanation of these overall behaviors in the subcellular and molecular description of muscle.

MUSCLE FIBERS HAVE A HIGHLY ORGANIZED STRUCTURE

Muscle fibers are typically large cells, some 20−100 μm in diameter and many centimeters long, with the longest fibers being about 12 cm. These cells are **multinucleated**, because they need many nuclei to govern protein synthesis and degradation. The nuclei are typically located near the periphery of the cell and often are more highly concentrated near the myoneural, or neuromuscular, junction. The most striking feature of muscle cells viewed under the light microscope is their banded appearance. The fibers have stripes, or **striations**, that result from the highly organized arrangement of proteins in the muscle fiber. These striations consist of alternating **A-bands** and **I-bands**, named because the I-bands are *isotropic* to polarized light (meaning that they appear the same from all directions) whereas the A-bands are *anisotropic* to polarized light. The cross-striations are perpendicular to the long axis of the muscle fiber. Figure 3.5.1 shows the microscopic appearance of frog skeletal muscle fibers using phase contrast microscopy.

Muscle cells are also striated longitudinally by the organization of contractile proteins into tiny threads called **myofibrils**. These are generally cylinders of material about 1 μm in diameter that also clearly show cross-striations. The myofibrils are kept in register across the entire cell to give rise to the cross-striated appearance. The electron micrograph in Figure 3.5.2 shows how the striations in the myofibrils line up across the cell.

Just as each muscle fiber contains many myofibrils, each myofibril is in turn composed of many filaments. These filaments come in two main varieties: the **thin filament** and the **thick filament**. The major constituent of the thin filament is **actin**; the main component of the thick filament is **myosin**. The microscopic striated appearance of the muscle is due to the way in which the filaments overlap each other.

The thick filaments define the beginning and end of the A-band. The myosin component of the A-band gives rise to the anisotropic behavior under polarized light. Because the thick filaments are 1.6 μm long, the A-band is also 1.6 μm long. Figure 3.5.3 shows a schematic illustration of the structure of the muscle fibers and myofibrils.

DOI: http://dx.doi.org/10.1016/B978-0-12-800883-6.00028-8

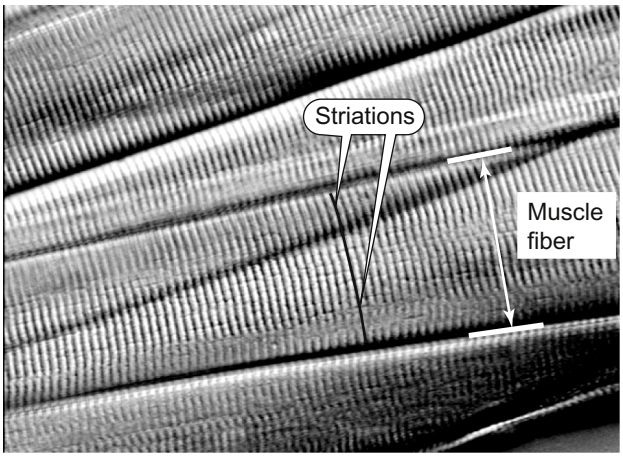

FIGURE 3.5.1 Microscopic appearance of skeletal muscle. A bundle of frog sartorius muscle fibers was teased out and viewed under phase contrast microscopy. Cross-striations are readily apparent in these unstained muscle fibers.

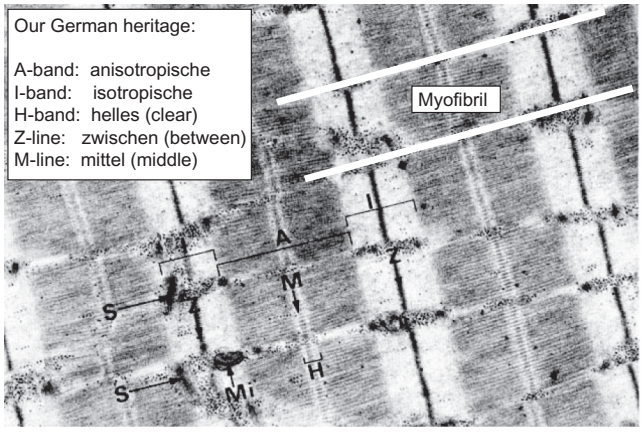

FIGURE 3.5.2 Electron micrograph of muscle. The spaces between myofibrils are filled with membranes of the sarcoplasmic reticulum, mitochondria, and glycogen granules. The myofibrils are bundles of filaments arranged longitudinally parallel to the long axis of the muscle fiber. The various bands are named according to their position, appearance, or by how they rotate the plane of polarized light.

The thin filaments are about 1.0 μm long but their length varies with muscle types and species. In human deltoid muscle biopsies, thin filament length averages 1.19 μm whereas in the pectoralis major it is 1.37 μm. Opposite thin filaments are connected, back to back, at a structure called the **Z-line** (from the German "zwischen" meaning "between"). Because the myofibrils are cylindrical, the Z-line is actually a disk of material and it is also called the **Z-disk**. The thin filaments typically overlap the thick filaments. The **H-zone** is a clearer area in the middle of the thick filaments. Its name derives from the German "helles," meaning "clear." This is the part of the A-band in which the thin filaments do not overlap the thick filaments. The thick filaments are connected in their center by material that forms the **M-line** (from the German "mittel" meaning "middle"; see Figures 3.5.2 and 3.5.3).

Electron micrographs show that the thick filaments form a hexagonal-centered lattice. The thin filaments also form a hexagonal lattice, but it is rotated 30° from the thick filament lattice. Each thick filament is in the center of a hexagon of thin filaments, whereas each thin filament is located equidistant from a triangle of three thick filaments. Thus each thin filament is surrounded by three thick filaments and each thick filament is surrounded by six thin filaments. In some electron micrographs, **cross-bridges** can be seen between the thick and thin filaments. The interaction of the filaments through these cross-bridges produces either shortening or force.

The functional unit of contraction or force production is the **sarcomere**, extending from one Z-disk to the next. The myofibrils consist of thousands of these sarcomeres strung end to end. The length of the sarcomere varies with muscle activation. Typically, the rest length is about 2.0–2.2 μm, depending on the length of the thin filament. This rest length is less than the thick filament (1.6 μm) plus two thin filaments (1.0–1.37 μm each) because of the overlap of the filaments at rest.

At regular intervals, the surface membrane of the muscle fiber, the **sarcolemma**, is invaginated to form a long tubule running perpendicular to the surface and penetrating into the farthest parts of the fiber's interior. These are the transverse tubules or **T-tubules**. The function of these T-tubules is to bring the action potential on the sarcolemma into the interior of the cell. The T-tubules allow for the rapid spread of the excitation to all parts of the sarcoplasm.

Adjacent to the T-tubules and surrounding each myofibril is a membranous network called the **sarcoplasmic reticulum** or **SR**. This organelle surrounds the myofibril like a loosely knit sweater surrounds your arm. It forms an internal compartment, the **lumen**, separate from the cytoplasm of the muscle fiber. The SR is divided into a **longitudinal SR** and **terminal cisternae**. The terminal cisternae are sacs that make contact with the T-tubules, whereas the longitudinal SR are thin tubes of membrane that connect the terminal cisternae from one side of the sarcomere to the other. The longitudinal SR and terminal cisternae are connected and form a single enclosed space. In skeletal muscle, the junction of the T-tubule and the SR forms a **triad**, because it is the junction of one T-tubule and two terminal cisternae. In mammalian skeletal muscle, the triads occur at the junction of the A-band and I-band, so that there are two triads per sarcomere. This arrangement of the T-tubules and SR junction varies with the species and muscle type. Figure 3.5.4 shows the anatomical relation of the SR to the myofibril.

THE SLIDING FILAMENT HYPOTHESIS EXPLAINS THE LENGTH–TENSION CURVE

One of the early observations of muscle contraction was that when a muscle shortens, the Z-disks move closer together but there is no change in the length of the A-band; all of the shortening appears to occur in the

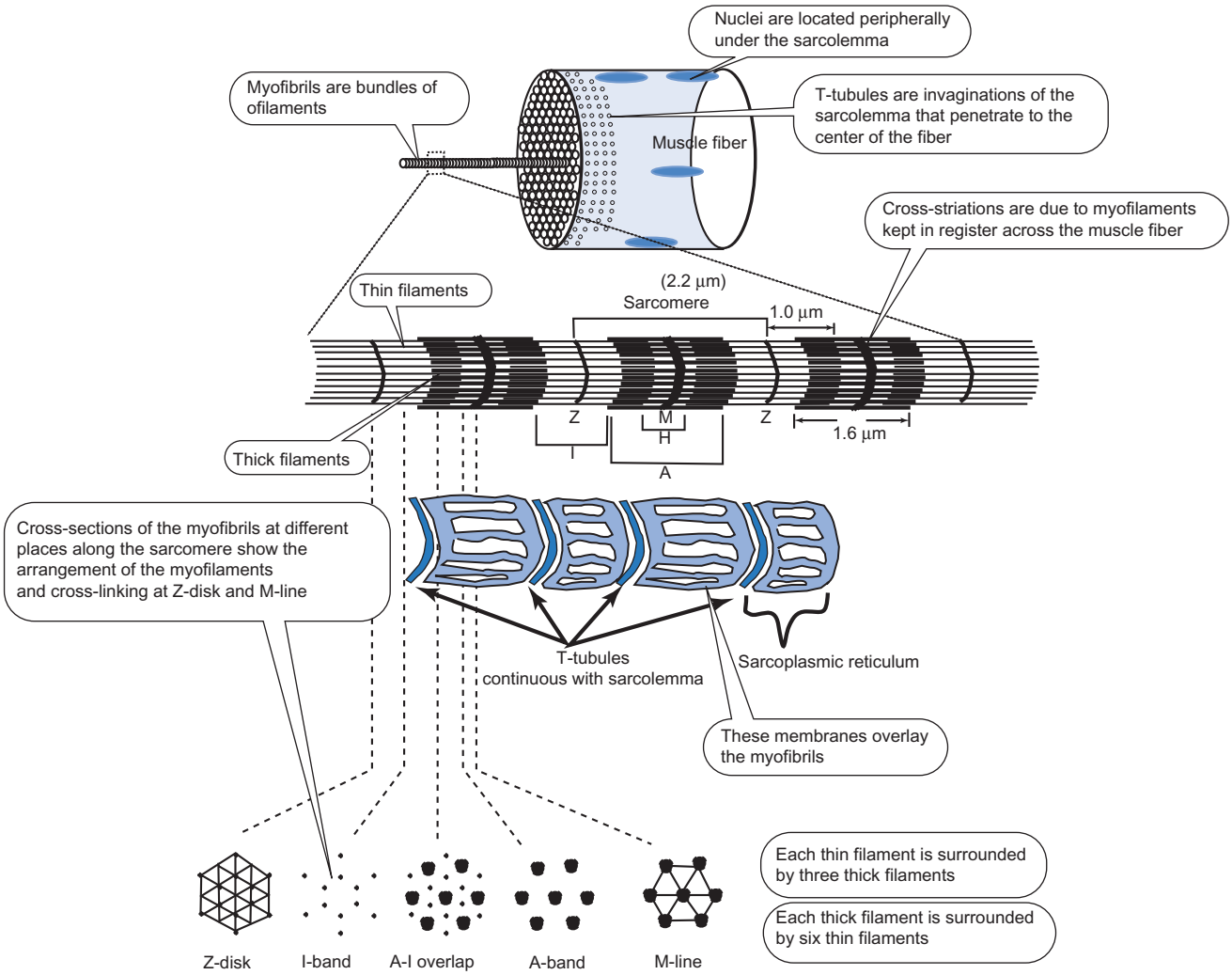

FIGURE 3.5.3 Structure of the muscle fiber and myofibrils. The A-band corresponds to the length of the thick filaments, 1.6 μm. The I-band corresponds to the thin filaments where they do not overlap with the thick filaments. Its width depends on the activation of the muscle. The Z-line or disk is where the thin filaments from opposite sarcomeres are attached. The M-line in the middle of the A-band keeps the thick filaments centered and in register. The clear zone in the middle of the A-band is the region where thin filaments do not overlap thick filaments.

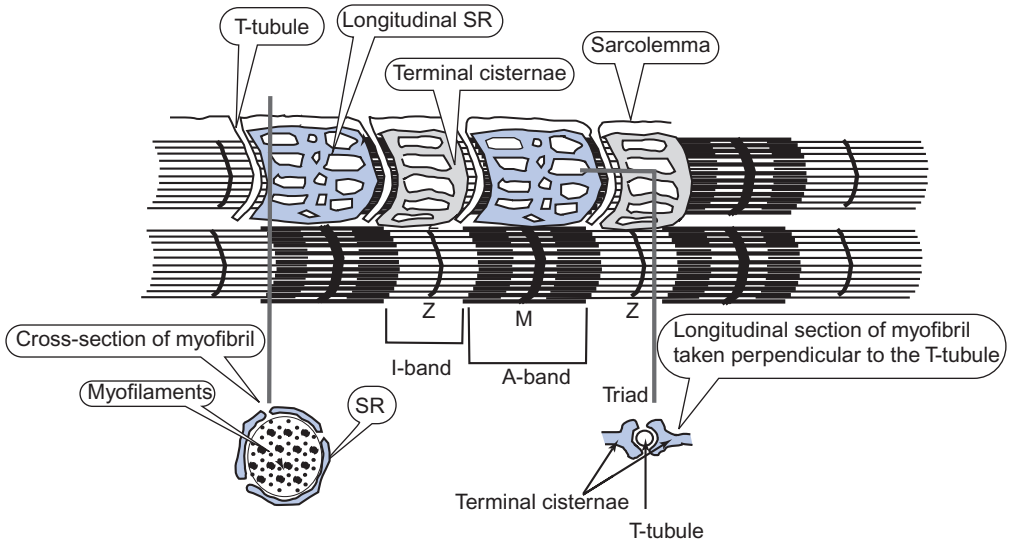

FIGURE 3.5.4 Structure of the SR around the myofibril.

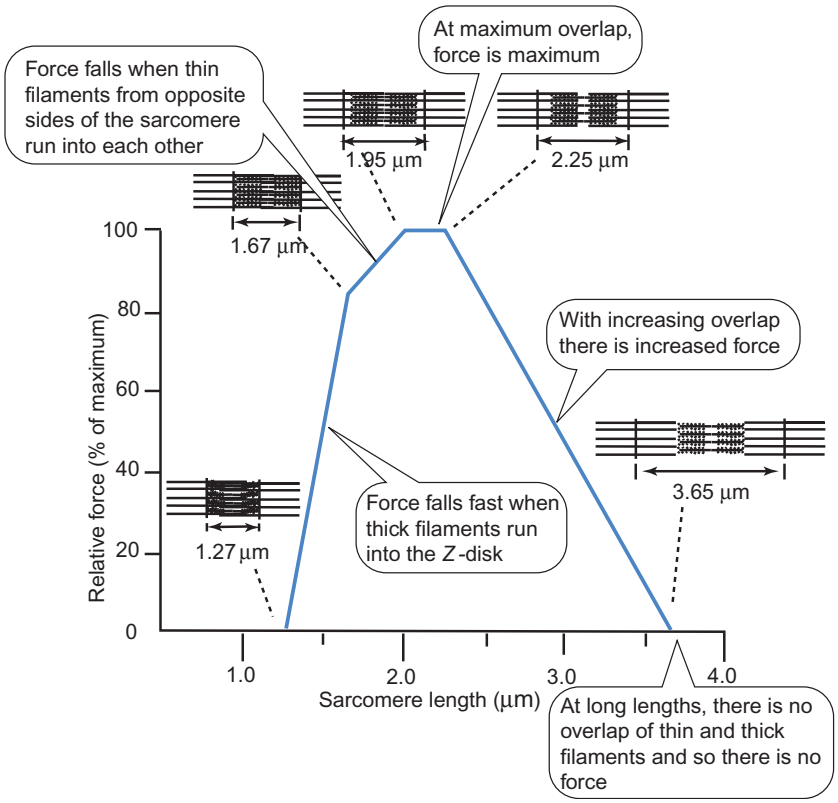

FIGURE 3.5.5 Dependence of tension on the degree of overlap of the thin and thick filaments.

I-bands. The sliding filament theory was first proposed by A.F. Huxley and R. Niedegerke in 1954 to explain these observations and the length–tension curve.

In Chapter 3.4, we showed that the active tension developed by a muscle is low at both extremes of length, and it peaks in the middle. This relationship also holds for individual muscle fibers where it is possible to measure the sarcomere length. The results are shown in Figure 3.5.5 for muscles with thin filaments 1.0 μm long. At long lengths of the muscle, the sarcomeres are proportionately long. At a sarcomere length of 3.65 μm, there is no overlap of the filaments (recall that the A-band is 1.6 μm and the I-band filament is 1.0 μm; thus the length of a sarcomere consisting of two I-band filaments and the A-band is 3.6 μm). At the point where the filaments do not overlap, the muscle cannot generate any force. At shorter lengths, the A-band and I-band filaments overlap increasingly so the force proportionately increases. At 2.25 μm, there is maximum overlap of the force generators and so the muscle generates maximal force. This force does not decrease until the sarcomere shortens to <1.95 μm. The reason for this is that there is still maximal overlap of those portions of the thick filaments that are engaged in force production: the thick filament has a central region that cannot produce force by interacting with the thin filament. When the overlap of the filaments increases, there is no further increase in the area of the thick filament that produces force. From the distances on the graph, we can infer that the bare zone is about 0.3 μm wide. When the sarcomeres shorten still further, the thin filaments begin to run into each other. This occurs at about twice the length of the thin filament or at about 1.95 μm. Although the thin filaments can slide past each other, they can do so only by using some of the force generated by the muscle. Thus the active force falls. At still shorter lengths, the thick filament begins to butt up against the Z-disk. This occurs at about the length of the A-band or 1.6 μm. At this point, further shortening seriously distorts the thick filament and force falls precipitously with further shortening.

FORCE IS PRODUCED BY AN INTERACTION BETWEEN THICK FILAMENT PROTEINS AND THIN FILAMENT PROTEINS

THE THICK FILAMENT CONSISTS PRIMARILY OF MYOSIN

Myosin is a complex of six proteins having a combined molecular weight of about 480,000 Da. It consists of two **heavy chains** of 200,000 Da each, and a total of two pairs of **myosin light chains**, with a molecular weight of about 20,000 and 16,000 Da. The two heavy chains have functionally distinct regions. They consist of a long, rod-shaped **tail** and a globular "**head**" region that contains the "business" end of the molecule. The head is attached to the tail by an arm section. This arm and its heads form the cross-bridges that interact with the thin filament to produce force. The tails of the two heavy chains are twisted together to form a supercoil.

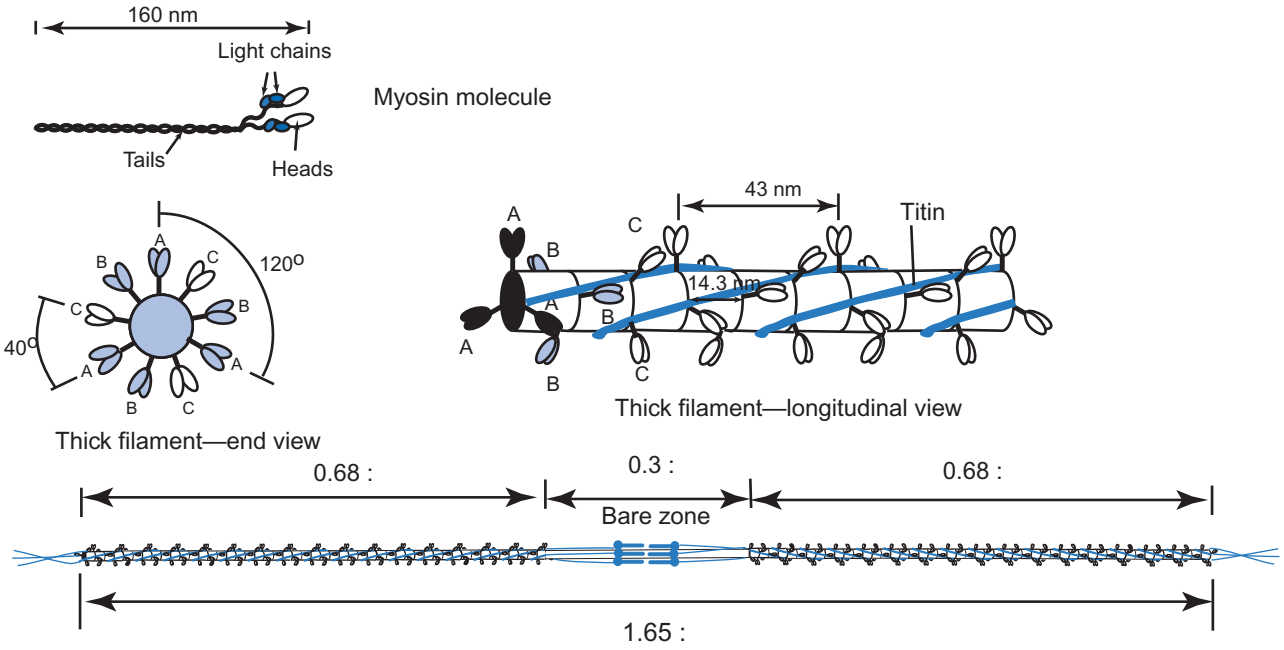

FIGURE 3.5.6 Structure of the thick filament.

Two distinct myosin light chains associate with each of the myosin heads. In smooth muscle, the activity of the myosin is regulated through phosphorylation and dephosphorylation of these light chains. In skeletal muscle, the phosphorylation state of these myosin chains is regulated, and appears to be responsible for "post-tetanic potentiation", in which isometric force increases immediately following a tetanic contraction.

The thick filaments also contain **titin**, a giant protein with a molecular weight of about 3.7 million Da. This protein is also called connectin and is the largest protein known to date. It is the third most abundant protein in skeletal muscle, comprising some 8–10% of the myofibrillar protein. The protein spans the distance from Z-disk to M-line and contains repeat regions for binding of α-actinin, a protein that anchors the thin filaments at the Z-disks. Titin also contains other repeat regions for binding myosin and M-protein, the one responsible for tying together the thick filaments at the M-line and myosin-binding protein C. Although uncertainties remain, the working hypothesis is that titin stabilizes mechanically active sarcomeres at the Z-disk, A-band, and M-line regions, and also serves in the cellular signaling of mechanical force to maintain the sarcomeres.

Myosin binds to titin and to other myosin molecules along its tail region. The myosin heads project out of the thick filaments at regular intervals of 14.3 nm. There appear to be three myosin molecules (six heads) at each plane oriented at right angles to the long axis of the filament. There is an identical repeat every 43 nm. Each thick filament has about 294 myosin dimers projecting from its surface (about 147 facing each of the neighboring Z-disks). Figure 3.5.6 shows myosin's basic structure. Because the myosin molecules lay themselves down along the titin template so that the tail regions point toward the middle of the filament, the middle of the thick filament contains only tails and the heads are located at the ends of the filament. Thus the thick filament is polarized with a "bare zone" in its middle. The titin molecule continues past the end of the myosin to make connections with other proteins in the Z-disk.

THE THIN FILAMENT CONSISTS PRIMARILY OF *ACTIN*

The actin in skeletal muscle is one of several types made by different cells. It can be isolated as a monomeric globular actin or **G-actin**. It has a molecular weight of 41,800 Da and a diameter of about 5.5 nm. Under proper conditions, G-actin will aggregate to form a filament or F-actin. This consists of two strands of actin molecules wound around each other. There are about seven G-actin molecules per half-turn, giving a half-turn distance of about 38.5 nm. Tropomodulin caps the filament at the minus end, which faces the M-line, and cap Z caps the filament at the plus end and anchors it at the Z-disk. The filament grows at the plus end but material can exchange at either end (see Figure 3.5.7).

Nebulin is another giant protein, with a molecular weight between 600,000 and 900,000 Da in different muscles. It makes up some 2–3% of the myofibrillar protein. Nebuin is anchored at its C-terminus at the Z-disk where it binds to cap Z, but it generally extends only 0.9 μm from the Z-line. It does not extend the entire distance of the thin filament. Human nebulin contains a string of 185 tandem repeats of 35 amino acids that are presumed to be actin binding domains. The central 154 of these repeats form 22 "super repeats"

of seven units. These are thought to bind tropomyosin and are about 38.5 nm long, corresponding to one-half turn of the actin helix. The 22 repeats of 38.5 nm do not extend to the end of the filament. There are 2 nebulin molecules per thin filament, and these are proposed to occupy the major groove of the filament. The exact location of nebulin is not known. The function of nebulin is not yet settled, but it appears to stabilize the thin filament against depolymerization, but it also interacts with Z-disk proteins.

Thin filaments also contain **tropomyosin**, which consists of two nonidentical polypeptide chains, each with a molecular weight of about 33,000 Da. They are long, rod-shaped molecules, and tropomyosin α and β wrap around each other to form a supercoil. The tropomyosin complex is 38.5 nm long, the same size as a half-turn of the F-actin helix. Along with **troponin**, tropomyosin participates in the regulation of the active state of muscle.

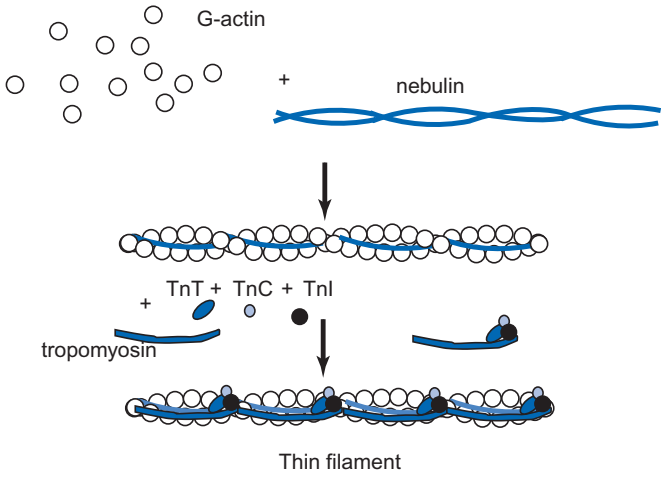

FIGURE 3.5.7 Structure of the thin filament. The actual location of nebulin is not known. The ends of the thin filament are shown. The plus, or barbed end, of the thin filament is anchored at the Z-disk. The minus, or sharp end, is typically in the A-band and is capped by tropomodulin.

Troponin consists of **troponin T**, a 37,000 Da protein, **troponin C** of 18,000 Da, and **troponin I** of 21,000 Da. These three derive their names from their functions: **TnT** binds to **tropomyosin**; **TnC** binds **calcium** ions (and thereby confers Ca^{2+} sensitivity onto the myofilaments), and **TnI inhibits** the interaction between the thick and thin filaments that cause force development or shortening. These three proteins form a complex at one end of each tropomyosin molecule. The troponin complex is responsible for the final regulation of the contractile state of the myofilaments.

α-*ACTININ* AT THE Z-DISK JOINS ACTIN FILAMENTS OF ADJACENT SARCOMERES

The Z-disk contains a number of proteins that can bind F-actin or other proteins found at the Z-disk. The precise way that these proteins align the thin filaments is not yet known. One of these proteins, α-actinin, consists of two subunits of 95,000 Da that is located in the Z-disk and anchors the thin filaments there. A proposed arrangement of the Z-disks shown in Figure 3.5.8 illustrates the complexity of these structures. Muscle cells ultimately connect the Z-disk to the surface membrane because the force generated by the myofilaments must eventually be transferred to the outside of the cell, through the sarcolemma.

MYOMESIN JOINS THICK FILAMENTS AT THE M-LINE OR M-BAND

Electron micrographs show a fine structure to the M-line that differs among muscle types. In particular, M-protein contributes to a central line that is present in fast-twitch

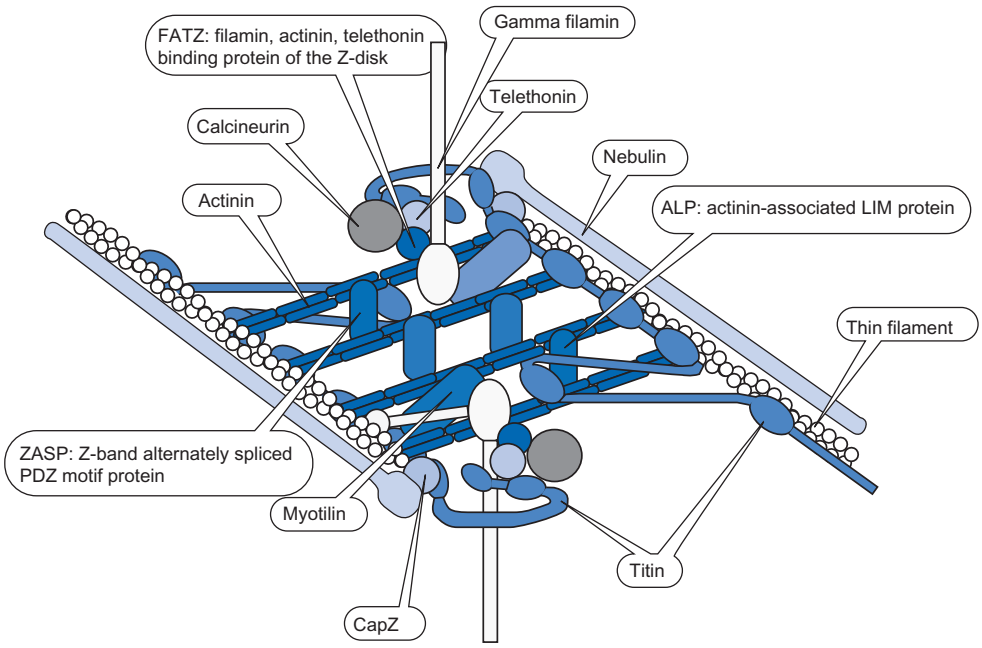

FIGURE 3.5.8 Proposed linkage of thin and thick filaments at the Z-disk. The thin filaments from adjacent sarcomeres have opposite polarity at the Z-disk. The Z-disk contains a variety of proteins that bind to other proteins in the disk. Gamma filamin is a cytoskeletal protein that links the Z-disk to the outside of the cell. Telethonin caps the titin filament. CapZ, a heterodimer, caps the plus or barbed end of the thin filament and also binds titin. Another name for FATZ is calsarcin, which binds telethonin, actinin, and filamin. ZASP is also named cypher and oracle. Actinin is an antiparallel homodimer. *Adapted from Faulkner, G., Lanfranchi, G., and Valle, G. Telethonin and other new proteins of the Z-disk of skeletal muscle.* IUBMB Life ***51***: *275–282, 2001.*

but not slow-twitch fibers. M-protein and another M-band protein, myomesin, share structural features, both consisting of a head region and 12 modules resembling fibronectin or IgG. Myomesin seems to be expressed in a fixed ratio to myosin in a variety of muscle types. Myomesin binds to myosin, titin, and to itself. A proposed structure for the M-band is shown in Figure 3.5.9.

OVERALL STRUCTURE OF THE SARCOMERE IS COMPLICATED

The overall structure of the sarcomere is shown in simplified form in Figure 3.5.10. The structures of the thin filaments, thick filaments, Z-disks, and M-line have already been reviewed in Figures 3.5.6–3.5.8 and 3.5.9, and their details are omitted in Figure 3.5.10. This figure emphasizes the disposition of the three giant proteins of the sarcomere: titin, nebulin, and obscurin. All of these proteins contain multiple repeats of immunoglobulin G-like and fibronectin-like domains. Titin extends from the M-line to the Z-line, but the portion in the I-band is differently constructed, consisting of a number of repeats that produce a spring-like behavior to the protein. Along with myomesin, it is thought that titin produces a restorative force after contraction and likely also centers the A-band in the sarcomere following force imbalances during contraction.

Nebulin is anchored in the Z-disk to Cap Z and it extends approximately 0.9 μm along the thin filament away from the Z-disk. In most skeletal muscles, this is not enough to reach the end of the thin filament. At the Z-disk, it is proposed that nebulin crosses from one thin filament to the one in the opposite sarcomere, binding only a short distance (0.1 μm or less).

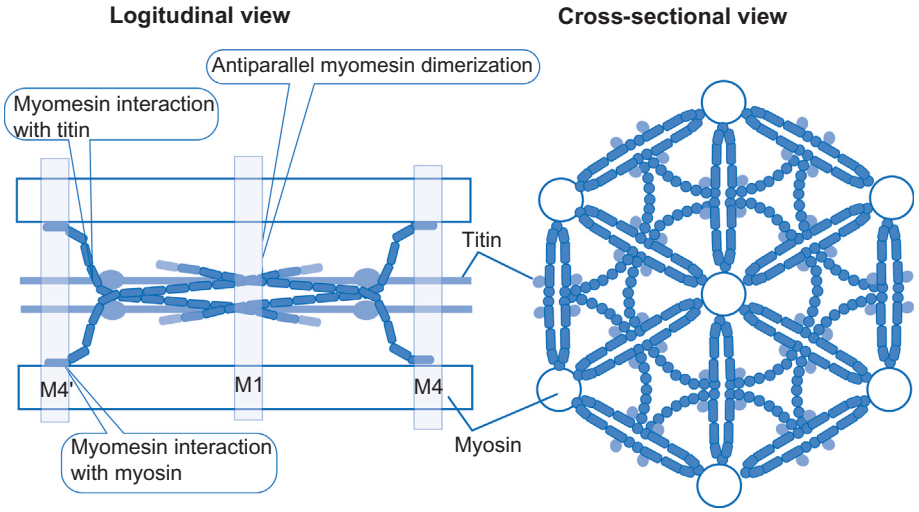

FIGURE 3.5.9 Proposed structure of the M-band. Myomesin binds to myosin, creating increased density on electron micrographs that make the M4 and M4′ lines. The dimerization of myomesin in the center of the A band contributes to the M1 line. Myomesin also binds to titin. Note that the central myosin is surrounded by 12 titin filaments. Six of these originate from the myosin filament on each side of the M-line. Thus, there are six titin filaments per myosin filament in each half sarcomere. Myosin is in white; titin is light blue, and myomesin is dark blue. *Adapted from Lange, S., et al., Dimerisation of myomesin: implications for the structure of the sarcomeric M-band.* J. Mol. Biol. ***345****: 289–298, 2005.*

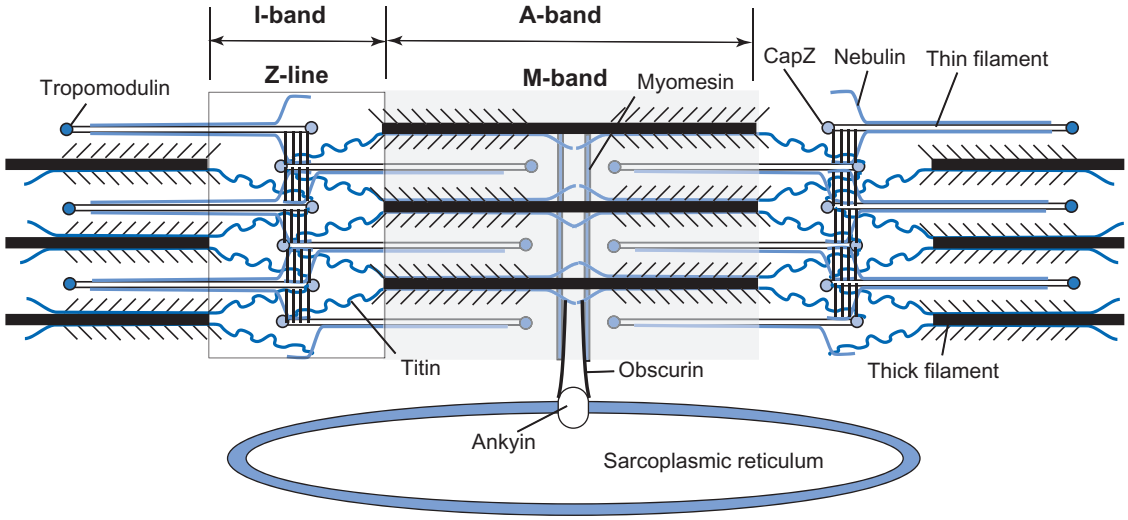

FIGURE 3.5.10 Distribution of the giant proteins titin, nebulin, and obscurin in the sarcomere. Titin extends from the center of the M-band to the Z-line. It interacts with the thick filament in the A-band and contains spring-like domains in the I-band. It binds to multiple proteins in the Z-disk. It is thought to provide a restorative force following contraction, and also centers the A-band filaments in the A-band. Nebulin is anchored in the Z-disk and follows the thin filament nearly to its end. It is thought to stabilize the think filament against depolymerization. Obscurin binds to titin at the M-band and to ankyrin on the sarcoplasmic reticulum membrane, thereby helping to stabilize the SR along the outside of the myofibril.

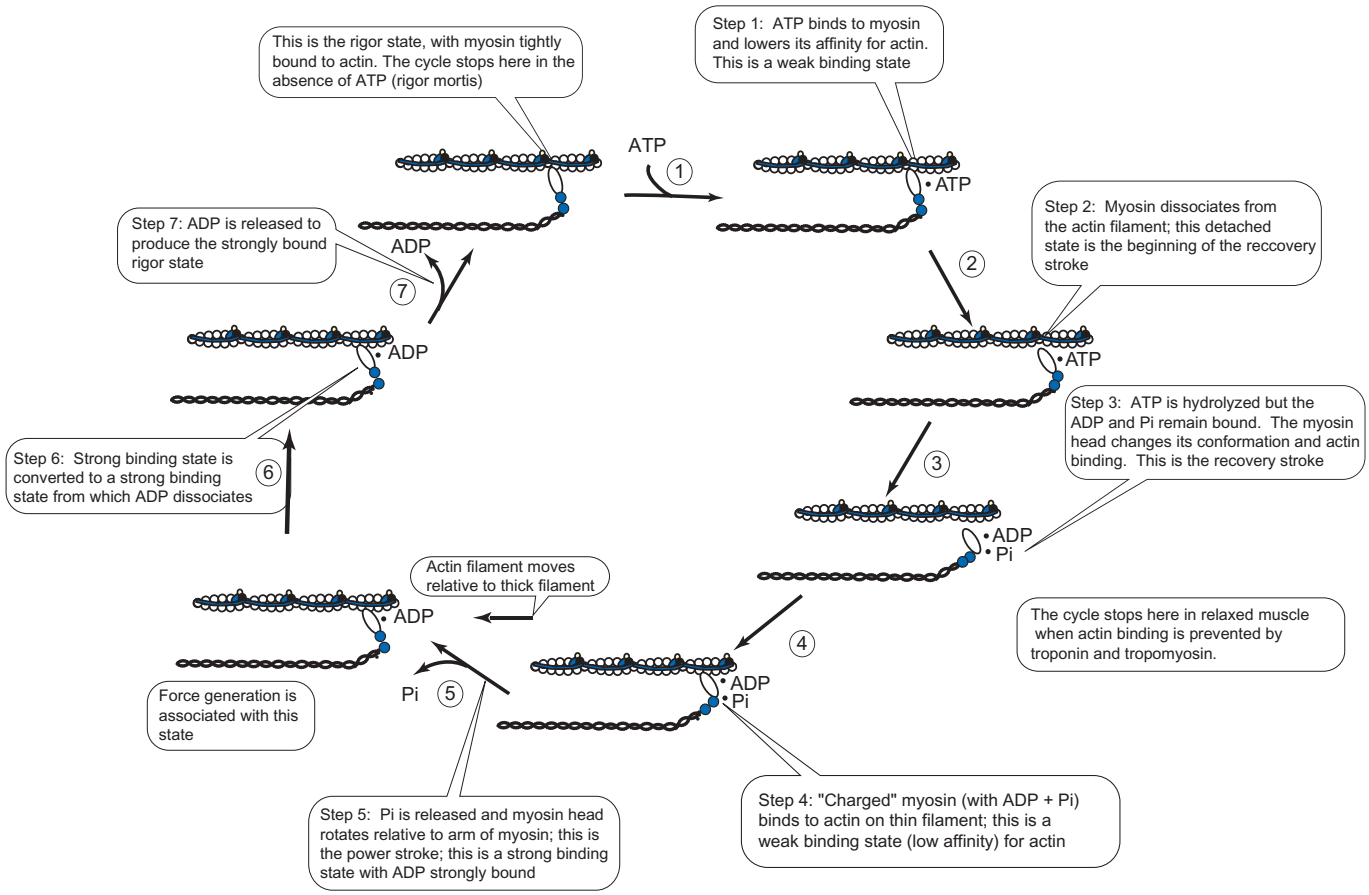

FIGURE 3.5.11 Reaction scheme for the acto-myosin ATPase cycle. Clockwise trace of the cycle shows ATP being converted to ADP + Pi over the entire cycle, steps 1–7. ATP binding to the rigor state in step 1 reduces the affinity of M for A, producing M* that dissociates from actin in step 2. ATP bound to M* is then hydrolyzed and the myosin head engages in a "recovery stroke" that re-establishes its resting position as M**. This form reattaches to A in step 4 and in step 5 it releases Pi and engages in the "power stroke" that moves the actin filament relative to the myosin filament. Here the complex is A•M*•ADP in which myosin has strong affinity for A and for ADP. In step 6, this is converted to A•M•ADP where myosin has strong affinity for A but from which ADP can dissociate. In step 7, ADP dissociates to form the rigor state where the cycle first began.

Obscurin (about 800 kDa) binds to titin and to ankyrin, an integral protein on the surface of the sarcoplasmic reticulum membrane. It is the only protein known to anchor membranes of the SR to the myofibrils. Obscurin is believed to be restricted to the surface of the myofibrils rather than penetrate through them as titin and nebulin do. It is located at the Z-line and M-band of developing muscle but seems to be restricted to the M-band in adult muscle.

CROSS-BRIDGES FROM THE THICK FILAMENT SPLIT ATP AND GENERATE FORCE

The interaction of myosin and actin has two consequences: hydrolysis of ATP to ADP and Pi, and the generation of force and/or shortening. The mechanism by which this happens can be viewed as a limited number of discrete states whose interconversions are governed by rate constants. There are several proposals for the overall mechanism of the acto-myosin ATPase, and a simplified form is shown in Figures 3.5.11 and 3.5.12. The overall reaction is called the **cross-bridge cycle**.

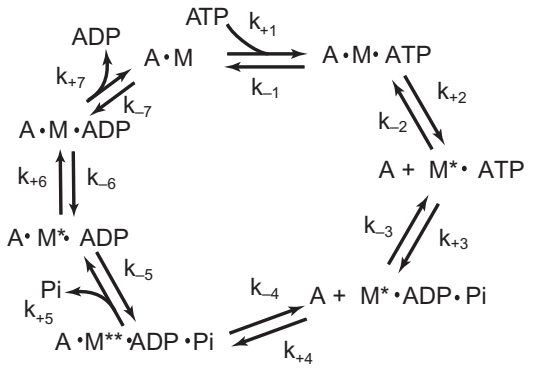

FIGURE 3.5.12 Alternate view of the acto-myosin reaction mechanism. Begin with A•M, the rigor state. Binding of ATP occurs first in step 1, which converts myosin to M* that has reduced affinity for actin and dissociates form A in step 2. ATP is hydrolyzed in step 3, causing strong binding to the actin filament in step 4. In step 5, phosphate (Pi) is released and myosin changes its conformation in the power stroke. In this state, myosin binds actin and ADP strongly. In step 6, this is converted to a myosin state that can dissociate ADP, which occurs in step 7. Each reaction step has forward and reverse rate constants that describe the rates of conversion in the forward and reverse partial reactions. A complete cycle results in the hydrolysis of ATP and the movement of the thin filament relative to the thick filament.

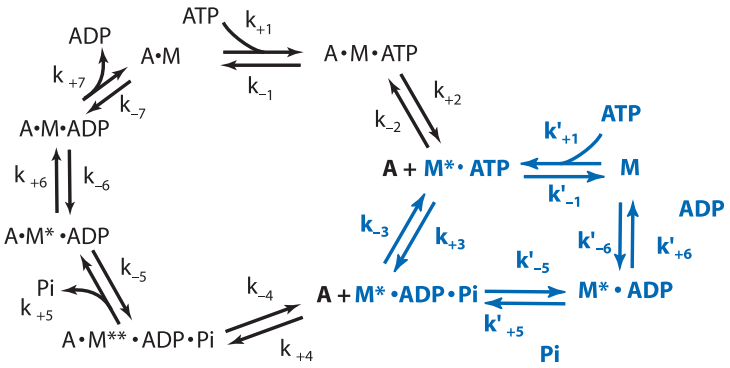

FIGURE 3.5.13 Myosin ATPase activity in the absence of actin. The acto-myosin cross-bridge mechanism is shown in black. Myosin-catalyzed ATPase activity is shown in blue. The rate constants for the transitions of myosin in the absence of actin are smaller than those in the presence of actin, particularly $k'_{+5} << k_{+5}$, so that ATPase activity in the absence of actin is much slower than in its presence: myosin is an actin-activated ATPase.

The most important concept of the acto-myosin cross-bridge cycle is that ATP hydrolysis is linked to sequential changes in the conformation, or 3D structure, of the myosin head and lever arm. This allows myosin to "walk" along the actin filament. Since myosin is connected to the thick filament, this movement of the myosin head corresponds to a movement of the thick filament relative to the thin filament.

The reaction scheme shown in Figures 3.5.11 and 3.5.12 is simplified and therefore incomplete. Myosin can split ATP all by itself, without actin, and this reaction is shown added on to the acto-myosin reaction scheme in Figure 3.5.13. However, the rate of ATP hydrolysis without actin is greatly reduced compared to the reaction with myosin in the presence of actin. Myosin is an actin-stimulated ATPase. Myosin binds to actin and this alters the rate of product release so that overall ATP hydrolysis is stimulated some 200- to 300-fold.

MYOSIN HEADS ARE INDEPENDENT BUT MAY COOPERATE THROUGH STRAIN ON THE CROSS-BRIDGE

Each myosin motor spends most of the time in the detached state and takes only one step along the actin filament before detaching again. The fraction of the time spent attached is generally referred to as the "duty cycle," symbolized as f, for the myosin motor. The efficient generation of force or shortening relies on a large number of these motors working together. Most models of muscle force generation assume that the two myosin heads work independently, meaning that the state of one myosin head does not directly affect the kinetics of any step involving a second myosin head. However, some of the reaction rates depend on the stress on the filaments, which in turn produces a strain. Because one myosin motor produces a strain on the thin and thick filaments, such strains can be communicated to nearby myosin heads and alter its kinetics through the effects of the strain.

For example, during eccentric contractions (contractions in which the muscle lengthens), the acto-myosin cross-bridge cycle is not completed. Instead, the myosin cross-bridges are strained and broken without the release of ADP—the myosin head detaches from the actin filament without releasing ADP. This transition is not shown in the reaction scheme of Figure 3.5.12. A consequence of this transition is that ATP hydrolysis is slowed during eccentric contractions. The value of k_{+6} in Figure 3.5.12 is believed to be dependent on the degree of strain, x. For low x, such as occurs in unloaded shortening, k_{+6} is large; for intermediate values of x, k_{+6} has intermediate values; for large x, k_{+6} is believed to be small. The consequence is that under loaded conditions the myosin head remains in the A•M*•ADP state for much longer. The duty ratio, f, could be 0.05 during unloaded shortening but f is much higher during eccentric or isometric contractions.

CROSS-BRIDGE CYCLING RATE EXPLAINS THE FIBER-TYPE DEPENDENCE OF THE FORCE—VELOCITY CURVE

The shortening of a muscle is the result of the shortening of its constituent sarcomeres. Thus if one sarcomere shortens by a distance Δx, and a sarcomere in series with it shortens by the same amount, the total shortening is $2 \times \Delta x$. Since both of these sarcomeres shorten in the same time interval, Δt, the velocity of shortening is $\Delta x/\Delta t$ for one sarcomere and $2 \times \Delta x/\Delta t$ for two sarcomeres in series. Thus the velocity of shortening is directly related to the number of sarcomeres in series. The velocity of shortening of a muscle for any given load should depend on its length.

Each cross-bridge cycle makes a single step estimated from 4 to 11 nm along the thin filament. Rapid turnover of the cross-bridge means that more of these cycles occur per second, and therefore the thin filament slides past the thick filament more quickly. Thus the rate of shortening of each sarcomere, and therefore of the entire muscle, depends on the **turnover rate** of the cross-bridges. The kinetics of the cross-bridges depends in large part on the kinetics inherent in myosin. Muscles make a variety of different **isoforms** of myosin. These are encoded by separate genes. In the adult skeletal muscle, there are two basic varieties: slow myosin and fast myosin that differ in their heavy chains. The slow type I fibers have MHCIβ, fast type IIA fibers have MHCIIa, and type IIB fibers have MHCIIb. Intermediate fibers have MHCIId. The catalytic mechanisms for the two

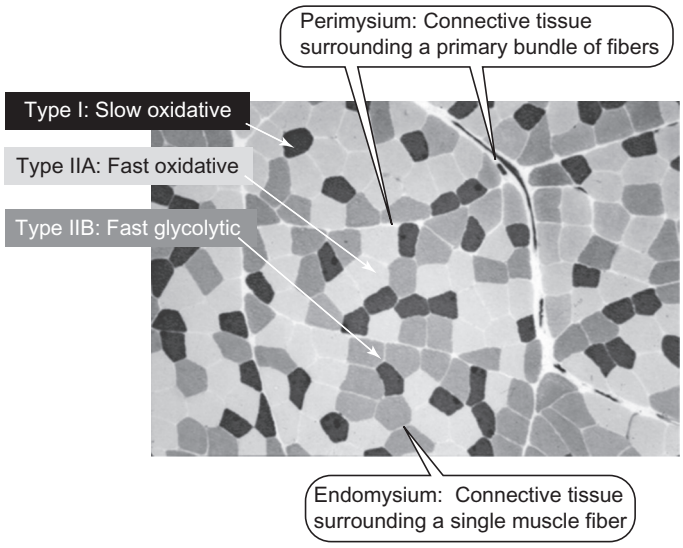

FIGURE 3.5.14 Histological staining of myosin reveals muscle heterogeneity. Myosin staining differentiates among various muscle types. This histological section shows three well-defined classes of staining but several intermediate fibers are also evident.

types of myosin, slow and fast, are similar. However, the turnover number (the number of completed reactions per second) is about $10\,s^{-1}$ for the fast myosin and $3\,s^{-1}$ for slow myosin. The kinetics of the cross-bridge cycling explains the different speeds of contraction of slow-twitch and fast-twitch muscle fibers.

The different myosin isoforms have different catalytic properties even though the basic mechanism is similar. These isoforms can be distinguished by staining for myosin ATPase activity by varying the incubation conditions such as the pH. On this basis, a variety of different staining patterns can be observed. Brooke has classified muscles as type I, type IIA, type IIB, and type IIC on the basis of myosin staining. The type I is the slow type of fiber and type II refers to different fast-twitch fiber types. Two types of myosin staining are available. The histological method uses differences in incubation conditions whereas myosin isoforms can be stained immunologically using antibodies directed against specific regions that vary among the isoforms. Figure 3.5.14 illustrates muscle heterogeneity as evidenced by myosin staining.

The force–velocity curve is also explained by the dependence of the reaction rate constants on strain. As force developed by the muscle increases, strain increases, and the duty cycle, the part of the time myosin stays attached, increases. This results in a decrease in turnover and a slower shortening velocity.

FORCE IS TRANSMITTED OUTSIDE THE CELL THROUGH THE CYTOSKELETON AND SPECIAL TRANSMEMBRANE PROTEINS

So far we have discussed the mechanism of force development or shortening as coming from the interaction of the thin filament protein actin, with the thick filament protein myosin. This force is ***inside*** the muscle fiber, whereas the force that is measured from muscle is ***outside*** the fibers. How does the force get outside? The short answer is this: the force of the myofilaments is transmitted laterally to the cytoskeleton elements, which then binds to discrete protein complexes at the **costameres**. Parts of this macromolecular complex also bind extracellular matrix proteins such as collagen and perlecan. Thus the myofilaments make indirect contact with the extracellular matrix through the cytoskeleton and the costameres.

Costameres were named because of their rib-like arrangement, revealed by the staining of the sarcolemma membrane with antibodies directed against specific proteins. These costameres are located at the Z-disk and M-line. In striated muscle, the myofilaments are connected to the costameres using either desmin intermediate filaments or actin microfilaments. There are three main types of complexes within costameres: (1) the **focal adhesion complex** involving transmembrane **integrins** linked to desmin through **talin** and **vinculin**, and linked to extracellular matrix fibers through **laminin**; (2) the **dystrophin–glycoprotein complex (DGC)** involving dystrophin to link γ actin on one end with syntrophin on the other, and a complex involving a number of proteins including α **dystroglycan** and β **dystroglycan** to link syntrophin to laminin, which makes final contact with the extracellular matrix; (3) a **spectrin-based filament network** making contact with membrane proteins through ankyrin. The structures of these costameres are still being worked out, and many more proteins than those listed here participate in it. Notable among these is **dystrophin**, a 427-kDa protein that is absent in **Duchenne muscular dystrophy**. This protein is localized to the periphery of the muscle fibers, on the cytosolic side of the sarcolemma, and binds to cytoskeletal elements. Although it is concentrated at the costameres it is not restricted to this

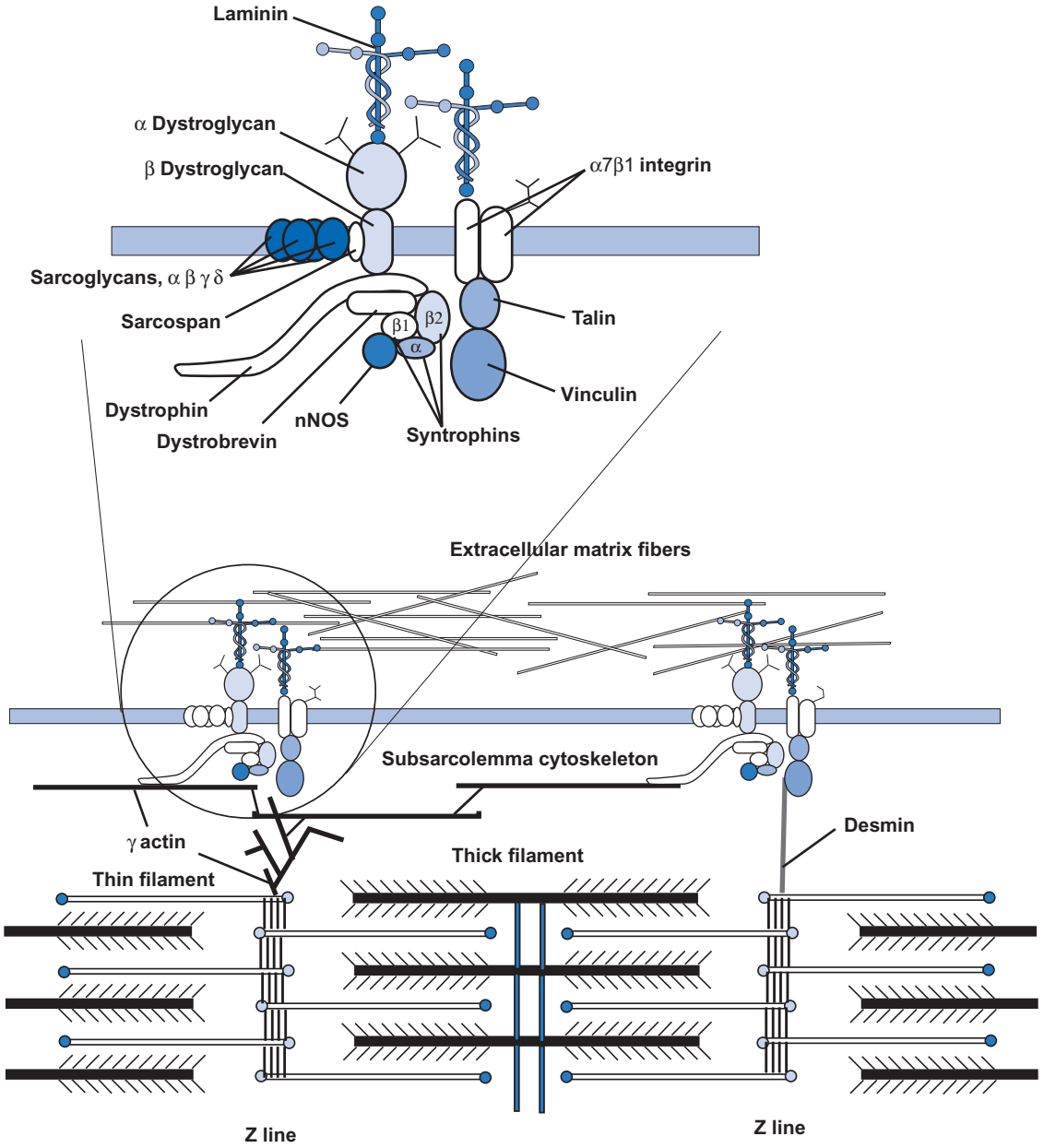

FIGURE 3.5.15 Proposed arrangement of proteins in costameres of skeletal muscle. The dystrophin–glycoprotein complex (DGC) consists of cytosolic proteins, transmembrane proteins, and extracellular proteins that link the myofilaments to the extracellular matrix through cytoskeleton filaments. In the DGC, Z-lines are linked to dystrophin through γ actin cytoskeletal filaments. Dystrophin binds to dystrobrevin and three syntrophins to form a complex with nNOS, neuronal nitric oxide synthetase. This complex thus participates in signaling of force either coming in (outside-in) or out (inside-out). Dystrophin also connects to β dystroglycan, a transmembrane protein that binds sarcospan and a complex of 4 sarcoglycans. The branched addendums to the proteins in the cartoon signify sugar residues added to the proteins. β dystroglycan forms a heterodimer with α dystroglycan, which binds laminin. Laminin is a heterotrimer that forms a cross-shaped protein that binds to collagen and other extracellular matrix proteins. The second major complex is connected to the Z-disks through desmin, an intermediate filament. This binds to vinculin, which binds talin, that binds the integral membrane protein, integrin. Integrin is a heterodimer that can be present in a variety of isoforms. The one in costameres is composed of α7 and β1. The integrin also binds laminin, which anchors the structure in the extracellular matrix. Integrins are associated with FAK (focal adhesion kinase) and ILK (Integrin-linked kinase), not shown.

location. The hypothetical disposition of cytoskeletal elements at the costameres is shown in Figure 3.5.15.

The function of the costameres is not yet established. The possibilities include: (1) transmit force from the contractile filaments to the outside of the cell (inside-out); (2) transmit force from the outside of muscle to the interior, involving signaling mechanisms (outside-in); (3) mechanically support the sarcolemma to protect it against damage, particularly during eccentric contractions; and (4) maintain uniform sarcomeric spacing between resting and active fibers of different motor units.

Clinical Applications: Muscular Dystrophy

The term "muscular dystrophy" includes a wide variety of inherited disorders characterized by progressive muscle weakness and wasting. The primary defect is in the muscle itself rather than in the nerve or neuromuscular junction. Part of this wide diversity of the disorder is summarized in Table 3.5.1.

What this list does is notify the observant student that there is much more to muscle than what we have discussed in the chapter. Some of the proteins in this list are not even mentioned in the chapter because a complete accounting of everything about muscle would occupy far too much space.

A single model to explain all of these muscular dystrophies does not yet exist. Many of the proteins whose defects cause muscular dystrophy are involved in linking the myofilament force generators to the extracellular matrix, and so they may be involved in the transmission of force or in protecting the integrity of the cell when exposed to large forces. They may also be involved in cellular signaling, particularly through nNOS, neuronal nitric oxide synthetase. Other proteins such as myotilin and telethonin may be involved in the integrity of the myofilaments themselves, rather than linking myofilaments to extracellular matrix. How mutations in the nuclear proteins emerin and lamin A/C can cause muscular dystrophy is less well understood. It may be that they participate in a larger karyocytoskeletal network (H.J. Spence, Y.-J. Chen, S.J. Winder, Muscular dystrophies, the cytoskeleton and cell adhesion, *Bio Essays* 24:542–552, 2002).

TABLE 3.5.1 Partial Listing of Muscular Dystrophies, with Their Mode of Inheritance, Gene Product, and the Normal Subcellular Locale of the Gene Product

Name	Mode of Inheritance	Gene Product	Subcellular Localization
Duchenne	XR	Dystrophin	Cytoskeleton
Emery–Dreifuss	XR	Emerin	Nucleus
Emery–Dreifuss	AD	Laminin A/C	Nucleus
LGMD 1A	AD	Myotilin	Myofilaments
LGMD 1B	AD	Lamin A/C	Nucleus
LGMD 1C	AD	Caveolin 3	Sarcolemma
LGMD 1E	AD	Filamin 2	Cytoskeleton
LGMD 2A	AR	Calpain 3	Cytosol
LGMD 2B	AR	Dysferlin	Sarcolemma
LGMD 2C	AR	γ-Sarcoglycan	Sarcolemma
LGMD 2D	AR	α-Sarcoglycan	Sarcolemma
LGMD 2E	AR	β-Sarcoglycan	Sarcolemma
LGMD 2F	AR	δ-Sarcoglycan	Sarcolemma
LGMD 2G	AR	Telethonin	Myofilaments
Congenital MD	AR	Laminin α2	Extracellular matrix

XR, X-linked recessive; AD, autosomal dominant; AR, autosomal recessive; LGMD, limb-girdle muscular dystrophy.

SUMMARY

Muscle fibers are large multinucleated cells whose most obvious histological feature is cross-striations. The cytoplasm contains many myofibrils—tiny cylinders consisting of bundles of myofilaments. The myofilaments include thick filaments, composed mainly of myosin, and thin filaments composed mainly of actin. The thick and thin filaments cause the cross-striations because of their regular overlap that is kept in register all across the diameter of the muscle fiber. The A-band corresponds to the thick filament. The I-band forms where the thin filament does not overlap the thick filament. Z-disks, from the German "zwischen," meaning "between," are centered in the I-band. The functional unit of contraction is the sarcomere, which extends from one Z-disk to the next. The M-line is in the middle of the A-band and corresponds to proteins that keep thick filaments in register and bound at their middle. It is surrounded by the H-zone, which is a clear zone corresponding to the part of the A-band where thin filaments do not overlap. During contraction, the A-band stays the same width whereas the I-band and H-zone both shrink.

The thick filament consists largely of myosin. Six proteins make up myosin: two heavy chains whose tails intertwine to form a supercoil and whose heads contain actin binding sites and a catalytic site for

ATP hydrolysis. Two myosin light chains bind to each head region. These myosin molecules assemble on a titin scaffold with their tails pointing toward the M-line. Because of this, there is a bare zone about 0.3 μm in the center of the thick filament that has no myosin heads.

Globular actin assembles to form a linear chain of filamentous actin. Two chains of F-actin intertwine to form the thin filament. The giant protein nebulin may set the length of the filament. Each half-turn of the filament binds a complex of tropomyosin and troponin. Troponin itself is a complex of: troponin T that binds tropomyosin; troponin I that inhibits actin availability to the thick filament; and troponin C that binds Ca^{2+}. Calcium binding to troponin C regulates the contractile state of the muscle.

Myosin is an actin-activated ATPase. When actin is available, myosin with ADP and Pi bound to it binds to the actin, followed by a release of ADP and Pi and a conformational change in the orientation of the head. Because myosin is bound to the thin filament, the conformational change exerts a force on the thin filament. ATP binding causes the head to dissociate from the actin, and subsequent hydrolysis of ATP to ADP and Pi converts the myosin back to its original form that can bind another actin. This cross-bridge cycle hydrolyzes ATP and develops force or shortens the sarcomere.

Myosin exists in multiple isoforms that can be distinguished by myosin staining. Slow-twitch muscles have myosin I and fast-twitch muscles have isoforms of myosin II. They differ in their turnover numbers. Faster cross-bridge cycling causes faster shortening.

The whole point of muscles is to deliver force to the outside of the muscle. This is accomplished by transferring the force to cytoskeletal elements that connect the internal myofilaments to special proteins that penetrate the membrane. These proteins transfer the force to the extracellular matrix at special regions called costameres. Dystrophin, the protein lacking in some kinds of muscular dystrophy, is concentrated at the costameres.

REVIEW QUESTIONS

1. What causes the cross-striations in skeletal muscle?
2. How large are muscle fibers? Why do they have lots of nuclei? Where are the nuclei located?
3. What is a myofibril? What are thick filaments? Thin filaments? What is the A-band? I-band? H-zone? Z-disk? M-zone? What happens to each during contraction?
4. Why is the top of the length–tension curve flat?
5. What proteins are found on the thick filament? Thin filament? What anchors the filaments?
6. What is the cross-bridge cycle? What hydrolyzes ATP?
7. What determines the speed of contraction? What is responsible for the different speeds of fast- and slow-twitch muscles?
8. How does muscle force, generated by the myofilaments, get out of the muscle fiber?