Muscle & the Cytoskeleton

49

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Proteins play an important role in movement at both the organ (eg, skeletal muscle, heart, and gut) and cellular levels. In this chapter, the roles of specific proteins and certain other key molecules (eg, Ca^{2+}) in **muscular contraction** are described. A brief coverage of **cytoskeletal proteins** is also presented.

Knowledge of the molecular bases of a number of conditions that affect muscle has advanced greatly in recent years. Understanding of the molecular basis of **Duchenne-type muscular dystrophy** was greatly enhanced when it was found that it was due to mutations in the gene encoding dystrophin. Significant progress has also been made in understanding the molecular basis of **malignant hyperthermia,** a serious complication for some patients undergoing certain types of anesthesia. **Heart failure** is a very common medical condition, with a variety of causes; its rational therapy requires understanding of the biochemistry of heart muscle. One group of conditions that cause heart failure are the **cardiomyopathies,** some of which are genetically determined. **Nitric oxide** (NO) has been found to be a major regulator of smooth muscle tone. Many widely used **vasodilators**—such as nitroglycerin, used in the treatment of angina pectoris—act by increasing the formation of NO. Muscle, partly because of its mass, plays major roles in the **overall metabolism** of the body.

MUSCLE TRANSDUCES CHEMICAL ENERGY INTO MECHANICAL ENERGY

Muscle is the major biochemical transducer (machine) that converts potential (chemical) energy into kinetic (mechanical) energy. Muscle, the largest single tissue in the human body, makes up somewhat less than 25% of body mass at birth, more than 40% in the young adult, and somewhat less than 30% in the aged adult. We shall discuss aspects of the three types of muscle found in vertebrates: **skeletal, cardiac,** and **smooth.** Both skeletal and cardiac muscle appear **striated** upon microscopic observation; smooth muscle is **nonstriated.** Although skeletal muscle is under voluntary nervous control, the control of both cardiac and smooth muscle is involuntary.

The Sarcoplasm of Muscle Cells Contains ATP, Phosphocreatine, & Glycolytic Enzymes

Striated muscle is composed of multinucleated muscle fiber cells surrounded by an electrically excitable plasma membrane, the **sarcolemma.** An individual muscle fiber cell, which may extend the entire length of the muscle, contains a bundle of many **myofibrils** arranged in parallel, embedded in intracellular fluid termed **sarcoplasm.** Within this fluid is contained glycogen, the high-energy compounds ATP and phosphocreatine, and the enzymes of glycolysis.

The Sarcomere Is the Functional Unit of Muscle

An overall view of voluntary muscle at several levels of organization is presented in Figure 49–1.

When the myofibril is examined by electron microscopy, alternating dark and light bands (anisotropic bands, meaning birefringent in polarized light; and isotropic bands, meaning not altered by polarized light) can be observed. These bands are thus referred to as **A and I bands,** respectively. The central region of the A band (the H band) appears less dense than the rest of the band. The I band is bisected by a very dense and narrow **Z line** (Figure 49–2).

The **sarcomere** is defined as the region between two Z lines (Figures 49–1 and 49–2) and is repeated along the axis of a fibril at distances of 1500–2300 nm depending upon the state of contraction.

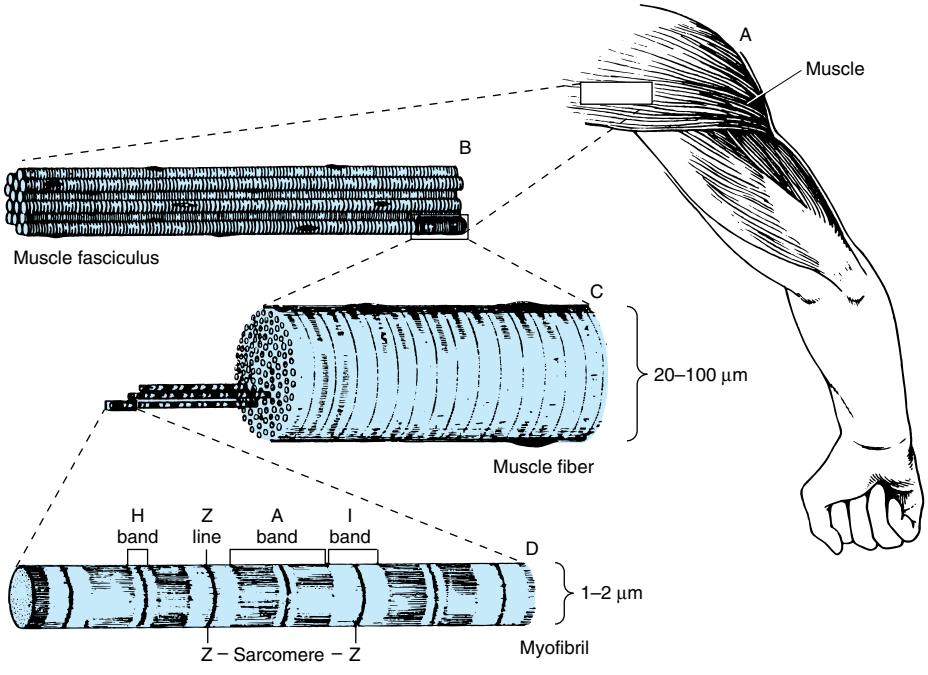

Figure 49–1. **The structure of voluntary muscle. The sarcomere is the region between the Z lines.** (Drawing by Sylvia Colard Keene. Reproduced, with permission, from Bloom W, Fawcett DW: *A Textbook of Histology,* 10th ed. Saunders, 1975.)

The striated appearance of voluntary and cardiac muscle in light microscopic studies results from their high degree of organization, in which most muscle fiber cells are aligned so that their sarcomeres are in parallel register (Figure 49–1).

Thick Filaments Contain Myosin; Thin Filaments Contain Actin, Tropomyosin, & Troponin

When myofibrils are examined by electron microscopy, it appears that each one is constructed of two types of longitudinal filaments. One type, the **thick filament,** confined to the A band, contains chiefly the protein myosin. These filaments are about 16 nm in diameter and arranged in cross-section as a hexagonal array (Figure 49–2, center; right-hand cross-section).

The **thin filament** (about 7 nm in diameter) lies in the I band and extends into the A band but not into its H zone (Figure 49–2). Thin filaments contain the proteins actin, tropomyosin, and troponin (Figure 49–3). In the A band, the thin filaments are arranged around the thick (myosin) filament as a secondary hexagonal array. Each thin filament lies symmetrically between three thick filaments (Figure 49–2, center; mid cross-section), and each thick filament is surrounded symmetrically by six thin filaments.

The thick and thin filaments interact via cross-bridges that emerge at intervals of 14 nm along the thick filaments. As depicted in Figure 49–2, the cross-bridges (drawn as arrowheads at each end of the myosin filaments, but not shown extending fully across to the thin filaments) have opposite polarities at the two ends of the thick filaments. The two poles of the thick filaments are separated by a 150-nm segment (the M band, not labeled in the figure) that is free of projections.

The Sliding Filament Cross-Bridge Model Is the Foundation on Which Current Thinking About Muscle Contraction Is Built

This model was proposed independently in the 1950s by Henry Huxley and Andrew Huxley and their colleagues. It was largely based on careful morphologic observations on resting, extended, and contracting muscle. Basically, when muscle contracts, there is no change in the lengths of the thick and thin filaments, but the H zones and the I bands shorten (see legend to Fig-

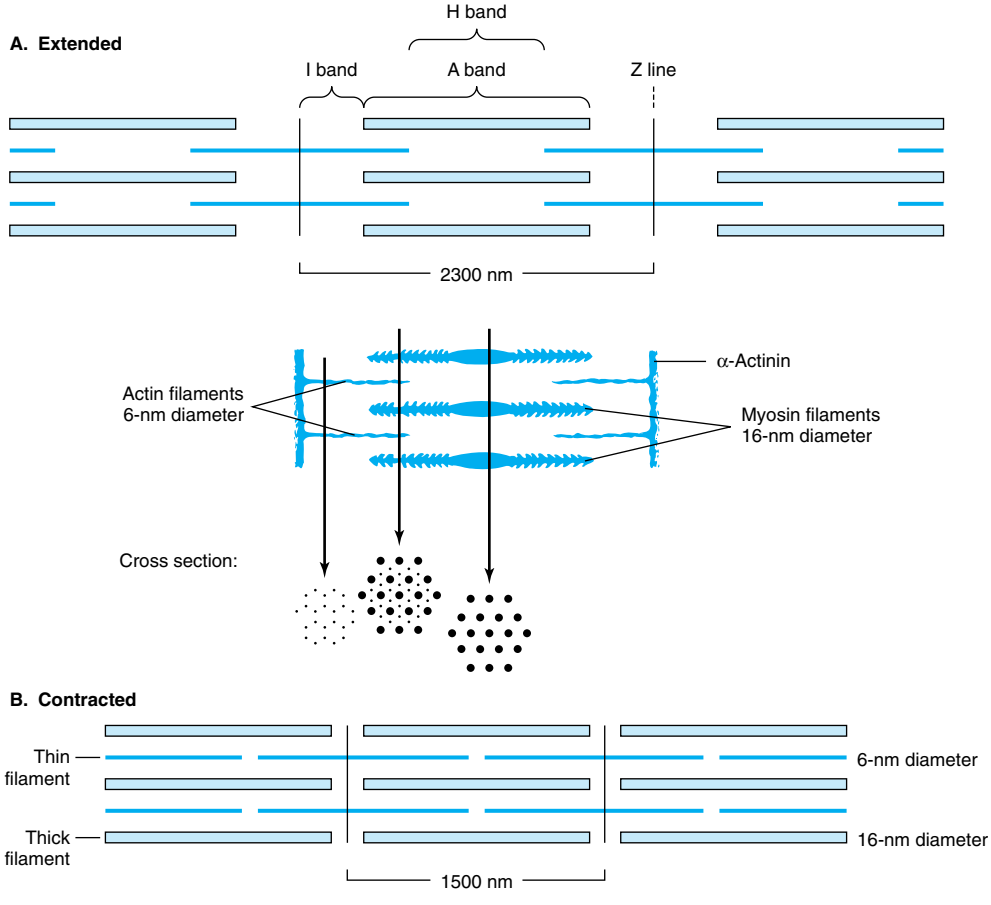

Figure 49–2. Arrangement of filaments in striated muscle. **A:** Extended. The positions of the I, A, and H bands in the extended state are shown. The thin filaments partly overlap the ends of the thick filaments, and the thin filaments are shown anchored in the Z lines (often called Z disks). In the lower part of Figure 49–2A, "arrowheads," pointing in opposite directions, are shown emanating from the myosin (thick) filaments. Four actin (thin) filaments are shown attached to two Z lines via α-actinin. The central region of the three myosin filaments, free of arrowheads, is called the M band (not labeled). Cross-sections through the M bands, through an area where myosin and actin filaments overlap and through an area in which solely actin filaments are present, are shown. **B:** Contracted. The actin filaments are seen to have slipped along the sides of the myosin fibers toward each other. The lengths of the thick filaments (indicated by the A bands) and the thin filaments (distance between Z lines and the adjacent edges of the H bands) have not changed. However, the lengths of the sarcomeres have been reduced (from 2300 nm to 1500 nm), and the lengths of the H and I bands are also reduced because of the overlap between the thick and thin filaments. These morphologic observations provided part of the basis for the sliding filament model of muscle contraction.

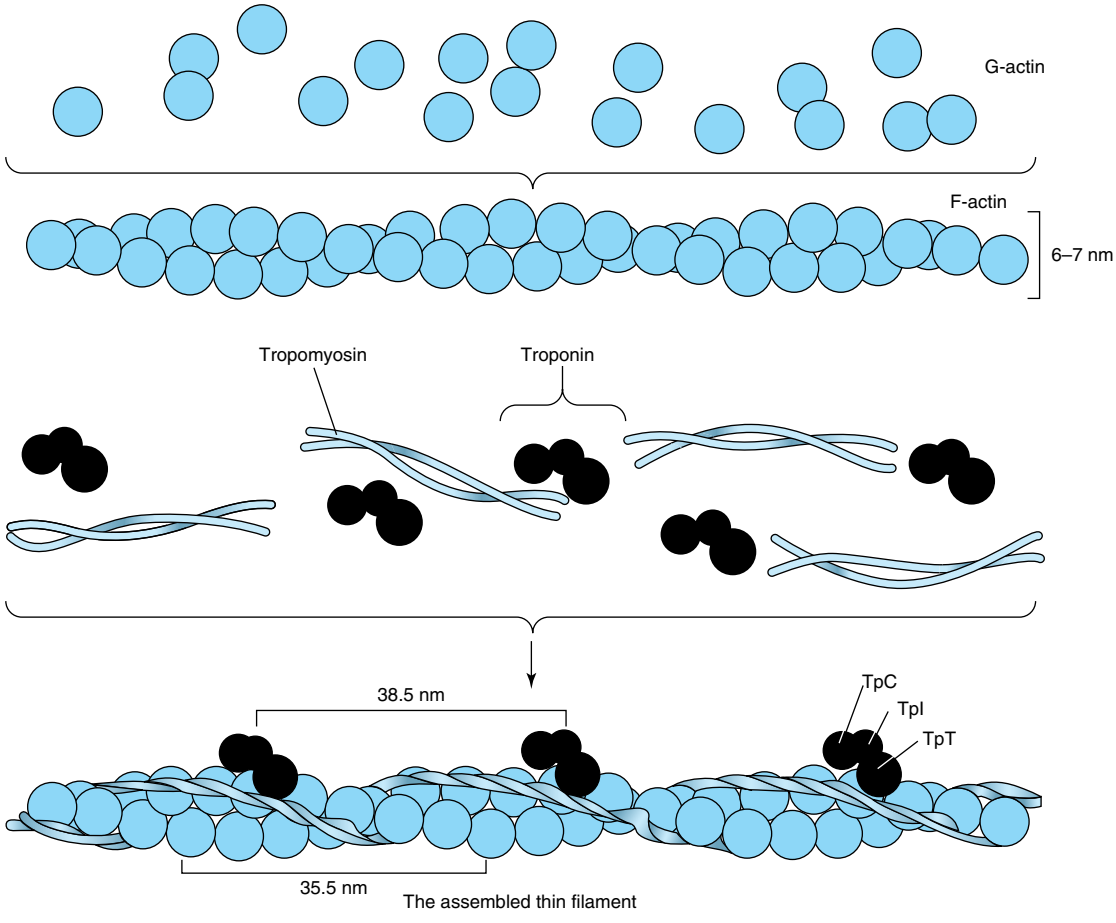

Figure 49–3. Schematic representation of the thin filament, showing the spatial configuration of its three major protein components: actin, myosin, and tropomyosin. The upper panel shows individual molecules of G-actin. The middle panel shows actin monomers assembled into F-actin. Individual molecules of tropomyosin (two strands wound around one another) and of troponin (made up of its three subunits) are also shown. The lower panel shows the assembled thin filament, consisting of F-actin, tropomyosin, and the three subunits of troponin (TpC, TpI, and TpT).

ure 49–2). Thus, the arrays of interdigitating filaments must slide past one another during contraction. Cross-bridges that link thick and thin filaments at certain stages in the contraction cycle generate and sustain the tension. The tension developed during muscle contraction is proportionate to the filament overlap and to the number of cross-bridges. Each cross-bridge head is connected to the thick filament via a flexible fibrous segment that can bend outward from the thick filament. This flexible segment facilitates contact of the head with the thin filament when necessary but is also sufficiently pliant to be accommodated in the interfilament spacing.

ACTIN & MYOSIN ARE THE MAJOR PROTEINS OF MUSCLE

The mass of a muscle is made up of 75% water and more than 20% protein. The two major proteins are actin and myosin.

Monomeric **G-actin** (43 kDa; G, globular) makes up 25% of muscle protein by weight. At physiologic ionic strength and in the presence of Mg^{2+}, G-actin polymerizes noncovalently to form an insoluble double helical filament called F-actin (Figure 49–3). The **F-actin** fiber is 6–7 nm thick and has a pitch or repeating structure every 35.5 nm.

Myosins constitute a family of proteins, with at least 15 members having been identified. The myosin discussed in this chapter is myosin-II, and when myosin is referred to in this text, it is this species that is meant unless otherwise indicated. Myosin-I is a monomeric species that binds to cell membranes. It may serve as a linkage between microfilaments and the cell membrane in certain locations.

Myosin contributes 55% of muscle protein by weight and forms the thick filaments. It is an asymmetric hexamer with a molecular mass of approximately 460 kDa. Myosin has a fibrous tail consisting of two intertwined helices. Each helix has a globular head portion attached at one end (Figure 49–4). The hexamer consists of one pair of **heavy (H) chains** each of approximately 200 kDA molecular mass, and two pairs of **light (L) chains** each with a molecular mass of approximately 20 kDa. The L chains differ, one being called the essential light chain and the other the regulatory light chain. Skeletal muscle myosin binds actin to form actomyosin (actin-myosin), and its intrinsic ATPase activity is markedly enhanced in this complex. Isoforms of myosin exist whose amounts can vary in different anatomic, physiologic, and pathologic situations.

The structures of actin and of the head of myosin have been determined by x-ray crystallography; these studies have confirmed a number of earlier findings concerning their structures and have also given rise to much new information.

Limited Digestion of Myosin With Proteases Has Helped to Elucidate Its Structure & Function

When myosin is digested with trypsin, two myosin fragments (meromyosins) are generated. **Light meromyosin** (LMM) consists of aggregated, insoluble α-helical fibers from the tail of myosin (Figure 49–4). LMM

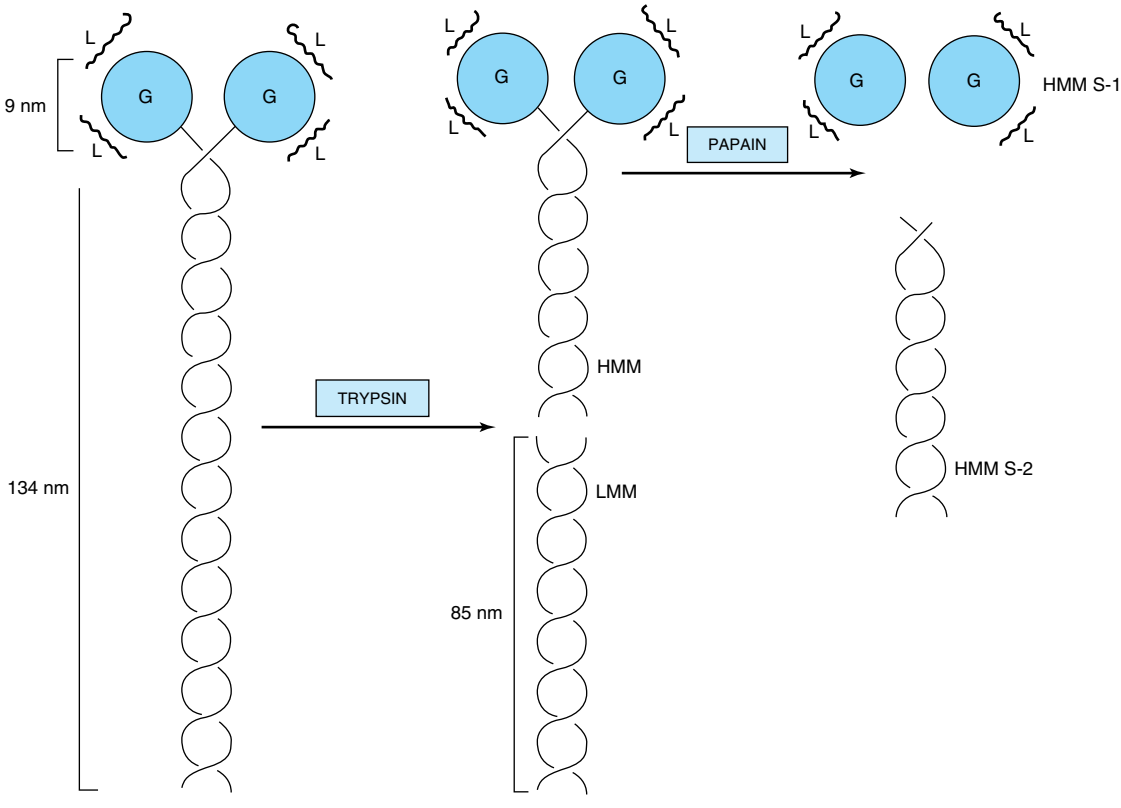

Figure 49–4. Diagram of a myosin molecule showing the two intertwined α-helices (fibrous portion), the globular region or head (G), the light chains (L), and the effects of proteolytic cleavage by trypsin and papain. The globular region (myosin head) contains an actin-binding site and an L chain-binding site and also attaches to the remainder of the myosin molecule.

exhibits no ATPase activity and does not bind to F-actin.

Heavy meromyosin (HMM; molecular mass about 340 kDa) is a soluble protein that has both a fibrous portion and a globular portion (Figure 49–4). It exhibits ATPase activity and binds to F-actin. Digestion of HMM with papain generates two subfragments, S-1 and S-2. The S-2 fragment is fibrous in character, has no ATPase activity, and does not bind to F-actin.

S-1 (molecular mass approximately 115 kDa) does exhibit ATPase activity, binds L chains, and in the absence of ATP will bind to and decorate actin with "arrowheads" (Figure 49–5). Both S-1 and HMM exhibit ATPase activity, which is accelerated 100- to 200-fold by complexing with F-actin. As discussed below, F-actin greatly enhances the rate at which myosin ATPase releases its products, ADP and P_i. Thus, although F-actin does not affect the hydrolysis step per se, its ability to promote release of the products produced by the ATPase activity greatly accelerates the overall rate of catalysis.

CHANGES IN THE CONFORMATION OF THE HEAD OF MYOSIN DRIVE MUSCLE CONTRACTION

How can hydrolysis of ATP produce macroscopic movement? Muscle contraction essentially consists of the cyclic attachment and detachment of the S-1 head of myosin to the F-actin filaments. This process can also be referred to as the making and breaking of cross-bridges. The attachment of actin to myosin is followed by conformational changes which are of particular importance in the S-1 head and are dependent upon which nucleotide is present (ADP or ATP). These changes result in the **power stroke,** which drives movement of actin filaments past myosin filaments. The energy for the power stroke is ultimately supplied by **ATP,** which is hydrolyzed to ADP and P_i. However, the power stroke itself occurs as a result of **conformational changes** in the myosin head when **ADP** leaves it.

The major biochemical events occurring during one cycle of muscle contraction and relaxation can be represented in the five steps shown in Figure 49–6:

(1) In the **relaxation phase** of muscle contraction, the S-1 head of myosin hydrolyzes ATP to ADP and P_i, but these products remain bound. The resultant ADP-P_i-myosin complex has been energized and is in a so-called high-energy conformation.

(2) When **contraction** of muscle is stimulated (via events involving Ca^{2+}, troponin, tropomyosin, and actin, which are described below), actin becomes accessible and the S-1 head of myosin finds it, binds it, and forms the actin-myosin-ADP-P_i complex indicated.

(3) Formation of this complex **promotes the release of P_i,** which initiates the power stroke. This is followed by release of ADP and is accompanied by a large conformational change in the head of myosin in relation to its tail (Figure 49–7), pulling actin about 10 nm toward the center of the sarcomere. This is the power stroke. The myosin is now in a so-called low-energy state, indicated as actin-myosin.

(4) Another molecule of ATP binds to the S-1 head, forming an actin-myosin-ATP complex.

(5) Myosin-ATP has a low affinity for actin, and **actin is thus released.** This last step is a key component of relaxation and is dependent upon the binding of ATP to the actin-myosin complex.

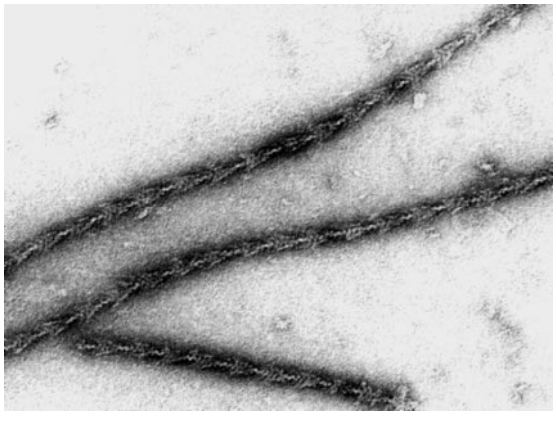

Figure 49–5. The decoration of actin filaments with the S-1 fragments of myosin to form "arrowheads." (Courtesy of JA Spudich.)

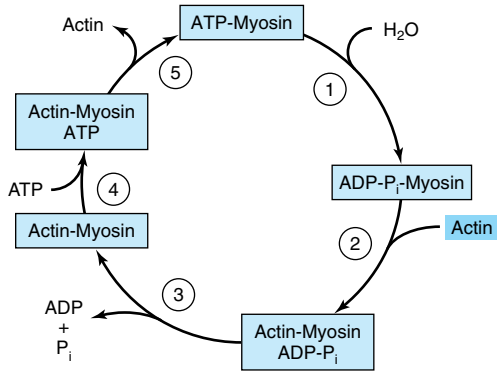

Figure 49–6. The hydrolysis of ATP drives the cyclic association and dissociation of actin and myosin in five reactions described in the text. (Modified from Stryer L: *Biochemistry,* 2nd ed. Freeman, 1981.)

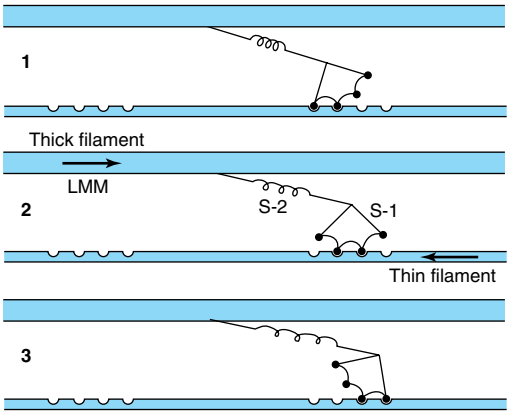

Figure 49–7. Representation of the active cross-bridges between thick and thin filaments. This diagram was adapted by AF Huxley from HE Huxley: The mechanism of muscular contraction. Science 1969;164:1356. The latter proposed that the force involved in muscular contraction originates in a tendency for the myosin head (S-1) to rotate relative to the thin filament and is transmitted to the thick filament by the S-2 portion of the myosin molecule acting as an inextensible link. Flexible points at each end of S-2 permit S-1 to rotate and allow for variations in the separation between filaments. The present figure is based on HE Huxley's proposal but also incorporates elastic (the coils in the S-2 portion) and stepwise-shortening elements (depicted here as four sites of interaction between the S-1 portion and the thin filament). (See Huxley AF, Simmons RM: Proposed mechanism of force generation in striated muscle. Nature [Lond] 1971;233:533.) The strengths of binding of the attached sites are higher in position 2 than in position 1 and higher in position 3 than position 2. The myosin head can be detached from position 3 with the utilization of a molecule of ATP; this is the predominant process during shortening. The myosin head is seen to vary in its position from about 90° to about 45°, as indicated in the text. (S-1, myosin head; S-2, portion of the myosin molecule; LMM, light meromyosin) (see legend to Figure 49–4). (Reproduced from Huxley AF: Muscular contraction. J Physiol 1974; 243:1. By kind permission of the author and the *Journal of Physiology*.)

Another cycle then commences with the hydrolysis of ATP (step 1 of Figure 49–6), re-forming the high-energy conformation.

Thus, hydrolysis of ATP is used to drive the cycle, with the actual power stroke being the conformational change in the S-1 head that occurs upon the release of ADP. The hinge regions of myosin (referred to as flexible points at each end of S-2 in the legend to Figure 49–7) permit the large range of movement of S-1 and also allow S-1 to find actin filaments.

If intracellular levels of ATP drop (eg, after death), ATP is not available to bind the S-1 head (step 4 above), actin does not dissociate, and relaxation (step 5) does not occur. This is the explanation for **rigor mortis,** the stiffening of the body that occurs after death.

Calculations have indicated that the efficiency of contraction is about 50%; that of the internal combustion engine is less than 20%.

Tropomyosin & the Troponin Complex Present in Thin Filaments Perform Key Functions in Striated Muscle

In striated muscle, there are two other proteins that are minor in terms of their mass but important in terms of their function. **Tropomyosin** is a fibrous molecule that consists of two chains, alpha and beta, that attach to F-actin in the groove between its filaments (Figure 49–3). Tropomyosin is present in all muscular and muscle-like structures. The **troponin complex** is unique to striated muscle and consists of three polypeptides. **Troponin T** (TpT) binds to tropomyosin as well as to the other two troponin components. **Troponin I** (TpI) inhibits the F-actin-myosin interaction and also binds to the other components of troponin. **Troponin C** (TpC) is a calcium-binding polypeptide that is structurally and functionally analogous to **calmodulin,** an important calcium-binding protein widely distributed in nature. Four molecules of calcium ion are bound per molecule of troponin C or calmodulin, and both molecules have a molecular mass of 17 kDa.

Ca^{2+} Plays a Central Role in Regulation of Muscle Contraction

The contraction of muscles from all sources occurs by the general mechanism described above. Muscles from different organisms and from different cells and tissues within the same organism may have different molecular mechanisms responsible for the regulation of their contraction and relaxation. In all systems, Ca^{2+} plays a key regulatory role. There are two general mechanisms of regulation of muscle contraction: **actin-based** and **myosin-based.** The former operates in skeletal and cardiac muscle, the latter in smooth muscle.

Actin-Based Regulation Occurs in Striated Muscle

Actin-based regulation of muscle occurs in vertebrate skeletal and cardiac muscles, both striated. In the gen-

eral mechanism described above (Figure 49–6), the only potentially limiting factor in the cycle of muscle contraction might be ATP. The skeletal muscle system is **inhibited** at rest; this inhibition is relieved to activate contraction. The inhibitor of striated muscle is the **troponin system,** which is bound to tropomyosin and F-actin in the thin filament (Figure 49–3). In striated muscle, there is no control of contraction unless the tropomyosin-troponin systems are present along with the actin and myosin filaments. As described above, **tropomyosin** lies along the groove of F-actin, and the three components of **troponin**—TpT, TpI, and TpC—are bound to the F-actin–tropomyosin complex. TpI prevents binding of the myosin head to its F-actin attachment site either by altering the conformation of F-actin via the tropomyosin molecules or by simply rolling tropomyosin into a position that directly blocks the sites on F-actin to which the myosin heads attach. Either way prevents activation of the myosin ATPase that is mediated by binding of the myosin head to F-actin. Hence, the TpI system blocks the contraction cycle at step 2 of Figure 49–6. This accounts for the inhibited state of relaxed striated muscle.

The Sarcoplasmic Reticulum Regulates Intracellular Levels of Ca^{2+} in Skeletal Muscle

In the sarcoplasm of resting muscle, the concentration of Ca^{2+} is 10^{-8} to 10^{-7} mol/L. The resting state is achieved because Ca^{2+} is pumped into the sarcoplasmic reticulum through the action of an active transport system, called the Ca^{2+} ATPase (Figure 49–8), initiating relaxation. The sarcoplasmic reticulum is a network of fine membranous sacs. Inside the sarcoplasmic reticulum, Ca^{2+} is bound to a specific Ca^{2+}-binding protein designated **calsequestrin.** The sarcomere is surrounded by an excitable membrane (the T tubule system) composed of transverse (T) channels closely associated with the sarcoplasmic reticulum.

When the sarcolemma is excited by a nerve impulse, the signal is transmitted into the T tubule system and a **Ca^{2+} release channel** in the nearby sarcoplasmic reticulum opens, releasing Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm. The concentration of Ca^{2+} in the sarcoplasm rises rapidly to 10^{-5} mol/L. The Ca^{2+}-binding sites on TpC in the thin filament are quickly occupied by Ca^{2+}. The TpC-4Ca^{2+} interacts with TpI and TpT to alter their interaction with tropomyosin. Accordingly, tropomyosin moves out of the way or alters the conformation of F-actin so that the myosin head-ADP-P_i (Figure 49–6) can interact with F-actin to start the contraction cycle.

The Ca^{2+} release channel is also known as the **ryanodine receptor** (RYR). There are two isoforms of this receptor, RYR1 and RYR2, the former being present in skeletal muscle and the latter in heart muscle and brain. **Ryanodine** is a plant alkaloid that binds to RYR1 and RYR2 specifically and modulates their activities. The Ca^{2+} release channel is a homotetramer made up of four subunits of kDa 565. It has transmembrane sequences at its carboxyl terminal, and these probably form the Ca^{2+} channel. The remainder of the protein protrudes into the cytosol, bridging the gap between the sarcoplasmic reticulum and the transverse tubular membrane. The channel is ligand-gated, Ca^{2+} and ATP working synergistically in vitro, although how it operates in vivo is not clear. A possible sequence of events leading to opening of the channel is shown in Figure 49–9. The channel lies very close to the **dihydropyridine receptor** (DHPR; a voltage-gated slow K type

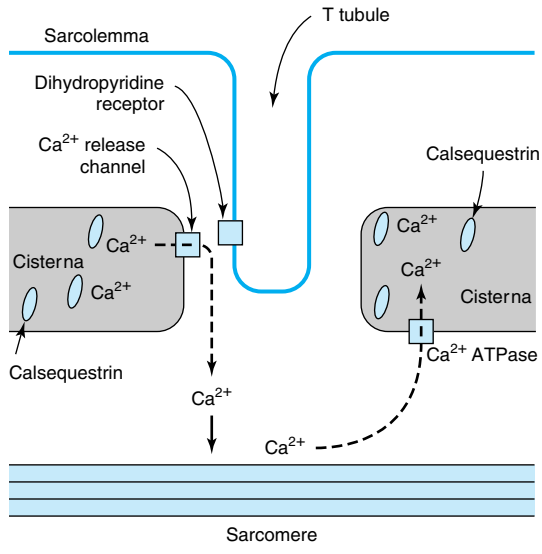

Figure 49–8. Diagram of the relationships among the sarcolemma (plasma membrane), a T tubule, and two cisternae of the sarcoplasmic reticulum of skeletal muscle (not to scale). The T tubule extends inward from the sarcolemma. A wave of depolarization, initiated by a nerve impulse, is transmitted from the sarcolemma down the T tubule. It is then conveyed to the Ca^{2+} release channel (ryanodine receptor), perhaps by interaction between it and the dihydropyridine receptor (slow Ca^{2+} voltage channel), which are shown in close proximity. Release of Ca^{2+} from the Ca^{2+} release channel into the cytosol initiates contraction. Subsequently, Ca^{2+} is pumped back into the cisternae of the sarcoplasmic reticulum by the Ca^{2+} ATPase (Ca^{2+} pump) and stored there, in part bound to calsequestrin.

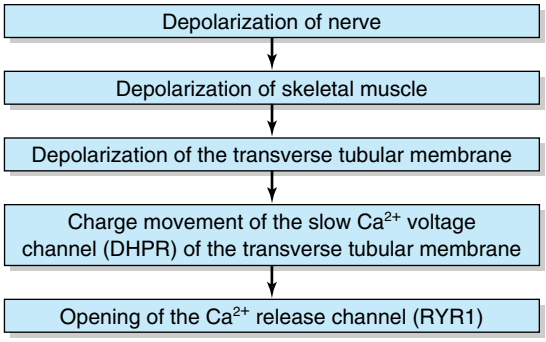

Figure 49–9. Possible chain of events leading to opening of the Ca^{2+} release channel. As indicated in the text, the Ca^{2+} voltage channel and the Ca^{2+} release channel have been shown to interact with each other in vitro via specific regions in their polypeptide chains. (DHPR, dihydropyridine receptor; RYR1, ryanodine receptor 1.)

Ca^{2+} channel) of the transverse tubule system (Figure 49–8). Experiments in vitro employing an affinity column chromatography approach have indicated that a 37-amino-acid stretch in RYR1 interacts with one specific loop of DHPR.

Relaxation occurs when sarcoplasmic Ca^{2+} falls below 10^{-7} mol/L owing to its resequestration into the sarcoplasmic reticulum by Ca^{2+} ATPase. TpC.4Ca^{2+} thus loses its Ca^{2+}. Consequently, troponin, via interaction with tropomyosin, inhibits further myosin head and F-actin interaction, and in the presence of ATP the myosin head detaches from the F-actin.

Thus, Ca^{2+} controls skeletal muscle contraction and relaxation by an allosteric mechanism mediated by TpC, TpI, TpT, tropomyosin, and F-actin.

A decrease in the concentration of **ATP** in the sarcoplasm (eg, by excessive usage during the cycle of contraction-relaxation or by diminished formation, such as might occur in ischemia) has two major effects: (1) The Ca^{2+} ATPase (Ca^{2+} pump) in the sarcoplasmic reticulum ceases to maintain the low concentration of Ca^{2+} in the sarcoplasm. Thus, the interaction of the myosin heads with F-actin is promoted. (2) The ATP-dependent detachment of myosin heads from F-actin cannot occur, and rigidity (contracture) sets in. The condition of **rigor mortis,** following death, is an extension of these events.

Muscle contraction is a delicate dynamic balance of the attachment and detachment of myosin heads to F-actin, subject to fine regulation via the nervous system.

Table 49–1 summarizes the overall events in contraction and relaxation of skeletal muscle.

Mutations in the Gene Encoding the Ca^{2+} Release Channel Are One Cause of Human Malignant Hyperthermia

Some genetically predisposed patients experience a severe reaction, designated malignant hyperthermia, on exposure to certain anesthetics (eg, halothane) and depolarizing skeletal muscle relaxants (eg, succinylcholine). The reaction consists primarily of rigidity of skeletal muscles, hypermetabolism, and high fever. A **high cytosolic concentration of Ca^{2+}** in skeletal muscle is a major factor in its causation. Unless malignant hyperthermia is recognized and treated immediately, patients may die acutely of ventricular fibrillation or survive to succumb subsequently from other serious complications. Appropriate treatment is to stop the anesthetic and administer the drug **dantrolene** intravenously. Dantrolene is a skeletal muscle relaxant that acts to inhibit release of Ca^{2+} from the sarcoplasmic reticulum into the cytosol, thus preventing the increase of cytosolic Ca^{2+} found in malignant hyperthermia.

Table 49–1. Sequence of events in contraction and relaxation of skeletal muscle.[1]

Steps in contraction

(1) Discharge of motor neuron
(2) Release of transmitter (acetylcholine) at motor endplate
(3) Binding of acetylcholine to nicotinic acetylcholine receptors
(4) Increased Na^+ and K^+ conductance in endplate membrane
(5) Generation of endplate potential
(6) Generation of action potential in muscle fibers
(7) Inward spread of depolarization along T tubules
(8) Release of Ca^{2+} from terminal cisterns of sarcoplasmic reticulum and diffusion to thick and thin filaments
(9) Binding of Ca^{2+} to troponin C, uncovering myosin binding sites of actin
(10) Formation of cross-linkages between actin and myosin and sliding of thin on thick filaments, producing shortening

Steps in relaxation

(1) Ca^{2+} pumped back into sarcoplasmic reticulum
(2) Release of Ca^{2+} from troponin
(3) Cessation of interaction between actin and myosin

[1]Reproduced, with permission, from Ganong WF: *Review of Medical Physiology,* 21st ed. McGraw-Hill, 2003.

Malignant hyperthermia also occurs in swine. Susceptible animals homozygous for malignant hyperthermia respond to stress with a fatal reaction **(porcine stress syndrome)** similar to that exhibited by humans. If the reaction occurs prior to slaughter, it affects the quality of the pork adversely, resulting in an inferior product. Both events can result in considerable economic losses for the swine industry.

The finding of a high level of cytosolic Ca^{2+} in muscle in malignant hyperthermia suggested that the condition might be caused by abnormalities of the Ca^{2+} ATPase or of the Ca^{2+} release channel. No abnormalities were detected in the former, but sequencing of cDNAs for the latter protein proved insightful, particularly in swine. All cDNAs from swine with malignant hyperthermia so far examined have shown a substitution of T for C1843, resulting in the substitution of Cys for Arg^{615} in the Ca^{2+} release channel. The mutation affects the function of the channel in that it opens more easily and remains open longer; the net result is massive release of Ca^{2+} into the cytosol, ultimately causing sustained muscle contraction.

The picture is more complex in humans, since malignant hyperthermia exhibits **genetic heterogeneity.** Members of a number of families who suffer from malignant hyperthermia have not shown genetic linkage to the *RYR1* gene. Some humans susceptible to malignant hyperthermia have been found to exhibit the same mutation found in swine, and others have a variety of point mutations at different loci in the *RYR1* gene. Certain families with malignant hypertension have been found to have mutations affecting the DHPR. Figure 49–10 summarizes the probable chain of events in malignant hyperthermia. The major promise of these findings is that, once additional mutations are detected, it will be possible to **screen,** using suitable DNA probes, for individuals at risk of developing malignant hyperthermia during anesthesia. Current screening tests (eg, the in vitro caffeine-halothane test) are relatively unreliable. Affected individuals could then be given alternative anesthetics, which would not endanger their lives. It should also be possible, if desired, to eliminate malignant hyperthermia from swine populations using suitable breeding practices.

Another condition due to mutations in the *RYR1* gene is **central core disease.** This is a rare myopathy presenting in infancy with hypotonia and proximal muscle weakness. Electron microscopy reveals an absence of mitochondria in the center of many type I (see below) muscle fibers. Damage to mitochondria induced by high intracellular levels of Ca^{2+} secondary to abnormal functioning of *RYR1* appears to be responsible for the morphologic findings.

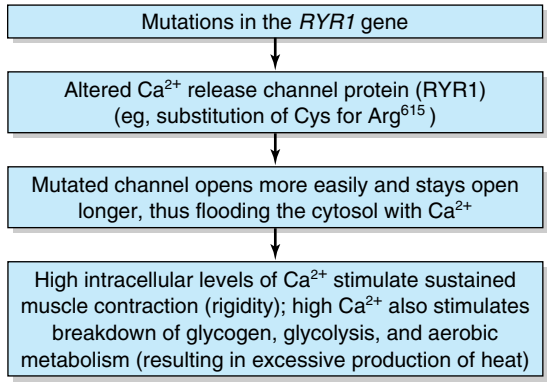

Figure 49–10. Simplified scheme of the causation of malignant hyperthermia (MIM 145600). At least 17 different point mutations have been detected in the *RYR1* gene, some of which are associated with central core disease (MIM 117000). It is estimated that at least 50% of families with members who have malignant hyperthermia are linked to the *RYR1* gene. Some individuals with mutations in the gene encoding DHPR have also been detected; it is possible that mutations in other genes for proteins involved in certain aspects of muscle metabolism will also be found.

MUTATIONS IN THE GENE ENCODING DYSTROPHIN CAUSE DUCHENNE MUSCULAR DYSTROPHY

A number of additional proteins play various roles in the structure and function of muscle. They include titin (the largest protein known), nebulin, α-actinin, desmin, dystrophin, and calcineurin. Some properties of these proteins are summarized in Table 49–2.

Dystrophin is of special interest. Mutations in the gene encoding this protein have been shown to be the cause of Duchenne muscular dystrophy and the milder Becker muscular dystrophy (see Figure 49–11). They are also implicated in some cases of dilated cardiomyopathy (see below). The gene encoding dystrophin is the largest gene known (≈ 2300 kb) and is situated on the X chromosome, accounting for the maternal inheritance pattern of Duchenne and Becker muscular dystrophies. As shown in Figure 49–12, dystrophin forms part of a large complex of proteins that attach to or interact with the plasmalemma. Dystrophin links the actin cytoskeleton to the ECM and appears to be needed for assembly of the synaptic junction. Impairment of these processes by formation of defective dystrophin is presumably critical in the causation of

Table 49–2. Some other important proteins of muscle.

Protein	Location	Comment or Function
Titin	Reaches from the Z line to the M line	Largest protein in body. Role in relaxation of muscle.
Nebulin	From Z line along length of actin filaments	May regulate assembly and length of actin filaments.
α-Actinin	Anchors actin to Z lines	Stabilizes actin filaments.
Desmin	Lies alongside actin filaments	Attaches to plasma membrane (plasmalemma).
Dystrophin	Attached to plasmalemma	Deficient in Duchenne muscular dystrophy. Mutations of its gene can also cause dilated cardiomyopathy.
Calcineurin	Cytosol	A calmodulin-regulated protein phosphatase. May play important roles in cardiac hypertrophy and in regulating amounts of slow and fast twitch muscles.
Myosin-binding protein C	Arranged transversely in sarcomere A-bands	Binds myosin and titin. Plays a role in maintaining the structural integrity of the sarcomere.

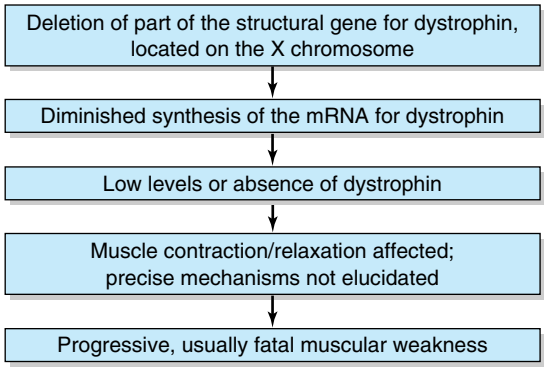

Figure 49–11. Summary of the causation of Duchenne muscular dystrophy (MIM 310200).

Duchenne muscular dystrophy. Mutations in the genes encoding some of the components of the sarcoglycan complex shown in Figure 49–12 are responsible for limb-girdle and certain other congenital forms of muscular dystrophy.

CARDIAC MUSCLE RESEMBLES SKELETAL MUSCLE IN MANY RESPECTS

The general picture of muscle contraction in the heart resembles that of skeletal muscle. Cardiac muscle, like skeletal muscle, is **striated** and uses the actin-myosin-tropomyosin-troponin system described above. Unlike skeletal muscle, cardiac muscle exhibits intrinsic rhythmicity, and individual myocytes communicate with each other because of its syncytial nature. The **T tubular system** is more developed in cardiac muscle, whereas the **sarcoplasmic reticulum** is less extensive and consequently the intracellular supply of Ca^{2+} for contraction is less. Cardiac muscle thus relies on **extracellular Ca^{2+}** for contraction; if isolated cardiac muscle is deprived of Ca^{2+}, it ceases to beat within approximately 1 minute, whereas skeletal muscle can continue to contract without an extracellular source of Ca^{2+}. **Cyclic AMP** plays a more prominent role in cardiac than in skeletal muscle. It modulates intracellular levels of Ca^{2+} through the activation of protein kinases; these enzymes phosphorylate various transport proteins in the sarcolemma and sarcoplasmic reticulum and also in the troponin-tropomyosin regulatory complex, affecting intracellular levels of Ca^{2+} or responses to it. There is a rough correlation between the phosphorylation of TpI and the increased contraction of cardiac muscle induced by catecholamines. This may account for the **inotropic effects** (increased contractility) of β-adrenergic compounds on the heart. Some differences among skeletal, cardiac, and smooth muscle are summarized in Table 49–3.

Ca^{2+} Enters Myocytes via Ca^{2+} Channels & Leaves via the Na^{+}-Ca^{2+} Exchanger & the Ca^{2+} ATPase

As stated above, extracellular Ca^{2+} plays an important role in contraction of cardiac muscle but not in skeletal muscle. This means that Ca^{2+} both enters and leaves myocytes in a regulated manner. We shall briefly consider three transmembrane proteins that play roles in this process.

A. Ca^{2+} Channels

Ca^{2+} enters myocytes via these channels, which allow entry only of Ca^{2+} ions. The major portal of entry is the

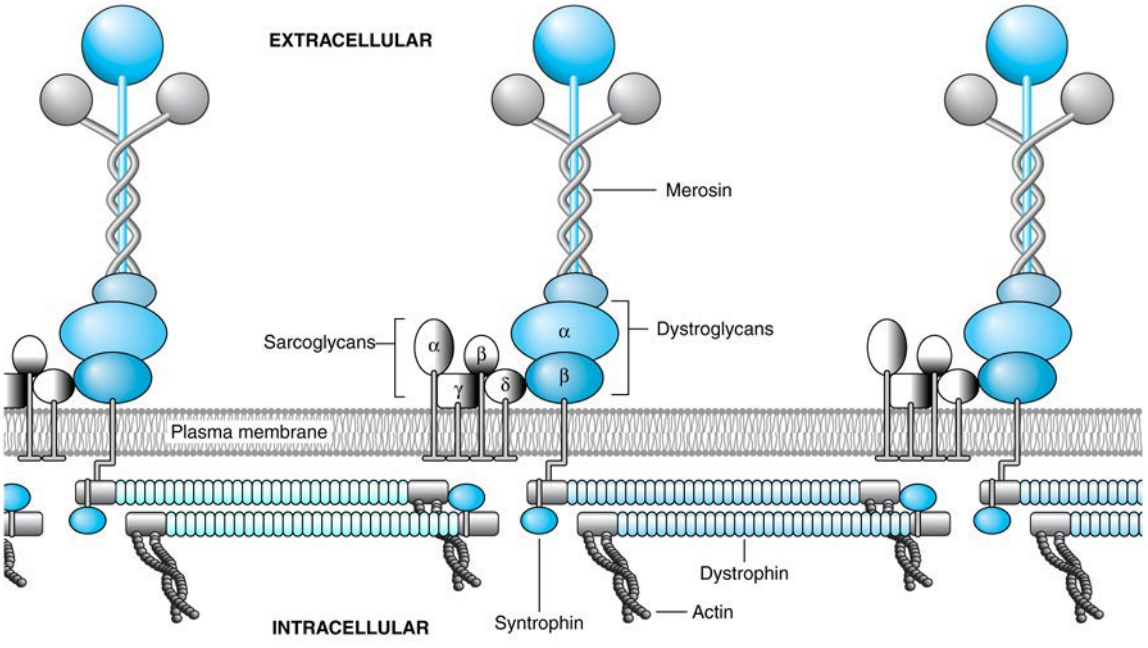

Figure 49–12. Organization of dystrophin and other proteins in relation to the plasma membrane of muscle cells. Dystrophin is part of a large oligomeric complex associated with several other protein complexes. The dystroglycan complex consists of α-dystroglycan, which associates with the basal lamina protein merosin, and β-dystroglycan, which binds α-dystroglycan and dystrophin. Syntrophin binds to the carboxyl terminal of dystrophin. The sarcoglycan complex consists of four transmembrane proteins: α-, β-, γ-, and δ-sarcoglycan. The function of the sarcoglycan complex and the nature of the interactions within the complex and between it and the other complexes are not clear. The sarcoglycan complex is formed only in striated muscle, and its subunits preferentially associate with each other, suggesting that the complex may function as a single unit. Mutations in the gene encoding dystrophin cause Duchenne and Becker muscular dystrophy; mutations in the genes encoding the various sarcoglycans have been shown to be responsible for limb-girdle dystrophies (eg, MIM 601173). (Reproduced, with permission, from Duggan DJ et al: Mutations in the sarcoglycan genes in patients with myopathy. N Engl J Med 1997;336:618.)

L-type (long-duration current, large conductance) or slow Ca^{2+} channel, which is voltage-gated, opening during depolarization induced by spread of the cardiac action potential and closing when the action potential declines. These channels are equivalent to the dihydropyridine receptors of skeletal muscle (Figure 49–8). Slow Ca^{2+} channels are regulated by cAMP-dependent protein kinases (stimulatory) and cGMP-protein kinases (inhibitory) and are blocked by so-called calcium channel blockers (eg, verapamil). Fast (or T, transient) Ca^{2+} channels are also present in the plasmalemma, though in much lower numbers; they probably contribute to the early phase of increase of myoplasmic Ca^{2+}.

The resultant increase of Ca^{2+} in the myoplasm acts on the Ca^{2+} release channel of the sarcoplasmic reticulum to open it. This is called Ca^{2+}-induced Ca^{2+} release (CICR). It is estimated that approximately 10% of the Ca^{2+} involved in contraction enters the cytosol from the extracellular fluid and 90% from the sarcoplasmic reticulum. However, the former 10% is important, as the rate of increase of Ca^{2+} in the myoplasm is important, and entry via the Ca^{2+} channels contributes appreciably to this.

B. Ca^{2+}-Na^+ Exchanger

This is the principal route of exit of Ca^{2+} from myocytes. In resting myocytes, it helps to maintain a low level of free intracellular Ca^{2+} by exchanging one Ca^{2+} for three Na^+. The energy for the uphill movement of Ca^{2+} out of the cell comes from the downhill movement of Na^+ into the cell from the plasma. This exchange contributes to relaxation but may run in the re-

Table 49–3. Some differences between skeletal, cardiac, and smooth muscle.

Skeletal Muscle	Cardiac Muscle	Smooth Muscle
1. Striated.	1. Striated.	1. Nonstriated.
2. No syncytium.	2. Syncytial.	2. Syncytial.
3. Small T tubules.	3. Large T tubules.	3. Generally rudimentary T tubules.
4. Sarcoplasmic reticulum well-developed and Ca^{2+} pump acts rapidly.	4. Sarcoplasmic reticulum present and Ca^{2+} pump acts relatively rapidly.	4. Sarcoplasmic reticulum often rudimentary and Ca^{2+} pump acts slowly.
5. Plasmalemma lacks many hormone receptors.	5. Plasmalemma contains a variety of receptors (eg, α- and β-adrenergic).	5. Plasmalemma contains a variety of receptors (eg, α- and β-adrenergic).
6. Nerve impulse initiates contraction.	6. Has intrinsic rhythmicity.	6. Contraction initiated by nerve impulses, hormones, etc.
7. Extracellular fluid Ca^{2+} not important for contraction.	7. Extracellular fluid Ca^{2+} important for contraction.	7. Extracellular fluid Ca^{2+} important for contraction.
8. Troponin system present.	8. Troponin system present.	8. Lacks troponin system; uses regulatory head of myosin.
9. Caldesmon not involved.	9. Caldesmon not involved.	9. Caldesmon is important regulatory protein.
10. Very rapid cycling of the cross-bridges.	10. Relatively rapid cycling of the cross-bridges.	10. Slow cycling of the cross-bridges permits slow prolonged contraction and less utilization of ATP.

verse direction during excitation. Because of the Ca^{2+}-Na^+ exchanger, anything that causes intracellular Na^+ (Na^+_i) to rise will secondarily cause Ca^{2+}_i to rise, causing more forceful contraction. This is referred to as a positive inotropic effect. One example is when the drug **digitalis** is used to treat heart failure. Digitalis inhibits the sarcolemmal Na^+-K^+ ATPase, diminishing exit of Na^+ and thus increasing Na^+_i. This in turn causes Ca^{2+} to increase, via the Ca^{2+}-Na^+ exchanger. The increased Ca^{2+}_i results in increased force of cardiac contraction, of benefit in heart failure.

C. Ca^{2+} ATPase

This Ca^{2+} pump, situated in the sarcolemma, also contributes to Ca^{2+} exit but is believed to play a relatively minor role as compared with the Ca^{2+}-Na^+ exchanger.

It should be noted that there are a variety of **ion channels** (Chapter 41) in most cells, for Na^+, K^+, Ca^{2+}, etc. Many of them have been cloned in recent years and their dispositions in their respective membranes worked out (number of times each one crosses its membrane, location of the actual ion transport site in the protein, etc). They can be classified as indicated in Table 49–4. Cardiac muscle is rich in ion channels, and they are also important in skeletal muscle. Mutations in genes encoding ion channels have been shown to be responsible for a number of relatively rare conditions affecting muscle. These and other diseases due to mutations of ion channels have been termed **channelopathies;** some are listed in Table 49–5.

Table 49–4. Major types of ion channels found in cells.

Type	Comment
External ligand-gated	Open in response to a specific extracellular molecule, eg, acetylcholine.
Internal ligand-gated	Open or close in response to a specific intracellular molecule, eg, a cyclic nucleotide.
Voltage-gated	Open in response to a change in membrane potential, eg, Na^+, K^+, and Ca^{2+} channels in heart.
Mechanically gated	Open in response to change in mechanical pressure.

Table 49–5. Some disorders (channelopathies) due to mutations in genes encoding polypeptide constituents of ion channels.[1]

Disorder[2]	Ion Channel and Major Organs Involved
Central core disease (MIM 117000)	Ca^{2+} release channel (RYR1) Skeletal muscle
Cystic fibrosis (MIM 219700)	CFTR (Cl^- channel) Lungs, pancreas
Hyperkalemic periodic paralysis (MIM 170500)	Sodium channel Skeletal muscle
Hypokalemic periodic paralysis (MIM 114208)	Slow Ca^{2+} voltage channel (DHPR) Skeletal muscle
Malignant hyperthermia (MIM 180901)	Ca^{2+} release channel (RYR1) Skeletal muscle
Myotonia congenita (MIM 160800)	Chloride channel Skeletal muscle

[1]Data in part from Ackerman NJ, Clapham DE: Ion channels—basic science and clinical disease. N Engl J Med 1997;336:1575.
[2]Other channelopathies include the long QT syndrome (MIM 192500); pseudoaldosteronism (Liddle syndrome, MIM 177200); persistent hyperinsulinemic hypoglycemia of infancy (MIM 601820); hereditary X-linked recessive type II nephrolithiasis of infancy (Dent syndrome, MIM 300009); and generalized myotonia, recessive (Becker disease, MIM 255700). The term "myotonia" signifies any condition in which muscles do not relax after contraction.

Inherited Cardiomyopathies Are Due to Disorders of Cardiac Energy Metabolism or to Abnormal Myocardial Proteins

An inherited cardiomyopathy is any structural or functional abnormality of the ventricular myocardium due to an inherited cause. There are nonheritable types of cardiomyopathy, but these will not be described here. As shown in Table 49–6, the causes of inherited cardiomyopathies fall into two broad classes: (1) disorders of cardiac energy metabolism, mainly reflecting mutations in genes encoding enzymes or proteins involved in fatty acid oxidation (a major source of energy for the myocardium) and oxidative phosphorylation; and (2) mutations in genes encoding proteins involved in or affecting myocardial contraction, such as myosin, tropomyosin, the troponins, and cardiac myosin-binding protein C. Mutations in the genes encoding these latter proteins cause familial hypertrophic cardiomyopathy, which will now be discussed.

Table 49–6. Biochemical causes of inherited cardiomyopathies.[1,2]

Cause	Proteins or Process Affected
Inborn errors of fatty acid oxidation	Carnitine entry into cells and mitochondria Certain enzymes of fatty acid oxidation
Disorders of mitochondrial oxidative phosphorylation	Proteins encoded by mitochondrial genes Proteins encoded by nuclear genes
Abnormalities of myocardial contractile and structural proteins	β-Myosin heavy chains, troponin, tropomyosin, dystrophin

[1]Based on Kelly DP, Strauss AW: Inherited cardiomyopathies. N Engl J Med 1994;330:913.
[2]Mutations (eg, point mutations, or in some cases deletions) in the genes (nuclear or mitochondrial) encoding various proteins, enzymes, or tRNA molecules are the fundamental causes of the inherited cardiomyopathies. Some conditions are mild, whereas others are severe and may be part of a syndrome affecting other tissues.

Mutations in the Cardiac β-Myosin Heavy Chain Gene Are One Cause of Familial Hypertrophic Cardiomyopathy

Familial hypertrophic cardiomyopathy is one of the most frequent hereditary cardiac diseases. Patients exhibit hypertrophy—often massive—of one or both ventricles, starting early in life, and not related to any extrinsic cause such as hypertension. Most cases are transmitted in an autosomal dominant manner; the rest are sporadic. Until recently, its cause was obscure. However, this situation changed when studies of one affected family showed that a **missense mutation** (ie, substitution of one amino acid by another) in the β-myosin heavy chain gene was responsible for the condition. Subsequent studies have shown a number of missense mutations in this gene, all coding for highly conserved residues. Some individuals have shown other mutations, such as formation of an α/β-myosin heavy chain hybrid gene. Patients with familial hypertrophic cardiomyopathy can show great variation in clinical picture. This in part reflects genetic heterogeneity; ie, mutation in a number of **other genes** (eg, those encoding cardiac actin, tropomyosin, cardiac troponins I and T, essential and regulatory myosin light chains, and cardiac myosin-binding protein C) may also cause familial hypertrophic

cardiomyopathy. In addition, mutations at different sites in the gene for β-myosin heavy chain may affect the function of the protein to a greater or lesser extent. The missense mutations are clustered in the head and head-rod regions of myosin heavy chain. One hypothesis is that the mutant polypeptides ("poison polypeptides") cause formation of abnormal myofibrils, eventually resulting in compensatory hypertrophy. Some mutations alter the charge of the amino acid (eg, substitution of arginine for glutamine), presumably affecting the conformation of the protein more markedly and thus affecting its function. Patients with these mutations have a significantly shorter life expectancy than patients in whom the mutation produced no alteration in charge. Thus, definition of the precise mutations involved in the genesis of FHC may prove to be of important prognostic value; it can be accomplished by appropriate use of the polymerase chain reaction on genomic DNA obtained from one sample of blood lymphocytes. Figure 49–13 is a simplified scheme of the events causing familial hypertrophic cardiomyopathy.

Another type of cardiomyopathy is termed **dilated cardiomyopathy.** Mutations in the genes encoding dystrophin, muscle LIM protein (so called because it was found to contain a cysteine-rich domain originally detected in three proteins: Lin-II, Isl-1, and Mec-3), and the cyclic response-element binding protein (CREB) have been implicated in the causation of this condition. The first two proteins help organize the contractile apparatus of cardiac muscle cells, and CREB is involved in the regulation of a number of genes in these cells. Current research is not only elucidating the molecular causes of the cardiomyopathies but is also disclosing mutations that cause **cardiac developmental disorders** (eg, septal defects) and arrhythmias (eg, due to mutations affecting ion channels).

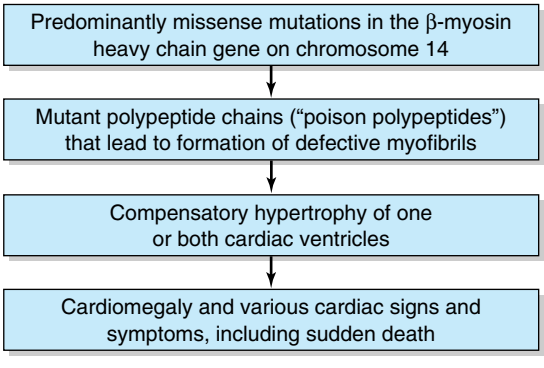

Figure 49–13. Simplified scheme of the causation of familial hypertrophic cardiomyopathy (MIM 192600) due to mutations in the gene encoding β-myosin heavy chain. Mutations in genes encoding other proteins, such as the troponins, tropomyosin, and cardiac myosin-binding protein C can also cause this condition. Mutations in genes encoding yet other proteins (eg, dystrophin) are involved in the causation of dilated cardiomyopathy.

Ca^{2+} Also Regulates Contraction of Smooth Muscle

While all muscles contain actin, myosin, and tropomyosin, only vertebrate **striated** muscles contain the troponin system. Thus, the mechanisms that regulate contraction must differ in various contractile systems.

Smooth muscles have molecular structures similar to those in striated muscle, but the sarcomeres are not aligned so as to generate the striated appearance. Smooth muscles contain α-actinin and tropomyosin molecules, as do skeletal muscles. They do not have the troponin system, and the light chains of smooth muscle myosin molecules differ from those of striated muscle myosin. Regulation of smooth muscle contraction is **myosin-based,** unlike striated muscle, which is actin-based. However, like striated muscle, smooth muscle contraction is regulated by Ca^{2+}.

Phosphorylation of Myosin Light Chains Initiates Contraction of Smooth Muscle

When smooth muscle myosin is bound to F-actin in the absence of other muscle proteins such as tropomyosin, there is no detectable ATPase activity. This absence of activity is quite unlike the situation described for striated muscle myosin and F-actin, which has abundant ATPase activity. Smooth muscle myosin contains light chains that prevent the binding of the myosin head to F-actin; they must be phosphorylated before they allow F-actin to activate myosin ATPase. The ATPase activity then attained hydrolyzes ATP about tenfold more slowly than the corresponding activity in skeletal muscle. The phosphate on the myosin light chains may form a chelate with the Ca^{2+} bound to the tropomyosin-TpC-actin complex, leading to an increased rate of formation of cross-bridges between the myosin heads and actin. The phosphorylation of light chains initiates the attachment-detachment contraction cycle of smooth muscle.

Myosin Light Chain Kinase Is Activated by Calmodulin-$4Ca^{2+}$ & Then Phosphorylates the Light Chains

Smooth muscle sarcoplasm contains a myosin light chain kinase that is calcium-dependent. The Ca^{2+} activation of myosin light chain kinase requires binding of **calmodulin-$4Ca^{2+}$** to its kinase subunit (Figure 49–14).

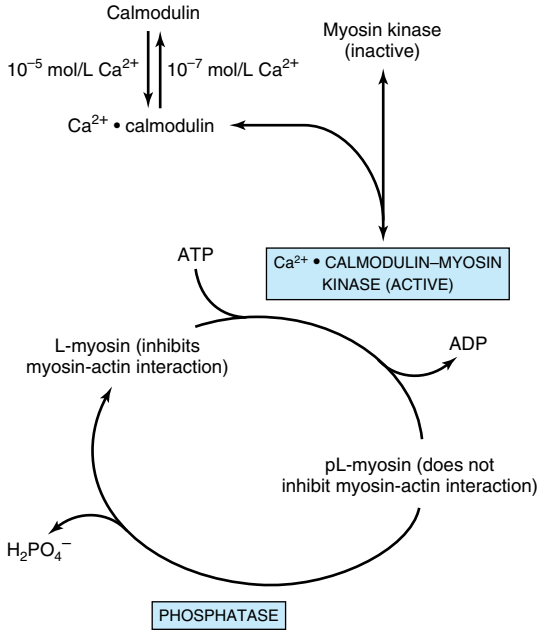

Figure 49–14. Regulation of smooth muscle contraction by Ca^{2+}. pL-myosin is the phosphorylated light chain of myosin; L-myosin is the dephosphorylated light chain. (Adapted from Adelstein RS, Eisenberg R: Regulation and kinetics of actin-myosin ATP interaction. Annu Rev Biochem 1980;49:921.)

The calmodulin-4Ca^{2+}-activated light chain kinase phosphorylates the light chains, which then ceases to inhibit the myosin–F-actin interaction. The contraction cycle then begins.

Smooth Muscle Relaxes When the Concentration of Ca^{2+} Falls Below 10^{-7} Molar

Relaxation of smooth muscle occurs when sarcoplasmic Ca^{2+} falls below 10^{-7} mol/L. The Ca^{2+} dissociates from calmodulin, which in turn dissociates from the myosin light chain kinase, inactivating the kinase. No new phosphates are attached to the p-light chain, and light chain protein phosphatase, which is continually active and calcium-independent, removes the existing phosphates from the light chains. Dephosphorylated myosin p-light chain then inhibits the binding of myosin heads to F-actin and the ATPase activity. The myosin head detaches from the F-actin in the presence of ATP, but it cannot reattach because of the presence of dephosphorylated p-light chain; hence, relaxation occurs.

Table 49–7 summarizes and compares the regulation of actin-myosin interactions (activation of myosin ATPase) in striated and smooth muscles.

The myosin light chain kinase is not directly affected or activated by cAMP. However, cAMP-activated protein kinase can phosphorylate the myosin light chain kinase (not the light chains themselves). The phosphorylated myosin light chain kinase exhibits a significantly lower affinity for calmodulin-Ca^{2+} and thus is less sensitive to activation. Accordingly, an increase in cAMP dampens the contraction response of smooth muscle to a given elevation of sarcoplasmic Ca^{2+}. This molecular mechanism can explain the relaxing effect of β-adrenergic stimulation on smooth muscle.

Another protein that appears to play a Ca^{2+}-dependent role in the regulation of smooth muscle contraction is **caldesmon** (87 kDa). This protein is ubiquitous in smooth muscle and is also found in nonmuscle tissue. At low concentrations of Ca^{2+}, it binds to tropomyosin and actin. This prevents interaction of actin with myosin, keeping muscle in a relaxed state. At higher concentrations of Ca^{2+}, Ca^{2+}-calmodulin binds caldesmon, releasing it from actin. The latter is then free to bind to myosin, and contraction can occur. Caldesmon is also subject to phosphorylation-dephosphorylation; when phosphorylated, it cannot bind actin, again freeing the latter to interact with myosin. Caldesmon may also participate in organizing the structure of the contractile apparatus in smooth muscle. Many of its effects have been demonstrated in vitro, and its physiologic significance is still under investigation.

As noted in Table 49–3, slow cycling of the crossbridges permits slow prolonged contraction of smooth muscle (eg, in viscera and blood vessels) with less utilization of ATP compared with striated muscle. The ability of smooth muscle to maintain force at reduced velocities of contraction is referred to as the **latch state;** this is an important feature of smooth muscle, and its precise molecular bases are under study.

Nitric Oxide Relaxes the Smooth Muscle of Blood Vessels & Also Has Many Other Important Biologic Functions

Acetylcholine is a vasodilator that acts by causing relaxation of the smooth muscle of blood vessels. However, it does not act directly on smooth muscle. A key observation was that if endothelial cells were stripped away from underlying smooth muscle cells, acetylcholine no longer exerted its vasodilator effect. This finding indicated that vasodilators such as acetylcholine initially interact with the endothelial cells of small blood vessels via receptors. The receptors are coupled to the phosphoinositide cycle, leading to the intracellular release of

Table 49–7. Actin-myosin interactions in striated and smooth muscle.

	Striated Muscle	Smooth Muscle (and Nonmuscle Cells)
Proteins of muscle filaments	Actin Myosin Tropomyosin Troponin (Tpl, TpT, TpC)	Actin Myosin[1] Tropomyosin
Spontaneous interaction of F-actin and myosin alone (spontaneous activation of myosin ATPase by F-actin	Yes	No
Inhibitor of F-actin–myosin interaction (inhibitor of F-actin–dependent activation of ATPase)	Troponin system (Tpl)	Unphosphorylated myosin light chain
Contraction activated by	Ca^{2+}	Ca^{2+}
Direct effect of Ca^{2+}	$4Ca^{2+}$ bind to TpC	$4Ca^{2+}$ bind to calmodulin
Effect of protein-bound Ca^{2+}	TpC · $4Ca^{2+}$ antagonizes Tpl inhibition of F-actin–myosin interaction (allows F-actin activation of ATPase)	Calmodulin · $4Ca^{2+}$ activates myosin light chain kinase that phosphorylates myosin p-light chain. The phosphorylated p-light chain no longer inhibits F-actin–myosin interaction (allows F-actin activation of ATPase).

[1]Light chains of myosin are different in striated and smooth muscles.

Ca^{2+} through the action of inositol trisphosphate. In turn, the elevation of Ca^{2+} leads to the liberation of **endothelium-derived relaxing factor (EDRF),** which diffuses into the adjacent smooth muscle. There, it reacts with the heme moiety of a soluble guanylyl cyclase, resulting in activation of the latter, with a consequent elevation of intracellular levels of cGMP (Figure 49–15). This in turn stimulates the activities of certain cGMP-dependent protein kinases, which probably phosphorylate specific muscle proteins, causing relaxation; however, the details are still being clarified. The important coronary artery vasodilator **nitroglycerin,** widely used to relieve angina pectoris, acts to increase intracellular release of EDRF and thus of cGMP.

Quite unexpectedly, EDRF was found to be the gas **nitric oxide (NO).** NO is formed by the action of the enzyme NO synthase, which is cytosolic. The endothelial and neuronal forms of NO synthase are activated by Ca^{2+} (Table 49–8). The substrate is arginine, and the products are citrulline and NO:

$$\text{Arginine} \xrightarrow{\text{NO SYNTHASE}} \text{Citrulline} + \text{NO}$$

NO synthase catalyzes a five-electron oxidation of an amidine nitrogen of arginine. L-Hydroxyarginine is an intermediate that remains tightly bound to the enzyme. NO synthase is a very complex enzyme, employing five redox cofactors: NADPH, FAD, FMN, heme, and tetrahydrobiopterin. NO can also be formed from **nitrite,** derived from vasodilators such as glyceryl trinitrate during their metabolism. NO has a very short half-life (approximately 3–4 seconds) in tissues because it reacts with oxygen and superoxide. The product of the reaction with superoxide is peroxynitrite ($ONOO^-$), which decomposes to form the highly reactive $OH^\bullet$ radical. NO is inhibited by hemoglobin and other heme proteins, which bind it tightly. Chemical inhibitors of NO synthase are now available that can markedly decrease formation of NO. Administration of such inhibitors to animals and humans leads to vasoconstriction and a marked elevation of blood pressure, indicating that NO is of major importance in the maintenance of blood pressure in vivo. Another important cardiovascular effect is that by increasing synthesis of cGMP, it acts as an inhibitor of platelet aggregation (Chapter 51).

Since the discovery of the role of NO as a vasodilator, there has been intense experimental interest in this substance. It has turned out to have a variety of physiologic roles, involving virtually every tissue of the body (Table 49–9). Three major isoforms of NO synthase have been identified, each of which has been cloned, and the chromosomal locations of their genes in hu-

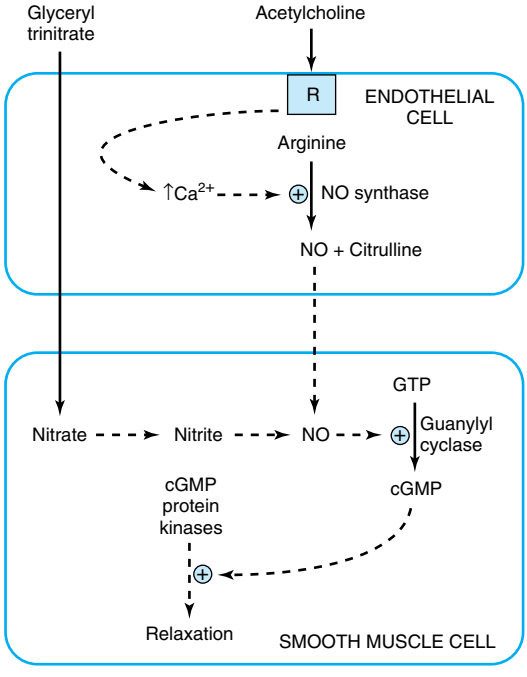

Figure 49–15. Diagram showing formation in an endothelial cell of nitric oxide (NO) from arginine in a reaction catalyzed by NO synthase. Interaction of an agonist (eg, acetylcholine) with a receptor (R) probably leads to intracellular release of Ca^{2+} via inositol trisphosphate generated by the phosphoinositide pathway, resulting in activation of NO synthase. The NO subsequently diffuses into adjacent smooth muscle, where it leads to activation of guanylyl cyclase, formation of cGMP, stimulation of cGMP-protein kinases, and subsequent relaxation. The vasodilator nitroglycerin is shown entering the smooth muscle cell, where its metabolism also leads to formation of NO.

mans have been determined. Gene knockout experiments have been performed on each of the three isoforms and have helped establish some of the postulated functions of NO.

To summarize, research in the past decade has shown that NO plays an important role in many physiologic and pathologic processes.

SEVERAL MECHANISMS REPLENISH STORES OF ATP IN MUSCLE

The ATP required as the constant energy source for the contraction-relaxation cycle of muscle can be generated (1) by glycolysis, using blood glucose or muscle glycogen, (2) by oxidative phosphorylation, (3) from creatine phosphate, and (4) from two molecules of ADP in a reaction catalyzed by adenylyl kinase (Figure 49–16). The amount of ATP in skeletal muscle is only sufficient to provide energy for contraction for a few seconds, so that ATP must be constantly renewed from one or more of the above sources, depending upon metabolic conditions. As discussed below, there are at least two distinct types of fibers in skeletal muscle, one predominantly active in aerobic conditions and the other in anaerobic conditions; not unexpectedly, they use each of the above sources of energy to different extents.

Skeletal Muscle Contains Large Supplies of Glycogen

The sarcoplasm of skeletal muscle contains large stores of glycogen, located in granules close to the I bands. The release of glucose from glycogen is dependent on a specific muscle glycogen phosphorylase (Chapter 18), which can be activated by Ca^{2+}, epinephrine, and AMP. To generate glucose 6-phosphate for glycolysis in skeletal muscle, glycogen phosphorylase b must be activated to phosphorylase a via phosphorylation by phosphorylase b kinase (Chapter 18). Ca^{2+} promotes the activation of phosphorylase b kinase, also by phosphorylation. Thus, Ca^{2+} both initiates muscle contraction and activates a pathway to provide necessary energy. The hormone epinephrine also activates glycogenolysis in muscle. AMP, produced by breakdown of ADP during muscular exercise, can also activate phosphorylase b without causing phosphorylation. Muscle glycogen phosphorylase b is inactive in **McArdle disease,** one of the glycogen storage diseases (Chapter 18).

Under Aerobic Conditions, Muscle Generates ATP Mainly by Oxidative Phosphorylation

Synthesis of ATP via oxidative phosphorylation requires a supply of oxygen. Muscles that have a high demand for oxygen as a result of sustained contraction (eg, to maintain posture) store it attached to the heme moiety of **myoglobin.** Because of the heme moiety, muscles containing myoglobin are red, whereas muscles with little or no myoglobin are white. Glucose, derived from the blood glucose or from endogenous glycogen, and fatty acids derived from the triacylglycerols of adipose tissue are the principal substrates used for aerobic metabolism in muscle.

Creatine Phosphate Constitutes a Major Energy Reserve in Muscle

Creatine phosphate prevents the rapid depletion of ATP by providing a readily available high-energy phosphate that can be used to regenerate ATP from ADP.

Table 49–8. Summary of the nomenclature of the NO synthases and of the effects of knockout of their genes in mice.[1]

Subtype	Name[2]	Comments	Result of Gene Knockout in Mice[3]
1	nNOS	Activity depends on elevated Ca^{2+}. First identified in neurons. Calmodulin-activated.	Pyloric stenosis, resistant to vascular stroke, aggressive sexual behavior (males).
2	iNOS[4]	Independent of elevated Ca^{2+}. Prominent in macrophages.	More susceptible to certain types of infection.
3	eNOS	Activity depends on elevated Ca^{2+}. First identified in endothelial cells.	Elevated mean blood pressure.

[1]Adapted from Snyder SH: No endothelial NO. Nature 1995;377:196.
[2]n, neuronal; i, inducible; e, endothelial.
[3]Gene knockouts were performed by homologous recombination in mice. The enzymes are characterized as neuronal, inducible (macrophage), and endothelial because these were the sites in which they were first identified. However, all three enzymes have been found in other sites, and the neuronal enzyme is also inducible. Each gene has been cloned, and its chromosomal location in humans has been determined.
[4]iNOS is Ca^{2+} -independent but binds calmodulin very tightly.

Creatine phosphate is formed from ATP and creatine (Figure 49–16) at times when the muscle is relaxed and demands for ATP are not so great. The enzyme catalyzing the phosphorylation of creatine is creatine kinase (CK), a muscle-specific enzyme with clinical utility in the detection of acute or chronic diseases of muscle.

SKELETAL MUSCLE CONTAINS SLOW (RED) & FAST (WHITE) TWITCH FIBERS

Different types of fibers have been detected in skeletal muscle. One classification subdivides them into type I (slow twitch), type IIA (fast twitch-oxidative), and type IIB (fast twitch-glycolytic). For the sake of simplicity, we shall consider only two types: type I (slow twitch, oxidative) and type II (fast twitch, glycolytic) (Table 49–10). The type I fibers are red because they contain myoglobin and mitochondria; their metabolism is aerobic, and they maintain relatively sustained contractions. The type II fibers, lacking myoglobin and containing few mitochondria, are white: they derive their energy from anaerobic glycolysis and exhibit relatively short durations of contraction. The proportion of these two types of fibers varies among the muscles of the body, depending on function (eg, whether or not a muscle is involved in sustained contraction, such as maintaining posture). The proportion also varies with training; for example, the number of type I fibers in certain leg muscles increases in athletes training for marathons, whereas the number of type II fibers increases in sprinters.

Table 49–9. Some physiologic functions and pathologic involvements of nitric oxide (NO).

- Vasodilator, important in regulation of blood pressure
- Involved in penile erection; sildenafil citrate (Viagra) affects this process by inhibiting a cGMP phosphodiesterase
- Neurotransmitter in the brain and peripheral autonomic nervous system
- Role in long-term potentiation
- Role in neurotoxicity
- Low level of NO involved in causation of pylorospasm in infantile hypertrophic pyloric stenosis
- May have role in relaxation of skeletal muscle
- May constitute part of a primitive immune system
- Inhibits adhesion, activation, and aggregation of platelets

A Sprinter Uses Creatine Phosphate & Anaerobic Glycolysis to Make ATP, Whereas a Marathon Runner Uses Oxidative Phosphorylation

In view of the two types of fibers in skeletal muscle and of the various energy sources described above, it is of interest to compare their involvement in a sprint (eg, 100 meters) and in the marathon (42.2 km; just over 26 miles) (Table 49–11).

The major sources of energy in the **100-m sprint** are **creatine phosphate** (first 4–5 seconds) and then **anaerobic glycolysis,** using muscle glycogen as the source of glucose. The two main sites of metabolic control are at glycogen phosphorylase and at PFK-1. The former is activated by Ca^{2+} (released from the sarcoplasmic reticulum during contraction), epinephrine, and

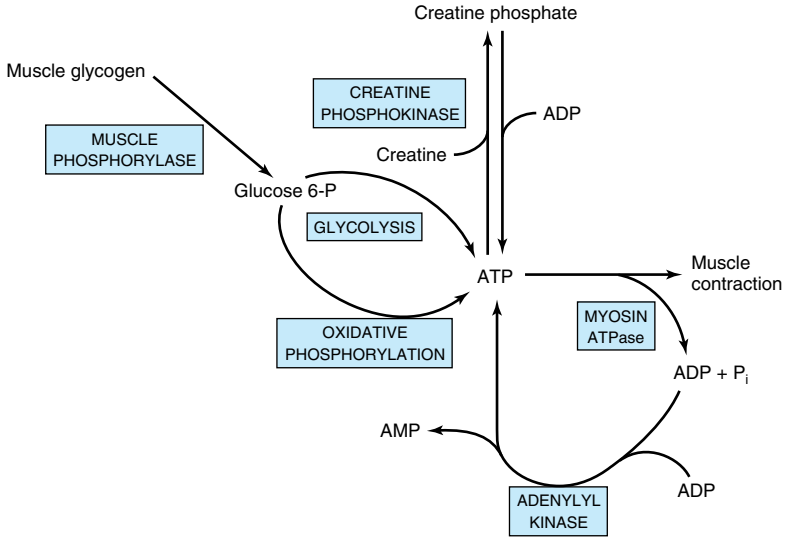

Figure 49–16. The multiple sources of ATP in muscle.

AMP. PFK-1 is activated by AMP, P_i, and NH_3. Attesting to the efficiency of these processes, the flux through glycolysis can increase as much as 1000-fold during a sprint.

In contrast, in the **marathon,** aerobic metabolism is the principal source of ATP. The major fuel sources are blood glucose and free fatty acids, largely derived from the breakdown of triacylglycerols in adipose tissue, stimulated by epinephrine. Hepatic glycogen is degraded to maintain the level of blood glucose. Muscle glycogen is also a fuel source, but it is degraded much more gradually than in a sprint. It has been calculated that the amounts of glucose in the blood, of glycogen in the liver, of glycogen in muscle, and of triacylglycerol in adipose tissue are sufficient to supply muscle with energy during a marathon for 4 minutes, 18 minutes, 70 minutes, and approximately 4000 minutes, respectively. However, the rate of oxidation of fatty acids by muscle is slower than that of glucose, so that oxidation of glucose and of fatty acids are both major sources of energy in the marathon.

A number of procedures have been used by athletes to counteract muscle fatigue and inadequate strength. These include carbohydrate loading, soda (sodium bi-

Table 49–10. Characteristics of type I and type II fibers of skeletal muscle.

	Type I Slow Twitch	Type II Fast Twitch
Myosin ATPase	Low	High
Energy utilization	Low	High
Mitochondria	Many	Few
Color	Red	White
Myoglobin	Yes	No
Contraction rate	Slow	Fast
Duration	Prolonged	Short

Table 49–11. Types of muscle fibers and major fuel sources used by a sprinter and by a marathon runner.

Sprinter (100 m)	Marathon Runner
Type II (glycolytic) fibers are used predominantly.	Type I (oxidative) fibers are used predominantly.
Creatine phosphate is the major energy source during the first 4–5 seconds.	ATP is the major energy source throughout.
Glucose derived from muscle glycogen and metabolized by anaerobic glycolysis is the major fuel source.	Blood glucose and free fatty acids are the major fuel sources.
Muscle glycogen is rapidly depleted.	Muscle glycogen is slowly depleted.

carbonate) loading, blood doping (administration of red blood cells), and ingestion of creatine and androstenedione. Their rationales and efficacies will not be discussed here.

SKELETAL MUSCLE CONSTITUTES THE MAJOR RESERVE OF PROTEIN IN THE BODY

In humans, skeletal muscle protein is the major nonfat source of stored energy. This explains the very large losses of muscle mass, particularly in adults, resulting from prolonged caloric undernutrition.

The study of tissue protein breakdown in vivo is difficult, because amino acids released during intracellular breakdown of proteins can be extensively reutilized for protein synthesis within the cell, or the amino acids may be transported to other organs where they enter anabolic pathways. However, actin and myosin are methylated by a posttranslational reaction, forming **3-methylhistidine.** During intracellular breakdown of actin and myosin, 3-methylhistidine is released and excreted into the urine. The urinary output of the methylated amino acid provides a reliable index of the rate of myofibrillar protein breakdown in the musculature of human subjects.

Various features of muscle metabolism, most of which are dealt with in other chapters of this text, are summarized in Table 49–12.

Table 49–12. Summary of major features of the biochemistry of skeletal muscle related to its metabolism.[1]

- Skeletal muscle functions under both aerobic (resting) and anaerobic (eg, sprinting) conditions, so both aerobic and anaerobic glycolysis operate, depending on conditions.
- Skeletal muscle contains myoglobin as a reservoir of oxygen.
- Skeletal muscle contains different types of fibers primarily suited to anaerobic (fast twitch fibers) or aerobic (slow twitch fibers) conditions.
- Actin, myosin, tropomyosin, troponin complex (TpT, TpI, and TpC), ATP, and Ca^{2+} are key constituents in relation to contraction.
- The Ca^{2+} ATPase, the Ca^{2+} release channel, and calsequestrin are proteins involved in various aspects of Ca^{2+} metabolism in muscle.
- Insulin acts on skeletal muscle to increase uptake of glucose.
- In the fed state, most glucose is used to synthesize glycogen, which acts as a store of glucose for use in exercise; "preloading" with glucose is used by some long-distance athletes to build up stores of glycogen.
- Epinephrine stimulates glycogenolysis in skeletal muscle, whereas glucagon does not because of absence of its receptors.
- Skeletal muscle cannot contribute directly to blood glucose because it does not contain glucose-6-phosphatase.
- Lactate produced by anaerobic metabolism in skeletal muscle passes to liver, which uses it to synthesize glucose, which can then return to muscle (the Cori cycle).
- Skeletal muscle contains phosphocreatine, which acts as an energy store for short-term (seconds) demands.
- Free fatty acids in plasma are a major source of energy, particularly under marathon conditions and in prolonged starvation.
- Skeletal muscle can utilize ketone bodies during starvation.
- Skeletal muscle is the principal site of metabolism of branched-chain amino acids, which are used as an energy source.
- Proteolysis of muscle during starvation supplies amino acids for gluconeogenesis.
- Major amino acids emanating from muscle are alanine (destined mainly for gluconeogenesis in liver and forming part of the glucose-alanine cycle) and glutamine (destined mainly for the gut and kidneys).

[1]This table brings together material from various chapters in this book.

THE CYTOSKELETON PERFORMS MULTIPLE CELLULAR FUNCTIONS

Nonmuscle cells perform mechanical work, including self-propulsion, morphogenesis, cleavage, endocytosis, exocytosis, intracellular transport, and changing cell shape. These cellular functions are carried out by an extensive intracellular network of filamentous structures constituting the **cytoskeleton.** The cell cytoplasm is not a sac of fluid, as once thought. Essentially all eukaryotic cells contain three types of filamentous structures: actin filaments (7–9.5 nm in diameter; also known as microfilaments), microtubules (25 nm), and intermediate filaments (10–12 nm). Each type of filament can be distinguished biochemically and by the electron microscope.

Nonmuscle Cells Contain Actin That Forms Microfilaments

G-actin is present in most if not all cells of the body. With appropriate concentrations of magnesium and potassium chloride, it spontaneously polymerizes to form double helical F-actin filaments like those seen in muscle. There are at least two types of actin in nonmus-

cle cells: β-actin and γ-actin. Both types can coexist in the same cell and probably even copolymerize in the same filament. In the cytoplasm, F-actin forms microfilaments of 7–9.5 nm that frequently exist as bundles of a tangled-appearing meshwork. These bundles are prominent just underlying the plasma membrane of many cells and are there referred to as stress fibers. The stress fibers disappear as cell motility increases or upon malignant transformation of cells by chemicals or oncogenic viruses.

Although not organized as in muscle, actin filaments in nonmuscle cells interact with myosin to cause cellular movements.

Microtubules Contain α- & β-Tubulins

Microtubules, an integral component of the cellular cytoskeleton, consist of cytoplasmic tubes 25 nm in diameter and often of extreme length. Microtubules are necessary for the formation and function of the **mitotic spindle** and thus are present in all eukaryotic cells. They are also involved in the intracellular movement of endocytic and exocytic vesicles and form the major structural components of cilia and flagella. Microtubules are a major component of axons and dendrites, in which they maintain structure and participate in the axoplasmic flow of material along these neuronal processes.

Microtubules are cylinders of 13 longitudinally arranged protofilaments, each consisting of dimers of α-tubulin and β-tubulin, closely related proteins of approximately 50 kDa molecular mass. The tubulin dimers assemble into protofilaments and subsequently into sheets and then cylinders. A microtubule-organizing center, located around a pair of centrioles, nucleates the growth of new microtubules. A third species of tubulin, **γ-tubulin,** appears to play an important role in this assembly. GTP is required for assembly. A variety of proteins are associated with microtubules (microtubule-associated proteins [MAPs], one of which is tau) and play important roles in microtubule assembly and stabilization. Microtubules are in a state of dynamic instability, constantly assembling and disassembling. They exhibit polarity (plus and minus ends); this is important in their growth from centrioles and in their ability to direct intracellular movement. For instance, in axonal transport, the protein **kinesin,** with a myosin-like ATPase activity, uses hydrolysis of ATP to move vesicles down the axon toward the positive end of the microtubular formation. Flow of materials in the opposite direction, toward the negative end, is powered by **cytosolic dynein,** another protein with ATPase activity. Similarly, **axonemal dyneins** power ciliary and flagellar movement. Another protein, **dynamin,** uses GTP and is involved in endocytosis. Kinesins, dyneins, dynamin, and myosins are referred to as **molecular motors.**

An absence of dynein in cilia and flagella results in immotile cilia and flagella, leading to male sterility and chronic respiratory infection, a condition known as **Kartagener syndrome.**

Certain **drugs** bind to microtubules and thus interfere with their assembly or disassembly. These include colchicine (used for treatment of acute gouty arthritis), vinblastine (a vinca alkaloid used for treating certain types of cancer), paclitaxel (Taxol) (effective against ovarian cancer), and griseofulvin (an antifungal agent).

Intermediate Filaments Differ From Microfilaments & Microtubules

An intracellular fibrous system exists of filaments with an axial periodicity of 21 nm and a diameter of 8–10 nm that is intermediate between that of microfilaments (6 nm) and microtubules (23 nm). Four classes of intermediate filaments are found, as indicated in Table 49–13. They are all elongated, fibrous molecules, with a central rod domain, an amino terminal head, and a carboxyl terminal tail. They form a structure like a rope, and the mature filaments are composed of tetramers packed together in a helical manner. They are important structural components of cells, and most are relatively stable components of the cytoskeleton, not undergoing rapid assembly and disassembly and not

Table 49–13. Classes of intermediate filaments of eukaryotic cells and their distributions.

Proteins	Molecular Mass	Distributions
Keratins		
Type I (acidic)	40–60 kDa	Epithelial cells, hair, nails
Type II (basic)	50–70 kDa	
Vimentin-like		
Vimentin	54 kDa	Various mesenchymal cells
Desmin	53 kDa	Muscle
Glial fibrillary acid protein	50 kDa	Glial cells
Peripherin	66 kDa	Neurons
Neurofilaments		
Low (L), medium (M), and high (H)[1]	60–130 kDa	Neurons
Lamins		
A, B, and C	65–75 kDa	Nuclear lamina

[1]Refers to their molecular masses.

disappearing during mitosis, as do actin and many microtubular filaments. An important exception to this is provided by the lamins, which, subsequent to phosphorylation, disassemble at mitosis and reappear when it terminates.

Keratins form a large family, with about 30 members being distinguished. As indicated in Table 49–13, two major types of keratins are found; all individual keratins are heterodimers made up of one member of each class.

Vimentins are widely distributed in mesodermal cells, and desmin, glial fibrillary acidic protein, and peripherin are related to them. All members of the vimentin-like family can copolymerize with each other. Intermediate filaments are very prominent in nerve cells; neurofilaments are classified as low, medium, and high on the basis of their molecular masses. **Lamins** form a meshwork in apposition to the inner nuclear membrane. The distribution of intermediate filaments in normal and abnormal (eg, cancer) cells can be studied by the use of immunofluorescent techniques, using antibodies of appropriate specificities. These antibodies to specific intermediate filaments can also be of use to pathologists in helping to decide the origin of certain dedifferentiated malignant tumors. These tumors may still retain the type of intermediate filaments found in their cell of origin.

A number of **skin diseases,** mainly characterized by blistering, have been found to be due to mutations in genes encoding various keratins. Three of these disorders are epidermolysis bullosa simplex, epidermolytic hyperkeratosis, and epidermolytic palmoplantar keratoderma. The blistering probably reflects a diminished capacity of various layers of the skin to resist mechanical stresses due to abnormalities in microfilament structure.

SUMMARY

- The myofibrils of skeletal muscle contain thick and thin filaments. The thick filaments contain myosin. The thin filaments contain actin, tropomyosin, and the troponin complex (troponins T, I, and C).
- The sliding filament cross-bridge model is the foundation of current thinking about muscle contraction. The basis of this model is that the interdigitating filaments slide past one another during contraction and cross-bridges between myosin and actin generate and sustain the tension.
- The hydrolysis of ATP is used to drive movement of the filaments. ATP binds to myosin heads and is hydrolyzed to ADP and P_i by the ATPase activity of the actomyosin complex.
- Ca^{2+} plays a key role in the initiation of muscle contraction by binding to troponin C. In skeletal muscle, the sarcoplasmic reticulum regulates distribution of Ca^{2+} to the sarcomeres, whereas inflow of Ca^{2+} via Ca^{2+} channels in the sarcolemma is of major importance in cardiac and smooth muscle.
- Many cases of malignant hyperthermia in humans are due to mutations in the gene encoding the Ca^{2+} release channel.
- A number of differences exist between skeletal and cardiac muscle; in particular, the latter contains a variety of receptors on its surface.
- Some cases of familial hypertrophic cardiomyopathy are due to missense mutations in the gene coding for β-myosin heavy chain.
- Smooth muscle, unlike skeletal and cardiac muscle, does not contain the troponin system; instead, phosphorylation of myosin light chains initiates contraction.
- Nitric oxide is a regulator of vascular smooth muscle; blockage of its formation from arginine causes an acute elevation of blood pressure, indicating that regulation of blood pressure is one of its many functions.
- Duchenne-type muscular dystrophy is due to mutations in the gene, located on the X chromosome, encoding the protein dystrophin.
- Two major types of muscle fibers are found in humans: white (anaerobic) and red (aerobic). The former are particularly used in sprints and the latter in prolonged aerobic exercise. During a sprint, muscle uses creatine phosphate and glycolysis as energy sources; in the marathon, oxidation of fatty acids is of major importance during the later phases.
- Nonmuscle cells perform various types of mechanical work carried out by the structures constituting the cytoskeleton. These structures include actin filaments (microfilaments), microtubules (composed primarily of α- tubulin and β-tubulin), and intermediate filaments. The latter include keratins, vimentin-like proteins, neurofilaments, and lamins.

REFERENCES

Ackerman MJ, Clapham DE: Ion channels—basic science and clinical disease. N Engl J Med 1997;336:1575.

Andreoli TE: Ion transport disorders: introductory comments. Am J Med 1998;104:85. (First of a series of articles on ion transport disorders published between January and August, 1998. Topics covered were structure and function of ion channels, arrhythmias and antiarrhythmic drugs, Liddle syndrome, cholera, malignant hyperthermia, cystic fibrosis, the periodic paralyses and Bartter syndrome, and Gittelman syndrome.)

Fuller GM, Shields D: *Molecular Basis of Medical Cell Biology.* Appleton & Lange, 1998.

Geeves MA, Holmes KC: Structural mechanism of muscle contraction. Annu Rev Biochem 1999;68:728.

Hille B: *Ion Channels of Excitable Membranes.* Sinauer, 2001.

Howard J: *Mechanics of Motor Proteins and the Cytoskeleton.* Sinauer, 2001.

Lodish H et al (editors): *Molecular Cell Biology,* 4th ed. Freeman, 2000. (Chapters 18 and 19 of this text contain comprehensive descriptions of cell motility and cell shape.)

Loke J, MacLennan DH: Malignant hyperthermia and central core disease: disorders of Ca^{2+} release channels. Am J Med 1998;104:470.

Mayer B, Hemmens B: Biosynthesis and action of nitric oxide in mammalian cells. Trends Biochem Sci 1998;22:477.

Scriver CR et al (editors): *The Metabolic and Molecular Bases of Inherited Disease,* 8th ed. McGraw-Hill, 2001. (This comprehensive four-volume text contains coverage of malignant hyperthermia [Chapter 9], channelopathies [Chapter 204], hypertrophic cardiomyopathy [Chapter 213], the muscular dystrophies [Chapter 216], and disorders of intermediate filaments and their associated proteins [Chapter 221].)