13
Cardiac Muscle

LEARNING OBJECTIVES

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

1. What structures in cardiac muscle facilitate coordinated contraction of the cardiomyocytes, which is critical for the pumping action of the heart?
2. What is the sequence of events and molecular interactions by which an action potential in the sarcolemma of cardiac muscle leads to muscle contraction?
3. What are the intrinsic mechanisms and the extrinsic mechanisms that increase the force of contraction of cardiac muscle?
4. How does sympathetic stimulation of cardiac muscle increase the force of contraction (positive inotropy) and the speed of relaxation (positive lusitropy)?
5. How does an increase in the frequency of contraction of cardiac muscle lead to an increase in the force of contraction (i.e., Treppe)?
6. What is the Frank-Starling law of the heart, and how does it relate to the effects of stretch on actin-myosin interactions in cardiac muscle?
7. What are the similarities and differences between hypertrophic cardiomyopathy, dilated cardiomyopathy, and pressure overload–induced left ventricular hypertrophy?

If the student has already completed Chapter 12 on skeletal muscle, the student will be able to compare cardiac muscle and skeletal muscle in terms of the organization of the muscle cells, excitation-contraction coupling, and the regulation of the force of contraction.

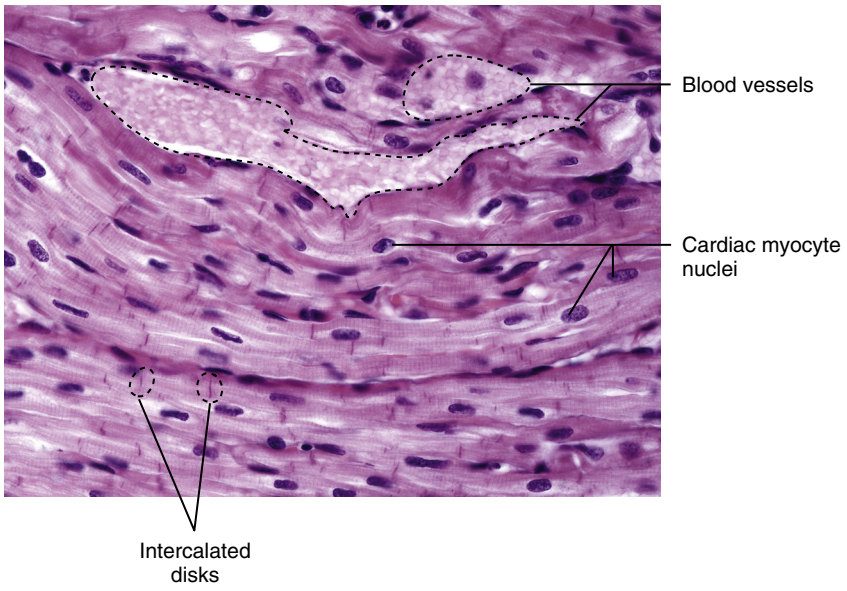
The function of the heart is to pump blood through the circulatory system, and this is accomplished by the highly organized contraction of cardiac muscle cells. Specifically, the cardiac muscle cells are connected together to form an electrical syncytium, with tight electrical and mechanical connections between adjacent cardiac muscle cells. An action potential initiated in a specialized region of the heart (e.g., the sinoatrial node) is therefore able to pass quickly throughout the heart to facilitate synchronized contraction of the cardiac muscle cells, which is important for the pumping action of the heart. Likewise, refilling of the heart requires synchronized relaxation of the heart; abnormal relaxation often results in pathological conditions.

This chapter begins with a description of the organization of cardiac muscle cells within the heart, including discussion of the tight electrical and mechanical connections. The mechanisms that underlie contraction, relaxation, and regulation of the force of contraction of cardiac muscle cells are also addressed. Although cardiac muscle and skeletal muscle are both striated muscles, they are significantly different in terms of organization, electrical and mechanical coupling, excitation-contraction coupling, and mechanisms to regulate the force of contraction. These differences are also highlighted.

Basic Organization of Cardiac Muscle Cells

Cardiac muscle cells are much smaller than skeletal muscle cells. Typically, cardiac muscle cells measure 10 μm in diameter and approximately 100 μm in length. As shown in Fig. 13.1A, cardiac cells are connected to each other through **intercalated disks,** which include a combination of mechanical junctions and electrical connections. The mechanical connections, which keep the cells from pulling apart when contracting, include the **fascia adherens** and **desmosomes. Gap junctions** between cardiac muscle cells, on the other hand, provide electrical connections between cells to allow propagation of the action potential throughout the heart. Thus the arrangement of cardiac muscle cells within the heart is said to form an electrical and mechanical syncytium that allows a single action potential (generated within the sinoatrial node) to pass throughout the heart so that the heart can contract in a synchronous, wave-like manner. Blood vessels course through the myocardium.

The basic organization of thick and thin filaments in cardiac muscle cells is comparable with that in skeletal muscle (see Chapter 12). Electron microscopy reveals repeating light and dark bands that represent I bands and A bands, respectively (see Fig. 13.1B and Chapter 12, Fig. 12.3). Thus cardiac muscle is classified as a striated muscle. The Z line transects the I band and represents the point of attachment of the thin filaments. The region between two adjacent Z lines represents the sarcomere, which is the contractile unit of the muscle cell. The thin filaments are composed of actin, tropomyosin, and troponin and extend into the A band. The A band is composed of thick filaments, along with some overlap of thin filaments. The thick filaments are composed of myosin and extend from the center of the sarcomere toward the Z lines.



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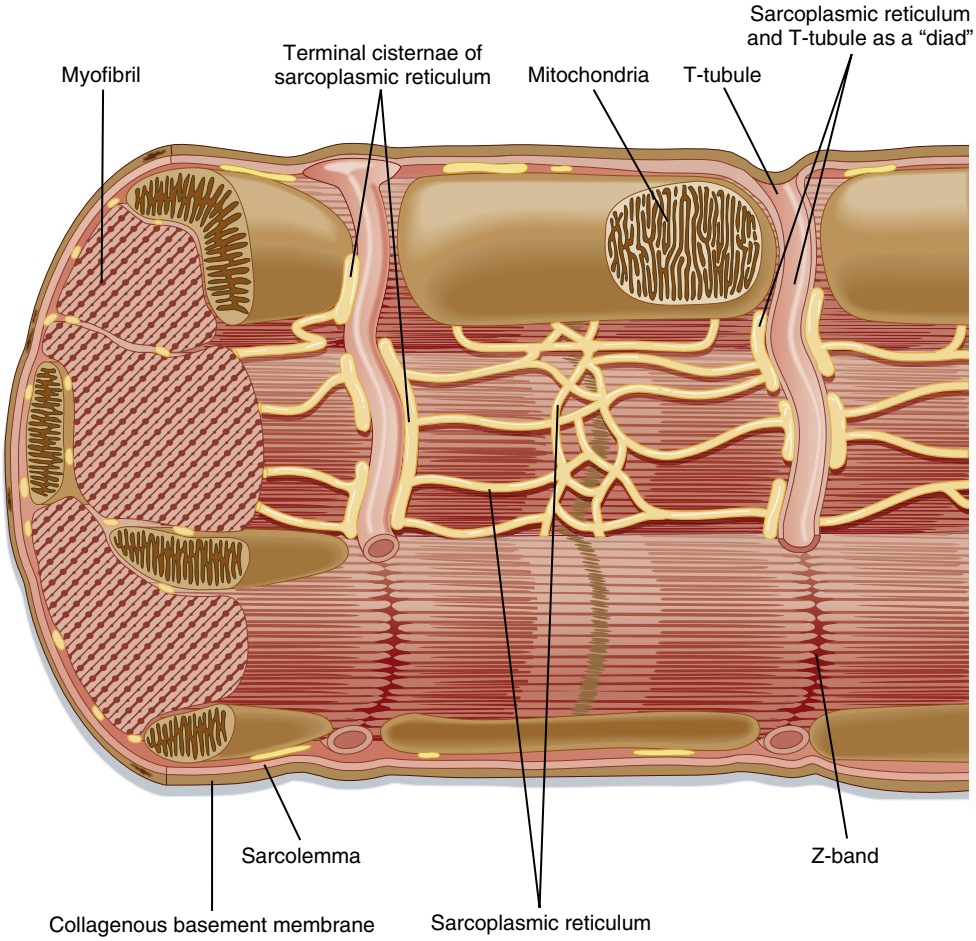


B

• **Fig. 13.1** **A,** Photomicrograph of cardiac muscle cells (magnification, × 210). Intercalated disks at either end of a muscle cell are identified in the lower left portion of the micrograph. The intercalated disk physically connects adjacent myocytes and, because of the presence of gap junctions, electrically couples the cells as well so that the muscle functions as an electrical and mechanical syncytium. **B,** Schematic representation of the organization of a sarcomere within a cardiac muscle cell. (**A,** From Telser A. *Elsevier's Integrated Histology.* St. Louis: Mosby; 2007. **B,** Redrawn from Fawcett D, McNutt NS. The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. *J Cell Biol.* 1969;42:1-45.)

Myosin filaments are formed by a tail-to-tail association of myosin molecules in the center of the sarcomere, followed by a head-to-tail association as the thick filament extends toward the Z lines. Thus the myosin filament is polarized and poised for pulling the actin filaments toward the center of the sarcomere. A cross-sectional view of the sarcomere near the end of the A band shows that each thick filament is surrounded by six thin filaments, and each thin filament receives cross-bridge attachments from three thick filaments. This complex array of thick and thin filaments is characteristic of both cardiac and skeletal muscle and helps stabilize the filaments during muscle contraction (see Fig. 12.3B for the hexagonal array of thick and thin filaments in the sarcomere of striated muscle).

Several proteins may contribute to the organization of the thick and thin filaments, including meromyosin and C protein (in the center of the sarcomere), which appear to serve as a scaffold for organization of the thick filaments. Similarly, nebulin extends along the length of the actin filament and may serve as a scaffold for the thin filament. The actin filament is anchored to the Z line by α-actinin, whereas the protein tropomodulin resides at the end of the actin filament and regulates the length of the thin filament. These proteins are present in both cardiac and skeletal muscle cells.

The thick filaments are tethered to the Z lines by a large elastic protein called **titin.** Although titin was postulated to tether myosin to the Z lines and thus prevent overstretching of the sarcomere, there is evidence indicating that titin may participate in cell signaling (perhaps by acting as a stretch sensor and thus modulating protein synthesis in response to stress). Such signaling by titin has been observed in both cardiac and skeletal muscle cells. Moreover, genetic defects in titin result in atrophy of both cardiac and skeletal muscle cells and may contribute to both cardiac dysfunction and skeletal muscle dystrophies (termed **titinopathies**). Titin is also thought to contribute to the ability of cardiac muscle to increase force upon stretch (discussed in the later section "Stretch").

Although both cardiac muscle and skeletal muscle contain an abundance of connective tissue, there is more connective tissue in the heart. The abundance of connective tissue in the heart helps prevent muscle rupture (as in skeletal muscle), but it also prevents overstretching of the heart. Length-tension analysis of cardiac muscle, for example, shows a dramatic increase in passive tension as cardiac muscle is stretched beyond its resting length. Skeletal muscle, in contrast, tolerates a much greater degree of stretch before passive tension increases to a comparable level. The reason for this difference between cardiac and skeletal muscle is not known, although one possibility is that stretch of skeletal muscle is typically limited by the range of motion of the joint, which in turn is limited by the ligaments/connective tissue surrounding the joint.

The heart, on the other hand, appears to rely on the abundance of connective tissue around cardiac muscle cells to prevent overstretching during periods of increased venous return. During intense exercise, for example, venous return may increase fivefold. However, the heart is capable of pumping this extra volume of blood into the arterial system with only minor changes in the ventricular volume of the heart (i.e., end-diastolic volume increases less than 20%). Although the abundance of connective tissue in the heart limits stretch of the heart during these periods of increased venous return, additional regulatory mechanisms help the heart pump the extra blood that it receives (as discussed in the section "Stretch"). Conversely, if the heart were to be overstretched, the contractile ability of cardiac muscle cells would be expected to decrease (because of decreased overlap of the thick and thin filaments), which would result in insufficient pumping, increased venous pressure, and perhaps pulmonary edema.

Within cardiac muscle cells, myofibrils are surrounded by the **sarcoplasmic reticulum (SR)**, an internal network of membranes (see Fig. 13.1B). This is similar to the SR in skeletal muscle except that the SR in the heart is less dense and not as well developed. Terminal regions of the SR abut the **T tubule** or lie just below the **sarcolemma** (or both) and play a key role in the elevation of intracellular Ca^{++} during an action potential. The mechanism by which an action potential initiates release of Ca^{++} in the heart differs significantly from that in skeletal muscle (as discussed in the section "Excitation-Contraction Coupling").

The heart contains an abundance of mitochondria; up to 30% of the volume of the heart is occupied by these organelles. The high density of mitochondria provides the heart with great oxidative capacity, more so than is typical in skeletal muscle.

The sarcolemma of cardiac muscle contains invaginations **(T tubules)** comparable to those seen in skeletal muscle. In cardiac muscle, however, T tubules are positioned at the Z lines, whereas in mammalian skeletal muscle, T tubules are positioned at the ends of the I bands. In cardiac muscle, the connections between the T tubules and the SR are fewer than, and not as well developed as, those in skeletal muscle. These junctional regions between the terminal portions of the SR and the T tubules in cardiac muscle are called dyads (as the junction consists of the T tubule membrane and one SR membrane), which contrasts with the triads in skeletal muscle where the T tubules are located between two SR terminal cisternae.

AT THE CELLULAR LEVEL

Familial cardiomyopathic hypertrophy (FCH) occurs in approximately 0.2% of the general population but is a leading cause of sudden death in otherwise healthy adults. It has been linked to genetic defects in a variety of proteins in cardiac sarcomeres, including myosin, troponin, tropomyosin, and myosin-binding protein C, a structural protein located in the middle of the A band of the sarcomere. FCH is an autosomal dominant disease, and transgenic studies indicate that expression of only a small amount of the mutated protein can result in development of the cardiomyopathic phenotype. Moreover, mutation of a single amino acid in the myosin molecule is sufficient to produce cardiomyopathic hypertrophy. The pathogenesis of FCH, however, is variable, even within a family with a single gene defect, in terms of both onset and severity; this variability suggests the presence of modifying loci.

Control of Cardiac Muscle Activity

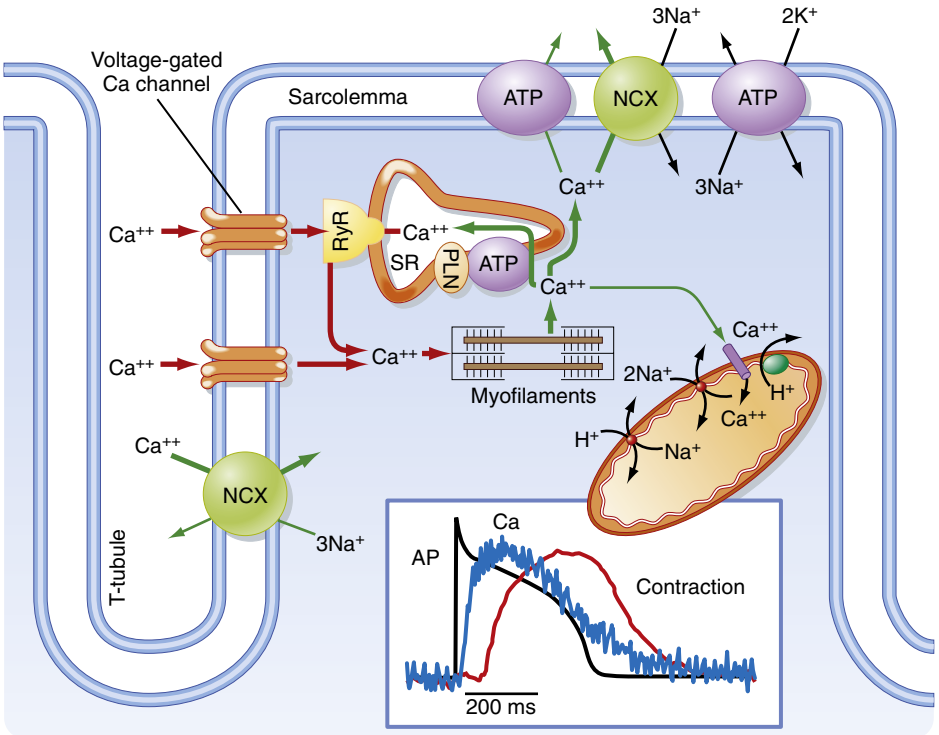
Cardiac muscle is an involuntary muscle with an intrinsic pacemaker. The pacemaker represents a specialized cell (located in the **sinoatrial node** of the right atrium) that is able to undergo spontaneous depolarization and generate action potentials. Of importance is that although several cells in the heart are able to depolarize spontaneously, the fastest spontaneous depolarizations occur in cells in the sinoatrial node. Moreover, once a given cell spontaneously depolarizes and fires an action potential, this action potential is then propagated throughout the heart (by specialized conduction pathways and cell-to-cell contact). Thus depolarization from only one cell is needed to initiate a wave of contraction in the heart (i.e., a heartbeat). The mechanisms underlying this spontaneous depolarization are discussed in depth in Chapter 16.

Once an action potential is initiated in the sinoatrial node, it is propagated between atrial cells via gap junctions, as well as through specialized conduction fibers in the atria. The action potential can pass throughout the atria within approximately 70 msec. For the action potential to reach the ventricles, it must pass through the **atrioventricular node,** after which the action potential passes throughout the ventricle via specialized conduction pathways (the **bundle of His** and the **Purkinje system**) and gap junctions in the intercalated disks of adjacent cardiac myocytes. The action potential can pass through the entire heart within 220 msec after initiation in the sinoatrial node. Because contraction of a cardiac muscle cell typically lasts 300 msec, this rapid conduction promotes nearly synchronous contraction of heart muscle cells. This is a very different scenario from that of skeletal muscle, in which cells are grouped into motor units that are recruited independently as the force of contraction is increased.

Excitation-Contraction Coupling

Blood and extracellular fluids typically contain 1 to 2 mmol/L of free Ca^{++}, and it has been known since the days of the physiologist Sidney Ringer (ca. 1882) that the heart requires extracellular Ca^{++} to contract. Thus an isolated heart typically continues to beat when perfused with a warm (37°C), oxygenated, physiological salt solution that contains approximately 2 mmol/L Ca^{++} (e.g., Tyrode's solution), but it stops beating in the absence of extracellular Ca^{++}. This cessation of contractions in Ca^{++}-deficient media is also observed in hearts that are electrically stimulated, which further demonstrates the importance of extracellular Ca^{++} for contraction of cardiac muscle. This situation is quite different from that of skeletal muscle, which can contract in the total absence of extracellular Ca^{++}.

Action potentials in cardiac muscle are prolonged, lasting 150 to 300 msec (Fig. 13.2, *inset*), which is substantially



• **Fig. 13.2** Excitation-contraction coupling in the heart requires Ca^{++} influx through L-type calcium channels in the sarcolemma and T tubules. See text for details. *Inset* shows time course of action potential *(AP)*, intracellular Ca transient *(Ca)*, and contraction. *ATP*, Adenosine triphosphate; *NCX*, sarcolemmal $3Na^+$-Ca^{++} antiporter; *PLN*, phospholamban; *RYR*, ryanodine receptor. (Modified from Bers DM. Cardiac excitation-contraction coupling. *Nature.* 2002;415:198-205. *Inset* modified from Mountcastle VB. *Medical Physiology*. 13th ed. St Louis: Mosby; 1974; Brooks CM, Hoffman BF, Suckling EE, Orias O. *Excitability of the Heart*. New York: Grune & Stratton; 1955.)

longer than the action potentials in skeletal muscle (≈5 msec). The long duration of the action potential in cardiac muscle is due to a slow inward Ca^{++} current through an **L-type voltage-gated calcium channel** in the sarcolemma. The amount of Ca^{++} coming into the cardiac muscle cell is relatively small and serves as a trigger for release of Ca^{++} from the SR. In the absence of extracellular Ca^{++}, an action potential can still be initiated in cardiac muscle, although it is considerably shorter in duration and unable to initiate a contraction. Thus, influx of Ca^{++} during the action potential is crucial for triggering release of Ca^{++} from the SR and thus initiating contraction.

The L-type voltage-gated calcium channel is composed of five subunits (α_1, α_2, β, γ, and δ). The α_1 subunit in cardiac muscle is also called $Ca_V1,2$. Historically, $Ca_V1.2$ was called the **dihydropyridine receptor (DHPR)** because it binds the dihydropyridine class of L-type voltage-gated calcium channel–blocking drugs (e.g., nitrendipine and nimodipine). Note that cardiac muscle contains $Ca_V1.2$, whereas skeletal muscle contains $Ca_V1.1$, which is very important, as it changes the mechanism by which an action potential in the T Tubule induces Ca^{++} release from the nearby SR (as discussed later).

In each cardiac muscle sarcomere, terminal regions of the SR abut T tubules and the sarcolemma (see Figs. 13.1B and 13.2). These junctional regions of the SR are enriched in calcium release channels called **ryanodine receptors** (RYR). The RYR2 isoform is the Ca^{++}-gated calcium release channel in cardiac SR. A critical point to appreciate is that during an action potential in the myocardial cell, the tiny amount of Ca^{++} passing through the L-type Ca^{++} channel in the T tubule stimulates the nearby RYR2 to release Ca^{++} from the terminal cisterna into the cytoplasm. The Ca release from the SR then promotes actin-myosin interaction, and hence contraction.

The amount of Ca^{++} released into the cytosol from the SR is much greater than that entering the cytosol from the T tubule or sarcolemma, although release of Ca^{++} from the SR does not occur without this entry of "trigger" Ca^{++}. Thus excitation-contraction coupling in cardiac muscle is termed **electrochemical coupling** (with voltage-induced Ca^{++} influx through $Ca_V1.2$ stimulating Ca^{++} release from RYR2), whereas excitation-contraction coupling in skeletal muscle is termed **electromechanical coupling** (with a voltage-induced conformational change in $Ca_V1.1$ promoting Ca^{++} release from RYR1 through protein-protein interactions). The basis for this difference in Ca^{++} release mechanisms appears to depend on the differences between $Ca_V1.1$ in skeletal muscle and $Ca_V1.2$ in the heart.

Contraction Mechanism

As in skeletal muscle, contraction of cardiac muscle is regulated by thin filaments, and an elevation in intracellular $[Ca^{++}]$ is necessary to promote actin-myosin interaction. At low (<50 nmol/L) intracellular $[Ca^{++}]$, binding of myosin to actin is blocked by tropomyosin. As cytosolic $[Ca^{++}]$ increases during an action potential, however, binding of Ca^{++} to troponin C results in a conformational change in the troponin/tropomyosin complex in which tropomyosin slips into the groove of the actin filament and exposes myosin-binding sites on the actin filament. As long as cytosolic $[Ca^{++}]$ remains elevated, and hence myosin-binding sites are exposed, myosin will bind to actin, undergo a ratchet action, and contract the cardiac muscle cell. Note that because myosin-binding sites on actin are blocked at low $[Ca^{++}]$ and exposed during a rise in intracellular $[Ca^{++}]$, contraction of cardiac muscle is termed *thin filament regulated*. This is identical to the situation in skeletal muscle; in smooth muscle, in contrast, contraction is thick filament regulated (see Chapter 14).

During a rise in intracellular $[Ca^{++}]$ and exposure of myosin-binding sites on actin, the myosin cross-bridges undergo a series of steps that result in contraction of the cardiac muscle cell. At rest, the myosin molecules are energized in that they have partially hydrolyzed adenosine triphosphate (ATP) to "cock the head" and are thus ready to interact with actin. An elevation in intracellular $[Ca^{++}]$ then exposes myosin-binding sites on actin and thus allows myosin to bind actin (step 1). The bound myosin subsequently undergoes a powerstroke in which the actin filament is pulled toward the center of the sarcomere (step 2). Adenosine diphosphate (ADP) and inorganic phosphate (P_i) are released from the myosin head during this step as the energy from ATP is used to contract the muscle. The myosin head moves approximately 70 nm during each ratchet action (cross-bridge cycle). Binding of ATP to myosin decreases the affinity of myosin for actin and thus allows myosin to release from actin (step 3). Myosin then partially hydrolyzes the bound ATP to reenergize ("cock") the head (step 4) and ready the cross-bridge for another cycle. This four-step cycle is identical to that described for skeletal muscle (see Chapter 12, Fig. 12.11).

Cardiac muscle and skeletal muscle differ, however, in the level of intracellular $[Ca^{++}]$ attained after an action potential and hence in the number of actin-myosin interactions. In skeletal muscle, intracellular $[Ca^{++}]$ rises and the number of actin-myosin interactions is high after an action potential. In cardiac muscle, the rise in intracellular $[Ca^{++}]$ can be regulated, which affords the heart an important means of modulating the force of contraction without recruiting more muscle cells or undergoing tetany. Recall that in the heart, all the muscle cells are activated during a contraction, and so recruiting more muscle cells is not an option. Moreover, tetany of cardiac muscle cells would prevent any pumping action and thus be fatal. Consequently, the heart relies on different means of increasing the force of contraction, including varying the amplitude of the intracellular Ca^{++} transient.

Relaxation of Cardiac Muscle

Relaxation of skeletal muscle simply requires reaccumulation of Ca^{++} by the SR through the action of the **sarcoplasmic**

endoplasmic reticulum calcium-ATPase (SERCA2), also known as the **SR Ca^{++} pump**. Although SERCA2 plays a key role in the decrease in cytosolic [Ca^{++}] in cardiac muscle, the process is more complex than that in skeletal muscle because some trigger Ca^{++} enters the cardiac muscle cell through the sarcolemmal calcium channels during each action potential. A mechanism must therefore exist to extrude this trigger Ca^{++}; otherwise, the amount of Ca^{++} in the SR would continuously increase, and Ca^{++} overload would result. In particular, some Ca^{++} is extruded from the cardiac muscle cell though the sarcolemmal **$3Na^{+}$-Ca^{++} antiporter** and a **sarcolemmal Ca^{++} pump** (Fig. 13.2). The extracellular [Ca^{++}] is in the millimolar range, whereas the amount of intracellular [Ca^{++}] is submicromolar, and so extrusion of Ca^{++} is accomplished against a large chemical gradient. Similarly, [Na^{+}] is considerably higher in the extracellular media than within the cell. The antiporter uses the Na^{+} gradient across the cell to power the uphill movement of Ca^{++} out of the cell. Because three Na^{+} ions enter the cell in exchange for one Ca^{++} ion, the $3Na^{+}$-Ca^{++} antiporter is electrogenic and creates a depolarizing current. The sarcolemmal Ca^{++} pump, on the other hand, uses the energy in ATP to extrude Ca^{++} from the cell. Both extrusion mechanisms and SERCA thus contribute to the relaxation of cardiac muscle by decreasing cytosolic [Ca^{++}].

Although the interaction of actin and myosin requires a relatively small increase in free intracellular [Ca^{++}], the abundance of Ca^{++}-binding proteins in the myoplasm necessitates a much larger increase in total intracellular [Ca^{++}]. The resting intracellular [Ca^{++}] is approximately 50 to 100 nmol/L; half-maximal force of contraction requires approximately 600 nmol/L of free Ca^{++}. However, because of Ca^{++}-binding proteins such as parvalbumin and troponin C, the total myoplasmic concentration must increase by 70 μmol/L. As already noted, much of this increase in total myoplasmic [Ca^{++}] occurs through release of Ca^{++} from the SR. In a number of species, including rabbits, dogs, cats, guinea pigs, and humans, uptake and release of Ca^{++} by the SR account for approximately 70% of the intracellular Ca^{++} transient. Thus, up to 30% of the rise in intracellular [Ca^{++}] may be attributable to influx of Ca^{++} through L-type voltage-gated calcium channels in the T Tubules and sarcolemma, and the $3Na^{+}$-Ca^{++} antiporter contributes significantly to Ca^{++} extrusion during relaxation.

The sarcolemmal Ca^{++} pump is in lower abundance than the $3Na^{+}$-Ca^{++} antiporter but has a higher affinity for Ca^{++} and thus may contribute more to the regulation of resting intracellular [Ca^{++}] (see Fig. 13.2). The relative contribution of the Ca^{++} extrusion mechanisms, however, varies between species. For example, rat and mouse myocytes rely primarily on Ca^{++} reuptake by the SR (i.e., the SR accounts for 92% of Ca^{++} transport).

Regulation of the Force of Contraction

Intracellular Calcium

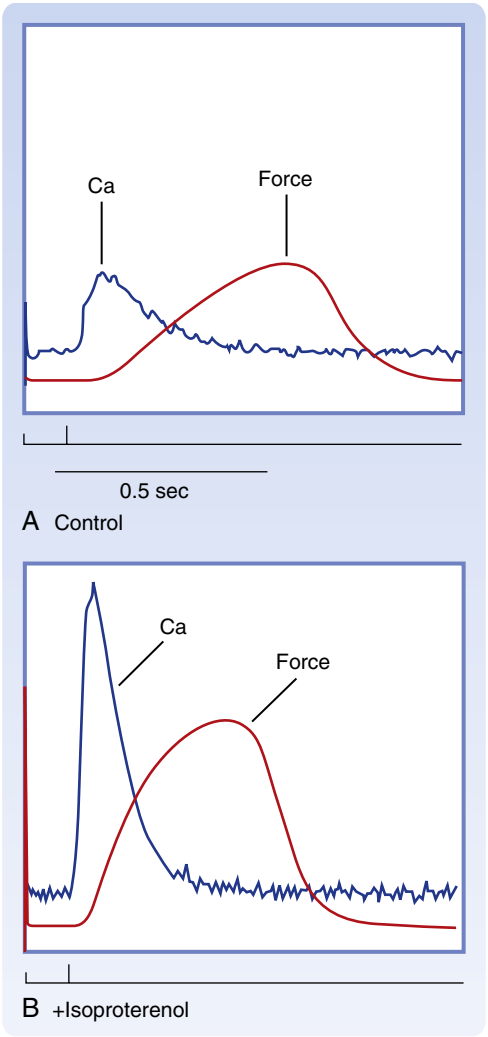
Because the heart represents an electrical syncytium, in which all the cardiac muscle cells contract during a single beat, it is not possible to increase the force of contraction by recruiting more muscle cells. Moreover, tetany of the heart would be lethal because it would defeat the critical pumping action of the heart. The heart has therefore developed alternative strategies to increase the force of contraction. The long duration of the action potential found in cardiac muscle, which is due to activation of the L-type voltage-gated calcium channel, results in a long refractory period, which in turn prevents tetany. Modulation of Ca^{++} influx through L-type calcium channels during an action potential, however, provides the heart with a mechanism to alter cytosolic [Ca^{++}] and hence the force of contraction.

A simple means of modulating the force of contraction of cardiac muscle cells in vitro is to vary extracellular [Ca^{++}]. As noted previously, contraction of the heart requires extracellular Ca^{++}. Decreasing extracellular [Ca^{++}] from a normal range of 1 to 2 mmol/L to 0.5 mmol/L, for example, reduces the force of the contraction. This reduction in force of contraction is not associated with a change in the duration of the contraction because the kinetic characteristics of Ca^{++} sequestration by the SR and Ca^{++} extrusion have not been modified. Although this approach of varying extracellular [Ca^{++}] to alter the force of contraction is demonstrable in vitro, it is not a common means of modulating the force of cardiac contraction in vivo.

In vivo, an increase in the size of the intracellular Ca^{++} transient and hence the force of contraction occurs in response to sympathetic stimulation (see the section "β-Adrenergic Agonists" and also Chapter 18). Sympathetic stimulation often occurs during periods of excitement or fright and involves activation of β-adrenergic receptors on the heart by norepinephrine (released from nerve terminals in the heart) or epinephrine (released from the adrenal medulla into the bloodstream). As shown in Fig. 13.3, the β-adrenergic agonist isoproterenol results in a dramatic increase in the size of the intracellular Ca^{++} transient and, consequently, a more forceful contraction. An increase in the force of contraction is termed **positive inotropy**. Typically, the rate of relaxation accompanying this β-adrenergic stimulation also increases, which results in a shorter contraction. The increase in the rate of muscle relaxation is termed **positive lusitropy**. The frequency of contractions of the heart also increases with β-adrenergic stimulation and is termed **positive chronotropy**. Thus β-adrenergic stimulation of the heart produces stronger, briefer, and more frequent contractions.

β-Adrenergic Agonists

The sympathetic nervous system is stimulated when a human or an animal becomes excited, and it is said to prepare the individual for "fight or flight." In the case of the heart, increased levels of the adrenal medullary hormone **epinephrine** or the sympathetic neurotransmitter **norepinephrine** activate β-adrenergic receptors on the cardiac muscle cells, which in turn activates **adenylate cyclase**, increases **cyclic adenosine monophosphate (cAMP)**, and thus promotes cAMP-dependent phosphorylation of numerous proteins in cardiac muscle cells (Fig. 13.4).



• **Fig. 13.3** Stimulation of β-adrenergic receptors in the heart increases the force of contraction. Electrical stimulation of myocardium results in a transient rise in intracellular [Ca^{++}] and production of force **(A)**. Isoproterenol (a β-adrenergic receptor agonist) increases the amplitude of the intracellular Ca^{++} transient and hence the amount of force generated **(B)**.

Both L-type voltage-gated calcium channels (responsible for the trigger Ca^{++}) and a protein associated with SERCA, called **phospholamban**, are phosphorylated by cAMP-dependent protein kinase. The combined action of these phosphorylations increases the amount of Ca^{++} in the SR. Specifically, phosphorylation of the sarcolemmal calcium channel causes more trigger Ca^{++} to enter the cell, and phosphorylation of phospholamban increases the activity of SERCA, thereby allowing the SR to accumulate more Ca^{++} before it is extruded by the $3Na^{+}$-Ca^{++} antiporter and the sarcolemmal Ca^{++} pump. The net result is that the SR releases more Ca^{++} into the cytosol during the next action potential, which promotes more actin-myosin interactions and hence greater force of contraction (see Fig. 13.3). The increased activity of SERCA after sympathetic stimulation also results in a shortened contraction because of the rapid reaccumulation of Ca^{++} by the SR. This in turn allows the heart to increase its rate of relaxation. An additional consequence of sympathetic stimulation is an increase in heart rate through a direct effect on the pacemaker cells (see Chapters 16 and 18).

Additional proteins and some micropeptides also appear to be associated with SERCA and influence SR calcium transport. This includes the 34–amino acid peptide **dwarf open reading frame (DWORF)**, which increases SERCA calcium affinity (Fig. 13.5), apparently by displacing phospholamban. DWORF was identified in putative noncoding RNA.

AT THE CELLULAR LEVEL

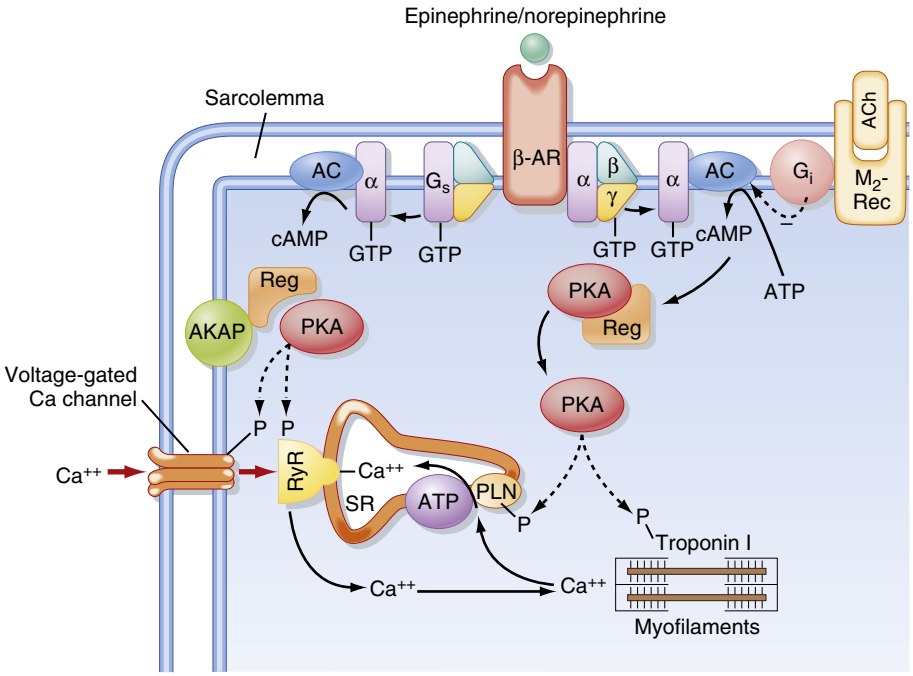
The mechanisms underlying the response of the heart to β-adrenergic stimulation are complex and involve cAMP-dependent phosphorylation of several proteins. An **A kinase anchoring protein (AKAP)** has been shown to be closely associated with the L-type voltage-gated calcium channel in the heart, thereby positioning **cAMP-dependent protein kinase** close to the channel and facilitating cAMP-dependent phosphorylation of this channel during sympathetic stimulation. How these cAMP-dependent phosphorylations increase the amplitude of the intracellular Ca^{++} transient and, in so doing, result in a more forceful, briefer cardiac contraction is discussed earlier (see also Chapter 18).

AT THE CELLULAR LEVEL

Mutations in the cardiac ryanodine receptor (RYR2) have been associated with cardiac arrhythmias. Specifically, catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited autosomal dominant disease that is typically manifested during childhood as an exercise-induced tachycardia that can progress to arrhythmias during exercise (or stress) and result in sudden death. Approximately 40% of patients with CPVT exhibit a defect in RYR2 that has been associated with increased release of Ca^{++} from the SR. The mutation in RYR2 may involve substitution of a highly conserved amino acid, which differs from malignant hyperthermia, in which splicing errors or deletions within RYR have been reported. It is hypothesized that during periods of exercise or stress, increased levels of intracellular Ca^{++} (because of the combined effects of β-adrenergic stimulation and increased activity of the mutated RYR2) promote the development of delayed afterdepolarizations and hence arrhythmias. Elevation of intracellular [Ca^{++}] during diastole is thought to promote the development of delayed afterdepolarizations through activation of the $3Na^{+}$-Ca^{++} antiporter, wherein Ca^{++} extrusion during diastole results in a net inward current sufficient to depolarize the cell to the threshold for an action potential. Treatment of CPVT involves antiadrenergic therapy (with β-adrenergic antagonists) or (for unresponsive patients) an implanted defibrillator.

Stretch

Stretching of the heart increases the force of contraction both in vivo and in vitro and is an intrinsic mechanism for regulating contractile force. In contrast, skeletal muscle typically exhibits maximal tension at resting length. Stretching



• **Fig. 13.4** Sympathetic stimulation of the heart results in an increase in cytosolic cyclic adenosine monophosphate *(cAMP)* and hence phosphorylation of several proteins by protein kinase A *(PKA)*. An A kinase anchoring protein *(AKAP)* adjacent to the L-type calcium channel facilitates phosphorylation of this channel and possibly nearby sarcoplasmic reticulum calcium channels. Other proteins phosphorylated by PKA include phospholamban *(PLN)* and troponin I. Muscarinic agonists (e.g., acetylcholine *[ACh]*), on the other hand, inhibit this sympathetic cascade by inhibiting the production of cAMP by adenylate cyclase *(AC)*. *β-AR*, β-Adrenergic receptor; *ATP*, adenosine triphosphate; G_i, inhibitory G protein; M_2*Rec*, muscarinic acetylcholine M_2 receptor; *Reg*, regulatory subunit of protein kinase A. (Redrawn from Bers DM. Cardiac excitation-contraction coupling. *Nature*. 2002;415:198.)

of the heart in vivo occurs during times of increased venous return of blood to the heart (e.g., during exercise or when the heart rate is slowed, or both). The **Frank-Starling law of the heart** refers to this ability of the heart to increase its force of contraction when stretched, which occurs at times of increased venous return (Fig. 13.6A; also see Chapter 16).

The importance of this mechanism is that it helps the heart pump whatever volume of blood it receives. Thus when the heart receives a lot of blood, the ventricles are stretched, and the force of contraction is increased, which ensures ejection of this extra volume of blood. Stretching of cardiac muscle also increases passive tension, which helps prevent overstretching of the heart. This passive resistance in the heart is greater than that in skeletal muscle and is attributed to both extracellular matrix (connective tissue) and intracellular elastic proteins (e.g., **titin**).

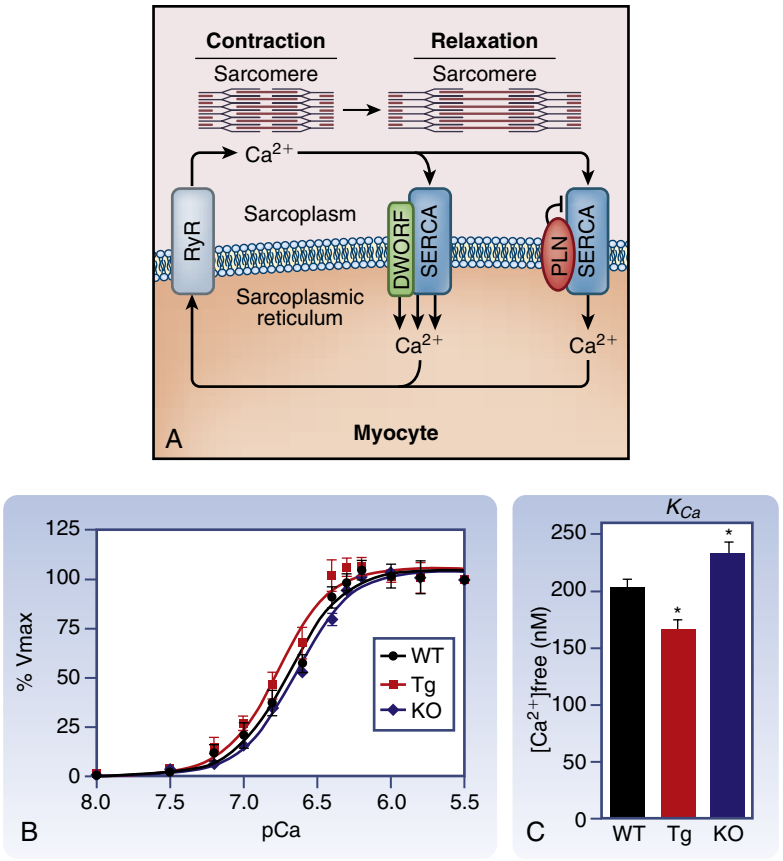
This stretch-induced increase in force of contraction of cardiac muscle occurs over a narrow range of sarcomere lengths (ca. 1.6–2.3 μm), resulting in a steep length-dependent activation of contraction. This ascending limb of the length-tension relationship in cardiac muscle is much steeper than that seen in skeletal muscle. It is important to note that this stretch-induced increase in force can occur within a single heartbeat.

The stretch-induced increase in force of contraction of cardiac muscle is associated with an increase in the Ca^{++}sensitivity of contraction (Fig. 13.6B). In rat ventricular trabecular muscle, approximately 60% of the stretch-induced increase in force of contraction has been attributed to an increase in Ca^{++} sensitivity, whereas the remaining 40% of the stretch-induced increase in force of contraction has been attributed to changes in the overlap of the thick and thin filaments. The changes in myofilament overlap, however, are less likely to contribute to the continued increase in force of cardiac contraction as sarcomere length increases from 2.0 μm to 2.3 μm, as this region is thought to represent a region of optimal overlap of myofilaments (and represents a plateau in the length-tension relationship in skeletal muscle).

The mechanism(s) contributing to the stretch-induced increase in Ca^{++} sensitivity of cardiac contraction are not clear, but has been reported to involve the following sarcomeric proteins (titin, troponin T and myosin-binding protein C).

Cardiac Muscle Metabolism

As in skeletal muscle, myosin uses the energy in ATP to generate force, so the ATP pool, which is small, must be continually replenished. Typically, this replenishment of ATP pools is accomplished by aerobic metabolism, including the oxidation of fats and carbohydrates. During times of ischemia, the **creatine phosphate** pool, which converts ADP



• **Fig. 13.5** A small (34–amino acid) peptide encoded by a long noncoding RNA appears to improve sarcoplasmic reticulum function and myocyte performance by countering the inhibitory effect of phospholamban *(PLN)* (as depicted in the working model shown in panel A). The ability of DWORF to increase cardiac SR Ca uptake is shown in panels B and C, where overexpression of DWORF in cardiomyocytes (labeled *Tg*) increased the Ca sensitivity of SR Ca transport, whereas knockout of DWORF (labeled *KO*) had the reverse effect. *DWORF*, Dwarf open reading frame; *KO*, knockout; *SERCA*, sarcoplasmic endoplasmic reticulum calcium-ATPase; *Tg*, transgenic; *WT*, wild-type. (From Nelson BR, Makarewich CA, Anderson DM, et al. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science.* 2016;351:271-275.)

to ATP, may decrease. As in skeletal muscle, the creatine phosphate pool is small.

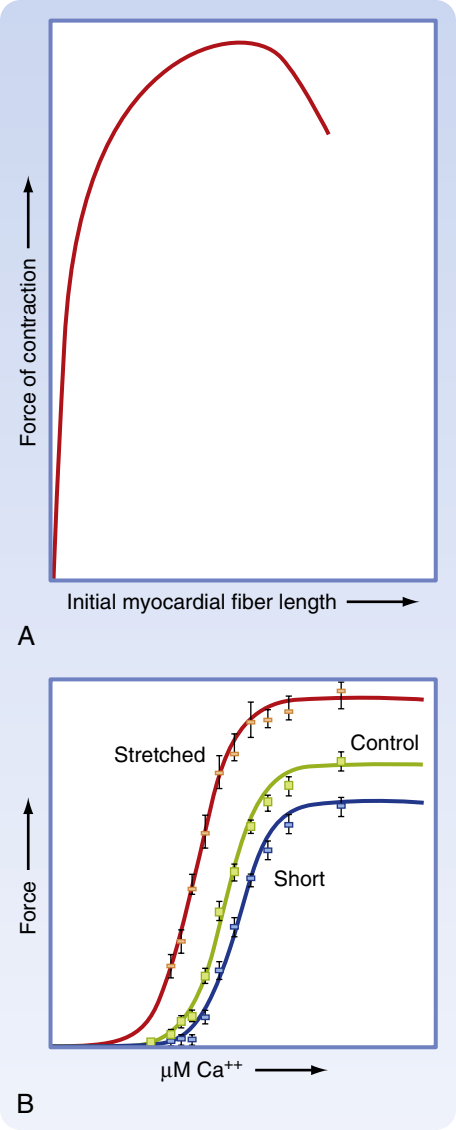
When cardiac muscle is completely deprived of O_2 because of occlusion of a coronary vessel (i.e., stopped-flow ischemia), contractions quickly cease (within 30 seconds). This is not due to depletion of either ATP or creatine phosphate because these levels decline more slowly. Even after 10 minutes of stopped-flow ischemia, when creatine phosphate levels are near zero and only 20% of the ATP remains, reperfusion can restore these energy stores, as well as contractile ability. However, prolonging the stopped-flow ischemia for 20 minutes results in further drops in ATP, so that reperfusion has considerably less effect, with only limited restoration of ATP and creatine phosphate levels or contractile activity.

Cardiac Muscle Hypertrophy

Exercise such as endurance running can increase the size of the heart as a result of hypertrophy of individual cardiac muscle cells. Concomitant with this enlarged so-called athlete's heart is improved cardiac performance, as assessed by an increase in stroke volume, increased oxygen consumption, and preserved relaxation. Thus the athlete's heart represents an example of "physiological hypertrophy," with beneficial contractile effects.

In contrast, if exposed to chronic pressure overload, the heart may undergo either **concentric left ventricular hypertrophy** or **dilated left ventricular hypertrophy**, which causes impairment of function.

Concentric hypertrophy is characterized by thickening of the left ventricular wall and represents a compensatory hypertrophy to the increased load. Dilated hypertrophy is characterized by increased ventricular volume (end-diastolic volume). Both concentric left ventricular hypertrophy and dilated left ventricular hypertrophy have been shown to exhibit decreased contractile response to β-adrenergic stimulation, which limits the contractile reserve. In dilated left ventricular hypertrophy, normal contractile function, along with the Frank-Starling response, may also be impaired.



• **Fig. 13.6** Stretching of the heart increases the force of contraction **(A).** This is attributable to both an increase in the maximal force of contraction and an increase in the sensitivity of contraction to Ca^{++} **(B)**. It reflects an intrinsic regulatory process referred to as the *Frank-Starling law of the heart.* (**B,** Redrawn from Dobesh D, Konhilas J, de Tombe P. Cooperative activation in cardiac muscle: impact of sarcomere length. *Am J Physiol Heart Circ Physiol.* 2002;282:H1055-H1062.)

The cellular and molecular mechanisms underlying the development of cardiac hypertrophy are not clear, although an elevation in intracellular [Ca^{++}] has been implicated.

The link or links between cardiac hypertrophy, decreased cardiac performance, and impaired β-adrenergic response during chronic pressure overload are unclear. Decreased cardiac performance has been attributed to dysregulation of intracellular [Ca^{++}]. Alterations in the level, activity, and phosphorylation status of a variety of proteins, including L-type voltage-gated calcium channels, phospholamban, SERCA2, and RYR2, have all been implicated in the Ca^{++} dysregulation associated with a failing heart (pathological hypertrophy).

A microRNA (miR-222) has been shown to be important for cardiac growth in response to exercise. It also appeared to inhibit maladaptive remodeling of the heart after ischemia/reperfusion injury.

AT THE CELLULAR LEVEL

A modest elevation in intracellular [Ca^{++}] (as a result of increased contractile activity, for example), has been proposed to activate a Ca^{++}/calmodulin-dependent protein phosphatase **(calcineurin)** that can dephosphorylate the transcription factor **nuclear factor of activated T cells (NFAT)**, thereby facilitating translocation of NFAT to the nucleus and ultimately promoting protein synthesis and thus hypertrophy. Activation of Ca^{++}/calmodulin-dependent protein kinase has also been implicated in activation of the transcription factor **myocyte enhancer factor 2 (MEF2)** by promoting the dissociation (nuclear export) of an inhibitor of MEF2 (namely, **histone deacetylase [HDAC])**. (These signaling pathways are analogous to those described for hypertrophy of skeletal muscle, as shown in Fig. 12.21)

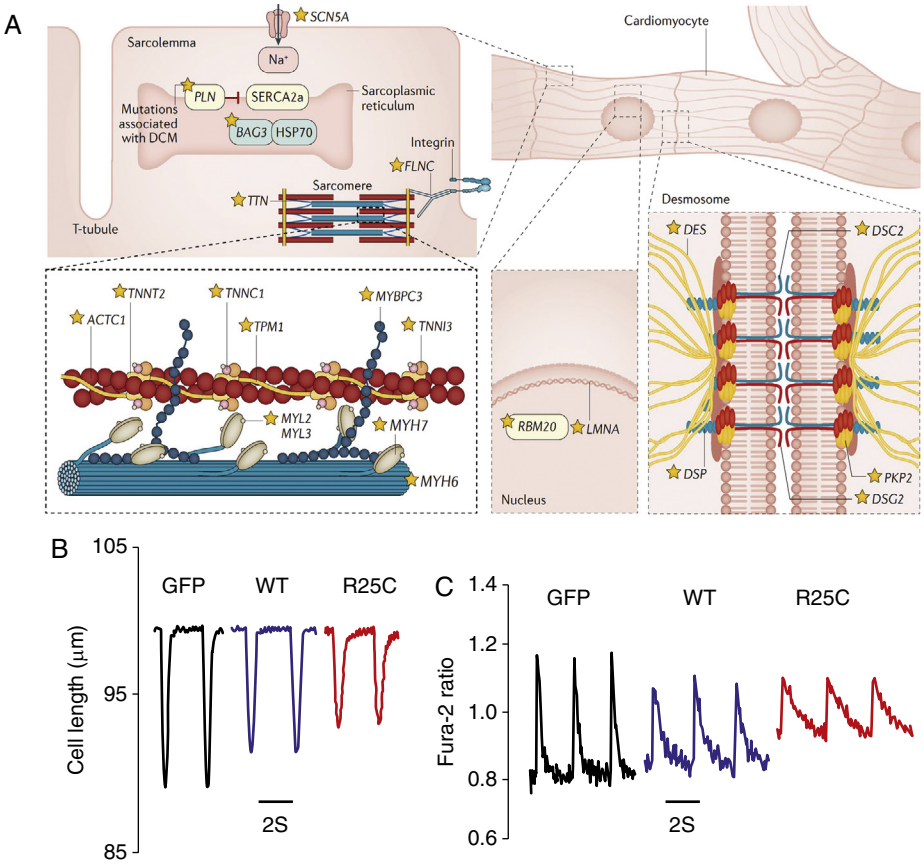
The impaired β-adrenergic response of cardiac muscle after chronic pressure overload involves, at least in part, a decrease in β-adrenergic receptors because of internalization. Both **phosphatidylinositol-3-kinase (PI3K)** and **β-adrenergic receptor kinase 1** have been implicated in the internalization of β-adrenergic receptors.

AT THE CELLULAR LEVEL

High blood pressure, defects in heart valves, and ventricular walls weakened as a result of myocardial infarction can all lead to heart failure, a leading cause of death. Heart failure may be seen with thickening of the walls of the ventricle or with dilation (i.e., increased volume) of the ventricles.

To monitor the development of left ventricular hypertrophy in response to pressure overload, and to assess strategies to reduce/prevent the associated myocardial hypertrophy and fibrosis, a rodent model using partial constriction of the aortic arch is commonly employed. Results from these studies have shown that the status of histone methylation and/or histone acetylation influences the development of cardiac hypertrophy. Specifically, inhibition for histone lysine dimethyl-demethylase (KDM3A) prevented the development of left ventricular hypertrophy and cardiac fibrosis in mice in response to aortic pressure overload. Inhibition of KDM3a also provided protection against ischemia/reperfusion injury. By contrast, overexpression of KDM3A induced hypertrophy of myocardium in vivo, and hypertrophy of cardiac myocytes in vitro. Inhibition of Class I and Class II histone deacetylases also preserved systolic function and inhibited left ventricular hypertrophy in response to aortic pressure overload, thus implicating histone methylation and histone acetylation in the response of the heart to pressure load, and raise the possibility of pharmacological targeting of KDM3A and/or HDACs to inhibit ventricular hypertrophy and/or reduce cardiac fibrosis.

There is evidence that cardiac hypertrophy may not be associated with some functional impairments. Intermittent aortic constrictions, for example, result in decreased β-adrenergic signaling, decreased capillary density, and decreased SERCA2 levels, without evidence of hypertrophy. Activation of PI3K appears to be involved in this response.



• **Fig. 13.7** Dilated cardiomyopathy *(DCM)* can result from mutations in sarcomeric proteins, channels, and/or desmosomes in myocardial cells **(A)**. Transfection of rat ventricular myocytes with a mutant phospholamban from a patient with Familial Dilated Cardiomyopathy resulted in decreased contraction **(B)** and decreased amplitude and kinetics of the intracellular [Ca^{++}] **(C)** following electrical stimulation at 0.5 Hz. *ACTC1*, Encodes actin; *BAG3*, encodes heat shock cognate 70 chaperone proteins; *DES*, encodes desmin; *DSC2*, encodes by desmocollin 2; *DSG2*, encodes desmoglein 2; *DSP*, encodes desmoplakin; *GFP*, myocardial cells overexpressed with the GFP vector (lacking phospholamban); *HSP70*, heat shock protein 70; *LMNA* encodes lamin A/C; *MYBPC3*, encodes myosin-binding protein C; *MYL2, MYL3, MYH6, and MYH7*, encode myosin chains; *PKP2*, encodes plakophilin 2; *PLN*, encodes phospholamban; *R25C*, myocardial cells overexpressed with the mutant DCM phospholamban vector; *SERCA2a*, sarcoplasmic/endoplasmic reticulum calcium ATPase 2a; *TNNT2, TNNC1 and TNNI3*, encode cardiac troponins; *TPM1*, encodes tropomyosin-α1 chain; *WT*, myocardial cells overexpressed with the wildtype phospholamban vector. **(A,** From Schultheiss HP, Fairweather D, Caforio ALP, et al. Dilated cardiomyopathy. Nat Rev Dis Primers. 2019;5:32. **B,** From Liu GS, Morales A, Vafiadaki E, et al. A novel human R25C-phospholamban mutation is associated with super-inhibition of calcium cycling and ventricular arrhythmia. Cardiovasc Res. 2015;107:164.)

IN THE CLINIC

Dilated cardiomyopathy (DCM) is characterized by dilation of the left ventricle or both ventricles, with impaired contraction that can lead to heart failure. There is also a risk of sudden death from an arrhythmia. The incidence of DCM is 0.4%. DCM is usually caused by a mutation in a sarcomeric protein or desmosome, though other causes include (1) inflammation, (2) exposure to drugs, toxins, or allergens, (3) systemic endocrine or autoimmune diseases, or (4) the cause may be idiopathic. Thus, DCM is not secondary to pressure overload. Mutations in DCM have been observed in the sarcomeric proteins titin, myosin 7, troponin, phospholamban, and tropomyosin, as well as the voltage-gated Na channel (Fig.13.7A). Data in Fig. 13.7B,C show the effects of a mutant phospholamban that was identified in exosomes from four members of a family with adult onset DCM. Left ventricular ejection fractions ranged from 17% to 40%, and they all experienced either atrial fibrillation or nonsustained tachycardia. The mutant phospholamban contained a change from arginine to cysteine at position 25. When the mutant phospholamban (R25C) was overexpressed in rat ventricular myocytes, the transfected myocytes exhibited decreased extent of

contraction (Fig. 13.7B) and decreased amplitude and kinetics of the intracellular Ca transient (Fig. 13.7C) in response to electrical stimulation at 0.5 Hz, compared to ventricular myocytes transfected with wildtype phospholamban (WT) or with a vector containing green fluorescent protein (GFP) instead of phospholamban. There was also a decrease in the Ca^{++} sensitivity of SR Ca transport in myocytes with the mutant phospholamban, along with increased passive Ca^{++} leak from the SR, both of which may have contributed to the increased basal intracellular Ca^{++} evident from the elevated baseline of R25C-PLN in Fig. 13.7C. The increased basal Ca^{++} could contribute to the development of arrhythmias in these DCM carriers.

Key Concepts

1. Cardiac muscle is an involuntary, striated muscle. Cardiac muscle cells are relatively small (10 μm × 100 μm) and form an electrical syncytium with tight electrical and mechanical connections between adjacent cardiac muscle cells. Action potentials are initiated in the sinoatrial node and spread quickly throughout the heart to allow synchronous contraction, a feature important for the pumping action of the heart.
2. Contraction of cardiac muscle involves the Ca^{++}-dependent interaction of actin and myosin filaments, as in skeletal muscle. However, unlike skeletal muscle, cardiac muscle requires an influx of extracellular Ca^{++}. Specifically, the influx of Ca^{++} during an action potential triggers release of Ca^{++} from the SR, which then promotes actin-myosin interaction and contraction.
3. Relaxation of cardiac muscle involves reaccumulation of Ca^{++} by the SR and extrusion of Ca^{++} from the cell via the $3Na^{+}$-Ca^{++} antiporter and the sarcolemmal Ca^{++} pump. The Ca^{++} pump of the SR is associated with numerous proteins (forming a regulosome), including some endogenous micropeptide inhibitors and activators.
4. The force of contraction of cardiac muscle is increased by stretch (Frank-Starling law of the heart) and by sympathetic stimulation. Skeletal muscle, in contrast, increases force by recruiting more muscle fibers or by tetany.
5. Hypertrophy of the heart can occur in response to exercise, chronic pressure overload, or genetic mutations. The cardiac hypertrophy resulting from exercise is typically beneficial, with improved cardiac performance, increased oxygen consumption, and normal relaxation. Chronic pressure overload, on the other hand, can result in cardiac hypertrophy that is initially associated with a decreased β-adrenergic response but may progress to dilated cardiac hypertrophy, characterized by decreased contractile ability. Genetic mutations resulting in cardiac hypertrophy include familial hypertrophic cardiomyopathy, in which a mutation in a single intracellular protein may alter contractile function and promote a hypertrophic response. Researchers have identified a microRNA that appears to contribute to the exercise-induced hypertrophy of the heart and to inhibit maladaptive remodeling after ischemia/reperfusion injury.