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Cardiac thin filament regulation

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Abstract

Myocardial contraction is initiated upon the release of calcium into the cytosol from the sarcoplasmic reticulum following membrane depolarization. The fundamental physiological role of the heart is to pump an amount blood that is determined by the prevailing requirements of the body. The physiological control systems employed to accomplish this task include regulation of heart rate, the amount of calcium release, and the response of the cardiac myofilaments to activator calcium ions. Thin filament activation and relaxation dynamics has emerged as a pivotal regulatory system tuning myofilament function to the beat-to-beat regulation of cardiac output. Maladaptation of thin filament dynamics, in addition to dysfunctional calcium cycling, is now recognized as an important cellular mechanism causing reduced cardiac pump function in a variety of cardiac diseases. Here, we review current knowledge regarding protein–protein interactions involved in the dynamics of thin filament activation and relaxation and the regulation of these processes by protein kinase-mediated phosphorylation.

Keywords

Cardiac function; Actin; Myocardial contractility; Muscle mechanics; Troponin; Thin filament

Introduction and scope

Myocardial force generation is the result of cyclic interactions between actin and myosin (a.k.a. cross-bridges) that are initiated upon the release of calcium ions from the sarcoplasmic reticulum [7,24]. The principal proteins involved in this process are illustrated schematically in Fig. 1a and structural estimates are shown in Fig. 1b. Actin monomers (globular actin) are polymerized into a double helical structure to form filamentous actin (F-actin). F-actin, together with two tropomyosin (Tm) strands, each binding a troponin (Tn) complex, forms the thin filament. Tm and the Tn complex regulate the affinity of F-actin towards myosin. Tn is composed of three subunits: troponin C (TnC), troponin I (TnI), and troponin T (TnT) with 1:1:1 molar ratio. Two alpha-helix Tm monomers dimerize to form a coiled-coil structure that overlaps partially end to end with neighboring Tm dimers (by about eight amino acid residues) to form a continuous Tm strand that lies in the two grooves of actin. Each Tm dimer binds one Tn complex such that the tail region of the Tn complex extends to the C-terminus of the Tm molecule at the Tm–Tm overlap region [83]. One Tm covers seven actin molecules, thereby forming one regulatory unit of the thin filament.

Due to the spatial constraints within the sarcomere lattice, it may be that the interaction between actin and myosin during physiological muscle contraction is limited to only a single cross-

bridge for each functional unit of the thin filament [34,60]. In addition, recent evidence suggests that the structural states of the seven actin monomers in the regulatory unit are not identical, supporting the notion of a preferential binding site for myosin halfway between the Tm–Tm overlap region close to the location of Tn on the actin filament [55,77,78]. Finally, whether simultaneous independent cross-bridge formation can take place on opposite sides of the F-actin filament is not known.

Unlike smooth muscle where formation of cross-bridges is regulated via Ca^{2+} -dependent phosphorylation of one of the light chains associated with the myosin molecule, Ca^{2+} activation in striated muscle is mostly regulated at the level of the thin filament [24]. Thus, Ca^{2+} binding to the regulatory troponin complex troponin induces a structural change in troponin that causes relocation of tropomyosin away from the actin groove towards a more peripheral location, thereby exposing the myosin binding site(s) on actin to allow for cross-bridge formation [12]. Further fine control of striated muscle contraction dynamics is exerted by pathways involving other contractile proteins such as the myosin light chains, myosin-binding protein C, and possibly, titin. In skeletal muscle, individual myocytes are fully activated via motor nerve activity and, thus, whole muscle force is principally regulated via motor unit recruitment. In contrast, in the heart, electrical stimulation readily spreads via low resistance gap junctions such that all myocytes contract each beat, and the strength of the heart is regulated by variation of the contractile strength of the cardiac myocytes. This is accomplished by two main cellular excitation–contractions mechanisms: (a) variation of the amount of Ca^{2+} released from the sarcoplasmic reticulum and (b) variation in the response of the myofilaments to activator Ca^{2+} [7,16,37,72]. For a discussion of factors that regulate myocardial Ca^{2+} fluxes, the reader is referred to [7]; it is important to note that physiologically, the level of Ca^{2+} activation in the heart is always less than saturated. The main modulators of myofilament Ca^{2+} sensitivity are sarcomere length (Frank–Starling relation) [40] and post-translational modification of sarcomere proteins, most notably by kinase-mediated phosphorylation [37,72].

In general, skeletal muscle contractile proteins lack the functional phosphorylation targets found in the cardiac contractile protein isoform counterparts, a finding that is consistent with the fundamentally different cellular mechanisms underlying regulation of contractile force between cardiac and skeletal muscle. Recent investigations have provided new insights into the molecular mechanisms that underlie thin filament activation and the role of contractile protein phosphorylation in modulating myofilament function in the heart [37,67]. Moreover, contractile protein phosphorylation has emerged as an important determinant of depressed myofilament function in cardiac diseases such as the syndrome of congestive heart failure, cardiac hypertrophy, and diabetic cardiomyopathy [5,6,27,67]. Accordingly, this review is focused on the cardiac thin filament and its role in regulating cardiac myofilament dynamics both in health and disease.

Thin filament structure and function

Biochemical and structural studies strongly indicate that the thin filament as allosteric regulator of muscle contraction can exist in multiple states [24,37]. Binding of the globular domain of myosin (S1) to reconstituted thin filaments has been found to be highly cooperative in the absence of Ca^{2+} [25]. In the presence of Ca^{2+} , the binding of S1 becomes less cooperative. This observation has been interpreted in a model where the thin filament can exist in two functional states, “on” or “off”. Thin filaments in the on state bind S1 strongly, whereas those in the off state bind S1 weakly. Strong S1 binding to a structural unit of the thin filament induces other structural units along the filament to be more permissive to enter the on state via a nearest neighbor effect, possibly communicated by Tm [31]. On the other hand, based on both equilibrium and transient kinetic studies of S1 binding to isolated thin filaments, Geeves's

group concluded that the binding of myosin S1 to actin occurs in two distinct molecular steps, leading to a three-state molecular model of thin filament activation [21,47]. These three states of the thin filament include a blocked state (B-state) reflecting steric block of cross-bridge formation, a closed state (C-state) reflecting cross-bridges that are only weakly bound and do not produce force, and an open or strong myosin binding state (M-state) in which force is generated. In this alternative model, the regulatory role of Ca^{2+} is to affect the equilibrium between these three thin filaments states. In cardiac muscle, it is estimated that in the absence of Ca^{2+} , 50% of thin filament regulatory units are present in the B-state and 40% in the C-state. Conversely, in the presence of Ca^{2+} , 75% of thin filaments are present in the C-state [46]. Although the thin filaments states were defined based on biochemical studies in terms of the ability of the thin filaments to interact with myosin heads (S1) in solution, recent electron microscopy analysis of thin filaments has indicated that these three states may indeed correspond to different physical positions of tropomyosin on the thin filament [57]. It should be mentioned that although Geeves' model predicts that S1 binding can fully activate the thin filaments irrespective of $[\text{Ca}^{2+}]$, it has been shown that in order for the thin filament to be fully activated, both Ca^{2+} and S1 binding are required [29].

Actin–tropomyosin

Actin forms two intertwined right-handed helical arrays with an approximate 770-Å repeat. Tm is composed of two polypeptide chains that form an alpha-coiled-coil structure 400 Å in length, which wraps around the actin filament. Head-to-tail interaction between Tm molecules is considered to play an important role in cooperative activation properties of the thin filament. For example, removal of the Tm overlap region by carboxypeptidase treatment was shown to induce partial loss of cooperativity of S1 binding to the thin filament [54]. Ser-283, which is the second residue from the C-terminus of Tm, can be phosphorylated by tropomyosin kinase and/or an as of yet unidentified protein kinase(s). Phosphorylation of Ser-283, as well as a charge mutation (Ser283Glu) believed to mimic the phosphorylated state of this residue, increases the viscosity of Tm in solution by increasing head-to-tail interactions between Tm molecules [30,64]. The NMR solution structure of the head-to-tail region, reported by Greenfield et al. [26], indicates that Ser283 is close to Lys7 or Lys12 of the next Tm molecule, although there are no specific NOEs defining such interactions. Phosphorylation of Ser283 may introduce an additional salt bridge between the phosphate group and the ϵ -amino group of Lys7 and/or Lys12 to stabilize the head-to-tail interaction. Recently, our group showed depression of both tension and ATPase activity in skinned cardiac fiber bundles isolated from a transgenic mouse model in which p38 MAPK was constitutively activated by overexpression of the upstream activator MKK6bE, and this was associated with a significant reduction in the phosphorylation level of Tm [79]. Together, these results suggest that the strength of Tm head-to-tail interaction is an important determinant of myofilament functional activity and that this mechanism is modulated by Tm phosphorylation at Ser238.

Troponin C

TnC is a dumbbell-shape molecule with two globular domains (the N-domain and the C-domain) connected with a central linker. TnC is a member of the broad class of E–F hand-type Ca^{2+} -binding proteins. It has four E–F hand motifs, two in each N- and C-domain. The Ca^{2+} -binding sites in the C-domain (sites III and IV) have a strong affinity for Ca^{2+} such that these sites do not control Ca^{2+} -dependent muscle contraction. Because of this, the C-domain is considered a structural domain. That is, it interacts with TnI and TnT irrespective of the prevailing cytosolic $[\text{Ca}^{2+}]$. The Ca^{2+} -binding site I in the N-domain of cardiac TnC (cTnC) cannot bind Ca^{2+} under physiological conditions due amino acid replacements in the key Ca^{2+} -coordinating position of this site. Thus, the Ca^{2+} -binding site II in the N-domain of cTnC is the only domain that is directly involved in Ca^{2+} regulation of cardiac muscle contraction,

in contrast to the two functional regulatory Ca^{2+} -binding sites in fast skeletal muscle TnC. Ca^{2+} binding to the N-terminal regulatory domain of TnC induces a series of structural transitions both in the Tn complex and in actin-Tm to induce activation of the thin filaments [24,37].

It is of note that Ca^{2+} binding to isolated cardiac troponin complex in solution is not cooperative, as would be expected based on the presence of a single-regulatory low-affinity Ca^{2+} -binding site on cTnC [13,38]. Furthermore, although the head-to-tail interactions between Tm molecules is considered to be important in cooperative S1 binding, this does not extend to Ca^{2+} binding to the Tn-Tm complex. Only when the Tn complex is incorporated into the thin filament does the single Ca^{2+} -binding site on TnC bind Ca^{2+} in a cooperative manner, indicating the presence of a positive cooperative feedback mechanism that is transmitted along the thin filament to neighboring regulatory units, a process that likely involves Tm-actin-Tn interaction [13,38]. Finally, we have found that the relationship between activator [Ca^{2+}] and myofilament force is highly cooperative in both cardiac and fast skeletal muscle but not in slow skeletal muscle despite the fact that the slow skeletal sarcomere contains cTnC [41].

Troponin I

TnI is the inhibitory subunit of the Tn complex; it has extensively been studied both by others and by us. For a detailed description of the structure/domain organization of cTnI, the reader is referred to a recent overview [70]. There are at least two actin-interacting sites along TnI sequence: the inhibitory region and a second actin-Tm binding site.

The inhibitory region is highly rich in basic amino acid residues, and its amino acid sequence is highly conserved among species. The minimum inhibitory region, which was originally identified by Talbot and Hodges for fsTnI [24], itself is capable of interacting with actin to inhibit actin-activated myosin ATPase activity. However, its inhibitory function is more pronounced in the presence of Tm. Several hypertrophic/restrictive cardiomyopathy-linked mutations have been reported within the inhibitory region [22,49]. As might be expected, these amino acid replacement(s) in the inhibitory region result in impaired inhibitory function at diastole. Furthermore, the weakened interaction between mutated TnI and actin-Tm causes sensitization of the thin filaments to Ca^{2+} [38] and associated slowed relaxation of force kinetics [44]. Consistent with this notion, thin filaments that contain cTnI with a HCM/RCM-linked mutation in the inhibitory region bind Ca^{2+} stronger than their wild-type counterpart [38,61]. Finally, the inhibitory region includes the PKC-specific phosphorylation site, Thr-144. The functional consequence of phosphorylation of this residue has not been fully resolved. On one hand, Burkart et al. found that by replacing Thr-144 by Glu to mimic phosphorylation, detergent-skinned cardiac fiber bundles containing mutant cTnI showed no significant difference in Ca^{2+} sensitivity and maximum force from those containing wild-type cTnI, whereas in the in vitro motility assay, cTnI with a Thr144Glu mutation displayed desensitization to Ca^{2+} [11]. On the other hand, Wang et al. showed that when cardiac myocytes expressing cTnI (Ser23Ala/Ser24Ala) were exposed to PKC- β II, phosphorylation of PKC-specific sites induced increased myofilament Ca^{2+} sensitization [81]. These investigators additionally found that the Thr-144 residue is the most permissive site for PKC-mediated phosphorylation, consistent with previous in vitro findings [39,53]. Finally, TnI, and in particular the inhibitory region of TnI, is emerging as an important molecule in relation to modulation of myofilament function by sarcomere length [40]. That is, cardiac sarcomeres containing the slow skeletal isoform of TnI (ssTnI) display a markedly blunted impact of sarcomere length on myofilament function [4,42]. The inhibitory region of ssTnI differs from cTnI by the presence of a proline in the equivalent position of Thr144 in cTnI. We recently found that TnI-Thr144 per se is able to impart length-dependent properties onto the cardiac

sarcomere, while a proline at that position virtually eliminates myofilament length dependency [75]. These results suggest that myofilament-length-dependent activation has, at its basis, a signal transduction pathway that includes Tn–Tm–actin signaling rather than a more direct mechanism involving myosin–actin affinity modulated by inter-filament spacing [19,20,40]. The underlying molecular mechanisms, however, are as of yet still largely unknown.

The second actin–Tm binding site is located in the C-terminal mobile domain of TnI. A structure of the mobile domain in solution has been proposed based on NMR NOE measurements [50]. However, the chemical shifts of amino acid residues in the mobile domain strongly indicate that this domain is intrinsically unfolded [10]. Furthermore, Hoffman et al. suggested that the mobile domain undergoes a disordered–ordered structural transition when it interacts with actin in the absence of Ca^{2+} [32], indicative of an association of this region with actin–Tm (termed “fly-casting mechanism”). It has been shown, at least in rodents, that moderate ischemia/reperfusion induces proteolytic cleavage of 17 residues from the C-terminal of TnI that includes the second actin-interacting sites [80]. Human myofibrils containing truncated cTnI display impaired force relaxation kinetics upon rapid solution switching Ca^{2+} deactivation [51]. In addition, presence of truncated cTnI induces a marked increase in myofilament Ca^{2+} sensitivity concomitant with a reduction in cooperativity in Ca^{2+} regulation of force. We recently showed that these phenomena require interaction between Tn and actin–Tm, consistent with the fly-casting mechanism [74]. In addition, we found a significant increase in cross-bridge cycling kinetics, suggestive of an inhibitory role for the C-terminus of cTnI [74]. However, the question as to whether this domain by itself inhibits myofilament function has not yet been resolved. Finally, hypertrophic/restrictive cardiomyopathy-linked mutations have also been found in this region and, as is the case with mutations found in the inhibitory region, mutations in the C-terminal mobile domain are associated with increased myofilament Ca^{2+} sensitivity [49].

At the distal end of the C-terminal mobile domain of TnI towards the N-terminal side of the molecule, cTnI has an alanine residue at position 164, while both in fsTnI and ssTnI this residue is substituted by a histidine. Neonatal myocardium is relatively insensitive to the decreased myofilament Ca^{2+} responsiveness that is normally seen under acidic conditions in adult myocardium [68]. This phenomenon is due to the presence of ssTnI that is predominantly expressed in the neonatal heart, being replaced by the adult cTnI isoform shortly after birth [43]. Recently, it has been demonstrated that the presence of the valine residue at the 164 position is largely responsible for this phenomenon such that transgenic mice that express A164V cTnI in the heart are resistant to depression of twitch force under conditions of cellular acidosis [14]. This “proof of concept” experiment indicates that alteration of cardiac contractile proteins, in this case cTnI, via gene therapy techniques may be a viable therapeutic approach in various cardiac diseases.

In between the inhibitory region and the second actin–Tm binding domain is a segment termed the “switch region”. It is a domain that interacts with a hydrophobic patch of the N-terminal regulatory domain of TnC only when Ca^{2+} occupies the regulatory site on TnC. The Ca^{2+} -induced interaction between the switch region of TnI and TnC pulls both the inhibitory region and the second actin–Tm site from the actin–Tm surface so as to release the inhibitory action of TnI on the thin filament. In addition, Hoffman et al. suggested that the switch region also undergoes a disordered–ordered structural transition when it binds to TnC in the presence of Ca^{2+} [32]. TnI also interacts with TnC near the N-terminal part in the IT–arm domain. This region forms an alpha-helix and strongly interacts with the C-domain of TnC. The very N-terminal end of this C-domain-interacting site of cTnI contains the other PKC-specific phosphorylation sites, Ser-43 and Ser-45. Despite the recent finding that these sites are poor substrates for PKC, phosphorylation of these sites has a strong impact on myofilament activity, in particular Ser-45 [72]. For example, substitution of Ser-43/Ser-45 with the charged residue

Glu induces a marked depression of actin-activated S1 ATPase activity in solution and a reduction in myofilament Ca^{2+} sensitivity [11,48]. This depression of myofilament activity by phosphorylation at these PKC-specific sites appears to be due to stabilization of the thin filament in the off-state [45]. Consistent with this notion, replacing Ser43/Ser45 with non-phosphorylatable Ala43/Ala45 residues in a transgenic murine model resulted in both increased cardiac contractility [62] and increased cross-bridge cycling kinetics in isolated skinned myocardium [58]. Finally, a TnT-interacting site follows the C-domain interacting site of TnI (termed the “IT-arm”). Until recently, this part of the Tn complex was considered structural rather than functional. However, recent data from our group suggest that the IT-arm region, in particular residues 33–80, is important for cross-bridge-dependent cooperative myofilament activation and its sensitivity to acidic pH [18].

Unlike the fast and slow skeletal isoforms of TnI, cTnI contains a unique N-terminal extension about 30 amino acids in length. This region contains the PKA-phosphorylation sites, Ser-23/Ser-24. However, other kinases such as PKC and PKD may also mediate phosphorylation at these sites [28,37,72]. The N-terminal extension is thought to be too flexible to be resolved by crystallography. Nevertheless, a partial structure of this segment has been proposed [33,36]. It has been shown that the cardiac-specific N-terminal extension interacts with the N-domain of cTnC near the non-functional Ca^{2+} -binding site 1 in a phosphorylation-dependent manner. That is, phosphorylation of Ser23/Ser24 results in a weakening of the interaction between the N-terminal extension of cTnI and the N-terminal domain of cTnC [1,82], concomitant with the characteristic reduction that is seen in myofilament Ca^{2+} sensitivity [69]. The underlying molecular mechanism of this phenomenon is the destabilization of the Ca^{2+} -bound state of cTnC. In addition, we recently found that phosphorylation of Ser23/Ser24, in isolation, induces an increase in cross-bridge cycling kinetics [9]. This result is in contrast to earlier findings indicating that PKA treatment of isolated skinned myocardium did not affect cross-bridge cycling rate [17,35], albeit that this has not been a universal observation [63,71]. Interestingly, phosphorylation of Ser-23/Ser-24 does not affect the maximum Ca^{2+} -dependent association rate of S1 to reconstituted thin filaments [59], indicating that the enhanced cross-bridge cycling kinetics must be the result of modulation of acto-myosin reaction steps subsequent to initial cross-bridge formation, most likely the rate of cross-bridge detachment. Thus, Ser-23/Ser-24 phosphorylation leads to decreased myofilament Ca^{2+} sensitivity and increased cross-bridge cycling rate. This notion is supported by a recent study of Biesiadecki et al. [9] investigating a dilated cardiomyopathy-linked mutation in cTnC (G159D). Using recombinant Tn exchange techniques in skinned myocardium, these investigators found no effect on myofilament function when this mutation was introduced in isolation. However, the Ser-23/Ser-24 phosphorylation-induced myofilament desensitization was virtually eliminated in the presence of the cTnC mutation. In contrast, the Ser-23/Ser-24 phosphorylation-induced accelerated cross-bridge cycling kinetics was not affected by the cTnC mutation. Thus, the G159D mutation of cTnC blunts only one part of the two separate functional effects of Ser23/Ser24 phosphorylation. A similar result has been reported for another cTnC mutation linked to hypertrophic cardiomyopathy (L29Q) [66]. How these two cTnC mutants that blunt the impact of PKA mediated phosphorylation of cTnI can lead to two entirely different disease states is yet to be resolved. Nevertheless, it is apparent that mishandling of the beta-adrenergic stimulus by the cardiac myocytes may play a key role in the development of cardiac disease.

Troponin T

TnT is a markedly polar molecule containing 30% acidic and 20% basic residues in its amino acid composition. TnT can be readily cleaved into two soluble fragments, T1 and T2, by mild chymotryptic treatment. The T1 region, which is N-terminal part of TnT, binds to tropomyosin strongly and is located closest to the Z-disk extending towards the head–tail interaction site of Tm. This region of TnT may play an important role in the cooperative activation of the thin

filament by modulating the Tm–Tm interaction in the head-to-tail junction of the Tm strand. In the human heart, alternative splicing of exons in the near N-terminal part of TnT produces at least four isoforms of TnT that are developmentally regulated [2]. In addition, it has been reported that human heart failure is associated with reexpression of the fetal TnT isoform profile [3]. Based on studies in the developing rabbit heart, it was originally thought that cardiac sarcomeres containing different isoforms of cTnT differ in terms of myofilament Ca^{2+} sensitivity [52]. More recent studies employing recombinant troponin exchange techniques, however, have demonstrated that the fetal TnT isoform profile only affects myofilament Ca^{2+} sensitivity in the presence of ssTnI [23], the fetal isoform of TnI expressed early after birth [43]. Similar to the proteolytic cleavage of C-terminus of cTnI, it is now apparent that the N-terminal hyper-variable region [8], a region that includes the exons that produce the splice variants mentioned above, is also cleaved by m-calpain during ischemia-reperfusion [84]. Of note and in contrast, m-calpain treatment of isolated cTnT in solution only results in non-specific protein degradation. The impact of cTnT N-terminal cleavage on myofilament function has not been studied in detail. Finally, the T2 region of cTnT, consisting of the C-terminal ~100 residues of TnT, binds both TnI and TnC and forms the globular domain of the Tn complex. The T2–TnI–TnC complex is sufficient to render S1 binding to actin–Tm Ca^{2+} -sensitive, albeit it not with a high level of cooperativity [65].

TnT is not a substrate for PKA. However, it does contain up to four potential PKC-specific phosphorylation sites [73]. Sumandea et al., employing recombinant Tn exchange techniques in skinned myocardium, demonstrated that Thr-206 is the functionally important site [73]. That is, either phosphorylation or a charge mutation phospho-mimic (Thr206Glu) at this residue significantly reduces maximum Ca^{2+} -saturated force, myofilament Ca^{2+} sensitivity, and cross-bridge cycling rate. Although the underlying molecular mechanisms are still largely unknown, it was noted that Thr-206 is located close to the end of a N-cap residue of an alpha-helix. Phosphorylation or Glu-substitution of this residue, therefore, could conceivably extend the alpha-helix up to Gly-200, causing either local or global conformational alterations of the Tn complex [73]. Regardless of the underlying mechanisms, it is clear that PKC-mediated phosphorylation of cTnT may play a significant role in the regulation of the cardiac thin filament.

Cardiac diseases

There are several factors that modulate cardiac myofilament activity, especially the activation state of the thin filament. One important factor that affects myofilament function that is not discussed in detail in this review is the genetic alteration of one more of the proteins that comprise the sarcomere. Over the past decade, a large number of such mutations (well over 50) have been reported that are causal to cardiac diseases such as familial hypertrophic cardiomyopathy, genetic dilated cardiomyopathy, and electrical abnormalities that lead to rhythm disturbances and sudden cardiac death [49,76]. As such, these diseases have now emerged as specific sarcomeric diseases of genetic origin. The clinical importance in comparison to that of other non-genetic cardiac diseases such as ischemic-dilated cardiomyopathy and ventricular hypertrophy is modest. Nevertheless, these genetic sarcomeric diseases provide important clues to identify regions of the contractile proteins that are pivotal to sarcomeric function in vivo.

Heart failure is characterized by a general decline in pump function of the heart that has, at its basis, a decline in contractile properties of the cardiac myocyte. Although it is well recognized that disturbances in calcium regulation constitute an important mechanism in this process [7], it is now clear that heart failure is also associated with a decline in myofilament response to activator Ca^{2+} [15,16,27,37,67,72]. Although isoform distribution and proteolytic cleavage of contractile proteins may play a role, (inappropriate) contractile protein phosphorylation has

emerged as an important determinant of depressed myofilament function in various etiologies of heart failure [5,6,27,67]. For example, in experimental heart failure in the rat, we found a depression in myofilament function that was causally linked to alterations in Tn; a follow-up study demonstrated that this was due to, most likely, up-regulation of PKC- α activity and the subsequent phosphorylation of cardiac contractile proteins [5,6]. Likewise, studies by van der Velden et al. indicate that depressed myofilament function in human heart failure is associated with alterations in Tn phosphorylation, as well as other contractile proteins such as myosin light chain and myosin-binding protein C [27]. Thus, maladaptive contractile protein function, and in particular contractile protein phosphorylation, may be a worthy signaling mechanism to target so as to restore contractile function of the ventricular myocyte in end-stage CHF.

Future perspectives

The coupling between excitation and contraction in the heart involves processes leading from membrane depolarization and subsequent release of calcium from the sarcoplasmic reticulum to the activation of the cardiac sarcomere. Although each of these physiological processes is subject to regulation, dynamics of thin filament activation has emerged as both a central and integral component of this control system. Accordingly, this review has focused on the molecular mechanisms and pathways that govern thin filament dynamics. The heart is an amazing biological mechanical pump capable of delivering cardiac output appropriate for a vast variety of loading conditions ranging from very low demand during sleep to very high demand during vigorous exercise. How the cardiac cell is able to tune its performance to such a vast range of physiological conditions and how the same signals may become maladaptive in cardiac diseases is only partly understood. Unraveling of this mystery will undoubtedly pave the way for the development of new therapeutic strategies to combat cardiac diseases.

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Fig. 1.

a Schematic diagram illustrating protein components of the cardiac sarcomere with a specific emphasis on the thin filament. The *top panels* illustrate the sarcomere in the resting condition in the absence of calcium bound to troponin C (cTnC, red). In this condition, troponin I (cTnI, green) acts to inhibit myosin cross-bridge formation (thick filament, purple) to actin (gray) via troponin T (cTnT, blue) and tropomyosin (orange). Binding of Ca²⁺ to cTnC (*bottom panels*) relieves this inhibition and allows movement of tropomyosin to the outer domain of the actin filament, thus exposing myosin-binding sites on actin. The *right panels* illustrate in greater detail the molecular protein–protein interactions in the cardiac troponin complex upon Ca²⁺ activation. **b** Estimated structure of the troponin complex (TnC, blue; TnI, pink; TnT, yellow), the tropomyosin strand (green), and actin filament (brown) in the Ca²⁺-free diastolic state (*top panel*) and Ca²⁺-bound systolic state (*bottom panel*). The approximate position of the inhibitory region of TnI is indicated in the *top panel* by the white arrow. The structure and relative positions employed to construct these projections are based on data obtained for rabbit skeletal muscle (actin) and bovine cardiac muscle (troponin and tropomyosin) by the Lehman group using electron microscopy data and helical and single particle reconstruction [55,56]