Protein Phosphorylation and Signal Transduction in Cardiac Thin Filaments*

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Homeostasis of cardiac function requires significant adjustments in sarcomeric protein phosphorylation. The existence of unique peptides in cardiac sarcomeres, which are substrates for a multitude of kinases, strongly supports this concept (1). We focus here on the troponin complex of the thin filaments, which contain two major proteins that participate in these phosphoryl group transfer reactions: the inhibitory protein (cardiac troponin (cTn)) and the tropomyosin (Tm)-(binding protein (cTnT)). We describe the relatively new understanding of the molecular mechanisms of thin filament-based control of the heartbeat and how these mechanisms are altered by phosphorylation. We discuss new concepts regarding the relation between the beat of the heart and the location of thin filament proteins and their long- and short-range interactions. We also discuss elucidation of mechanisms by which these phosphorylations exacerbate or ameliorate effects of mutations in the myofilament proteins that are linked to familial cardiomyopathies.

Thin Filaments during the Relaxed State

Fig. 1 depicts an A-band region of the cardiac thin filament functional unit in the diastolic and systolic states. In diastole, force-generating reactions of cross-bridges with actin are inhibited, ATP hydrolysis is relatively low, and the sarcomere is relatively extensible (2). Properties of the giant protein titin dominate the compliance of the relaxed sarcomere (3). Interactions of thin filament regulatory proteins, the troponin heterotrimeric complex, and Tm hinder the actin-cross-bridge reaction and establish the B-state. Calcium binding to a single regulatory site on cTnC triggers a release from this inhibited state by modifications of interactions among actin, Tm, and Tn.

Evidence derived from the core crystal structure of cardiac Tn (4), from elucidation of the structures by NMR (5), from biochemical investigations of protein-protein interactions (6, 7), and from reconstructions and single-particle analysis of electron micrographs of reconstituted myofilament preparations (8, 9) provided the basis for the illustration in Fig. 1 (see Ref. 6 for a review). Apart from the lack of a thin filament lattice in the Tn core crystal structure, there was no structural information on significant regions, including the tail region of cTnT, an inhibitory peptide (Ip; which tethers cTnI to actin), the unique N-terminal peptide (~30 amino acids), and portions of the far C-terminal domain of cTnI. Thus, Fig. 1 (upper) shows binding of cTnI to actin via two regions, the highly basic Ip and a second actin-binding region. Importantly, these regions flank a switch peptide, which binds to cTnC when Ca2+-binds to the N-terminal lobe of cTnC, which houses the regulatory Ca2+-binding site, thereby participating in the mechanism by which Tn releases the thin filament from inhibition.

The C-terminal mobile domain of cTnI beyond the second actin-binding site may also participate in establishing the relaxed state. Two observations point to this possibility. The first is a report of results from reconstructions and single-particle analysis of electron micrographs indicating that the C-terminal mobile domain of cTnI lays across azimuthally localized actins and potentially binds directly to Tm (9, 10). These structural studies on the role of the mobile domain indicated that the cTnI C terminus is an important element in driving Tm to its blocking state along the actin outer domain. The second is a report by Mudalige et al. (11) providing direct evidence for the proximity between the C-terminal mobile domain and Tm from photochemical cross-linking studies with Tm labeled at position 146 or 147. Tm-146, but not Tm-174, cross-linked to the fast skeletal TnI peptide 157–163 (DVGDRWK), which corresponds to a nearly identical C-terminal peptide in cTnI (EVGDWRK), in a Ca2+-dependent manner.

There is now strong evidence that the hypervariable N-terminal tail of cTnT is also an important element in establishing the diastolic state. A study by Tobacman et al. (12) emphasized the importance of the N-terminal tail in the relaxed state by demonstrating that the tail domain cTnT-1–153 alone is able to induce a blocked state of the myofilaments in the complete absence of cTnI. Single-particle analysis and reconstructions of electron micrographs also demonstrated that, at low Ca2+, Tm is wedged between cTnI actin-binding peptides and the tail of cTnT from the Tn complex in register on the opposite actin strand (8, 9). Although binding of cTnI to Tm gave this Tn component its name, interactions with other components of the cTn complex are highly dependent on cTnT, which acts as a scaffold by directly binding to both cTnI and cTnC through a rigid coiled coil, the I-T arm (4). The stability of the I-T arm predicted from the core crystal structure has been verified by studies of dynamic mapping of amide hydrogens employing hydrogen/deuterium exchange, which show the I-T arm as one of a few tightly folded regions in the solution structure of cTn (13). The other region is helix 1 of cTnI, which interacts with the C-terminal lobe of cTnC.

Thin Filaments during the Active States

Ca2+-triggered protein-protein interactions engage a complex process releasing thin filaments from inhibition and actively promoting force-generating interactions between
myosin cross-bridges and actin. The Ca\(^{2+}\) sensor is cTnC, which contains EF-hands, calcium-binding sites with consensus sequences for Ca\(^{2+}\) and Mg\(^{2+}\) binding. The N-terminal lobe of cTnC contains nonfunctional site I and functional site II (regulatory site). The C-terminal lobe contains sites III and IV (14). Sites III and IV bind Ca\(^{2+}\) or Mg\(^{2+}\) with relatively high affinity and slow exchange (15, 16). Metal binding to these sites anchors cTnC to the myofilaments by its tight interaction with the N-terminal region of cTnI (34–71). Site II binds Ca\(^{2+}\) with relatively low affinity and exchange fast enough to occur within the beat of the heart (17, 18).

Calcium binding to the single regulatory site triggers conformational changes in thin filament structure and state. Upper left, relaxed state; upper right, active state. In the relaxed state, Tm is immobilized and positioned to impede force-generating reactions of myosin cross-bridges with actin. This position of Tm is imposed by actin-binding peptides (hatched) of cTnI (green). The C-terminal mobile domain of cTnI may also bind directly to Tm. The immobilization of Tm is also imposed by the tail region (blue stripes) of the cTnT N-terminal tail arising from a Tn complex on the opposite actin strand. With Ca\(^{2+}\) binding to cTnC (red), a hydrophobic patch is formed, which promotes an interaction with the cTnC switch peptide (SwP), which is flanked by actin-binding peptides and which triggers release of the tethering of cTnI to actin-Tm and cTnT. The release of Tm from an immobilized state exposes sites on actin for force-generating reactions with cross-bridges. Sites of phosphorylation are indicated as stars (upper right) and also in the illustration of the Tn complex (lower panel). See text for a further description.

Thin Filament Signaling and Cooperative Activation

An important aspect of thin filament signaling and signal transduction is that despite control by a single regulatory Ca\(^{2+}\)-binding site on cTnC, the dependence of tension on Ca\(^{2+}\) is steeper than predicted by a non-cooperative binding isotherm. Hill coefficients for the steady-state relation are commonly 3–5. One possible mechanism for this steep relation is the interaction between neighboring functional units consisting of actin/Tm/Tn at a 7:1:1 ratio. Thus, the signal generated with the Ca\(^{2+}\) bound to Tn spreads along the thin filament or may even spread to the Tn complexes in register on adjacent actin strands (21). Detailed balance dictates that the promotion of energies of interaction in the direction from calcium-cTnC to actin-myosin also occur in the direction from actin-cross-bridge back to cTnC. It is well known that binding of rigor cross-bridges to the thin filament increases the Ca\(^{2+}\)-binding affinity of cTnC (17). A dominant and appealing theory is that force-generating cross-bridges reacting with the thin filament also promote interactions within a functional unit and between near-neighbor functional units. However, most of the evidence for this has been developed not with force-generating but with rigor (22) or strongly bound non-cycling cross-bridges in the form of N-ethylmaleimide-modified myosin heads (2, 23, 24). Recent studies by Sun et al. (25) have emphasized the possibility that rigor or N-ethylmaleimide-modified cross-bridges may not affect the thin filament the same as strong force-generating cross-bridges (reviewed in Refs. 26 and 27). The theory advanced by Sun et al. is that cooperative spread of activation is dominated by processes at the level of the thin filaments. Their studies employed fluorescent probes to sense the on-state of specific sites of cTnC in force-generating skinned fiber bundles. These states remained the same whether or not cross-bridges were reacting in the presence MgATP with the thin filament. Data demonstrating cooperative binding isotherms of calcium binding to cTnC in reconstituted thin filaments support this concept (28, 29).

Phosphorylation of cTnl: Cardiac-specific N-terminal Extension

Phosphorylation of cTnl has been extensively reviewed (1, 30); we focus here on recent data indicating novel intra- and intermolecular interactions associated with these phosphorylations in thin filament signaling. PKA, as well as PKD and PKG, phosphorylates cTnl at Ser\(^23\) and Ser\(^24\) (1), whereas PKC\(\beta\) and PKC\(\delta\) phosphorylate these as well as other cTnl sites (31–34). These two sites appear to be the only sites that are phosphorylated at basal physiological levels of activity in mouse and pig.
hearts (35, 36). Earlier studies using synthetic peptides derived from the PKA sites in the N-terminal extension of cTnI demonstrated that the phosphorylation of these residues is ordered with Ser\(^{24}\) as the first residue to be phosphorylated (37–39); monophosphorylation of the peptide at Ser\(^{23}\) was not detected. Zhang et al. (40) also reached the same conclusion in experiments in which a cTnI mutant in which one of these Ser residues was replaced with Ala was treated with PKA. More recently, however, studies using top-down electron capture dissociation and electron transfer dissociation mass spectrometry (41) demonstrated that phospho-Ser\(^{24}\) was found only in the bisphosphorylated species of cTnI isolated from human tissue. Although more studies are needed to address this issue, the involvement of phosphatases at basal phosphorylation levels under physiological conditions in vivo has been considered as a possibility. Phosphatases may dephosphorylate Ser\(^{24}\) faster than Ser\(^{23}\), resulting in a faster turnover rate of the phosphorylation/dephosphorylation state of Ser\(^{24}\). It has been well documented that phosphorylation of these two sites results in calcium desensitization, an increase in the relaxation rate, and an increase in the cross-bridge cycling of heart muscle myofilaments (40, 42–45), although studies of skinned myocardium from transgenic mice lacking myosin-binding protein C (MyBP-C), another substrate for PKA in myofilaments, and/or expressing non-PKA-phosphorylatable cTnI indicate that PKA phosphorylation of cTnI is responsible for calcium sensitivity and that phosphorylation of MyBP-C is responsible for enhanced cross-bridge cycling (46).

It is critical to delineate the structural information on the N-terminal extension relative to other Tn components to understand molecular mechanisms underlying these functional consequences. The N-terminal extension of cTnI interacts with the N-terminal lobe of TnC and modifies the conformational states of the N-terminal lobe of TnC (47, 48). With phosphorylation, there is a depression of the affinity of the cTnI N-terminal extension and the N-terminal lobe of TnC (47–50). Evidence also indicates that the N-terminal extension of cTnI is located close not only to the N-terminal lobe of TnC but also to the switch region of cTnI. Warren et al. (51) reported intramolecular cross-linking between cTnI at amino acid 5 or 18 and the switch region of TnI in the Tn complex. Ward et al. (52) determined which amino acid residues of the N-terminal extension participate in the interaction with cTnC. They generated a series of N-terminal deletion mutants of cTnI and measured calcium-dependent ATPase activities before and after PKA treatment. The deletion mutants up to position 18 were phosphorylated by PKA at the same rate and to the same extent as wild-type cTnI. The \(\Delta P_{\text{Ca}_{2+}}\) values of ATPase activities before and after PKA treatments were similar and substantial for all of the deletion mutants up to position 15, indicating that residues 1–15 of cTnI play only a minor role in transmitting the phosphorylation signal to other myofilament proteins. Ward et al. (53) also showed that peptide residues 1–18 of cTnI do not bind to cTnC. NMR studies (53, 54), as well as previous studies (37), demonstrated that PKA phosphorylation at Ser\(^{23}\) and Ser\(^{24}\) produced only localized structural effects in segment residues \(\sim 25–35\).

A more detailed picture of the intermolecular interactions between the N-terminal extension and the N-terminal lobe of cTnC and the intramolecular interactions within TnI was proposed by Howarth et al. (5). They determined the solution structure of the bisphosphorylated N-terminal extension (residues 1–32) of cTnI and modeled the N-terminal extension into the crystal structure of the core domain of cTn (4) based on bioinformatics analysis and previous small angle scattering experiments. According to their model, the N-terminal extension interacts with cTnC near Leu\(^{29}\), as also demonstrated in peptide array experiments (55) with cTnI in a non-phosphorylated state. Upon bisphosphorylation, cTnI undergoes relatively drastic conformational transitions, resulting from a hinge-like movement within residues 33–42. Molecular docking experiments designed for maximum interactions of the N-terminal extension with opposite polarity showed that the acidic residues in the N-terminal end, such as Asp\(^{3}\), Glu\(^{4}\), Asp\(^{7}\), and Glu\(^{11}\), interact with the basic residues in the inhibitory region, suggesting intramolecular interactions. Photo-cross-linking experiments in the presence of Ca\(^{2+}\) showed that benzophenone attached to Cys\(^{5}\) or Cys\(^{19}\) cross-linked to the switch region of cTnI in the non-phosphorylated cTnI complex, indicating that the N-terminal extension exists in an equilibrium between these two proposed structural states without phosphorylation (51).

Although induction by phosphorylation of cTnI at Ser\(^{23}\)/Ser\(^{24}\) of a straightforward depression of Ca\(^{2+}\) affinity of the cTnC regulatory site has been proposed to account for increased release of Ca\(^{2+}\) from cTnC and enhanced relaxation, another mechanism was proposed by Baryshnikova et al. (56). As indicated above, phosphorylation of Ser\(^{23}\) and Ser\(^{24}\) modifies the conformational states of the N-terminal lobe of cTnC. They found that the presence of phosphate groups at Ser\(^{23}\)/Ser\(^{24}\) does not affect calcium binding to the regulatory site of cTnC when measured in the presence of peptide residues 1–29 of the N-terminal extension of cTnI. With or without the peptide or the bishphosphopeptide, the association constant for the regulatory site was determined to be \(\sim 2 \times 10^5\) M\(^{-1}\). Baryshnikova et al. proposed that bisphosphorylation of peptide residues 1–29 modifies the affinity of the switch region of cTnI for the N-terminal lobe of cTnC and hence the relaxation rate. Another possibility was proposed on the basis of solution ATPase activity assays. Deng et al. (57) and Lu et al. (33) demonstrated that PKA treatment of the cTn complex resulted in a reduced maximum ATPase activity in the presence of Ca\(^{2+}\). These observations are supported by results of studies on the relation between thin filament length and velocity as determined by \textit{in vitro} motility assay in which the apparent binding affinity of myosin for thin filaments was reduced after PKA treatment (58). Thus, it is plausible that PKA phosphorylation of cTnI modifies the affinity between cross-bridges and thin filaments.

**The Inhibitory Region and Intramolecular Interactions between the cTnI N-terminal Extension and Inhibitory Region**

Thr\(^{144}\) is located in the middle of the inhibitory region of cTnI. The equivalent position is Pro in skeletal muscle TnI. The
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The functional consequence of phosphorylation of Thr\(^{144}\) by itself has not been well established. Burkart et al. (59) reported that the T144E mutation, which mimics the phosphorylation state, does not affect calcium sensitivity or maximum tension when introduced into skinned cardiac fiber bundles, whereas in the in vitro motility assay, it desensitizes to Ca\(^{2+}\). On the other hand, when skinned cardiomyocytes or small myocyte bundles harboring cTnI S23A/S24A to prevent phosphorylation were treated with PKC\(\beta\), Wang et al. (34) found that the isometric tension of these myocytes was sensitized to Ca\(^{2+}\) compared with non-PKC-treated myocytes. Because Thr\(^{144}\) of cTnI was phosphorylated mainly by PKC treatment in their preparations, they concluded that phosphorylation of Thr\(^{144}\) was responsible for the calcium sensitization. Data reported by Westfall et al. (60) indicated that the phosphorylation of Thr\(^{144}\) accelerates relaxation of cardiomyocytes, whereas it does not affect shortening. These divergent observations may be explained by recent findings that Thr\(^{144}\) is involved in length-dependent activation of tension development in skinned fiber bundles (61) and strong cross-bridge-dependent activation of the thin filaments in solution acto-S1 ATPase activity (62).

The bisphosphorylation of cTnI at its N terminus induces a lengthening of the adjacent helix TnI-(21-30) with induction of a bend in the extension, which contains acidic residues (5). On the basis of these structural modifications, we predicted that the acidic N-terminal regions of cTnI might interact intramolecularly with the basic inhibitory region of cTnI. Cross-linking studies supported this prediction (51). Functional evidence for this interaction came from the study of a phosphomimetic mutation at Ser\(^{22}/\text{Ser}^{24}\) in the N-terminal extension and Thr\(^{144}\) in the inhibitory region (33). It was demonstrated that pseudophosphorylation at Thr\(^{144}\) of cTnI depressed the cooperative activation of cardiac thin filaments exclusively in the presence of the S23D/S24D mutation of cTnI. The calcium-binding properties of the thin filaments regulated by cTnI with the T144E mutation were the same as those with wild-type cTnI. The cTnI S23D/S24D mutation desensitized the thin filaments to Ca\(^{2+}\), as expected. Thin filaments with the S23D/S24D/T144E mutation retained the same calcium sensitivity as those with the S23D/S24D mutation, yet the Hill coefficient, indicative of cooperativity, was significantly smaller. Similar trends followed the tension development of skinned cardiac muscle fiber bundles. Moreover, studies of the cTnI R145G mutation linked to hypertrophic cardiomyopathy also indicated an interaction between the cTnI N terminus and the Ip (55). These studies demonstrated that compared with controls, calcium sensitivity is enhanced in myofilaments regulated by cTnI R145G but not when cTnI Ser\(^{23}\) and Ser\(^{24}\) are bisphosphorylated. One interpretation is that the loss of the basic residue not only depresses inhibition by the Ip but also alters electrostatic interactions with the N terminus. Transgenic mouse hearts in which residues 2-11 of cTnI were deleted showed a significant decrease in contractility and relaxation upon basal and \(\beta\)-adrenergic stimulation, whereas the calcium sensitivity of force development was not altered, suggesting the importance of the N-terminal part of cTnI in regulation and modification (63). These experiments provide strong indications of modulation of cTn function by an intramolecular interaction between the N-terminal extension and inhibitory region of cTnI.

TnT Phosphorylation

Several kinases that phosphorylate cTnT have been identified, but the functional significance of the phosphorylation in thin filament signaling and integrated control of cardiac function remains unclear. Evidence for phosphorylation of cTnT by a “specific” cTnT kinase (64) and by phosphorylase kinase (65, 66) has not been followed up. Early studies (67) identified a site of phosphorylation in the N terminus at Ser\(^{1}\), but the significance of this site remains unknown. Sites identified as PKC substrates in the C-terminal region have been more extensively documented. cTnT PKC-dependent phosphorylation sites are Thr\(^{197}\), Ser\(^{201}\), Thr\(^{206}\), and Thr\(^{287}\). We (68) mutated these residues to glutamate or alanine and exchanged single, double, triple, and quadruple mutants into skinned fiber bundles of mouse heart. We also exchanged the Tn complex reconstituted with cTnT phosphorylated by PKC\(\alpha\). Our studies of isometric tension development and actomyosin Mg-ATPase activity as a function of Ca\(^{2+}\) concentration identified Thr\(^{206}\) as a functionally critical cTnT PKC phosphorylation residue. Compared with wild-type controls, exclusive phosphorylation by PKC\(\alpha\) or replacement by Glu induced a significant decrease in myofilament maximum tension, actomyosin Mg-ATPase activity, calcium sensitivity, and cooperativity. Tension cost was also reduced. Subsequent studies also determined that phosphorylation of cTnT depresses sliding speed in the motility assay (69). Inasmuch as PKC\(\alpha\) activation promotes cardiac growth, engagement of this pathway coordinates hypertrophic signaling and contractile dynamics. Along these lines, Thr\(^{206}\) also appears to be a substrate for Raf kinase (70).

Thin Filament Protein Phosphorylation as an Important Modifier in Effects of Mutations Linked to Familial Cardiomyopathies

Modifications in the interaction of the N terminus of cTnI with cTnC and with the Ip region of cTnI appear to be an important mechanism in the linkage of Tn mutations to cardiomyopathies. As discussed above, studies of the cTnI R146G mutation, which is linked to hypertrophic cardiomyopathy, provide not only further indications of an interaction between the cTnI N terminus and the Ip but also an important role for phosphorylation of cTnI Ser\(^{23}/\text{Ser}^{24}\) in the course of the disorder. Our studies (42) with cTnC G159D, which is linked to dilated cardiomyopathy (DCM), provide another example of an effect of phosphorylation of these cTnI residues on the functional effects of a mutant thin filament protein. In this case, the interaction appears to exacerbate the functional effects of the mutations. We (42) reported little or no effect of the mutation under base-line conditions without cTnI phosphorylation. However, with PKA-dependent phosphorylation or pseudophosphorylation of cTnI, there was a significant depression of the decrease in the calcium sensitivity of tension development compared with controls. This depression in the effect of cTnI Ser\(^{23}/\text{Ser}^{24}\) phosphorylation was correlated with a reduced effect on calcium binding to the cTnC regulatory site. Another region of phosphorylation of cTnI, Ser\(^{17}/\text{Ser}^{45}\), appears to mod-
ify the functional effects of a familial hypertrophic cardiomyopathy-linked mutation, Tm E180G (26). Tension developed by myofilaments controlled by Tm E180G was significantly more sensitive to Ca^{2+} compared with controls. In skinned fibers with wild-type thin filaments, pseudophosphorylation of cTnI Ser^{3}/Ser^{45} in the anchoring domain interacting with the cTnC C-terminal lobe induced a depression in force and calcium sensitivity (59). This depression was significantly enhanced in skinned fibers controlled by Tm E180G. Interactions between the Ip and the R145G mutation linked to hypertrophic cardiomyopathy and the Ca^{2+}-saturated C-terminal lobe of cTnC were also altered. These interactions have been determined employing NMR chemical shift mapping (71). There was a significant 14-fold decrease in the affinity with Thr^{144} phosphorylation and a 4-fold decrease in the presence of the R146G mutation in the peptide. Phosphorylation (Fig. 1) at cTnI residue 150, which is poorly understood, may also affect the response to the R146G mutation.

A potentially important aspect of phosphorylation of cTnT is the proximity of Thr^{206} to a region linked with DCM in which there is a deletion of Lys^{210}. Comparison of the state of phosphorylation of myofilaments from hearts expressing the cTnTΔLys^{210} mutant with that of controls demonstrated significant net decreases in phosphorylation of cTnT, cTnI, and MyBP-C (72). However, there was an increase in site-specific phosphorylation of Thr^{206}. Moreover, there was an increased rate of phosphorylation of Thr^{206} by PKCα. Evidence that cTnTΔLys^{210} has enhanced affinity for cTnI provides further evidence for the long-range effects of this mutation (72).

Dephosphorylation of Thin Filament Proteins

Although there has been progress in understanding the phosphatases that control phosphorylation of TnI and TnT, signaling cascades controlling these phosphatases remain poorly understood. Moreover, site-specific dephosphorylation, which is likely to be an important mechanism, has been understudied and is poorly understood. The major phosphatases controlling thin filament protein phosphorylation are PP1 and PP2A. Both of these phosphatases have been reported to have a Z-disc localization (73, 74), placing them in close proximity to the A- and I-band regions of the sarcomere and indicating that their localization may be strain-sensitive. In fact, recent studies (74) indicate that that the B56a unit of the PP2A complex localizes to the Z-disc but moves away with β-adrenergic stimulation, whereas light chain 2 phosphatase does not. Our studies (26) showing a decrease in tropomyosin phosphorylation in hearts with constitutively active p38α demonstrated co-localization of this MAPK with α-actinin at the Z-disc, as well as protein phosphatases (PP2α and PP2β). Few studies have directly compared the substrate specificities of PP2A and PP1, but there is some agreement that PP1 has preference for cTnI over cTnT (73, 75) but with no effect on dephosphorylation of Tm (73). Moreover, Jideama et al. (75) employed phosphopeptide mapping to show that PP2A induced uniform dephosphorylation of cTnI in preparations previously treated with both PKA and PKC. On the other hand, Ser^{23} and Ser^{24} were the preferred substrates for PP1. In the case of cTnT, Thr^{199} and an unidentified residue were the least favorable for dephosphorylation by PP1. These studies thus demonstrate specificity for dephosphorylation, a mechanism not generally taken into account when considering the integrated effects of signaling to the thin filaments. In our studies of the effects of constitutively active PKCζ (76), we observed a significant decrease in Thr phosphorylation of cTnI and cTnT notably by PKCζ T560E. To explain the apparent Thr dephosphorylation of cTnI and cTnT, we hypothesized that PKCζ exists in a complex with Pak1 and PP2A, and this was confirmed by immunoprecipitation and Western blotting. Reviewed elsewhere (77, 78) are our studies on a signaling cascade to PP2A via G_{i} coupling to small G-proteins (e.g. bradykinin) and potential sphingolipid signaling via p21-activated kinase.

Conclusions

New data advance our understanding of the molecular mechanism by which thin filament proteins control the state of the sarcomere. Understanding these molecular control mechanisms in thin filament regulation of the heartbeat has taken on new significance with the identification of many mutations linked to hypertrophic cardiomyopathy and DCM. There is also strong evidence for a role of alterations at the level of the thin filaments in acquired cardiac disorders and sudden death. The results summarized here indicate that apart from the direct effects of these mutations and modifications on function and indirect effects on stress signaling, it is important to consider the pathological process in the context of post-translational modifications of the sarcomeric proteins. The design of new diagnostics and therapies, as well as preventative measures, requires advancement of our understanding of these processes and their relative significance.

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