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Cardiac Troponin Structure-Function and the Influence of Hypertrophic Cardiomyopathy Associated Mutations on Modulation of Contractility

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Abstract

Cardiac troponin (cTn) acts as a pivotal regulator of muscle contraction and relaxation and is composed of three distinct subunits (cTnC: a highly conserved Ca^{2+} binding subunit, cTnI: an actomyosin ATPase inhibitory subunit, and cTnT: a tropomyosin binding subunit). In this mini-review, we briefly summarize the structure-function relationship of cTn and its subunits, its modulation by PKA-mediated phosphorylation of cTnI, and what is known about how these properties are altered by hypertrophic cardiomyopathy (HCM) associated mutations of cTnI. This includes recent work using computational modeling approaches to understand the atomic-based structural level basis of disease-associated mutations. We propose a viewpoint that it is alteration of cTnC-cTnI interaction (rather than the Ca^{2+} binding properties of cTn) *per se* that disrupt the ability of PKA-mediated phosphorylation at cTnI Ser-23/24 to alter contraction and relaxation in at least some HCM-associated mutations. The combination of state of the art biophysical approaches can provide new insight on the structure-function mechanisms of contractile dysfunction resulting cTnI mutations and exciting new avenues for the diagnosis, prevention, and even treatment of heart diseases.

Graphical abstract

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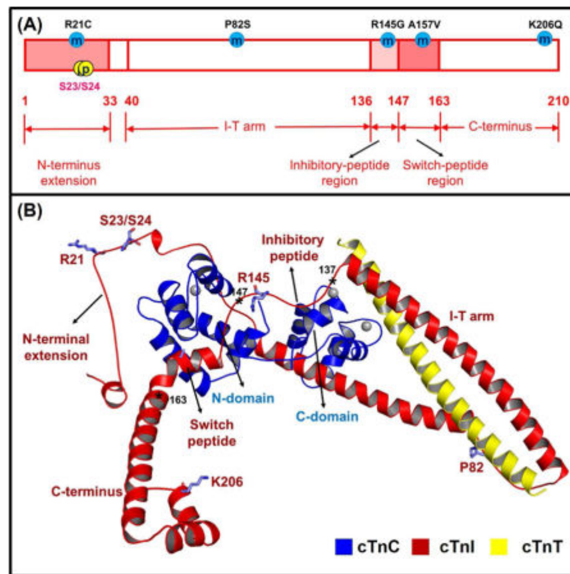

Keywords

Cardiac troponin; PKA phosphorylation; hypertrophic cardiomyopathy; mutation; cTnC-cTnI interaction; structure-function relationship

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Familial hypertrophic cardiomyopathy (HCM) is a common autosomal dominant disease, resulting in increased ventricular wall size that can cause outflow tract obstruction and unexpected sudden cardiac death [1, 2]. It was recently estimated that the prevalence of HCM-associated mutations is 1 per 500 individuals [3, 4]. To date, over 900 mutations associated with HCM have been identified in 24 genes [5, 6]. A large number of these HCM-related mutations reside in thick filament associated proteins (cardiac β-myosin heavy chain, cardiac myosin binding protein C (cMyBP-C), and titin) with fewer in the thin filament proteins actin, tropomyosin (Tm), and cardiac troponin (cTn)) [7-20]. For cTn, the effect of HCM-related mutations has been investigated using various biochemical and physiological approaches. For instance, recombinant cTn subunits containing mutations have been incorporated into reconstituted cTn complexes or thin filaments to study the effects on protein-protein interaction and ATPase activity *in vitro* [21-23]; adenovirus containing mutation genes have been constructed and transduced into isolated cardiomyocytes to examine effects on intact cardiomyocyte shortening and intracellular Ca^{2+} handling [24]; and transgenic animal models have been developed to study the disease progression [25-33]. In general, functional studies of many of the HCM-related mutations have been shown to increase the Ca^{2+} sensitivity of contractile (tension) and ATPase activity, with some exceptions for mutations which produced complex and contradictory results [23, 34].

There is growing evidence that the Ca^{2+} dependence of contractile properties may not be the only modulatory mechanism affected, for at least some mutations. During β-adrenergic stimulation, protein kinase A (PKA) phosphorylates multiple myofilament proteins, including the residues Ser-23 and Ser-24 of cardiac troponin I (cTnI) [35]. This phosphorylation reduces the Ca^{2+} binding affinity of cTn and the strength of cTnI

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interaction with cardiac troponin C (cTnC) [36]. In this review, we specifically focus on HCM-associated mutations of cTnI, and recent studies indicating disruption of the role of PKA-mediated modulation of contraction and relaxation. We speculate that introduction of particular mutations in cTnI may either directly affect the cTnC-cTnI (C-I) interaction (mutations located at the inhibitory/switch peptide regions) or cTnI-actin interaction (mutations located at the C-terminal domain), and thus modulate myosin binding (cross-bridge formation), tension generation and relaxation. We further propose that for at least some mutations, it disrupts the ability of PKA-mediated phosphorylation at Ser-23/Ser-24 of cTnI to alter these contractile properties. Below we first review the structure-function relationship of the cTn and its subunits, and the effects of cTnI phosphorylation by PKA. We then focus on recent studies of how mutations in cTnI that are associated with HCM may alter the regulation of cTnC-cTnI interaction and contractile function. We finish with a short perspective on how a combination of state of the art biophysical approaches can provide new insight on the structure-function mechanisms of contractile dysfunction resulting cTnI mutations and exciting new avenues for studying familial/genetic based disease development in the heart.

Cardiac Troponin – The Gate-Keeper of Contraction

Heart muscle cells (cardiomyocytes) contain a high density of the organelles of contraction, myofibrils, which consist of repeating structural units-named as sarcomeres. Sarcomeres, the smallest contractile units of muscle, are composed of thick filaments that contain the motor protein myosin, and thin filaments composed of double helical strands of actin monomers, a coiled-coil dimer strand of tropomyosin (Tm) and the troponin ternary complex [37]. The cardiac troponin complex (cTn) is a critical regulator of contraction and is composed of three distinct subunits named according to their functions: a highly conserved Ca^{2+} binding subunit (cTnC); an actomyosin ATPase inhibitory subunit (cTnI) and a Tm binding subunit (cTnT) [37].

Troponin Structure

Troponin C (TnC)

Troponin C (TnC, the Ca^{2+} binding subunit), is a dumbbell-shaped protein with N- and C-terminal globular domains connected by a long central helix linker, and belongs to the super-family of calmodulin [38-41]. The two globular domains each consist of two helix-loop-helix motifs that bind divalent metal ions [39, 42]. The C-terminal domain of TnC (CTnC) has two high-affinity Ca^{2+}/Mg^{2+} binding sites (site III and site IV), which are primarily occupied by Mg^{2+} when muscle cells are at rest. The affinity for these sites for Ca^{2+} is 10^7 M^{-1} [43, 44], which is higher than that for Mg^{2+} (10^3 M^{-1}) [43], but the 5×10^3-5×10^4-fold greater intracellular Mg^{2+} levels (0.5-5 mM) [45], compared with Ca^{2+} (< 100 nM) in resting cardiac muscle insures that mostly Mg^{2+} is bound to sites III and IV. During the cardiac twitch, when intracellular Ca^{2+} levels can rise to 1-10 μM [46], there is likely some transient exchange of Mg^{2+} for Ca^{2+}. The CTnC domain primarily plays a structural role in anchoring the proteins within the whole Tn complex and to the thin filament [47].

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The N-terminal domain of cTnC, found in cardiac and slow-twitch skeletal muscle, only has one active Ca^{2+}-binding site (site II), compared with two active sites in fast-twitch skeletal TnC (sTnC) [48]. These sites are lower-affinity (10^5 M^{-1}) metal ion binding sites that are highly specific for Ca^{2+} [43, 44]. N-terminal Ca^{2+} binding is responsible for initiating thin filament activation and the subsequent generation of force and contraction [48-50]. Several loop residue substitutions in site I of cTnC are responsible for the loss of the ability to bind Ca^{2+}. Studies using demembranated cardiac muscle preparations have demonstrated that replacement of native cTnC with a variant that eliminates site II Ca^{2+} binding (via mutagenesis) also eliminates the ability of Ca^{2+} to initiate contraction [51]. Interestingly, replacing native cTnC with an engineered cTnC that has a dysfunctional site II, but repaired Ca^{2+} binding at site I does not restore the ability of Ca^{2+} to activate force generation, suggesting site II (other than site I) is critical for the regulatory function of cTnC [50]. However, when cTnC is engineered such that both site I and site II bind Ca^{2+}, it increases Ca^{2+} sensitivity of contraction over that of native or wild-type cTnC, suggesting site II is not completely effective in activation of cardiac muscle [50].

The structure of the regulatory domain of TnC has been determined in both Ca^{2+}-saturated and Ca^{2+}-free states, as well as in complex with the switch-peptide of TnI [52-56]. Information obtained from these different structures is critical to understanding how Ca^{2+} binding changes the conformation within the N-terminus domain that initiates the signaling process leading to thin filament activation. In the apo-state, the regulatory domain stays in a closed conformation with most of the hydrophobic residues buried [52]. When Ca^{2+} binds to the regulatory domain of TnC it induces an opened structure of the hydrophobic patch, facilitating increased interaction with TnI [54]. Interestingly, structural changes in the N-terminal domain of cTnC (NcTnC) in response to Ca^{2+} binding are much smaller than for NsTnC, such that most of the hydrophobic patch residues remain un-exposed [52]. It has been demonstrated that both Ca^{2+} binding at site II and interaction with the cTnI switch peptide are required to maintain NcTnC in a stable open state [53, 57]. Ca^{2+} binding to NcTnC induces a conformational change in the A- and B- helices ("open" conformation) that increases the affinity of this region for the switch peptide of cTnI. The cTnI switch-peptide is adjacent to the cTnI inhibitory region, which binds to actin in the absence of Ca^{2+} and is release when Ca^{2+} binds to TnC, allowing increased Tm mobility and myosin interaction with actin to form force generating cross-bridges [37].

Troponin I (TnI)

Troponin I (TnI, the inhibitory subunit) is a highly flexible protein that is able to adapt favorable conformations to interact with both TnC and TnT, as well as with actin [58]. There are three isoforms of TnI expressed in vertebrate striated muscles: the fast and slow skeletal isoforms (sTnI) and the cardiac-specific isoform [59-61]. For cardiac muscle, slow sTnI (ssTnI) is expressed during early embryonic development, but there is a progressive increase in cTnI which completely replaces ssTnI shortly after birth [62]. cTnI is the sole isoform present in the adult heart and it does not switch under pathological conditions [63]. Several studies of rodents and rabbits have demonstrated that the presence of ssTnI results in a higher Ca^{2+} binding affinity of Tn compared with cTnI [64-67]. Reiser *et al.* reported that ssTnI is expressed in neonatal myocardium and transitions into cTnI in adult myocardium,

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and it is associated with the decreased Ca^{2+} sensitivity in adult myocardium [64]. Westfall *et al.* reported that ssTnI plays an important role in the reduced pH sensitivity observed in fetal cardiac and slow skeletal muscle with respect to the adult cardiac myocytes, and further demonstrated that the isoform transition of TnI during development may result in decreased Ca^{2+} sensitivity and increased pH responsiveness of tension development of cardiomyocytes [65]. Using cardiomyocytes from adult transgenic mice, F'entzke *et al.* found that the overexpression of slow sTnI in the cardiac muscle impaired relaxation (diastolic function) and suggested this was due to enhanced Ca^{2+} binding affinity [67].

In terms of the structure-function relationship, human cTnI (NCBI protein ID: NP_000354.4) can be divided into five functional regions [36, 59]: (1) the cardiac-specific N-terminal extension (residues 1-33), containing two PKA-dependent phosphorylation sites not present in either fast and slow sTnI; (2) the I-T arm region (residues 40-136), which is composed of two α–helices that interact with the α–helices of C-terminal domain of TnT and the C-lobe of TnC; (3) the inhibitory-peptide region (residues 137-146), that interact with actin-Tm and perhaps C-lobe of TnC; (4) the switch-peptide region (residues 147-163), that interact with the hydrophobic patch of NcTnC; and (5) the C-terminus mobile domain (residues 164-210), which has a second actin-Tm binding site [68-71]. Although the solution nuclear magnetic resonance (NMR) and/or X-ray crystal structures of each cTnI fragment is available, the entire structure of cTnI in the whole cTn complex has still has not been solved.

The cardiac-specific N-terminal extension—There is no crystal structure available for the cardiac-specific N-terminal extension of cTnI ($cTnI_{1-32}$), however Howarth *et al.* [72] used solution NMR to determine the structures of both non-phosphorylated and *bis*-phosphorylated species. In the absence of phosphorylation, the N-terminus extension is less structured, and interacts with the NcTnC. Upon the *bis*-phosphorylation on Ser-23/Ser-24, the C-terminal α–helix (residues 21-30) is stabilized by electrostatic interactions between the negatively charged phosphorylated serine and the neighboring basic residues. This weakens the interaction between $cTnI_{1-32}$ and NcTnC, allowing it to be more mobile and increasing potential interaction with other regions of cTn.

The I-T arm region—The I-T arm region ($cTnI_{40-136}$), which has been considered to play a more structural rather than regulatory function on anchoring the troponin complex onto the thin filament, may form a helical coiled-coil with a portion of C-terminal domain of TnT [73]. The first half part of the I-T arm region ($cTnI_{40-80}$) has been demonstrated to bind with the hydrophobic cleft of the C-lobe of cTnC [74].

The inhibitory-peptide region—The inhibitory-peptide region ($cTnI_{137-146}$), is considered a key region of cTnI as it interacts strongly with actin in the absence of Ca^{2+} and stabilizes Tm in a position that blocks strong myosin binding to actin that is required for contraction [75, 76]. The structure of $cTnI_{137-146}$ is missing in the core crystal structure of cTn complex solved by Takeda et al. [77], and studies on the structures of this region are not in good agreement [78-80]. Using site-directed spin labeling electron paramagnetic resonance (SDSL-EPR), Brown *et al.* determined the structure of the inhibitory region of cTnI in the intact cardiac troponin ternary structure, which demonstrated that $cTnI_{129-137}$ forms a regular 3.6 residues/turn a–helix, and the following region $cTnI_{138}$-145 displayed no

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secondary structure [78]. Using the cross-linking and fluorescence-detected resonance energy transfer (FRET) approaches, Tung *et al.* proposed the inhibitory region is in a (3-hairpin structure [79]. In contrast, Lindhout *et al.* reported the NMR structure of $cTnI_{128-147}$ with $cTnC_{89-161}$, in which $cTnI_{134-139}$ forms α–helix structure, and $cTnI_{140-147}$ displayed an extended conformation that potentially interacts with the C-lobe of cTnC [80].

The switch-peptide region—The switch-peptide region ($cTnI_{147-163}$) is located just next to the inhibitory-peptide region, and acts as another crucial region in cTnI, since it is required to stabilize NcTnC in the "open" conformation. In contrast to the regulatory N-domain of sTnC, binding of Ca^{2+} to cTnC does not induce an "open" state of the regulatory domain in order to interact with cTnI [52, 55]. Using multinuclear multidimensional NMR spectroscopy, Li *et al.* determined the solution structure of the $NcTnCCa^{2+}cTnI_{147-163}$ complex [53]. In this NMR structure, the N-terminus of $cTnI_{147-163}$ binds to the hydrophobic patch (interface of helices A and B) of NcTnC, thus stabilizing the "open" conformation of NcTnC, similar to that of the Ca^{2+}-saturated NsTnC [54, 81]. Despite the relative position of the switch-peptide region in this binary cTn complex [53] being different than that in the ternary cTn crystal structure (PDB: 1J1E) provided by Takeda *et al.* [77], the orientation and conformation of switch-peptide with respect to the NcTnC are in a very good agreement.

The C-terminus region—The C-terminus region ($cTnI_{164-210}$), also known as the mobile region, serves as the second actin-Tm binding site [68, 69]. This region is the most conserved part in TnI among different species and isoforms [56, 70, 71]. According to the core domain of X-ray structure of the whole troponin complex, the C-terminus has an α–helical structure and is free of interaction with either cTnC or cTnT.

Troponin T (TnT)

Troponin T (TnT, the Tm binding subunit), a striated-muscle specific protein with ~250-300 amino acids with a molecular weight range from 31-kDa to 36-kDa, serves as the structural "glue" that holds the Tn-Tm-actin complex together [82]. Similar to TnI, TnT also has three homologous genes evolved in mammalian striated muscle: the fast and slow skeletal isoforms and the cardiac specific isoform [71, 83]. The expression of the three isoforms in adult striated muscle is strictly regulated by a specific manner according to muscle-fiber types [83]. In addition to expression in cardiomyocytes [84, 85], cardiac TnT (cTnT) also presents in the embryonic skeletal muscle, Duchenne muscular dystrophy and myopathic skeletal muscle [86].

TnT is regarded as a highly asymmetric elongated protein, and can be divided into two functionally distinct regions: the N-terminus T1 region that interacts with Tm [87], and the globular C-terminus T2 region that is integrated into the Tn complex, interacting with both TnC and TnI and anchoring the Tn complex onto the thin filament [82, 83, 88, 89]. The sequences of the middle and the C-terminus portions of TnT are highly conserved among species for all three isoforms, however, the size/length of the N-terminus region is variant among species and isoforms [83]. There is no solved crystal/NMR structure for whole TnT, and only limited structural data are available for partial regions of TnT. The high resolution

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structure of the T2 region is present in Tn complex structures, showing that the main TnI-TnT interface is the coiled-coil α-helices bundle containing residues Phe-90 to Arg-136 of cTnI and residues Leu-224 to Val-274 of cTnT in the human cTn complex [77], or residues Gly-55 to Leu-102 of fast sTnI and residues Glu-199 to Gln-245 of fast sTnT in the chicken fast skeletal muscle troponin complex [90]. For the T1 region, Ertz-Berger *el al.* computationally built up an α-helix structure for residues 70-170 (part of the T1 region) of murine cTnT [91]. Manning *el al.* then docked this cTnT structure onto a known Tm structure and performed large-scaled computational modeling studies [92].

Cardiac Troponin Function

In the absence of Ca^{2+} (diastole), cTnC exists in its "closed" conformation, and cTnI binds actin tightly, inhibiting actin-myosin interaction (Fig. 1A) [37, 55]. During systole, depolarization of cardiomyocyte membranes results in Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR). In turn, the rise in intracellular Ca^{2+} results in Ca^{2+} binding to cTnC and this initiates a chain of events involving dynamic and structural changes in cTn, Tm, actin and myosin (Fig. 1B-D) [37]. Ca^{2+} binding to site II of cTnC reduces the stability of the "closed" conformation of the N-terminus half of the protein and increases interaction with the cTnI switch-peptide [37, 52]. In turn, this results in an "open" conformation of cTnC, exposing hydrophobic residues that increase interaction with the switch-peptide of cTnI, while decreasing the interaction between the (adjacent) inhibitory-peptide domain of cTnI with actin (Fig. 1B) [37]. Consequently, this allows increased Tm mobility and exposure of myosin binding sites on actin, allowing the formation of actin-myosin "cross-bridges" (Fig. 1C) [37]. In cardiac muscle, strong cross-bridge binding increases Ca^{2+} binding to cTn, allowing additional myosin binding, resulting in strong allosteric (cooperative) cross-bridge formation, cycling and force generation (Fig. 1D) [93-96]. When Ca^{2+} dissociates from cTn, it is primarily re-sequestrated into the SR, thus when myosin dissociates from thin filaments there may be a negative feedback decrease in cTn Ca^{2+} affinity that accelerates relaxation. Thus, cTn acts as a gate-keeper of contraction and a logical point for fine control of cardiac contractile performance, so investigating its structure and function relationship can be very informative for understanding contractile dysfunction with at least some mutations associated with cardiac disease.

Post-translational Modification of cTnI

One method of modulating contraction via cTn is phosphorylation of key amino acids. Post-translational modification of cTnI by phosphorylation plays a key role in regulating the function of cTnI by precipitating structural changes that alter its interaction with cTnC or actin and thus influence cardiac muscle function. It is important to point out that the sequence and length of cTnI varies among species, thus the discrepancy in residue number may actually refer to the same residue. For example, in rodent, there is one redundant residue (Ala-25) in the N-terminus of cTnI, thus the residue number of cTnI after this residue will change (+1) correspondingly. There are multiple sites on human cTnI (NCBI protein ID: NP_000354.4) that can be phosphorylated by various kinases (Fig. 2B), including Ser-5/Ser-6, Ser-23/Ser-24, Tyr-26, Thr-31, Ser-42/Ser-44, Thr-51, Ser-77/Thr-78, Thr-143, Ser-150, Ser-166, Thr-181 and Ser-199 (the numbering includes the initial Met-1),

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in which Thr-31 can only be phosphorylated *in vitro*, and phosphorylation of Ser-150 has never been observed in the human heart. Ser-42/Ser-44, Thr-143 and Ser-199 are all substrates of protein kinase C (PKC). Phosphorylation of Ser-42/Ser-44 has been reported to depress maximal tension, decrease the cross-bridge cycling kinetics, and reduce thin filament sliding velocity in the motility assay and ATPase rate [97-99]. Wang *et al.* reported that phosphorylation of Thr-144 in mouse heart (NCBI protein ID: NP_033432.1) increase the Ca^{2+} sensitivity of contraction [100], while Burkart *et al.* and Lu *et al.* reported Thr-144 phosphorylation or glutamic acid substitution (T144E) in mouse heart did not change the thin filament Ca^{2+} sensitivity [98, 101]. It has also been reported that phosphorylation of this site depressed the acto-myosin ATPase activity and contractility [102, 103], and depressed the cooperative activation of the thin filament [101]. Ser-150 can be phosphorylated by either P21-activatedkinase (Pak) or AMP-activated Protein Kinase (AMPK), which increased the Ca^{2+} sensitivity of force development [104], prolonged the relaxation [105], and increased the adrenergic-induced myocardial hypertrophy [106]. Thr-31, Thr-51 and Thr-181 of human cardiac cTnI can all be phosphorylated by mammalian sterile 20-like kinase 1 (Mst1) *in vitro*, and Thr-31 serves as the preference site [107]. Recently, Zhang *et al.* identified several novel phosphorylation sites on human cTnI (Ser-5/Ser-6, Tyr-26, Thr-51, Thr-181 and Ser-199), and found that in heart failure patient there was a decrease in the extent of phosphorylation sites Ser-5/Ser-6/Tyr-26 that located in the N-terminus extension, and an increase in the phosphorylation sites Ser-77/Thr-78 at the I-T arm region and Ser-166/Thr-181/Ser-199 resided at the C-terminal domain [108]. Henze *et al.* reported that aspartic acid substitution of Ser-6 (S6D) in mouse heart significantly depressed the maximal force, ATPase rate, kinetics of force redevelopment and did not change the Ca^{2+} sensitivity of force development, whereas aspartic acid substitution of Ser-5 (S5D) had no effect on all those parameters [109]. Salhi *et al.* demonstrated that Tyr-26 phosphorylation, glutamic acid substitution (Y26E) or aspartic acid substitution (Y26D) decreased the Ca^{2+} binding to thin filament, decreased the Ca^{2+} sensitivity of force development (by exchanging the recombinant human cTn into skinned rat myocyte), and accelerated the Ca^{2+} dissociation rate from cTnC [110]. Wijnker *et al.* found that pseudo-phosphorylation of Ser-199 (S199D) in human cardiomyocytes increased the Ca^{2+} sensitivity of force development, and did not affect the maximal and passive forces, and the rate of force redevelopment [111].

Among all the phosphorylation sites, Ser-23/Ser-24 (S23/S24) in the cardiac-specific N-terminus of cTnI have been the most studied and their modulatory function is best understood. β-adrenergic stimulation serves as a major physiological mechanism to meet the increase in circulatory demand, acting through positive inotropic and lusitropic effects [35, 112]. It is well established that during β-adrenergic stimulation, cTnI is phosphorylated by PKA at sites S23/S24, and that this affects Ca^{2+}-mediated contraction of cardiac muscle [35]. However, S23/S24 can be phosphorylated by other kinases *in vitro*, including PKC [113], protein kinase D (PKD) [114-116] and GMP-dependent protein kinase (PKG) [117]. Phosphorylation at these sites has been shown to increase the Ca^{2+} dissociation rate from cTnC, weaken the interaction between cTnI and the N-terminus of cTnC, reduce the Ca^{2+} sensitivity (pCa_{50}) of cardiac muscle tension production, increase cross-bridge cycling kinetics, and accelerate cardiac muscle cell relaxation [35, 36, 118-124]. Zhang *et al.* demonstrated that PKA phosphorylation of a cardiac skinned muscle results in decreased

Ca^{2+} sensitivity of the contractile apparatus, and increased the rate of relaxation [35]. Kentish *et al.* also reported that PKA phosphorylation accelerated myofibrillar relaxation rate and suggested this was due, at least in part, to faster cross-bridge cycle kinetics [119]. In support of this, we reported that either PKA phosphorylation of isolated rat cardiac myofibrils or exchange of cTn containing cTnI with phosphor-mimetic S23D/S24D accelerates and shortens the initial slow-phase of relaxation that is thought to reflect the rate cross-bridge detachment, the rate limiting transition in loaded cross-bridge cycling [125]. Additionally, we have demonstrated that reduction of cTn Ca^{2+} binding affinity and/or cTnC-cTnI binding affinity reduces the duration of the initial slow-phase of relaxation [125]. Thus, together the data suggests that accelerating relaxation is a primary functional role of phosphorylation at Ser-23/Ser-24.

cTn Mutations Associated with Familial Hypertrophic Cardiomyopathy (HCM)

Since the first mutations associated with HCM were detected in 1993 [10, 20], there has been a dramatic increase in the number of cTn mutations reported. Thus far, a total of at least 69 mutations have been identified in cTn subunit proteins that have been reported to be associated with HCM, including 6 in cTnC (Fig. 2A) [19, 126, 127], 30 in cTnI (Fig. 2B) [5, 16, 128-137], and 34 in cTnT (Fig. 2C) [9, 15, 129, 137-143]. All of the mutations are single nucleotide polymorphisms (SNPs or variants), and the degree of cardiac pathology associated with them is highly variable as is verification of their causal nature. There is also considerable variation in the extent to which these variants have been studied. Functional studies have been performed using tissue from patients, transgenic animal models and recombinant proteins in solution or exchanged into cardiac tissue. Measurements have primarily been made of Ca^{2+} binding affinity and ATPase activity in solution and the Ca^{2+} sensitivity of steady-state contraction in demembranated cardiac muscle, though an increasing number of studies are reporting on the kinetics of contractile activation and relaxation. Many (but not all) of the HCM-associated cTn mutations increase the Ca^{2+} binding affinity of cTn (K_{Ca}), paralleled with enhanced Ca^{2+} sensitivity of ATPase activity and thin filament activation (force development). Considering the central role of cTn in muscle regulation and contraction, introduction of a mutation into cTn may potentially influence interactions between cTnC-cTnI-cTnT, and the activation signal pathway transmitted from cTn to the whole thin filament. In this section, we focus primarily on HCM-associated mutations identified in cTnI. It is interesting that most of the cTnI mutations are located at the inhibitory/switch-peptide region and the C-terminus segment of cTnI, which suggests mutations at the inhibitory/switch-peptide regions may directly influence the interaction of cTnI with NcTnC, while the mutations at the C-terminal domain may directly affect the cTnI-actin interaction (thus indirectly influencing cTnC-cTnI interaction). As mentioned above, when Ca^{2+} binds to the regulatory domain of cTnC, it strengthens the cTnC-cTnI interaction, and weakens the cTnI-actin interaction, thus allowing the formation of actin-myosin cross-bridge and force generation. As such, all the mutations in the inhibitory/switch-peptide and C-terminus regions of cTnI could directly influence the equilibrium between cTnC-cTnI and cTnI-actin interaction, and thus modulate myosin binding (cross-bridge formation), tension generation and relaxation.

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It is important to mention that the sequence and length of cTn subunits vary among species, thus the discrepancy in residue number may actually refer to the same residue. For example, in rodent, there is one redundant residue (Ala-25) in the N-terminus of cTnI, thus the residue number of cTnI after this residue will change (+1) correspondingly. Using skinned muscle fibers, Takahashi-Yanaga *et al.* studied the function of six HCM-associated cTnI mutations (R145G, R145Q, R162W, K183, G203S, and K206Q) and found that, with the exception of cTnI^{G203S} (which showed a tendency, but without statistical significance), all the other mutations significantly increased the Ca^{2+} sensitivity of myofibrillar ATPase activity and force generation [144]. Elliott *et al.* reported that both cTnI^{R145G} and cTnI^{R162W} mutations reduced the inhibition of actin-Tm-activated myosin ATPase, and significantly increased the Ca^{2+} sensitivity of ATPase activity, suggesting both mutations may impair relaxation in addition to enhancing contraction [145]. Using the *in-vitro* motility assay with recombinant rat cTn subunits, Kohler *et al.* investigated the effect of three HCM-associated cTnI mutations (K183, G203S, and K206Q) on the Ca^{2+} regulation, and found that all three mutants enhanced the Ca^{2+} sensitivity and maximal speed of filament sliding [146]. Among all HCM-related cTnI mutations, the cTnI^{R145G} (cTnI^{R146G} in rodent), located in the inhibitory-peptide of cTnI, has received the most prominent attention [29, 102, 144, 145, 147-156]. There are complex and sometimes contradictory results from the literatures for the effects of cTnI^{R145G} on the maximal force production and the Ca^{2+} sensitivity of force generation. For example, James *et al.* reported that cTnI^{R146G} Tg mice showed a significant increase in the Ca^{2+} sensitivity of force and a significant depress in maximal tension when compared with the NTG littermate controls [156]. Takahashi-Yanaga *et al.* also reported that the cTnI^{R145G} resulted in an increase in the Ca^{2+} sensitivity of force generation and myofibrillar ATPase activity in skinned muscle fibers [144]. However, incorporation of recombinant human cTnI^{R145G} into guinea-pig cardiac trabeculae skinned fibres had no effect on Ca^{2+} sensitivity [155]. Similarly, using recombinant human cTnI^{R145G} exchanged into murine myofibrils, Kruger *et al.* reported no change in the Ca^{2+} sensitivity of tension development [151]. They also reported a slightly decreased Ca^{2+} sensitivity of force in myofibrils from transgenic cTnI^{R146G} mice [151]. The reasons for different results reported for this mutation (cTnI^{R145G}) on the Ca^{2+} sensitivity of tension are unclear. However, many factors can affect the Ca^{2+} sensitivity of tension, such as species-specific differences, exchange efficiency (mutant to wild-type ratio), solution pH and temperature. Additionally, using the reconstituted actin-tropomyosin activated myosin ATPase assay, Lang *et al.* [148], Takahashi-Yanaga *et al.* [144], and Elliot *et al.* [145] reported that cTnI^{R145G} decreased maximal ATPase in the presence of Ca^{2+}, and reduced inhibition of actomyosin ATPase activity in the absence of Ca^{2+}. cTnI^{R21C} is the only identified HCM-associated mutation located at the cardiac-specific N-terminus of cTnI [30, 157-159]. Using transgenic mice, Wang *el al.* reported that the cTnI^{R21C} mutation prevented PKA-mediated phosphorylation *in vivo* [30]. By replacing the recombinant human cTnI into porcine muscle, Gomes *et al.* reported that cTnI^{R21C} significantly increased the Ca^{2+} sensitivity of force development with respect to the cTnI^{WT} [157]. He also reported that compared to cTnI^{WT}, PKA phosphorylation of fibers containing cTnI^{R21C} resulted in a significantly smaller decrease in the Ca^{2+} sensitivity of force generation, which is attribute to the decreased ability of PKA to phosphorylated cTnI^{R21C} at S23/S24 [157]. Dweck *et al.* found that the isolated cardiac myocytes from R21C transgenic mice (with ages older than 12 months) significantly delayed

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Ca^{2+} transient decay and relaxation [158]. The phenotype of $cTnI^{R21C}$ mutation supports the regulatory role of cTnI N-terminus in diastolic function of the heart. Very recently, Ramirez-Correa *et al.* studied the $cTnI^{P82S}$ mutation, which was initially considered as a disease-causing mutation, however, later studies suggested the contrary [28]. Using transgenic mice, they found that $cTnI^{P83S}$ prolonged the isovolumetric relaxation time, impaired ejection and relaxation time, as well as blunted the β–adrenergic response and impaired myofilament cooperativity [28]. Similar to these cTnI mutations, several (but not all) HCM-associated mutations in cTnC (A8V, L29Q, A31S, C84Y and D145E) and cTnT (F110I, R92L/W/Q, R94L, A104V, R130C, 160E, E163R, S179F and E244D) have been demonstrated to increase Ca^{2+} sensitivity of ATPase activity and/or force development [31-33, 126, 160-184]. Pinto *et al.* reported that $cTnC^{A8V}$ and $cTnC^{D145E}$ significantly slowed Ca^{2+} dissociation kinetics (k_{off}) in solution, suggesting both mutations may alter muscle relaxation properties [178]. Considering that most of the findings of these HCM mutations were focused on the Ca^{2+} sensitivity of ATPase activity and/or force development, it is of importance to also investigate how they influence the muscle relaxation.

Recently, we reported that both $cTnI^{R146G}$ and $cTnI^{R21C}$ mutations (based on rat sequence) alter not only normal contractile properties, but also blunted the modulatory capacity of S23/S24 PKA-mediated phosphorylation during β-adrenergic stimulation [185]. We [185] and others [30, 158] have demonstrated that $cTnI^{R21C}$ disrupts the PKA phosphorylation on S23/S24 of cTnI, and thus results in a “blunted” β-adrenergic stimulation effects, which may be the actual physiologic/pathogenic mechanism of $cTnI^{R21C}$. So, to understand whether it is the $cTnI^{R21C}$ *per se* that is altering function or just the inability to get S23/S24 phosphorylated, we constructed the *bis*-phosphomimic substitutions S23D/S24D into $cTnI^{R21C}$ ($cTnI^{R21C/S23D/S24D}$) to mimic effect of PKA phosphorylation. Using steady-state fluorescence measurements, we found that both mutations significantly increased Ca^{2+} binding affinity to cTn (K_{Ca}) and the affinity of cTnC for cTnI (K_{C-I}). PKA phosphorylation of cTnI (or introduction of *bis*-phosphomimic substitutions on cTnI, $cTnI^{S23D/S24D}$) resulted in a similar reduction of K_{Ca} for all complexes, but the reduction in K_{C-I} seen with $cTnI^{WT}$ did not occur for either mutation in cTnI. When recombinant cTn containing either mutation was exchanged into isolated adult rat cardiac myofibrils they caused an increased Ca^{2+} sensitivity of tension (pCa_{50}) as reported by others [29, 144, 145] and prolonged the duration of the early, slow-phase of relaxation. Interestingly, PKA phosphorylation of cTnI resulted in decreased pCa_{50} for $cTnI^{WT}$ exchanged myofibrils, but not for either mutation. PKA phosphorylation also accelerated the early, slow-phase relaxation for myofibrils with $cTnI^{WT}$, especially at Ca^{2+}-levels that the heart operates *in-vivo*. Importantly, this effect was blunted for $cTnI^{R146G}$ and $cTnI^{R21C}$ exchanged myofibrils. Interestingly, this blunting of PKA-mediated effects has also been reported for another HCM-associated mutation, $cTnC^{L29Q}$. Dong *et al.* found that $cTnC^{L29Q}$ abolished the enhancement of closing rate of NcTnC (that is triggered by Ca^{2+} dissociation) upon PKA phosphorylation of cTnI [186]. In addition, Schmidtmann *et al.* [181] and Li *et al.* [179] reported that $cTnC^{L29Q}$ hindered the effect of PKA phosphorylation of cTnI on transduction the signal from cTnC to cTnI. Together these findings suggest that, for at least some HCM-related mutations, an additional contractile abnormality may be blunting (or uncoupling) of myofilament response to the PKA-mediated phosphorylation of cTnI during β-adrenergic stimulation. The solution

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measurements of cTnI affinity for cTnC suggest this may be due to an inability of S23/S24 phosphorylation to weaken this interaction, an idea that is supported by molecular dynamics modeling studies of cTn discussed in the next section.

Computational Studies on the cTn Structure and HCM-associated cTn Mutations

Understanding the structural changes in proteins that result in changing function is beneficial in gaining a firm understanding of the molecular mechanisms involved in modulating contraction and its dysfunction with disease. The correlation between specific alterations in the cTn structure by introduction of a single amino-acid mutation that results in complex functional phenotypes remains poorly understood. NMR and X-ray crystallography have shed light on the atomic level structures of the cTnC regulatory domain and the cTn complex [52-56, 77]. Li *et al.* solved the NMR structures of the regulatory domain of cTnC (NcTnC, residues 1-89) in both Ca^{2+}-saturated and Ca^{2+}-free states, and the regulatory domain of cTnC in complex with the switch-peptide of cTnI [53]. Based on these NMR structures, Wang *et al.* applied integrated experimental-computational approaches to study the interactions of cTnC variants with altered Ca^{2+} binding affinities with the switch peptide of cTnI [187, 188]. Lindert and Kekenes-Huskey *et al.* studied the dynamics and Ca^{2+} association to NcTnC via both conventional and accelerated molecular dynamics (MD) simulations [189, 190], as well as the exposure dynamics and kinetics of cTnC hydrophobic residues via micro-second MD simulations [191]. In 2003, Takeda *et al.* determined the first crystal structure of the human cTn complex (with residues cTnC 1-161, cTnI 40-191, cTnT 202-276) in the Ca^{2+}-saturated form [77], making it possible for the first time to study detailed interactions between cTn subunit proteins. Using FRET techniques in combination with MD simulations, Jayasundar *et al.* studied the molecular details of how a Ca^{2+} signal, received at cTnC, is transmitted to cTnI [192]. Varughese *et al.* also applied this structure to study the binding of the drug Bepridil to cTnC [193], designed a set of potential cTnC-binding ligands [194], and studied the interactions and correlated motions among the three components of cTn [195].

All of the above studies used either partial models of cTnC in the presence/absence of Ca^{2+} and/or the cTnI switch-peptide, or the cTn model without the N-terminus of cTnI. Recently, using solution NMR, Howarth *et al.* [72] solved the structure of the N-terminus of cTnI, for both the non-phosphorylated and *bis*-phosphorylated (at position Ser-23/Ser-24) species, thus making it feasible to study PKA-mediated phosphorylation of cTnI employing computational models. We recently built up the structure of the cTn complex (including residues cTnC 1-161, cTnI 1-210, and cTnT 236-285) containing the cardiac-specific, N-terminus of cTnI (Fig. 3) [196]. This structure also included the inhibitory-peptide (residues 138-148) and the C-terminus (residues 192-210) of cTnI, and the C-terminal residues (residues 282-285) of cTnT that were not present in Takeda's crystal structure 1J1E or Howarth's NMR structure. Using this constructed model, we performed triplicate MD simulations of cTnI-WT and cTnI-S23D/S24D containing cTn models to elucidate the effect of PKA phosphorylation on cTn structure and Ca^{2+} binding. The most significant finding was that introduction of the *bis*-phosphomimic substitutions onto Ser-23/Ser-24 (S23D/

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S24D) led to formation of an intra-subunit interaction between the N-terminus and the inhibitory-peptide of cTnI that may destabilize the interaction of NcTnC hydrophobic residues with the switch-peptide residues of cTnI [196].

This intra-subunit interaction of cTnI has previously been suggested by Solaro and colleagues based on solution biochemical and spectroscopic studies [36, 72, 197]. Howarth *et al.* applied the comparative docking to determine the low energy structure between the NMR structure of $cTnI_{1-32}$ in the *bis*-phosphorylated state and the Takeda's crystal structure of cTn [72]. They proposed that in the low energy structure, $cTnI_{1-32}$ resulted in weakening interaction with NcTnC and re-positioning the $cTnI_{1-32}$ for favorable interactions with basic regions of cTnI, and most likely the inhibitory-peptide region of cTnI [72]. Specifically, the acidic amino acids (Asp-3, Glu-4, Asp-7 and Glu-11) of N-terminus interacted with residues Arg-142 and Arg-146 of the inhibitory-peptide region [72]. Sadayappan *et al.* generated a cardiac-specific transgenic mice in which residues 2-11 of cTnI (cTnI (2-11)) were deleted, and tested the hypothesis that the acidic N' region of cTnI help regulate myocardial function [197]. Compared to the non-transgenic heart, the cTnI (2-11) transgenic mice had significantly decreased contraction and relaxation under basal and β-adrenergic stress, accompanied by a reduction in the maximal Ca^{2+}-dependent tension and Ca^{2+}-activated Mg^{2+}-ATPase activity. However, the Ca^{2+}-sensitivity of force development and PKA phosphorylation of cTnI-S23/S24 were not affected, suggesting that the acidic N' region of cTnI does not play a direct role in the Ca^{2+}-induced transition in NcTnC. The cTnI (2-11) transgenic mice also had decrease in myocardial contractility, demonstrating the importance of acidic N' region in modulating contractility and mediating the response of the heart to the β-adrenergic stimulation. These results provide further support for a role of the acidic N-terminus in regulating cardiac contractility and mediating the response of the heart to β-adrenergic stimulation by interacting directly with the inhibitory-peptide region of cTnI. Based on these finding and the NMR structural data, Solaro *et al.* predicted that the phosphorylated N-terminal extension of cTnI can interact with the inhibitory-peptide region of cTnI [36].

Our MD simulations also suggest a bending at the N-terminal extension of cTnI and a more compact cTn structure upon introducing the *bis*-phosphomimic substitutions that is consistent with previous biochemical studies studying *bis*-phosphorylation of cTnI S23/S24 ($cTnI^{pS23/pS24}$) on overall cTn structure [198-200]. Using fluorescence studies, Dong *et al.* demonstrated that *bis*-phosphorylation resulted in a reduction of the axial ratio of cTnI and the formation of a more compact structure [198]. Using small-angle neutron scattering, Heller *et al.* found that $cTnI^{pS23/pS24}$ induced a dramatic bending of the rod-like cTnI at its N-terminus that binds with cTnC, resulting in a dramatic decrease in the axial ratio of cTnI and the cTn complex overall [199]. In addition, using surface plasmon resonance (SPR), Reiffert *et al.* determined that upon PKA phosphorylation, the shape of cTnI changed from an asymmetrical structure to a more symmetrical one, which is consistent with the bending that results in a shorter and broader structure [200]. All of the above experimental findings suggest the formation of an alternative binding pattern for the *bis*-phosphorylated cardiac-specific N-terminal extension.

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The relationship between cTnC-cTnI interaction and the blunted PKA effects for some HCM-related cTnI mutations

As mentioned above, cTnC-cTnI (C-I) interaction plays a 'gatekeeper' role in transmitting the Ca^{2+} signal to other myofilament proteins to initiate cardiac muscle contraction. Structural changes of the N-domain in cTnC in response to Ca^{2+} binding are different from those found for sTnC [52-54, 57]. For the sTnC, Ca^{2+} binding to its regulatory domain induces a transition from "closed" to "open" structure of the hydrophobic patch, facilitating sTnI binding and muscle activation [54]. However, Ca^{2+} binding to cTnC does not result in a similar structural alteration (hydrophobic patch exposure is minimal), and interaction with the cTnI switch-peptide is also required to expose and stabilize the hydrophobic patch [52, 53, 57]. Studies of C-I interaction also offer insight into how mutations in troponin may result in cardiac contractile dysfunction, and provides insight into potential targets for therapeutic agents that could mediate the Ca^{2+} sensitivity of the myofilaments in diseased hearts.

Using steady-state fluorescence measurements, we determined the effects of PKA phosphorylation (or *bis*-phosphomimic substitutions) on cTn-Ca^{2+} binding affinity (K_{Ca}) and cTnC-cTnI affinity ($K_{C\text{-}I}$) in the absence of confounding influences of other myofilament proteins [125]. We found that both K_{Ca} and $K_{C\text{-}I}$ were decreased upon PKA phosphorylation of cTnI or introduction of the *bis*-phosphomimic substitutions on cTnI ($cTnI^{S23D/S24D}$) [125]. By exchanging the recombinant cTn complex into demembranated trabeculae and isolated myofibrils, we studied the effects on myofibril activation/relaxation kinetics [125, 201]. We demonstrated that PKA phosphorylation of cTnI-S23/S24 (or *bis*-phosphomimic substitutions) decreased the Ca^{2+} sensitivity of force production and, most importantly, accelerated the initial slow-phase relaxation for rat left ventricular myofibrils, especially at the sub-maximal Ca^{2+} levels that heart operates *in vivo* [125, 201]. These findings suggest the strength of cTnC-cTnI interaction modulates the kinetics of thin filament activation, the magnitude of tension development and the initial phase of myofibril relaxation. Our computational results suggested that this may be the result of formation of an intra-subunit interaction between the N-terminus and the inhibitory-peptide of cTnI [196]. We hypothesized that this intra-subunit interaction may weaken interactions between the switch-peptide of cTnI and NcTnC, allowing stronger interaction between the cTnI inhibitory-peptide and actin (move the equilibrium towards thin filament deactivation), and this may provide the structural basis for how PKA phosphorylation of cTnI modulates cross-bridge activity and relaxation.

Based on our findings, we further hypothesized that introduction of a mutation located in either the inhibitory-peptide or the N-terminus of cTnI may disrupt the formation of this intra-subunit interaction and blunt the effects of PKA-mediated phosphorylation during β-adrenergic stimulation. Recently, we tested this hypothesis by studying two HCM-associated mutations, $cTnI^{R146G}$ and $cTnI^{R21C}$ that are located in the inhibitory-peptide and the N-terminus of cTnI (respectively) using combined steady-state fluorescence measurements, myofibril kinetics/mechanics measurements and computational modeling [185, 202]. We found that both mutants increased K_{Ca} and $K_{C\text{-}I}$ in solution, increased the Ca^{2+} sensitivity of

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myofibril tension development (pCa_{50}), and also prolonged the early, slow-phase of relaxation. Importantly, both mutants blunted the ability of PKA to reduce K_{C-I} and pCa_{50} and speed relaxation of myofibrils [125]. It is worth mentioning that cTnI^{R21C} disrupts the PKA phosphorylation on S23/S24 of cTnI [30, 158, 185], and thus results in a "blunted" β-adrenergic stimulation response, which may be the actual physiologic/pathogenic mechanism of cTnI^{R21C}. Therefore, to further understand whether it is the cTnI^{R21C} *per se* that is altering function or just the inability to get S23/S24 phosphorylated, we introduced the *bis*-phosphomimic substitutions S23D/S24D into cTnI^{R21C} (cTnI$^{R21C/S23D/S24D}$) to mimic effect of PKA phosphorylation. Our results indicated that even with *bis*-phosphomimic substitutions ('forced phosphorylation'), the phosphorylation mediated effects on K_{C-I} and myofibril relaxation were still blunted, suggesting that the cTnI^{R21C} mutation *per se* results in the cardiac dysfunction of modulation by phosphorylation, similar to our results for the cTnI^{R146G} mutation. Our computational results of cTn suggested that introduction of either mutation inhibited the formation of the intra-subunit interaction between the N-terminus and the inhibitory-peptide of cTnI that normally seen for cTn with the *bis*-phosphomimic substitutions of S23/S24 [196]. Additionally, both mutations abrogated the destabilization of contacts between the cTnI switch-peptide and hydrophobic residues in the N-terminal lobe of cTnC. This suggests a potential structure-based mechanism of how both mutations can impair PKA regulation of contraction and relaxation. Furthermore, it suggests that the degree of cTnC-cTnI interaction may act as a regulator that is modulated by changes in either Ca^{2+} or cTnI phosphorylation during β-adrenergic stimulation of the heart.

Summary and Future Directions

The cTn ternary complex is the key regulatory protein complex of myofilament contraction and tension generation. Studying how disease-related mutations affect conformational changes in cTn provides insight as to how Ca^{2+}-mediated signal transduction may be altered and result in contractile dysfunction associated with cardiac disease (the correlations between genotype and phenotype). Mounting evidence obtained from the biochemical/biophysical, physiological and computational studies have shed light on understanding the structure-function relationships, regulation, post-translational modification and pathogenicity of mutations from cTn subunits.

Our recent findings suggest that for at least two HCM-associated cTnI mutations (cTnI^{R146G} and cTnI^{R21C}) there is a blunting of the ability of PKA-mediated phosphorylation to modulate both contraction and relaxation [185]. Considering the specific locations of both mutations (cTnI^{R146G} in the inhibitory-peptide and cTnI^{R21C} in the N-terminus), it was not surprising that both mutations inhibited formation of the intra-subunit interaction between the N-terminus and the inhibitory-peptide of cTnI that normally seen with phosphorylation. Whether this is the case for other mutations in cTnI or the other cTn subunits remains to be determined. We are currently studying three additional HCM-associated mutations (cTnI^{P82S} in the I-T arm region, cTnI^{A157V} in the switch-peptide region and cTnI^{K206Q} in the C-terminus region) using the combined protein biochemistry, myofibril kinetics/mechanics measurements, and computational modelling approach.

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Computational models with detailed structural and temporal information can provide invaluable mechanistic interpretations of experimental data, enabling extended understanding of how disease-associated mutations result in functional changes of the thin filament or whole sarcomere at an atomic-based structural level. Even more complete models, such as the models of Schwartz and colleagues [92] that computationally study the whole thin filament (with cTn, Tm, and actin) and potential future models with atomistic details and dynamics of multiple thin and thick filaments in a sarcomere can be even more enlightening. This more complete overall picture, based on the view from the structural basis, will assist in the interpretation of the physiological findings.

Computationally studies can also act as a powerful strategy to assist and guide the design of engineered troponin variants as a strategy to treat muscle disease. Based on the current computational models, we and others have designed engineered cTnC variants with altered Ca^{2+} binding affinities (to cTn) and demonstrated that those variants alter the contractile function in cardiomyocytes and correct or prevent disease-related aberrant Ca^{2+} binding and contractile function [163, 187, 188, 203-209]. These studies also provide important implications with respect to the design of Ca^{2+} sensitizing or desensitizing small molecules or drugs. Altering cTnC-cTnI interaction may be a promising target to reduce or reverse the hypertrophic signaling that results from the pathological myofilament protein mutations, or strengthen contraction to counter reduced systolic function in hearts with dilated cardiomyopathy. Taken together, considering the central role of troponin in muscle contraction and regulation, further elucidation the structure-function relationship can provide powerful strategies for the diagnosis, prevention, and even treatment of heart diseases.

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References

1. Maron BJ, Roberts WC, McAllister HA, Rosing DR, Epstein SE. Circulation. 1980; 62:218–229. [PubMed: 6446987]
2. Teare D. Br Heart J. 1958; 20:1–8. [PubMed: 13499764]
3. Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. Circulation. 1995; 92:785–789. [PubMed: 7641357]
4. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, Dubourg O, Hl UKR, Maisch B, McKenna WJ, Monserrat L, Pankuweit S, Rapezzi C, Seferovic P, Tavazzi L, Keren A. Eur Heart J. 2008; 29:270–276. [PubMed: 17916581]
5. Mogensen J, Murphy RT, Kubo T, Bahl A, Moon JC, Klausen IC, Elliott PM, McKenna WJ. J Am Coll Cardiol. 2004; 44:2315–2325. [PubMed: 15607392]
6. Tester DJ, Ackerman MJ. Annu Rev Med, Annual Reviews, Palo Alto. 2009:69–84.
7. Bonne G, Carrier L, Bercovici J, Cruaud C, Richard P, Hainque B, Gautel M, Labeit S, James M, Beckmann J, Weissenbach J, Vosberg HP, Fiszman M, Komajda M, Schwartz K. Nat Genet. 1995; 11:438–440. [PubMed: 7493026]
8. Watkins H, Conner D, Thierfelder L, Jarcho JA, Macrae C, McKenna WJ, Maron BJ, Seidman JG, Seidman CE. Nat Genet. 1995; 11:434–437. [PubMed: 7493025]
9. Ho CY, Lever HM, DeSanctis R, Farver CF, Seidman JG, Seidman CE. Circulation. 2000; 102:1950–1955. [PubMed: 11034944]

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10. Thierfelder L, Macrae C, Watkins H, Tomfohrde J, Williams M, McKenna W, Bohm K, Noeske G, Schlepper M, Bowcock A, Vosberg HP, Seidman JG, Seidman C. Proc Natl Acad Sci U S A. 1993; 90:6270–6274. [PubMed: 8327508]
11. Geisterfer-Lowrance AAT, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, Seidman JG. Cell. 1990; 62:999–1006. [PubMed: 1975517]
12. Poetter K, Jiang H, Hassanzadeh S, Master SR, Chang A, Dalakas MC, Rayment I, Sellers JR, Fananapazir L, Epstein ND. Nat Genet. 1996; 13:63–69. [PubMed: 8673105]
13. Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, Odonoghue A, Spirito P, Matsumori A, Moravec CS, Seidman JG, Seidman CE. New Engl J Med. 1995; 332:1058–1064. [PubMed: 7898523]
14. Moolman JC, Corfield VA, Posen B, Ngumbela K, Seidman C, Brink PA, Watkins H. J Am Coll Cardiol. 1997; 29:549–555. [PubMed: 9060892]
15. Nakajima Taniguchi C, Matsui H, Fujio Y, Nagata S, Kishimoto T, Yamauchi Takihara K. J Mol Cell Cardiol. 1997; 29:839–843. [PubMed: 9140840]
16. Kimura A, Harada H, Park JE, Nishi H, Satoh M, Takahashi M, Hiroi S, Sasaoka T, Ohbuchi N, Nakamura T, Koyanagi T, Hwang TH, Choo TA, Chung KS, Hasegawa A, Nagai R, Okazaki O, Nakamura H, Matsuzaki M, Sakamoto T, Toshima H, Koga Y, Imaizumi T, Sasazuki T. Nat Genet. 1997; 16:379–382. [PubMed: 9241277]
17. Mogensen J, Klausen IC, Pedersen AK, Egeblad H, Bross P, Kruse TA, Gregersen N, Hansen PS, Baandrup U, Borglum AD. J Clin Invest. 1999; 103:R39–R43. [PubMed: 10330430]
18. Satoh M, Takahashi M, Sakamoto T, Hiroe M, Marumo F, Kimura A. Biochem Biophys Res Commun. 1999; 262:411–417. [PubMed: 10462489]
19. Hoffmann B, Schmidt-Traub H, Perrot A, Osterziel KJ, Geßner R. Hum Mutat. 2001; 17:524–524. [PubMed: 11385718]
20. Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP, Seldman JG, Seidman CE. Cell. 77:701–712. [PubMed: 8205619]
21. Albury ANJ, Swindle N, Swartz DR, Tikunova SB. Biochemistry. 2012; 51:3614–3621. [PubMed: 22489623]
22. Liu B, Tikunova SB, Kline KP, Siddiqui JK, Davis JP. Plos One. 2012; 7:11.
23. Robinson P, Griffiths PJ, Watkins H, Redwood CS. Circul Res. 2007; 101:1266–1273.
24. Rust EM, Albayya FP, Metzger JM. J Clin Invest. 1999; 103:1459–1467. [PubMed: 10330428]
25. Tardiff JC, Factor SM, Tompkins BD, Hewett TE, Palmer BM, Moore RL, Schwartz S, Robbins J, Leinwand LA. J Clin Invest. 1998; 101:2800–2811. [PubMed: 9637714]
26. Tardiff JC, Hewett TE, Palmer BM, Olsson C, Factor SM, Moore RL, Robbins J, Leinwand LA. J Clin Invest. 1999; 104:469–481. [PubMed: 10449439]
27. Javadpour MM, Tardiff JC, Pinz I, Ingwall JS. J Clin Invest. 2003; 112:768–775. [PubMed: 12952925]
28. Ramirez-Correa GA, Frazier AH, Zhu GS, Zhang PB, Rappold T, Kooij V, Bedja D, Snyder GA, Lugo-Fagundo NS, Hariharan R, Li YJ, Shen XX, Gao WD, Cingolani OH, Takimoto E, Foster DB, Murphy AM. J Appl Physiol. 2015; 118:212–223. [PubMed: 25324519]
29. Wen YH, Pinto JR, Gomes AV, Xu YY, Wang YC, Wang Y, Potter JD, Kerrick WGL. Journal of Biological Chemistry. 2008; 283:20484–20494. [PubMed: 18430738]
30. Wang YC, Pinto JR, Solis RS, Dweck D, Liang JS, Diaz-Perez Z, Ge Y, Walker JW, Potter JD. Journal of Biological Chemistry. 2012; 287:2156–2167. [PubMed: 22086914]
31. Guinto PJ, Haim TE, Dowell-Martino CC, Sibinga N, Tardiff JC. Am J Physiol-Heart Circul Physiol. 2009; 297:H614–H626.
32. Jimenez J, Tardiff JC. Am J Physiol-Heart Circul Physiol. 2011; 300:H627–H635.
33. Moore RK, Grinspan LT, Jimenez J, Guinto PJ, Ertz-Berger B, Tardiff JC. J Mol Cell Cardiol. 2013; 58:188–198. [PubMed: 23434821]
34. Willott RH, Gomes AV, Chang AN, Parvatiyar MS, Pinto JR, Potter JD. J Mol Cell Cardiol. 2010; 48:882–892. [PubMed: 19914256]
35. Zhang R, Zhao J, Mandveno A, Potter JD. Circul Res. 1995; 76:1028–1035.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

36. Solaro RJ, Rosevear P, Kobayashi T. Biochem Biophys Res Commun. 2008; 369:82–87. [PubMed: 18162178]
37. Gordon AM, Homsher E, Regnier M. Physiol Rev. 2000; 80:853–924. [PubMed: 10747208]
38. Kawasaki H, Nakayama S, Kretsinger RH. BioMetals. 1998; 11:277–295. [PubMed: 10191494]
39. Herzberg O, James MNG. Nature. 1985; 313:653–659. [PubMed: 3974698]
40. Li MX, Wang X, Sykes BD. J Muscle Res Cell Motil. 2004; 25:559–579. [PubMed: 15711886]
41. Dvoretsky A, Abusamhadneh EM, Howarth JW, Rosevear PR. Journal of Biological Chemistry. 2002; 277:38565–38570. [PubMed: 12147696]
42. Collins JH. J Muscle Res Cell Motil. 1991; 12:3–25. [PubMed: 2050809]
43. Holroyde MJ, Robertson SP, Johnson JD, Solaro RJ, Potter JD. Journal of Biological Chemistry. 1980; 255:1688–1693.
44. Potter JD, Gergely J. Journal of Biological Chemistry. 1975; 250:4628–4633. [PubMed: 124731]
45. Brinley FJ, Scarpa A, Tiffert T. J Physiol-London. 1977; 266:545–565. [PubMed: 17002]
46. Evenas J, Malmendal A, Forsen S. Curr Opin Chem Biol. 1998; 2:293–302. [PubMed: 9667926]
47. Zot HG, Potter JD. Journal of Biological Chemistry. 1982; 257:7678–7683. [PubMed: 6211445]
48. Van Eerd JP, Takahashi K. Biochem Biophys Res Commun. 1975; 64:122–127. [PubMed: 1170846]
49. Sheng ZL, Strauss WL, Francois JM, Potter JD. Journal of Biological Chemistry. 1990; 265:21554–21560. [PubMed: 2254314]
50. Sweeney HL, Brito RMM, Rosevear PR, Putkey JA. Proc Natl Acad Sci U S A. 1990; 87:9538–9542. [PubMed: 2263608]
51. Putkey JA, Sweeney HL, Campbell ST. Journal of Biological Chemistry. 1989; 264:12370–12378. [PubMed: 2745448]
52. Spyracopoulos L, Li MX, Sia SK, Gagne SM, Chandra M, Solaro RJ, Sykes BD. Biochemistry. 1997; 36:12138–12146. [PubMed: 9315850]
53. Li MX, Spyracopoulos L, Sykes BD. Biochemistry. 1999; 38:8289–8298. [PubMed: 10387074]
54. Gagne SM, Tsuda S, Li MX, Smillie LB, Sykes BD. Nat Struct Biol. 1995; 2:784–789. [PubMed: 7552750]
55. Sia SK, Li MX, Spyracopoulos L, Gagne SM, Liu W, Putkey JA, Sykes BD. Journal of Biological Chemistry. 1997; 272:18216–18221. [PubMed: 9218458]
56. Vassylyev DG, Takeda S, Wakatsuki S, Maeda K, Maeda Y. Proc Natl Acad Sci U S A. 1998; 95:4847–4852. [PubMed: 9560191]
57. Dong WJ, Xing J, Villain M, Hellinger M, Robinson JM, Chandra R, Solaro RJ, Umeda PK, Cheung HC. Journal of Biological Chemistry. 1999; 274:31382–31390. [PubMed: 10531339]
58. Martins SM, Chapeaurouge A, Ferreira ST. Eur J Biochem. 2002; 269:5484–5491. [PubMed: 12423346]
59. Perry SV. Mol Cell Biochem. 1999; 190:9–32. [PubMed: 10098965]
60. Hastings KEM. Cell Struct Funct. 1997; 22:205–211. [PubMed: 9113408]
61. Chong SM, Jin JP. J Mol Evol. 2009; 68:448–460. [PubMed: 19365646]
62. Saggin L, Gorza L, Ausoni S, Schiaffino S. Journal of Biological Chemistry. 1989; 264:16299–16302. [PubMed: 2777792]
63. Sasse S, Brand NJ, Kyprianou P, Dhoot GK, Wade R, Arai M, Periasamy M, Yacoub MH, Barton PJR. Circul Res. 1993; 72:932–938.
64. Reiser PJ, Westfall MV, Schiaffino S, Solaro RJ. Am J Physiol-Heart Circul Physiol. 1994; 267:H1589–H1596.
65. Westfall MV, Lee AM, Robinson DA. Journal of Biological Chemistry. 2005; 280:41324–41331. [PubMed: 16236710]
66. Solaro RJ, Lee JA, Kentish JC, Allen DG. Circul Res. 1988; 63:779–787.
67. F'Entzke RC, Buck SH, Patel JR, Lin H, Wolska BM, Stojanovic MO, Martin AF, Solaro RJ, Moss RL, Leiden JM. J Physiol-London. 1999; 517:143–157. [PubMed: 10226156]
68. Rarick HM, Tu XH, Solaro RJ, Martin AF. Journal of Biological Chemistry. 1997; 272:26887–26892. [PubMed: 9341121]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

69. Foster DB, Noguchi T, VanBuren P, Murphy AM, Van Eyk JE. Circul Res. 2003; 93:917–924.
70. Jin JP, Yang FW, Yu ZB, Ruse CI, Bond M, Chen A. Biochemistry. 2001; 40:2623–2631. [PubMed: 11327886]
71. Jin JP, Zhang ZL, Bautista JA. Crit Rev Eukaryot Gene Expression. 2008; 18:93–124.
72. Howarth JW, Meller J, Solaro RJ, Trewhella J, Rosevear PR. J Mol Biol. 2007; 373:706–722. [PubMed: 17854829]
73. Pearlstone JR, Smillie LB. Canadian Journal of Biochemistry and Cell Biology. 1985; 63:212–218. [PubMed: 3986667]
74. Gasmi-Seabrook GMC, Howarth JW, Finley N, Abusamhadneh E, Gaponenko V, Brito RMM, Solaro RJ, Rosevear PR. Biochemistry. 1999; 38:8313–8322. [PubMed: 10387077]
75. Syska H, Wilkinson JM, Grand RJA, Perry SV. Biochem J. 1976; 153:375–&. [PubMed: 179535]
76. Talbot JA, Hodges RS. Journal of Biological Chemistry. 1981; 256:2798–2802. [PubMed: 6451620]
77. Takeda S, Yamashita A, Maeda K, Maeda Y. Nature. 2003; 424:35–41. [PubMed: 12840750]
78. Brown LJ, Sale KL, Hills R, Rouviere C, Song LK, Zhang XJ, Fajer PG. Proc Natl Acad Sci U S A. 2002; 99:12765–12770. [PubMed: 12239350]
79. Tung CS, Wall ME, Gallagher SC, Trewhella J. Protein Sci. 2000; 9:1312–1326. [PubMed: 10933496]
80. Lindhout DA, Sykes BD. Journal of Biological Chemistry. 2003; 278:27024–27034. [PubMed: 12732641]
81. Slupsky CM, Sykes BD. Biochemistry. 1995; 34:15953–15964. [PubMed: 8519752]
82. Perry SV. J Muscle Res Cell Motil. 1998; 19:575–602. [PubMed: 9742444]
83. Wei B, Jin JP. Archives of Biochemistry and Biophysics. 2011; 505:144–154. [PubMed: 20965144]
84. Anderson PAW, Malouf NN, Oakeley AE, Pagani ED, Allen PD. Circul Res. 1991; 69:1226–1233.
85. Jin JP, Wang J, Zhang JY. Gene. 1996; 168:217–221. [PubMed: 8654947]
86. Ricchiuti V, Apple FS. Clin Chem. 1999; 45:2129–2135. [PubMed: 10585344]
87. Jin JP, Chong SM. Archives of Biochemistry and Biophysics. 2010; 500:144–150. [PubMed: 20529660]
88. Heeley DH, Golosinska K, Smillie LB. Journal of Biological Chemistry. 1987; 262:9971–9978. [PubMed: 3611073]
89. Schaertl S, Lehrer SS, Geeves MA. Biochemistry. 1995; 34:15890–15894. [PubMed: 8519745]
90. Vinogradova MV, Stone DB, Malanina GG, Karatzaferi C, Cooke R, Mendelson RA, Fletterick RJ. Proc Natl Acad Sci U S A. 2005; 102:5038–5043. [PubMed: 15784741]
91. Ertz-Berger BR, He HM, Dowell C, Factor SM, Haim TE, Nunez S, Schwartz SD, Ingwall JS, Tardiff JC. Proc Natl Acad Sci U S A. 2005; 102:18219–18224. [PubMed: 16326803]
92. Manning EP, Tardiff JC, Schwartz SD. Biochemistry. 2011; 50:7405–7413. [PubMed: 21797264]
93. Martyn DA, Regnier M, Xu D, Gordon AM. Biophys J. 2001; 80:360–370. [PubMed: 11159408]
94. Martyn DA, Freitag CJ, Chase PB, Gordon AM. Biophys J. 76:1480–1493. [PubMed: 10049329]
95. Fuchs F, Wang YP. American Journal of Physiology - Cell Physiology. 1991; 261:C787–C792.
96. Wang X, Li MX, Spyracopoulos L, Beier N, Chandra M, Solaro RJ, Sykes BD. Journal of Biological Chemistry. 2001; 276:25456–25466. [PubMed: 11320096]
97. Pyle WG, Sumandea MP, Solaro RJ, De Tombe PP. Am J Physiol-Heart Circul Physiol. 2002; 283:H1215–H1224.
98. Burkart EM, Sumandea MP, Kobayashi T, Nili M, Martin AF, Homsher E, Solaro RJ. Journal of Biological Chemistry. 2003; 278:11265–11272. [PubMed: 12551921]
99. Mathur MC, Kobayashi T, Chalovich JM. Biophys J. 2008; 94:542–549. [PubMed: 17872964]
100. Wang H, Grant JE, Doede CM, Sadayappan S, Robbins J, Walker JW. J Mol Cell Cardiol. 2006; 41:823–833. [PubMed: 17010989]
101. Lu QW, Hinken AC, Patrick SE, Solaro RJ, Kobayashi T. Journal of Biological Chemistry. 2010; 285:11810–11817. [PubMed: 20164197]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

102. Lindhout DA, Li MX, Schieve D, Sykes BD. Biochemistry. 2002; 41:7267–7274. [PubMed: 12044157]
103. Li MX, Wang X, Lindhout DA, Buscemi N, Van Eyk JE, Sykes BD. Biochemistry. 2003; 42:14460–14468. [PubMed: 14661957]
104. Nixon BR, Thawornkaiwong A, Jin J, Brundage EA, Little SC, Davis JP, Solaro RJ, Biesiadecki BJ. Journal of Biological Chemistry. 2012; 287:19136–19147. [PubMed: 22493448]
105. Oliveira SM, Zhang YH, Solis RS, Isackson H, Bellahcene M, Yavari A, Pinter K, Davies JK, Ge Y, Ashrafian H, Walker JW, Carling D, Watkins H, Casadei B, Redwood C. Circul Res. 2012; 110:1192–1201.
106. Taglieri DM, Monasky MM, Knezevic I, Sheehan KA, Lei M, Wang X, Chernoff J, Wolska BM, Ke YB, Solaro RJ. J Mol Cell Cardiol. 2011; 51:988–996. [PubMed: 21971074]
107. You B, Yan GJ, Zhang ZL, Yan L, Li J, Ge QY, Jin JP, Sun JX. Biochem J. 2009; 418:93–101. [PubMed: 18986304]
108. Zhang PB, Kirk JA, Ji WH, dos Remedios CG, Kass DA, Van Eyk JE, Murphy AM. Circulation. 2012; 126:1828–U1123. [PubMed: 22972900]
109. Henze M, Patrick SE, Hinken A, Scruggs SB, Goldspink P, de Tombe PP, Kobayashi M, Ping PP, Kobayashi T, Solaro RJ. Biochimica Et Biophysica Acta-Molecular Cell Research. 2013; 1833:823–832.
110. Salhi HE, Walton SD, Hassel NC, Brundage EA, de Tombe PP, Janssen PML, Davis JP, Biesiadecki BJ. J Mol Cell Cardiol. 2014; 76:257–264. [PubMed: 25252176]
111. Wijnker PJM, Li YJ, Zhang PB, Foster DB, dos Remedios C, Van Eyk JE, Stienen GJM, Murphy AM, van der Velden J. J Mol Cell Cardiol. 2015; 82:93–103. [PubMed: 25771144]
112. Solaro RJ, Moir AJG, Perry SV. Nature. 1976; 262:615–617. [PubMed: 958429]
113. Kobayashi T, Yang XF, Walker LA, Van Breemen RB, Solaro RJ. J Mol Cell Cardiol. 2005; 38:213–218. [PubMed: 15623438]
114. Haworth RS, Cuello F, Herron TJ, Franzen G, Kentish JC, Gautel M, Avkiran M. Circul Res. 2004; 95:1091–1099.
115. Cuello F, Bardswell SC, Haworth RS, Yin XK, Lutz S, Wieland T, Mayr M, Kentish JC, Avkiran M. Circul Res. 2007; 100:864–873.
116. Bardswell SC, Cuello F, Rowland AJ, Sadayappan S, Robbins J, Gautel M, Walker JW, Kentish JC, Avkiran M. Journal of Biological Chemistry. 2010; 285:5674–5682. [PubMed: 20018870]
117. Layland J, Li JM, Shah AM. J Physiol-London. 2002; 540:457–467. [PubMed: 11956336]
118. Kirkpatrick KP, Robertson AS, Klaiman JM, Gillis TE. J Exp Biol. 2011; 214:1981–1988. [PubMed: 21613513]
119. Kentish JC, McCloskey DT, Layland J, Palmer S, Leiden JM, Martin AF, Solaro RJ. Circul Res. 2001; 88:1059–1065.
120. Pi YQ, Kemnitz KR, Zhang DH, Kranias EG, Walker JW. Circul Res. 2002; 90:649–656.
121. Chandra M, Dong WJ, Pan BS, Cheung HC, Solaro RJ. Biochemistry. 1997; 36:13305–13311. [PubMed: 9341222]
122. Turnbull L, Hoh JFY, Ludowyke RI, Rossmanith GH. J Physiol-London. 2002; 542:911–920. [PubMed: 12154188]
123. Nixon BR, Walton SD, Zhang B, Brundage EA, Little SC, Ziolo MT, Davis JP, Biesiadecki BJ. J Mol Cell Cardiol. 2014; 72:177–185. [PubMed: 24657721]
124. Hanft LM, Biesiadecki BJ, McDonald KS. J Physiol-London. 2013; 591:4535–4547. [PubMed: 23836688]
125. Rao V, Cheng YH, Lindert S, Wang D, Oxenford L, McCulloch AD, McCammon JA, Regnier M. Biophys J. 2014; 107:1196–1204. [PubMed: 25185555]
126. Landstrom AP, Parvatiyar MS, Pinto JR, Marquardt ML, Bos JM, Tester DJ, Ornmen SR, Potter JD, Ackerman MJ. J Mol Cell Cardiol. 2008; 45:281–288. [PubMed: 18572189]
127. Rani DS, Nallari P, Priyamvada S, Narasimhan C, Singh L, Thangaraj K. BMC Med Genet. 2012; 13:8. [PubMed: 22284537]
128. Baudenbacher F, Schober T, Pinto JR, Sidorov VY, Hilliard F, Solaro RJ, Potter JD, Knollmann BC. J Clin Invest. 2008; 118:3893–3903. [PubMed: 19033660]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

129. Ashrafian H, Redwood C, Blair E, Watkins H. Trends Genet. 2003; 19:263–268. [PubMed: 12711218]
130. Alcalai R, Seidman JG, Seidman CE. J Cardiovasc Electrophysiol. 2008; 19:104–110. [PubMed: 17916152]
131. Fatkin D, Graham RM. Physiol Rev. 2002; 82:945–980. [PubMed: 12270949]
132. Niimura H, Patton KK, McKenna WJ, Soults J, Maron BJ, Seidman JG, Seidman CE. Circulation. 2002; 105:446–451. [PubMed: 11815426]
133. Konno T, Shimizu M, Ino H, Fujino N, Hayashi K, Uchiyama K, Kaneda T, Inoue M, Masuda E, Mabuchi H. J Intern Med. 2005; 258:216–224. [PubMed: 16115294]
134. Van Driest SL, Ellsworth EG, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Circulation. 2003; 108:445–451. [PubMed: 12860912]
135. Arad M, Penas-Lado M, Monserrat L, Maron BJ, Sherrid M, Ho CY, Barr S, Karim A, Olson TM, Kamisago M, Seidman JG, Seidman CE. Circulation. 2005; 112:2805–2811. [PubMed: 16267253]
136. Frazier A, Judge DP, Schulman SP, Johnson N, Holmes KW, Murphy AM. Pediatr Cardiol. 2008; 29:846–850. [PubMed: 18175163]
137. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet JP, Millaire A, Desnos M, Schwartz K, Hainque B, Komajda M, Project EHF. Circulation. 2003; 107:2227–2232. [PubMed: 12707239]
138. Varnava AM, Elliott PM, Baboonian C, Davison F, Davies MJ, McKenna WJ. Circulation. 2001; 104:1380–1384. [PubMed: 11560853]
139. Anan R, Shono H, Kisanuki A, Arima S, Nakao S, Tanaka H. Circulation. 1998; 98:391–397. [PubMed: 9714088]
140. Torricelli F, Girolami F, Olivotto I, Passerini I, Frusconi S, Vargiu D, Richard P, Cecchi F. Am J Cardiol. 2003; 92:1358–1362. [PubMed: 14636924]
141. Ackerman MJ, VanDriest SL, Ommen SR, Will ML, Nishimura RA, Tajik AJ, Gersh BJ. J Am Coll Cardiol. 2002; 39:2042–2048. [PubMed: 12084606]
142. Song L, Zou YB, Wang JH, Wang ZM, Zhen YS, Lou KJ, Zhang Q, Wang XJ, Wang H, Li J, Hui R. Clin Chim Acta. 2005; 351:209–216. [PubMed: 15563892]
143. Van Driest SL, Ackerman MJ, Ommen SR, Shakur R, Will ML, Nishimura RA, Tajik AJ, Gersh BJ. Circulation. 2002; 106:3085–3090. [PubMed: 12473556]
144. Takahashi-Yanaga F, Morimoto S, Harada K, Minakami R, Shiraishi F, Ohta M, Lu UW, Sasaguri T, Ohtsuki I. J Mol Cell Cardiol. 2001; 33:2095–2107. [PubMed: 11735257]
145. Elliott K, Watkins H, Redwood CS. Journal of Biological Chemistry. 2000; 275:22069–22074. [PubMed: 10806205]
146. Köhler J, Chen Y, Brenner B, Gordon AM, Kraft T, Martyn DA, Regnier M, Rivera AJ, Wang CK, Chase PB. Physiological Genomics. 2003; 14
147. Deng Y, Schmidtmann A, Redlich A, Westerdorf B, Jaquet K, Thieleczek R. Biochemistry. 2001; 40:14593–14602. [PubMed: 11724573]
148. Lang R, Gomes AV, Zhao JJ, Housmans PR, Miller T, Potter JD. Journal of Biological Chemistry. 2002; 277:11670–11678. [PubMed: 11801593]
149. Westfall MV, Borton AR, Albayya FP, Metzger JM. Circul Res. 2002; 91:525–531.
150. Reis S, Littwitz C, Preilowski S, Mugge A, Stienen GJM, Pott L, Jaquet K. Pflugers Arch. 2008; 457:17–24. [PubMed: 18548271]
151. Kruger M, Zittrich S, Redwood C, Blaudeck N, James J, Robbins J, Pfitzer G, Stehle R. J Physiol-London. 2005; 564:347–357. [PubMed: 15718266]
152. Kobayashi T, Solaro RJ. Journal of Biological Chemistry. 2006; 281:13471–13477. [PubMed: 16531415]
153. Zhou ZQ, Rieck D, Li KL, Ouyang YX, Dong WJ. Archives of Biochemistry and Biophysics. 2013; 535:56–67. [PubMed: 23246786]
154. Brunet NM, Chase PB, Mihajlovic G, Schoffstall B. Archives of Biochemistry and Biophysics. 2014; 552:11–20. [PubMed: 24418317]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

155. Burton D, Abdulrazzak H, Knott A, Elliott K, Redwood C, Watkins H, Marston S, Ashley C. Biochem J. 2002; 362:443–451. [PubMed: 11853553]
156. James J, Zhang Y, Osinska H, Sanbe A, Klevitsky R, Hewett TE, Robbins J. Circul Res. 2000; 87:805–811.
157. Gomes AV, Harada K, Potter JD. J Mol Cell Cardiol. 2005; 39:754–765. [PubMed: 16005017]
158. Dweck D, Sanchez-Gonzalez MA, Chang AN, Dulce RA, Badger CD, Koutnik AP, Ruiz EL, Griffin B, Liang JS, Kabbaj M, Fincham FD, Hare JM, Overton M, Pinto JR. Journal of Biological Chemistry. 2014; 289:23097–23111. [PubMed: 24973218]
159. Liang JS, Kazmierczak K, Rojas AI, Wang YC, Szczesna-Cordary D. Biomed Res Int. 2015:9.
160. Pinto JR, Parvatiyar MS, Jones MA, Liang JS, Ackerman MJ, Potter JD. Journal of Biological Chemistry. 2009; 284:19090–19100. [PubMed: 19439414]
161. Liang B, Chung F, Qu Y, Pavlov D, Gillis TE, Tikunova SB, Davis JP, Tibbits GF. Physiological Genomics. 2008; 33:257–266. [PubMed: 18285522]
162. Parvatiyar MS, Landstrom AP, Figueiredo-Freitas C, Potter JD, Ackerman MJ, Pinto JR. Journal of Biological Chemistry. 2012; 287:31845–31855. [PubMed: 22815480]
163. Parvatiyar MS, Pinto JR, Liang J, Potter JD. Journal of Biological Chemistry. 2010; 285:27785–27797. [PubMed: 20566645]
164. Nakaura H, Yanaga F, Ohtsuki I, Morimoto S. J Biochem. 1999; 126:457–460. [PubMed: 10467159]
165. Harada K, Potter JD. Journal of Biological Chemistry. 2004; 279:14488–14495. [PubMed: 14722098]
166. Harada K, Takahashi-Yanaga F, Minakami R, Morimoto S, Ohtsuki I. J Biochem. 2000; 127:263–268. [PubMed: 10731693]
167. Manning EP, Guinto PJ, Tardiff JC. Journal of Biological Chemistry. 2012; 287:14515–14523. [PubMed: 22334656]
168. Cuinto PJ, Manning EP, Schwartz SD, Tardiff JC. J Theor Comput Chem. 2007; 6:413–419. [PubMed: 26500385]
169. Haim TE, Dowell C, Diamanti T, Scheuer J, Tardiff JC. J Mol Cell Cardiol. 2007; 42:1098–1110. [PubMed: 17490679]
170. Chandra M, Tschirgi ML, Tardiff JC. Am J Physiol-Heart Circul Physiol. 2005; 289:H2112–H2119.
171. Schober T, Huke S, Venkataraman R, Gryshchenko O, Kryshtal D, Hwang HS, Baudenbacher FJ, Knollmann BC. Circul Res. 2012; 111:170–U120.
172. Hernandez OM, Szczesna-Cordary D, Knollmann BC, Miller T, Bell M, Zhao JJ, Sirenko SG, Diaz Z, Guzman G, Xu YY, Wang Y, Kerrick WGL, Potter JD. Journal of Biological Chemistry. 2005; 280:37183–37194. [PubMed: 16115869]
173. Hinkle A, Tobacman LS. Journal of Biological Chemistry. 2003; 278:506–513. [PubMed: 12409295]
174. Szczesna D, Zhang R, Zhao JJ, Jones M, Guzman G, Potter JD. Journal of Biological Chemistry. 2000; 275:624–630. [PubMed: 10617660]
175. Yanaga F, Morimoto S, Ohtsuki I. Journal of Biological Chemistry. 1999; 274:8806–8812. [PubMed: 10085122]
176. Moore RK, Abdullah S, Tardiff JC. Archives of Biochemistry and Biophysics. 2014; 552:21–28. [PubMed: 24480310]
177. Matsumoto F, Maeda K, Chatake T, Maeda Y, Fujiwara S. Biochem Biophys Res Commun. 2009; 382:205–209. [PubMed: 19275886]
178. Pinto JR, Reynaldo DP, Parvatiyar MS, Dweck D, Liang J, Jones MA, Sorenson MM, Potter JD. Journal of Biological Chemistry. 2011; 286:1005–1013. [PubMed: 21056975]
179. Li AY, Stevens CM, Liang B, Rayani K, Little S, Davis J, Tibbits GF. Plos One. 2013; 8:13.
180. Dweck D, Hus N, Potter JD. Journal of Biological Chemistry. 2008; 283:33119–33128. [PubMed: 18820258]
181. Schmidtmann A, Lindow C, Villard S, Heuser A, Mugge A, Gessner R, Granier C, Jaquet K. FEBS J. 2005; 272:6087–6097. [PubMed: 16302972]

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182. Pinto JR, Siegfried JD, Parvatiyar MS, Li DX, Norton N, Jones MA, Liang JS, Potter JD, Hershberger RE. Journal of Biological Chemistry. 2011; 286:34404–34412. [PubMed: 21832052]
183. Swindle N, Tikunova SB. Biochemistry. 2010; 49:4813–4820. [PubMed: 20459070]
184. Little SC, Biesiadecki BJ, Kilic A, Higgins RSD, Janssen PML, Davis JP. Journal of Biological Chemistry. 2012; 287:27930–27940. [PubMed: 22718768]
185. Cheng Y, Rao V, Tu Ay, Lindert S, Wang D, Oxenford L, Mc Culloch AD, Mc Cammon JA, Regnier M. Journal of Biological Chemistry. 2015; 290:27749–27766. [PubMed: 26391394]
186. Dong WJ, Xing J, Ouyang YX, An JL, Cheung HC. Journal of Biological Chemistry. 2008; 283:3424–3432. [PubMed: 18063575]
187. Wang D, Robertson IM, Li MX, Mc Cully ME, Crane ML, Luo Z, Tu AY, Daggett V, Sykes BD, Regnier M. Biochemistry. 2012; 51:4473–4487. [PubMed: 22591429]
188. Wang D, McCully ME, Luo Z, McMichael J, Tu AY, Daggett V, Regnier M. Archives of Biochemistry and Biophysics. 2013; 535:75.
189. Lindert S, Kekenes-Huskey PM, McCammon JA. Biophys J. 2012; 103:1784–1789. [PubMed: 23083722]
190. Kekenes-Huskey PM, Lindert S, McCammon JA. PLoS Comp Biol. 2012; 8:11.
191. Lindert S, Kekenes-Huskey P, McCammon JA. Biophys J. 2013; 104:331A–332A.
192. Jayasundar JJ, Xing J, Robinson JM, Cheung HC, Dong WJ. PLoS ONE. 2014; 9:e87135. [PubMed: 24558365]
193. Varughese JF, Baxley T, Chalovich JM, Li YM. J Phys Chem B. 2011; 115:2392–2400. [PubMed: 21332124]
194. Varughese JF, Li YM. Journal of Biomolecular Structure & Dynamics. 2011; 29:135.
195. Varughese JF, Chalovich JM, Li YM. Journal of Biomolecular Structure & Dynamics. 2010; 28:159–173. [PubMed: 20645650]
196. Cheng YH, Lindert S, Kekenes-Huskey P, Rao VS, Solaro RJ, Rosevear PR, Amaro R, McCulloch AD, McCammon JA, Regnier M. Biophys J. 2014; 107:1675–1685. [PubMed: 25296321]
197. Sadayappan S, Finley N, Howarth JW, Osinska H, Klevitsky R, Lorenz JN, Rosevear PR, Robbins J. The FASEB Journal. 2008; 22:1246–1257. [PubMed: 17984178]
198. Dong WJ, Chandra M, Xing J, She MD, Solaro RJ, Cheung HC. Biochemistry. 1997; 36:6754–6761. [PubMed: 9184157]
199. Heller WT, Finley NL, Dong WJ, Timmins P, Cheung HC, Rosevear PR, Trewhella J. Biochemistry. 2003; 42:7790–7800. [PubMed: 12820888]
200. Reiffert SU, Jaquet K, Heilmeyer LMG, Herberg FW. Biochemistry. 1998; 37:13516–13525. [PubMed: 9753437]
201. Rao VS, Korte FS, Razumova MV, Feest ER, Hsu H, Irving TC, Regnier M, Martyn DA. The Journal of Physiology. 2013; 591:475–490. [PubMed: 23129792]
202. Lindert S, Cheng YH, Kekenes-Huskey P, Regnier M, McCammon JA. Biophys J. 2015; 108:395–407. [PubMed: 25606687]
203. Feest ER, Korte FS, Tu AY, Dai J, Razumova MV, Murry CE, Regnier M. J Mol Cell Cardiol. 2014; 72:219–227. [PubMed: 24690333]
204. Korte FS, Feest ER, Razumova MV, Tu AY, Regnier M. Am J Physiol-Heart Circul Physiol. 2012; 303:H863–H870.
205. Kreutziger KL, Piroddi N, McMichael JT, Tesi C, Poggesi C, Regnier M. J Mol Cell Cardiol. 2011; 50:165–174. [PubMed: 21035455]
206. Longyear TJ, Turner MA, Davis JP, Lopez J, Biesiadecki B, Debold EP. J Appl Physiol. 2014; 116:1165–1174. [PubMed: 24651988]
207. Liu B, Lee RS, Biesiadecki BJ, Tikunova SB, Davis JP. Journal of Biological Chemistry. 2012; 287:20027–20036. [PubMed: 22511780]
208. Norman C, Rall JA, Tikunova SB, Davis JP. Am J Physiol-Heart Circul Physiol. 2007; 293:H2580–H2587.

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209. Alves ML, Dias FAL, Gaffin RD, Simon JN, Montminy EM, Biesiadecki BJ, Hinken AC, Warren CM, Utter MS, Davis RT, Sadayappan S, Robbins J, Wieczorek DF, Solaro RJ, Wolska BM. Circ-Cardiovasc Genet. 2014; 7:132–143. [PubMed: 24585742]

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Highlights

- cTn, one component of the thin filament, plays a central role of muscle regulation and contraction.
- During β-adrenergic stimulation, cTnI is phosphorylated by PKA at sites S23/S24 located in the cardiac-specific N-terminus.
- This phosphorylation weakens the interaction between cTnI and the N-terminus of cTnC, reduces the Ca^{2+} sensitivity (pCa_{50}) of tension production, increases crossbridge cycling kinetics and accelerates cardiac muscle cell relaxation.
- Mutations in cTnI that have been identified as associated with hypertrophic cardiomyopathy may disrupt some or all of these PKA mediated modulatory effects.
- For at least some HCM mutations, the “blunting” of effects that PKA-mediated phosphorylation on contraction and relaxation is associated with a loss of the ability of cTnI Ser-23/24 phosphorylation to reduce the affinity of cTnI for cTnC.

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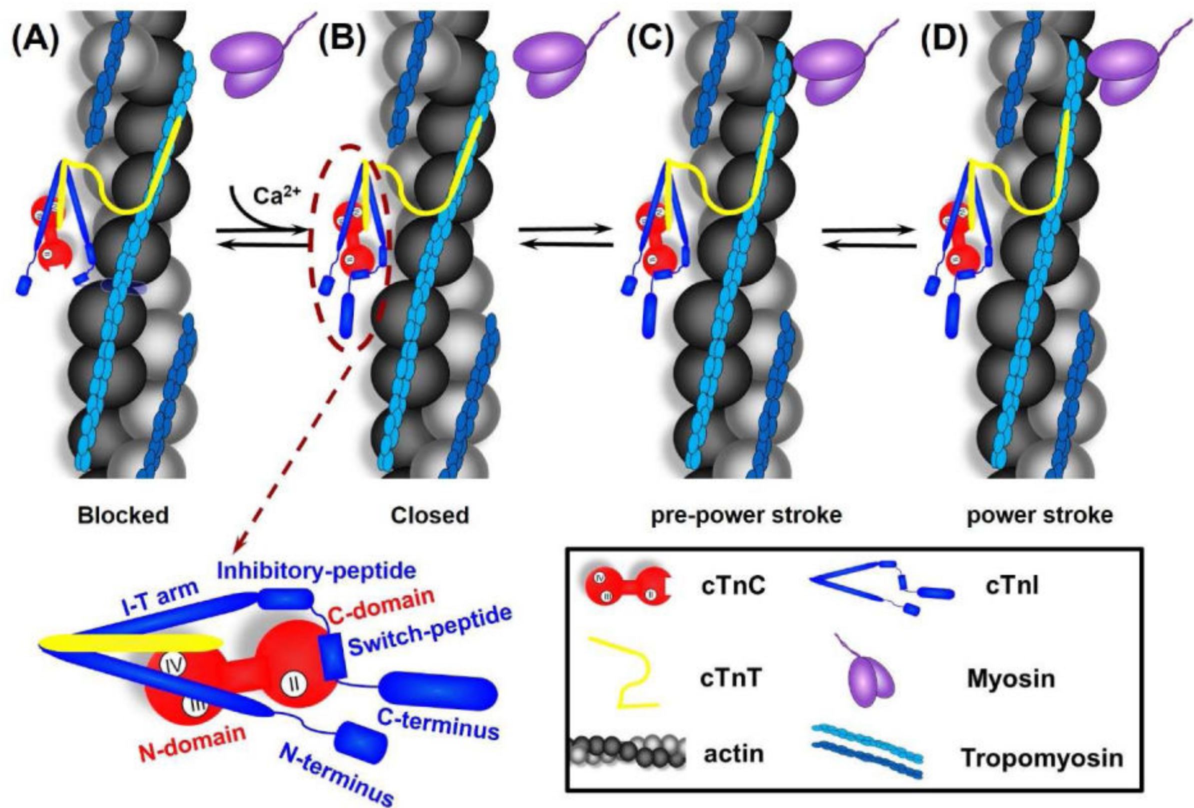

Figure 1.

Cartoon model of the signaling pathway for thin filament activation. From *left* to *right* are: (A) In diastole (in the absence of Ca^{2+}), cTnC exists in its "closed" conformation, and the inhibitory-peptide of cTnI binds actin tightly, inhibiting actin-myosin interaction. (B) In systole, Ca^{2+} binding to site II of cTnC induces an "open" conformation and increases interaction between NcTnC and cTnI switch-peptide, which pulls the adjacent inhibitory-peptide of cTnI from binding with actin. (C) Consequently, this leads to increased Tm mobility, exposes the myosin binding site on actin and myosin weakly interaction with actin (pre-power stroke), and (D) This weakly interaction further pushes Tm, and myosin strongly binds with actin, allowing the formation of cross-bridges and thus force generation (power stroke). The *left-bottom* is the close-up of cTn complex with the labeling of specific region of cTnC and cTnI.

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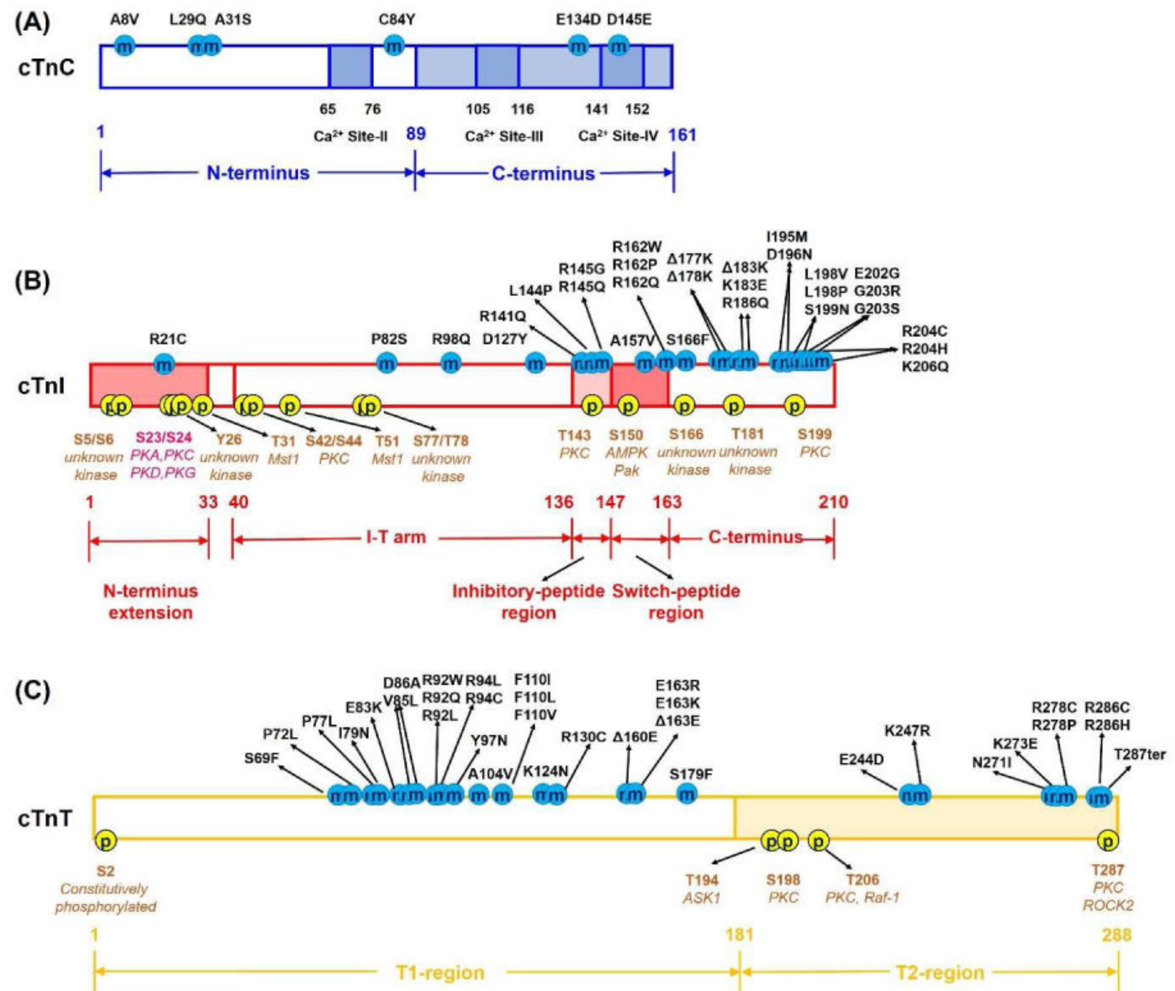

Figure 2.

The structural/functional domain and HCM-associated mutations in human (A) cTnC, (B) cTnI and (C) cTnT subunits. Here, the residue number of cTnI corresponds to that human sequence including Met-1.

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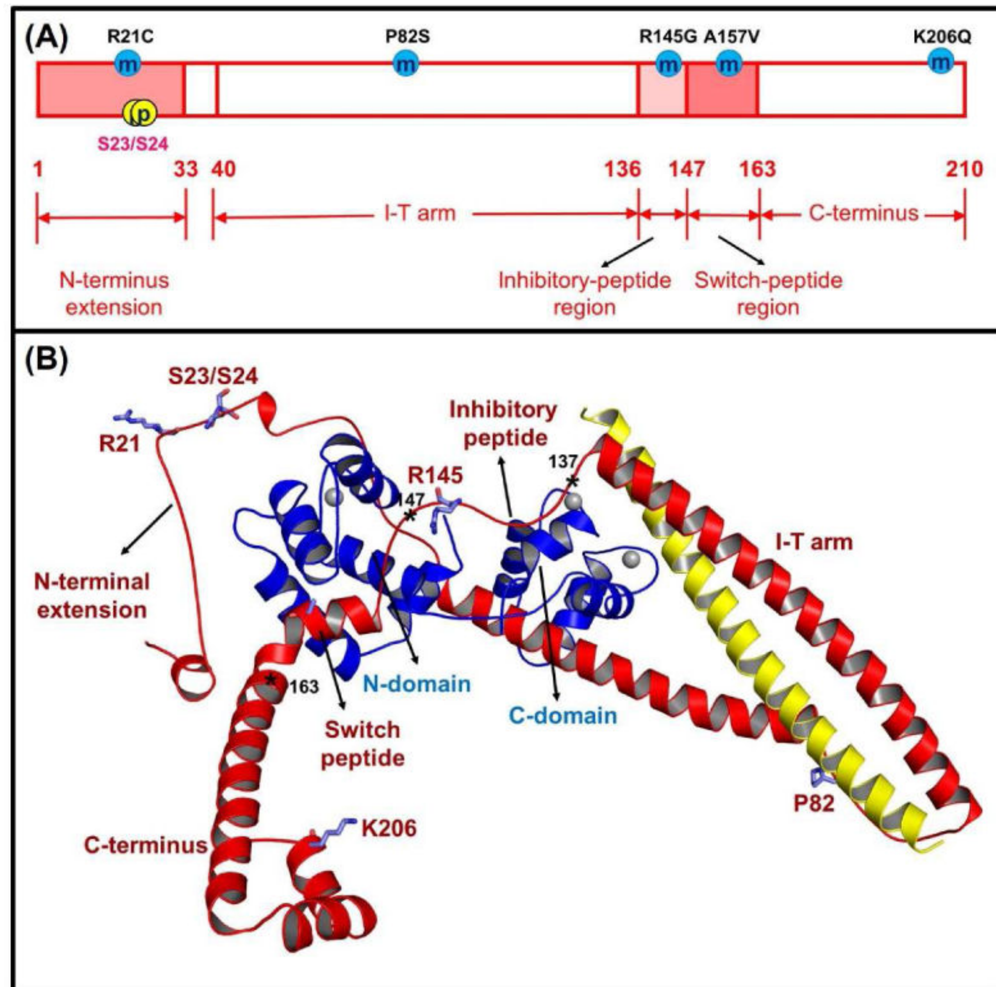

Figure 3.

Human cTnI (A) sequence and (B) ternary structure with the some HCM-related mutation sites located at different regions of cTnI (R21C, P82S, R145G, A157V and K206Q) and PKA phosphorylation sites (S23/S24). In the ternary structure, the cTnC (1-161) is shown in blue, the cTnI (1-210) is in red, and the cTnT (236-285) is in yellow. The asterisks indicate three key positions in human cTnI, for which residues 137-146 is the inhibitory-peptide region of cTnI, residues 147-163 is the switch-peptide region of cTnI, and residues 164-210 belong to the C-terminus of cTnI.