difference is greatly accentuated during acidosis, which is often associated with ischemia of myocardial infarction. *In vitro* and *in vivo* functional studies have shown that replacement of cTnI with ssTnI results in a marked enhancement of myofilament Ca^{2+}-sensitivity at acidic pH conditions. Recent reports have indicated that this effect can be ascribed to amino acid sequence differences in ssTnI and cTnI and in particular to a critical A162H substitution in the switch region. In this study, we have used NMR spectroscopy to examine the binding of the switch regions of ssTnI ($sTnI_{115-131}$) and cTnI ($cTnI_{147-163}$) to the N-domain of cardiac troponin-C (cNTnC) at physiological and acidic pH conditions. The results show that the affinity of $sTnI_{115-131}$ for cNTnC•Ca^{2+} (K_D ~50uM) is ~3-fold stronger than that of $cTnI_{147-163}$ (K_D ~150uM), but neither are affected by a pH change from 7 to 6. The pKa of H130 in $sTnI_{115-131}$ is 6.2 when free and 6.7 when bound to cNTnC•Ca^{2+}. We have also used {^{1}H, ^{15}N}-HSQC NMR spectroscopy to monitor the pKa changes of cNTnC•Ca^{2+} from peptide free to peptide bound states. The implications of these results will be discussed in the context of structure and function of myofilament protein interactions.

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Structure and Dynamics of Cardiac Troponin C using Paramagnetic Relaxation Enhancement Derived Distances

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We have prepared four spin-labeled single cysteine mutants of isolated cardiac TnC (cTnC) in order to examine the conformation of the Ca^{2+}-loaded *N*-domain and the interdomain dynamics using PRE-NMR. The long-range PRE distances (10 - 30 Å) measured within the regulatory *N*-domain were compared to TnC structures in the Protein Data Bank. Q-factor statistics were used to rank all available TnC PDB structures according to their agreement with our experimentally derived distances with scores ranging from 0.16 (best) to 0.30 (worst). The energy minimized solution structure of isolated human cTnC (1AJ4, Sia *et al*, JBC 1997) demonstrated the best correlation with our PRE data for the *N*-domain. Interdomain dynamics of our isolated cTnC were also examined by comparing PRE distances to available ensembles of TnC structures. Our results indicate that isolated cTnC is more compact than the skeletal TnC isoform, and that the central domain linker is highly flexible with a defined range of relative domain orientations. We also present a simple approach for modeling of the spin label position and mobility using PRE distances which is applicable to all spin-labeled systems for which there are existing structural models.

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Protein Kinase a Phosphorylation of Cardiac Troponin I Prevents Cardiac Hypertrophy in Mice

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R21C is the only FHC-associated mutation located in the N-terminal domain of cardiac TnI (cTnI) and is within the consensus sequence for PKA phosphorylation (RR_{21}RSS). We have developed an R21C cTnI knock-in (KI) mouse model and have evaluated the mouse hearts for biochemical, biophysical, structural and functional changes. Our results show that the R21C KI mice (heterozygous;R21C+/- and homozygous;R21C+/+) developed the FHC phenotype as evidenced by the presence of hypertrophy and fibrosis. Some hypertrophic markers such as, ANP, BNP and β-MHC were found elevated at a late age (18 months). The R21C+/- and R21C+/+ mice had decreased phosphorylation at Ser23/24 (~18% and 90%, respectively) compared to WT mice. Top down mass spectrometry of cTnI from the R21C+/- mice demonstrated a molar ratio of 1:4 R21C:WT in the hearts. Using three different methods to sacrifice the WT and mutant mice, we did not find any significant decrease in the Ca^{2+} sensitivity of force upon PKA treatment. Western blot analysis of these mice showed that the endogenous cTnI in the WT and R21C+/- mice is completely phosphorylated at Ser23/24. However, when mice were treated with propanolol (β-adrenergic receptor antagonist) before sacrifice, the Ca^{2+} sensitivity was decreased after PKA treatment of the WT (0.25 pCa) and R21C+/- mice (0.14 pCa). In contrast the R21C+/+ mice did not show a significant decrease in Ca^{2+} sensitivity after PKA treatment. No significant changes were found in the maximal force in all three mice, before and after PKA treatment. Our results suggest that the primary mechanism for producing hypertrophy in the R21C mice results from the impaired ability of the myofilament to respond to the desensitizing effects of PKA phosphorylation. Supported by NIH grant HL042325

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Structure and Dynamics of the Mobile Domain of Troponin I by SDSL-EPR

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The Troponin (Tn) molecular switch contains highly dynamic regions which allow for Ca^{2+} induced conformational changes to be propagated through to the thin filament. The Mobile domain (Md) of TnI, a secondary thin filament binding domain, is a key player in this process. The functional importance of the Md is also highlighted through the clustering of cardiomyopathy mutations. Structural elucidation of this region by traditional methods is often limited by the absence of key thin filament binding partners. Current Md models describe a highly dynamic region with either a nascent or a well-defined structure. We have utilized Site-Directed Spin Labeling Electron Paramagnetic Resonance (SDSL-EPR) to elucidate the structure of the Md upon interaction with the thin filament. EPR mobility measurements from cysteine scanning of the Md (res. 175-206) in the reconstituted thin filament show a highly dynamic domain in the $+Ca^{2+}$ (ON) state. A decrease in the mobility occurs in the $-Ca^{2+}$ (OFF) state, indicating interaction with the thin filament. Further, trends in the mobility of the EPR label reveal two helical structural components within the Md (res. 175-179 & 192-202). Conventional EPR methods were used to measure three interspin distances (176/178, 176/179 & 176/180) which further confirm this assignment. Double Electron-Electron Resonance (DEER) was used to measure the longer interspin distance (178/206) and found that the Md exists in an extended conformation (34 ± 26Å). An extended helical structural model for the interaction of the Md with the thin filament through electrostatic bonding is proposed. Residues involved in cardiomyopathy are found clustered at the interacting interface.

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Desensitizing Effect of N-terminal Truncated cTnI In RCM Myofibrils

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Cardiac TnI (cTnI) mutations have been associated with the development of restrictive cardiomyopathy (RCM) characterized by a Ca^{2+} hypersensitivity and diastolic dysfunction in cardiac myofibrils. Whereas cTnI N-terminal deletion (cTnI-ND) caused by restricted proteolysis in cardiac adaptation to stress manifests a lower left ventricular end diastolic pressure and an enhanced ventricular diastolic function. By crossing the RCM cTnI R193H transgenic mice ($cTnI^{193His}$) with cTnI-ND transgenic mice (cTnI-ND) that contain 100% cTnI-ND in the heart, we have obtained double TG mice containing both the cTnI R193H mutant and cTnI-ND. In this study, by using these TG mouse lines, we have investigated the desensitizing effect of cTnI-ND on the RCM cTnI mutant mice and myofibrils. Our survival data for these mice indicated that cTnI-ND greatly reduced the mortality of the RCM $cTnI^{193His}$ mice. Ca^{2+} sensitivity measured in skinned myofibrils confirmed that increased myofibril Ca^{2+} sensitivity was the major mechanism that resulted in impaired relaxation and diastolic dysfunction in RCM $cTnI^{193His}$ mice and that cTnI-ND could reverse the cellular dysfunction by desensitizing the myofibrils to Ca^{2+}. The PKA stimulation assays showed that $cTnI^{193His}$ myofibrils were able to respond to PKA activation, resulting in a right-shift of pCa curve after PKA treatment. However, since the myofibrils from cTnI-ND hearts lacked Ser residues 23 and 24, they had no response to PKA stimulation, showing a similar pCa curve before or after PKA. Our data have, for the first time, demonstrated a desensitizing effect by an endogenous myofibril protein proteolysis without the intervention of β-adrenergic stimulation mediated cTnI phosphorylation. The desensitizing function in cTnI-ND hearts indicates that the removal of cTnI N-terminal extension by restricted proteolysis represents a novel mechanism to improve myofibril relaxation and cardiac diastolic function in cardiac adaptation to hemodynamic and inotropic stresses.

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Effects of Pseudo-Phosphorylation of cTnI by P^{21} Activated Kinase-3 (PAK3) on Structure and Kinetics of Ca^{2+}-Induced Cardiac Thin Filament Regulation

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Residue Ser151 of rat cardiac troponin I (cTnI) can be phosphorylated by P21-activated kinase 3 (PAK3). It has been found that PAK3 phosphorylation of cTnI induces an increase in Ca^{2+} sensitivity of myofilament, but detailed mechanism is unknown. We investigated the structural and kinetic effects of phosphorylation of cTnI PAK3 site Ser151 on the Ca^{2+}-induced thin filament regulation. Using