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Structural Dynamics of Actin-Myosin Bound and Unbound States of Cardiac Myosin Binding Protein-C Detected by Dipolar EPR
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We have used site-directed spin labeling and pulsed dipolar electron-electron paramagnetic resonance (DEER) to resolve the structure and dynamics of flexible and disordered regions of myosin binding protein-C (MyBP-C)'s cardiac isoform, with implications for the pathophysiology of hypertrophic cardiomyopathy (HCM). N-terminal domains of cMyBP-C contain binding domains for several interaction partners in the myofilament, including myosin heavy chain subfragment 2 (S2) and actin. We engineered pairs of labeling sites in protein fragments of mouse cMyBP-C to measure with high resolution distance and disorder between (1) domains C0 and C1, flanking the flexible Pro/Ala-rich linker, and between (2) domains C1 and C2, flanking the partially disordered phosphorylation motif, using DEER. Changes in distance and disorder were assessed for double-Cys mutant cMyBP-C's free in solution and when bound to myosin S2 or actin, with or without cMyBP-C phosphorylation by protein kinase A (PKA). Understanding conformational transitions in the flexible and dynamic portions of cMyBP-C upon actin-myosin binding and phosphorylation provide new molecular insight into defining its modulatory role in muscle force development. (NIH-F32 to BAC; NIH-R01 to DDT)

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Obscurin: A New Player in Cardiac Hypertrophy
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Obscurins, encoded by the single OBSCN gene, comprise a family of giant (~890-810 kDa) and small (~550-50 kDa) proteins of vertebrate striated muscles composed of adhesion and signaling motifs. Giant obscurins intimately surround sarcomeres at the level of M-bands and Z-disks where they participate in the assembly, stabilization, and integration of the contractile cytoskeleton with other sarcoplasmic structures. Consistent with this, the immunoglobulin (Ig) domains 58 and 59 of obscurins interact directly with Ig domains 9 and 10 of titin located at the periphery of Z-disks. Genomic linkage analysis has recently revealed a missense mutation (R4344Q) in obscurin Ig58 that is causally linked to hypertrophic cardiomyopathy (HCM). To examine how the R4344Q mutation leads to the development of HCM, we generated two animal models: a knock-in model that contains full length obscurins carrying the R4344Q mutation, and a partial knock-out model that lacks Ig 58 and 59. Immunoblot and immunofluorescence analysis indicated that both mutant and truncated obscurins are readily expressed in cardiac muscles, and properly incorporated into sarcomeres. Homozygous partial knock-out mice developed overt cardiac hypertrophy by 12 months of age, as measured by echocardiography; notably, hypertrophy was exacerbated in the female homozygous animals. While a hypertrophic trend was apparent in homozygous knock-in animals, phenotypic and functional alterations of the affected hearts were not statistically significant from those of wild-type animals. Importantly, trans-aortic constriction of ~2 months old knock-in and partial knock-out male and female mice led to severe cardiac hypertrophy within 4-8 weeks post-surgery, as evaluated by echocardiography. We are currently examining the cellular and biochemical manifestations of mutant and truncated obscurins, as related to the development of HCM. Our studies provide the first in vivo models to study the molecular defects that underlie HCM due to altered obscurins.

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The HCM-Associated Cardiac Troponin T Mutation K280N Increases the Energetic Cost of Tension Generation in Human Cardiac Myofibrils
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A novel homozygous mutation in the *TNNT2* gene encoding cardiac troponin T (cTnT K280N) was identified in one HCM patient undergoing cardiac transplantation. mRNA and Mass Spectrometry analyses revealed expression of the mutant alleles without evidence of haploinsufficiency. Kinetics of contraction and relaxation of myofibrils from a frozen left ventricular sample of the K280N HCM patient were compared to those of "control" myofibrils (from donor hearts, from aortic stenosis patients, and from HCM patients negative for sarcomeric protein mutations). Preparations, mounted in a force recording apparatus (15 °C), were maximally Ca^{2+}-activated (pCa 4.5) and fully relaxed (pCa 9) by rapid (<10 ms) solution switching. The rate constant of active tension generation following maximal Ca^{2+} activation (k_{ACT}) was markedly faster in K280N myofibrils (ca. 1.7 s^{-1}) compared to controls (0.7-1 s^{-1}). The rate constant of isometric relaxation (slow k_{REL}) was 2-3-fold faster in K280N myofibrils than in controls, evidence that the apparent rate with which cross-bridges leave the force generating states is accelerated in the mutant preparations. The results suggest that the energy cost of tension generation is increased in the K280N sarcomeres. Simultaneous measurements of maximal isometric ATPase and Ca^{2+}-activated force in Triton-permeabilized left ventricular muscle strips from the K280N sample demonstrated that tension cost was much higher in the K280N than in control myocardium. Replacement of the mutant protein by exchange with wild-type recombinant human Tn in the K280N myofibrils reduced both k_{ACT} and slow k_{REL} close to control values. This indicates that the HCM-associated *TNNT2* mutation K280N primarily alters apparent cross-bridge kinetics and impairs sarcomere energetics. Supported by the 7th Framework Program of the European Union, "BIG-HEART" grant agreement 241577.

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Additive Compensatory Effects of Cardiac Troponin I and Cardiac Troponin T N-Terminal Truncations on the Disease Phenotypes of a Familial Hypertrophic Cardiomyopathy Mutation (E180G) of α-Tropomyosin
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A naturally occurring proteolytic N-terminal truncation of cardiac troponin I (cTnI-ND) has been shown to compensate for functional abnormalities caused by cardiomyopathic mutations. A proteolytic N-terminal truncation of cardiac TnT (cTnT-ND) elongates ventricular ejection time to compensate for cardiac output against high pressure load. To investigate the mechanisms for these troponin modifications to modulate the function of cardiac muscle thin filament, we produced transgenic mouse lines co-expressing cTnI-ND and/or cTnT-ND with a family hypertrophic cardiomyopathy mutation of cardiac α-tropomyosin (TM-E180G). The over expression of TM-E180G resulted in ~90% replacement of endogenous α-tropomyosin in the cardiac muscle with normal total stoichiometry. Functional studies in ex vivo working hearts of 2-month-old TM-E180G mice showed lower diastolic velocity and higher left ventricular diastolic pressure, indicating higher Ca^{2+} sensitivity. β-MHC expression together with myocardial fibrosis was found in the left ventricle of 28-day and 2-month-old TM-E180G mice with early failing phenotypes (lower stroke volume and lower systolic velocity). In double and triple transgenic mouse hearts, expression of cTnI-ND and/or cTnT-ND decreased the occurrence of β-MHC in TM-E180G mouse hearts. Functional studies showed cTnI-ND+cTnT-ND did not override the contractile phenotype of TM E180G mutation but had beneficial effects on improving stroke volume and reducing fibrosis, with additive effects in the triple transgenic hearts. While TM-E180G mice usually die between 4 and 5 months of age, cardiac function of TM-E180G+cTnI-ND+cTnT-ND triple transgenic mice remained apparently normal at 10-11 months of age as shown by the compensated heart function and cardiac efficiency with minimal β-MHC occurrence. These results demonstrated compensatory effects of posttranslational modifications of troponin on the functional abnormality of tropomyosin for potential applications in the treatment of heart failure.

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Effects of Cardiomyopathy-Related Troponin T Mutations on the Ubiquitin-Proteasome System
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Familial hypertrophic cardiomyopathy (FHC) is a disease of the myocardium that can be caused by a mutation in a sarcomeric gene. TNNT2 encodes cardiac troponin T (cTnT), a sarcomeric protein important for cardiac muscle contraction. Mutations in TNNT2 are frequently linked to sudden cardiac death (SCD); however their role in heart failure and cardiomyopathy is unclear. We hypothesize that changes in the ubiquitin-proteasome system (UPS) are related to increased risk of cardiac death, and that changes in the UPS may be related to calcium sensitization caused by TNNT2 mutations. We examined two mutations in TNNT2, R278C, which is associated with mild effects and late-onset heart disease, and the calcium-sensitizing mutation I79N, which is early-onset and has severe cardiac effects, including SCD. In 3-month-old transgenic mice expressing the I79N mutant form of troponin T, expression of the proteasome subunit PSMA6 was decreased by approximately 35% in I79N mice when