

32-Plat**Calcium-Induced Conformational Changes in the Cardiac Isoform of the Troponin Complex Monitored By Hydrogen/deuterium Exchange and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry**George M. Bou-Assaf^{1,2}, Jean E. Chamoun¹, Piotr G. Fajer^{1,2}, Mark R. Emmett², Alan G. Marshall^{1,2}.¹Florida State University, Tallahassee, FL, USA, ²National High Magnetic Field Laboratory, Tallahassee, FL, USA.

Calcium-induced conformational changes in the cardiac troponin complex have been determined from solvent accessibility data measured by solution-phase hydrogen/deuterium exchange (HDX). The troponin complex was digested with protease XIII and the peptides identified by FT-ICR mass spectrometry. To uniquely assign proteolytic fragments of the same mass, experiments were performed with troponin subunits isotopically depleted in ¹³C and ¹⁵N. For instance, the peptide with monoisotopic m/z = 592.98³⁺ could be either TnT fragment 76-91 or to TnI fragment 155-170, also known as the switch peptide. Isotopic depletion uniquely identified the TnT fragment.

Comparison of the HDX rates in the isolated subunits and those observed in the reconstituted complex identified inter-subunit interfaces, in general agreement with the x-ray structure of the core complex (Takeda *et al.* Nature, 2003 **424** (6944)). HDX experiments for the complex in the presence and in the absence of calcium revealed a multitude of conformational changes, some of which were not apparent from comparison of Ca-saturated and Ca-free x-ray structures of skeletal troponin (Vinogradova *et al.* PNAS, 2005 **102** (14)). For example, the C-lobe of TnC, which was not expected to change upon Ca binding, showed different HDX rates in the presence and in the absence of Ca for peptides 105-119 and 150-153. Similar effects were observed for residues 24-27 of TnC, but smaller differences were found for the TnI switch peptide (158-162), the N-terminus of TnI, and the IT coiled-coil. These observed changes will be discussed in the context of the cardiac and skeletal crystal structures. This work was supported by NIH (R01 GM78359), NSF Division of Materials Research through DMR-06-54118, and the State of Florida.

33-Plat**Structure of Troponin Complex: FRET and Molecular Dynamic Simulations**Jayant J. Jayasundar¹, Jun Xing¹, John M. Robinson², Herbert C. Cheung³, Wen-ji Dong¹.¹Washington State Univ, Pullman, WA, USA, ²University of Alabama at Birmingham, Birmingham, AL, USA, ³University of Alabama at Birmingham, Birmingham, AB, USA.

Cardiac troponin regulates the functioning of the heart. Ca²⁺ binding to cTnC and PKA-phosphorylation of the unique N-extension (Nxt) of cTnI play critical roles in regulation. However details of the Ca²⁺ and PKA phosphorylation signal transduction is still elusive. To acquire detailed structural information of troponin during activation/deactivation, we performed molecular dynamic simulations of the cardiac troponin complex combined with FRET distance measurements as restraints. Over one hundred inter-site distances were obtained from time-resolved FRET measurements at Ca²⁺-free and Ca²⁺-saturating conditions with/without PKA phosphorylation. These distance distributions were used as distance constraints during the simulations to resolve the detailed structure, particular the previously unresolved key functional regions of troponin. Results show that under Ca²⁺-free condition the Nxt of cTnI interacted with the Ca²⁺-binding sites on the N-domain of cTnC. The inhibitory region of cTnI and the D/E linker of cTnC were disordered. Upon Ca²⁺ binding in the absence of PKA phosphorylation, the Nxt of cTnI interacted with cTnC helix A and the cTnI mobile domain. The inhibitory region of cTnI and the D/E linker of cTnC become ordered. The results suggest that Ca²⁺-induced folding and unfolding of the D/E linker of troponin C, coupled with a change in orientation of the regulatory region of troponin I within the N-domain hydrophobic pocket of troponin C, regulates the dynamics of the inhibitory region of troponin I. When Ser23/24 was phosphorylated, the Nxt of cTnI moved away from the surface of the cTnC, perturbing the interaction between the Nxt and cTnC helix A, leading to significant unfolding of helices A and D of cTnC. The D/E linker of cTnC and the inhibitory region of cTnI became mobile. These finding shed new light onto the modulation role of PKA phosphorylation of cTnI on thin filament regulation.

34-Plat**Functional and Structural Changes Induced By cTNT-Related FHC Mutations in TNT1 Alter Actomyosin Binding Interactions**

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Familial Hypertrophic Cardiomyopathy (FHC) is a primary disease of the cardiac sarcomere. Many FHC mutations in hcTnT are found within the TNT1 do-

main, with a mutational hotspot at residues 160-163. These residues fall within a highly charged region (158-RREEENRR-166), which may create a flexible hinge necessary for function, the structure and function of which is affected by FHC mutations. We are investigating the effects of these hotspot mutations using in vitro motility (IVM) assays, SDSL-EPR, and transgenic mouse models. IVM data indicate that mutations Δ160E and E163R disrupt actin binding to heavy meromyosin under standard assay conditions. By reducing the ionic strength of the motility solutions, thin filament binding and sliding are restored suggesting that mutations in this region cause disease by disrupting the weak electrostatic interactions between the thin filament and myosin necessary for crossbridge formation. CW-EPR spectra show an increase in spin label isotropic rotational rate at hcTnT residue 153 (upstream of the putative hinge region) between Troponin ternary complexes containing Δ160E versus WT hcTnT, suggesting an increase in flexibility due to backbone changes induced by the mutation. We are expanding our SDSL-EPR experiments with additional cysteine substitutions superimposed onto 160-163 mutant proteins to provide further data regarding secondary structural changes imposed by these mutations. These results correspond with our Δ160E mouse model showing dose dependent myofilament disarray. Preliminary observations of an E163R model suggest that this mutant is less severely affected, tolerating a higher transgene dose. The structural and functional changes observed in vitro may contribute to the structural impairment seen in vivo. By correlating our IVM and SDSL-EPR findings with in vivo data generated from the Δ160E and E163R models, a mechanism of disease for these hotspot mutations can be determined.

35-Plat**PKA Phosphorylation Has No Effect on the Force-PCA Relationship or Length Dependent Activation Following L48Q cTnC-Tn Exchange in Rat Demembranated Trabeculae**

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The molecular mechanism behind the Frank-Starling law of the heart is thought to be due in part to changes in myofilament lattice spacing and/or increased myosin proximity to actin binding sites at longer sarcomere lengths (SL). We previously showed that cardiac trabeculae have a left-shifted force-pCa relationship and eliminated SL-dependence of this relationship following passive exchange with cTn containing a mutant (L48Q) cTnC (with enhanced TnC-TnI interaction strength). This result suggests L48Q cTnC reduces the cross-bridge dependent component of thin filament activation. Experiments here were designed to test the hypothesis that cTnI phosphorylation can restore SL dependence in L48Q cTnC exchanged trabeculae by decreasing Tn Ca²⁺ affinity and/or TnC-TnI interaction strength. Interestingly, PKA treatment did not reduce Ca²⁺ sensitivity of force or restore SL dependence in L48Q cTnC-cTn exchanged trabeculae. PKA treatment of native and WT cTn exchanged trabeculae right-shifted and also reduced SL dependence of the force-pCa relationship. Other laboratories investigating the effect of PKA on SL dependence have produced discordant results, possibly due to PKA-induced phosphorylation of proteins other than TnI (i.e., MyBP-C and titin). Therefore, planned future experiments will investigate exchange of PKA pre-treated cTn complexes, S23/24D (phosphomimic) cTnI, and S23/24A (non-phosphorylatable) cTnI with WT and L48Q cTnC. Experiments will be coupled with solution measurements cTnI-cTnC affinity using spectrofluorimetry to provide a more detailed understanding of how PKA phosphorylation and cTnI-cTnC interaction strength influence SL-dependence in cardiac muscle. Support provided by NIH HL65497 (MR) and T32 HL07828 (FSK).

36-Plat**The Effect of Hypertrophic Cardiomyopathy (HCM) Mutations of Tropomyosin on Force Generation and Cross-Bridge Kinetics in Thin-Filament Reconstituted Bovine Cardiac Muscle Fibers**Fan Bai¹, Adam Weis¹, Aya Takeda², Bryant Chase², Masataka Kawai¹.¹Department of Anatomy and Cell Biology, The University of Iowa, Iowa City, IA, USA, ²Department of Biological Science, Florida State University, Tallahassee, FL, USA.

Three HCM mutations (V95A, D175N and E180G) of Tropomyosin (Tm) were examined using the thin-filament extraction and reconstitution technique. Effects of Ca²⁺, ATP, phosphate and ADP concentrations on force and its transients were studied at 25°C and compared to WT. E180G showed larger isometric tension (1.89 ± 0.11) than WT (1.59 ± 0.08). Tension of two other mutants (V95A 1.53 ± 0.09, D175N 1.47 ± 0.07) was not different from WT. pCa₅₀ (Ca²⁺ sensitivity) of V95A (6.20 ± 0.06) and E180G (6.51 ± 0.02) was larger than WT (5.85 ± 0.03), while that of D175N (5.88 ± 0.05) remained the same. The cooperativity was reduced in all three mutants (V95A 1.70 ± 0.11, D175N 1.87 ± 0.09, E180G 1.91 ± 0.14) compared to WT (2.79 ± 0.25). Four equilibrium constants were deduced using sinusoidal analysis. The largest effect was on K_s (Pi association constant) which was reduced to ~0.5X in all