181-Plat
Impact of Familial Hypertrophic Cardiomyopathy-Linked Mutations in the N-Terminus of the Myosin Regulatory Light Chain on the Calcium Based Motility
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Calcium binding to the Regulatory Light Chain (RLC) has been shown to alter the structure and function of isolated RLC and when it is bound to the myosin heavy chain (MHC) in muscle. Furthermore, mutations associated with hypertrophic cardiomyopathy (FHC) have been shown to modify the affinity of the RLC for calcium binding (Szczesna et al., 2001). Here we studied the effect of calcium binding to the three N-terminal RLC mutants associated with FHC on the β-myosin mechano-chemistry using in vitro motility assays. To generate mutant β-myosin, native pig RLC was depleted from porcine cardiac MHC and reconstituted with the human cardiac WT (wild-type) or mutant (A13T, F18L & E22K) RLC. We measured the actin-activated myosin ATPase, actin sliding velocity and rotational stiffness of the mutant vs. WT myosin. Our results demonstrate that as pCa levels increased from 10 to 5.5, WT sliding velocity increased ~20% while two of the mutants (A13T and E22K) decreased ~10% respectively. Further increase in calcium to pCa 4 restored sliding velocity of all strains of RLC toward values observed at pCa 10. In contrast the actin-activated myosin ATPase of WT was lower ~20% as calcium was increased from pCa 10 to 5.5 while that of A13T and E22K was increased by ~5.5%. The velocity-pCa relationship concurs with the previously reported decreased calcium affinity values of the mutant RLC vs. WT (Szczesna et al., 2001). These results suggest that calcium binding to the RLC in the β-myosin can affect its contractile activity. The function of RLC as a calcium sensor allowing the heads to respond to the initial phases of activation and a greater strong cross bridge formation is largely compromised by FHC-linked RLC mutations.

182-Plat
Phosphorylation Modulates the Dynamics of the N-Terminal Tail in Cardiac RLC
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Background: Phosphorylation of the Regulatory Light Chain (RLC) in cardiac myosin has been shown to be critical for the normal heart performance. In particular, different cardiomyopathy-related mutations have been found to be associated with decreased levels of RLC phosphorylation. RLC is part of the myosin lever arm, whose power stroke is the result of the amplification of conformational changes occurring in the motor domain during the contractile cycle. Several findings indicate that RLC phosphorylation can have significant effects on myosin mechanics. However, the associated molecular events are still unknown.
Methods and Results: Here, we use computational methods to model the dynamics of human cardiac RLC bound to a fragment of myosin heavy chain (MyBS). Molecular Dynamics simulations are used to explore the space of conformations accessible to the N-terminal tail, which contains the RLC phosphorylation site. The conformational free energy landscape is reconstructed and the dynamic behaviour is analysed in terms of probability distributions of key residues of the tail around RLC and MyBS. Remarkably, simulations of phosphorylated RLC show significantly modified tail distributions, associated with a shifting in the population of the preferred tail conformations.
Results from simulations are compared with experimental NMR data and the effects of mutations implicated in the pathogenesis of hypertrophic cardiomyopathy (HCM) are discussed.
Conclusions: Molecular Dynamics simulations of cardiac RLC reveal phosphorylation-induced changes in the dynamics of the N-terminal tail and in its interaction with the rest of the molecule. These modifications can potentially regulate the interactions between RLC and other myosin components and interactors.
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Familial Hypertrophic Cardiomyopathy-Linked Mutation (K104E) in the Myosin Regulatory Light Chain Affects Sarcomeric Structure and Function in Tg-Mice
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Structural and functional effects of the familial hypertrophic cardiomyopathy (FHC)-linked mutation (Lysine 104-to-Glutamic Acid, K104E) in the human ventricular regulatory light chain (RLC) were studied in transgenic (Tg) mice. Small angle X-ray diffraction measurements on freshly skinned papillary muscle fibers demonstrated a mutation-induced increase in the interfilament lattice spacing at both short (2.3 μm) and long (2.5 μm) sarcomere lengths (SL) compared with Tg-WT. The intensity ratio (I1,1/I1,0) in WT fibers increased upon stretch (from SL=2.3 to SL=2.5 μm) indicating a shift in the cross-bridge mass distribution toward the thin filaments. In contrary, the K104E fibers demonstrated no change in I1,1/I1,0 on stretch. In line with X-ray data, K104E fibers developed higher passive tension at pCa 8 when consecutively stretched by 10%, 20%, 30% and 40% of their length compared to WT. Furthermore, the K104E mutation significantly decreased the endogenous RLC phosphorylation (~2-fold) compared to WT myocardium. Histopathological changes included signs of hypertrophy and severe fibrotic lesions in Tg-K104E hearts. The higher mitochondrial content seen in the left ventricles of K104E mice by electron microscopy was supported by significantly increased myosin ATPase activity in Tg-K104E mice indicating higher energy demand and ATP consumption by the mutant hearts. Studies using single molecule detection demonstrated significantly decreased cross-bridge dissociation rates with lower duty cycle (indicative of lower force production) in Tg-K104E cardiac myofibrils compared to WT. Our results in Tg-K104E mice demonstrated cardiomyocyte hypertrophy and fibrosis, and significant alterations in sarcomere structure and function. Morphological abnormalities, increased passive stiffness and a slower cross bridge dissociation rate in Tg-K104E mice mirror the diastolic dysfunction phenotype observed in K104E positive patients. Supported by AHA12PRE12030412 (to WH), and NIH HL-071778 and HL-108343 (to DSC).

Symposium: Cellular Stress, Protein Folding, and Disease

184-Symp
Spectroscopic Studies of Membrane Protein Folding: Changes in Hydration
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We have investigated the folding reaction of a model beta-barrel membrane protein, OmpA, using vibrational and electronic spectroscopy. Our primary focus is to interrogate changes in hydration during insertion and folding of the protein into the bilayer. The results suggest that expulsion of water and residual denaturant from the bilayer and/or protein core may give rise to the long-time component in folding kinetics. This finding is discussed in the context of in vitro mechanisms for membrane protein folding.

185-Symp
Protein Interactions and Transition Times that Influence the Pathogenesis of Protein Folding Diseases
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Protein folding diseases occur when a specific protein fails to fold into its correct functional state as a consequence of mutation in the protein amino acid sequence. In this talk, I present a model of the folded and misfolded protein expression, processing and their interactions, which we have used to investigate how protein folding disease phenotypes develop from mutated genotypes. Modeling protein processing as a continuous flow reactor, we found that the pathogenesis of protein folding diseases can be modulated by a combination of the transition time of folded and misfolded proteins in the reactor, the ratio of folded and misfolded protein inflow rates in the reactor and a chemical interaction parameter between folded and misfolded proteins. Our analysis reveals therapeutic strategies targeting the modulation of protein folding diseases, which have been recently explored in cellular and animal models of Mutant INS-gene Induced Diabetes of Youth and Congenital Hypothyroidism with deficient thyroglobulin.

186-Symp
Post-Translational Modifications Promote Formation of SOD1 Oligomers with Potential Toxicity in ALS
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Mutation of the ubiquitous cytosolic enzyme Cu/Zn superoxide dismutase (SOD1) causes a subset of cases of familial amyotrophic lateral sclerosis (FALS) through structural destabilization of SOD1, which leads to misfolding and aggregation. The non-heritable (sporadic) nature of most cases of ALS and