phosphorylation. Molecular modeling of cTnT-ΔK210 structure reveals changes in the electrostatic environment of cTnT helix (residues 203-224) that lead to a more basic environment around Thr^{203}, which enhances PKC-dependent phosphorylation. In addition, yeast two-hybrid assays indicate that cTnT-ΔK210 has enhanced binding to cTnI compared with cTnT-wt, and may impair Ca^{2+} sensing/transmission leading to myofilament desensitization. Collectively, our observations suggest that cardiomyopathy-causing ΔK210 has far-reaching effects influencing posttranslational modifications of key sarcomeric proteins, and potentially cTnI-cTnT interaction.

1828-Pos

Em and Single Particle Analysis of Troponin at Low and High Ca^{2+}

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Crystal structures of the troponin ''core-domain'' formed in the presence and absence of Ca^{2+} display a bilobed TnC subunit mounted on a semi-rigid scaffold formed from major stretches of TnI and TnT. A central coiled-coil of TnI and TnT is flanked by single TnI and TnT helices to form the W-shaped supporting structure, which appears to be little changed by the binding of Ca^{2+} (Takeda et al., 2003; Vinogradova et al., 2005). In contrast, at low Ca^{2+}, the central helix joining C- and N-lobes of TnC melts, and the ''regulatory'' C-terminal domain of TnI dissociates from the N-lobe of TnC (Vinogradova et al., 2005). Consistent with biochemical studies, the C-terminal TnI sequences in the thin filament are thought to latch onto actin and constrain tropomyosin in the blocked state at low-Ca^{2+}. Their dissociation from actin at high-Ca^{2+} and association with the N-terminal lobe of Ca^{2+}-saturated TnC may relieve the constraint (Galinksa-Rakoczy et al., 2008). These conclusions remain uncertain, however, because troponin is only semi-rigid (so crystal packing forces may have influenced the structure) and the troponin complex used for crystallization contained truncated subunits. Here we have studied isolated, intact troponin molecules using negative stain electron microscopy and single-particle image processing. Averaged projection views and 3D reconstructions of the isolated molecules show many of the same features seen in the crystal structures. Comparison of reconstructions of low and high Ca^{2+} data suggests that the TnC N-lobe of cardiac troponin may be further from the core domain in the EM than in the crystal structure.

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1829-Pos

Nanobiology of the Cardiac Myofilament

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The cardiac myofilament is a protein assembly that provides Ca-regulated force development enabling the heart to undergo alternating periods of contraction and relaxation. Troponin (Tn), a three-member protein assembly within the myofilament, acts as a Ca-sensitive switch. Here, using single pair FRET in freely diffusing assemblies of Tn, we show that Tn incompletely activates after binding regulatory Ca. The reserved population of inactive Tn appears to function as a nanoscopic form of cardiac reserve that can be can be manipulated by cell signaling mechanisms to fine-tune cardiac contractility. The results are discussed in terms of an energetic model of the cardiac myofilament.

1830-Pos

Investigating the Effect of Cardiomyopathy-Causing Mutations in Cardiac Troponin-T on Calcium Buffering *In Situ*

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In vitro investigation of the effects of cardiomyopathy-causing mutations in thin filament regulatory proteins has demonstrated that hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are caused by distinct primary alterations of cardiac contractility and myofilament calcium affinity. We hypothesise that chronically altered calcium-buffering by mutant thin filaments leads to altered calcium handling and, via calcium-dependent signalling pathways, contributes to disease pathogenesis. We aim to study the *in situ* effect on calcium flux of a HCM and a DCM causing mutation in human cardiac troponin T (cTnT) (R92Q, R131W respectively), by adenoviral mediated expression of mutant protein in adult guinea pig cardiomyocytes. The adenoviral vectors co-express GFP and western blot analysis of FACS-sorted, GFP-expressing cells showed that recombinant cTnT comprised 45-50% of the total cTnT in these cardiomyocytes, 48 hours after infection. Analysis of unloaded sarcomere shortening showed that at an excitation frequency of 2 Hertz, cardiomyocytes infected with R131W cTnT elongated the time to 50% relaxation and reduced the magnitude of contraction, whilst R92Q cTnT reduced the time to 50% relaxation and increased the contractile magnitude compared to wild type. Analysis of calcium transients of the same cells using fura-2 loading, indicates that the R92Q mutation reduces calcium transient amplitude, whilst the R131W mutation increases the time to complete calcium reuptake, with no change to the transient amplitude, despite the observed decrease in contraction. We are currently assessing the caffeine transients of these cells to investigate alterations to overall SR load and measuring alterations to components of calcium-dependent signalling cascades which may link the acute effects of cTnT mutations to macroscopic remodelling observed in the pathological disease states of HCM and DCM.

1831-Pos

The Small Molecule Smooth Muscle Myosin Inhibitor, CK-2018571, Selectively Inhibits ATP Hydrolysis and Relaxes Smooth Muscle *In Vitro*

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Smooth muscle contraction is driven by cyclical, nucleotide-dependent changes in myosin conformation that alter its affinity for actin, produce force, and generate movement. We used a high throughput screen to identify compounds that inhibit the ATPase activity of smooth muscle myosin; optimization of the initial hit compounds has resulted in compounds with nanomolar affinity. A potent representative of this chemical series, CK-2018571, inhibits the steady-state ATPase activity of human smooth muscle myosin at low nanomolar concentrations, approximately 10-fold lower than are required to inhibit non-muscle myosin, the most closely related myosin II. Selectivity between smooth and striated myosin IIs are >100-fold. Transient kinetic studies demonstrate that CK-2018571 inhibits the myosin-catalyzed hydrolysis of the γ-phosphate group of ATP, with no effect on nucleotide binding or release from the enzyme. Actin co-sedimentation assays indicate that CK-2018571 stabilizes a weak actin-binding conformation of myosin in the presence of ATP. Consistent with this mechanism, CK-2018571 relaxes skinned rat tail artery muscle tissue at low micromolar concentrations. Importantly, this relaxation occurs regardless of whether the skinned muscle has been activated by calcium or by thiophosphorylation of the myosin regulatory light chain, supporting evidence that CK-2018571 relaxes smooth muscle tissue by direct inhibition of activated smooth muscle myosin. The ability of CK-2018571 to relax intact tracheal smooth muscle and aortic ring preparations at micromolar concentrations suggests this mechanism may prove useful in diseases of smooth muscle hypercontractility, such as hypertension and asthma.

1832-Pos

Direct Interaction between the C-terminus of the Myosin Light Chain Phosphatase Targeting Subunit and Myosin Phosphatase-Rho Interacting Protein

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Both the Ca^{2+} signal and the alteration of the Ca^{2+} sensitivity of the contractile apparatus regulate smooth muscle contraction. Myosin light chain kinase (MLCK) phosphorylated the 20 kDa regulatory myosin light chain (MLC20) resulting in contraction. Myosin light chain phosphatase (MLCP) dephosphorylates MLC20 causing relaxation. Thus, the balance between the activities of MLCK and MLCP determines the level of MLC20 phosphorylation.

MLCP consists of a 38 kDa catalytic subunit (PP1cδ), a 110 kDa targeting subunit (MYPT1), and a 21 kDa small subunit (M21). MYPT1 provides the substrate specificity and the regulation of phosphatase activity. It was reported that myosin phosphatase-Rho interacting protein (M-RIP) bound MYPT1 and thus targeted MLCP to the actomyosin contractile filament based on yeast-two hybrid and cell biological assays.

To determine if MYPT1 binds to M-RIP directly, we performed analytical ultracentrifugation (AUC) study using purified peptides of MYPT1 and M-RIP.