lipid order parameters due to the presence of rhodopsin were much larger in cholesterol-containing membranes than in cholesterol-free membranes suggesting strongly that the perturbation in the lipid matrix from protein insertion reaches much further away from the protein. This is expected for membranes that are stiffer due to the presence of cholesterol. The consequences of these findings for lipid mediated shifts in rhodopsin function and rhodopsin-rhodopsin interactions will be discussed.

**Platform: Cardiac Muscle: Myosin Binding Proteins**

2207-Plat Cardiac Myosin Binding Protein-C Slows Cross-Bridge Kinetics and Increases MgATP Sensitivity

Bertrand C.W. Tanner1, Yuan Wang2, Jeffrey Robbins2, Bradley M. Palmer3.

1University of Vermont, Burlington, VT, USA, 2Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA.

Cardiac myosin binding protein-C (cMyBP-C) is a thick filament protein that augments filament stiffness and modulates cardiac contractility through its phosphorylation. We investigated the effect of cMyBP-C on myosin cross-bridge kinetics in demembranated papillary muscle strips from nontransgenic (NTG) and transgenic homozygous mice lacking cMyBP-C (t/t). Both groups expressed ~100% β-myosin heavy chain isoform due to hypothyroidism. Papillary muscle cross-bridge perturbation analysis at maximal Ca²⁺-activation (pCa 4.8 at 17°C, 0 mM P) showed that MgATP-dependent myosin detachment rate (g) increased hyperbolically with MgATP (0.025 to 5 mM), with saturating values of 2.5±0.2 and 3.9±0.2 s⁻¹ (P<0.01) for NTG and t/t, respectively. Kinetic analysis of g vs. [MgATP] resulted in a lower [MgATP]₅₀ for NTG than t/t (187±45 vs. 361±58 μM, P<0.05). As myosin attachment time (tₐₐₐ) is inversely related to g, tₐₐₐ correspondingly decreased as [MgATP] increased for both groups. Saturation values of 71.3±5.0 and 44.2±2.3 ms (P<0.01) for NTG vs. t/t, respectively. At saturating MgATP, tₐₐₐ decreased as [P] increased in both groups (0-12 mM), although tₐₐₐ remained ~25% longer at each [P] for NTG vs. t/t. Protein kinase-A (PKA) incubation reduced tₐₐₐ for t/t. The longer tₐₐₐ for NTG at saturating MgATP suggests a longer lived MgADP myosin cross-bridge state when cMyBP-C is present. The lower [MgATP]₅₀ for NTG further suggests that cMyBP-C elevates the nucleotide binding pocket affinity for MgATP in addition to MgADP. cMyBP-C phosphorylation by PKA may diminish this affinity resulting in a shorter tₐₐₐ. These results support a functional role for cMyBP-C slowing myosin kinetics, possibly through altered strain-dependent myosin kinetics due to cMyBP-C supporting a stiffness of the thick filament or myofilament lattice that is diminished with PKA phosphorylation of cMyBP-C.

2208-Plat Activation of Cardiac Thin Filaments by N-Terminal Domains of Cardiac Myosin Binding Protein C

Howard D. White1, Mohanitha Harris1.

1Eastern Virginia Medical School, Norfolk, VA, USA, 2University of California, Davis, CA, USA.

We have used double mixing stopped-flow fluorescence to study the effect of the soluble N-terminal C1C2 domain of cMyBP-C on the activation of product dissociation from myosin-S1-mdADP-Pi by native porcine thin filaments. Product dissociation from cardiac actomyosin is an ordered process in which phosphate dissociation is rate limiting (AM-mdADP-Pi → AM-mdADP → AM) and can therefore be measured by the rate of mdADP dissociation. Myosin-S1 was mixed with an equal concentration of the fluorescent ATP anologue, mdATP, and held in a delay line 2 seconds for the nucleotide to bind and be hydrolyzed (M+mdATP → M-mdATP → M). The mixture was subsequently mixed with native cardiac thin filaments, 1 mM MgATP, 1 mM EGTA and expressed mouse cMyBP-C domains C1C2. In control experiments, cMyBP-C was either replaced with NEM-S1 (a catalytically inactive form of myosin-S1, modified with N-ethylmaleimide, which binds to actin in the absence of ATP), rigor myosin-S1 (or the absence of ATP) or 0.1 mM CaCl₂. Maximal activation of the rate of product dissociation by cMyBP-C domains C1C2 is the same as observed with saturating rigor- myosin-S1 or 0.1 mM calcium and greater than observed with NEM-S1. These experiments were also performed with physiological ionic strength (0.18 M KAc, 10 mM Mops, 2 mM MgCl₂, pH 7) to eliminate non specific ionic interaction that might occur at lower ionic strength. Cardiac cMyBP-C domains C1C2 not only bind to actin at a similar position to rigor myosin-S1 but substoichiometric concentrations (1 cMyBP-C C1C2 per 7 actin subunits) are as effective as rigor myosin-S1 at activating cardiac thin filament in the absence of calcium.

2209-Plat Effects of Myosin Binding Protein-C Isoform, Phosphorylation, and Domains on the Rotational Dynamics of Actin Filaments

Brett A. Colson1, Iima N. Rybakova2, Richard L. Moss2, Eva Prochniewicz2, David D. Thomas3.

1University of Minnesota, Minneapolis, MN, USA, 2University of Wisconsin School of Medicine & Public Health, Madison, WI, USA.

We have determined the effects of Myosin binding protein-C (MyBP-C) and its domains, the quantitative time-scale rotational dynamics of actin, using time-resolved phosphorescence anisotropy (TPA). MyBP-C is a multi-domain thick filament-associate protein of striated muscle contraction, spanning the filament spacing to contact both myosin and actin. Cardiac (c-) and slow skeletal (ss-) MyBP-C are known substrates for Protein kinase-A (PKA), and phosphorylation of cMyBP-C alters contractile properties and myofilament structure. To determine the effects of MyBP-C on actin’s microdynamic rotational dynamics, we labeled actin at C374 with either iodoacetamide and performed TPA experiments. The interaction of all three MyBP-C isoforms with actin increased the final anisotropy (τₐₐₐ) of the TPA decay in a concentration-dependent manner, indicating restriction of the rotational amplitude of actin dynamics. While PKA phosphorylation had little effect on fast skeletal (fs-) MyBP-C, phosphorylation of cMyBP-C and ssMyBP-C nearly eliminated the effects of these proteins to restrict actin dynamics, despite no change in myosin affinity with phosphorylation. Skeletal MyBP-C (C1-C10) affected actin anisotropy at lower concentrations than cMyBP-C (C0-C10), suggesting that skeletal and cardiac N-terminal MyBP-C interactions with actin have distinct properties. The effects of truncated cMyBP-C on actin anisotropy determined that C-terminal domains are important for restricting rotational dynamics, whereas N-terminal domains are important for regulating this effect. These MyBP-C-induced changes in actin dynamics may play a role in the known effects of MyBP-C on the functional actin-myosin interaction. This work was funded by grants from NIH (F32 HL107039-01 to BAC and T32 AR007612 to DDT).

2210-Plat Myosin Binding Protein-C Slow: An Intricate Subfamily of Phosphoproteins

Maegen Ackermann, Jane Valenti, Aikaterini Kontogianni-Kanitopoulos.

University of Maryland, Baltimore, MD, USA.

Myosin Binding Protein-C (MyBP-C), a family of accessory proteins of striated muscles, contributes to the assembly and stabilization of thick filaments, and regulates the formation of actomyosin cross-bridges. Three distinct MyBP-C isoforms have been characterized; cardiac, slow skeletal and fast skeletal. The slow isoform, sMyBP-C, consists of four alternatively spliced forms, variants 1-4 (v1-4) that share common structures and sequences. Variants 1-4 differ, however, in three regions; v1 and v2 contain a 25-residue long insertion within the N2H2-terminus, v3 carries an 18-amino acid long segment within immunoglobulin domain C10 and v4 contains a unique C1H2-terminus consisting of 26 amino acids, while v4 does not possess any of these insertions. Variants 1-4 are expressed in variable amounts among skeletal muscles, exhibiting different topographies and potentially distinct functions. To date, the regulatory mechanisms that modulate the activities of sMyBP-C are unknown. Using an array of proteomic approaches, we show that sMyBP-C comprises a family of phosphoproteins that are substrates for PKA and PKC. The levels of phosphorylated skeletal MyBP-C proteins (i.e. slow and fast) are increased in mouse dystrophic muscles, although their overall amounts are decreased. In vitro binding and motility assays indicate that sMyBP-C modulates the formation of actomyosin cross-bridges through interactions with both actin and myosin. Importantly, the presence of the novel insertions differentially affects the ability of sMyBP-C variants 1-4 to modulate cross-bridges formation. In summary, our studies are the first to show that sMyBP-C comprises a subfamily of phosphoproteins that differentially regulate actomyosin crossbridges.

2211-Plat Identification and Characterization of a New Phosphorylation Site on Cardiac Myosin Binding Protein C

Diederik W.D. Kuster1, Steven B. Marston2, Cris G. dos Remedios3, Jeroen A.A. Demmers4, Jolanda van der Velden1.

1Dept of Physiology, VU University Medical Center, Institute for Cardiovascular Research, Amsterdam, Netherlands, 2Cardiovascular Science, National Heart and Lung Institute, Imperial College London, London, United Kingdom, 3Muscle Research Unit, Bosch Institute, University of Sydney, Sydney, Australia, 4Proteomics Centre, Erasmus University Medical Centre, Rotterdam, Netherlands.

During recent years it has become increasingly evident that cardiac myosin binding protein C (cMyBP-C) exerts an important role in regulation of