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Myofilament Modulation of Contraction

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The interaction of myosin with actin is necessary to the development of force and striated muscle contraction. Whether in skeletal or cardiac striated muscles, the number of myosin molecules that are able to bind to actin is therefore critical to the development of muscle force and modulating these numbers is essential to match the contractile needs of the body to its everyday tasks. While increased intracellular calcium is required to initiate muscle contraction and can be altered to indirectly modulate muscle contraction, it is the myofilament regulatory proteins that respond to this increased calcium to directly regulate the number of myosin molecules that can bind actin and thus contractile force development. Since the identification of the myofilament regulatory proteins tropomyosin, the troponin complex (Tn), myosin binding protein C (MyBP-C) and the myosin regulatory light chain (RLC), much has been learned about the structure-function of these myofilament regulatory proteins and their role in modulating contraction, however significant questions regarding the molecular mechanism of myofilament contractile modulation remain. This Highlight Issue of *Achieves of Biochemistry and Biophysics* brings together of a broad spectrum of both reviews and original research works that further the current understanding of the diverse molecular mechanisms involved in the modulation of striated muscle contraction through the myofilament regulatory proteins.

Reviews in this Highlight Issue summarize current knowledge regarding the role of the Tn regulatory complex to translate the initiating calcium signal into force and its function in modulating muscle contraction. The review by **Jonathan Davis** and colleagues summarizes the role of calcium as a second messenger signaling molecule and the calcium binding motifs that the downstream sensing proteins employ to respond to this signal by binding calcium (1). This understanding is applied to discuss current knowledge of how the Tn calcium sensor is involved in the myofilament regulation of muscle contraction with a focus on the calcium binding subunit of the Tn complex troponin C (TnC). Finally, the review addresses current state of the art approaches to engineer the TnC calcium binding EF-hand motif as a mechanism to modulate muscle contraction. The review from **Michael Regnier** and colleague summarizes the calcium regulated structure-function of the Tn complex with a focus on the role of the Tn inhibitory subunit (TnI) (2). This review discusses current knowledge of how protein kinase A (PKA) mediated phosphorylation signaling to TnI and TnI hypertrophic cardiomyopathy associated mutations affect the modulation of cardiac muscle contraction. Finally, the review addresses current data supporting a significant role for the TnC-TnI interaction as a myofilament mechanism to modulate cardiac contraction.

Upon physiological or pathological increase in body blood demand the heart responds by initiating signaling pathways that result in the modulation of cardiac muscle contraction to match this new demand. Towards better understanding the molecular mechanisms involved in this demand induced modulation of cardiac contraction original works in this Highlight Issue focus on defining the signaling induced myofilament modifications responsible for altered cardiac contraction. Work from the laboratory of **Kerry McDonald** investigated the molecular mechanisms of beta-adrenergic induced PKA mediated myofilament phosphorylation to increase cardiac contraction (3). Results from *in vivo* heart experiments demonstrated that beta-adrenergic induced phosphorylation of both TnI and MyBP-C correlated with increased levels of ventricular power. Experiments measuring the power of skinned muscles with manipulated myofilament PKA phosphorylation further demonstrated TnI phosphorylation resulted in increased cooperativity, while MyBP-C phosphorylation increased cross-bridge cycling. These findings support a combined molecular role for both TnI and MyBP-C to augment cardiac contractile function in response to beta-adrenergic stimulation through these respective mechanisms. Work from the laboratory of **John Konhilas** investigated the role of the Liver Kinase B1 (LKB1) complex localization to the myofilament to modulate contraction (4). Experiments in cardiac muscle demonstrated that energetic stress induced LKB1 association with the myofilament to decrease calcium sensitive force production. These findings support an energy sensing role of LKB1 induced myofilament phosphorylation to modulate cardiac contraction. The use of phosphorylated residue substitution has been critical to understanding the molecular mechanism of myofilament protein phosphorylation to modulate contractile. Work from the laboratory of **Margaret Westfall** sought to develop a novel phosphorylation null substitution for TnI residues Ser-43 and Ser-45 that did not affect contractile function as noted with traditional null substitutions (5). Adenoviral expression in primary cardiomyocytes demonstrated the myofilament expression of TnI S43/45N did not alter intact myocyte calcium, or contractile function. These findings identify the TnI S43/45N phosphorylation null substitution as a significant tool to investigate the myofilament effects of protein kinase C (PKC) mediated signaling on cardiac contraction.

The length-tension relationship is a basic property of striated muscle to increase force development upon increased sarcomere length. The molecular mechanisms responsible for this increased force have not been fully elucidated to date. To further identify the mechanisms that contribute to length dependent changes in muscle contraction a collection of original works in this Highlight Issue investigated the myofilament mechanisms involved in the length tension relationship. Work from the laboratory of **Paul Janssen** investigated the length-dependent effects of PKA or PKC signaling on myosin cross-bridge cycling kinetics (6). Intact human cardiac trabeculae force development measurements demonstrated that the presence of PKA or PKC inhibitors did not alter length dependent myosin cross-bridge cycling. These data suggest that the rapid length-dependent effects on myosin cycling kinetics result from intrinsic properties of the myofilament rather than PKA or PKC signaling mediated phosphorylation. Work from the laboratory of **Bertrand Tanner** investigated the effects of length on myosin RLC phosphorylation regulated contractile function (7). Measurements in skinned cardiac muscle preparations demonstrated both RLC phosphorylation and sarcomere length increased calcium sensitivity and decreased the cross-

bridge cycling rate, however the effect of RLC phosphorylation to slow the rate of cross-bridge MgATP binding was greater at increased sarcomere lengths. These findings support a role for the length dependent effects of RLC phosphorylation as a myofilament mechanism to match contractile kinetics across the ventricular wall to demand. Work from the laboratory of **Wenji Dong** investigated the structure-function regulatory effects of the TnI C-terminal domain in response to altered calcium, cross-bridge and sarcomere lengths (8). Simultaneous measurement of TnI-TnC structure and force development in skinned cardiac muscle preparations demonstrated that calcium, cross-bridges and length all have different effects on the structural transition of the TnI C-terminal domain, with the effect of cross-bridges and length imparting the most significant changes in the absence of calcium. These data support the significance of the TnI C-terminal domain in length dependent myofilament contractile regulation of muscle.

The role of the TnI inhibitory peptide in Tn mediated calcium regulation of contraction has long been established, however it has recently become clear that additional domains of TnI also contribute to the myofilament regulation of contraction. A collection of original works in this Highlight Issue further investigated the molecular role of the TnI C-terminus in contractile regulation. Work from the laboratory of **Bryant Chase** sought to investigate the structure-function contribution of the TnI C-terminus in Tn mediated contractile regulation (9). Employing recombinant TnI molecules in the *in vitro* motility assay results demonstrated that entirely removing or disrupting the structure of the TnI C-terminal mobile domain resulted an increased calcium sensitivity of motility. Additionally, deletion of the mobile domain further resulted in the inability to fully inhibit filament sliding in the absence of calcium. These findings support the TnI mobile domain as significant in calcium regulation of the myofilament and necessary for full inhibition of contraction in the absence of calcium. Work from the laboratory of **Aldrin Gomes** investigated the significance of the last 5 TnI C-terminal residues to the myofilament regulation of contraction (10). Removal of the last 3 or last 5 TnI C-terminal residues resulted in decreased TnI binding to TnC and troponin T (TnT) as determined by two-hybrid studies. Troponin calcium binding experiments further demonstrated that while only the removal of the last 5 residues increased calcium sensitivity, removal of the last 1, 3 or 5 residues all increased calcium dependent motility in the *in vitro* motility assay. These findings support the significance of the last 5 TnI C-terminal residues in the myofilament regulation of contraction.

Functional characterization of cardiomyopathy linked point mutations in myofilament proteins has served as a significant tool to provide vital information regarding the structure-function role of these proteins in the modulation of muscle contraction. A collection of original works in this Highlight Issue has therefore focused on the structure-function effects of myofilament protein mutations linked to cardiomyopathy. Work from the laboratory of **Jose Pinto** sought to identify the mechanism by which the cardiomyopathy linked A8V TnC mutation results in increased myofilament calcium sensitivity (11). Results demonstrated that although the TnC A8V mutation does not directly alter calcium binding to isolated TnC, the measurement of TnC-TnI binding and modeling identified the A8V mutation sensitized the myofilament to calcium by increasing the affinity of the TnI C-terminus for TnC. These findings support the TnC A8V mutation disrupts muscle function by altering the TnC-TnI

interaction and provide further support for the significance of this interaction in the myofilament regulation of cardiac contraction. Work from the laboratory of **Murali Chandra** sought to investigate the myofilament mechanism responsible for differential phenotypes observed for the TnT R97L hypertrophic cardiomyopathy linked mutation (12). Mechanic measurements of R97L TnT muscles containing α - or β -myosin heavy chain (MHC) demonstrated that while the R97L mutation increased calcium sensitivity similarly in the presence of both the α - or β -MHC, cross-bridge recruitment was dependent upon the MCH isoform co-expressed. These findings support the significance of TnT in the regulation of myosin cross-bridge formation and therefore the myofilament regulation of contraction. Work from the laboratory of **Andrew Messer** sought to investigate if there was a common mechanism involved in the contractile dysfunction of 7 cardiomyopathy linked TnT mutations (13). Experiments employing purified and reconstituted Tn in the *in vitro* motility assay demonstrated that these 7 hypertrophic cardiomyopathy linked TnT mutations all increase calcium sensitivity and were also not responsive to TnI phosphorylation mediated calcium desensitization. These findings support a role for the TnT mutation induced inhibition of TnI phosphorylation desensitization in the cardiomyopathy phenotype and the significance of disrupting TnI phosphorylation to cardiac contractile function. Work from the laboratory of **Danuta Szczesna-Cordary** sought to determine the mechanism for myosin RLC mutation linked cardiomyopathy by investigating altered gene expression (14). Gene expression profiling of malignant and benign RLC cardiomyopathy mutation mouse models demonstrated that all mutation models exhibited varied gene expression from that of non-transgenic animals with differences in unique gene expression clusters between the 3 models. These findings support a role for different myofilament protein mutations to cause cardiomyopathy through diverse mechanisms. Finally, work from the laboratory of **Samatha Harris** investigated the molecular mechanism of the A31P MyBP-C cardiomyopathy linked mutation in cats (15). Localization and expression data in ventricular tissue from affected cats demonstrated that while the A31P MyBP-C did not disrupt protein sarcomeric localization or alter total MyBP-C, the A31P mutation decreased the amount of mutant protein and altered the local MyBP-C structure. Although the A31 alters protein stability to decrease mutant protein levels, the normal total MyBP-C protein levels suggest the A31P mutation results in cardiomyopathy through the disruption of MyBP-C mediated contraction and not haploinsufficiency.

While the collection of diverse works in this Highlight Issue of *Achieves of Biochemistry and Biophysics* has elucidated broad and significant new insight towards understanding the structure-function role of the myofilament regulatory proteins to the modulation of striated muscle contraction, new questions have now arisen and much work remains. It is my hope that the collection of new insight presented in this issue will serve to further energize future investigations into these myofilament mediated mechanisms responsible for the regulation of striated muscle contraction. On behalf of all the contributing authors, I offer great thanks to the Editorial Office and Executive Editors for supporting the publication of this Highlight Issue emphasizing the significance of the myofilament regulatory proteins in striated muscle contraction.

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