Platform: Cardiac Muscle Regulation

1812-Plat
Overexpression of Foxo in the Heart Ameliorates Performance Decline through Enhanced UPS Processing in Aging Drosophila
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Heart performance declines with age. A likely contributor to age-associated cardiac dysfunction is reduced protein quality control due to decreased function of the ubiquitin/proteasome system (UPS) and the autophagy lysosomal pathway (ALP). The transcription factor, FOXO, has been shown to be involved in the regulation of genes related to both of these interrelated pathways as well as a host of other cellular processes. Here, we investigated the effects of cardiac-restricted overexpression of dFOXO in Drosophila melanogaster, an ideal model for aging studies, exploiting the tissue-specific UAS-GAL4 expression system. Using high-speed video microscopy and motion analysis and atomic force microscopy, we showed that with age, mild heart-specific overexpression of dFOXO significantly attenuated senescence-associated cardiac functional decline and stiffening, respectively. We also determined that differing amounts of heart-specific dFOXO overexpression elicited disparate effects, the strongest driver proving fatal. Overexpression of dFOXO in all Drosophila muscle has been shown to increase lifespan, likely owing to systemic expression of autophagy related proteins and reduced ubiquitin content. Similarly, we found that dFOXO-mediated improvement in heart function with age was also accompanied by a significant decrease in ubiquitinated myocardial proteins as determined by quantitative western blot analysis. However, microarray data suggested that this reduction was caused by increased expression of genes associated with the UPS rather than autophagy, indicating that FOXO may perform its function differently in the heart than in other striated muscles. Because FOXO transcriptionally regulates genes associated with many facets of cellular life, we have established a list of specific targets that dFOXO may affect when its expression is increased to attenuate cardiac dysfunction with age. We will systematically investigate these candidates to pinpoint how mild FOXO overexpression ameliorates the natural decline in heart performance in Drosophila.

1813-Plat
Obscurins' Mechanistic Involvement in Signal Transduction at the Cardiac Intercalated Disc
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The intercalated disc (ID) of cardiac muscle embodies a highly ordered, multifunctional network, which is essential for the transmission of electrical stimuli and mechanical force resulting in the synchronous contraction of the heart. Recently, a plethora of proteins have been identified as novel components of the ID. The challenge now lies in their characterization. Here we focus on the molecular and functional description of two novel members of the ID, obscurin-80 and obscurin-40.
Obscurins are a family of proteins expressed in striated muscles where they localize to distinct subdomains. The members of the obscurin family are multidomain proteins composed of adhesion modules and signaling domains, resulting from extensive alternative splicing of transcripts arising from the single OBSCN gene. Recent work from our laboratory has demonstrated that complex splicing at the 3' end of the obscurin transcript gives rise to at least two novel obscurins, obscurin-80 (obsc-80) and obscurin-40 (obsc-40), named after their predicted molecular weights.
Using immunofluorescence and immunoelectron microscopy, we show that obsc-80 and obsc-40 localize to the ID of developing and adult murine cardiomyocytes. Using biochemical assays we further demonstrate that both obsc-80 and obsc-40 exist in a complex with major ID proteins, including N-cadherin, connexin-43, vinculin, and ankyrin-G. The PH domain present in both obsc-80 and obsc-40 binds specifically and directly binds to phosphatidylinositol 3,4 and 4,5 bisphosphates, likely targeting both proteins to the ID membrane. Overexpression of the obscurin PH domain results in decreased phosphorylation of Akt, therefore reducing Akt activation. This suggests a potential role for obsc-80 and obsc-40 in the regulation of cell growth and proliferation via the Akt pathway. Further experiments are underway to examine the functional activities of obsc-80 and obsc-40 at the ID and their regulation in health and disease.

1814-Plat
The N-Terminal Hypervariable Region of Troponin T Differentially Modulates the Affinity of Tropomyosin-Binding Sites
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The troponin complex plays a central role in the allosteric function of sarcomeric thin filaments by enacting conformational changes during the Ca^{2+}-regulated contraction and relaxation of striated muscle. The troponin subunit T (TnT) has two binding sites for tropomyosin (Tm) and is responsible for anchoring the troponin complex to the thin filament. Although the C-terminal and middle regions of the TnT polypeptide chain are highly conserved among the three muscle type isoforms, the hypervariable N-terminal region has evolutionarily diverged significantly among isoforms. Previous studies have shown that the N-terminal variable region fine-tunes Ca^{2+} regulation of muscle contractility via modulation of the overall molecular conformation of TnT, and its interactions with Tm. In the present study, we engineered intact TnT and representative fragments of TnT, expressed them in E. coli, and prepared purified proteins for functional studies. Tropomyosin binding affinity was analyzed using affinity chromatography and solid phase protein binding assays to investigate the modulatory effects of the N-terminal variable region. The results demonstrated that in the absence of the N-terminal variable region, TnT's conserved middle region and C-terminal T2 region Tm-binding sites showed comparable Tm-binding affinities across isoforms. The data demonstrate that without the modulatory effect of the N-terminal variable region, the intrinsic Tm-binding affinities of the two sites are both high. In contrast, the presence of the isoform specific N-terminal variable region differentially reduces the binding affinity of TnT for Tm, primarily at the middle region binding site. These novel findings indicate that the N-terminal variable region plays a key role in the functional difference of muscle fiber type-specific, developmental, splice variant, and pathogenic TnT isoforms by modulating the interactions with Tm during the contraction and relaxation of cardiac and skeletal muscle.

1815-Plat
Constitutive Phosphorylation of Myosin Regulatory Light Chain (RLC) in vivo is Maintained by Low Kinase and Phosphatase Activities
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The importance of RLC phosphorylation in enhancing cardiac myofibrillar contraction is well-known. In anesthetized mice the extent of RLC phosphorylation (45%) was not changed by changes in sympathetic tone with prolonged infusion of the beta-agonist dobutamine or treatment with the beta-blocker propranolol. The goal of this study was to determine if the constitutive RLC phosphorylation in vivo was limited to half of the RLC due to: a) negative cooperativity for phosphorylation of two heads in myosin; or b) due to a steric constraint in myofibrils blocking access of soluble cardiac myosin light chain kinase (cMLCK) to RLC. We measured the kinetic properties of RLC phosphorylation in native myosin filaments and myofibrils. Results showed that RLC was phosphorylated by a pseudo-first order rate in both preparations with maximal phosphorylation over 90%. Thus, RLC for each head in myosin was readily available for phosphorylation. Pacing trabeculae at 1.5 Hz for 30 minutes increased RLC phosphorylation from 20 ± 1 % to 43 ± 3 %. Consistent with biochemical results, RLC phosphorylation increased to 91 ± 3 % when myosin light chain phosphatase activity was inhibited with calyculin A while pacing. Together, these results exclude negative cooperativity and steric blocking as mechanisms limiting RLC phosphorylation. We determined that the heart has a high cMLCK content (2.4 ± 0.1 μM) compared to the MLCK present in fast skeletal muscle (0.5 ± 0.03 μM), but similar to the MLCK content in smooth muscle (3.4 ± 0.2 μM). However cMLCK has a low specific activity compared to the other MLCKs. In conclusion, the extent of RLC phosphorylation in a normally beating heart is limited by cMLCK with its low activity in balance with low myosin light chain phosphatase activity. RLC phosphorylation is insensitive to sympathetic activation or inhibition in vivo.

1816-Plat
Epigallocatechin-3-Gallate Reverses the Defects in Modulation of Ca^{2+}-Sensitivity by Troponin I Phosphorylation Caused by Hypertrophic and Dilated Cardiomyopathy Mutations in Cardiac Muscle
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Heart muscle contraction is regulated *via* the β-adrenergic response that leads to phosphorylation of Troponin I (TnI) at Ser 22/23, which changes the Ca^{2+}-sensitivity of the cardiac myofilament. Previously it has been shown that mutations found in Dilated Cardiomyopathy (DCM) and Hypertrophic