represent KO titin (exons 219-225 represent ~30kDa, which is insufficient to cause a mobility difference). The smaller isoform, found in both EDL and soleus KO muscle, is likely to represent a splicing adaption that correlates with increased passive tension. We are currently investigating the identity of these exons using titin exon microarray analysis. In summary, excision of titin exons 219-225 in skeletal muscle results in elevated passive stress with the underlying cause likely being due to an adaption in titin splicing.

2812-Pos

Developmentally Regulated Differential Splicing of the Large Sarcomeric Proteins Titin and Nebulin

Danielle Buck[1], Adam Hoying[1], Bryan Hudson[1], Coen Ottenheijm[2], Henk Granzier[1].
[1]University of Arizona, Tucson, AZ, USA, [2]VU University Medical Center, Amsterdam, Netherlands.

Titin (3-4MDa) and nebulin (700-900kDa) are large sarcomeric proteins of skeletal muscle. Both proteins contain sites of differential splicing throughout their length. To study splicing during development we used a home-made exon microarray representing all of titin's and nebulin's exons and compared exon expression between mice of different ages in numerous muscle types. We found that a gradual reduction in titin's transcript size in exons encoding the elastic PEVK segment located in the I-band correlates with a reduced titin protein (typically ~0.2 MDa smaller in adults) and stiffer sarcomere in all skeletal muscles studied (soleus, m. gastrocnemius, tibialis cranialis, extensor digitorum longus, and quadriceps). Nebulin undergoes less dramatic differential splicing than titin, but it was found that exons encoding the M177/M178 repeat in the z-disc are upregulated in the adult versus embryonic samples. Additionally, the soleus muscle exhibited a 4-fold increase in expression level of the SH3 domain of nebulin and a 3-fold increase in the serine rich exons. These findings correlate with structural changes in the z-disc which are wider in the adult (133nm) versus the neonatal soleus samples (121nm, pvalue 0.04). Thus titin and nebulin transcripts are tuned during development with changes in titin altering the I-band properties and changes in nebulin affecting the Z-disc.

2813-Pos

MybP-C Slow: a Novel Phosphoprotein of Skeletal Muscles

Maegen A. Ackermann, Aikaterini Kontrogianni-Konstantopoulos.
University of Maryland, Baltimore, MD, USA.

Myosin Binding Protein-C slow (MyBP-C slow) is a thick filament associated protein, consisting of seven immunoglobulin (Ig) and three fibronectin-III (Fn-III) motifs, arranged in tandem. Four alternatively spliced forms of MyBP-C slow, referred to as variants 1-4, have been characterized in skeletal muscles. These differ in three regions; variants 1 and 2 contain a 25-residues long insertion at the extreme, non-modular NH2-terminus, variant 3 carries an 18-amino acid long segment within Ig7 and variant 1 contains a unique COOH-terminus consisting of 26-amino acids, while variant 4 does not possess any of these insertions. Our recent work has indicated that variants 1-4 are expressed in different amounts in distinct skeletal muscles and exhibit diverse topographies. To date, the regulatory mechanisms that modulate the activities of MyBP-C slow are unknown. We used proteomics tools to examine the profile of MyBP-C slow in flexor digitorum brevis (FDB) muscle. To this end, protein homogenates prepared from adult FDB muscles of mouse origin were treated with different kinases, phosphatases or their inhibitors, and analyzed by isoelectric focusing and 2-dimensional SDS-PAGE. We found that MyBP-C slow, similar to its cardiac counterpart, is phosphorylated by Protein Kinase A, and additional, yet unidentified, kinases. Using high-stringency phosphorylation prediction software, we also found that the three novel insertions present in variants 1-3 contain consensus phosphorylation motifs for PKA and other Ser/Thr kinases. We are currently mutagenizing the predicted sites and examining their ability to be phosphorylated in vitro by PKA, as well as two other major kinases associated with the thick filaments: titin kinase and obscurin kinase. Our studies are the first to show that MyBP-C slow is a phosphoprotein and a substrate of PKA, and to suggest a novel mechanism that may regulate its activities in skeletal muscle.

2814-Pos

A C-terminal Truncation of Flightin Slows Actomyosin Cycling, Elevates Passive Tension, and Decreases Power Output in Drosophila Flight Muscle Fibers

Bertrand C.W. Tanner, Mark S. Miller, Becky M. Miller, Panagiotis Lekkas, David W. Maughan, Jim O. Vigoreaux.
University of Vermont, Burlington, VT, USA.

Flightin is a 182 amino acid myosin rod binding protein that is essential for thick filament stability and function in the indirect flight muscles (IFM) of *Drosophila melanogaster*. Flightin is not homologous to any known protein in vertebrates, but recent phylogenetic analyses revealed that flightin is widespread among insects and crustaceans. Amino acid sequence analysis suggests that flightin consists of three functional domains: a fast evolving and highly phosphorylated N-terminal domain (residues 1-83), a conserved central domain (residues 84-134) and a semi-conserved C-terminal domain (residues 135-182). To interrogate the function of the C-terminal domain, we created a transgenic Drosophila that expresses a truncated flightin, missing the last 43 residues (fln^{142t}). These transgenic flies are unable to beat their wings, but their myofilament lattice and sarcomere structure appear normal compared to a flightin-null rescued control line (fln^{R}). Active (pCa 5) and rigor isometric tensions in skinned IFM fibers were higher for fln^{142t} versus fln^{R}, which occurs due to a 2-fold increase in passive (pCa 8) isometric tension. Small amplitude sinusoidal perturbation analysis showed that fln^{142t} fibers produced approximately 30% of the oscillatory work and power of fln^{R} fibers. The fln^{142t} also showed a decreased frequency of maximum work (123 Hz vs. 154 Hz) and power (139 Hz vs. 187 Hz) compared to fln^{R}, suggesting slower myosin kinetics even though myosin attachment time (=0.5 ms) was unchanged. These results suggest that the C-terminus of flightin plays a limited role in thick filament integrity and normal sarcomere structure of the IFM, but is essential for maintaining the muscle's passive properties and tuning the kinetic properties during contraction.

2815-Pos

Intermediate Filament and Ecm Mechanics Deduced from Desmin Knockout Muscles

Gretchen A. Meyer, Andrew D. McCulloch, Samuel R. Ward, Richard L. Lieber.
University of California, San Diego, San Diego, CA, USA.

Desmin is the skeletal muscle intermediate filament protein that forms a mesh-like network around Z-disks and helps transmit force to the extracellular matrix (ECM). It generally functions as a mechanical integrator of the cell, maintaining lattice connectivity and enabling efficient force transmission. Desmin related myopathies have debilitating effects that range from muscle weakness and atrophy to cardiac and respiratory failure. Studies performed on desmin knockout muscles have begun to elucidate the physiological and biological roles of desmin, but the mechanical properties of the desmin network are still unknown.

We performed incremental stress-relaxation tests on fibers and fiber bundles from desmin knockout and wildtype mouse EDL muscles to investigate how the absence of desmin affects the fiber and fiber bundle viscoelastic properties. Using these data, we developed a structural model with explicit elastic and viscous elements representing the desmin, the rest of the fiber and the ECM. Single fibers from desmin knockout muscles were significantly more compliant (linear modulus $= 122 \pm 61$ kPa) compared to wildtype fibers (176 ± 49 kPa, $p<0.05$). These data demonstrate that the desmin matrix bears 30-40% of the passive load in muscle cells, a much greater fraction than previously believed. In contrast to fibers, bundles of fibers were nonlinear and demonstrated the opposite trend_bundles from desmin knockout muscles were over twice as stiff (440 ± 237 kPa) compared to wildtype bundles (214 ± 97 kPa) at 60% strain ($p<0.0001$). Time constants for stress-relaxation were larger for fibers than bundles and were significantly larger for knockout bundles compared to wildtype bundles. These data suggest a biological and biomechanical interaction between muscle cells and the ECM and may indicate that the ECM becomes stiffer in desmin knockout muscles in response to the more compliant fibers.