

the most significant stress change in response to stretching. The distribution of filamin was very similar to dystrophin forming longitudinal tracks along the contacted surfaces of the myotube.

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Structure-Function of Synthetic Membrane-Sealant Copolymers for Dystrophic Muscle

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¹University of Minnesota, Department of Integrative Biology and Physiology, Minneapolis, MN, USA, ²University of Minnesota, Department of Chemical Engineering and Materials Science, Minneapolis, MN, USA. Muscle membrane vulnerability is a hallmark of Duchenne Muscular Dystrophy (DMD), an X-linked neuromuscular disease that results in progressive skeletal muscle weakness and significant cardiomyopathy. We propose the use of membrane-sealant copolymers as an innovative and unique potential therapeutic for DMD. The tri-block copolymer family comprises molecules made of a hydrophobic polypropylene oxide (PPO) core flanked bilaterally by linear chains of hydrophilic polyethylene oxide (PEO) chemical moieties. Block copolymers exist at various molecular weights and PPO/PEO ratios and it is currently not known what structural properties confer membrane sealing capacity to copolymers of this family. We and other groups have shown that one such sealant, poloxamer P188, protects dystrophic hearts in vitro and in vivo in both small and large animal models of DMD but its apparent efficacy is significantly reduced in skeletal muscle in vivo. This underscores the importance of discovering more potent membrane sealants to treat all striated muscles in DMD. We have initiated a collaborative structure-function approach by implementing and refining an in vitro membrane injuring osmotic and shear stress assay to analyze the membrane sealing functions of these tri-block copolymer family members on dystrophic skeletal muscle to systematically determine the effects of PPO/PEO ratio and molecular weight on membrane protection. We will present progress on copolymer structure-function understanding and discuss how these new data will shed light into the structural requisite for more efficacious and potent membrane sealants.

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Multiple Antibody Colocalization Imaging of Skeletal Muscle

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Anatomical distribution of multiple proteins within complex tissue is predicted to aid in determination of musculodystrophy pathophysiology. Dysferlin is a large (~230 kDa), membrane-anchored, calcium-binding protein localized to plasmalemma and t-tubules of muscle fibers. In fibers lacking dysferlin, these structures exhibit ultrastructural abnormalities. As a means to identify the possible role(s) of dysferlin in maintenance of fiber ultrastructure and calcium homeostasis, we are using array tomography (ATomo) to detect differences in protein co-localization in dysferlin-negative vs. dysferlin-normal muscle fibers. We have screened and optimized antibodies for specific labeling of major structural components: contractile apparatus (actin, myosin, desmin), plasmalemma (dystrophin, caveolin), t-tubules (dysferlin, DHPR- α 1, DHPR- α 2, ryanodine receptor) and basement membrane (collagen A1, elastin) and calnexin and α -smooth muscle actin observe the fiber anatomy. Testing different temperatures for labeling incubation, we found better labeling and deeper penetration at 37°C. This labeling procedure was not associated with increased noise from non-specific labeling. We optimized labels in ATomo by comparing their specificity for anatomical structures over a range of antibody concentrations, from the minimum required for observable signal to very high concentrations demonstrating maximum signal and high noise levels. We have observed a consistent pattern between appropriate antibody concentrations for ATomo relative to other immunolabeling methods such as western blotting and immunohistochemistry that roughly encapsulates a high S:N ratio plateau, forming good first approximations for optimized labeling. We have combined up to four antibodies against epitopes colocalized within the same structure to label the complete anatomy of the T-tubule and triad architecture across the volume of a muscle fiber. We will use ATomo to colocalize dysferlin with other proteins, to assess the number, distribution and localization of L-type Ca²⁺-channels, SERCA-type re-uptake Ca²⁺-pumps, Ca²⁺-leak channels and Ca²⁺ buffering proteins located in the t-tubules in normal and dysferlin-null myofibers.

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3D Palm Shows Distinct Distributions of Z-Disc Proteins with the Z-Discs in Cardiomyocytes

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Z-discs are important structural and signalling structures that form the boundaries of each muscle sarcomere in striated muscle. Within the Z-disc, electron microscopy indicates that actin filaments, arranged in an anti-parallel organization, are cross-linked by α -actinin dimers arranged at ~20 nm intervals. At least 30 different proteins reside in the Z-disc, and their organization is not well understood. Conventional light microscopy cannot resolve the localisation of proteins within the Z-disc, as it is too narrow ~100 nm wide.

We have developed the use of 3-D Photoactivated Localisation Microscopy (PALM) to image two specific proteins within the Z-disc; α -actinin2 and Lasp-2 (LIM and SH3 containing protein 2), which binds to α -actinin. α -actinin and LASP fused to mEos2 were expressed in cultured embryonic mouse or isolated adult rat cardiomyocytes. Fixed cells were imaged using PALM, in which a weak cylindrical lens in the light path between the specimen and the camera was used to obtain 3D information from a single 2D plane.

The resulting images show individual molecules of mEos2- α -actinin2 and mEos2-LASP within the Z-disc, using light microscopy, for the first time. The localization precision was 20nm (X,Y) and 50nm in Z. The density of mEos2- α -actinin2 molecules was higher than that for mEos2-LASP, and the densities of both molecules was non-uniform throughout the Z-disc structures. A quantitative analysis of these molecules provides new insight into the organisation of these molecules within the Z-disc structure. These results demonstrate that PALM can be used to localise specific proteins within the narrow Z-disc and thus it has great potential for investigating the organisation of component proteins within this structure.

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The M-Line Protein Obscurin in the early Development of Drosophila Flight Muscle

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Obscurin is a 475 kDa protein in the M-line of Drosophila muscles. The protein is made up of Ig and Fn3 domains, a Rho-GEF domain near the N-terminus and two kinase domains near the C-terminus. The expression of obscurin was reduced by a P-element insertion, or by RNAi. Knockdown of obscurin by RNAi was targeted to all muscles, or specifically to the flight muscle (IFM). In P-element mutants and RNAi lines, embryo, larva and pupa developed normally; adults could walk and jump, but were flightless. In the wild-type pupa, obscurin in the IFM appeared in striations at 30 hours after puparium formation, when kettin (a Z-disc protein) and myosin were in amorphous strands. Thus, obscurin in the M-line precedes the regular assembly of the Z-disc and A-band. In the IFM of flies with reduced obscurin, the sarcomere length was normal but the M-line was missing and H-zone irregular. Isolated thick filaments were asymmetrical with the bare zone shifted from the middle of the filaments. In the sarcomere, the length and polarity of thin filaments depended on the position of the bare zone in adjacent thick filaments. Thus, the early expression of obscurin nucleates the assembly of a symmetrical thick filament, which leads to thin filaments of uniform length. Ligands of the kinase domains are: ball (another kinase) binding to kinase 1 and MASK (an ankyrin-repeat protein) binding to kinase 2. Confirmation of these interactions by injecting embryos with kinase constructs will be described. The abnormalities in the IFM sarcomere in RNAi lines with reduced ball or MASK were similar to those in obscurin knockdown flies. The expression of obscurin was normal in RNAi lines of both ligands, demonstrating the importance of obscurin, ball and MASK to sarcomere assembly.

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In Vivo Time Resolved X-Ray Diffraction Reveals Radial Motions of Myofilaments in Insect Flight Muscle

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The constraint of constant volume for a contracting muscle cell implies a radial expansion that occurs during axial shortening will lead to increases in the radial spacing of the lattice of myofilaments. That change in filament spacing, in turn,