**3185-Pos Board B290**

**A Novel Assay of Mechano-Transduction in Single Muscle Cells**

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In striated muscle the tight integration of contractility and biomechanical properties with electrical, metabolic and hormonal signaling are defined as mechano-transduction signaling pathways. The great interest to define the molecular basis for mechano-transduction is underpinned by the growing number of loss-of-function disorders and diseases in which altered mechano-transduction has been identified.

Intact, single cell preparations are attractive for studying mechano-transduction, however the mechanical loading of single cells requires technically challenging methods. We have recently developed MyoTak, a tenacious biological adhesive, which enables us to attach single dissociated striated myocytes (skeletal and cardiac) to force transducer and length controller units. The myocytes thus prepared can be readily imaged using an inverted microscope equipped with confocal, widefield and multiphoton fluorescence imaging systems. With this preparation we have been able to control myocyte length while assaying passive tension and electrically evoked contractility. In recent experiments we have explored the interaction of myocyte stretch and contractility with $Ca^{2+}$ signaling, mitochondrial function and ROS production. We will present these new findings, the novel methods and additional experiments examining the role of microtubules as mechano-transducer elements that contribute to these interactions.

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**3186-Pos Board B291**

**Dynamic Interactions between the Myocyte and Extracellular Matrix Promote Myocyte Differentiation and Myofibril Assembly**

Maide O. Raeker, **Mark W. Russell**.

During development, skeletal myoblasts differentiate into myocytes and skeletal myotubes with mature contractile structures that are precisely oriented with respect to surrounding cells and tissues. Establishment of this highly ordered structure requires reciprocal interactions between the differentiating myocytes and the surrounding extracellular matrix to form correctly positioned and well organized connective tissue attachments from the skeletal muscle to the bony skeleton. Using the developing zebrafish embryo as a model, we examined the relationship between new myofibril assembly and the organization of the membrane domains involved in cell-extracellular matrix interactions. We determined that apical clustering of integrins was associated with changes in cell morphology which included myoblast elongation along the body axis. Through the analysis of zebrafish embryos depleted of obscurin A that have impaired formation of the myotendinous junctions, we determined that cell elongation and, as a result, new myofibril formation was delayed in regions that lacked integrin clustering and fibronectin matrix organization. In addition, it was noted that striated myofibrils first form at the cell periphery and are associated with the early patterning of the lateral sarcolemma. These specialized membrane domains will ultimately form mature lateral connections between the myofibril and the extracellular matrix at the costameres. We have also determined that the giant cytoskeletal and sarcomeric protein obscurin has an important role in the organization and maturation of the MTJ and in the maturation of the costamere. As myocyte-extracellular matrix connections have critical roles in force transduction and tension-mediated signaling defining the processes that promote and sustain these connections will have important implications for the understanding the pathogenesis of, and developing new treatment strategies for, a range of myopathies and muscular dystrophies.

**3187-Pos Board B292**

**Muscle Giants Create Order from Chaos with Force**

**Kuan Wang**, Jeffrey G. Forbes.

Titin and nebulin possess perhaps the longest span of intrinsically disordered protein segments in the human proteome. We have implemented an integrated platform of nanomechanics to investigate the elasticity of intrinsically disordered segments and its functional manifestations. Our titin studies have indicated that the titin PEVK region is an elastic spring, with charge pairing as a new mechanism of creating diversity and modulating molecular elasticity. Significantly, PEVK also serves a dual role as a giant scaffold for SH3-containing signaling proteins. Many novel hybrid and overlapping motifs for SH3 domains are embedded in the seemingly random proline-rich sequences throughout the titin molecule. We now propose that titin PEVK directly couples the force sensing and response, by controlling the accessibility of SH3 receptor proteins via the opening and closing the access to binding sites upon mechanical stress, reversibly and elastically. In other words, titin's elastic PEVK appears to act both as an analog force sensor and as a transducer that converts the force input directly into biochemical signals of the SH3 pathways. Our nebulin studies indicated that native full length nebulin and nebulin modules are intrinsically disordered and elastic. We proposed that that nebulin is an "elastic or adjustable ruler" that has to be stretched and lengthened properly to interact and stabilize actin filaments. The continued presence of compressive force exerted by stretched nebulin may well be a requirement for thin filament assembly, integrity and maintenance. Additionally, since skeletal muscle thin filaments is thought to be "dually-regulated" where calcium activates contraction by targeting both troponin and nebulin-bound calcium sensor proteins (Root and Wang 1994), we envisage that nebulin tethers elastically myosin heads to actin in the inactivated state and then releases and de-inhibits myosin heads upon calcium activation.

## Excitation Contraction Coupling II

**3188-Pos Board B293**

**Electron Microscopy of Cryo-Sectioned Skeletal Muscle by Focused ion Beam Milling**

**Terence Wagenknecht**, Chyong-ere Hsieh, Michael Marko, Clara Franzini-Armstrong.

Knowledge of the detailed three-dimensional architecture of the couplon, the fundamental functional unit of excitation-contraction coupling (ECC), is essential for understanding the mechanism of ECC in striated muscle. Transmission electron microscopy of conventional thin-sectioned skeletal muscle resolves some structural features of the couplon: (1) the sarcoplasmic reticulum (SR) -associated ryanodine receptors whose cytoplasmic regions (''foot'' structures) bridge the junctional gap between the transverse (T-) tubule, and (2) density in the SR lumen attributable to calsequestrin. However, many structures are surely not resolved due to resolution and specimen preparation limitations. To advance further, we propose to image the native ECC machinery by cryo-electron microscopy and tomography of vitreously frozen thin sections of muscle. Obtaining suitable vitreous sections by ultramicrotomy is difficult and artifact-prone, and so we are investigating the application of focused ion beam (FIB) milling to obtain cryo-sections of 200 nm or less in thickness. The first cryo-micrographs of FIB-milled skeletal muscle from toadfish swimbladder (glutaraldehyde fixed) have been obtained, and they show structural features seen previously, as well as some additional ones (e.g., mass densities within the T-tubule lumen, densities associated with the T-tubule in the vicinity of RyRs). The micrographs obtained from FIB-milled specimens appear comparable to those obtained by cryo-ultramicrotomy, but without the defects, such as specimen compression, associated with the latter technique. Our goal is to extract many RyR-containing sub-volumes from tomograms of cryo-sectioned muscle, and then to average them so as to reproducibly resolve additional fine structure within the couplon.

**3189-Pos Board B294**

**Inducible Silencing of Junctophilins in Skeletal Muscle Leads to Reversible Remodeling of the Triad Junction Structure and Compromised Store-Operated Calcium Entry**

**Jae-Kyun Ko**, Kyoung-Han Choi, Xiaoli Zhao, Shinji Komazaki, Zui Pan, Noah Weisleder, Jianjie Ma.

Junctophilins (JPs) play an essential role in muscle excitation-contraction (E-C) coupling by contributing to the formation of junctional membrane complexes (JMCs). However, the lethality associated with germ-line ablation of either JP1 or JP2 prevents physiological evaluation of their function in the maintenance of calcium homeostasis in adult muscle fibers. To investigate the physiological role of JP genes, we developed a novel transgenic system for tissue-specific and inducible control of gene expression in mouse models. This system employs a Tet-response CMV promoter that controls expression of a small-hairpin (sh) RNA against JP1 and JP2, which is non-functional until an interrupting reporter gene cassette is excised by the Cre recombinase. Insertion of the natural *Dicer* and *Drosha*-RNAse processing sites within the shRNA sequence allows for generation of specific siRNA probe for efficient knockdown of JP1 and JP2. Under the tight control of doxycycline (Dox), tissue- or lineage-specific expression of siRNA is achieved by the use of inducer mice that expresss Cre in a given tissue. Transgenic mice with muscle-specific expression of shRNA against JP showed no apparent change of JP expression before treatment with Dox; inducible and reversible knockdown of JP in skeletal muscle