muscles. In this research, we investigated localization and binding partner of SESTD1 in striated muscles. SESTD1 was detected in the whole extracts from skeletal but not cardiac muscles by western blot using anti-SESTD1 monoclonal antibody. Immunofluorescence microscopy using the antibody revealed that SESTD1 is localized at Z-line of sarcomere. To search the binding partners of SESTD1, we carried out yeast two-hybrid screening with skeletal muscle cDNA libraries. Several proteins located in Z-line were obtained as candidates for SESTD1 C-terminus binding proteins and alpha-actin was obtained as a SESTD1 N-terminus binding protein. These results indicate that SESTD1 is the novel Z-line protein of the sarcomere in skeletal muscle and may function through binding to both Z-line structural proteins and actin filaments. We are currently investigating the role of SESTD1 in Z-line formation and actin filament assembly.

3873-Pos Board B601
The Role of Myopalladin in Skeletal Muscle
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The half-sarcomere, the functional unit of skeletal muscle, is able to produce power at high efficiency or resist a sudden increase in load with low metabolic cost, due to the combination of the function of the myosin II motors in the thick filament overlapped with the thin filament and the meshwork of cytoskeleton proteins acting as a scaffold. The importance of cytoskeleton proteins is illustrated by the identification of mutations in many of the corresponding human genes in patients with skeletal myopathies. Here we studied the role of myopalladin (MYPN), a protein located in the Z-line and the I-band. MYPN gene mutations have been identified in patients with limb-girdle dystrophy as well as dilated, hypertrophic and restrictive cardiomyopathy (Duboscq-Bidot et al. Cardiovasc Res 77:118, 2008; Purevjav et al. Hum Mol Genet 21:2039, 2012). To provide insights into the physiological role of this protein and the mechanisms leading to myopathy, the mechanical performances of skeletal muscles from wt and KO mice were determined. In EDL muscle, the absence of MYPN (i) decreases the isoformic force (T0) by 48% and the cross-sectional area (CSA), calculated by the muscle wet weight, by 21%; (ii) decreases the power at any load and (iii) does not affect the curvature of the force-velocity relation. In skinned fibers from the same muscles T0 is reduced in proportion to CSA, indicating that the CSA of EDL muscle is overestimated in KO mice with respect to control. Thus the reduced muscle performance in KO mice is due to the reduction in fiber dimension while the kinetics of actin-myosin interaction is unaffected. Injection of an adeno-associated virus (AAV) vector expressing the wt form of MYPN results in a substantial rescue of muscle performance. Supported by Ministero della Salute, Telethon and MIUR (Italy).

3874-Pos Board B602
Triadin Function in Sarcoplasmic Reticulum Structure
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Muscle contraction is achieved when an efficient excitation signal at the plasma membrane triggers intracellular calcium release. This process called “excitation-contraction (E-C) coupling” relies on a macromolecular protein complex, spanning the plasma membrane and the sarcoplasmic reticulum (SR), containing the calcium channel of the SR, the ryanodine receptor (RyR). This calcium release complex is present exclusively in highly organized muscle structures called triads. A triad is composed of two SR terminal cisternae surrounding a plasma membrane transverse-tubule. This architecture is essential to sustain the activity of the calcium channel RyR1, which is located in the membrane of SR terminal cisternae. However, little is known about the molecular mechanisms allowing the formation and maintenance of SR terminal cisternae. Triadin is a member of this complex, present in the SR membrane and interacting with RyR1. Deletion of the triadin gene leads to partial disorganisation of SR membranes in skeletal muscles, with abnormal orientation of part of the triads. We have shown in a non muscle cell model that triadin expression leads to important modification of the endoplasmic reticulum (ER) morphology, already observed with the expression of proteins regulating ER morphology, and known as “rope-like structures”. These modifications of ER morphology are correlated to alteration of the microtubule network. It thus suggests that in skeletal muscle, triadin could play a role in the structure of sarcoplasmic reticulum to allow efficient E-C coupling. For the present work, using mass spectrometry analysis of the proteins co-immunoprecipitated with triadin, we have identified a putative triadin partner which could interact with triadin and with the microtubules, and therefore anchor the sarcoplasmic reticulum to the microtubule network. Using different deletion mutants of both proteins we identified the domains of each protein important for this interaction.

3875-Pos Board B603
In Vitro Smooth Muscle Hypercontractility Induced by Cd4 + T Cells is Transient
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Smooth muscle (SM) hypercontractility is a characteristic of several pathologies, including asthma. Inflammatory cells and/or the cytokines they release may alter contractile protein expression, thereby also altering the SM mechanics. We characterized the maximal unloaded shortening velocity (Vmax), stress (force/cross sectional area), and contractile protein expression of Brown Norway rat airway SM co-cultured with activated CD4+ T cells for 24h and 48h. After 24h incubation, Vmax (mean ± SEM) was significantly increased (0.15 ± 0.01 l/s vs. 0.29 ± 0.02 l/s; control vs. T cells, p = 0.0012). However, this alteration in Vmax was transient and disappeared after 48h of co-incubation (0.21 ± 0.01 vs. 0.25 ± 0.01 l/s, p = 0.099). No significant differences were observed at 24h or 48h. Western blot analysis showed a significant increase in the levels of MLCK (0.20 ± 0.03 vs. 0.41 ± 0.09; control vs. T cells; p<0.05). The (+) isomer SM myosin isoform (0.55 ± 0.04 vs. 0.81 ± 0.05; p = 0.0023) after 24h incubation with the CD4+ T cells. These alterations were also transient; all values returned to baseline after 48h exposure to the CD4+ T cells. Thus, the increase in Vmax was correlated with the increase in MLCK and (+) isomer isoform expression. Conversely, there were no differences in levels of total SM myosin heavy chain or -smooth muscle actin, suggesting there was no increase in muscle mass. These results show that indeed inflammatory cells and/or their mediators can alter SM contractile protein expression and SM function, but that these effects may be transient.

This transient behavior may be important to consider when studying SM function in disease. Supported by: CIHR, NIH-R01HL103145.

3876-Pos Board B604
The Interaction of AMP-Activated Protein Kinase and its Upstream Activator, Lkb1/Mo25/Strad, Modifies Contractile Function in Rat Cardiac Trabeculae
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AMP-activated protein kinase (AMPK) is an energetic regulator that is activated by alterations in cellular AMP. In addition, as well as others, have shown that AMPK modifies both metabolic and contractile function of the heart. However, to become fully activated, it is necessary for AMPK to be phosphorylated by the upstream AMPK kinase complex, LKB1/MO25/STRAK. Yet, the relationship between this upstream kinase complex, AMPK activation, and myofilament contractile function is not known. We hypothesize that a high LKB1/MO25/STRAK to AMPK ratio will have a different impact on myofilament function than a low LKB1/MO25/STRAK to AMPK ratio. Accordingly, demembranated rat cardiac trabeculae were pre-incubated with varying ratios of LKB1/MO25/STRAK to AMPK and contractile function was measured. Surprisingly, at sarcomere length of 2.2 μm, cardiac fibers pre-incubated with the LKB1/MO25/STRAK complex alone are desensitized to Ca2+ (EC50 4.16 ± 0.12 μM [n=6] vs 2.56 ± 0.11 μM [n=13] p<0.05) and have lowered maximum tension (20.02 ± 2.68 mN/mm2 [n=6] vs 39.18 ± 4.27 mN/mm2 [n=13] p<0.05). A high LKB1/MO25/STRAK:AMPK (2.5 mol LKB1/MO25/STRAK: 1 mol AMPK) decreases Ca2+-sensitivity of tension (EC50 3.71 ± 0.17 μM [n=8] vs. 2.56 ± 0.11 μM [n=13] p<0.05). Tension generation is also somewhat -sensitivity of tension (EC50 1.36 ± 0.06 μM [n=8] vs. 2.56 ± 0.11 μM [n=13] p<0.05). Tension generation also is restored to control values when length dependent activation, cooperativity, and rate of tension redevelopment remain unaltered regardless of the presence of AMPK or LKB1/MO25/STRAK.
In conclusion, the LKB1/MO25/STRAD complex has been identified as a potential novel regulator of myofilament function. The interaction between LKB1/MO25/STRAD and its target kinase AMPK can also alter contractile function.

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LKB1 and MO25 Demonstrate Significant Interaction with Myofilament Proteins

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AMP-activated protein kinase (AMPK) is an energetic regulator in the heart that is activated by alterations in energetic status. AMPK has been shown to alter both metabolic status as well as contractile function in the heart. However, to become fully activated, the upstream kinase complex LKB1/MO25/STRAD must phosphorylate AMPK. Recent evidence from our lab clearly illustrates that pre-incubation of demembranated cardiac trabeculae with the LKB1/MO25/STRAD complex (alone) can alter Ca\textsuperscript{2+}-sensitivity of isometric tension development. The mechanism underlying the ability of the LKB1/MO25/STRAD complex to alter myofilament contractility is currently unknown. Accordingly, we hypothesize that the LKB1/MO25/STRAD complex alters myofilament contractility through a direct interaction with myofilament proteins. To test this hypothesis, cardiac trabeculae or cut papillary tissue was harvested from 2 month old male Sprague-Dawley rats following a Krebs-Henselet perfusion. Cardiac trabeculae or papillary strips were demembranated overnight and washed. Myofilibrils were isolated and solubilized. Using western blot analysis we show that MO25 remained associated with myofilaments in the absence of LKB1. Next, we incubated demembranated muscle strips with the LKB1/STRAD/MO25 complex (0.02 U/µL) for thirty minutes at 30 C. Following incubation, myofilibrils were isolated and solubilized. By western blot analysis, we show a significant increase in the amount of MO25 as well as LKB1 associated with myofilibrillar proteins over unincubated controls (p=0.0354 and p<0.0001 for MO25 and LKB1, respectively). We conclude that the myofilibrillar proteins can interact with the LKB1/MO25/STRAD complex and that this may underlie the impact of this complex on contractile function. Future studies will be designed to address the physiological relevance of this interaction as well as determine the specific proteins that are interacting with the LKB1/MO25/STRAD complex.

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A Molecular Simulation Study to Investigate Actin Filament Elongation Mechanism

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Actin is a protein responsible for numerous cellular functions, for example, it is associated with the muscle contraction. Globular actin (G-actin) polymerizes into filamentous actin (F-actin). In the elongation process of the filament, actin hydrolyzes ATP into ADP. The rates of elongation at the pointed and barbed ends are different, and the latter end is the main elongation direction. The two planes defined by two near-axial pairs of domains are known to be relatively twisted (propeller angle ~20°) in G-actin whereas those in F-actin are flat (~5°). The atomic structure of F-actin has recently been determined by electron cryomicroscopy; however, the filament elongation mechanism is not fully understood yet at molecular level. In this work, we performed molecular dynamics simulations of G- and F-actins to investigate the elongation mechanism. As models for the terminal regions of F-actin, pentamer, hexamer, and heptamer of actin protomers were adopted. We examined the conformational changes of G-actin at the terminal of F-actin. The analysis of the propeller angle showed that G-actin bound to ATP tended to take the twisted form whereas ADP-bound G-actin was relatively flat. In addition, the salt bridge patterns near the hinge regions were significantly different between the ATP and ADP bound forms. Therefore, the propeller angle rotation is correlated with these salt bridges. The pentamer consisting of ADP-bound protomers was found to maintain the flat form. No notable change was observed in the propeller angles of the oligomer when one actin bound ATP was attached to the barbed end but significant change was induced for actin at the end when two ATP-bound actin molecules were attached to the pentamer at the barbed end, suggesting that protomer-protomer interaction between ATP-bound actin might be key for the elongation.

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X-Ray Diffraction Pattern of Non-Uniformly Stretched Actin Filament

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Helical structures within living cells are exposed to mechanical forces and are consequently deformed. However, interpretations of X-ray fiber diffraction patterns of such structures assume Fourier transform of helices with fixed periodicities. Huxley et al. and Wakabayashi et al. (Biophys. J. 67: 2422-2435 and 2411-2421, 1994) calculated the extensibility of actin filaments in contracting muscle fibers from the X-ray diffraction patterns using mean values of strain. Single value of strain corresponds to symmetric, narrow meridional peak profile, while, non-uniform deformation of actin filament provides profile similar to the observations, noticeably changing elastic constant for actin filament. Due to discrete, stochastic attachments of myosin heads to actin filaments the strain along the filament increases stepwise from the free end of the actin filament to the Z-line. We have developed a methodology for predicting X-ray diffraction patterns with stepwise increases of strain along actin filament. Using PDB data for the crystal structures of G-actin and rules for constructing actin fibers we reconstructed the geometry of actual deformed fibers. Fiber deformations are determined by Monte Carlo calculations using the computational platform, MUSICO. Predicted X-ray diffraction patterns show smeared layer lines, caused by different pitch of helices, in contrast to distinct layer lines originating from constant pitch of undeformed helices. Calculated meridional X-ray diffraction peak profiles from deformed helices are skewed and closely resemble the observed profiles by Huxley et al. and Wakabayashi et al. The proposed methodology for analyzing deformed helices provides realistic intensity profiles and spacing values of molecular structures in inverse space. This enables the extraction of much more information concerning molecular structure from the X-ray diffraction data from any helical structures in living cells.

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Adding Regulation to Cross-Bridge Muscle Models

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For over fifty years, cross-bridge models have helped us understand muscle contraction. These models describe both steady state and transient force measurements. Being computationally efficient, they allow parameter estimation from data fits. Unfortunately, they only apply to maximal activation. At sub-maximal activation, tropomyosin inhibits myosin binding. If a myosin molecule binds to actin, it locally displaces tropomyosin thereby facilitating the binding of adjacent myosin. Since myosin and actin are arranged in filaments, cooperativity violates the mean-field approximation. This approximation is fundamental to any cross-bridge model, so models of activation typically use Monte-Carlo methods. Such models, though useful for some applications, are orders of magnitude slower than cross-bridge models and so parameter estimation is difficult. To address this problem, we have developed a method to efficiently incorporate activation into cross-bridge models.

The basis of our theory is that, at sub-maximal activation, some parts of the thin filament are locally active, while the rest is inactive. A set of differential equations can keep track of these locally activated regions as they form, grow, shrink and disappear. Inside these active regions, myosin obeys a cross-bridge model; outside these active regions, myosin remains unbound. We have validated this model by comparisons to Monte-Carlo simulations. The model successfully fits both steady state and transient experiments at all levels of activation. We have compared the model to measurements of in vitro motility at low calcium and low ATP (rigor activation), showing that the model reproduces the measurements, and can estimate parameters of activation. This parameter estimation is possible because the model is roughly 10,000-fold faster than Monte-Carlo simulations. We are now fitting the model to experiments performed under conditions of variable calcium and ATP. This work represents a step toward a complete multi-scale description of muscle contraction.