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^{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. Myofibrils were isolated and solubilized. Using western blot analysis we show that MO25 remained associated with myofibrillar proteins. Next, we incubated demembranated muscle strips with the LKB1/STRAD/M025 complex (0.02 U/μL) for thirty minutes at 30 C. Following incubation, myofibrils were isolated and solubilized. By western blot analysis, we show a significant increase in the amount of Mo25 as well as LKB1 associated with myofibrillar proteins over unincubated controls ($p=0.0354$ and $p=<0.0001$ for MO25 and LKB1, respectively). We conclude that the myofibrillar 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.