provide useful parameters for modeling studies and suggest that the actin-binding loop of myosin contributes to both the binding and unbinding of myosin from actin.

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Tropomyosin Movement on F-actin Analyzed by Energy Landscape Determination

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Muscle contraction is regulated by movement of tropomyosin over the surface of actin filaments. At low-Ca^{2+}, tropomyosin blocks myosin-binding on actin, whereas, in a two-step process, Ca^{2+}-saturated troponin and myosin open the myosin-binding site leading to contraction. No obvious steric obstructions or geometrical barriers on actin limit such tropomyosin movement. However, lacking atomic models for these transitions, the pathways taken by tropomyosin during regulatory movements are uncertain. Here, end-points for regulatory transitions were determined by fitting tropomyosin to EM reconstructions. Reconstructions of negatively-stained low-Ca^{2+} thin filaments yield an atomic model very close to that described for troponin-free actin-tropomyosin by Li et al. (2011), while comparable high-Ca^{2+} filament maps suggest an azimuthal sliding of tropomyosin parallel to its superhelical path. Additionally, Raunser et al. (2012-Biophys. Soc. abst.) describe an atomic model of tropomyosin based on high-resolution cryo-EM reconstructions of myosin-decorated filaments, showing expected azimuthal movement and pronounced ~15Å axial displacement of tropomyosin toward the pointed-end of the thin filament. To evaluate transitions between these various regulatory positions, we explored the energy landscape between ''end-states'' over a comprehensive grid of 832 tropomyosin locations relative to F-actin coordinates. The position of tropomyosin was varied azimuthally and axially relative to F-actin, and then the structure energy-minimized. The resulting electrostatic energy landscape shows a wide energy basin with a minimum centered near the blocked-state. The width of this basin indicates that large azimuthal and axial oscillations of tropomyosin are possible. By contrast, in the myosin-induced open-position, tropomyosin is located at an energy peak, representing a region with no obvious complementary electrostatic interactions between tropomyosin and F-actin. Our results therefore suggest that the open-position is reached only because of tropomyosin interaction with myosin, while binding of tropomyosin to the F-actin surface becomes less important.

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Structural Changes in Both the Troponin Complex and the Thick Filament May Underlie Myofilament Length Dependent Activation

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The main cellular mechanism that underlies the so-called ''Frank-Starling Law of the Heart'' is an increase in the responsiveness of cardiac myofilaments to activating Ca^{2+} at longer sarcomere lengths (SL). The structural basis of this ''Length Dependent Activation'' (LDA) is not known. 2D X-ray diffraction patterns were obtained using the BioCAT beamline 18ID at the Advanced Photon Source from electrically stimulated (0.2 Hz) intact, twitching papillary muscle isolated from rat hearts during a 10 ms time window in diastole just prior to electrical stimulation. Diffraction patterns were compared from muscles that were stretched to L_{max} (SL= ~2.3 μm) to those taken following a quick release to slack length (SL=~1.9 μm). We previously reported that myosin heads moved radially inwards at longer SL suggesting that an increased radial extent of crossbridges at longer length cannot explain increased calcium sensitivity so other explanations must be sought. It is known that changes in isoform composition of the troponin complex can markedly affect calcium sensitivity but the role of troponin in the length sensing mechanism underlying LDA is not clear. Here we analyzed the meridional patterns which showed that the 3rd order troponin repeat distance, the 3rd - order troponin reflection intensity and the 2nd order myosin (''forbidden'') meridional reflection all increased significantly ($P < 0.01$) at L_{max} as compared to slack length. Thus, stretching intact heart muscle in diastole induces changes in the structure of both the thick filaments and the thin filaments. It appears, then, that the length sensing mechanism underlying LDA must involve connections of some kind that transmit strain between the thick and thin filament that alter the structure of the troponin complex and, presumably, myofilament contractile properties. Supported by **NIH HL075494** and RR08630.

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Myosin Heavy Chain Isoforms Influence the Magnitude of Stretch Activation in Drosophila Muscles

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The stretch activation (SA) mechanism, a general property of all muscle types, is most prominent in muscles that generate power through cyclical contractions, such as insect indirect flight muscles (IFM) and vertebrate cardiac muscles. SA is defined as phase 3, a delayed tension increase, of the tension transient following a muscle stretch. We are testing our hypothesis that myosin heavy chain isoforms help set SA magnitude and hence power generating ability in different muscle types. The *Drosophila* thorax contains two muscle types, IFMs and jump muscles. In IFMs, the magnitude of SA is great enough to enable net positive power generation when it is fully calcium activated. In contrast, jump muscles display very little stretch activation and cannot generate net positive power when fully activated. We found that when an embryonic myosin isoform (EMB) is transgenically expressed in the jump muscle, the muscle is transformed and behaves like a moderately stretch-activatable muscle. The transformed muscle can now generate positive power and SA magnitude is increased by ~60% at 0mM Pi and ~350% at 16mM Pi. This shows that SA magnitude is influenced by myosin isoforms. We found that power generation is [Pi] dependent as $\geq$ 2mM Pi is required for power production in jump muscles expressing the EMB isoform. Expressing the IFM isoform in jump muscles did not enable positive power generation or increase SA magnitude. To further test our hypothesis, we are expressing the jump myosin isoform in IFMs to determine if this reduces IFM SA magnitude. We conclude that myosin isoforms can influence the magnitude of SA, but are not the only mechanism responsible for natural differences in SA magnitude.

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Half-Sarcomere Mechanics and Energetics Indicate that Myosin Motors Slip Between Two Consecutive Actin Monomers during their Working Stroke

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The coupling between chemical and mechanical steps of actomyosin ATPase cycle was studied in situ by using fast mechanical protocols in Ca2+-activated demembranated fibres from rabbit psoas under sarcomere length control (sarcomere length 2.4 μm, temperature 12°C). We determined the effects of the concentration of inorganic phosphate (Pi) on the force-velocity relation (T-V), on the stiffness-velocity relation (e-V) and on the isotonic velocity transient following a stepwise drop in force from the isometric plateau force (T0) (Piazzesi et al. J Physiol 545:145, 2002). With respect to control (no added Pi), the increase of [Pi] to 10 mM, i) reduced T0 by 50-60%, decreased the curvature of the T-V relation by 30% and increased the unloaded shortening velocity (V0) by 19%; ii) decreased the relative half-sarcomere stiffness at each shortening velocity by an extent that indicates that Pi has little effect on the force per attached myosin motor; iii) did not change the rate of early rapid shortening (phase 2) following the stepwise drop in force, while reduced its size and made the subsequent pause of shortening (phase 3) briefer. Steady state and transient mechanical responses and the known related energetics (Potma and Stienen J Physiol 496:1, 1996) are simulated with a kinetic-mechanical model of the actomyosin ATPase cycle that incorporates Huxley and Simmons mechanism of force generation. Muscle power and efficiency during isotonic shortening at high and intermediate loads can be predicted only if myosin motors at an intermediate stage of both the working stroke and product release can slip to the next Z-ward actin monomer. Supported by MIUR, Ministero della Salute and Ente Cassa di Risparmio di Firenze (Italy).

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Myosin-Induced Detachment Causes Differences Between Ensemble and Single Molecule Myosin Kinetics

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Single molecule measurements of mechanochemistry have greatly increased our understanding of muscle contraction. However, since trillions of myosin molecules work together in muscle, extrapolation to in vivo function requires additional understanding of how motors behave in an ensemble. Early findings suggested that myosin behaves similarly at both the single molecule and ensemble levels; but more recent experiments suggest otherwise. Using a combination of simulation and theory, we show that the force-dependence of ADP release