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Drug Effects and Mechanism Underlying the Force-velocity Relationship of Skeletal Muscle
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Amrinone is a bipyridine drug with characteristic effects on the force-velocity relationship of fast skeletal muscle. Here we combined *in vitro* motility assays, transient biochemical kinetics and optical tweezers studies to elucidate the mechanisms underlying the drug effects. Amrinone (1-2 mM) reduced the sliding velocity of heavy meromyosin (HMM) propelled actin filaments by 31.0 ± 2.5% (n = 15) at different ionic strengths of the assay solution (20 - 160 mM). The drug also reduced (by 2 - 18%) the sliding velocity of actin filaments propelled by subfragment 1 (S1). Stopped-flow studies of myofibrils, acto-HMM and acto-S1 showed no amrinone-induced reduction in the rate of MgATP induced actomyosin dissociation and optical tweezers studies detected no changes in the working stroke length. In contrast, the ADP affinity of acto-HMM (but not acto-S1) was increased about two-fold by 1 mM amrinone. Our results are consistent with inhibition of a strain-dependent MgADP-release step as the basis for amrinone induced reduction in sliding velocity. Modeling suggests that such an effect may also account for most other amrinone-induced changes of the force-velocity relationship of muscle (*e.g.* in isometric force and in shape of the force-velocity curve). Moreover, the results point to the possible importance of cooperative interactions between the two myosin heads in muscle contraction.

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Role Of Myosin Binding Proteins On The Structural Stability And Flexural Rigidity Of Thick Filaments
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Despite the fundamental role of thick filaments in muscle contraction, little is known about the mechanical behavior of these filaments and how myosin associated proteins dictate differences between muscle types. Insect flight muscle (IFM) and vertebrate cardiac muscle share common physiological properties such as their cyclical contraction for producing either a wing beat or a heart beat, as well as their reliance on a pronounced stretch activation response to produce oscillatory power. We used atomic force microscopy (AFM) to study the morphological and biomechanical properties of native thick filaments from age-matched normal (+/+) and mutant (t/t) mice heart lacking cardiac myosin binding protein C (cMyBPC) and from IFM of normal (*fln*+) and mutant (*fln*^{0}) *Drosophila* lacking flightin. AFM images of these filaments were evaluated with an automated analysis algorithm that identified filament position and shape. The t/t thick filament length (1.48 ± 0.02 μm) was significantly ($P < 0.01$) shorter than +/+ (1.56 ± 0.02 μm). To determine if cMyBP-C contributes to the mechanical properties of thick filaments, we used statistical polymer chain mechanics to calculate a per filament specific persistence length (PL), an index of flexural rigidity directly proportional to Young's modulus. PL in the t/t (373 ± 62 μm) was significantly lower than +/+ (639 ± 101 μm). Accordingly the Young's modulus of t/t thick filaments was approximately 60% of +/+. Thick filaments from newly eclosed *fln*^{0} IFM have longer contour length (3.90 ± 1.33 μm) than *fln*+ filaments from same age flies (3.00 ± 0.38 μm), and a PL less than half that of IFM filaments from *fln*+ flies. These results provide a new understanding for the critical role of myosin binding proteins in defining normal cardiac and IFM output by sustaining force and muscle stiffness.

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Thin Filament Regulation of Relaxation in 3D Multi-Sarcomere Geometry
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The dynamics of muscle relaxation in physiologically relevant situations is complex due to interactions among crossbridge kinetics and thin filament regulation by Ca^{2+}. To quantitatively study behavior of muscle relaxation we have developed stochastic model of muscle contraction and its regulation in the 3D multi-sarcomere geometry. The model includes a simple three state and comprehensive nine state actomyosin cycle, extensibility of thick and thin filaments, and McKillop-Geeves and the flexible continuous tropomyosin chain (CFC) models of thin filament regulation. Loading conditions include isometric force development for prescribed Ca^{2+} concentrations and Ca^{2+} transients. We tested the hypothesis that the observed heterogeneity of shortening of individual sarcomeres is the principal mechanism causing rapid decrease in overall force upon sudden decrease of Ca^{2+} concentrations. We quantitatively determined the effect of heterogeneity of sarcomere lengths on the speed of muscle relaxation. The model predicted slow early relaxation caused by multiple myosin bindings within a single troponin-tropmyosin (TnTm) unit which keeps the unit open and allows the reattachment of detached crossbridges except at very low Ca^{2+} concentrations. At later times rapid shortening of some sarcomeres is observed due to force fluctuations caused by stochasticity of myosin binding. This inhomogenous shortening dramatically increases speed of the slow phase of relaxation. The combination of the effects of inhomogeneous shortening and the filaments extensibility mechanistically explains the observed two phase relaxation. Both regulatory models predict well the force-pCa relationship, but CFC model better fits the experimental data. The principal mechanism underlying this better fit is reduced size of the flexible TmTn regulatory unit upon myosin detachment which prevents reattachment of the crossbridges by partially covering actin sites within the unit. For the same reason the flexible chain model better predicted the twitch dynamics. Supported by NIH grant R01 AR048776.

1038-Plat
Molecular Dynamics of Tropomyosin: Implications for the Assembly and Regulation of Thin Filaments
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The molecular switching mechanism governing skeletal and cardiac muscle contraction couples the binding of Ca^{2+} on troponin to the movement of tropomyosin (Tm) on actin filaments. By shifting position around thin filaments in response to changing Ca^{2+}, Tm either blocks or exposes myosin-binding sites on actin, thereby regulating myosin-crossbridge cycling and consequently contraction. Tm lies over actin at a ~39 angstrom radius with considerable water between the two surfaces. Lorenz *et al.* (1995) and later Poole *et al.* (2006) proposed that Tm has a distinctive coiled coiled-coil shape designed to match the contours of F-actin. This arrangement might facilitate binding of Tm on F-actin and movement between regulatory states. In contrast, others have suggested that Tm flexibility is needed for binding and regulatory movements. To understand transitions of Tm between regulatory states better, the structure and flexibility of Tm was assessed by Molecular Dynamics performed in implicit water. A full-length Tm atomic structure was constructed by fitting different crystal structures of Tm segments (PDBs: 2D3E, 1IC2, and 2B9C) to the coordinates of the Lorenz coiled coiled-coil model. The Tm stretches and the model fitted to each other very well. Tm showed delocalized but pronounced anisotropic bending during 11ns MD, with no evidence of localized kinking, suggesting that Tm lacks discrete domains that flex. A persistence length several times the length of Tm was calculated, indicating that the molecule is only semi-flexible. Although Tm bends away from its initial supercoiled shape, it revisits the contours of the Lorenz model multiple times during simulation, implying that Tm may assume this shape when binding to F-actin. The results indicate that Tm is flexible enough to coil around actin, yet stiff enough to act as a cooperative unit during regulatory movements.

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Obscurin, A Large Modular Protein, Regulating Sarcomere Formation In Drosophila Muscle
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Drosophila obscurin is a modular muscle protein of ~ 420 kDa. The sequence, derived from the genome, contains two C-terminal serine-threonine kinases, both of which are predicted to be inactive, as well as 21 Ig and two Fn3 domains. A Rho/GEF domain has been identified in the N-terminal region. There are four obscurin isoforms, two of which are exclusively expressed in the indirect flight muscle (IFM). Obscurin is in the M-line throughout IFM- development and in the adult fly. In Drosophila IFM, the protein is across the M-line, unlike the vertebrate, where obscurin is at the periphery of the myofibril. A P-element insertion in the first intron of the gene leads to severely reduced obscurin protein levels and a flightless phenotype in homozygous mutant flies.