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Actin Sliding Velocities Are Influenced By The Chemical Driving Force Of Actin-myosin Binding

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It is widely assumed that muscle shortening velocity, *V*, is solely limited by actin-myosin detachment kinetics; however, it was recently shown (Hooft et al., 2007) that *V* is influenced by a driving force modulated by actin-myosin attachment kinetics and energetics. To further test this hypothesis, we have developed a novel *in vitro* motility and force assay that allows us to correlate changes in internal driving force with changes in *V*. We alter the driving force by varying inorganic phosphate, P_i, and blebbistatin concentrations using an *in vitro* motility assay to measure corresponding changes in *V*. We estimate changes in driving force by calculating the rate, k_{break}, at which actin filaments break during this assay. We observe that at 30 μM ATP, both *V* and k_{break} decrease by approximately 50% (*V* from 1.5 to 0.88 $\mu m \cdot sec^{-1}$ and k_{break} from 0.04 to 0.02 sec^{-1}), demonstrating that actin-myosin driving forces in an *in vitro* motility assay decrease with *V* upon addition of P_i. Similarly upon addition of 50 μM blebbistatin, a small molecule known to decrease actin-myosin mechanics by inhibiting actin-myosin binding kinetics, both *V* and k_{break} decrease by approximately 50% (*V* from 1.5 to 0.62 and k_{break} from 0.04 to 0.02 sec^{-1}). These results support the hypothesis that in an *in vitro* motility assay internal forces modulated by actin-myosin binding energetics influence actin sliding velocities, supporting a new paradigm for the mechanism of muscle shortening.

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Flexibility of Actin Filaments During Myosin Induced Sliding

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The bending flexibility of actin filaments, freely suspended in solution or propelled by heavy meromyosin (HMM) in the *in vitro* motility assay, was studied under different experimental conditions using both phalloidin-stabilized and phalloidin-free filaments (visualized by N-Hydroxysuccinimide-Rhodamine). For actin filaments propelled by HMM, the persistence length (inversely related to the bending flexibility) was obtained from exponential fits to the cosine correlation function for sliding paths. In solution (in the absence of HMM) both phalloidin free and phalloidin stabilized filaments exhibited a nearly rectilinear increase (slope ≈ 0.046 μm/mM) in persistence length upon increased ionic strength of the observation solution (from 40 to 130 mM). For phalloidin-free and phalloidin-stabilized actin filaments (80 mM ionic strength), L_P in solution was 9.2 ± 0.8 μm (mean ± 95 % confidence interval) and 15.7 ± 1.1 μm, respectively (> 80 filaments). For phalloidin free filaments, the persistence length was similar whether the filaments were propelled by HMM or freely suspended in solution in the absence of HMM. In contrast, the persistence length for phalloidin stabilized actin filaments was reduced to the same level as for phalloidin free filaments during HMM induced sliding. The results accord with the idea that a high flexibility structural state of the actin filament is a perquisite for actomyosin motility. However, the results do not support ideas that a low-to-high flexibility transition of the filament should be an important component of the force-generating step *per se*. Finally, our results challenge the general view that phalloidin stabilized filaments behave as native actin filaments in their interaction with myosin.

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The Molecular Effects of Skeletal Muscle Fatigue on Myosin Mechanics and Kinetics

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Skeletal muscle, during periods of exertion, experiences several different fatigue based changes in contraction including reductions in force, velocity, power output, and energy usage. The physiological bases of fatigue induced changes in contractility stem from many different factors including changes in neuronal activity, excitation-contraction coupling, and actomyosin contraction. The direct changes in the actomyosin contractile complex have previously been shown to be the result of alterations in the levels of metabolites, oxidative damage, and an increase in myosin regulatory light chain phosphorylation. Here, we measured the direct molecular effects of fatigue-like conditions on actomyosin velocity and force generation using the in vitro motility assay. We examined how changes in ATP, ADP, Pi, and pH affect the ability of the myosin to translocate actin. We show that fatigue-induced increases in metabolites decrease unloaded shortening velocity, with ADP and pH contributing the most to the observed changes in velocity. We also examined whether myosin regulatory light chain phosphorylation alters the sensitivity of the myosin to fatigue-like conditions. We show that under fatigue-like conditions, phosphorylation of the myosin regulatory light chian enhances force production and reduces actin sliding velocity, similar to the effects of phosphorylation under fatigue-like conditions observed in both muscle fiber and in vitro motility studies. Furthermore, we found that force production by dephosphorylated myosin is very sensitive to fatigue-like conditions whereas force production by phosphorylated myosin is rather fatigue insensitive. These results suggest that phosphorylation of the myosin regulatory light chain in skeletal muscle may serve as a protective mechanism against fatigue. Supported by NIH-HL077280, AHA-0435434T (J.R.M.), AHA-0815704D (to M.J.G.), and NIH-HL071778 (D.S-C.).

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Different Molecular Mechanisms of Force Enhancement by Myosin Head Domain Mutations R723G and R453C

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The myosin head transforms chemical energy from ATP hydrolysis into mechanical work to generate isometric force or to drive muscle shortening. During the power stroke, small changes in the catalytic ATPase site of the actin-bound myosin head are coupled to tilting of the light-chain binding domain relative to the catalytic domain. In this mechanism distortion of an elastic element within the actomyosin complex is essential for strain to develop prior to movement.

We have studied missense mutations naturally occurring in Familial Hypertrophic Cardiomyopathy (FHC) to test molecular mechanisms and functional roles of specific domains of the myosin head. Slow soleus muscle fibers (expressing the ventricular myosin isoform) of FHC-patients were used for these studies. Previously we found that mutation R723G which is located in the converter domain of the myosin head increases active force and resistance to elastic distortion (fiber stiffness) during contraction, relaxation, and rigor, while cross-bridge cycling kinetics were unchanged. This indicated that the converter is the part of the actomyosin complex where most of the elastic distortion occurs.

We now included mutation R453C located near the nucleotide binding pocket of myosin in our functional studies. This mutation was found to also increase active force, however, without affecting fiber stiffness. Instead, mutation R453C increased fiber ATPase activity and the rate constant of force redevelopment (kredev) significantly, which were unchanged by mutation R723G. Thus, both FHC mutations cause force enhancement in muscle fibers from FHC patients, however, by distinctly different mechanisms. R723G affects resistance to elastic distortion of the myosin head while R453C alters cross-bridge cycling kinetics. The data underline the different functional roles of the domains within the myosin head and point to an involvement of residue R453 in human ventricular myosin ATPase activity.

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Kinetics Of Two Single Point Mutants Of Drosophila Myosin S1

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We investigated the kinetic properties of two homozygous-viable *Drosophila* myosin mutants, *D45* ($A^{261}T$) and *Mhc*^{5} ($G^{200}D$). The *D45* mutation is located near β7, while the *Mhc*^{5} mutation is located at the N-terminal side of loop 1. *D45* was originally identified as a suppressor of the troponin I (TnI) *hdp*^{2} mutation, which causes muscle hypercontraction, resulting in a wings-up phenotype. The *Mhc*^{5} mutation is lethal when co-expressed with *hdp*^{2}, presumably enhancing the hypercontraction phenotype. *Drosophila* hearts, carrying either the *D45* or *Mhc5* mutation, demonstrated depressed or enhanced motor function and this evoked a dilatory or restrictive response, similar to that seen with vertebrate dilated or restricted cardiomyopathy myosin mutations. Using flash photolysis, our transient kinetics results suggest that suppression of the TnI hypercontraction phenotype is accomplished by expression of a less-active myosin (*D45*), whereas enhancement of muscle hypercontraction can be achieved with the expression of an overactive myosin isoform (*Mhc5*). Both