shows that myosin X adopts a left-handed helical path along these cytoskeletal structures, consistent with its step size. The radius of the helical path followed by the quantum dot increases between labeling sites on the CaMs and the C-terminus and between single filaments and bundles. The radii suggest flexibility in the tail. These features of the motility, in conjunction with membrane and microtubule binding domains, enable myosin X to operate on varied actin structures in multiple cellular functions. Supported by NSF NSEC grant DMR04-25780 and NIH grant GM086352.

752-Pos

Influence of Actin Mutant to Processive and Non-Processive Myosin Motility

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The myosin family is an ATP driven molecular motor that interacts with an actin filament via ionic bonds. In particular, there are eight specific, negative charged amino acids in actin that match with eight positively charged amino acids in the myosin loop2 region. However, how these ionic bonds relate to the disparate stepping mechanism of processive myosins like myosin-V and non-processive ones like myosin-II remains to be explained. To clarify these points, we constructed several actin mutants in which the number of negatively charged amino acids were decreased (0 to 6) or increased (10 to 12).

To clarify the functional properties of each actin mutant, we performed actin gliding assays using myosin-II and -V, separately. The actin gliding velocity on myosin-V was accelerated with a decrease in negative actin charge, although we did not see processive movement in single molecule imaging measurements. On the other hand, actin gliding on myosin-II decreased regardless of increasing or decreasing the number of negative charges in actin relative to WT. These results indicate that the number of negative charges in WT actin is well tuned for processive and non-processive myosin motility. At present, we are planning to perform additional analysis including biochemical assays and single molecule measurements to further test this hypothesis. Additionally, we are invesitgating differences in the stepping mechanism between processive and non-processive myosins with respect to the actomyosin interaction.

753-Pos

Differential Effects of Alpha Vs Beta Myosin Heavy Chain on the Kinetcs and Mechanics of Familial Hypertrophic Cardiomyopathy Mutations in the Myosin Regulatory Light Chain

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Cardiac muscle myosin is comprised of two heavy chains (MHCs), two essential light chains, and two regulatory light chains (RLCs). The MHC contains both the ATPase and actin binding domains. It has been shown that the contractile properties of myosin can be tuned by the MHC isoform and that the MHC isoform distribution in the human heart changes during heart failure from predominantly beta isoform to all beta. One cause of heart failure is familial hypertrophic cardiomyopathy (FHC) which is triggered by mutation of sarcomeric proteins including the RLC. Although the RLC is spatially separated from the myosin active site, it appears to have a role in tuning myosin kinetics. In order to examine how two RLC mutations implicated in FHC, N47K and R58Q, affect the kinetic and mechanical properties of beta isoform myosin, we exchanged porcine cardiac RLC with recombinant mutant RLC. We examined the contractile properties of these mutants using the *in vitro* motility assay and compared these results to our earlier results with mutant RLCs on the alpha -MHC background. Regardless of MHC isoform, the mutations cause reductions in force and power output. However, on the alpha MHC backbone, R58Q shows differences in calcium handling and an elevated ATPase rate which is not seen on the beta backbone. Also, both mutants show increases in duty cycle on the alpha MHC but not the beta. These data suggest that small changes in the myosin structure, far from the active site, can disrupt the contractile properties of the motor depending on the MHC isoform.

754-Pos

Temperature Dependence of MgATP and MgADP Affinity of Fast and Slow Rat Myosin Isoforms: An in Vitro Motility Assay Approach

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It has been suggested that the rate of ADP release from acto-myosin and the rate of acto-myosin dissociation by ATP may play different roles to define unloaded shortening velocity of slow and fast myosins and tha their role might change with temperature (Nytray et al. 2006; Iorga et al. 2007). In this study, the in vitro motility assay (IVMA) approach was used to study the effect of MgATP and MgADP on actin sliding velocity (Vf) on slow and fast skeletal myosin isoforms at different temperatures. The velocity of actin filaments sliding on pure slow (myosin 1) and pure fast (myosin 2B) myosin isoforms from the rat was determined in a range of [MgATP] (0.01-2mM) and in the presence or absence of 2mM MgADP. Experiments were performed at 20, 25 and 35 °C. The rate constants of ADP release and ATP binding to acto-myosin were calculated. The inhibition of Vf by MgADP was greater in slow then fast isoforms and the rate constant of ADP release was higher in fast than slow isoform. The results suggest that, in slow isoforms, the maximum velocity could be limited by the rate of ADP dissociation from actomyosin. The ADP inhibitory effect decreased and the rate constant of ADP release increased in each isoform with temperature accounting for the increase in Vf. No differences were found between isoforms and among temperatures in the rate constant of ATP binding. The possibility that the rate of acto-myosin dissociation induced by ATP could play a role in defining Vf in fast isoforms will be discussed.

755-Pos

Heavy Meromyosin Head-Surface Distance and Geometrical Arrangement on a Silanized Surface

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In the *in vitro* motility assay, actin filaments are propelled by myosin motor fragments, e.g. heavy meromyosin (HMM) adsorbed to nitrocellulose or silanized surfaces. Even though the *in vitro* motility assay is used on a regular basis in fundamental studies of actomyosin function, very little is known about the geometry of the surface-adsorbed myosin fragments. Here, we have taken a multi-technique approach to elucidate the mechanism of HMM adsorption on silanized surfaces (trimethylchlorosilane [TMCS] derivatized SiO_2) with high quality actomyosin motility. Data obtained using quartz crystal microbalance with dissipation (QCM-D) and fluorescence interference contrast (FLIC) microscopy suggest a dynamic HMM layer with a thickness in the range of 20 - 50 nm where the actin filaments are held 38 $\pm$ 2.3 nm (mean $\pm$ SEM from error propagation) above the surface. This is considerably more than the distance of 10-15 nm between the thin and thick filaments in skeletal muscle. However, the QCM-D and FLIC data, taken together with total internal reflection fluorescence spectroscopy based ATPase assays, suggest that HMM is attached to the TMCS-derivatized surface at the C-terminal end corresponding to the attachment of the hinge region to the thick filament backbone.

756-Pos

A Simple Model to Explore Half-Sarcomere Inhomogeneity in a Myofibril

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Given the possible half-sarcomere interactions in a myofibril and the complexity of cross-bridge models, a model adequate to explore all the possible properties that might emerge from interactions among half-sarcomeres in series would be complex and computationally costly. On the premise that even a simple model can be useful, we explore the stability of half-sarcomere (hs) lengths in a model of a rabbit psoas myofibril consisting of N half-sarcomeres in series. Each hs includes an element that can produce forces proportional to myosin cross-bridge-actin filament overlap and an elastic element, simulating titin. The nodes of the network are the centers of A-bands of myosin filaments common to adjoining half sarcomeres. We use the model to explore how variability in hs properties leads to inhomogeneities in hs lengths along the myofibril. Experiments show that activation increases hs length inhomogeneity but does not lead to 'popped sarcomeres' or unlimited A-band shifts. The model demonstrates the minimal assumptions needed to explain the growth of hs length inhomogeneity with activation, and the constraints imposed on non-cross-bridge force-producing elements by the limits on hs length dispersion. Recently published measurements of A-band shifts during activation of single sarcomeres strongly suggest that titin can produce force adequate to compensate for imbalances between cross-bridge-generated hs forces. Experiments show that stretching fully-activated myofibrils does not increase the variability in hs length. The model demonstrates that the force balance achieved by A- band movement during activation leads to a reduction in hsl dispersion for half sarcomeres at or beyond maximum cross-bridge overlap.