perturbations were used. Experiments were performed in identical solutions with 0.2M ionic strength at pH 7.00. The concentration of MgATP was varied to detect kinetic constants of the ATP binding step 1 (K_1: dissociation constant), the cross-bridge detachment step 2 (k_2, k_{-2}: rate constants), and the ATP cleavage step 3 (k_3, k_{-3}). By following the fast rate constant at 20°C, we found in myofibrils: k_2/K_1=1.0∈ $\mu M^{-1}s^{-1}$, K_1=0.3mM, k_2=300s^{-1}, and $k_{-2} \approx 0$; in fibers: k_2/K_1=0.23∈ $\mu M^{-1}s^{-1}$, K_1=1.58mM, k_2=363s^{-1}, k_{-2}=180s^{-1}. From these results we conclude that (1) ATP binding is ~5X stronger in myofibrils than in fibers, (2) cross-bridge detachment rate is just about the same, and (3) its reversal step is almost absent in myofibrils, but it is finite in fibers. Consequently, we found a good agreement in the results obtained from myofibrils and fibers, indicating that phase 2 of tension transients from step analysis in fibers (Huxley and Simmons, 1971) represents the cross-bridge detachment step. We also studied actin-myosin cross-linked myofibrils and found no difference, indicating that cross-linking does not significantly modify steps 1-3 kinetics. We further studied the Pi effect in myofibrils, and found that Pi is a competitive inhibitor of MgATP with the inhibitory dissociation constant of 7-8mM. To deduce the kinetic constants of the ATP cleavage step, we measured the slower rate constant in fluorescence in myofibrils and found that k_3+k_{-3}=10.7s^{-1} at 4°C. From the Pi burst experiments using radioactive ATP, we found that K_3=6.1 at 4°C. From these, k_3=9.2s^{-1} and k_{-3}=1.50s^{-1} were deduced.

747-Pos

Myosin ATP Turnover Rate: A Mechanism Involved in Thermogenesis in Resting Skeletal Muscle Fibers

Melanie Stewart, Kathleen Franks-Skiba, **Roger Cooke.**
Univ california, San Francisco, CA, USA.

Thermogenesis by resting muscle varies with conditions and plays an active role in homeostasis of body weight. The low metabolic rate of living resting muscles requires that ATP turnover by myosin be inhibited relative to the purified protein in vitro. This inhibition has not been previously seen in in vitro systems. We used quantitative epifluorescence microscopy of fluorescent nucleotides to measure single nucleotide turnovers in relaxed permeable skeletal muscle fibers. We observed two lifetimes for nucleotide release by myosin, a fast component with a lifetime of 0.2- 0.3 minutes, similar to that of purified myosin, and a slower component with a lifetime of 3.8 ± 0.4 minutes. We define the latter component to be the ''super relaxed state''. The fraction of myosins in the super relaxed state was decreased at lower temperatures, by substituting GTP for ATP or by increased levels of myosin phosphorylation. All of these conditions have also been shown to cause increased disorder in the structure of the thick filament. We propose a model in which the structure of the thick filament modulates the nucleotide turnover rates of myosin in relaxed fibers. Modulation of the relative populations of the super relaxed and conventional relaxed states would have a profound effect on muscle thermogenesis, with the capacity to significantly alter whole body metabolic rate. The mechanism proposed provides a new target for therapeutics with the potential to treat obesity or help in controlling high blood sugar levels.

748-Pos

Structural Impact Of Myosin Methionine Oxidation

Jennifer C. Klein, Nicole Piechowski, Margaret A. Titus, David D. Thomas.
Univeristy of Minnesota, Minneapolis, MN, USA.

We have examined the structural and functional consequences of methionine (Met) oxidation in Dictyostelium (Dicty) myosin II using a three-pronged approach that includes Met mutagenesis, site-directed spectroscopy, and molecular dynamics simulations. Protein oxidation by reactive oxygen species (ROS) is a critical element of cell function, but in the context of oxidative stress, has been implicated in disease progression and biological aging. Our goal is to bridge our understanding of protein oxidation and muscle dysfunction with molecular-level insights into actomyosin interaction. A Cys-lite version of Dicty myosin II serves as our model system for examining site-specific Met oxidation. Peroxide treatment to mimic oxidative stress induced a two-fold decline in Vmax and KATPase for actin-activation, consistent with the decline in actomyosin interaction observed for biologically aged or peroxide-treated skeletal myosin. We tested the oxidation sensitivity of previously characterized myosin labeling sites in the force-producing region and actin-binding interface and found that spin label mobility and distance measurements in the actin-binding cleft are particularly sensitive to Met oxidation, but only in the presence of actin. Moreover, we conclude that the oxidation-induced structural change in myosin includes a redistribution of structural states involved in the weak to strong actin-binding transition, the step associated with muscle force production. Site-specific Met substitutions combined with functional measurements have allowed us to pinpoint which Met is responsible for the observed structural change. Lastly, we will examine Met oxidation in silico to gain mechanistic knowledge of how residue-specific oxidation translates into changes in both local and global myosin structural dynamics. We expect that our results will be applicable to the many biological and pharmaceutical contexts in which a detailed understanding of protein oxidation, function and structure relationships are sought. This work is supported by the NIH training grant ''Functional Proteomics of Aging'' (T32AG029796).

749-Pos

Novel Approach Applied to IVMA to Study the Modulation of the Actomyosin Interaction by MgATP In Fast Skeletal Muscle

Monica CANEPARI[1], Manuela Maffei[1], Emanuela Longa[1],
Antonio Sabatini[2], Alberto Vacca[2], Roberto Bottinelli[1], **Stefano Iotti[3,4]**.
[1]University of Pavia, Pavia, Italy, [2]University of Firenze, Firenze, Italy, [3]University of Bologna, Bologna, Italy, [4]National Institute of Biostructures and Biosystems, Roma, Italy.

Table of Contents

In this study we used an ''in vitro motility assay'' (IVMA) approach to investigate the effect of the variation of [MgATP] in determining the number and the sliding velocity (Vf) of actin filaments moved by fast skeletal myosin. Vf was studied on type 2B HMM from rats at 25C°, 100mM ionic strength and at varius [MgATP], [MgADP] and [Pi]. We designed a new experimental set-up to perform experiments at lower ionic strength and in buffers that had no interference with the ionic environment, in order to mimic physiological condition. This set-up allowed a complete and chemical speciation of the solutions opening the possibility to perform an accurate thermodynamic study. Therefore, along with kinetic measurements also quantitative thermodynamics measurements were carried obtaining the $\in''G$ of MgATP hydrolysistaking into account pH and [Mg^{2+}]. We correlate the thermodynamics property of the system to Vf and to the number of sliding actin filaments which were assessed by a purpose-designed software. Preliminary results indicate: **a)** no straightly correlation between values of $\in''G_{ATP}$and the velocity of actin filaments, **b)** an increase in thenumberof sliding actin filaments at low [MgATP] and no changes when the ratio [MgATP] / [HMM] was kept constant **c)** a decrease in the velocity of actin filaments at [Pi]=30mM**.** More investigations are required to confirm the unexpected results that indicate a complex role of MgATP and its metabolites in the modulation of actomyosin interaction.

750-Pos

The Effects of Head-Head Interactions on Myosin-Based Actin Sliding Velocities

Del R. Jackson Jr., Travis J. Stewart, Josh E. Baker.
University of Nevada, Reno, Reno, NV, USA.

Myosin generates force with its weak-to-strong actin binding transition and senses force through a strain-dependent step closely associated with ADP release. We have developed both analytical and computational models of the cooperative interplay between these force-generating and force-sensing biochemical transitions. These models make several novel predictions for unloaded shortening muscle, such as rate constants, k_D, for ADP release that are [ADP]- and [ATP]-dependent. The model also predicts that the acceleration of k_{-D} is associated with a dissipation of interhead strain. To test these model predictions, we use an in vitro motility assay to determine the effects of [ADP] and [ATP] on actin sliding velocities, V, and to determine changes in interhead strain by measuring the rate of actin filament breaking. Our results show a non-hyperbolic nucleotide-dependence of V and a nucleotide-dependence of the rate of actin filament breaking that are both consistent with our cooperative model.

751-Pos

Single Molecule Stepping and Structural Dynamics of Myosin X

Yujie Sun[1], Osamu Sato[2], Felix Ruhnow[3], Mark E. Arsenault[1],
Mitsuo Ikebe[2], Yale E. Goldman[1].
[1]Pennsylvania Muscle Institute, Philadelphia, PA, USA, [2]University of Massachusetts Medical School, North Worcester, MA, USA, [3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Myosin X is an unconventional myosin motor protein with puzzling motility properties that are under debate. We studied the motility, angular motions and stepping of myosin X in vitro using single molecule fluorescence of rhodamine bound to the lever arm calmodulins (CaMs) and quantum dots on the CaMs and at the C-terminus of the heavy chain. Myosin X walks processively both on single actin filaments and actin bundles. The average step size, measured by FIONA, is 34 nm, supporting the postulate that an α-helical domain extends the lever arm beyond the binding region of myosin X for its three CaMs per head. The step size and velocity are smaller on actin bundles than individual filaments, suggesting that myosin X often steps onto neighboring actins in a bundle. Alternating larger and smaller steps with FIONA and alternating axial angles of the lever arm measured with polTIRF imply that myosin X steps in a hand-over-hand manner. Single molecule 3-dimensional (3D) tracking by Parallax of quantum dot-labeled myosin X on actin filaments and bundles suspended above the coverslip, by flow over ridges or by dielectrophoresis

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

Tomotaka Komori[1], Hiroaki Takagi[2], Masatoshi Nishikawa[3], Atsuko H. Iwane[1], Toshio Yanagida[1].
[1]Osaka University, Suita Osaka, Japan, [2]Nara medical university, Nara, Japan, [3]Hiroshima University, Hiroshima, Japan.

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

Michael J. Greenberg[1], James D. Watt[1], Katarzyna Kazmierczak[2], Michelle Jones[2], Danuta Szczesna-Cordary[2], **Jeffrey R. Moore**[1].
[1]Boston University, Boston, MA, USA, [2]University of Miami Miller School of Medicine, Miami, FL, USA.

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

Monica Canepari[1], Manuela Maffei[1], Emanuela Longa[1], Mike Geeves[2], **Roberto Bottinelli**[1].
[1]University of Pavia, Dept Physiology, Pavia, Italy, [2]University of Kent, Dept of Biosciences, Canterbury, United Kingdom.

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

Malin Persson[1], Nuria Albet-Torres[1], Leonid Ionov[2], Mark Sundberg[1], Fredrik Hook[3], Stefan Diez[2], Alf Mansson[1], Martina Balaz[1].
[1]School of Pure and Applied Natural Sciences, University of Kalmar, Kalmar, Sweden, [2]Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [3]Dept. of Applied Physics, Chalmers University of Technology, Gothenburg, Sweden.

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

Steven L. Lehman.
University of California, Berkeley, CA, USA.

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.