sequential processes desensitization and inactivation. Here we separate the properties of these two distinct but kinetically entangled processes. Dose-response curves measured with saturating pressure ramps ~ −200 mmHg of varied speed revealed 2 different activation regimes. Ramps >=1s (1–90s) displayed the lowest and stable $P_{1}/_{2}$ consistently near −120 mmHg. With a machine-limited speed of 10ms, we applied shorter ramps (20–500ms), which revealed an increasing shift of $P_{1}/_{2}$ to higher values apparently due to the slow kinetics of MscS opening. Holding the channel population at intermediate pressures was found to drive desensitization and revealed a third regime, a right-shift of dose-response curves by ~25 mmHg. A new double-pulse protocol revealed that desensitization occurs both above and below the channel's opening threshold. Recovery of desensitized channels to the closed state occurs quickly at zero pressure however this process is strongly inhibited by any residual tension. In contrast to desensitization, inactivation of MscS requires opening followed by transition to a desensitized closed state while tension is present. Inactivated channels that recover under low tension re-enter the desensitized state, not the closed state. Opposite tension dependencies of these two processes suggest that desensitization is associated with a reduction of channel in-plane area, whereas inactivation requires additional expansion. Finally, using triangular stimuli we show that MscS displays prominent hysteresis with ~20–30 mmHg rightward shift (lower pressure) during closing. Mutant channels that change the hydrophobic properties of the pore were shown to either abolish or make worse this hysteresis. It this appears that the open probability of MscS depends critically on the channel's tension prehistory.

Muscle Mechanics & Ultrastructure - I

611-Pos Key-Intermediate in Actomyosin Crossbridge-Cycle during Sliding: A Candidate for Pre-Power Stroke Configuration

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We are studying the conformational changes of myosin crossbridges during in vitro sliding, utilizing a novel image analysis to quantitatively compare freeze-replica microscopic images with the atomic models in various conformations. We reported that the lever-arm moiety of crossbridges there is mostly kinked to the opposite side of ADP/Vi-bound structure, implying the need for complete revision of conventional tilting-lever-arm hypothesis. Similar reversely-kinked heads were observed in SH1-SH2 crosslinked myosin. We generated a realistic model of that novel structure corresponding to sliding-intermediate by modifying known crystal structures. pPDM-crosslinked (between SH2 and Lys705) species was used as a start, since the EM image of the motor-domain was the closest among reported structures. Gly695, between oppositely-oriented SH1 and 2, is likely to be the hinge, considering the occurrence of chemical-crosslink between them. Assuming rotation around Gly695 with additional one at Gly765, we could generate a new model that explains all the observed images during sliding. We also tried to reconstruct the 3-D structure of SH-crosslinked analogue of the key-intermediate, from a series of classified and averaged freeze-replica images. Tentative result showed comma-shaped body, compatible to the kinked lever-arm structure abundantly observed during in vitro motility. We noticed the presence of small population of crossbridges in the same configuration but attached to actin filament in a different angle. Since the orientation of the motor-domain of that species relative to actin was identical to that in rigor, we assumed that could be a good candidate for the real pre-power stroke primed configuration, ready to convert to the rigor structure by a simple extension of the lever-arm. Assigning that new intermediate as pre-power stroke, primed configuration, as well as weakly-bound one, we can comprehensively explain all the observed images and previous experimental results.

612-Pos Conserved Glycine Mutants of Myosin

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Board B457

Myosin heavy chain has several highly conserved glycines including G362 and G407. G362 is found at the end of a helix-turn-helix segment on the C-loop known to communicate with the ATP binding site. G407 is part of the myopathy loop, imbedded in a cluster of potential heart disease causing mutations. G407 mutated to valine in β-cardiac myosin has been implicated in hypertrophic cardiomyopathy. Two mutant proteins (G362A and G407V) were constructed in smooth muscle HMM. ATPase, actin-activated myosin ATPase, actin binding, and *in vitro* motility assays were preformed to determine the functional significance of their conservation. The mutations did not affect strong actin binding compared to wild type HMM and Ca^{2+} and Mg^{2+} ATPase assays showed minimal differences among the three proteins, however, EDTA ATPase activity was reduced in both mutants. G407V showed a decreased actin-activated ATPase and a corresponding lower actin motility velocity. In contrast, G362A showed a marked increase in both the actin-activated ATPase and the motility velocity.

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613-Pos R777 Modulates Intrinsic Fluorescence Changes in Smooth Muscle Myosin

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Tryptophan 512, located in the rigid relay loop, is the principle endogenous tryptophan sensitive to both nucleotide binding and

hydrolysis during the ATPase cycle of smooth muscle myosin. There is a significant fluorescence enhancement observed in the presence of ATP without a significant change in the peak emission maximum, suggesting the polarity of the environment around this side chain changes very little during the ATPase cycle. Solvent accessibility and analysis of the solvent accessible surface of the crystal structure suggest W512 remains predominantly buried in all states of the ATPase cycle. Furthermore, both the steady state and time-resolved quantum yields of W512-MDE are similarly dependent on nucleotide, suggesting that excited state mechanisms are primarily responsible changes in the intrinsic fluorescence of W512. Time-resolved lifetime decays from W512-MDE are best fit to three exponentials in the apo state but only two exponentials in the presence of nucleotide. The additional fast relaxation process observed in the absence of nucleotide suggests a specific interaction of W512 with a nearby side chain residue. Examination of available crystal structures reveals that R777 is close enough and properly oriented to interact with the pi-electrons of W512 in the apo-state, but not in the presence of nucleotide. Consistent with this hypothesis, a mutant lacking R777 (R777A-MDE) displayed a significant decrease in the steady state nucleotide-dependent fluorescence emission enhancement in the wild-type background. Thus our evidence suggests that nucleotide dependent changes in W512 fluorescence are associated with the close proximity of R777 in smooth muscle myosin.

614-Pos The Flight Muscle of Cicada Is Synchronous, But Is Crystalline and Stretch-Activatable

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Board B459

Asynchronous flight muscles of waterbugs, bees, flies, etc. are characterized by their ability of stretch activation (SA) and the crystalline arrangement of sarcomeric proteins as is evident from the sharply lattice-sampled layer-line reflections in the X-ray diffraction pattern. On the other hand, such features are much less pronounced in the synchronous flight muscles of moths, dragonflies, etc. Here we show that the flight muscle of cicadas (known to be synchronous) exhibits small but clear SA, and that its layer-line reflections are sharply lattice-sampled as in asynchronous flight muscles. The resting stiffness per cross-sectional area of the flight muscle fibers of a cicada, *Meimuna*, is about 1/10 of that of a giant waterbug, *Lethocerus* (the most extensively studied insect with asynchronous flight muscle), and the amount of stretch-activated force is proportionately small. As a typical synchronous reference, the flight muscle of a giant silkworm moth, *Antheraea*, shows even smaller resting stiffness. Its amount is about half of that of *Meimuna* and the amount of stretch-activated force is also even smaller. Therefore, the ability of SA seems to be common among flight muscles, synchronous or asynchronous, and its magnitude seems to be related to the amount of resting stiffness, which originates from elastic proteins that connect the end of the thick filaments to the Z-lines. Cicadas may be in the process of developing asynchrony, or alternatively, in the process of losing the features of its smaller, once asynchronous ancestor as a result of increased body size.

615-Pos Myosin Phosphorylation Inhibits the Velocity of Skeletal Muscle Fibers in the Presence of Blebbistatin

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Phosphorylation of the skeletal myosin regulatory light chain occurs during heavy use and fatigue, and may play a role in the inhibition of fiber velocity observed in vivo. We studied permeable rabbit psoas fibers in the presence of the myosin inhibitor blebbistatin, which inhibited tension by 75%. Shortening velocities are decreased by 40% in fibres where myosin is phosphorylated compared with those in fibres with dephosphorylated myosin (3.1±0.3 lengths/sec vs. 5.7±0.5 lengths/s; 30°C). No difference in shortening velocities was found with dephosphorylated myosin fibers with or without blebbistatin and phosphorylated myosin fibers in the absence of blebbistatin. Isometric tensions are equally decreased in fibres with phosphorylated and dephosphorylated myosin by the presence of blebbistatin. The Km for the maximum velocity of shortening as a function of [ATP] for fibers with phosphorylated myosin was significantly lower than that from dephosphorylated fibers (50±20μMATP versus 330±84μMATP) indicating that the binding of ATP is much stronger in phosphorylated fibers. Similar results (a 40% decrease in velocities and smaller values of Km) for phosphorylated fibers compared with dephosphorylated fibers have been found in the presence of the phosphate analogue vanadate (Franks-Skiba, et al., 2007). Blebbistatin is thought to form a complex with myosin and ADP the structure of which may be similar to that of the complex myosin. ADP.Pi; this complex forms prepower stroke cross bridges that mimic a state in the actomyosin cycle populated during fatigue. Myosin phosphorylation destabilizes the binding of pre-power stroke myosin heads to the thick filament, and it is possible this allows them to bind to actin and exert a drag that inhibits velocity. Alternatively the lower Km of phosphorylated fibers suggests a direct effect on binding and release of nucleotides.

616-Pos Sarcomere Length Dependence Of Power Output Is Increased After Pka Treatment In Rat Cardiac Myocytes

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Board B461

The Frank-Starling relationship of the heart yields increased stroke volume with greater end-diastolic volume and this relationship is steeper following β-adrenergic stimulation. The underlying basis for the Frank-Starling mechanism involves length dependent changes in Ca^{2+} sensitivity of myofibrillar force and power output. In this study, we tested the hypothesis that PKA-induced phosphor-

ylation of myofibrillar proteins would increase length dependence of myofibrillar power output, which would provide a myofibrillar basis to, in part, explain steeper Frank-Starling relations following β-adrenergic stimulation. For these experiments, adult rat left ventricles were mechanically disrupted and permeabilized cardiac myocyte preparations were attached between a force transducer and position motor and length dependence of loaded shortening and power output were measured before and after treatment with PKA (Upstate, 0.015U/μl). PKA increased phosphorylation of myosin binding protein-C (MyBP-C) and cardiac troponin I (cTnI) as assessed by autoradiography. In terms of myocyte mechanics, PKA decreased Ca^{2+} sensitivity of force and increased loaded shortening and power output at all relative loads when the myocyte preparations were at long sarcomere length (~2.30 μm). PKA had less of an effect on loaded shortening and power output at short sarcomere length (~2.0 μm). These changes resulted in greater length dependence of myocyte power output after PKA treatment; peak normalized power output increased ~20% with length before PKA and ~50% after PKA. These results suggest that PKA-induced phosphorylation of myofibrillar proteins explains, in part, the steeper ventricular function curves (i.e., Frank-Starling relationship) following β-adrenergic stimulation of the left ventricle.

617-Pos During Lengthening Contractions Of Permeabilized Single Muscle Fibers, The Regions At The Longest Initial Sarcomere Lengths Undergo The Greatest Elongation

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Board B462

Steady-state models of muscle contraction predict that when maximally activated fibers are lengthened, longer sarcomeres stretch more than shorter sarcomeres, increasing the likelihood that the longer sarcomeres will become damaged by overstretch. We tested the hypothesis that regions of activated fibers that contain sarcomeres at longer lengths prior to a lengthening contraction undergo greater increases in the lengths of sarcomeres (L_s) during the lengthening contraction. To test this hypothesis, we used a laser diffraction technique to make rapid measurements (500 s^{-1}) of the L_s in 20 contiguous regions of fibers undergoing lengthening contractions with a strain of 27 % and a strain rate of 54 % s^{-1}. Fiber segments of length 1.39 ± 0.13 mm (n = 12) were obtained from soleus muscles of rats and the central 1.04 ± 0.11 mm were investigated. During steady-state activation prior to the lengthening, the fibers produced a peak isometric stress of 132 ± 27 kN m^{-2} at a mean L_s of 2.54 ± 0.12 μm. The lengthening contractions resulted in a 17 ± 8 % loss in isometric stress. For each of the 20 regions, the pre-stretch L_s and the increase in L_s at the peak of the stretch were computed and compared, revealing that:

(i) the stretch was not distributed uniformly along the length of a fiber, and

(ii) the regions that were at long L_s prior to the stretch elongated relatively more during the stretch than did the regions with shorter L_s.

The findings support the hypothesis that during contraction-induced injury to serially connected sarcomeres, longer sarcomeres are more likely to be damaged due to excessive lengthening.

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618-Pos Enthalpy/Entropy Compensation Renders Tension Generation in Muscle Unresponsive to Strain

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The kinetics of tension generation can be studied by applying small perturbations to single muscle fibers contracting under isometric conditions and analyzing the resultant multi-exponential tension transients. Forward and reverse rate constants of tension generation in the absence of movement were recently determined from laser temperature-jump tension transients (Davis & Epstein Biophys J. 2865–74, ***92***, (2007)). Here, step-changes in length were applied to fibers to probe the consequences of movement on the tension-generating isomerization. Increasing temperature accelerates the forward, and slows the reverse rate constant of tension generation by phase 2_{slow}: kinetics, similar to the laser temperature-jump data. Length-jump and temperature-jump reaction amplitudes, however, respond quite differently to increasing temperature. Direct perturbation of tension generation by temperature results in a bell-shaped dependency. The linear dependency of the length-jump data excludes a direct perturbation mechanism. Apparently, movement activates actin-attached non-tension-generating A.M.ADP cross-bridges during the step-release to later generate tension with the kinetics of phase 2_{slow}. This unexpected insensitivity of tension generation to strain arises from a specific molecular mechanism: Length-jump activation enthalpy and entropy values obtained from Arrhenius plots of the forward rate constant of tension generation for small (−0.75) and larger (−1.50 nm/half sarc.) step-releases show a decrease in the contribution of $\Delta H^{\ddagger}$, and a matched increase in the contribution of $\Delta S^{\ddagger}$ to the $\Delta G^{\ddagger}$ for tension generation the lower the average strain. The reverse reaction shows matching reciprocal changes in activation parameters. This near complete entropy/enthalpy compensation results in a strain-independent equilibrium constant and thus free energy (ΔG°) for tension generation. Contrary to expectation, a drop in crossbridge strain does not result in a compensatory rise in tension by a shift from the pre-tension to the tension generating state.

619-Pos Targeting Myosin ATPase to Increase Cardiomyocyte Contraction Without Affecting Relaxation

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Many heart failure therapies can have significant functional side-effects, often by directly or indirectly affecting intracellular [Ca^{2+}] ([Ca^{2+}]$_i$). In this study, we directly targeted the cardiac actin-myosin 'crossbridge' cycle to enhance intact cardiomyocyte contraction independent of altered [Ca^{2+}]$_i$. Direct crossbridge enhancement was targeted by increasing intracellular 2-deoxy-ATP ([dATP]) in cells via over-expression of the ribonucleotide reductase complex (RRM1-RRM2) that converts ATP to dATP. In demembranated cardiomyocytes we have demonstrated that substitution of ATP with dATP increases the magnitude and rate of force production at all levels of Ca^{2+}-mediated activation. Neonatal cardiomyocytes over-expressing RRM1/2 had significantly increased rate (RRM1/2 = 1.43 ± 0.36 ML/s; control = 0.65 ± 0.17 ML/s) and extent of shortening (RRM1/2 = 10.6 ± 2.7 %; control = 5.0 ± 1.2 %) as compared to control (GFP-expression only), with no affect on the rate of relaxation (RRM1/2 = 0.57 ± 0.16 ML/s; control = 0.40 ± 0.12 ML/s. HPLC analysis indicated a significant increase of intracellular [dATP] in RRM1/2 transfected myocytes that reflected an increase from ~0.01% (in GFP-expression only) to ~1% of the total nucleotide pool. Intriguingly, this relatively low dATP content that is concomitant with increased contractility suggests that produced dATP may have higher affinity for myosin ATPase than other cellular ATPases. These experiments demonstrate the feasibility of directly targeting the actin-myosin crossbridge to enhance cardiac contractility without altering diastolic function.

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620-Pos Actin in Intermediate States of ATP Hydrolysis Cycle in Psoas Muscle Fibres by EPR and DSC

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The effects of beryllium and aluminium fluoride in the presence of ATP were studied on glycerinated muscle fibres prepared from psoas muscle of rabbit. ADP. and ADP. states model the transition states of the ATP hydrolysis cycle which precede the powerstroke of the muscle fibres. Analysis of transition temperatures by DSC showed that beryllium and aluminium fluorides, similar to vanadate affected strongly the thermal stability of fibres. The largest change could be ascribed to the interaction between the nucleotide and the catalytic domain of myosin as well as between nucleotide and actin, whereas the interaction between other domains was relatively small. Electron paramagnetic resonance spectra of spin-labelled muscle fibres recorded in intermediate states showed that ADP. and ADP.V_i states differed slightly from each other, indicating similar dynamic states, whereas the strong binding states of myosin to actin could be determined by the orientation dependence of the attached probe molecules. The EPR spectrum of ADP. state was a superposition of two spectra, characterising different dynamic states of myosin heads in muscle fibres. The thermal denaturation experiments support the results of Muhlrad et al. (J. Biol. Chem. *269*: 11852–11858, 1994; Biophys. J. *91*: 4490–4499, 2006) for the cooperativity of BeF_x binding to F-actin.

621-Pos Effect of Orthovanadate on the Kinetics of Actin-Myosin Interaction Studied in Skinned Fibers from Rabbit Psoas Muscle

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Ortovanadate (Vi), an inorganic phosphate (Pi) analogue, reduces the isometric force (T_0) and the half-sarcomere (hs) stiffness and inhibits the myosin ATPase because it forms a stable inactive myosin-ADP-Vi complex (Goodno, *Proc Natl Acad Sci 76*:2620–2624, 1979; Dantzig and Goldman, *J Gen Physiol 86*:305–327, 1985; Chase et al., *J Physiol 460*:231–246, 1993). After correction for myofilament compliance, the observed changes in stiffness show that the reduction in T_0 is explained by a proportional reduction in the myosin motors attached to actin (Caremani et al., *Biophys J* 480a/2289-Pos, 2007). We investigated the effects of Vi on the mechanics and kinetics of the actin-myosin interaction in skinned fibres from rabbit psoas (sarcomere length 2.4 μm) at different [Pi] (range 0–15 mM added Pi), by determining T_0 and the rate constant of force development following a period of unloaded shortening (k_D). Fibers were activated by using a system that allows arapid rise in fiber temperature from ~1°C to the test temperature (12 °C), with preservation and control of sarcomere length (Linari et al., *J Physiol 554*:335–352, 2004). At each [Pi], addition of 0.1 mM Vi reduced T_0 to ~0.4 of T_0 in the absence of Vi and k_D to ~0.9 of k_D in the absence of Vi. These results suggest that Vi acts as a competitive inhibitor of Pi for the actin-myosin-ADP state.

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622-Pos ADP Modulation Of Actin Sliding Velocity On Slow And Fast Skeletal Myosin Isoforms At Different Temperatures

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It is generally believed that the rate of ADP release from acto-myosin defines unloaded shortening velocity (Vo) of skeletal myosin. However, we have recently suggested that at least at low temperature (12 °C) the rate of acto-myosin dissociation by ATP could play a significant role in defining Vo of fast, and not of slow skeletal myosins. Interestingly, the temperature dependence of ATP induced dissociation and of ADP affinity of acto-myosin for fast and slow isoforms suggested that, at temperatures above ~25°C, the ADP release can limit velocity of both isoforms. In this study we have used an in vitro motility assay (IVMA) approach and studied the effect of MgADP on the sliding velocity of actin (Vf) on slow and fast skeletal myosin isoforms from the rat in the absence and in the presence of 2mM MgADP. MgATP concentrations were varied in the range 0.01mM and 2mM, at 25 and 15°C and 50mM ionic strength. At 25°C the presence of MgADP decreased Vf of fast and slow myosin (Km of 73 and 32µmol/L respectively) and shifted the substrate concentration dependence toward higher MgATP concentrations (Ki of 215 µmol/L and 66 µmol/L respectively). At 15°C the effect of MgADP on fast isoforms yielded a Ki of 293µmol/L. The results suggests a more significantly role of ADP release in defining velocity in slow than fast myosin and, in fast isoforms, at high than at low temperature. The analysis on rat slow myosin at 15°C is ongoing.

623-Pos Effects of Elevated Solvent Viscosity on Myofilament Contractility of Single Skinned Porcine Cardiomyocytes

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We have previously shown with skinned skeletal muscle fibers at maximum Ca^{2+} activation and unregulated *in vitro* motility assays that solvent viscosity modulates actomyosin function in a manner consistent with diffusional limitation of a kinetic process. To further assess the viscosity effect on myofilament contractility, especially at submaximum Ca^{2+} level, single skinned porcine cardiomyocytes were used to measure steady-state force and the kinetics of tension redevelopment (k_{TR}) when viscosity within the myofilament lattice was elevated by adding the low molecular weight sugar sucrose to the bathing solutions. Maximum Ca^{2+}-activated force decreased as sucrose was increased above 0.3 M ($\dagger/\dagger_0 \sim 1.4$). Maximum k_{TR} decreased steeply and monotonically as sucrose was increased. Ca^{2+}-sensitivity of isometric force (pCa_{50}) decreased by ~ 0.3 pCa units with 0.3 M sucrose. While 0.3 M sucrose lowered k_{TR} at high [Ca^{2+}], there was little or no effect at low [Ca^{2+}]. To investigate observed effects of elevated solvent viscosity, we explored a three-state numerical model of Ca^{2+}-regulation in cardiac muscle (Hancock, et al., 1997). To simulate elevated viscosity, we lowered the rate constants for Ca^{2+}-induced activation of thin filament regulatory units (k_{on}) and cross-bridge association (f) by assuming that rate constants changed proportional to $(\dagger/\dagger_0)^{-1}$. This simulation predicted steady state and kinetic changes in the direction observed experimentally, but did not fully explain the extent of inhibition of Ca^{2+}-dependent myofilament function. Modeling therefore suggests sucrose may modulate kinetic processes in addition to Ca^{2+} turning on regulatory units and cross-bridge association.

624-Pos Mechanisms For The Effects Of Viscous Solutes On Actin Sliding Velocities

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Board B469

Sugars and other viscous solutes have been shown to slow myosin-based actin sliding velocities, *V*, *in vitro*, yet the mechanism for this effect remains unclear. It has been suggested that the mechanism is not a drag force on actin but rather a more local effect on myosin dynamics. To better understand the mechanisms by which viscous solutes affect actin sliding velocities, we use an *in vitro* motility assay to study the effects of sucrose on the myosin-, ATP-, and P_i-dependence of actin sliding velocities. We observe that actin sliding velocities, *V*, are slowed approximately 60% upon addition of 50% sucrose, and that *V* exhibits a strong P_i-dependence in the presence but not in the absence of sucrose. Our results indicate that sucrose imposes a load capable of reversing the weak-to-strong binding, P_i-release step. These results demonstrate that viscous solutes might provide a useful tool for mechanically loading in vitro motility assays.

625-Pos Single Molecule Measurement of the Stiffness of the Rigor Myosin Head

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The force-extension curve of single myosin subfragment-1 molecules, interacting in the rigor state with an actin filament, has been investigated in the presence of low [ATP] by applying a triangle-wave movement to the optical traps holding a bead-actin-bead dumbbell and, consequently, a linearly increasing force on the myosin head while it is bound to actin. In combination with a measurement of the overall stiffness of the dumbbell, this allowed characterisation of the three extensible elements, i.e. the actin-bead links and the myosin. Their stiffnesses were compared with those deduced from a previously reported covariance method (Smith *et al.*, Biophys. J. ***81***, 2795) and were in reasonably good agreement. Using the latter method, a larger data set from 166 myosin molecules gave a mean estimate for the myosin stiffness of 1.79 pN/nm with a standard deviation of 0.7 pN/nm and (SEM 0.06 pN/nm). These values are consistent with the most recent report (1.7 pN/nm) for a rabbit myosin head in a muscle fibre (Linari et al, Biophy. J. ***92***, 2476). In the triangle-wave protocol, the motion of the trapped beads

during interactions was linear over the physiological range of force applied to myosin (±10 pN), consistent with the myosin stiffness being Hookean, but the data set did not allow the limitations on linearity to be confidently assigned. It was also observed that the maximum size of the force excursions in the direction that resisted the working stroke were smaller than those in the direction that assisted it, indicative of the effect of strain on the rate of dissociation.

626-Pos ATPase Activity Of Myofibrils Immobilized On A Substrate

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Board B471

We have developed a method for more accurate measurement of ATPase activities in myofibrils. Myofibrils were immobilized on a glass surface, which preserved a well-ordered sarcomere array during measurement of ATPase activities, and allowed multiple sequential assays. In conventional assays, unfixed myofibrils shorten; or conventional cross-linking techniques, which prevent shortening, fix a fraction of the myofibrillar proteins. Myofibrils were first bound to a polylysine coated glass surface. Cross-linking was achieved using polyglutamic acid activated by EDC, which reacts with both the polylysine and with lysines in the myofibrils thus attaching the myofibrils covalently to the glass surface. The size, 61kD, and charge of the polyglutamic acid prevent it from diffusing into the myofibril and reacting with proteins in the interior. The sarcomere patterns of the covalently attached myofibrils did not change upon activation at 23°C. The ATPase activity of the myofibrils could be measured and remained stable for up to 5 sequential, 30 second measurements. In contrast to myofibrils fixed by conventional techniques, the myofibrils had a lower ATPase activity in relaxing solutions, suggesting that the reaction of the polyglutamic acid with the myofibrils is confined to the surface. Reaction of the polyglutamic acid with myofibrils that were free in solution, did not prevent subsequent shortening upon activation showing that the reaction between the myofibril and the poly lysine coated surface is required to stabilize the sarcomere pattern. The system developed here allows measurement of multiple sequential ATPase activities on a system with a well defined sarcomere array.

627-Pos Number Fluctuation of Cross-Bridges in Muscle Revealed by Rupture Force Measurements at the Single-Sarcomere Level

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Board B472

We investigated the number fluctuation of working cross-bridges in skeletal myofibrils from rupture response of individual sarcomeres against an externally applied load. A single myofibril was held with a pair of glass micro-needles under a phase-contrast microscope, so that both the length-response of individual sarcomeres and the active force development were simultaneously recorded. To emphasize the dynamics of cross-bridge binding to the thin filament, the myofibrils were activated in the absence of Ca^{2+}, by adding exogenous MgADP (2–4 mM) with 1 mM MgATP, in which the thin filament activity was directly regulated only by the ADP-bound cross-bridges. Against a quick stretch, actively contracting sarcomeres abruptly lengthened by more than 300 nm within 30 ms, accompanied by a rapid force decay. This indicates that the ADP-bound cross-bridges, which function as activators under these conditions, were forcibly ruptured by external loads. In addition, this phenomenon occurred locally in a myofibril and stochastically at different regions from trial to trial when the external load was within ~10% of the active isometric force, while the frequency of its occurrence was independent of sarcomere length. These results suggest that the stability of individual sarcomeres is fluctuating within a myofibril. From the load- and [ADP]-dependent frequency of the rupture in individual sarcomeres we estimated the degree of fluctuation in the force-generating capacity around the active isometric force. The rupture force measurements at the single-sarcomere level revealed that the cross-bridges work stochastically in a higher ordered lattice structure, sarcomere.

628-Pos Direct Observation of Rotations of Actin Monomer During Contraction of Skeletal Muscle Myofibrils

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Board B473

During interaction of actin with myosin, cross-bridges impart mechanical impulses to thin filaments resulting in rotations of actin monomers. It is believed that splitting of one molecule of ATP leads to a single impulse. In order to test whether this is so in working muscle, individual impulses were visualized during isometric contraction of skeletal muscle myofibrils. Myofibrillar actin was labeled with a 10^5:1 mixture of non- fluorescent: fluorescent phalloidin, assuring that on average less than one molecule of actin per half-sarcomere was fluorescent. The intensities of the polarized TIRF images of the half-sarcomeres of isometrically contracting, but not of rigor, myofibrils were oscillating. The average frequency of oscillations was consistently less than ATPase, suggesting that in isometrically working muscle the bulk of the energy of ATP hydrolysis was used for purposes other than performance of mechanical work.