Muscle Mechanics & Ultrastructure - II

629-Pos Stabilisation Of The Helical Order Of Myosin Filaments By Blebbistatin

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Blebbistatin (Straight, A.F. et al. Science *299*, 1743 (2003)) inhibits nonmuscle myosin II and certain isoforms of muscle myosin II (Kovacs, M. et al., J. Biol. Chem. *279*, 35557, (2004)). It binds in a hydrophobic pocket at the apex of the 50-kD cleft (Allingham, J.S. et al., Nat. Struct. & Mol. Bio. *12*:378 (2005)). In the present study, its structural effects in muscle fibers were investigated by X-ray diffraction. It was previously shown that X-ray diffraction patterns originating from the thick filaments directly reflect the conformations of the myosin molecules (Xu, S. et al., Biochem. *42*, 390 (2003)). The equilibrium between the disordered and ordered states of the filament, as measured by the intensities of the myosin layer lines, directly correlates with the fraction of myosin heads in the switch-II closed conformation. Present results show that significant increases in the intensities of myosin layer lines with the addition of blebbistatin were observed over the temperature range of 5C to 35 C in the presence of MgATP, MgADP or even in the absence of nucleotide. Normally, the equilibrium with either ADP bound or in the absence of bound nucleotide is overwhelmingly in favor of the switch-II open conformation. These results indicate that blebbistatin produces a shift in the equilibrium towards the switch-II closed conformation and support the idea that myosin can assume multiple conformations in equilibrium regardless of ligand bound at the active site.

630-Pos Head-head Interaction Characterizes the Relaxed State of Scallop and *Limulus* Muscle Myosin Filaments

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Muscle contraction is regulated by switches associated with the actin and myosin filaments. Myosin is regulated either by phosphorylation of the regulatory light chain or Ca^{2+} binding to the essential light chain. The switched-off state of vertebrate smooth muscle myosin molecules (phosphorylation-regulated) involves interaction between the two myosin heads, blocking actin-binding and ATP hydrolysis sites (Wendt et al., 2001). Similar interactions are seen in native myosin filaments from tarantula muscle (also phosphorylation-regulated; Woodhead et al., 2005). Our goal was to determine whether head-head interaction is a general regulatory structural motif that is present in other phosphorylation-regulated filaments and also in filaments regulated by direct Ca^{2+} binding to myosin. Filaments were purified from *Limulus* (phosphorylation-regulated) and scallop (Ca^{2+}-regulated) striated muscles and examined under relaxing ionic conditions by cryo-EM. Three-dimensional reconstructions were carried out by single particle methods. Reconstructions of *Limulus* filaments revealed intramolecular head-head interactions that were essentially identical to those in tarantula. Interaction between different myosin molecules, and head interaction with S2 were also similar. The myosin tails appeared to be arranged in twelve parallel subfilaments forming the filament backbone, as seen in tarantula. Similar intramolecular head interactions were also seen in scallop filaments, although the head-head motif as a whole was differently arranged on the filament surface. The myosin tails appeared to be organized into seven groups of subfilaments following the path of the myosin head helices, different from the organization in the arthropod muscles. We conclude that head-head interaction is a common mechanism for switching off myosin filaments in muscles with different regulatory mechanisms, in agreement with studies of single molecules (Jung et al., 2004; Jung and Craig, 2006).

631-Pos

No Abstract

632-Pos Cardiac Thick Filaments: Indirect Evidence Suggesting that the Density of cMyBP-C Contributes to the "Forbidden" Meridional Reflections in Fourier Transforms

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Although the filaments appear similar, the Fourier transforms of cardiac thick filaments isolated from cMyBP-$C^{-/-}$ knockout mice differ from the transforms of thick filaments from wildtype hearts in the weakness or absence of the "forbidden" meridionals on the 2nd, 4th, 5th, and 8th layer lines (Kensler and Harris, 2007, Biophys. J. *92*. 297a.). These "forbidden"meridional reflections are thought to arise either from the presence of a perturbation from ideal helical symmetry in the crossbridge array or from the accessory proteins along the filament (Huxley and Brown, 1967, J. Mol. Biol. *30*:383–434). The weakness of the reflections could result either from the crossbridges assuming a more helical arrangement in the absence of cMyBP-C or if the density associated with cMyBP-C directly contributes to the reflections and in its absence the reflections are weakened. To test the hypothesis that the density of cMyBP-C contributes directly to the "forbidden" meridional reflections, we calculated Fourier transforms of the wildtype filaments and masked them to either include or exclude the data from the "forbidden"

meridional reflections. Fourier filtered images were calculated from the masked transforms. The difference image between the filtered images was calculated to reveal any changes that occurred in the filtered images as a result of exclusion of the "forbidden" meridional reflections. A consistent change in density near the level 1 cross-bridge crown was found that correlated well with the difference between filtered images from the wildtype and cMyBP-C$^{-/-}$ knock-out mouse cardiac thick filaments. The results suggest that this density correlates with the presence of the "forbidden" meridional reflections, and is the location of cMyBP-C on the filament.

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633-Pos Structural dynamics of the skeletal muscle fiber in different physiological states by Second Harmonic Generation

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The high degree of intrinsic structural order in skeletal muscle allows imaging of this tissue by Second Harmonic Generation (SHG). As previously shown by fractional extraction of proteins (Vanzi *et al.*, J. Muscle Cell Res. Motil. 2006), myosin is the source of SHG signal. The characterization of the polarization-dependence of the SHG signal provides very selective information on the orientation of the emitting proteins and their dynamics during contraction. We developed a line scan polarization method in order to perform measurements of a full polarization curve in intact muscle fibers from frog skeletal muscle (Rana esculenta, 10°C). These measurements allow the characterization of the dependence of the SHG polarization on different physiological states (resting, rigor and isometric tetanic contraction) over a wide range of sarcomere lengths (between 2.0 um and 4.0 um). The polarization data have been interpreted by means of a model in terms of the average orientation of SHG emitters. The different physiological states are characterized by distinct patterns of SHG polarization and the variation of the orientation of emitting molecules in relation to the physiological state of the muscle demonstrates that one part of SHG signal arises from the globular head of the myosin motor that cross-links actin and myosin filaments. The dependence of the SHG modulation on the degree of overlap between actin and myosin filaments during an isometric contraction provides the constraints to estimate the fraction of myosin motors generating the isometric force in the active muscle fiber.

634-Pos Quick-Freezing Muscle In Slush-cooled Liquid Nitrogen For Combined X-ray Cryodiffraction And Electron Microscopy

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Glycerinated Lethocerus insect flight muscle (IFM) fibers, 65–70 μm x 12–16 mm, can be cryo-vitrified for x-ray and EM study by plunge-freezing in slush-cooled liquid nitrogen (SC-LN2, -210°C) using thin enough bundles with 15–20% glucose or methyl-pentanediol (MPD) as cryoprotectant (CP). Plunging thermocouples (TCs) report cooling rates 4-9X faster in SC-LN2 than in boiling nitrogen (B-LN2, -196°C), probably because "film boiling" insulates TCs in B-LN2 but not SC-LN2 (MacKenzie, 1969, Biodynamica *10*:341–351). Our present best: 38,000-55,000°K/s (40μm Type E TC), is 2-3x faster than in B-LN2. Walker etal (1998, J Appl Cryst *31*:954–956) measured equal cooling rates in B-LN2 and propane, so SC-LN2 may yet match/exceed the 300,000°K/s reported by 30 μm TCs (Type T) plunged into propane (−190°C) (Costello etal, 1984, p105-115 in Science Biol Specm'n Prep'n for Microsc). Maximum cooling-rate protocols measured by 30 μm TCs should allow lowest cryoprotectant percentage, and would likely underestimate freeze-trapping rates at depths 0–10 μm. If cryo-vitrified, such depths could permit exhaustive cryo-microdiffraction (2μm x-ray beam) and thin-section EM. In our 8-fiber bundle (MPD-CP) frozen in B-LN2, X-ray cryo-diffraction (−160°C) showed hexagonal ice absent, but amorphous plus cubic ice present; thin-section EM after freeze-substitution showed apparent vitrification across 2/3 of 200μm diameter, and modest freeze-damage across the rest. A 160 μm bundle (glucose-CP) cryodiffracted mainly as amorphous ice, but was lost before EM. Gently exhaling into a falling shroud that arrests 1–2 mm above coolant can avoid gas pre-cooling (Warkentin etal, 2006, J Appl Cryst *39*:805–811) and prevent evaporative self-cooling, but risks depositing water droplets that can freeze on specimen surfaces. A proven SC-LN2 plunge freeze design, and a planned design for time-resolved freezing of mechanically monitored fiber bundles, using a 50–250 ml jet of SC-LN2, will be shown.

635-Pos Visualization of Active Muscle Cross-bridges Using Electron Tomography (ET): The Challenge of Obtaining 3-D Images of Highly Heterogeneous Structures

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As a vehicle for visualizing macromolecules, a raw tomogram is very noisy. Retrieval of molecular conformations requires intensive image processing. We use ET and sub-volume processing to obtain 3-D images of active cross-bridges of quick-frozen *Lethocerus* flight muscle. Sub-volume contains a 38.7nm length of thin filament and its bound cross-bridges. After alignment, the global average reveals actin monomers, bound catalytic domains and predicted locations of troponin complexes. To characterize whole cross-bridges, particularly the lever arm, is a great challenge.

Active cross-bridges are distributed over the entire catalytic cycle and over the entire actin filament with a variety of orientations. To improve signal-to-noise-ratio by averaging requires precise grouping of sub-volumes with similar features. Multivariate-data-analysis (MDA) identifies patterns in data. By convention, we perform cluster analysis after MDA. Class averages are computed over a mosaic of sub-regions within each sub-volume. Final averages are reassembled from the mosaic. However, with various experiments, the intra-class signal-related variation is always too big to neglect, implying "signal" itself is completely heterogeneous. Therefore, we turn to eigen-images generated from MDA. We take advantage of the condensation of signal-related variance in the highest-ranking eigen-images and reconstitute each sub-volume through a linear combination of these eigen-images.

Both methods exhibit similar details of thin filament, but the reconstituting method obtains clearer definition of lever arms. Analysis of actin occupancy by myosin heads indicates the target-zone is mid-way between troponins and spans two actins on each long-pitch strand. Variable links other than target-zone cross-bridges are also preserved: 45% are myosins on non-target-zone non-troponin actins, 19% troponin-attached-bridges, and the rest, too thin to be myosin, might be the floating extensions from troponin-H or regular light chain N-terminus.

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636-Pos Single Skeletal Muscle Fiber Mechanics and Myosin Kinetics in Humans

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We have modified existing techniques for small amplitude sinusoidal length perturbation analyses to measure the mechanical and kinetic properties of human skeletal muscle fibers obtained from needle biopsies of the *vastus lateralis* (quadriceps). Humans have three myosin heavy chain (MHC) isoforms (I, IIa, IIx) with unloaded shortening velocities that increase in the order: I < IIa < IIx. Under maximally Ca^{2+}-activated (pCa 4.5) conditions, we found that the characteristic frequencies of cross-bridge work and oscillatory power production from fibers expressing exclusively one type of MHC isoform increased in the same order as unloaded shortening velocity. Myosin attachment times, calculated from sinusoidal analysis curve-fitting techniques (Palmer et al., Biophys. J. *93*, 760, 2007), decreased in the order of I > IIa > IIx, as expected. These measurements represent the first cross-bridge kinetic measurements from single human skeletal muscle fibers. Under relaxed conditions (pCa 8), MHC-I fibers had increased elastic and viscous modulus compared to IIa and IIx fibers, indicating MHC-I fibers are stiffer longitudinally. The higher stiffness could be due to:

1. stiffer passive elements in the thick, thin or connecting filaments,
2. the presence of weakly bound myosin heads or
3. a combination of both.

Interestingly, preliminary experiments on fibers osmotically compressed to estimated *in vivo* lattice spacing with 5% w/v Dextran T-500 showed an increase in both attachment time and passive stiffness for all fiber types compared to uncompressed fibers, suggesting that mechanical and kinetic properties of the fibers are dependent on myofilament lattice spacing. Our experiments highlight the utility of sinusoidal analysis to examine the fundamental mechanical and kinetic properties of human skeletal muscle.

637-Pos A Second Population of ATP-dependent Myosin Crossbridges Arises with Acidosis in Human Skeletal Muscle

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Acidosis is thought to reduce force production in fatiguing skeletal muscle. We examined the role of pH on force production and myosin crossbridge kinetics. A *vastus lateralis* sample was taken via needle biopsy from a human volunteer. Single fibers (n=3) were chemically-skinned, attached between a length motor and a force transducer, and exposed to solutions of varying pH (7.4 and 6.4) and ATP (3 and 0.1 mM). Tension at pH 7.4 rose as ATP was lowered (76.1±16.0 mN.mm^{-2}, 3 mM ATP; 100.5±20.1 mN.mm^{-2}, 0.1 mM ATP). A single population of cycling myosin crossbridges was evident from sinusoidal analysis at pH 7.4. Crossbridge mean time attached (time-on) was prolonged as ATP was lowered (38.6±3.2 ms, 3 mM ATP; 66.3±18.7 ms, 0.1 mM ATP), which could explain the increased tension with lower ATP. Tension was reduced at pH 6.4 compared to pH 7.4 and rose as ATP was lowered (63.5±8.5 mN.mm^{-2}, 3 mM ATP; 75.5±11.5 mN.mm^{-2}, 0.1 mM ATP). A second population of myosin crossbridges was evident from sinusoidal analysis at pH 6.4. The mean time-on for the "first" population at pH 6.4 responded to ATP similarly to that detected at pH 7.4 (36.5±16.6 ms, 3 mM ATP; 59.9±19.9 ms, 0.1 mM ATP), while the "second" population demonstrated slower ATP-sensitive kinetics (111.4±47.7 ms, 3 mM ATP; 165.8±33.2 ms, 0.1 ATP). These data suggest that a second, possibly non-force producing population of myosin crossbridges arise at lower pH. Such a state of myosin could be in a post-power stroke state in which ATP has bound, but is able to

re-attach to actin. A similar model has been proposed based on data from the laser trap.

638-Pos Factors Modulating Recovery Rate After Intermittent Titanic Fatigue In Atrophic Soleus

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To clarify factors modulating recovery rate after intermittent tetanic fatigue in soleus and to seek reasons for the decrease of recovery rate in atrophic soleus, we observed the recovery time course of different types of fatigue in isolated muscle strips. A 10 % or 50 % decrease in maximal tetanic tension was defined respectively as slight or moderate fatigue. After short-term (S10P) and long-term (L10P) slight fatigue, tetanic tension recovered to nearly 100% of the maximal tetanic tension (P_o) at the twentieth minute. In both groups, perfusion with 10 μmol/L of ruthenium red (an inhibitor of Ca^{2+} release channels in sarcoplasmic reticulum) decreased recovery rate. This suggested that slight fatigue only inhibited myofibril. After short term (S50P) or long term (L50P) moderate fatigue, recovery rate at the twentieth minute was about 95 % P_o (S50P) and 90% P_o (L50P) respectively. The difference showed that the recovery rate after moderate fatigue was related to the fatigue duration. After two kinds of moderate fatigue, perfusion with 5 mmol/L of caffeine (an opener of Ca^{2+} release channels in sarcoplasmic reticulum) made near 100% recovery at the fifth minute. This suggested that moderate fatigue inhibited both myofibril and sarcoplasmic reticulum Ca^{2+} release channels. In one-week unloaded soleus, the recovery rate was declined to 94 % P_o (S10P), 95% P_o (L10P), 92 %P_o (S50P), 84 %P_o(L50P) at the twentieth minute, respectively. There were significant decreases in all of the fatigue groups as compared with their synchronous groups. These results suggest that both slight and moderate fatigue inhibit myofibrils and sarcoplasmic reticulum Ca^{2+} release channels in one-week unloaded soleus.

639-Pos Acidosis Affects The Kinetics Of Single Skeletal Muscle Myosin Molecules In The Laser Trap

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Muscle loses much of its ability to generate force and motion following repeated, intense contractile activity. Acidosis is believed to contribute to this phenomenon, but the molecular mechanisms underlying its depressive effects are unknown. Therefore, we studied the effect of acidosis on the molecular mechanics and kinetics of skeletal muscle myosin using *in vitro* motility and single molecule laser trap assays. Decreasing the pH from 7.4 to 6.4 at 1 mM ATP decreased actin filament velocity (V_{actin}) by 65%. In the laser trap at 1 μM ATP, myosin's average step size (d) decreased from 10 ± 2 nm (pH 7.4) to 4 ± 4 nm (pH 6.4). The reduced average step size was consistent with a population of 10 nm steps and population of non-productive interactions that was centered at zero nm at low pH. However, at 1mM ATP and pH 6.4, d was restored to 10 ± 3 nm, suggesting that the lifetime of the non-productive binding events is ATP-dependent and that acidosis depresses d by altering the kinetics of myosin rather than directly affecting rotation of the lever arm. In addition, at 1 mM ATP, pH 6.4 the duration of strong actin binding (t_{on}) was 31 ± 9 ms, ~3-fold greater than estimated values at 1 mM ATP, pH 7.4 (Baker et al. 2002). Assuming that $V_{actin} \sim d/t_{on}$, the observed increase in t_{on} may fully explain the acidosis-induced decrease in V_{actin}, however, the increased proportion of non-productive binding events may also contribute to the slowing of V_{actin} by increasing viscous drag on the actin filament.

640-Pos Disuse-induced Changes In Fatigability In Rat Soleus Muscle

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We reported that long-term hindlimb immobilization (6 weeks) lowers the expression of the giant protein titin in the soleus muscle of the rat, resulting in reduced active force production via abnormal sarcomeric organization (Biophysical Society, 2007). In the present study, we investigated how immobilization affected fatigability by using Triton X-100-treated single skinned fibers taken from the same animal model. The intracellular concentrations of inorganic phosphate (Pi) and H^+ are known to increase in skeletal muscle during intense exercise, resulting in a fall in active force production. Therefore, we tested the effects of changes in pH and Pi concentration on maximal Ca^{2+}-activated force production in control vs. immobilized fibers. We found that lowering pH from 7.0 to 6.2 decreased maximal force in both muscles, with a greater magnitude in immobilized fibers. Likewise, the inhibitory effect of Pi up to 20 mM was more pronounced in immobilized fibers. These results suggest that fatigability is enhanced in disused muscle and that the mechanism includes a decrease in the fraction of force-generating cross-bridges coupled with abnormal sarcomeric organization.

641-Pos Preliminary Analysis of Tarantula Muscle-Related Genes Based on EST Approach

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Tarantula has been used as a model system for studying striated muscle structure and function, yet data on the genes expressed in

tarantula muscle are lacking. We constructed a cDNA library from tarantula (*Aphonopelma* spp.) skeletal muscle and sequenced over 2500 randomly picked clones. Expressed sequence tag (EST) analysis showed 305 unigenes, among which 81 had more than 2 ESTs. Twenty abundant unigenes had matches to skeletal muscle-related genes including actin, myosin, tropomyosin, troponin-I, T and C, paramyosin, muscle LIM protein, muscle protein 20, and α-actinin. Sequence similarities suggested several isoforms of some proteins, including actin, myosin heavy chain and troponin I. Molar ratios of several components expected from the EST counts (e.g. TnC:I:T) differed from the measured protein ratios in arthropods, possibly due to the presence of different isoforms. Matches to MLCK, calponin, and Ig/Fn motifs (found in giant sarcomere-related proteins) were also identified. These results support the existence of both actin-linked and myosin-linked regulation in tarantula skeletal muscle. We have predicted full-length cDNA sequences both experimentally and computationally. The RLC is deduced to be 196 amino acids long (21.8 kDa) and to have two MLCK phosphorylation sites. Conserved glycines involved in RLC-HC binding are present, while other HC-binding residues differ from those in most RLCs. The ELC is predicted to be 156 amino acids long (17.6 kDa) and lacks the calcium-binding residues conserved in scallop. Sequences exhibiting similarities to different parts of the myosin heavy chain were 78%–86% identical to each other, suggesting possible isoforms. Sequence analysis showed one skip residue site different from scallop, T replacing Q, as seen in vertebrate skeletal muscle. These and other new insights should greatly facilitate structural understanding of muscle in the model tarantula system.

642-Pos The Function of Costameres in EDL Muscle: A biomechanical approach

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Costameres connect superficial myofibrils to the sarcolemma in striated muscles, align the sarcolemma with myofibrils, and transmit contractile force laterally through the sarcolemma to the extracellular matrix. Dystrophin, desmin, intermediate filaments and actin are some of the proteins that form costameres. We have used an elastimeter to investigate the biomechanical properties of the sarcolemma and costameres in myofibers of wild type (WT), desmin −/− and dystrophin null (mdx) mice. Negative pressure (**P**) was applied through a micropipette to the sarcolemma, generating a bleb whose height **h** depended on **P** and sarcomere length (SL) obtaining the displacement-pressure curves at different SL. Tension (γ), stiffness (**k**), **P** at which links between costameres and myofibrils breaks ($\mathbf{P_c}$), and **P** at which the sarcolemma ruptures after being detached from myofibrils ($\mathbf{P_r}$), were calculated using Laplace′s and Hooke′s equations. The range of values found for $\mathbf{P_c}$, **γ, k,** and $\mathbf{P_r}$ for WT, des−/−, and mdx muscles at different SL are shown in Table 1.

	Pc dyne/cm^2 $\times 10^3$	γ dyne/cm	k dyne/cm	Pr dyne/cm^2$\times 10^3$
WT	(238–496)	(70,460)	(100,580)	(635,741)
des −/−	(184,463)	(60,280)	(110,430)	
mdx	(101,295)	(40,190)	(200,260)	(349,402)

Values depend on SL varying from (2.5,4.9) μm. Our results show that dystrophin plays a larger mechanical role than desmin, illustrating how changes in costameric proteins linked to disease contribute to sarcolemmal instability.

643-Pos Contractile response impairment in skeletal muscles of Ank1.5 deficient mice

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Vertebrate genome contains three ankyrin genes (Ank1, Ank2, and Ank3) from which by differential splicing multiple transcripts originate resulting in a large number of expressed proteins that participate in the organization of specific membrane domains by linking specific membrane proteins with intracellular cytoskeleton. Recently, a muscle specific variant of Ank1, ank1.5 has been shown to link obscurin, a myofibrillar protein, with the sarcoplasmic reticulum in striated muscles. To clarify the role of Ank1.5 in skeletal muscles we generated mice carrying null mutations that selectively affect the expression of the ank1.5 mRNA. Homozygote Ank1.5 mice are vital and fertile. To further understand the role of ank1.5 protein, the structure of the SR and the contractile properties of skeletal muscles from ank1.5 KO mice were analysed. Although staining of SR proteins revealed no apparent alteration in the organization of SR proteins ank1.5 KO mice, the contractile performance of the diaphragm was however weaker than in wild type animals and their fatigue resistance in an endurance test on the treadmill reduced. These findings suggest that ank1.5 play an important, but yet not completely defined role, in muscle contraction.

644-Pos Obscurin - Position and Function of a Large Modular Muscle Protein in Drosophila melanogaster

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Obscurin is a large modular protein of ~800 kDa that has been well characterized in vertebrate skeletal and cardiac muscle. Vertebrate

obscurin is involved in sarcomere assembly and muscle turnover. It contains immunoglobulin and signalling domains. There are two C-terminal kinase domains and one Rho/GEF domain. The *Drosophila* homologue is ~420 kDa and there are four isoforms, one of which is exclusively expressed in the indirect flight muscle (IFM). *Drosophila* IFMs are striated muscles that are not required for survival and are therefore easily accessible for genetic manipulation. Obscurin is expressed at late embryonic stage 16 and is present in the M-line during all embryonic and larval stages. It is present in the M-line of developing IFM in early pupal stages, and may be needed for assembly of the IFM sarcomere. *Drosophila* obscurin has two kinase domains in the C-terminal region and sequence of a Rho/GEF domain in the N-terminal region, which lacks the usual PH sequence. The kinase domains are conserved in 11 *Drosophila* species and show some divergence from vertebrate and nematode sequences. A conserved aspartic acid residue in the catalytic loop of eukaryotic kinase domains, which is essential for phosphorylation, is missing in all *Drosophila* species; therefore, the kinases are predicted to be inactive. Obscurin in IFM was shown to bind to myosin, possibly in a complex with another protein. Obscurin mutant flies which have a P-element inserted upstream to the promoter region are homozygous viable, but flightless. Flies carrying this P-element crossed over a deficiency in the obscurin region show the same phenotype. Detailed examination of these phenotypes will contribute to understanding the function of obscurin in the IFM.

645-Pos Computational Estimation of the Energy Requirements for Conformational Transitions in Skeletal Muscle Myosin

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In order to determine the energy consumed during transitions between critical conformational states of myosin S1, we used molecular mechanics and dynamics simulations in which the conformational transition is induced using a spring constant of 16.7 N/m. A simulated force-distance curve is calculated based on the difference between the actual distance and target distance after successive energy minimizations and the area under the force distance plot is determined.

The energy required to open 50 kDa cleft relative to the crystal structure is less than the energy required to close 50 kDa cleft when nucleotide binding site is closed relative to the crystal structure. Whereas, with nucleotide binding site open, opposite results are observed. Cleft closing is independent of the opening/closing of nucleotide binding site whereas cleft opening is dependent on the nucleotide binding site. The energy required to open the 50 kDa cleft is less than closing the 50 kDa cleft when reactive cysteines are computationally crosslinked with nucleotide binding site closed. Crosslinking reactive cysteines with nucleotide binding site closed creates a barrier to cleft closure.

In contrast, opening 50 kDa cleft requires more energy than closing 50 kDa cleft when reactive cysteines are crosslinked and nucleotide binding site is opened. Crosslinking reactive cysteines makes 50 kDa cleft opening easier whereas opening nucleotide binding site makes closing 50 kDa cleft easier. Crosslinking reactive cysteines with nucleotide binding site open requires more energy than crosslinking the reactive cysteines with nucleotide binding site closed. These results are consistent with experimental studies on crosslinking reactive cysteines in presence and absence of a nucleotide and provide molecular level detail of the complex internal interactions of myosin.

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646-Pos Analysis of Mechano-chemical Coupling in Muscle through Force Step Simulations

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This works aims to elucidate mechano-chemical coupling in muscle, i.e. the strain dependency of biochemical transition rates, on the molecular level through computer simulations. An isometric cardiac muscle contraction model that includes titin, troponin I and a potential Frank-Starling mechanism (Schneider et al., J Mol Cell Cardiol *41*: 522–536, 2006) was combined with the crossbridge (Xb) mechanics part as described by Negroni and Lascano (J Mol Cell Cardiol *28*: 915–929, 1996; $dX/dt = B*(L-X-h_c)$). A newly developed passive tension equation was added which splits the contribution from titin, collagen and intermediate filaments. Force steps simulation experiments were carried out as described by Piazzesi et al. (J Physiol *545*:145–51, 2002). Simulation results of a sudden decrease of load from a muscle in a tetanic steady state show 4 distinct phases (P1 to P4) as found experimentally for frog skeletal muscle at 5°C. The temperature dependency of the elastic P1 (Decostre et al., Proc Natl Acad Sci U S A *102*:13927–32, 2005) could be exactly reproduced through a variation of the average Xb elongation, h_c in the Xb mechanics equation. After taking X-ray experimental findings into account (Reconditi et al., Nature *428*:578–81, 2004) which suggest that no de- and reattachment of Xbs occurs in P1 and P2, experimental force step time courses could be repeated by simulations. The fast shortening P2 is characterized by the load dependent proportionality factor B of the Xb mechanics equation, which determines the maximum stroke length of the myosin heads. The slow shortening P3 and the steady state shortening P4 depend on the ADP release rate (k_5), which is changing with load. To reproduce experimental force step time courses a simultaneous detachment of Xbs with a high k_5 was necessary at the start of P3.

647-Pos Multi-segmental Model Of A Myofibril Incorporating Chemo-mechanical Coupling

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The importance of inter-sarcomere dynamics during muscular contraction has been shown in different contributions (e.g. [1], [2]). A generic framework regarding the muscle as a linear motor composed of serially coupled subunits has been presented to analyze inter-sarcomere dynamics [3].

In the present study we extent the former Hill-type description of the contractile element with a Huxley formalism to account for 'real' transient forces based on cross-bridge kinetics. The formalism allows calculation of a myofibril for any length- and force-controlled protocol.

We performed numerical simulations of a system of eight half-sarcomeres (hS) in series under physiological conditions taking into account a biological variability of the force capacities in single hS. The simulations showed time-sequential relaxation, i.e. lengthening of one hS after another, and moreover, the relationship between initial movement of hS (hS dynamics) and force development upon activation. The hS dynamics during relaxation depends strongly on the stiffness of the whole system. Furthermore we analyzed the dependence of the commonly used force kinetics parameters (k_{act}, k_{lin}, $t_{lin,}$ k_{rel} [4]) on the hS dynamics. Simulations of standard length-/force-clamped experiments showed considerable effect of hS dynamics on the force outcome. We conclude that a steady-state approximation does not hold in any case.

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648-Pos An Alternative Approach To Mass-Action Models

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In 1957 A.F. Huxley proposed a model for describing the mechanical properties of muscle fibers, based on the idea that muscle motion originates from myosin heads attaching to and detaching from the binding sites along the actin filament. Huxley's work introduced a simple partial differential equation to describe the crossbridge dynamics, which later was extended to more complex models of the actomyosin cycle.

We have described a Monte Carlo simulation of muscle contraction, which included the discrete structure of sarcomeres and therefore provided a very realistic description of the coupled biochemical and mechanical processes inside muscle fibers. A comparison of the crossbridge distributions from the Monte Carlo simulation and the classical mass-action approach revealed differences that were caused by a non-obvious difference in the model assumptions. In the Monte Carlo simulation a global pool of myosin heads is available for attachment; however, this pool is local in the standard mass-action approach. In certain situations, such as during initial force development, fast transients and under ramp shortening, these limitations significantly alter the crossbridge dynamics. Our new approach modifies mass-action models such that the model assumptions match those of our Monte Carlo simulation. As a consequence, it resembles the actual discrete structure of the muscle filaments and consequently leads to results that compare well with those found by the Monte Carlo simulation.

This new approach was tested with a two- and three-state model of the actomyosin cycle to demonstrate how the different mathematical formulation affects the model predictions. Furthermore, using this approach we sought a new set of rate functions in the actomyosin cycle to match experimental data.

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649-Pos Some Aspects of the Theory of Muscle Contactility

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1. **Behavior of an ensemble of cross - bridges** should be described in terms of the theory of Markovian death and birth processes.

– - Each cross - bridge has some probability μ for engaging with actinic filament and some probability λ for disengaging with it;

– -at some arbitrary moment of time some number *i* of any cross

– - bridges are in engaged state with a probability of *pi(t)*.

This process can be described using Markovian death-and-birth graph for which one can easily write a set of Kolmogorov differential equations for probabilities *pi(t)*.

1. **Behavior of myosin system** which is actually act as a support for cross - bridges is fundamentally important. Each myosinic filament is subjected to the force that is provided by a cross - bridge and is equal to the force being applied by that cross - bridge to an actinic filament. Since its mass is not infinite it will vibrate in some complex manner. These vibrations consume some part of the energy provided by the hydrolysis of ATP thereby providing a significant effect on the process of force generation.
2. We have succeeded to define the energy of myosinic system in terms of parameters μ and λ.
3. **Biochemical processes** are defined by the reaction rates that we have identified here as μ and λ. Thus our approach make it possible to correlate chemical and physical processes occurring in the muscle.
4. **Thermodynamics of the muscle** is strongly defined by the presence of myosinic system vibrations. Indeed it is clear that vibrating myosinic filament will exchange its energy with sarcoplasmic solution. We state that this interaction is controlled by the Brownian mechanism (fluctuations), that is it depend on the temperature.