flight muscle fibers from *Drosophila melanogaster* indicate faster cross-bridge cycling kinetics during lengthening and slower cycling during shortening, compared to isometric contraction. During isometric contraction we estimate a myosin attachment time of 5.0 ms, and 4.6 versus 5.5 ms during the lengthening and shortening transients, respectively. These initial applications of the white-noise system analysis technique show promise for future studies probing molecular processes that underlie complicated length transients associated with normal muscle contraction.

1804-Pos

The Isotonic Velocity Transient Following a Sudden Rise in Force Imposed on the Muscle Sarcomere During Unloaded Shortening Reveals a Rate Limiting Step in Detached Myosin Motors

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Energy balance studies indicate that ATP splitting by myosin motors during rapid shortening of skeletal muscle is not sufficient to account for the energy (mostly heat) output (Rall et al., *J Gen Physiol* 68(1), 13, 1976; Homsher et al., *J Physiol* 321, 423, 1981). We investigated the kinetic step of the myosin ATP-ase cycle related to this phenomenon in single muscle fibers from *Rana esculenta* (~2.15 μm sarcomere length, 4°C), by recording the isotonic velocity transient following a force step from zero to the isometric tetanic value (T_0). Once the isometric tetanus had developed, the force was first clamped to zero for a range of times from 10 ms to 18 ms (during which the fiber shortened at the maximum velocity by 30 nm hs^{-1} to 50 nm hs^{-1}) and then raised again to T_0 in a stepwise manner (~120 μs). The elastic lengthening induced by the force step was followed by a transient isotonic lengthening, the size of which ranged from 40 to 60 nm hs^{-1} depending on the size of the preceding shortening. The lengthening velocity was larger for larger shortening size and progressively decreased to approach the isometric condition with a half-time of 2-3 ms. Similarly, the half-sarcomere stiffness recovered the isometric value e_0 from the unloaded shortening value of 0.4 e_0 with an exponential time course with τ ~3 ms. We conclude that during rapid shortening a ~3 ms-transition between detached states of the myosin motor, likely related to the ATP hydrolysis, becomes rate limiting. Accumulation of motors in the state preceding the hydrolysis step can account for the unexplained energy during rapid shortening. Supported by MIUR (Italy).

1805-Pos

Effect of Inorganic Phosphate on the Rate of ADP Release During Ramp Shortening in Activated Permeabilized Fibers from Rabbit Psoas Muscle

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A coumarin-labeled recombinant phosphorylated nucleoside diphosphate kinase (P~NDPK-IDCC; West et al., 2009; Biophys.J. 96:3281-3294), was used as a fluorescence probe for time-resolved measurement of changes in [MgADP] during steady shortening of single permeabilized rabbit psoas fibers at 12°C (pCa 4.5, pH 7.1, ATP 5.7mM). A fiber contracted from the relaxed state by immersion into a Ca^{2+} activation solution at 0°C. Temperature activation was then initiated by immersion of the fiber into silicone oil at 12°C. The activation solutions were prepared with either zero added P_i or with 10 mM added P_i at constant ionic strength (0.15 M). The decline in fluorescence intensity emission (470nm) associated with MgADP-dependent dephosphorylation of P~NDPK-IDCC (60 μM) was monitored during activation and during a period of isovelocity shortening. Fluorescence emission (580 nm) of a rhodamine dye was measured simultaneously to correct the P~NDPK-IDCC signal for the effects of fiber movements and volume changes. The rate of MgADP release in the absence of added P_i increased from 0.7 ± 0.07 mM·s^{-1} at 0.2 fiber-lengths·s^{-1} to approximately 3.4 ± 0.25 mM·s^{-1} for shortening velocities between 1 and 2 fiber-lengths·s^{-1}. When 10 mM P_i was added, the rate of ADP release at 0.2 fiber-lengths·s^{-1} was 0.48 ± 0.05 mM·s^{-1} and 2.6 ± 0.4 mM·s^{-1} at 1-2 fiber-lengths·s^{-1}. In the absence of added Pi, the rate of ATP hydrolysis calculated from the appearance of ADP is similar to that calculated previously from the appearance of P_i using MDCC-labeled phosphate binding protein, over the same range of fiber shortening speeds (He et al., 1999, J. Physiol. 517: 839-854). Thus P_i slows the ATPase rate by 25-30%, both in the isometric and isotonic state. The energetic consequences will be discussed.

1806-Pos

Cross-Bridges and Sarcomere Stiffness in Single Intact Frog Muscle Fibers

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The number of cross-bridges formed in activated skeletal muscles is a key information for both energetics and mechanics of muscle contraction. In this study we determined the cross-bridge number in single fibers by measuring the tension $P_c$which forcibly detached the cross-bridge by fast stretches (Bagni et al. 2005, *J. Physiol* 565). Fibers, isolated from the tibialis anterior muscle of *Rana esculenta*, were mounted between an electromagnetic motor and a fast force transducer. Sarcomere length was measured by means of a striation follower device. Measurements were made during tetanus rise in normal Ringer and in sub-maximal tetanic contractions in Ringer-BTS (*N*-benzyl-p-toluene sulphonamide, 1 μM) at 5°C at sarcomere length of 2.1 μm.
The results were compared with fiber stiffness, another indicator of cross-bridge number, measured with 4 kHz sinusoidal length oscillations (1 $nmhs^{-1}$ p-p amplitude). The stiffness-tension relation was the same both during the tetanus rise and Ringer-BTS and showed the non-linearity expected from the myofilament compliance. However, the data could not be fitted satisfactorily with a simple model made of cross-bridge and linear filament compliances in series. A good fit was obtained by assuming that a fraction (~14%) of attached bridges at tetanus plateau was generating no-force. Relative myofilament and cross-bridge compliance resulted 0.37 and 0.63 respectively. The stretch data showed a linear relation between $P_c$and tension with a slope consistent with the presence of the non-force generating bridges suggested by stiffness data. These results suggests the existence of a possible non-linearity between cross-bridge force and stiffness and show that the relation between fiber stiffness and cross-bridge number is not as simple as usually assumed.

1807-Pos

Ultrafine Striations in Skeletal Muscle Revealed by 3D Super-Resolution Fluorescence Microscopy

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Super-resolution three-dimensional imaging was achieved using newly synthesized photoactivatable quantum dot probes. Semiconductor quantum dots are nanoparticles with high photostability and brightness. They were modified with a novel quencher system to make them photoactivatable. The unique properties of these photoactivatable quantum dots enable single-fluorophore localization in three dimensions using a confocal microscopy optical sectioning method with a piezo scanner. To image skeletal muscle at resolutions exceeding that of the standard confocal microscope, the photoactivatable quantum dots were conjugated to secondary antibodies. Myofibrilar bundles were dual- labeled using both a primary antibody to myosin rod with the secondary antibody-conjugated photoactivatable 655 nm quantum dot and a primary antibody against tropomyosin with the secondary antibody-conjugated photoactivatable 525 nm quantum dot. During the 3D acquisition on a spinning disk confocal with piezo scanner, different individual quantum dots were photoactivated during each of hundreds of cycles. A sufficient number of single quantum dots were localized, reduced to their center of mass and then reconstructed to a super-resolution image. The resulting super-resolution image shows a sub-diffraction resolution in both lateral and axial directions. The broad absorbance band of quantum dots enables the excitation of both quantum dots with the same laser type. This technique enables the relative localization of two different myofibril proteins at nanometer scale resolutions in solution demonstrates ultrafine striations in the staining pattern with widths less than 70 nm in axial and lateral dimensions that are not evident by conventional confocal microscopy due to its resolution constraints. The bands appear to be related to the presentation of epitopes at the surface of thin and thick filaments and may be related to thick and thin filament binding proteins and/or structural variations in the actin and myosin filaments.

1808-Pos

Structural and Functional Gradients with Temperature in the Flight Muscle of Manduca Sexta

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The force/extension curve of the flight muscle of the Hawkmoth, *Manduca sexta* is remarkably similar to that of mammalian cardiac muscle suggesting that it may serve as a useful model system for certain aspects of cardiac muscle structure and function (J Exp Biol. 2004;207:2455). More recently, it was discovered that these animals maintain an astonishing thermal gradient of 8.8 C in the 5 mm distance dorsal to ventral in their dorso-longitudinal flight muscles (DLMs). Does the existence of this thermal gradient necessarily imply a functional gradient? Do these changes in function have, as their basis, changes in structure? Twitch dynamics of individual fibers within the DLM in intact animals are temperature dependent so that mechanical power output (and it's phase dependence) varies with depth in the tissue. A surprising observation was that all five sub-units in the DLM were simultaneously activated. Cooler

muscles subjected to 25 hz stimulation (flight frequency) are partially fused with (likely) little crossbridge turnover. To assess the structural correlates of temperature gradients, we performed small-angle x-ray fiber diffraction measurements as a function of position along the dorsal-ventral thermal gradient in intact moth thoraces. The equatorial intensity ratio (I_{20}/I_{10}) in unstimulated muscle increased by ~25% in the first 1 −1.5 mm traversing from dorsal to ventral, implying that increased temperature was associated with increased association of the myosin heads with the thin filaments presumably predisposing them towards more productive acto-myosin interaction. Interestingly, X-ray patterns from skinned muscle preparations improved with increasing temperature indicating better structural order. Together, these observations suggest that cooler, superficial muscles may act mainly as elastic energy storage, whereas warmer deeper muscles may do the bulk of the mechanical work.

1809-Pos

Positioning of Myosin-Binding Protein C in Skeletal Muscle

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Frog striated muscle gives a pair of X-ray meridional reflections at spacings of ~419Å and ~442Å, which Offer (CSH Symp.37, 83-97, 1972) and Rome (ibid., 331-339) have shown are related to the disposition of C-protein in two sets of bands at ~430Å intervals on either side of the H-zone, giving rise to interference fringes that sample the underlying 430Å reflection. However, there are problems with this simple interpretation.

We have studied these reflections at high resolution on the BioCAT beam line at the Argonne National Lab., in both relaxed and contracting muscles, and during the onset of activation. In resting muscle, two main peaks can generally be seen in the relevant region, usually at ~419Å and ~442Å as previously described, but the latter peak is about 4 times more intense than the former, which would require an underlying sampled peak at ~437Å. It seems unlikely that the C-protein repeat is different from the helical repeat of the myosin filament to which it is attached (429.6Å), and more probable that some second component is involved, namely a ''forbidden'' first order myosin meridional reflection, as discussed by Malinchik and Lednev (JMRCM 13, 406-419, 1992), The interference fringes generated by this repeat would interact in a complex way with those from C-protein, since the reflections would in general have different phases. We find that the observed patterns, with very strong ~442Å reflections, can be modeled very satisfactorily even when both underlying repeats are kept at 429.6Å.

Passive stretch of semitendinosus muscles to sarcomere lengths up to the 3.2 - 3.5 μm range, where overlap between the C-protein bands and actin becomes zero, has little effect on the spacing of these reflections. However, that does not mean there is no interaction between C-protein and actin.

1810-Pos

Different Orientation of Two Heads of a Myosin Crossbridge in Full-Filament Overlapped and Overstretched Muscles Obtained by X-Ray Fiber Diffraction

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A novel method using the cylindrically averaged difference Patterson function was applied to correct a sampling effect due to the hexagonal filament array on the thick filament-based layer-line intensities from frog skeletal muscles at the full-filament overlap length. Using the corrected intensity data and the mixed structural model of a thick filament with two different axial periodicities of the myosin crossbridges, we performed an optimum search of azimuthal orientation of two heads of a myosin crossbridge and compared the optimum orientation to that from muscles stretched beyond filament overlap reported previously. The result showed that the myosin crossbriges in the regular repeating region had a similar configuration in both muscles. Two heads of a myosin crossbridge formed a windmill-shape when seen from the top of the filament and one head of a myosin crossbridge seemed to be almost in contact with another head in a pair at an adjacent crown level along the filament axis. One head was toward the converter domain of the other head, similar to regulated myosin heads in Tarantula muscles in which the intramolecular head-head interaction occurs. In the perturbed region, however, myosin crossbridges had different configurations in these muscles. In top view, two heads of a myosin crossbridge showed a U-shape structure in the overstreched muscles while a cross-shape structure in muscles with the full-filament overlap. One myosin head seemed to be in contact with the other head at the same axial crown level. The models suggest that the disposition of two-headed myosin crossbridges is stabilized by the head-head interaction at same or different axial crown levels. Probably this would be related to the inhibition mechanism of actomyosin interaction in the relaxed muscles.

1811-Pos

Relative Contribution of Attached and Detached Myosin Heads to the X-Ray Pattern from Skeletal Muscle

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In the X-ray diffraction pattern from skeletal muscle the third order myosin-based meridional reflection, M3, originates from the axial repeat of myosin heads along the thick filament. Changes in the intensity (I_{M3}), spacing (S_{M3}), and fine structure (R_{M3}) of the M3 reflection in contracting skeletal muscle at full filament overlap (~2.1 μm sarcomere length) have been measured in many different protocols (Piazzesi *et al. Nature***415**:659,2002; Reconditi *et al. Nature***428**:578**,** 2004; Linari *et al. J. Physiol.***567**:459, 2005; Huxley *et al. J. Mol. Biol.***363**:743, 2006; Huxley *et al. J. Mol. Biol.***363**:762, 2006; Brunello *et al. PNAS***104**:20114, 2007; Piazzesi *et al. Cell***131**:784, 2007). These studies showed the presence of a fixed periodic mass that is insensitive to filament sliding and attributed to detached myosin heads, but estimates of the relative contribution of the detached heads to the M3 reflection ranged from 0.3 to 0.6. Here we show that this parameter can be constrained by the dependence of the M3 reflection on sarcomere length (*sl*). When *sl* is increased from 2.1 to 3.20 μm, decreasing the fraction of myosin heads that are overlapped by actin filaments from 1 to 0.3 (and thus, according to Piazzesi *et al.* 2007, the fraction of actin-attached myosin heads from 0.3 to 0.09), force and I_{M3} decrease in proportion to filament overlap, while S_{M3} and R_{M3} are approximately constant (Linari *et al*. *PNAS***97**:7226, 2000). These results suggest that in isometric contraction at full filament overlap the contribution to I_{M3} of detached myosin heads is no more than 35% of that of attached heads and that there is very little axial offset between the two head populations.

1812-Pos

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

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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 vivo. This inhibition has not been previously seen in in vitro systems. We used quantitative epifluorescence microscopy of fluorescent nucleotides to measure single molecule 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 to obesity or help in controlling high blood sugar levels.

Muscle Regulation II

1813-Pos

A Disulfide Bond at Cys 190 of Tropomyosin Alters Tryptic Cleavage Pattern

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Native tropomyosin (Tm), an α-helical coiled-coil, possesses a charged acidic amino acid (Asp 137) that occurs in a hydrophobic position which destabilizes the coiled-coil. This region is sensitive to tryptic cleavage and is important in the proper regulation of the myosin activate ATPase, (Sumida, John P., Wu, Eleanor, Lehrer, Sherwin S, JBC 283, 2008). Thermal stability measurements of Tm suggest a long-range interaction between the Asp 137 position and the Cys 190 position. In the current work, we present further evidence of long-range interactions along the length of tropomyosin.