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.