rotations. The unknown effects of this disorder on diffraction by muscle fibres have so far prevented a rigorous analysis of x-ray fiber diffraction patterns in terms of the structure of this complex system. We report a quantitative model of the disorder and its incorporation into calculations of x-ray fiber diffraction patterns from model structures. This allows rapid calculation of the diffraction and does not involve numerical averaging over the disorder. Calculations show that the disorder modulates the Bragg reflections in diffraction patterns and introduces diffuse diffraction. The results of this analysis will allow the effects of the disorder to be included in muscle structure refinement programs, allowing more accurate structure determination from x-ray fiber diffraction data.

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The Intensity Of The 2.7nm Reflection As A Constraint For Models Of Myosin Docking To Actin

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Previous workers have proposed high resolution models for the docking of the myosin heads on actin on the basis of combined crystallographic and electron microscopy data (Mendelson and Morris, 1997 *PNAS* **94**:8533; Holmes *et al.* 2003 *Nature* **425**:423). We have used data from small angle X-ray fiber diffraction from living muscle to check the predictions of these models. Whole *sartorius* muscles from *Rana pipiens* were mounted in a chamber containing Ringer's solution at 10°C and at rest length at the BioCAT beamline (18 ID, Advanced Photon Source, Argonne, IL-U.S.A.). The muscles were activated by electrical stimulation and the force was recorded with a muscle lever system type 300B (Aurora Scientific). X-ray patterns were collected with 1s total exposures at rest and during isometric contraction out to 0.5 nm^{-1} in reciprocal space, as the higher angle reflections are expected to be more sensitive to the arrangement of myosin heads on actin. We observed that during isometric contraction the meridional reflection originating from the 2.73nm repeat of the actin monomers along the actin filament increases its intensity by a factor 2.1 ± 0.2 relative to rest. Among the models tested, Holmes *et al.* fits the data when the actin filament is decorated with 30-40% the total available myosin heads, a fraction similar to that estimated with fast single fiber mechanics by Piazzesi *et al.* (2007, *Cell* **131**:784). However, when the mismatch between the periodicities of actin and myosin filaments is taken into account, none of the models can reproduce the fiber diffraction data. We suggest that the fiber diffraction data should be used as a further constraint on new high resolution models for the docking of the myosin heads on actin.

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New X-ray Data about Myosin-binding Protein C in Frog Muscle

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Frog striated muscle gives many meridional X-ray reflections at spacings greater than 400Å which are still incompletely understood. Some come from C-protein, as discovered by Offer (CSH Symp.37, 83-97, 1972) and by Rome (ibid,331-339). Others may come from a ''forbidden'' first order myosin meridional reflection, as discussed by Malinchik and Lednev (JMRCM.13, 406-419, 1992). In both cases the reflections will be split by interference fringes from the two half A -bands. Squire has suggested that the apparent C-protein repeat of ~ 435Å (rather than 429Å) in relaxed muscle may be due to interaction with actin (JMB.331, 713-724, 2003).

We have studied these reflections at high resolution on the BioCAT beam line at the Argonne National Lab., in relaxed and contracting muscle. In resting muscle, two main peaks occur in the relevant region, at ~419Å and ~442Å, the latter being about 4 times more intense than the former, indicating an underlying repeat of ~437Å and an apparent interference distance of ~8200Å (and an actual one of ~7100Å). In contracting muscle, the pattern is very much weaker, and the corresponding spacings are 412Å, 447Å (i.e. a wider doublet), 440Å underlying spacing, and 5300Å apparent interference distance. Part of the change may be ascribed to a weakening of the presumed contribution from the myosin ''forbidden'' meridional reflection, but the apparent interference distance would now indicate an actual distance of ~4460Å, short compared to the expected value (~5900Å) for C-protein ; the long C repeat spacing (440Å) is also notable.

At lower angles still, the observed reflections index as the higher even orders of the sarcomere repeat, as reported by Bordas and colleagues (J. Cell. Biol.105, 1311-1318, 1987), and which shorten in contraction. But why only even orders appear is still an intriguing puzzle.

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An Automated Apparatus for Isometric Force Analysis of Skinned Muscle Fibers

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Skinned muscle fibers provide a powerful means to assess the functional effects of compounds that modulate the sarcomere. The main drawback of this preparation as an assay system is its low-throughput nature. As part of an effort to optimize small molecule activators of the skeletal sarcomere for therapeutic applications in conditions where muscle weakness is a feature, an automated system was designed that can simultaneously run multiple types of isometric force assays. Six identical units, controlled through a single software interface, run a variety of assay protocols. Each unit independently measures the force of a single suspended fiber as it is submerged into various solutions in a temperature controlled block. Assay protocols are unique for each tissue type and desired measurement. Fiber quality is automatically assessed by switching between fully contracting and fully relaxing pCa solutions. If sufficiently robust, fibers are tested by indexing between solutions of varying pCa or compound concentration. In each new solution, the software monitors the rate of force generation and when the fiber has reached a force plateau, automatically moves to the next solution. Control pCa profiles of rabbit psoas fibers measured over a year and a half period show typical variation of < 0.1 pCa unit from historic values. This capability has allowed characterization of several hundred compounds aiding with the selection of a troponin activator as a development candidate for diseases characterized by muscle weakness.

3179-Pos Board B226

A White Noise Approach To System Analysis In Demembranated Muscle Mechanics

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Measuring the force response to sinusoidal length perturbations in muscle enables calculating the viscoelastic properties of the tissue over a wide range of frequencies. Coupling these empirical results with complementary mathematical and computational models describes the kinetics of force-generating actomyosin cross-bridges. This sinusoidal analysis requires system linearity, a constraint confining length stimuli to very small amplitudes in demembranated (skinned) muscle preparations because larger length perturbations produce a non-linear force response. Therefore, it becomes difficult to examine cross-bridge cycling kinetics during length transients that are comparable with sarcomeric strains experienced during contraction in living muscles. Here we introduce a white noise method of system analysis that facilitates extracting the linear and non-linear components of the system response. Building upon Wiener theory, this method estimates the system response to a band-limited Gaussian white noise length stimulus through cross-correlation techniques (Lee-Schetzen approach). To examine and develop this approach, we computer simulated the response of a predefined system consisting of both linear and non-linear components and were able to estimate the expected linear response of the system. These simulations demonstrate the powerful utility of this technique to separate the linear and non-linear system responses in both the time or frequency domains. We also examined the experimental applicability of these methods using small strips of skinned muscle tissue, from which we estimated the linear and non-linear components of the system response in calcium-activated muscle. This linear component is consistent with the linear system response calculated from comparable measurements using sinusoidal length perturbation analysis. These computational and experimental methods provide a platform for characterizing cross-bridge cycling behavior, and permits distinguishing between linear and non-linear components of the complicated force responses following length transients associated with normal muscle contraction.

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Repetitive Contractions at Short and Long Lengths: Do Not Subtract Passive Force!

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Several reports have compared the consequence of repetitive contractions at long and short lengths, with the goal of gaining an understanding of factors causing muscle fatigue: metabolic vs ion distribution. This is traditionally done calculating active force as peak force - passive force. Alternatively, it has recently been shown that during contraction of whole muscle, fascicle length shortens, and it would be more appropriate to subtract the passive force associated with the fascicle length at the peak of the contraction. These two approaches will give different results, for contractions at long length. Contractions of the rat medial gastrocnemius muscle were obtained at 0.3Hz (trains

of 50 Hz, 400 ms duration) at a length either 3.6mm shorter or 3.6mm longer than the reference length. During the repetitive contractions, muscle length was changed periodically to the other length to observe 1-2 contractions, then returned to the test length. Initial active force was 2.53 ± 0.4 (mean $\pm$ SD) and 6.26 ± 1.2 N at short and long lengths respectively. Active force at the long length would be similar to that of the short length if active force was calculated in the traditional manner. Active force decreased to 1.90 ± 0.5 and 1.8 ± 1.0 N at the short and long lengths respectively. During repetitive contractions at the short length, active force was 3.6 ± 1.1 N, when measured at the long length. During repetitive contractions at the long length, active force was 0.67 ± 0.4 N, when measured at the short length. Clearly, the long length resulted in substantially greater fatigue than the short length. There would be no explanation for this if active force was calculated in the traditional manner. The higher real active force and therefore metabolic demand of contractions at the long length can explain the greater fatigue.

3181-Pos Board B228

Lengthening Contractions Produce Strain-Dependent Regional Changes in the Passive Length-Tension Properties of Permeabilized Single Fibers

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During forced lengthening of an activated skeletal muscle fiber (a ''lengthening contraction''), the applied strain is not distributed uniformly along the length of the fiber. Instead, regions having the longest sarcomere lengths (L_s) prior to the stretch are strained the most during the stretch (Panchangam et al. Biophys. J. 95:1890-1901, 2008). These differences in regional strain result in differences in strain history that could influence the subsequent resting L_s of the regions. We hypothesized that the change in resting L_s of a region following a lengthening contraction correlates positively with the strain of the region during the lengthening contraction. This hypothesis was tested on permeabilized fibers (n=15) obtained from *soleus* muscles of adult rats (8-9 mo, n=5). A laser diffraction technique was used to make rapid measurements (500 s^{-1}) of the L_s in 20 contiguous regions of fibers before, during, and after a single lengthening contraction (strain, 27 %; strain rate, 54 % s^{-1}; temperature, 15°C). During steady-state activation prior to lengthening, fibers produced an isometric stress of 133 ± 29 kPa at a mean L_s of 2.54 ± 0.16 μm. The lengthening contractions resulted in a 19 ± 9 % loss in isometric stress. For each of the 20 contiguous regions, the difference between the resting L_s 5 min before and 10 min after the lengthening contraction was plotted as a function of the increase in L_s at the peak of the lengthening contraction. The increase in resting L_s correlated positively (r=0.71) with the increase in L_s during lengthening contractions. We conclude that lengthening contractions produce regional changes in the passive length-tension properties of permeabilized single fibers and that these changes can be attributed to the recent strain history of the fiber regions. Support: NIH AG-13283; AG-015434.

3182-Pos Board B229

Crossbridge Properties During The Quick Force Recovery In Single Frog Muscle Fibers

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Fast stretches (~25 nmhs^{-1} amplitude and ~400 μs duration) which induced the forced rupture of the crossbridge ensemble were applied to intact muscle fibers to investigate the actomyosin bond properties during the force recovery following a step length change (release or stretch of 2 or 4 nm amplitude). Force and sarcomere length were measured with a fast force transducer (~50 kHz natural frequency) and a striation follower device. To reduce fiber damaging by the stretches and to reduce the influence of myofilament compliance on the measurements, experiments were made on the tetanus rise at tension of about 0.5 the maximum plateau tension. Fast stretches were applied before and at progressively increasing times (up to 20 ms) after the step length change. The rupture force of the crossbridge ensemble (P_c) and the sarcomere elongation at P_c (L_c) were measured. In contrast with the data obtained previously on the tetanus rise (Bagni et al. J. Physiol., 2005; 565). The results showed that: (1) P_c was almost independent of the tension developed by the fiber and (2), L_c was not constant but increased immediately after the release and decreased after the stretch. These changes were still present 2 ms later when the quick recovery was almost complete and disappeared completely within 15-20 ms. Data analysis suggests that: 1) crossbridge number remains almost constant during the quick force recovery; 2) crossbridge detachment by the fast stretch is preceded by the reversal of the myosin head power stroke and, 3) the extent of the power stroke can be measured by the changes in L_c occurring during the quick recovery.

3183-Pos Board B230

Active And Passive Myofibrils Lengthened Beyond Acto-myosin Filament Overlap Produce Different Forces

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We stretched myofibrils actively and passively beyond myofilament overlap and measured forces. We expected active myofibrils stretched beyond myofilament overlap to produce the same force as equally long passively stretched myofibrils. Actively stretched myofibrils produced approximately four times more force than passively stretched myofibrils (Figure 1). Titin deletion with active and passive stretching resulted in complete force loss suggesting titin plays a crucial role in active and passive force production. Calcium activation and force inhibition through BDM reproduced the passive force curve, suggesting that titin and active force and not just Ca^{2+} activation was required for the large force of actively stretched myofibrils at lengths beyond myofilament overlap. Based on these results, we suggest that titin is a molecular spring whose stiffness is regulated by changes in effective length which in turn are controlled by force-dependent actin-titin interactions.

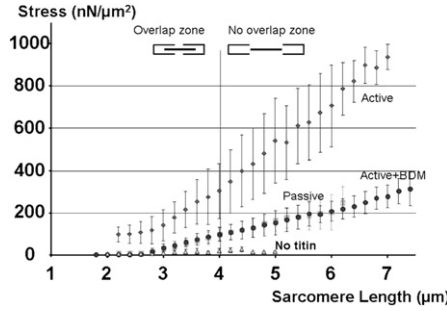

Figure 1. Actively stretched myofibrils show greater force beyond myofilament overlap than either passively stretched, or BDM actively stretched myofibrils. This suggests that passive titin forces increase with active force production and/or cross-bridge attachment but not with calcium activation.

3184-Pos Board B231

The Extent And Speed Of The Myosin Motor Recruitment Following 1-5 Nm Stretch Per Half-sarcomere Of Single Frog Muscle Fibers

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The contracting muscle exhibits a quite high resistance to sudden increase in load up to twice the isometric force (Katz, *J. Physiol.* 96:45,1939). The increase in half-sarcomere (hs) stiffness and the changes in the x-ray interference of myosin-based reflections during muscle stretch indicate that the second motor domain of the myosin molecules with the first motor domain already attached to actin in the isometric contraction attaches within 2 ms following the stretch (Brunello et al., *Proc. Natl. Acad. Sci. USA*, 104:20114, 2007). The mechanism is further investigated here by using single frog fiber mechanics (*Rana esculenta*, 4 °C, 2.1 μm sarcomere length). Stretches between 2 and 8 nm hs^{-1}, complete within 100 μs, were applied at the tetanus plateau (T_0) and the fraction of new motors relative to the isometric number (f) was determined either at the peak of the force response to stretch (T_1) or at the end of the quick phase of force recovery, 2 ms after the stretch (T_2). We show that: 1) for stretches <5 nm, independently of the phase of the force transient elicited by the stretch, f depends solely on the size of the axial distortion (Δz) of the attached motors; 2) for stretches >5nm at T_1 f reaches a maximum value of 0.3, while at T_2 f reaches a maximum value of 1. These results support the idea that the distortion of the attached motor domain of one myosin molecule promotes the attachment of the partner motor domain and indicate an upper limit (~10^4 s^{-1}) for the rate of the recruitment process. Supported by NIH (Grant no. 5R01AR49033-4) and MiUR, Italy.

3185-Pos Board B232

Load Dependence of Structural Changes in the Myosin Filament during Muscle Activation

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The M3 and M6 X-ray reflections from the myosin filaments of skeletal muscle correspond to axial periodicities SM3 and SM6 which are 14.34 and 7.19 nm at rest and 14.58 and 7.31 nm at the tetanus plateau (force T_0). This ~1.5% periodicity increase is much larger than the instantaneous filament compliance, and is probably due to an activation-dependent change in filament structure. SM6