1035-Plat
Drug Effects and Mechanism Underlying the Force-velocity Relationship of Skeletal Muscle
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Amrinone is a bipyridine drug with characteristic effects on the force-velocity relationship of fast skeletal muscle. Here we combined *in vitro* motility assays, transient biochemical kinetics and optical tweezers studies to elucidate the mechanisms underlying the drug effects. Amrinone (1-2 mM) reduced the sliding velocity of heavy meromyosin (HMM) propelled actin filaments by 31.0 ± 2.5% (n = 15) at different ionic strengths of the assay solution (20 - 160 mM). The drug also reduced (by 2 - 18%) the sliding velocity of actin filaments propelled by subfragment 1 (S1). Stopped-flow studies of myofibrils, acto-HMM and acto-S1 showed no amrinone-induced reduction in the rate of MgATP induced actomyosin dissociation and optical tweezers studies detected no changes in the working stroke length. In contrast, the ADP affinity of acto-HMM (but not acto-S1) was increased about two-fold by 1 mM amrinone. Our results are consistent with inhibition of a strain-dependent MgADP-release step as the basis for amrinone induced reduction in sliding velocity. Modeling suggests that such an effect may also account for most other amrinone-induced changes of the force-velocity relationship of muscle (*e.g.* in isometric force and in shape of the force-velocity curve). Moreover, the results point to the possible importance of cooperative interactions between the two myosin heads in muscle contraction.

1036-Plat
Role Of Myosin Binding Proteins On The Structural Stability And Flexural Rigidity Of Thick Filaments
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Despite the fundamental role of thick filaments in muscle contraction, little is known about the mechanical behavior of these filaments and how myosin associated proteins dictate differences between muscle types. Insect flight muscle (IFM) and vertebrate cardiac muscle share common physiological properties such as their cyclical contraction for producing either a wing beat or a heart beat, as well as their reliance on a pronounced stretch activation response to produce oscillatory power. We used atomic force microscopy (AFM) to study the morphological and biomechanical properties of native thick filaments from age-matched normal (+/+) and mutant (t/t) mice heart lacking cardiac myosin binding protein C (cMyBPC) and from IFM of normal (*fln*+) and mutant (*fln*^{0}) *Drosophila* lacking flightin. AFM images of these filaments were evaluated with an automated analysis algorithm that identified filament position and shape. The t/t thick filament length (1.48 ± 0.02 μm) was significantly ($P < 0.01$) shorter than +/+ (1.56 ± 0.02 μm). To determine if cMyBP-C contributes to the mechanical properties of thick filaments, we used statistical polymer chain mechanics to calculate a per filament specific persistence length (PL), an index of flexural rigidity directly proportional to Young's modulus. PL in the t/t (373 ± 62 μm) was significantly lower than +/+ (639 ± 101 μm). Accordingly the Young's modulus of t/t thick filaments was approximately 60% of +/+. Thick filaments from newly eclosed *fln*^{0} IFM have longer contour length (3.90 ± 1.33 μm) than *fln*+ filaments from same age flies (3.00 ± 0.38 μm), and a PL less than half that of IFM filaments from *fln*+ flies. These results provide a new understanding for the critical role of myosin binding proteins in defining normal cardiac and IFM output by sustaining force and muscle stiffness.

1037-Plat
Thin Filament Regulation of Relaxation in 3D Multi-Sarcomere Geometry
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The dynamics of muscle relaxation in physiologically relevant situations is complex due to interactions among crossbridge kinetics and thin filament regulation by Ca^{2+}. To quantitatively study behavior of muscle relaxation we have developed stochastic model of muscle contraction and its regulation in the 3D multi-sarcomere geometry. The model includes a simple three state and comprehensive nine state actomyosin cycle, extensibility of thick and thin filaments, and McKillop-Geeves and the flexible continuous tropomyosin chain (CFC) models of thin filament regulation. Loading conditions include isometric force development for prescribed Ca^{2+} concentrations and Ca^{2+} transients. We tested the hypothesis that the observed heterogeneity of shortening of individual sarcomeres is the principal mechanism causing rapid decrease in overall force upon sudden decrease of Ca^{2+} concentrations. We quantitatively determined the effect of heterogeneity of sarcomere lengths on the speed of muscle relaxation. The model predicted slow early relaxation caused by multiple myosin bindings within a single troponin-tropmyosin (TnTm) unit which keeps the unit open and allows the reattachment of detached crossbridges except at very low Ca^{2+} concentrations. At later times rapid shortening of some sarcomeres is observed due to force fluctuations caused by stochasticity of myosin binding. This inhomogenous shortening dramatically increases speed of the slow phase of relaxation. The combination of the effects of inhomogeneous shortening and the filaments extensibility mechanistically explains the observed two phase relaxation. Both regulatory models predict well the force-pCa relationship, but CFC model better fits the experimental data. The principal mechanism underlying this better fit is reduced size of the flexible TmTn regulatory unit upon myosin detachment which prevents reattachment of the crossbridges by partially covering actin sites within the unit. For the same reason the flexible chain model better predicted the twitch dynamics. Supported by NIH grant R01 AR048776.

1038-Plat
Molecular Dynamics of Tropomyosin: Implications for the Assembly and Regulation of Thin Filaments
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The molecular switching mechanism governing skeletal and cardiac muscle contraction couples the binding of Ca^{2+} on troponin to the movement of tropomyosin (Tm) on actin filaments. By shifting position around thin filaments in response to changing Ca^{2+}, Tm either blocks or exposes myosin-binding sites on actin, thereby regulating myosin-crossbridge cycling and consequently contraction. Tm lies over actin at a ~39 angstrom radius with considerable water between the two surfaces. Lorenz *et al.* (1995) and later Poole *et al.* (2006) proposed that Tm has a distinctive coiled coiled-coil shape designed to match the contours of F-actin. This arrangement might facilitate binding of Tm on F-actin and movement between regulatory states. In contrast, others have suggested that Tm flexibility is needed for binding and regulatory movements. To understand transitions of Tm between regulatory states better, the structure and flexibility of Tm was assessed by Molecular Dynamics performed in implicit water. A full-length Tm atomic structure was constructed by fitting different crystal structures of Tm segments (PDBs: 2D3E, 1IC2, and 2B9C) to the coordinates of the Lorenz coiled coiled-coil model. The Tm stretches and the model fitted to each other very well. Tm showed delocalized but pronounced anisotropic bending during 11ns MD, with no evidence of localized kinking, suggesting that Tm lacks discrete domains that flex. A persistence length several times the length of Tm was calculated, indicating that the molecule is only semi-flexible. Although Tm bends away from its initial supercoiled shape, it revisits the contours of the Lorenz model multiple times during simulation, implying that Tm may assume this shape when binding to F-actin. The results indicate that Tm is flexible enough to coil around actin, yet stiff enough to act as a cooperative unit during regulatory movements.

1039-Plat
Obscurin, A Large Modular Protein, Regulating Sarcomere Formation In Drosophila Muscle
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Drosophila obscurin is a modular muscle protein of ~ 420 kDa. The sequence, derived from the genome, contains two C-terminal serine-threonine kinases, both of which are predicted to be inactive, as well as 21 Ig and two Fn3 domains. A Rho/GEF domain has been identified in the N-terminal region. There are four obscurin isoforms, two of which are exclusively expressed in the indirect flight muscle (IFM). Obscurin is in the M-line throughout IFM- development and in the adult fly. In Drosophila IFM, the protein is across the M-line, unlike the vertebrate, where obscurin is at the periphery of the myofibril. A P-element insertion in the first intron of the gene leads to severely reduced obscurin protein levels and a flightless phenotype in homozygous mutant flies.

Myofibrils are thinner and electron microscopy shows a disrupted M-line and shifted H-zones. This phenotype was rescued by precise P-element excision using a transgene fly stock carrying a transposase. Non-flight muscles are not affected by the mutation. Obscurin RNAi lines driven with an IFM specific Gal4 driver lead to a flightless phenotype, and the specific reduction of obscurin IFM isoforms. Electron microscopy shows the phenotype is more severe than in the P-element mutant. Co-immunoprecipitation showed that obscurin is associated with myosin. It is likely that obscurin is needed for normal alignment and symmetry of thick filaments. In yeast two-hybrid screens, a 400 kDa protein, MASK, was identified as a binding partner of obscurin kinase 2. MASK co-localises with obscurin in the M-line. MASK RNAi lines show a flightless phenotype. A possible binding partner for obscurin kinase 1 is ball, a kinase of unknown specificity. MASK and ball can both be linked to signalling pathways involved in muscle development.

Workshop 1: Advanced Single Molecule Fluorescence Techniques in Vitro and in Vivo

1040-Wkshp
Single-Molecule Analysis of Transcription
Richard Ebright[1], Shimon Weiss[2], Anirban Chakraborty[1], Dongye Wang[1], You Korlann[2], Achillefs Kapanidis[2], Emmanuel Margeat[2].
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We are using single-molecule fluorescence resonance energy transfer to define fundamental aspects of transcription initiation, elongation, and termination.
In published work, we have shown that initial transcription proceeds through a "scrunching'' mechanism, in which RNA polymerase (RNAP) remains fixed on promoter DNA and pulls downstream DNA past its active center. We have shown further that putative alternative mechanisms for RNAP-active-center translocation in initial transcription, involving "transient excursions" of RNAP or "inchworming" of RNAP, do not occur. The results support a model in which a stressed intermediate, with DNA-unwinding stress and DNA-compaction stress, is formed during initial transcription, and in which accumulated stress is used to drive breakage of RNAP-promoter interactions during promoter escape.
In unpublished work, we are assessing opening and closing of the RNAP active-center-cleft, movements of modules of sigma relative to RNAP in transcription initiation, movements of modules of the RNAP active center in transcription elongation, and movements of RNAP relative to DNA in transcription termination.
In further unpublished work, carried out in support of these studies, we have developed reagents and procedures that permit incorporation of a fluorescent probe at any position of interest within a transcription complex.

1041-Wkshp
In vitro and in vivo; kinesin and myosin moving one (or a few) at a time
Paul Selvin.
University of Ilinois, Urbana, Urbana, IL, USA.

1042-Wkshp
In-Vivo Super-Resolution Microscopy by Structured Illumination
Mats G.L. Gustafsson.
HHMI Janelia Farm Research Campus, Ashburn, VA, USA.
Periodically structured illumination light can extend the resolution of fluorescence microscopy beyond the classical limit through spatial frequency mixing. The amount of resolution extension, set by the spatial frequency of the illumination pattern, is normally about a factor of two, because the pattern frequency is limited by the diffraction in the same way as the conventional resolution.
Dramatically greater resolution extension is possible, however, if a nonlinearity can be introduced between the incoming illumination intensity and the outgoing emission rate, because such a nonlinearity can create harmonics of the illumination frequency. Reversible photo-switching of fluorophores constitutes one promising form of such nonlinearity.
Structured-illumination microscopy typically uses data reconstruction algorithms that assume that the entire data set represents a single unchanging structure. It has therefore been largely confined to fixed, unmoving samples. If a data set can be acquired in a time that is short compared to sample movement speeds, however, live imaging becomes possible. Here we present live imaging with ~100 nm lateral resolution at multi-Hz rates for hundreds of time frames, using linear structured illumination with a rapid pattern-generating system in the TIRF mode.

1043-Wkshp
Advanced Fluorescence Microscopy Of Single, Living Cells: Using Optical Proteomics To Study Native Biochemistry One Molecule At A Time
Mark C. Leake.
Oxford University, Oxford, United Kingdom.
What is the molecular basis of the cell? How do single-molecule properties in a living organism scale up to effect whole-organism functionality? Can we bridge our gap in understanding between molecular biology and cell science in a rational, predictive context? These questions pose some of the hardest and most fundamental challenges to the future of biological research. Full understanding of processes in living organisms is only achievable if all molecular interactions are considered, though to date the shear complexity of biological systems has caused precise single-molecule experimentation to be far too demanding, instead focusing on studies of single systems using relatively crude bulk ensemble-average measurements. What I will discuss are some experiments that are leading us to being able to monitor several biological systems simultaneously in a single living, functioning cell using ultra-sensitive single-molecule techniques.

1044-Wkshp
Elucidating Mechanisms in Complex Systems by Multi-wavelength Single-molecule Fluorescence
Jeff Gelles.
Brandeis Univ, Waltham, MA, USA.
Many biological systems function through multiple non-covalent interactions between different proteins or nucleic acids. Even when only a few different kinds of macromolecules are involved, it is often true that a large number of different non-covalent complexes can form. This combinatorial complexity can make using conventional biochemical approaches to elucidate the kinetic mechanisms of these systems intractably difficult. Multi-wavelength single-molecule fluorescence is powerful approach to mechanistic analysis of these complex systems. By following individual molecules, this method can define reaction pathways and measure kinetics even in mixtures as complex as whole cell extracts. This talk will illustrate this approach with examples taken from basic processes in molecular biology including transcription and pre-mRNA splicing.

Workshop 2: Channelopathies of Nerve and Muscle

1045-Wkshp
Mechanistic Diversity for Channelopathies of Brain and Skeletal Muscle
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Mutations in the coding sequence of voltage-gated ionic channels are known to cause a wide variety of diseases affecting muscle and brain. Biophysical studies on the functional consequences of these defects are now revealing an equally diverse spectrum of mechanisms that underlie the disruption of cellular excitability, synaptic transmission, or neuronal survival. This Workshop on Channelopathies highlights recent advances in understanding the mechanistic connection between altered channel behavior and disease pathogenesis. New knock-in mouse models of Familial Hemiplegic Migraine illustrate how subtle gain-of-function changes in P/Q-type CaV2.1 channels enhance excitatory synaptic transmission and promote cortical spreading depression. A transmembrane protein linked to Familial Alzheimer Disease (presenilin) has recently been shown to form an unconventional Ca2+ leak channel that accounts for 80% of the divalent conductance of the ER. Finally, new insights have emerged in the past two years on a possible common pathomechanism by which mutations in either NaV1.4 or CaV1.1 channels of skeletal muscle may cause periodic paralysis. In both cases, mutations are clustered at arginine residues of the S4 voltage-sensor domain. Mutant channels conduct small ''omega'' currents through a voltage-regulated gating pore and may be the source of the inward current that renders affected fibers susceptible to sustained depolarized shifts during attacks of weakness.

1046-Wkshp
Neuronal calcium channels and migraine
Daniela Pietrobon.
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Migraine is a common disabling brain disorder of unknown etiology. A subtype of migraine with aura (familial hemiplegic migraine type 1: FHM1) is caused