showed that although local, near-to-equilibrium systems may be interlocked. Thus, for example, if the act of proton pumping were reversibly connected to ATP synthesis, the ATP could then be used as a key component of other near-to-equilibrium systems such as metabolism and/or cytoskeletal activities. I propose a specific mechanism for the act of proton pumping that qualifies as a near-to-equilibrium system. It embodies motion perpendicular to the membrane plane for ion pumping. The mechanism is both cooperative and synchronized. Each transport event results in a compaction of the protein across the membrane provoking a neighboring pump to expand and *visa versa*. The mechanism implies that pumps be dimers or multimers in living membranes although they could pump individually when reconstituted into bilayers. It is the nature of the pumping mechanism that it can be restarted with Brownian motion if it falters.

Platform U: Muscle: Fiber & Molecular Mechanics & Structure

1095-Plat

X-Ray Diffraction "Movie" Of Complete Oscillatory Work Cycles Myogenically Produced In Glycerinated Insect Flight Muscle (IFM)

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When slightly calcium-activated (pCa ~5.7, gives ~0.2 peak isometric force), glycerinated *Lethocerus* insect flight muscle (IFM) can be mechanically stretch-activated at constant [Ca^{2+}] to give a delayed active rise to peak force, a myogenic response typical of asynchronous IFM. Continuous sine-wave length-oscillations elicit sinusoidally cycling force traces, delayed ~45° behind length cycles. Force-length x-y plots therefore follow anti-clockwise Lissajous loops of elliptical form, enclosing an area proportional to oscillatory work output per cycle, which peaked at ~2%X~2Hz for 10-20-fiber bundles. A Pilatus 100K detector collected 64 synchrotron-x-ray fiber-diffraction frames per full cycle (8ms time resolution), throughout an 11-cycle run (704 frames). Summing successive cycles and adjacent frames produced a 16-frame movie (32ms time resolution) showing weaker details. The movie shows clear within-cycle peak-to-valley intensity changes in multiple reflections, some signaling crossbridge mass shifts toward (and away from) thin-filament lattice positions, others cross-bridge shifts between binding to and detaching from actin target zones, still others signaling crossbridge shifts between tilt angles mostly near 90° versus mostly dispersed to non-90° angles. Surprises include: 1) Maximum 90° angles occur near force peak in *Drosophila* but near force valley in *Lethocerus*. 2) Although the force sine-wave varies smoothly, two structural signals of crossbridge attachment show biphasic profiles as force rises and again as force falls, as if outer and inner myosin heads (AL-Khayat etal model) attach/detach in separate cohorts during the 2% (26 nm/half-sarcomere) length changes. 3) Structural signals of crossbridge action are variably phase-coupled to the force sine-wave; some x-ray signals even differ in phase lag between force peak and valley. Maximum tropomyosin shift spans ~5 frames around force peak. Overall results strongly constrain possible mechanisms of stretch-activation, suggesting complementary approaches for revealing it. (Support: NIH, DOE).

1096-Plat

Photoactivatable Quantum Dots in Super-Resolution 3D Microscopy of Myofibrils

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To image the relationships between immune-labeled myofibrilar proteins at sub-diffraction-limited resolutions, highly photostable quantum dots were chemically modified to make them photoactivatable. Although previous reports have used photoactivation of cyanine dyes and GFP variants for 2D super-resolution microscopy, photoactivatable quantum dots (PAQ dots) have sufficient brightness and photostability to enable 3D acquisitions of signals from individual quantum dots. The chemical synthesis of PAQ dots caused only minor changes in the spectroscopic properties and brightness of the activated PAQ dots relative to unmodified quantum dots as assessed by fluorescence lifetime imaging of single quantum dots. The PAQ dots were conjugated to Fab fragments for immunostaining of myofibrils. After optimizing conditions so that a balance between photoactivation and photobleaching of the PAQ dots occurred during 3D acquisition in a spinning disk confocal microscope, 3D images of individual quantum dots were reduced to the 3D center of mass and accumulated until sufficient data for a full image was generated. Initial results demonstrate sub-diffraction resolutions in XY and even more striking resolution improvements in Z. The superresolution images reveal finer structural details in the myofibrils than conventional confocal imaging. Unlike electron microscopy, all measurements are made in aqueous solutions. Furthermore, the ability to make PAQ dots with a variety of emission wavelengths enables multicolor 3D labeling that can be used for protein mapping at super-resolutions in myofibrils and other samples.

1097-Plat

Structural Changes in the Myosin Motors During Activation of Skeletal Muscle

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Structural changes in the myosin motors during the transition from the resting state to the plateau of isometric contraction were investigated by X-ray interference from single fibers of frog skeletal muscle. Isolated intact fibers (2.1μm sarcomere length, 4°C) were mounted vertically at beamline ID2 of the ESRF synchrotron (Grenoble, France) between a loudspeaker motor and a capacitance force transducer. 2D diffraction patterns were collected on a CCD detector 10 m from the preparation with 5 ms time resolution. During the development of the isometric tetanus, the intensity of the M3 reflection, originating from the axial repeat of the myosin motors, first decreases to 30% (at 50 ms) of its resting value, then increases to a steady value 70% of that at rest. The M3 reflection has a major peak with spacing 14.34 nm at rest and two peaks with mean spacing 14.57 nm at the tetanus plateau (Linari *et al.*, *Proc. Natl. Acad. Sci. USA* 97:7226, 2000). The changes in the fine structure of the M3 reflection during activation were best fit by a structural model in which (1) all thick filaments have the same mean spacing at a given time during activation (2) the number of active motors increases in proportion to the isometric force (Brunello *et al.*, *J. Physiol.* 577:971 2006), (3) the conformation of the active motors is independent of the level of force and strain in the thick filament.
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1098-Plat

Measurement Of ATPase Activity During Ramped Stretches In Contracting Skeletal Muscle Fibers Of The Rabbit

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Skeletal muscle force response to small amplitude and low velocity ramp stretches is biphasic. An initial fast increase in force, where myosin heads are forcibly detached is followed by a slower increase in force with net re-attachment of myosin heads to actin. The average ATPase rate is very low during stretch (Curtin & Davies,1973), but due to lack of time resolution the two phases to match force changes have never been resolved. We therefore examined and modeled tension and ATPase responses to ramp stretches (5% and 1% of fiber length, Lo) at low velocities (0.1 and 0.5 Lo/s) in permeabilised fiber bundles of rabbit psoas at 12 and 20°C. We show that ATPase activity drops to near zero during the initial fast phase of the stretch and increases slightly but still remains lower than isometric during the second part of the stretch phase, returning to normal post-stretch. The response was not as marked at 12 as at 20°C, although ATPase rate was still reduced in both the fast initial and slow secondary phase of the force response. During the initial phase the myosin heads are forcibly removed from actin, the cross-bridge cycle is not complete and release of hydrolysis products is interrupted. In the second phase, myosin heads re-attach whilst the muscle is still lengthened and the cross-bridge cycle is truncated for a fraction of attached heads, leading to slower Pi release than during isometric conditions. These effects are less marked at 12 than at 20°C because the fraction of strongly bound cross-bridges is reduced at the lower temperature.
Curtin, N.A. & Davies, R.E. (1973). Cold Spring Harbor Symp. on Q. Biol., 37, 619-626.

1099-Plat

Disrupting Myosin Relay-Converter Domain Communication Impairs Drosophila Muscle Mechanical Performance and Flight Ability

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We evaluated the mechanical properties of *Drosophila* indirect flight muscle (IFM) fibers expressing a myosin converter domain R759E mutation. The interaction of R759 with relay loop residue I508 is thought to be critical for relay-converter inter domain communication. By changing the charge on residue 759, we are testing if this inter domain interaction is important for the mechanical performance of muscle fibers. Electron microscopic examination of muscle fibers from young adult R759E flies indicates normal myofibril assembly. Using the work loop analysis technique we found that the maximum power (P_{max}) generated by the mutant R759E fibers from two day old flies was significantly reduced by 50% compared to control fibers while the frequency at which maximum power is generated (f_{max}) was reduced to 67%. Maximum power occurred at peak-to-peak strain amplitude of 2% resting muscle fiber length. Varying ATP concentration at 15°C revealed no significant difference in K_m for P_{max} or f_{max} between control and mutant R759E fibers, suggesting that the mutation does not affect ATP affinity. Small amplitude sinusoidal analysis revealed a significant reduction in complex stiffness by 48% compared to control fibers, with elastic modulus, E_e, reduced by 31% and viscous modulus, E_v, reduced by 45%. This reduction in power and mechanical performance of the flight muscle fibers led to a decrease in wing beat frequency from 140 ± 2 Hz for control flies to 127 ± 2 Hz. The reduction in wing beat frequency contributed to a decrease in flight index from 2.31 ± 0.1 for control flies to 1.25 ± 0.1 at 15°C. Thus, this study suggests that the interaction between relay loop I508 and converter domain R759 is critical for myosin inter domain communication, muscle fiber power generation and *Drosophila* flight performance.

1100-Plat
Single Skeletal Muscle Fiber Performance is Altered in Heart Failure Patients
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A decrease in whole skeletal muscle performance is common in heart failure patients. We examined the viscoelastic properties of individual human skeletal muscle fibers using small amplitude sinusoidal analysis to test the hypothesis that heart failure affects skeletal muscle mechanics and kinetics at the single fiber level. We obtained *vastus lateralis* (quadriceps) muscle from needle biopsies of 7 heart failure patients and 4 sedentary controls. At low [Ca^{2+}] (pCa 8, 25°C), Type I (slow contraction velocity) and Type IIA (fast contraction velocity) muscle fibers from heart failure patients had lower isometric tensions as well as lower elastic and viscous moduli. Notably, Type I and IIA fibers produce positive oscillatory work and power at pCa 8. Type I fibers from heart failure patients at low [Ca^{2+}] produced less oscillatory work and had a higher frequency of maximum work, indicating an increase in myosin kinetics, compared to controls. At high [Ca^{2+}] (pCa 4.5, 25°C), Type I and IIA fibers from heart failure patients showed similar isometric tensions and myosin kinetics parameters as controls. In contrast to low [Ca^{2+}], at high [Ca^{2+}] Type I and IIA fibers from heart failure patients had a larger elastic modulus at low oscillation frequencies and consistently produced greater oscillatory work and power than control fibers. Together, these results indicate that heart failure modifies single skeletal muscle fiber performance at the level of the myosin-actin cross-bridge, although the effect differs between low and high [Ca^{2+}]. The relevance of these differences to reduced whole muscle function in heart failure patients awaits further studies.

1101-Plat
Skeletal Muscle Lacking the Extreme C-Terminal SH3 Domain of Nebulin Exhibits Heightened Vulnerability to Eccentric Contraction-Induced Injury
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Nemaline myopathy is a congenital myopathy afflicting roughly 1 in 50,000 children. Nemaline myopathy is a disease of the thin filament, and mutations in the giant thin filament template nebulin contribute to its etiology. A clinical case report has demonstrated that loss of the extreme C-terminal Src homology 3 (SH3) domain of nebulin can cause nemaline myopathy. The nebulin SH3 domain is believed to anchor the thin filament to the Z-disk through its interaction with myopalladin. To further elucidate the physiological roles of the nebulin SH3 domain, the skeletal muscle phenotype of wild-type (*nebulin*$^{+/+}$) mice was compared to that of mice homozygous for the I6611X mutation in the nebulin gene (*nebulin*$^{I6611X/I6611X}$). The I6611X mutation introduces a premature truncation of the nebulin transcript and eliminates the SH3 domain from the nebulin protein. Contractile measurements revealed that baseline isometric stress production was identical in *nebulin*$^{I6611X/I6611X}$ and *nebulin*$^{+/+}$ muscle (247 ± 6 kPa vs. 253 ± 6 kPa, respectively; P=0.50). However, *nebulin*$^{I6611X/I6611X}$ muscle exhibited a greater vulnerability to eccentric contraction-induced injury compared to *nebulin*$^{+/+}$ muscle, where "injury" was defined as a decline in isometric stress production across a series of 10 eccentric contractions (39.3 ± 0.8% vs. 29.1 ± 1.6%, respectively; P<0.01). The corresponding decline in passive stiffness was identical in *nebulin*$^{I6611X/I6611X}$ and *nebulin*$^{+/+}$ muscle (13.5 ± 2.4% vs. 14.4 ± 2.1%, respectively; P=0.79). Muscle fiber type distributions and cross-sectional areas were also identical in *nebulin*$^{I6611X/I6611X}$ and *nebulin*$^{+/+}$ muscle. These data indicate that the nebulin SH3 domain is dispensable for isometric stress production in skeletal muscle but necessary for protecting muscle from injurious eccentric contractions. It is conceivable that heightened vulnerability to eccentric contraction-induced muscle injury, or to other types of biomechanical challenges, explains the pathology observed in children with nemaline myopathy.

1102-Plat
Extremely Low Maximal Force-Generating Ability in Hummingbird Flight Muscle Fibers
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Hummingbird flight muscle has the highest mass-specific mechanical power output among all vertebrates. The wingbeat kinematics and aerodynamics of hummingbird flight have been studied in multiple species, but little is known about fundamental contractile properties of these remarkable muscles. The objective of this study was to measure the maximal force-generating ability (maximal force per unit of fiber cross-sectional area, P_o/CSA) of single muscle fibers from the pectoralis muscle, which powers the wing downstroke, in adult hummingbirds and in another similarly-sized species, zebra finch, which does not hover but also has a very high wingbeat frequency during routine flight. Single, skinned pectoralis fibers were maximally calcium-activated and P_o/CSA was measured across a range of temperatures. P_o/CSA in hummingbird pectoralis fibers was 1.1 ± 0.4 (mean ± SEM), 5.2 ± 1.6, and 10.8 ± 2.4 kN/m^2, at 10, 15, and 20°C, respectively. P_o/CSA in zebra finch pectoralis fibers was 2.0 ± 0.4 (mean ± SEM), 10.4 ± 1.6, and 21.6 ± 3.2 kN/m^2, at 10, 15, and 20°C, respectively. For comparison, P_o/CSA in adult mammalian limb muscle fibers at 15°C is typically 100-120 kN/m^2. The mean P_o/CSA in hummingbird leg muscles fibers, which are used for perching, was 73.4 ± 11.6 kN/m^2 at 10°C. These results indicate that hummingbird pectoralis fibers have an extremely low force-generating ability, compared to mammalian limb muscle fibers and hummingbird leg muscle fibers, even when maximally activated, and have an unusually high temperature-dependence of force generation. The unusually low force-generating ability of hummingbird and zebra finch pectoralis fibers may reflect a constraint imposed by a need for extremely high contraction frequencies, especially during hovering flight in hummingbirds. Supported by the National Science Foundation.

Symposium 9: Sensing the Membrane

1103-Symp
Mechanisms of Signaling and Regulation of Membrane Properties by a Bacterial thermosensor
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The ability of bacteria to control the biophysical properties of their membrane phospholipids allows them to thrive in a wide range of physical environments. When bacteria are exposed to temperatures below those of their normal conditions, the lipids of their membrane become rigidified, leading to a suboptimal functioning of cellular activities. These organisms can acclimate to such new conditions by an increase in the desaturation of the acyl chain of membrane phospholipids. Phospholipids containing unsaturated fatty acids have a much lower transition temperature than those lipids made of saturated fatty acids. As a result, the physical properties (fluidity) of the membrane lipids return to their original state, or close to it, with restoration of normal cell activity at the lower temperature. We discovered that in the model Gram-positive bacterium *Bacillus subtilis* the transcription of the *des* gene, coding for an acyl lipid desaturase, is controlled by a two component system that senses changes in the membrane properties due to abrupt temperature change. The membrane component, named DesK, of this transcriptionally regulatory system is a thermosensor with histidin kinase and phosphatase activities that senses membrane biophysical properties and transmits this signal to the transcriptional