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The compensatory mutation suppresses the assembly defects in pupal muscle that are seen in both *E499R* and *R714E* and reduces disrupted myofibril morphology in two-hour old indirect flight muscles. However, the *E499R-R714E* mutant myosin was unable to maintain sarcomere organization in two-day-old flies or to restore flight ability. Overall, our results reveal that interaction of residues at the relay/SH1-SH2 helix interface is important for myosin and muscle function.

**739-Pos Board B525**

**Kinetic Characterization of Converter and Relay Loop Domain Interaction in Drosophila Myosin Sub-Fragment 1**

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We investigated the kinetic properties of Drosophila myosin sub-fragment 1 (S-1) mutant R759E and its suppressor R759E/N509K. The R759E mutation is located in the converter domain of the myosin head whereas N509K is present at the relay loop. Steady-state measurements for R759E S-1 show more than 50% reduction in calcium ATPase as well as ~40 % reduction in basal magnesium ATPase compared to wild type whereas actin-stimulated Mg-ATPase of R759E (Vmax) is reduced ~70 %. Homology models of myosin S-1 suggest the R759E mutation can disrupt the interface between the converter and the relay loop and that the suppressor (R759E/N509K) can restore this interaction. This prediction was confirmed by our steady-state ATPase data, which demonstrated that the suppressor mutation restored calcium, basal Mg-ATPase and Vmax back towards wild type values. Calcium and basal Mg-ATPase activity of R759E/N509K increased ~30 % and ~25 % respectively compared to mutant R759E S-1. Actin-stimulated Mg-ATPase activity of R759E/N509K was ~40 % higher compared to R759E mutant S-1. Using flash photolysis, our transient kinetics results showed no significant change in the ATP-induced dissociation of acto-S-1 (K1k+2′) and ADP-affinity (KAD) of acto-S-1 for R759E and R759E/N509K compared to wild-type. Using stopped-flow we measured ATP-binding to S-1 (R759E and R759E/N509K). Compared to wild-type we find for R759E a 30% reduction in the rate constant of ATP-binding (K1k+2) and a 25% reduction in the rate constant of ATP-hydrolysis (k+3+k-3) whereas for R759E/N509K ATP-hydrolysis is restored to wild-type level. This demonstrates the significance of relay-loop and converter domain interaction for S-1's enzymatic ability. The ability to suppress many of the mutant defects in S-1 kinetics corroborates the dramatically improved muscle function we previously found in R759E/N509K double mutants and provides mechanistic insight into this improvement.

**740-Pos Board B526**

**A Method for the Transgenic Expression and Purification of Skeletal Muscle Myosin II Isoforms using Drosophila Melanogaster**

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There are many biologically interesting myosin isoforms that are expressed at levels too low for direct purification from *in vivo* sources. Efforts aimed at recombinant expression of functional striated muscle myosin isoforms in bacterial or insect cell culture have largely met with failure, although high level expression in muscle cell culture has recently been achieved at significant expense. We are optimizing a novel method for the use of *Drosophila melanogaster* that has been genetically engineered to produce histidine-tagged recombinant muscle myosin isoforms. This method relies on several facts. First, that the *Drosophila* genome contains only one gene encoding all skeletal muscle myosin isoforms. Second, that this DNA can be manipulated with molecular biology techniques and then be inserted into a specific location in the *Drosophila* genome. Third, that the $Mhc^{10}$ fly line has endogenous myosin knocked out in the indirect flight muscles. Fourth, that the *Actin88F* promoter facilitates high-level expression of accessible myosin in the thoracic indirect flight muscles. Finally, that cost of fruit fly colony propagation is relatively low. Employing these advantages, we have created a system for the production of extremely pure skeletal muscle myosin. We demonstrate this method by expressing and purifying a recombinant histidine-tagged variant of embryonic body wall skeletal muscle myosin II from an engineered fly strain. This myosin shows the expected ATPase activity and is of sufficient purity and homogeneity for crystallization. Our technique may prove useful for the expression and isolation of mutant myosins linked with skeletal muscle diseases and cardiomyopathies for their biochemical and structural characterization.

**741-Pos Board B527**

**Instability of the S1/S2 Hinge is not Affected by the Myosin Filament**

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The S1/S2 hinge of myosin is thought to be unstable in some species of myosin such as myosin VI and certain scallop muscle myosin. To examine the stability of the S1/S2 hinge of rabbit skeletal muscle myosin, gravitational force spectrometry was used to measure the separation of myosin heads bound in rigor to actin immobilized on glass microspheres. The molecular lengths of this separation were measured at different levels of applied force up to the limit that the rigor bond would sustain of about 10 pN in these experiments. The molecular lengths were found to increase linearly with the applied force up to lengths exceeding 100 nm which requires a partial unraveling of the S2 coiled coil in the single myosin molecule. Free-fall force spectrometry on such single myosin molecules confirmed that the coiled coil could reversibly unravel and refold at forces less than 13 pN. In order to test whether thick filament formation would affect this instability under force, synthetic cofilaments of proteolytically isolated myosin rod and intact myosin were prepared at different ratios of myosin to rod up to ratios where it is expected that only one intact myosin would be present per myosin rod cofilament. The force-distance relationships for myosin in the cofilament were not substantially different from those of single myosin molecules. These data indicate the possibility that vertebrate skeletal muscle myosin may also utilize S1/S2 hinge unraveling to provide flexibility during crossbridge formation so that a productive binding orientation is achieved. The reversibility of the unraveling of the coiled coil might contribute to the mechanism of motility under some circumstances as has been proposed for non-muscle myosin VI.

**742-Pos Board B528**

**Manganese Cation Reports on the Number of Coordinated Phosphates from the Nucleotide at the Active Site in Myosin ATPase**

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ATP is an important cofactor for myosin ATPase. ATP binding initiates myosin conformation change (recovery stroke), priming it for force generation. In the presence of a divalent cation (usually $Mg^{2+}$), myosin hydrolyzes ATP to ADP and phosphate, and their subsequent release presumably triggers the power stroke in actomyosin. What is the role of ATP hydrolysis in myosin? What is the relation between the kinetics of ATP hydrolysis and those of myosin conformation changes? To address these problems, we propose to use pulsed electron paramagnetic resonance (EPR) techniques and study the coordination environment of the divalent cation complexed with a nucleotide at the myosin active site. To enable the EPR investigations, $Mn^{2+}$ can be used instead of $Mg^{2+}$ without interruption of the myosin ATPase activity. In this preliminary work, we studied the myosin.Mn complexes with non-hydrolyzable nucleotide analogs, AMPPNP (triphosphate) and ADP.$AlF_4$ (diphosphate), as well as Mn.nucleotide complexes without myosin. $Mn^{2+}$ binding to myosin was monitored by pulsed electron-electron double resonance (ELDOR), which detected the magnetic dipole interaction between $Mn^{2+}$ and a nitroxide spin probe attached to the A639C myosin mutant. The number of coordinated phosphates in myosin.Mn.AMPPNP complex determined by $^{31}P$ pulsed electron-nuclear double resonance (ENDOR) was twice that detected for the myosin.Mn.ADP.$AlF_4$ complex. These results demonstrate the potential of pulsed EPR for distinguishing between the myosin.ATP and myosin.ADP biochemical states and study the role of ATP hydrolysis in myosin structural kinetics.

**743-Pos Board B529**

**3-Dimensional Structure of Human Cardiac Muscle Myosin Filaments by Electron Microscopy and Single Particle Analysis**

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In all muscles the interaction between actin and myosin filaments leads to muscle contraction and force production mediated by the hydrolysis of ATP. Actin filament structure is well understood to high resolution, but myosin filament structure is much less well defined. Myosin filaments are formed from complicated arrangements of myosin molecules and accessory proteins (e.g. C-protein [MyBP-C] and titin). We compare the arrangement of the myosin heads in the different species and how these arrangements change in diseased muscle. We have developed a method to solve the 3-dimensional (3D) structure of myosin

filaments by single particle analysis of electron microscope (EM) data. We already defined the 3D structure of myosin filaments of various muscles from different species by both X-ray diffraction modelling and EM and single particle analysis including insect flight muscle, scallop striated muscle, fish skeletal muscle and rabbit cardiac muscle. We are now studying the 3D structure of myosin filaments isolated from human heart muscles.
Mutations in cardiac myosin, C-protein and titin are known to be associated with cardiomyopathies (e.g. hypertrophic cardiomyopathy and dilated cardiomyopathy). In order to understand myosin-associated heart disease, it is important to understand the 3D structure of myosin filaments in normal heart muscle. Recently we have developed procedures to isolate human cardiac muscle myosin filaments preserving their highly ordered pseudo-helical structure thus making them amenable, for the first time, to EM and single particle image analysis. We have collected EM data from myosin filaments isolated from both normal and failing hearts, and have so far processed the data from normal heart muscle. Analysis of the 3D structure of myosin filaments in normal heart muscle will permit the structural effects of known myosin filaments-associated mutations to be investigated in detail.

**744-Pos Board B530**
**Functional and Structural Impact of Site-Directed Methionine Oxidation in Myosin**
**Rebecca J. Moen**[1], Jennifer C. Klein[2], Evan A. Smith[1], Margaret A. Titus[1], David D. Thomas[1].
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We have examined the functional and structural perturbations of methionine (Met) oxidation in Dictyostelium (Dicty) myosin II. Protein oxidation by reactive oxygen species (ROS) is a critical element of cell function. However, elevated production of ROS can lead to the accumulation of oxidatively modified proteins, contributing to a decline of function in biologically aged or diseased muscle. For Dicty myosin containing all native methionines, peroxide-treatment (used as an oxidative agent) decreases actin-activated myosin ATPase activity, consistent with the decline in actomyosin function previously observed in biologically aged or peroxide-treated muscle. We have recently identified a single Met that is responsible for this functional decline, Met 394, located near the myosin cardiomyopathy loop in the actin-binding interface. Oxidation of this Met also induces a redistribution of existing myosin structural states of the actin-binding cleft. We have now expanded the understanding of how oxidation at M394 affects myosin function and structure by using site-directed mutagenesis (from M to Q) to mimic oxidation. We then characterized actomyosin interaction and changes in structural states of both the force-generating region and actin-binding cleft of myosin. Our goal is to bridge our understanding of site-specific Met oxidation and muscle dysfunction with molecular-level insights into actomyosin interaction.

## Cell & Bacterial Mechanics & Motility I

**745-Pos Board B531**
**Study Controlling Factors of Gram-Negative Bacterial Cell Shape**
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Peptidoglycan (PG) sacculus has an important role in determining the shape of many bacterial cells. It is composed of glycan strands cross-linked by peptides into a net enclosing the cell and acting as a mechanical shield which protects the cell from bursting due to high internal (turgor) pressure. Importantly, how peptidoglycan is organized in the sacculus is the key controlling the cell shape. Many cells lose their rod shape and become spherical as perturbation of certain proteins such as MreB is introduced - a process believed to change the PG remodeling. Although much effort has been devoted, the knowledge of how the PG remodeling is controlled is still little. It has been proposed that penicillin-binding proteins (PBPs) - the peptidoglycan synthesizers - are grouped into a synthesis machinery which is associated with MreB. However the exact role of MreB in such synthesis machinery, if existed, is still a mystery. By using a computational approach, we aim to understand how the cell maintains its rod shape and what conditions lead to cell rounding so that the role of MreB in remodeling of peptidoglycan can be revealed.

**746-Pos Board B532**
**Growth of Curved and Helical Bacterial Cells**
**Hongyuan Jiang**, Sean X. Sun.
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A combination of cell wall growth and cytoskeletal protein action give rise to the observed bacterial cell shapes. Aside from the common rod-like and spherical shapes, bacterial cells can also adopt curved or helical geometries. To understand how curvature in bacteria is developed or maintained, we examine how Caulobacter crescentus obtains its crescent-like shape. Crescentin is an intermediate filament like protein. Caulobacter cells with or without the cytoskeletal bundle crescentin exhibit two distinct growth modes, curvature maintenance that preserves the radius of curvature and curvature relaxation that straightens the cell. Using a proposed mechanochemical model, we show that bending and twisting of the crescentin bundle can influence the stress distribution in the cell wall, and lead to the growth of curved cells. In contrast, after crescentin bundle is disrupted, originally curved cells will slowly relax towards a straight rod over time. Furthermore, we showed that the shape anisotropy of the cross section of a curved cell is never greater than 3%, even in the presence of crescentin.

**747-Pos Board B533**
**Spatially-Resolved Measurements of Bacillus Subtilis Cell Wall Growth Dynamics during Elongation and Division**
**Yi Deng**, Joshua W. Shaevitz.
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The cell wall, a cross-linked 3D network of peptidoglycan, is the key structural element that dictates cell shape and counter-balances turgor forces in prokaryotes. During cell growth and division, the wall undergoes a coordinated series of addition and remodeling events that enable the cell shape to change in a stereotyped manner. Recent studies suggest that the spatial insertion pattern of new material into the peptidoglycan network, guided by the bacterial cytoskeleton, is critical for cell shape maintenance. In this work, we use a high-density of quantum dots (QDs) on the B. subtilis cell surface to track the local motions of the wall in three-dimensions during cell growth and division. From a 2D projection of the QD distribution onto the cylindrical cell surface, we obtain the local stretching and shearing rates on multiple spatial scales. The homogeneity of insertion and cell wall twist during elongation and division can then be quantified from these growth maps.

**748-Pos Board B534**
**Coupling of Type III Secretion to the Membrane Potential**
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The bacterial flagellum and the virulence-associated bacterial needle are intricate nanomachines, which possess a type III secretion system for export of external components. The type III secretion system contains five highly conserved, essential membrane proteins. In the flagellum, these proteins are named: FlhA, FlhB, FliP, FliQ, and FliR. To understand how the secretion system functions, it will be necessary to characterize how these proteins interact and are regulated. This study reveals that modulating the level of the electron-carrier ubiquinone (Coenzyme Q) in the membrane can control protein export through flagellar secretion system. It is also shown that the N-terminal transmembrane regions of FlhA and FlhB are functionally associated. In the *Salmonella* flagellum, wild-type FlhB was replaced with a homologous FlhB chimera, made of the N-terminal transmembrane region of *Aquifex aeolicus* FlhB fused to the C-terminal cytoplasmic domain of *Salmonella* FlhB. Cells expressing the FlhB chimera were mostly non-motile and only rare flagella were made. Flagellar biogenesis was recovered in motile suppressor mutants isolated from soft-typtone agar, expressing the FlhB chimera and bearing spontaneous suppressor mutations. The identified suppressor mutations belonged to two categories: the first category of mutation were found in non-flagellar genes, affecting enzymes for the biosynthesis of ubiquinone; and the second category of mutation were encoded by the *flhA* gene. The mutations in the genes for the biosynthesis of ubiquinone decreased levels of ubiquinone and this reduced the membrane potential. Consequently, efficient flagellar assembly requires coupling between export through the type III secretion system and the energized state of the membrane.

**749-Pos Board B535**
**Observation of Bacterial Type I Pili Extension under Fluid Flow**
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Type I pili which mediate bacterial adhesion of E coli, are thought to help bacteria resist drag forces caused by fluid flow. The mechanical properties of these pili have already been studied by AFM force spectroscopy: They were found to elongate significantly at forces above ~50pN and contract at forces below ~25pN due to uncoiling and coiling of the quaternary structure of pili. It was speculated that these coiling forces could help bacteria move in opposition to the direction of fluid flow.
We show here that pili extend when E coli bacteria are exposed to physiological shear stresses in a flow chamber. Moreover, we observe that pili can indeed help bacteria move against flow. We compare the results obtained in the flow chamber with those obtained by AFM and optical tweezers measurements