can have a profound effect on the attachment rate of crossbridges (Williams et al. (2010) PLoS Comput Biol 6:e1001018.). At the same time, cross-bridges attaching to thin filaments and generating axial (longitudinal) forces and may simultaneously restrict the extent to which the filament lattice can expand or contract in the radial direction as muscle shortens. Here we examined radial changes in the lattice of the flight muscle of the hawk-moth Manduca sexta using high speed time resolved X-ray diffraction to directly measure the time course of changes in filament spacing as a function both the length of muscle and the timing of activation. Interestingly, the measured lattice spacing (1) strongly reflected activation timing and (2) varied considerably during the cycle of shortening and lengthening and (3) did not follow the pattern predicted by a constant lattice volume. Three key issues arise from these data. (1) Large changes in lattice spacing suggest that models of cross-bridge force generation should consider radial separation of thick and thin filaments; (2) cross-bridges may restrict the expansion of the filament lattice and may experience considerable radial force; and (3) the mismatch between measured and predicted radial motions of myofilaments indicates that there is fluid movement between subcellular compartments that has not been considered in the mechanics and energetics of force generation by muscle. Finally, radial tensions may play a key role in elastic energy storage for insect flight. Supported by NSF IOS-1022058 and NIH P41 GM103622-17.

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Coordination of Two Antagonistic Flight Muscles during Wing-Beat of Bumblebee Visualized by Ultrafast X-ray Diffraction Movies

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In bees and other insects, their wings are driven by the alternating actions of two antagonistic flight muscles, dorsal longitudinal muscle (DLM) and dorsoventral muscle (DVM). Here we recorded X-ray diffraction patterns simultaneously from these two muscles during tethered flight, as well as live images of the bees, by using two fast CMOS video cameras at a 5,000/s frame rate. The length changes of the two muscles, as probed by equatorial reflections, are almost perfectly anti-phase, and the DVM is shortest when the wings are upright. In both muscles, force-generating myosin heads, as probed by the 102, 211 and 311 reflection spots, build up slowly during the entire lengthening phase, and their peaks are substantially delayed behind the length. The 111 reflection spot (the first reflection to respond to stretch in skinned flight muscle fiber preparations: 2011 Annual meeting), is conspicuously enhanced in both muscles, well ahead (~20°) of the aforementioned reflection spots. Previously we interpreted this intensity change to reflect troponin structural changes, but its magnitude and the clear concomitant diminution of the 201 reflection spot are better explained by an azimuthal twist of attached myosin heads. Locating the stretch-sensing mechanism is crucial for understanding the mechanism for stretch activation, and the present results provide evidence that a population of myosin heads respond to stretch in a specific manner. This population is present in the lengthening phase in which fewer heads generate force. Therefore this population is likely to represent low-force myosin heads non-stereospecifically bound to actin. Stretch-induced conversion from low-force to high-force states has been proposed for vertebrate skeletal muscle (Iwamoto, 1995, Biophys. J.), and insects might have evolved to maximally utilize the preexisting function of myosin.

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Exploration and Suppression of Tau-Induced Cardiac and Skeletal Muscle Defects in a Drosophila Model

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Hyperphosphorylation and aggregation of the microtubule-associated protein tau into tangles occurs in several neurodegenerative diseases referred to as tauopathies. Several tau mutations, including tauR406W and tauV337M, have been associated with tau hyperphosphorylation and neurodegeneration. Accumulation of phosphorylated tau in cardiac and skeletal muscle biopsy samples suggests that striated muscle tissue generates tau-amyloid which causes the destruction/malfunctioning of myocytes. However, it is unknown how mutations in the tau gene lead to myopathies and moreover, there is no experimental model to understand tau-mediated striated muscle dysfunction. To test the effects of tau mutations on cardiac structure and function, we developed a novel Drosophila model that expresses pathological human tau in the heart using the UAS-Gal4 expression system with a cardiac specific driver. Cardiac physiology was assessed using high-speed video recording of heart tube contractile parameters from semi-intact heart preparations. Compared to control human tau (h-tau), expression of mutant tau (h-tauR406W and h-tauV337M) resulted in progressive cardiac dysfunction and ultrastructural abnormalities. Expression of h-tauR406W in 4 week-old hearts resulted in severe cardiac dilation, reduced contractility, increased arrythmia, and ultrastructural defects, including myofibrillar degeneration and mitochondrial elongation. Expression of h-tauV337M in 4 week-old hearts resulted in reduced contractility, increased arrythmia, and fragmented mitochondria. Interestingly, cardiac dilation and ultrastructural defects in 4 week-old h-tauR406W flies were suppressed by cardiac overexpression of DRP-1 (a regulator of mitochondrial biogenesis), or TRAP-1 (a mitochondrial chaperone). Additionally, expression of mutant tau (h-tau R406W and h-tau V337M) in indirect flight muscles using Actin88F driver resulted in reduced flight ability and ultrastructural defects, including myofibrillar disorganization, bulk Z-disk accumulation, and mitochondrial abnormalities. Our data demonstrate pathological consequences for tau mutations in striated muscle and a link between tau-induced myopathies and mitochondrial defects.

Electron and Proton Transfer

2484-Pos Board B503

Hydrogenase 4 Activity at pH 6.5 and Fhla Protein Interaction with F0F1-ATPase during Mixed Carbon Fermentation by Escherichia Coli

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Escherichia coli is able to produce molecular hydrogen (H2) via special enzymes - hydrogenases (Hyd) which catalyze simple redox reaction H2 to 2H+. H2 production during mixed carbon (glucose and glycerol) fermentation at slightly acidic pH (pH 6.5) was investigated. During mixed carbon fermentation in glucose assays, wild type cells produced ~2 fold less H2 than cells grown on glucose only as a sole carbon source. In fhlA, hyfG and double fhlA hyfG mutants H2 production decreased ~2 fold, compared to the wild type, but in hyaB, hybC, and double hyaB hybC mutants H2 evolution was lowered by ~1.5 fold.

In the assays supplemented with glycerol, no H2 production could be detected. Taken together, these results suggest that Hyd-3 and Hyd-4 are the H2 producing Hyd enzymes during mixed carbon fermentation. This is absolutely novel finding about Hyd-4 activity at pH 6.5. The decrease of H2 production in the strains with defects in Hyd-1 and Hyd-2 was mostly due to an interaction between the different Hyd enzymes and their organization in the membrane. In the assays with adding glucose, in the wild type cells H2 production was inhibited ~2 fold by 0.3 mM N,N′-dicyclohexylcarbodiimide (DCCD), an inhibitor of F0F1-ATPase. This inhibition was approximately the same for fhlA and hyfG fhlA mutants but not hyaB, hybC, hyfG or hyaB hybC mutants. These results indicate that the FhlA protein coded by the fhlA gene, which is transcriptional activator for FHL complex, might interact with the F0F1-ATPase. We suggest that this interaction is mediated due to mixed carbon fermentation.

2485-Pos Board B504

Proton Cycle trough the Membrane in Bacteria under Fermentation: Dependence of Proton Fluxes on some Physico-Chemical Factors

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Proton (H^+) circles through the bacterial membranes are represented, relationship with ATP synthesis or hydrolysis is considered. The data on passive and active H^+ fluxes through the membranes are analyzed and their relationship with membrane H^+ conductance (GmH^+) and permeability for H^+ (PH^+) are analyzed. Different ways and mechanisms of passive and active H^+ fluxes, including a role of membrane lipids in H^+ transfer, importance of phase transitions in lipid bilayers, operation of protonophores as well as H^+ translocation via the F0F1-ATPase, are discussed.

Dependence of GmH^+ for Escherichia coli, Enterococcus hirae, Streptococcus lactis and the other bacteria under fermentation on some external physico-chemical factors, particularly, on growth pH and oxidation-reduction potential (ORP) as well as influence of oxygen and osmotic stress on GmH^+ and H^+ active fluxes have been shown [1–3]. Acid pulse method for determination of GmH^+ was used. The effects were different with E. coli atp mutant pointing out the role of the F0F1-ATPase in GmH^+ change. The relationship between GmH^+, PH^+ and active H^+ fluxes (GmH^+ -> proton-motive force, pH -> ORP) is proposed, mechanisms for these changes relationship are discussed. Increase of GmH^+ result in decrease of internal pH in E. coli without change in ATPase activity [4].

The results are of significance for understanding of structure-function properties of bacterial membranes determining H^+ cycle operation and of H^+ fluxes mechanisms important in adaptation of bacteria to environment conditions.