stalk region. ABDM and WSA crosslink two cysteine residues located on the surface of kinesin inter- or intra-molecularly. On the other hand, AB-NTA crosslinks inter-molecularly His-tagged kinesin. Photo-reversible alteration in the microtubule dependent ATPase activities and motor activity of cross-linked kinesins were examined.

2279-Pos Board B423

Aplip1 Controls the Processivity of Neurexin Axonal Transport

Ulises Rey[1], Mehmet Ucar[1], Reinhard Lipowsky[1], Stephan Sigrist[2].
[1]Max Planck Institute of Colloids and Interfaces, Berlin, Germany, [2]Institute for Biology/Genetics, Freie Universität Berlin, 14195 Berlin, Germany, Berlin, Germany.

The long-range transport of synaptic components along axons (which can exceed one meter for human motorneurons) is known as a mechanistic bottleneck widely discussed in the etiology of developmental diseases of the nervous system. In particular, mutations in the human neurexin loci have been found to cause autism in human patients, a process that remains poorly understood. Rather subtle deficits of Neurexin at the synapse are known to cause cognitive diseases of the nervous system.

We recently published a study were we found that Aplip1, [Ref] a protein known to bind to Kinesin and Dynein is necessary for neurons to start building up the synapse at the axon terminal. Aplip1 mutants were unable to transport the main organizers of the synapse to the end of the axon and started the ectopic assembly of synaptic proteins at the initial segments of the axon. In the present study we want to characterize how Neurexin, an early assembly protein necessary for proper synapse formation, is transported in the absence of Aplip1.

For that, we have been extracting trajectories of Neurexin GFP tagged vesicles in the living Drosophila larvae using confocal Microscopy. To start with, we found a reduced number of Neurexin vesicles. We also show that Aplip1 regulates the processivity of both anterograde and retrograde transport: the remaining vesicles show extended pauses during transport and are more likely to change their directionality (from anterograde to retrograde or vice-versa). Using this unique in vivo protocol and the striking behavior of this mutant we are currently refining the theory for bi-directional transport by teams of molecular motors. Comparative analysis of kinetic parameters of the wild-type and the Aplip1 mutant will help to elucidate this complex process.

Cardiac Muscle Mechanics and Structure II

2280-Pos Board B424

High-Speed, High-Performance Real-Time Imaging of Physiological Sarcomere Dynamics in the Beating Mouse Heart in Vivo

Fuyu Kobirumaki-Shimozawa[1], Kotaro Oyama[2], Togo Shimozawa[3], Takashi Ohki[2], Takako Terui[4], Shin'ichi Ishiwata[2,5], Norio Fukuda[1].
[1]Cell Physiology, The Jikei University School of Medicine, Tokyo, Japan, [2]Department of Physics, Waseda University, Tokyo, Japan, [3]Department of Life Science and Medical Bioscience, Waseda University, Tokyo, Japan, [4]Department of Anaesthesiology, The Jikei University School of Medicine, Tokyo, Japan, [5]Waseda Bioscience Research Institute in Singapore, Waseda University, Singapore, Singapore.

Active force in cardiac muscle is highly dependent on sarcomere length (SL), highlighting the need for real-time SL imaging at high spatial and temporal resolution in vivo. In the present study, we expressed α-actinin-AcGFP in the Z-disks in cardiomyocytes of the left ventricle in adult mice, and applied SL nanometry (see Shintani et al., J Gen Physiol 2014) for the measurement of SL displacement at 100 fps under a fluorescence microscope (combined with a spinning disk confocal unit and an EMCCD camera), simultaneously with the measurements of left ventricular pressure (LVP) and the electrocardiogram (ECG). SL changed between ~1.93 and ~1.73 μm during diastole and systole, respectively, and varied by ~300 nm in both phases, even in the same myocyte. LVP was positively correlated with the SL change between diastole and systole ($P<0.05$), but not with diastolic SL. Therefore, under physiological auxotonic conditions in the beating heart, the greater the magnitude of SL shortening, the higher the output of ventricular function. We then reconstructed the Z-sectioning images of single sarcomeres, and successfully observed sarcomeric motions during the entire cardiac cycle, with a precision of 20 nm. The present cardiac nano-imaging system has a broad range of application possibilities for revealing sarcomere dynamics at high precision in relation to changes in LVP or ECG in health and disease. Here, we discuss 1) sarcomere dynamics as a key determinant of ventricular function, and 2) the technical aspects of the first application of nano-imaging to the in vivo heart under true physiologic conditions.

2281-Pos Board B425

Imagings of Sarcomeres in Rat Neonatal Cardiomyocytes Expressing Stress Fiber-Like Structures

Teruyuki Fujii.
Department of Cell Physiology, The Jikei University School of Medicine, Tokyo, Japan.

In the present study, we investigated whether sarcomeric dynamics is influenced by the development of stress fiber-like structures, and observed sarcomeres of rat neonatal cardiomyocytes by time-lapse imaging. Ventricular myocytes were isolated from 1-day-old Wistar rats, and cultured on collagen-coated glass bottom dishes. We observed a decrease in the number of sarcomeres 100 hrs) and development of stress fiber-like structures likely to be formed via degradation of sarcomeric ≥ upon long-term culture (proteins. Sarcomere length (SL) nanometry (Shintani et al. J Gen Physiol. 2014; Shintani et al., BBRC 2015) was performed at 37 ± 0.5°C. The formation of stress fiber-like structures did not significantly alter the lengths of remaining sarcomeres at rest, and shortening velocity was increased while lengthening velocity was decreased in single sarcomeres, coupled presumably with a change in the tug-of-war between sarcomeres in myofibrils. We hereby conclude that in neonatal cardiomyocytes, 1) intracellular stress fiber-like structures develop, and 2) the formation of stress fiber-like structures did not significantly alter the average lengths of remaining sarcomeres at rest, 3) the contractility of remained sarcomeres is maintained.

2282-Pos Board B426

Oscillatory Behavior in Muscle Myosin

Lorenzo Marcucci[1], Takumi Washio[2], Toshio Yanagida[3].
[1]Department of Biomedical Sciences, Padova University, Padova, Italy, [2]Graduate School of Frontier Sciences, Tokyo University, Tokyo, Japan, [3]QBiC, Riken, Osaka, Japan.

Muscle contraction is generated by cyclical interactions of myosin heads with actin filaments to form the actomyosin complex. The stable configurations of the actomyosin complex have been described in detail, but whether the in vivo configurations at physiological temperatures are fixed to those observed in cryomicroscopy (at low temperature) or undergo thermal oscillations is unknown and not generally considered in mathematical modeling.

By comparing three mathematical models, we analyze whether thermal oscillations of the actomyosin complex affect muscle contraction at three levels; namely, single cross-bridge, single fiber and organ levels, in a textit{ceteris paribus} analysis.

We observed that state fluctuations allow the lever arm of myosin to easily and dynamically explore all possible minima in the energy landscape, generating several backward and forward jumps between states during the lifetime of the actomyosin complex, whereas the rigid case is characterized by fewer force generating events. Therefore, dynamical oscillations enable an efficient contraction mechanism, in which a higher force is sustained by fewer attached cross-bridges.

2283-Pos Board B427

Cross-Bridge Group Ensembles Describing Cooperativity in Thermodynamically Consistent Way

Mari Kalda, Pearu Peterson, Marko Vendelin.
Laboratory of Systems Biology, Institute of Cybernetics, Tallinn University of Technology, Tallinn, Estonia.

The aim of this work is to incorporate cooperativity into Huxley-type cross-bridge model in thermodynamically consistent way. While the Huxley-type models assume that cross-bridges act independently from each other, we take into account that each cross-bridge is influenced by its neighbors and cooperativity is induced by tropomyosin movement. For that, we introduce ensembles of cross-bridge groups connected by elastic tropomyosin. By taking into account that the mechanical displacement of tropomyosin induces free energy change of the cross-bridge group ensemble, we develop the formalism for thermodynamically consistent description of the cooperativity in muscle contraction. An example model was composed to test the approach. The model parameters were found by optimization from the linear relation between oxygen consumption and stress-strain area as well as experimentally measured stress dynamics of rat trabecula. We have found a good agreement between the optimized model solution and experimental data. Simulations also showed that it is possible to study cooperativity with the approach developed in this work.

2284-Pos Board B428

Cardiac Length-Dependent Activation: Weak Binding Hypothesis Tested by a Computational Sarcomere Model

William C. Hunter, Alison L. Schroeder.
Biomedical Engineering, New Jersey Inst. of Technology, Newark, NJ, USA.

Experiments suggest that length changes alter activation in cardiac sarcomeres within a few milliseconds (Mateja & deTombe, 2012). Thus, the mechanism