Subgroup: Motility

24-Subg
Effect of Non-Linear Elasticity of Skeletal Myosins on Force Generation in Muscle
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During muscle force generation, an elastic energy stored at the compliant region of myosin head is a source of mechanical work against the external environment. Thus, this elastic distortion of myosin molecule was modeled as an essential mechanical element of the force generation driven by the crossbridges (Huxley, 1957). In the crossbridge model, the central assumption of the constant stiffness implies that the negatively-strained myosins must be detached at much higher rate than the positively-strained myosins to avoid the significant drag effect. However, the molecular studies on processive motors, such as kinesin and unconventional myosins, show that the assemblies of these motors do not seem to impede the molecular interactions, although they have much higher duty ratio and thus, a higher chance to cause the molecular interference. Recently, we found that skeletal myosins have the non-linear elasticity, in which stiffness is much higher when they are positively-strained and lower when negatively-strained. Therefore, this non-linear elasticity seems to be the essential and complementary property of motors to avoid the drag force generation in the motor assembly. We have currently worked on the theoretical model to investigate the effect of non-linear elasticity on the force generation, particularly the force-velocity relationships and the T1-T2 curves obtained from the muscle fiber quick release experiment.

25-Subg
Life of a Single Dynein during Meiotic Nuclear Oscillations
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Cytoplasmic dynein is a motor protein that exerts force on microtubules and in doing so, drives a myriad of intracellular activities from mitotic spindle positioning to chromosome movements in meiotic prophase. To exert force on microtubules, dynein needs anchorage, which is typically found at the cell cortex. The key question is how dynein finds the sites where a microtubule and an anchor protein are close enough for dynein to link them and subsequently pull on the microtubule. Here we directly observe single dyneins in fission yeast and show that they attach in two steps, first from the cytoplasm to a microtubule and then also to cortical anchors. Upon attachment to the microtubule, dynein moves in a diffusive manner along the microtubule. This is a surprising finding for a minus end directed motor and may help dynein to find cortical anchors. Our work demonstrates that dynein performs three-dimensional diffusion in the cytoplasm and one-dimensional diffusion along the microtubule to find sites where it can exert pulling force on the microtubule.

26-Subg
Half-Site Inhibition of Kinesin-1 by a Single Tail Domain, Sometimes One is Enough
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Isolated kinesin-1 is autoinhibited through binding of a region in the C-terminal tail domain to the N-terminal head (motor) domains. Although the heavy chain dimer has two head and two tail domains, the stoichiometry of binding when expressed separately in trans is one tail peptide per two heads. Both monomeric and dimeric tail domain constructs have similar affinity for a dimer of heads, indicating that the second peptide in a tail dimer does not contribute significant additional free energy to the interaction. The recent solution of the X-ray structure (Kaan et al., Science 333, 883 (2011)) of a complex of one tail domain and a dimer of heads suggested a 'double lockdown' mechanism in which ADP release is likely inhibited because the coupled undocking of the neck linker cannot occur while maintaining both the cross link between heads at the coiled coil neck and the new cross link provided by the tail domain that bridges two heads. This result stimulates a number of new questions that will be addressed. If only one tail is interacting strongly with the heads, what is the other tail peptide doing? Can it bind to cargo or regulators while the other peptide remains bound to the heads? One approach is to produce heterodimer tails with mutations that allow only one peptide to bind to heads, while introducing probes into the other tail to test for its binding interactions.

27-Subg
Myosin V as a Point-to-Point Organelle Transporter
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Mice that lack myosin Va (*dilute* mice) exhibit two striking phenotypes-a dilution of coat color and severe ataxia. Regarding the first phenotype, we previously demonstrated that the myosin cooperates with long-range, bidirectional, microtubule-based transport to position melanosomes at the melanocyte's dentritic tips, the site of melanosome transfer from the melanocyte to the keratinocyte (the Cooperative-Capture Model) (*J. Cell Biol.* 1998, 143:1899). These and other results have contributed to the current debate as to whether type V myosins in higher eukaryotes function as vesicle transporters or as dynamic vesicle tethers. Regarding the second phenotype, we have now demonstrated that myosin Va serves as a point-to-point transporter to carry the ER into the dendritic spines of cerebellar Purkinje neurons following its long-range transport out dendrites via microtubule motors (*Nature Cell Biol.* 2011, 13:40). That the myosin actually moves the ER in vivo is supported by several results, the most convincing of which is that in *dilute* Purkinje neurons rescued with slow versions of myosin Va (lever arm truncations, a switch 1 mutant), the speed of ER tubule transport into spines is slowed correspondingly. Moreover, we show that this myosin Va-dependent ER targeting is required for the local calcium transient downstream of strong spine activation that underlies synaptic plasticity/learning and, hence, normal coordination. These results, together with another recent study from our lab demonstrating that the *Dictyostelium* type V myosin MyoJ drives the cortical translocation of contractile vacuole membrane tubules (*J. Cell Biol.* 2009, 186:555), argue that, as in yeast, type V myosins in higher eukaryotes can serve as point-to-point organelle transporters.

28-Subg
Building Complexity to Understand Myosin V Cargo Transport
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There is a large gap between the conditions we use to study molecular motors *in vitro*, and the motor walking in a cellular environment. For simplicity, *in vitro* experiments on myosin V generally use single constitutively active truncated motor constructs that walk on individual bare actin filaments. Within the cell, however, actin generally has bound tropomyosin. Full-length motors are regulated by a folded-to-extended conformational transition, and adapter proteins that link the motor to cargo can affect this conformational transition. Adapter proteins, or the cargo itself, can also recruit multiple motors to improve the efficiency of cargo movement. The theme of this talk is that by re-creating a more native myosin complex and actin track, unexpected properties of the motor emerge that are likely to be important for cellular cargo transport. Several different class V myosins will be used to illustrate these principles.

Subgroup: Biological Fluorescence

29-Subg
Drug and Gene Delivery with "Smart" Nanoparticles and Live Cell Imaging
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Highly sensitive fluorescence techniques, such as single molecule and single particle tracking are used to follow the uptake and trafficking of nanoparticles in living cells. Due to the high temporal and spatial resolution of the particle trajectories, recorded in real time, mechanistic details of these processes are revealed. Nanoparticles consisting of DNA complexed by cationic polymers (polyplexes) and functionalized with cell-specific ligands for targeting are investigated[1]. Furthermore, mesoporous silica nanoparticles[2] are observed, which contain the drug inside the porous network of nanometer-sized channels and show triggered release upon e.g. cell signalling[3].
In all these applications live cell imaging is used to understand the mechanistic processes of uptake, trafficking and interactions of the nanoparticles with cell components in order to improve the efficiencies of the nanoparticles as drug-delivery systems.
[1] K. de Bruin et al., Mol. Therapy, 15 (2007), 1297; K. de Bruin et al., J. Contr. Release, 130 (2008), 175; A. Sauer et al., J. Contr. Release, 137 (2009), 136; F.M. König et al., J. Contr. Release, (2011), accepted.