contractile reserve decreases with consideration of protein characteristics governing the force-frequency relationship. However, the molecular alterations involved in the beta-adrenergic response lead to an increase in sensitivity. By following contractile function over time, and assessing the impact of physiologically relevant modulators of function, we will obtain a temporal resolution of cardiac function in its transition from the healthy to the diseased state.

3746-Pos

A Novel Pleiotropic Effect of Statins: Enhanced Cardiomyocyte β2-Adrenoceptor Responsiveness

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Pleiotropic effects of statins on endothelial cells, vascular smooth muscle cells and fibroblasts are well-established and contribute to reduced cardiovascular morbidity and mortality. Here we test whether these effects extend to the cardiomyocyte. Adult rat ventricular cells were maintained in culture +/- 10 μM simvastatin (SIMV). After 48h, shortening and $[Ca^{2+}]_i$ responses to β1-adrenoceptor stimulation were identical in SIMV and control cells, but a marked SIMV potentiation of the β2 response was seen (Figure 1). Statins mediate their effects through cholesterol-dependent and -independent pathways. Caveolae are cholesterol-dependent signalosomes that limit the magnitude of β2, but not β1, responses in the adult cardiomyocyte. Therefore we surmised that SIMV's effects could be mediated through caveolar disruption. Indeed, the mean density of caveolae (visualised by electron microscopy) was reduced in SIMV-treated cells (0.63 ± 0.08 vs. 0.86 ± 0.11 μm^{-1}; P<0.05; n=9 cells from 3 hearts). This is the first demonstration of effects of statins on the β responsiveness of the adult cardiomyocyte. These data suggest a novel mechanism for the beneficial effects of statins in heart failure - enhancing the contractile reserve of the failing heart, through effects on the caveolar signalosome.
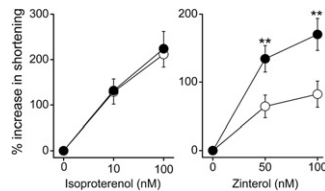

Figure 1. Simvastatin (●) selectively enhances the inotropic response of the cardiac myocyte to β2, but not β1, adrenoceptor stimulation compared with controls (○). Similar effects on [Ca$^+$]$_i$ responses to β-stimulation were seen. Data are from n=24-28 cells; 7 hearts. ** P< 0.01 vs control; Student's t-test.

Intracellular Cargo Transport

3747-Pos

Real Time Visualization of Axonal Transport of GTPase Rab7 in Rat Embryonic Dorsal Root Ganglia

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Charcot-Marie-Tooth (CMT) neuropathy, characterized by severe sensory neuron loss, is the most common inherited disorder of the peripheral nervous system. Several GTPase Rab7 protein mutants, mainly targeted to the highly conserved amino acid, have been identified in CMT type 2B. Exact mechanism of how such point mutations cause malfunction of neurons is not well understood. Here, we studied how those Rab7 mutations affect their axonal transport in primary rat dorsal root ganglia neurons. Real time fluorescence imaging revealed that Rab7-containing endosomes engage in bi-directional transport in axons, similar to that of TrkA receptors in the same culture. However, the speed of Rab7 transport is significantly slower than that of TrkA. In addition, there is a clear variation in the speed of axonal transport between wild-type Rab7 and mutated Rab7 proteins. Our work suggested that point mutations of Rab7 proteins could potentially cause or contribute to CMT2B neurodegenerative disease by regulating its axonal transport process.

3748-Pos

**Modeling Cytoskeletal Dynamics and Vesicle Movements in Growing Pollen Tubes**

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Intracellular cargo transport is a crucial process in growing plant cells. Since cellular expansion in walled cells entails the continuous assembly of new wall material, enormous amounts of polysaccharides need to be delivered to the growth site. In the rapidly elongating pollen tube the spatio-temporal movement pattern of exocytotic vesicles is precisely targeted and controlled by the continuously polymerizing actin cytoskeleton in the subapical region of the cell. Remarkably, the cone-shaped target region at the apical pole of the cylindrical cell does not contain much filamentous actin. We model the vesicular trafficking using as boundary conditions the expanding cell wall and the actin

array forming the subapical actin fringe. Dynamic advancement of this actin fringe was obtained by imposing a steady shape and constant polymerization rate of the actin filaments. Letting vesicle flux into and out of the apical region be determined by the orientation of the actin microfilaments was sufficient to generate a flow that corresponds in magnitude and orientation to that observed experimentally. This model explains how the cytoplasmic streaming pattern in the apical region of the pollen tube can be generated without the presence of filamentous actin.
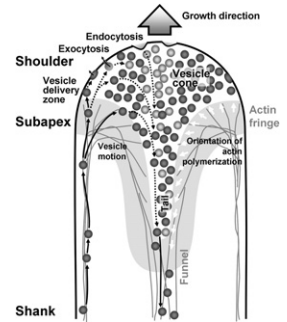
3749-Pos

Velocities of Microtubule-Based Motors in Living *Chlamydomonas*

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Reports in the published literature suggest that the velocities of vesicle transport in living neurons are discrete and quantal (multiples of a fundamental velocity), with the instantaneous velocity being dependent upon the number of molecular motors driving transport (Shtridelman *et al.*, *Cell Biochem. Biophys*, 2008). We similarly observed discrete changes in the velocity of microspheres undergoing saltatory transport on the flagella of *Chlamydomonas*, and that these velocities appeared to be dependent upon location along the flagellum. We therefore studied the movements of adherent microspheres on flagella, driven by the intracellular motors kinesin-2 and dynein-2, to determine whether transport is driven at multiple, discrete velocities and whether they are spatially correlated. We measured separately the translational velocities of unconstrained and optically trapped microspheres as a function of position along the flagellum. The velocities of unconstrained microspheres were on average about two-fold higher than those of trapped microspheres. Unconstrained microsphere velocities in the anterograde and retrograde directions were not spatially correlated except at turn-around points near the beginning and end of the flagellum where velocities were consistently lower. Histograms of these data showed a broad distribution of velocities and suggested no strong evidence for quantized velocities. For trapped microspheres, for a given anterograde or retrograde transport event we often saw at least two discrete velocities; however, any two transport events can have different 'slow'and 'fast'velocities. Thus even when velocities are measured from a single microsphere at a specific position on a flagellum, combining multiple velocity histograms results in an apparently non-quantized, broad distribution of velocities. What causes the abrupt change between discrete velocities during movements of a microsphere is yet unknown.

3750-Pos

How the Flagellum Measures Its Length

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The intraflagellar transport (IFT) particle injector controls eukaryotic flagellar length. The injector works by restricting the availability of new material for growth of the organelle, analogous to a fuel injector controlling the speed of a single piston engine by limiting fuel in the piston. Using quantitative TIRF microscopy and computational image processing we measure GFP-tagged IFT proteins KAP and IFT27 in Chlamydomonas reinhardtii flagella over a range of cellular and flagellar states (i.e. regenerating cell vs steady state cell and short flagellum vs long flagellum). From measuring the IFT particles in the flagellum, we then back-calculate the behavior of the IFT particle injector. We then derive mathematical models for the system that controls the IFT particle injector, finding that our data are consistent with a two-state time of flight model and not a diffusing signal or a constant IFT particle number model as previous studies have suggested. These results indicate that the group of proteins responsible for the injector behavior includes a two-state protein, such as a GTPase, that travels the length of the flagellum to measure the flagellar length. A mutant in this putative protein with either a constitutive excited state or a constitutive ground state would then have abnormally long or abnormally short flagella respectively. Our results further indicate that the flagellar length is set by the flagellum rather than the cell, which implies that the organelle can self-regulate, to some extent independent of the cell.

3751-Pos

On the Movement of Cargo Driven by Molecular Motors and the Asymmetric Exclusion Processes

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We consider the dynamics of particles driven by a collection of interacting molecular motors in the context of asymmetric simple exclusion processes (ASEP). The model is formulated to account for i) excluded volume interactions, ii) the observed asymmetry of the stochastic movement of individual motors and iii) interactions between motors and particles. Items (i) and (ii) form the basis of ASEP models and have already been considered in the literature to study the behavior of a collection of interacting motors in the absence of cargo. Item (iii) is new. It is introduced in this context as an attempt to describe explicitly the dependence of particle dynamics on the movement of motors [C.Goldman, E.Sena, Physica A 388(2009)]. The steady-state solutions to this model indicate that the system undergoes a phase transition of condensation type that can explain why, for sufficiently high motor densities, cargo velocity becomes independent of density in concert with the data obtained by Beeg et al [Biophys.J. 94(2008)]. The model predicts also that if more than one cargo-particle is present in the system these may exhibit inversion in movement direction. This means that the model suggests an alternative to explain the origin of the so called bidirectional movement of cargo according to which, inversions may happen in the presence of just one kind of motor.

3752-Pos

Surface Adsorption of Protein Corona Controls the Cell Internalization Mechanism of Multicomponent Lipoplexes in Serum

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Designer multicomponent lipoplexes have recently emerged as especially promising transfection candidates, since they are from 10 to 100 times more efficient than binary complexes usually employed for gene delivery purposes. Here, we show, for the first time, that after internalization binary complexes of lower transfection potency remain in compact perinuclear endosomes, while multicomponent systems have intrinsic endosomal rupture properties that allow plasmid DNA to escape from endosomes with extremely high efficiency. Endosomal rupture results in an extraordinarily homogeneous distribution of unbound plasmid DNA throughout the cytoplasm and in the nucleus. Serum has often been reported as a barrier to efficient lipid-mediated transfection. Here we found that the transfection efficiency of multicomponent lipoplexes increases in serum. To provide insight into the mechanism of lipoplex-serum interaction, several state-of-the-art methodologies have been applied. The nanostructure of lipoplexes was found to be serum-resistant as revealed by high resolution synchrotron small angle X-ray scattering, while dynamic light scattering measurements showed a marked size increase of complexes. Proteomics experiments showed that serum proteins competed for the cationic surface of lipid membranes leading to the formation of a rich a 'protein corona'. Combining structural results with proteomics findings, we suggest that such a protein corona can promote large aggregation of intact lipoplexes. According to a recently proposed size-dependent mechanism of lipoplex entry within cells, protein corona-induced formation of large aggregates most likely results in a switch from a clathrin-dependent to caveolae-mediated entry pathway into the cells which is likely to be responsible for the observed transfection efficiency boost. As a consequence, we suggest that surface adsorption of protein corona can have a high biological impact on serum-resistant cationic formulations for in vitro and in vivo lipid-mediated gene delivery applications.

3753-Pos

More Freight, More Engines: Force Scales with Cargo Size in a Living Cell

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The protein FMG-1 is transported in a saltatory manner within the flagellar membrane of *Chlamydomonas* by intracellular kinesin-2 and dynein-2 motors. Three models have been proposed to explain how these movements are coordinated: transport complex, biased accumulation, and molecular clutch (Laib et al., PNAS, 2009). To help discriminate between these models, we attached microspheres of varying diameter (0.5-1.4 μm) but identical surface chemistry to *Chlamydomonas* flagella and used a laser trap to determine whether the forces imposed on the microspheres by intracellular motors were dependent upon bead size. Larger microspheres are expected to accumulate larger patches of FMG-1. The transport complex and biased accumulation models suggest that force should be dependent on FMG-1 patch size; in contrast, the molecular clutch model suggests that force should be roughly independent of patch size. Our data shows that larger microspheres result in larger anterograde and retrograde

forces. With microspheres ranging from 0.5 to 1.4 um in diameter, anterograde forces respectively ranged from 6.9 to 30.6 pN and retrograde forces from 7.7 to 48.8 pN. This size dependency is consistent with either the transport complex or biased accumulation model, but not with the molecular clutch model. The forces measured at the smallest microsphere size were roughly equivalent to the *in vitro* stall forces of single kinesin and dynein motor molecules. These data show the feasibility of *in vivo* single motor mechanics experiments for understanding motor regulation and coordination.

3754-Pos

In Vivo Organelle Tracking, Calibration, and Force Measurement with an Optical Trap

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We report our results on calibrating an optical trap in vivo while simultaneously trapping and tracking organelles with it. We have built an optical trap with microsecond time resolution and nm spatial resolution with a trapping laser that is positioned with an Acousto-Optic Deflector (AOD). This AOD is of fundamental importance to the in vivo stiffness calibration technique, as this technique requires high speed oscillation of the trap position (on the order of 10's of kHz), in order to compare an active spectrum (the system's response to laser driving) with a passive spectrum (the system's response to Brownian motion). In addition, we have an AOD in our detection laser's path, allowing us to calibrate our position detection Quadrant Photo Diode's (QPD) volts to nanometers conversion. Our system allows calibration of every necessary trap parameter in the cell on each individual organelle we trap. We currently trap lipid droplets in Cos-7 cells, and are planning on measuring motor stall forces, motor number, and looking at switching between kinesin based transport and dynein based transport.

3755-Pos

Effects of Actin Filaments on NGF Retrograde Transport

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Actin filament is an essential component of the cell cytoskeletal system under the physiological conditions. In addition to their roles in supporting cell shape, actin filaments act as molecular tracks for myosin motors that are involved in the movement of organelles such as mitochondria in the axon. However, how actin filaments regulate axonal transport processes are yet to be fully elucidated.

Nerve growth factor binds and activates its receptor located at axon terminus, which intriguers the complex to be endocytosed sorted into signaling endosomes. NGF-containing endosomes are retrogradely transported from the axon terminus to the cell body. In this study, we investigated the effects of actin filaments on axonal transport by tracking the transport of single NGF modified with the quantum dot using microfluidic device.

Embryonic DRG neurons were cultured in the microfluidic nerve cell chambers made by PDMS. The microfluidic chamber allows us to apply latrunculin B, an actin de-polymerization inhibitor, exclusively to the middle segment of axon. This treatment would not affect signaling processes in the cell body or the endocytosis process that happens at the axon termini. We monitored the retrograde transport of Qdot-NGF in actin-depleted axons using TIRF microscopy. We found that NGF axonal transport continues in axons that are depleted of actin filaments, confirming previous reports that NGF transport is a microtubule-based process. However, we found that the average speed of axonal transport slowed down in Latrunculin B treated axons. Detailed analysis of why actin de-polymerization affects axonal transport is still in progress.

3756-Pos

Mean Time and Probability to Reach a Structured Target

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In cellular biology, reaching a target before being degraded or trapped is a ubiquitous problem. In particular, the communication between the cytoplasm and the nucleus is ensured by many small nuclear pores located on the nuclear membrane. DNA viruses hijack the cell replication and transport machinery to reach one of these nanopores before being trapped or degraded through the ubiquitin-proteasome system. In this article we provide general formula for the conditioned mean first passage time and the probability a particle (such as a virus) reaches a structured target (such as a nucleus covered by many small absorbing pores). Because the particle can be intermittently actively transported on the microtubules network, the asymptotics formulas account for a drift term in the Langevin description of trajectories. Mean first passage time questions are crucial and will help to understand quantitatively the cell biology at a molecular level.