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rodent beta-cells. Hence, from a clinical perspective and to obtain insight into the defects in insulin secretion relevant for diabetes mellitus, it is important to study human beta-cells.
To investigate the role of small conductance calcium-activated potassium SK-channels, we build on a recently developed mathematical model of electrical activity based entirely on detailed ion channel characteristics of human beta-cells.
We show that the inclusion of SK-channels allows us to investigate various patterns of electrical activity, and interpret atypical and non-intuitive responses to ion channel blockers in human beta-cells. However, our simulations suggest that the effect on electrical activity of SK channel antagonists is minimal. This is supported by preliminary experimental data.

2428-Pos Board B447
Changes in STIM1 Interactions during Activation of Store-Operated Ca^{2+} Entry Monitored by FRET
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Oligomerization of STIM1 and co-clustering with the Ca^{2+} channel protein, Orai1, at ER-plasma membrane (PM) junctions activates Ca^{2+} influx known as store-operated Ca^{2+} entry (SOCE) that is associated with visible puncta formed in the plane of the PM. Current information suggests that STIM1 in the resting state is minimally dimeric due to interactions between cytoplasmic CRAC activation domain (CAD) segments. We previously identified a basic sequence inside CAD responsible for activation of Orai1, and an acidic inhibitory region flanking CAD that controls a conformation change during activation. In recent studies we developed a fluorimetry-based fluorescence resonance energy transfer (FRET) method to monitor STIM1 oligomerization, both at the luminal (N-terminal) side, as well as at the cytoplasmic (C-terminal) side of STIM1, and we probed these interactions with mutations in STIM1 that are tagged with fluorescent protein reporterss. We found significant differences in stimulated FRET during Ca^{2+} store depletion depending on where the fluorescent proteins were placed: N-terminally tagged STIM1s exhibits stronger stimulated FRET than C-terminally tagged derivatives. Co-expressing untagged Orai1 largely eliminates stimulated FRET between C-terminally-tagged STIM1, but does not reduce stimulated FRET at the luminal side. Functional coupling between AcGFP-Orai1 and STIM-mApple can be monitored either by FRET or by Ca^{2+} influx using the Ca^{2+} sensor GCaMP3. Sequential truncations of STIM1 from the C-terminus support a role for CAD as a oligomer-promoting part of STIM1. Our FRET results showing nanometer-scale re-arrangements of STIM1 oligomers during activation provide a higher resolution picture that is distinctive from micrometer scale clustering that is visualized as puncta formation.

Microtubular Motors II

2429-Pos Board B448
JIP1 Sustains Long Distance Anterograde Transport via Direct Regulation of Kinesin Autoinhibition and Coordination of Retrograde Motor Association
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Analysis of the highly processive vesicular cargo APP (amyloid precursor protein) in primary mammalian neurons shows axonal transport characterized by long run lengths with few pauses or directional switches. In order to elucidate the molecular mechanism for sustaining long-distance processive motility, we focused on the adaptor protein JIP1, which binds directly to APP and KLC (kinesin light chain). Upon siRNA depletion of JIP1 from primary neurons, we observed significant defects in both anterograde and retrograde APP transport. We further investigated the association of JIP1 with kinesin-1 and identified novel and distinct interactions between JIP1 and both kinesin heavy chain (KHC) stalk and tail. Using *in vitro* TIRF motility assays with single-molecule resolution, we found that addition of JIP1 relieves autoinhibition of KHC, leading to activation of motility and increased run frequency, run length and speed. Interestingly, truncated constructs of JIP1 that only bind KHC stalk or KHC tail were sufficient to initiate processive runs, but could not fully recapitulate the enhancement of KHC run length or speed observed with full-length JIP1. In addition, JIP1 can coordinate both kinesin- and dynein-driven motility, as we identify a novel interaction between JIP1 and the $p150^{Glued}$ subunit of dynactin. Both *in vitro* and in primary neurons, addition of the JIP1-binding region of $p150^{Glued}$ competitively inhibits JIP1-mediated enhancement of KHC processivity. In contrast to a stochastic tug-of-war model, our data suggest that JIP1 sustains long-distance transport by coordinating the formation of two distinct motile complexes - an anterograde complex that binds directly to KHC and a retrograde complex via its interaction with dynactin.

2430-Pos Board B449
Huntingtin and HAP1 Regulate Microtubule Motors during Autophagosome Transport
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Autophagy is a critical homeostatic process whereby autophagosomes engulf and degrade cellular cargo. Live cell imaging assays in neurons recently demonstrated autophagosome formation is restricted to the axon tip, followed by robust retrograde transport of the autophagosome towards the cell body. While >75% of autophagosome motility is directed towards microtubule minus ends, both the minus end-directed motor dynein and the plus end-directed motor kinesin colocalize to autophagosomes in neurons and copurify with isolated autophagosomes. Thus, it still remains unclear what mechanisms promote autophagosome transport, activating the retrograde motor dynein while inactivating the anterograde motor kinesin. One key candidate for regulating autophagosome transport is huntingtin (htt), which directly binds to dynein and binds to kinesin and dynactin through the adaptor HAP1 (huntingtin associated protein 1). Using live cell imaging in primary neurons, we found that depleting endogenous htt disrupted the retrograde motility of autophagosomes resulting in shorter run lengths and slower velocities. While these defects could be rescued by expression of full-length htt, deletion constructs of htt that could not bind either dynein or HAP1 were unable to rescue the defects in autophagosome motility upon depletion of endogenous htt. To further test HAP1's role in regulating autophagosome transport, we depleted endogenous HAp1 and observed a similar disruption in the retrograde motility of autophagosomes. Finally, we found that autophagosome transport is disrupted in a neuronal model of Huntington's disease, a genetic neurodegenerative disorder characterized by polyglutamine expansions in htt. Our results suggest a model where htt regulates autophagosome transport by binding to dynein and enhancing motor processivity, as well as to HAP1 whose interactions with kinesin and dynactin may further promote retrograde autophagosome transport. Further, out data suggest that misregulation of this transport may contribute to the pathogenesis of Huntington's disease.

2431-Pos Board B450
Dynamic Force Adaptation of Lipid Droplets during Intracellular Transport in Cos1 Cells
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Most bidirectional motion of organelle towards and away from the nucleus (Minus and Plus end of microtubules) inside cell is carried out by the combination cytoplasmic dynein and a Kinesin family of motors on the microtubule tracks. It is generally assumed that transport is static, and does not respond to local opposition to motion. However we observe that this is not the case: lipid droplets stalled by the application of external load adapt, and dynamically increase their force production. The average time for the adaptation is under ten seconds and it improved the ability of LDs to overcome the opposition to motion between three and four-fold. Although there is no observation of such a phenomena at the individual cargo level there is a well known adaptive behavior of the heart muscle explained by the Starling's law of heart[1] wherein the more the heart is stretched, the stronger the cardiac muscle contracts. We hypothesize the load dependent adaptation in our case could involve reactivation process by a kinase that controls the activation and inactivation of motors through the switching complex. Adaptation occurs only in the minus-end direction, and requires the presence of NudE/L and Lis1.
1. Solaro, R. J. Mechanisms of the Frank-Starling law of the heart: the beat goes on. Biophysical journal 93, 4095- 4096, doi:10.1529/biophysj.107.117200 (2007).

2432-Pos Board B451
Lipid Droplets Purified from Drosophila Embryos as an Endogenous Handle for Precise Single Molecule Measurements
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Single molecule studies of molecular motors are typically carried out by attaching a motor to a plastic bead and manipulating it via an optical trap. While this method had phenomenal success in investigating the mechanochemical properties of motors, it suffers from shortcomings that limit its suitability for studies of motor regulation: the motor-cargo attachment as well as the number and distribution of similar and dissimilar motors and motor co-factors on the cargo can be crucial for cargo transport. Here, we present a method for isolating lipid droplets from Drosophila embryos and using them in an ''ex vivo'' motility

assay. We show that the isolated droplets have excellent optical properties and can be used in high-precision and high temporal resolution optical trapping experiments on par with plastic beads used in in vitro assays. Using a Photonic Force Microscope (PFM), we determine their size precisely in situ. Moreover, using the high bandwidth (MHz) provided by the PFM, we are able to detect single lipid-droplet-bound motors attaching to the microtubule before they start moving. Analysis of the Brownian motion of the tethered droplet provides the mechanical properties of the tether. Given the disparate structures of the different motors, this in situ measurement of tether properties will allow us to identify the motor type and subsequently to study multiple motor dynamics with high temporal resolution.

2433-Pos Board B452

Testing the Tug-of-War Model of Bidirectional Transport In Vivo

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Molecular motor proteins are responsible for force generation in myriad cellular processes. Much of our understanding of how motors function has benefited from force measurements and manipulation at the single molecule level in vitro. We report on novel optical trapping methodology capable of precise in vivo stall-force measurements of individual endogenous cargoes hauled by molecular motors in their native environment. We present unprecedented stall force histograms of motor-driven lipid droplets in Drosophila embryos. Force measurements show that equal numbers of kinesin-1 and cytoplasmic dynein haul each cargo in opposite directions. Critically, by measuring cargo dynamics in the optical trap, we find that there is memory: it is more likely for a cargo to resume motion in the same direction -rather than reverse direction- after the motors transporting it detach from the microtubule due to the trap force. This suggests that only motors of one polarity are active on the cargo at any instant in time and is not consistent with the tug-of-war models of bidirectional transport where both polarity motors can bind the microtubules at all times. We further use the optical trap to measure, in vivo, the detachment rates from microtubules of kinesin-1 and dynein-driven lipid droplets. Unlike what is commonly assumed, we find that dynein's but not kinesin's detachment time in vivo increases with opposing load. This suggests that dynein's interaction with microtubules behaves like a catch bond and further argues in favor of a regulatory model for bidirectional transport.

2434-Pos Board B453

Photo-Regulation of Cell Division using Photochromic Inhibitors for Kinesin Eg5

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Previously it has been demonstrated that some kinesins known as an ATP driven motor protein are directly involved in regulation of the cell division cycle. Eg5 is one of the kinesin and its biochemical properties and structure were well studied. It is suggested that that Eg5 is activated in M phase of cell cycle and performs stabilizing and positioning of spindle. It is also known that Eg5 is overexpressed in tumor cell and induce significant cell division. Monastrol and STLC, which are potent inhibitor specific for kinesin Eg5, shut off mitotic division and result in apotosis. Therefore, these inhibitors are attracting as anti-cancer drug. Azobenzene and spiropyran, a widely studied photochromic compound, can be reversibly isomerized between the cis and trans forms by ultra-violet (UV) and visible (VIS) light irradiation, respectively. We have recently demonstrated the Monastrol and STLC analogues composed of photochromic molecules inhibit ATPase activity of Eg5 reversibly upon UV and VIS light irradiation in vitro. Therefore, it is strongly expected that the photochromic inhibitors are applicable to reversible photo-regulation of cell mitosis. In this study, we have examined the effects of the photochromic inhibitors for mammalian cells. Effects of the photochromic inhibitors were evaluated by survival number of mammalian cells, HeLa cells or A172 cells. Survival numbers of cells were quantitatively analyzed by MTS reagent and cell divisions were observed by phase-contrast microscope. spindle behavior of the cells cultured in the presence of photochromic inhibitors were also examined using immunofluorescent staining. These experimental results suggested that the effects of photochromic inhibitors for the cell division were controlled by UV and VIS light irradiations reversibly.

Cell Mechanics & Motility III

2435-Pos Board B454

The Molecular Landscape of Mechanosensing by the Cell Cortex

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Mechanical forces direct a host of cellular and tissue processes. While much emphasis is placed on cell adhesion complexes as force sensors, these forces must be transmitted through the cortical cytoskeleton. However, how the cortex senses and transmits forces and how cytoskeletal proteins interact in response to these forces are poorly understood. Here, by combining molecular and mechanical experimental perturbations with theoretical multi-scale modeling, we decipher cortical mechanosensing from molecular to cellular scales. Forces are shared between myosin II and different actin crosslinkers with myosin having potentiating or inhibitory effects on certain crosslinkers. Furthermore, different types of cell deformations elicit distinct responses: myosin and α-actinin respond to dilation while filamin mainly reacts to shear. The kinetics of each protein's accumulation may be explained by its molecular mechanisms. Finally, protein accumulation, along with the cell's viscoelastic state, can also explain the monotonic or oscillatory contraction against mechanical load in different mutant strains.

2436-Pos Board B455

Intracellular Microrheology in the Presence of Myosin-Generated Forces in Living Cells

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The mechanics of cells are governed by a network of cytoskeletal filaments and molecular motor forming a dynamic mechanical entity. A recent experimental study by Mizuno showed local shear modulus of a synthesized cytoskeletal network could increase in the presence of myosin-generated internal stresses. It was speculated whether similar behaviors could also take place in cells. To examine this, we used an experimental method similar to Mizuno's that combined active and passive microrheology to measure myosin-generated fluctuating force and the local intracellular stiffness. Our data obtained from measurements in 15 HeLa cells showed a linear relationship between the magnitude of the fluctuating forces and the time-averaged shear modulus. Moreover, when myosin activities were inhibited by ML7, we saw a dramatic decrease in the intracellular forces, but surprisingly no significant changes were observed in the time-averaged intracellular stiffness by ML7. While we did not see a direct correlation between the mean intracellular shear modulus and the motor-generated fluctuating force, we did observe an increase in the fluctuation of the shear modulus with increasing motor forces.

While it is not clear why results from the intracellular study were so different from the actin construct, some differences between the two systems may be attributed to the difference. First, the intracellular fluctuating forces were found to be weaker than that in the actin construct, thus perhaps not strong enough to modulate the cell stiffness. Secondly, the steady-state internal tension, which could dictate mechanical proprieties of a network, may be different between the two systems. Unfortunately, the experimental method we used could only measure fluctuating forces but not the steady-state tension in the system. Further studies that measure both fluctuating and steady-state forces at the same time may be required to address this problem.

2437-Pos Board B456

The Role of Myosin-II in Cell Spreading on Soft Hyaluronan-Fibronectin Substrates

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Studies using polyacrylamide (PAA) substrates show that cells respond to the resistance of the substrate by modifying spread area, focal adhesions (FA) and cytoskeletal morphology. Cells on a soft substrate (E~300 Pa) generate low traction forces, which do not support cell growth, FA formation and maturation or the assembly of actin stress fibers. We show that soft hyaluronan-fibronectin (HA-Fn) substrates support cell growth and survival similarly to rigid substrates, suggesting that the link between increased tension at the cell-substrate interface and cell growth could be altered by the substrate composition.

To address this question we measured epithelial and endothelial cell growth and morphology on soft HA-Fn substrates in the presence of agents that affect the myosin II-dependent contractile mechanism, such as the ROCK inhibitor Y-27632, and using myosin II knockdown cells.