likely to undergo exocytosis than those without such associations. When endocytosis was inhibited with Dynasore (inhibitor of dynamin 1 and 2), membrane *p/s* changes following fusion were long-lived (10s of seconds). This is consistent with endocytosis occuring at the site of exocytosis. These experiments are the first to directly visualize plasma membrane changes occuring with secretion. They reveal an association of the granule and plasma membrane for seconds before exocytosis and provide direct evidence for maintained curvature of the plasma membrane after exocytosis.

2937-Plat

Actin Cytoskeleton Controls Movement Of Intracellular Organelles In Epithelia

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Background: Intracellular organelles move along microtubules and filamentous actin (F-actin) using molecular motors. Previously we reported that movement of secretory granules was hindered by Ca^{2+}-dependent formation of a dense cytoplasmic F-actin meshwork in pancreatic duct epithelial cells (PDEC). **Aim:** Here we addressed whether other organelles such as mitochondria and lysosomes also are controlled by the same mechanism. **Methods:** Mitochondria and lysosomes were labeled with fluorescent MitoTracker Red and LysoTracker Red, respectively. Their movements were monitored every 1.2 s with high resolution fluorescence microscopy and measured by comparing subsequent images. Cells were stimulated with UTP to activate an endogenous $P2Y_2$ G-protein coupled receptor to increase intracellular Ca^{2+}. **Results:** Mitochondria and lysosomes moving actively at rest stopped rapidly ($\tau \sim 2.6$ s) after Ca^{2+} rise. This organelle 'freezing' was accompanied by the formation of F-actin in the whole cytoplasm as stained with phalloidin-Alexa 488. EGFP-actin binding domain 2 (ABD2) expressed in PDEC, indicated a rapid formation of cytoplasmic F-actin ($\tau \sim 7.8$ s). In addition, the freezing of the organelles was blocked by latrunculin B, an inhibitor of F-actin formation. **Conclusion:** Ca^{2+}-dependent formation of a fine F-actin meshwork reduces the movement of intracellular organelles physically in PDEC. The freezing mechanism controls exocytosis of secretory granules and may affect local ATP supply by controlling mitochondrial distribution in PDEC.

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Platform BG: Microtubular Motors

2938-Plat

Crystal Structure of Nucleotide-free Kinesin-1 Motor Domain Explains Coordinated Walking Mechanism

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Kinesin is a highly processive motor that moves along microtubules in a hand-over-hand manner. To understand structural basis for the walking mechanism, atomic-detailed structural information of kinesin at various nucleotide states are essential, however the nucleotide-free crystal structure has been unavailable. Here we report the first crystal structure of kinesin-1 motor domain without bound nucleotide solved at a 2.8Å resolution. The structure fits very well to the 9Å cryo-EM density map of kinesin-microtubule complex without nucleotide (Sindelar et al. 2007), demonstrating that the structure represents the ADP-released microtubule-bound kinesin head. The nucleotide-free structure showed marked differences from the previously solved ATP- and ADP-like crystal structures. First, compared to ADP-like structure, the switch II helix extends and this extension is stabilized through the interactions with both switch I/II loops and tubulin subunits. Furthermore, switch I uncoils and extends toward the nucleotide pocket. We suggest that microtubule-binding is sensed via the extension of switch II helix, which then promotes ADP release through switch I/II loops. Another striking feature is that α6 helix that directly connects to the neck linker moved such that the beginning of the neck linker collides with the switch II helix. The neck linker itself is mobile but this steric hindrance prohibits extension of the neck linker to the forward direction. Using crystal structures docked to the EM density map, we modeled dimeric kinesin on microtubule and found that kinesin cannot adapt two-head-bound state when both heads are nucleotide-free due to the constraint posed on the neck linker, and the tethered head can bind only to the forward tubulin-binding site after the trailing head becomes ATP-like structure. These findings provide structural basis for the coordinated processive movement of two kinesin motor domains.

2939-Plat

Conformational Specificity in Allosteric Signaling: High-throughput Measurement of Modular Secondary Structural Changes within Human Eg5 Kinesin

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Communication within allosteric enzymes requires long-range interactions. The ATPase activity of human Eg5 kinesin (HsEg5) is allosterically inhibited upon noncovalent binding of monastrol or S-trityl-L-cysteine (STC) with the L5 loop and subsequent distal conformation changes. Our hypothesis is that the E116 and E118 carboxylates, flanking an isolated β-bridge in the L5 loop, are required for allosteric signaling during small chemical inhibition. Surprisingly, assessment by molecular and biochemical methods revealed that substitution of these carboxylates, irrespective of its nature, has a positional dependence on HsEg5 ATPase rates. Single-site substitution of E118 increased basal ATP hydrolysis rates of Eg5, whereas substitution of E116 resulted in lower ATPase rates. Thus, sequence variation at residues 116 and 118 of the L5 loop can drive upregulation and downregulation of ATP hydrolysis *in vitro,* respectively. We conducted high-throughput infrared measurements of the secondary structure composition of the upregulated and downregulated proteins in solution. Vibrational signatures from upregulated proteins were distinct from the net changes measured from downregulated proteins. Compared to control samples, in HsEg5 motors with E118 substitutions, amide I′ components between 1642-1620 cm^{-1} decreased in normalized area, whereas proteins with alterations of E116 exhibited an increased contribution in the amide I′ area for modes between 1684 and 1646 cm^{-1}. Moreover, the spectral changes exhibited by E116 variants were similar to those of wildtype HsEg5 during allosteric inhibition by monastrol or STC. We conclude that allosteric inhibition by amino acid substitution or by small molecules both result in convergent steady-state changes to the secondary structure of the HsEg5 motor domain in solution, and these rapid methods will provide insight into how long-range structural changes impact motor function.

2940-Plat

The Motility of Monomeric and Dimeric Variants of Eg5 studied in the Presence of the Kinesin-5-specific Inhibitor Monastrol

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The homo-tetrameric motor-protein Eg5 from Xenopus laevis drives relative sliding of anti-parallel microtubules, most likely by the processive action of its two sets of dimeric motor domains at each end. As recently shown by Kwok et al. (NCB 2006) and Kapitein et al. (JCB 2008) , tetrameric motors move on a single microtubule in a fashion including diffusional and directional episodes, while motors moving between anti-parallel microtubules act in a highly directional and processive fashion. We have studied the processive behavior of a dimeric chimera (Eg5Kin) carrying the Eg5-motor and neck-linker and the Kinesin-1 neck and stalk. While Eg5Kin displays essentially the same motile properties as a truncated Eg5 (Eg5-513 his, Krysziak et al., JBC 2006, Valentine et al., NCB, 2006) its processivity is 40x increased to about 240 consecutive 8nm-steps on average, at a velocity of 95 nm/s. With increasing monastrol concentrations we find a dose-dependent and cooperative reduction in run length, but not in speed, indicating that two monastrol molecules are required to terminate a processive run. To further study the allosteric effect of monastrol on the motility of Eg5-motors, we generated monomeric and dimeric Eg5-constructs and compared their surface gliding-velocities in the presence of increasing concentrations monastrol.

2941-Plat

Cross-Species Analysis of Kinesin-14s: Human HSET Functions in Fission Yeast to Regulate Spindle Bipolarity

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The discovery of Kinesin-like proteins in 1990, five years after conventional Kinesin, has blossomed into the identification of hundreds of Klps that are classified into fourteen families and one orphan family. Not all Klp families are conserved between eukaryotes, however the Kinesin-14 Klps are ubiquitous and key regulators of bipolar spindle assembly and microtubule dynamics and organization. Kinesin-14 Klps associate with microtubules through ATP-dependent binding of their motor domains, but an additional ATP-independent microtubule binding site in the stalk domain is proposed to allow bundling of microtubules. Kinesin-14 members represent some of the most extensively studied Klps, however limited analysis has been done to examine conserved