results suggest that the tension through the tail domain does not play a critical role in the processive motion. The direct interaction of the two AAA rings in the motor domain may be responsible for the ''mechanical gating'' to sustain alternative steps of the two motor domains on MT.

2608-Pos Board B578

In Vitro Reconstitution of Dynamic, ER-like, Nanotubular Networks, and of Small, Tubulo-Vesicular Transport Entities by Interactions of Cytoplasmic Dynein and Spectrin with Liposomes

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Cells contain intricate networks of membrane tubes of nanoscale dimensions, such as the endoplasmic reticulum (ER). Much smaller tubular entities, derived by extraction from donor compartments (e.g., those emerging from recycling endosomes or the trans-Golgi network) or generated by vesicle fusion (e.g., the ER-to-Golgi transport units), function in intracellular transport. Different mechanisms are thought to underlie the morphogenesis of the complex, tubular ER network, and the formation of the small tubular transport entities that travel along microtubules. Here, we show that the molecular machinery that powers retrograde vesicle motility in neurons can interact with membranes to generate these different types of tubes. We reconstituted in vitro elaborate networks of interconnected membrane tubes (with ER-characteristic ring closures and three-way junctions), as well as freely moving, stable tubes and tubulo-vesicular clusters, from mixtures of the minus-end motor, cytoplasmic dynein, its regulatory complex, dynactin, the anchoring protein, spectrin, and liposomes containing acidic phospholipids, in the presence of microtubules and ATP. The tubulo-vesicular clusters contained trains of spherical liposomes attached to a small tube via elastic linkers, likely maintained together through a supravesicular spectrin meshwork that encompasses both the tube and the associated vesicles. Recruitment of dynein-dynactin and spectrin from the cytosol to liposomes was stimulated by phospholipase D-induced conversion of neutral phospholipids to acidic forms, and by activation of small GTPases. We conclude that similar mechanisms underlie the generation of ER-like tube networks and small tubular transport entities. Both may be generated and maintained by the action of soluble microtubule motor complexes and anchoring proteins, which bind to phospholipids, and do not require membrane proteins. Supported by March of Dimes grant 1-FY04-240 and NIH grant R01GM068596.

2609-Pos Board B579

Dynein Stepping Flexibility as a Mechanism for Optimal Trafficking in the Cell

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Single molecule experiments have revealed that even under conditions of no load, backward stepping constitutes about 20 percent of cytoplasmic dynein's steps. Sideward steps are also common, and the motor's step size distribution is very broad. Such stepping flexibility might allow dynein to efficiently navigate the crowded cellular environment and avoid obstacles. However, the high speed and processivity of the motor implies strong coordination of its two heads. The idea of head coordination through a direct physical interaction seems plausible based on structural considerations, but such a mechanism raises the question of how tight coordination and stepping flexibility are simultaneously accomplished. We use physical reasoning and mathematical modeling to explore mechanisms that optimize these two opposing motor properties.

2610-Pos Board B580

Drag-brake Mechanism Of A Spindle Motor kinesin-5

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In most eukaryotic systems, kinesin-5 motors are absolutely required for mitosis and meiosis, where they drive engagement and subsequent sliding apart of the antiparallel half-spindles, whilst antagonising and/or collaborating with other motors. It was previously demonstrated that the kinesin-5 motor efficiently slows down other motors such as kinesin-1 and Ncd, suggesting it can generate resistive force as well as motive force (Crevel, I.M., Alonso, M.C., and Cross, R.A. 2004. Curr. Biol. 14, R411-412 and Tao, L., Mogilner, A., Civelekoglu-Scholey, G., Wollman, R., Evans, J., Stahlberg, H., and Scholey, J.M. 2006. Curr. Biol. 16, 2293-2302). Subsequent in vivo work has born this out: depletion of kinesin-5 causes faster spindle extension in Caenorhabditis elegans (Saunders, A.M., Powers, J., Strome, S., and Saxton, W.M. 2007. Curr. Biol. 17, R453-454) and causes up to 5 times longer and much more branched axons in neurons (Myers, K.A., and Baas, P.W. 2007. J. Cell Biol. 178, 1081-1091). The molecular mechanisms of these dual functions are poorly understood. Using mutagenesis, we show here that we can increase or decrease the drag-force component, with reciprocal effects on microtubule sliding velocity. In particular, we report a microtubule-binding-deficient mutant of Xenopus Eg5 with substantially reduced drag that slides microtubules 50% faster than wild type. Another mutant, in the kinesin-5 neck linker, shows increased drag together with decreased velocity. Our results suggest that whilst strongly-bound to the microtubule, Eg5 crossbridges are tuned to divide their time between force-generating states that drive microtubule sliding, and force-holding states that resist sudden or over-rapid microtubule sliding in cells.

2611-Pos Board B581

Millisecond Time-lapsed Monitoring of ATP Hydrolysis by Human Eg5 Kinesin: Real-time Dynamics of Conformation and Chemistry *in vitro*

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The hydrolysis of ATP is one of the key chemical reactions in life. Its mechanistic details in biological systems have been a challenge to unravel, as chemical changes in proteins during ATP hydrolysis go hand-in-hand with a series of large-scale conformational alterations. Monitoring the dynamics of chemistry and structure has been experimentally intractable, and requires application of novel methods. Herein, we present time-lapsed monitoring of an *in vitro* ATP hydrolysis reaction by a kinesin motor protein with 180-millisecond time resolution. Kinesin proteins are one of three major categories of motor proteins, capable of using ATP hydrolysis to power force generation and subsequent movement along cytoskeletal elements in cells. Our model protein system is human Eg5 (HsEg5), a Kinesin-5 motor protein participating in the spindle pole segregation during mitosis in higher eukaryotes. Truncated to its monomeric motor domain, we purified active HsEg5 and confirmed its ability to hydrolyze ATP. To monitor dynamic structural and chemical changes during ATP hydrolysis *concomitantly*, we used difference Fourier-transform infrared (FTIR) spectroscopy with HsEg5 kinesin samples, triggering initiation of the ATPase reaction by UV-photolysis of caged ATP. Interpretation of these biological data was guided by model compound data on ATP derivatives. The time-lapse data highlighted resolution of two distinct sets of conformational changes: a series of HsEg5 structural changes that precedes ATP hydrolysis and a set of structural alterations that occurs upon onset of ATP hydrolysis. Thus, we conclude that we have the first direct observation of dynamic conformational changes caused by the ATP binding in any kinesin motor protein. Secondly, the structural modifications that occur HsEg5 when ATP hydrolysis is initiated are different than those in the substrate-binding step.

2612-Pos Board B582

Crystal Structure of HsEg5 in Complex with S-trityl-L-cysteine

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Eg5 is a motor kinesin involved in the formation of the bipolar mitotic spindle which is essential for the completion of mitosis. The discovery of a class of allosteric Eg5 inhibitors has raised the possibility of a novel approach for the treatment of cancer. Monastrol and S-trityl-$_L$-cysteine (STC) are two well-characterized inhibitors of Eg5 known to prevent ADP release by the motor domain, with the latter compound being a more potent inhibitor of Eg5 than the former. We have determined the 2.5 Å resolution crystal structure of the Eg5 motor domain in complex with STC and Mg•ADP. STC interacts with Eg5 *via* a pocket formed by helices α2, α3 and loop L5, and induces conformational changes within the Eg5 motor domain similar to those seen with bound monastrol. The necklinker is positioned in the ''docked'' conformation seen in the monastrol-bound Eg5 structures, and the switch I and II regions also adopt conformations similar to those observed for bound monastrol. Moreover, STC contacts with Eg5 differ from those seen in monastrol-bound crystal structures. Monastrol contacts with Eg5 are largely mediated by nonpolar surfaces of the drug. During STC binding, contact between the motor domain and polar surfaces of the drug is increased in relation to that of monastrol by over 20 Å^2. STC binding occludes approximately 80 Å^2 more of the Eg5 surface from solvent access than does monastrol. Site-directed mutagenesis and coupled biochemical assays monitoring ATP hydrolysis were used to examine whether these residues involved in polar interactions between STC and Eg5, and residues altered in solvent accessibility upon STC binding to Eg5,

were critical in the increased efficacy of Eg5 inhibition by this small molecule.

2613-Pos Board B583
The Homotetrameric Kinesin-5, KLP61F, Preferentially Crosslinks Antiparallel Microtubules
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The segregation of the genetic material during mitosis is coordinated by the mitotic spindle, whose mechanism of action depends upon the polarity patterns of its constituent microtubules (MTs). Homotetrameric mitotic kinesin-5 motors are capable of crosslinking and sliding adjacent spindle MTs, but it is unknown if they, or other motors, contribute to the establishment of these MT polarity patterns. Here we explored if the *Drosophila* embryo kinesin-5, KLP61F, which is thought to crosslink both parallel and anti-parallel MTs, displays a preference for the parallel or anti-parallel orientation of MTs. In motility assays, KLP61F was observed to crosslink and slide adjacent MTs, as predicted. Remarkably, KLP61F displayed a three-fold higher preference for crosslinking MTs in the antiparallel, relative to the parallel orientation. This polarity preference was observed in the presence of ADP or in ATP plus AMPPNP, but not in AMPPNP alone, which induces instantaneous rigor binding. Also, a purified motorless tetramer containing the C-terminal tail domains displayed an antiparallel orientation preference, confirming that motor activity is not required. The results suggest that, during the morphogenesis of the *Drosophila* embryo mitotic spindle, the crosslinking and sliding activities of KLP61F could facilitate the gradual accumulation of KLP61F within antiparallel interpolar (ip) MTs at the equator, where the motor could then generate force to drive poleward flux and pole-pole separation.

2614-Pos Board B584
Three-dimensional Nanometer Resolution Optical Tracking Reveals A Torque Component Present In Single-headed Kinesin
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We have developed a novel method for tracking microtubule rotation in three dimensions, which uses only one optical component, a prism, without modification of other aspects of a standard microscope. We applied our method to a conventional *in vitro* sliding assay by tracking streptavidin-coated quantum dots that are bound to a sparsely-biotinylated microtubule sliding across lawns of kinesin motors. Our method achieves nanometer accuracy and returns three-dimensional positional information. Using this method, we found that surface-attached Eg5 monomeric fragments (a member of the kinesin-5 sub-family of microtubule-based motors, which is essential for the assembly and maintenance of the bipolar spindle architecture *in vivo*) drove counterclockwise rotation of sliding microtubules around their axis. These corkscrewing motions have not been seen previously for kinesin-5, and it demonstrates that single kinesin-5 heads produce torsional force as well as axial sliding force. We also found that the rotational pitch was insensitive to microtubule geometry [1]. This short-pitch rotation by single-headed kinesin-5 molecules is strikingly similar to both that of a plus-end directed, non-processive single-headed kinesin-1 molecules [1, 2], which have a N-terminal motor domain, and that of a minus-end directed, non-processive double-headed kinesin-14 molecules [3], which have a C-terminal motor domain. A value of ~ 0.3 μm for the rotational pitch generated by theses three motors appears to represent a characteristic signature for non-processive motors. This suggests the possibility that a kinesin head possesses in common an inherent torque component. [1] Yajima J., Mizutani K. & Nishizaka T. Nat. Struct. Mol. Biol. (2008), [2] Yajima J. & Cross RA. Nat. Chem. Biol. 1 (2005) 338-41., [3] Walker RA., Salmon ED. & Endow SA. Nature 347 (1990) 780-2.

2615-Pos Board B585
Structure of the Kinesin13-Microtubule Ring Complex
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Kinesin-13 proteins are a group of motors that are not motile on microtubules, but instead catalyze the ATP-dependent depolymerization of microtubules *in vivo* and *in vitro*. Their functions are important for accurate chromosome segregation in mitosis. Our goal is to elucidate the structural basis of the mechanism-of-action of these motors by studying the interaction of kinesin-13s with microtubules.
Previously we have shown that the kinesin-13 motor domain (MD) in ATP-bound state has the unusual property to form rings/spirals around microtubules. We have recently obtained a medium resolution three-dimensional (3D) density map of the kinesin13-ring-microtubule complex by cryo-electron microscopy and image analysis. An atomic model of the complex has been built by docking the crystal structures of tubulin and a kinesin13 MD into the 3D map. Our model reveals a snapshot of the depolymerization mechanism by providing a 3D view of the complex formed between the kinesin13 MDs and a curved tubulin protofilament. It suggests that contacts mediated by kinesin13 class-specific residues in the putative microtubule-binding site stabilize intra-dimer tubulin curvature. In addition, a new tubulin-binding site on the kinesin13 MD was identified. Mutations at this class-conserved site selectively disrupt the formation of microtubule-associated ring complexes.

2616-Pos Board B586
Studies of the Interaction of a Kinesin-13 Protein with Microtubules
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Kinesin-13s are motor proteins involved in microtubule (MT) depolymerization and are important for regulating of MT dynamics during chromosome segregation in mitosis. Recently, it was proposed based on single molecule experiments that Kinesin-13s use diffusion on the lattice to reach the MT ends, where they accumulate and induce the depolymerization in an ATP dependent manner. Besides, we showed by Electron Microscopy that Kinesin-13s form rings and spirals around MT in AMPPNP state. Such behavior was never reported for others Kinesin family, which shows only regular MT decoration in identical conditions. Furthermore, observation that kinesin-13s accumulate on depolymerizing ends of a MT *in vivo* suggests that such rings might work by keeping kinesin-13s associated with the MT ends.
Here, we are using BSR-labeled KLP10A to investigate changes in orientation and mobility of Kinesin-13 bound to MT at different steps in the ATP hydrolysis cycle by Fluorescence Polarization Microscopy. Our results show that KLP10A is more disordered than Kinesin in all nucleotide conditions, except ADP state. We observed diffusion of KLP10A neck-motor constructs on MT in all nucleotide states except by Non-Nucleotide conditions. Further experiments with KLP10A motor-only constructs are important for the identification of the regions of the protein necessary for the diffusion movement. Interestingly, we observed events of KLP10A oligomerization during our diffusion experiments, which can be an evidence of the rings structures. A comparison between the diffusion profile of KLP10A in AMPPNP state with MT attached and detached from the slide bottom is important to reinforce the evidence for KLP10A oligomerization. Also, the accumulation of KLP10A on the depolymerizing MTs ends by single molecule measurements would favor the hypothesis of rings facilitating MT depolymerization. Overall, this work will provide a better understanding of the interaction of Kinesin-13 with MT.

2617-Pos Board B587
Measurement Of The Protein Friction Between The Yeast Kinesin-8 Kip3p And Microtubules
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Several proteins have been shown to undergo 'one-dimensional' diffusion along the surface of microtubules. Diffusion is thought to enhance the rate of targeting of proteins to the microtubule end for the depolymerizing kinesin-13 and the polymerase XMAP215, or to increase the processivity of kinesin-1 and dynein. According to the Einstein-Smolukowski relation, the diffusion coefficient, D, is related to the friction coefficient, *gamma*, according to $D = kT/gamma$. This relation, however, has not been experimentally tested for individual bio-molecules. We measured both the diffusional and frictional properties of single yeast kinesin-8 motor proteins,