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,

Kip3p, interacting with microtubule filaments in the ADP state. Using single molecule fluorescence we found that that the diffusion coefficient was 5400 ± 1400 nm^2/s with an average lifetime on the microtubule lattice of 8 s. Using an optical trap to drag a microsphere coated with Kip3p along microtubules, we measured a single molecule drag coefficient of 790 ± 230 nNs/m at low speeds. Thus we verified the Einstein-Smolukowski relation. For larger speeds and drag forces, we measured a non-linear force-velocity relation which was well fit by a model in which Kip3p is diffusing in a periodic potential with an 8-nm periodicity and a barrier height between binding sites of 14 ± 2 *kT*. This finding of an 8-nm periodicity is supported by an analysis of the positional fluctuations. Our measurements are a step towards resolving the molecular mechanism underlying protein friction an important parameter for active protein locomotion limiting the efficiency.

2618-Pos Board B588

Role of cortical rigidity in spindle positioning in C. elegans

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The first cell division in C. elegans is asymmetric. Asymmetric cell division requires correct positioning of the mitotic spindle. Prior to metaphase, the nuclear-centrosome complex, the precursor of the mitotic spindle, is positioned in the cell center. During anaphase, the spindle is displaced towards the posterior so that bisection of the spindle during cytokinesis leads to daughter cells of unequal sizes. Forces that center and position the spindle come from cortical force generators that pull on astral microtubules. In order to generate force, the cortex needs to provide a stiff anchoring platform. However, a role for the cortex in C. elegans has only been described with respect to polarity establishment. We perturbed the acto-myosin cortex by RNAi of non-muscle-myosin II (nmy-2) using conditions that allowed us to avoid disturbing polarity. Strikingly, in nmy-2(RNAi), membrane tubes are pulled from the plasma membrane into the cell. They were seen after RNAi against other actin cytoskeleton proteins and members of force generation complex, suggesting that the cortical force generators pull the invaginations, and a weakening of the cortex. As expected, we observed an increase in the variance of spindle position and orientation in nmy-2(RNAi). We used the oscillations of the centrosomes during anaphase as a reporter of spindle mechanics, and measured an increase in oscillations frequency but only a marginal decrease in amplitude. In order to understand this phenotype, we used our previously published model to analyze the results. Only by including the cortex into the model, we were able to fully describe the role of NMY-2. In summary, the occurrence of tubes after nmy-2(RNAi) strongly points towards a weakening of the cortex and the analysis of the spindle positioning suggests that the cortex provides a rigid platform for anchoring the force generators.

2619-Pos Board B589

Microtubule Binding and Rotation of the Kinesin-14 Stalk

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Movement of motors along cytoskeletal filaments is thought to be driven by small structural changes that are amplified by a large rotation of the alpha-helical coiled coil. The coiled-coil stalk of the kinesin-14 motor, Ncd, has been visualized in a rotated conformation in a crystal structure and proposed to act like a lever to amplify force produced by the motor, resulting in a working stroke that directs the motor to the microtubule minus end. We show here that an Ncd mutant that is trapped in a stalk-rotated conformation binds tightly to microtubules and shows fluorescence resonance energy transfer between the end of the stalk and microtubule, indicating that rotation of the stalk towards the microtubule is coupled to motor binding to microtubules. A mutant blocked in stalk rotation binds weakly to microtubules and shows no energy transfer, demonstrating that binding by the motor to microtubules requires movement of the stalk. Energy transfer assays show that wild-type Ncd binds to microtubules without added nucleotide with the end of the stalk more than ~9 nm from the microtubule, rotating less than 50 degrees from a position perpendicular to the microtubule. Upon binding with a non-hydrolysable ATP analogue, the stalk lies within ~6 nm of the microtubule surface, representing a rotation of ~70 degrees. These findings are consistent with previous reports by cryoEM that the Ncd stalk rotates when the microtubule-bound motor binds ATP. However, our results indicate that stalk rotation is initiated by filament binding and ADP release, and completed upon ATP binding, rather than triggered by ATP binding. Initiation of the Ncd stalk rotation by microtubule binding and ADP release, and its completion on ATP binding is reminiscent of the two-step working stroke of myosin I, revealing an unexpected similarity between the motors.

2620-Pos Board B590

A Common Microtubule Activation Mechanism for Plus- and Minus-End Directed Kinesin Motor Proteins

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Interactions with the microtubule drive the working cycle of all kinesins, yet the the structure of the kinesin-microtubule complex remains poorly characterized. We solved a series of 8Å-resolution cryo-EM structures representing the microtubule-bound hydrolysis cycles of two different kinesin family members: plus-end directed conventional kinesin, and minus-end directed NCD. These structures reveal that microtubule binding transforms part of the poorly ordered loop L11, located within the switch II nucleotide response element of these kinesins, into a stable extension of the so-called "switch II helix." We show how this extension likely enables the helix to function as a rigid "relay" element, driving the "power stroke" of both conventional kinesin and NCD. Moreover, binding of ATP analogs in either kinesin variant was associated with a 3% lengthwise contraction of the microtubule lattice. This latter effect may link conventional kinesin and NCD to the microtubule-depolymerizing kinesins.

2621-Pos Board B591

Mechanistic Analysis of Kar3Cik1 for Mitotic Function

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Kar3Cik1 is a *S. cerevisae* Kinesin-14 motor protein that promotes microtubule (MT) shortening during karyogamy yet acts to crosslink interpolar MTs (ipMTs) during anaphase. The Kar3 head contains both an ATP and MT binding site, yet there is no nucleotide binding site in Cik1. Presteady-state and steady state experiments have been pursued to define the mechanism by which Kar3Cik1 performs its mitotic function to crosslink and stabilize antiparallel ipMTs. We have developed an approach to begin our experiments with a homogenous population in which the Cik1 head binds to the MT first followed by the Kar3 motor domain (Kar3MD). The MT association kinetics at 2.6 $\mu M^{-1}s^{-1}$ are fast followed by Kar3MD association and rapid ADP release at 26 s^{-1}. ATP binding to the Kar3MD is also a fast step at 4 $\mu M^{-1}s^{-1}$ with $k_{off} = 12$ s^{-1}. Dissociation of the MT-Kar3Cik1 complex occurs as a slow step at 3.8 s^{-1}. These initial results suggest a model in which Kar3-Cik1 interacts with the MT through an alternating cycle of Cik1 binding followed by Kar3MD binding. Because Cik1 does not have a nucleotide binding site, we propose that head-head communication is mediated by a strain-dependent mechanism.

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2622-Pos Board B592

Multi-functional Dynamic Control of Bipolarity, Chromosome segregation and Spindle Elongation by a Novel Essential Chromatin Binding Klp in Fission Yeast

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A subset of all known kinesin-like microtubule motor proteins (Klps) regulates spindle function for fidelity in chromosome segregation. While some families are ubiquitous, such as Kinesin-5 and Kinesin-14 Klps, others families seem restricted to multi-cellular eukaryotes, such as chromokinesins and MKlp1-like passenger proteins. We report a novel essential chromatin-binding Klp in fission yeast *Schizosaccharomyces pombe* Ckk1 (Cho1 and Kid-like kinesin). *S. pombe* Ckk1p has an N-terminal motor domain and carboxy-terminal tandem basic-Zip DNA binding domains. It localizes to chromosome arms in prometaphase and kinetochores in metaphase. In prometaphase to anaphase it also associates with overlapping anti-parallel microtubules of the spindle midzone. Passenger proteins show dynamic relocalization from chromosome arms to kinetochores to spindle midzone and regulate cytokinesis. Although Ckk1p has the conserved Cho-domain, it does not have actin binding domains and exhibits no cytokinesis defects.