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.

Ckk1p microtubule localization regulates microtubule sliding but not spindle midzone integrity, a role attributed to Ase1p in fission yeast. A single microtubule-binding site within the motor domain suggests that like MKlp1, protein association with the stalk is needed to mediate other microtubule interactions. Premature overexpression of Ckk1 can stabilize bipolar spindles in a compromised Kinesin-5 *cut7-22ts* strain, overriding the opposing effect of Kinesin-14 Pkl1. However in wild type cells overexpressed Ckk1p results in spindle collapse. Upon removal of *pkl1,* the ability of Ckk1p overexpression to cause spindle collapse is reduced, however pre-prophase spindle elongation occurs. The result is non-central, off-side anaphase A segregation of chromosomes. Ckk1p represents a novel chromatin-binding Klp in fission yeast, absent in budding yeast, that has multiple roles in stabilizing spindle assembly, equatorial chromosome alignment and spindle elongation and does not fall cleanly into either Kinesin-6 or Kinesin-10 families of Klps.

2623-Pos Board B593

Binding Dimeric Kinesin-like proteins to Tubulin: Analysis of Microtubule and Pole Determinants

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The interaction of kinesin-like proteins (Klps) with microtubules or α/β-tubulin heterodimers is fundamental to the mitotic mechanism through regulation of microtubule organization and dynamics. Since the discovery of conventional Kinesin in 1985, and kinesin-like proteins (Klps) beginning in 1990, information on the molecular nature of this interaction has been vague. In part its analysis has awaited elegant crystallographic data that now allows us to visualize, albeit still at low resolution, the site of interaction of monomeric and dimeric Klps on microtubules. In addition the availability of sequenced genomes provides bioinformatic profiling of conserved tubulin elements. This lab is investigating determinants of dimeric Klp/tubulin interactions. We recently defined for the first time, since the discovery of kinesin over 20 years ago, the binding site on tubulin for a Klp. That tubulin is the microtubule organizing center (MTOC) protein γ-tubulin and the Klp, dimeric Kinesin-14 Pkl1. Kinesin-14 Klps are ubiquitous in eukaryotes and oppose spindle bipolarity mediated by the Kinesin-5 family. The Kinesin-14/γ-tubulin interaction is effective as a prominent mechanism to oppose spindle bipolarity. We demonstrated that its disruption, by mutating the γ-tubulin site for Kinesin-14 binding, restored spindle bipolarity and viability in a normally impaired Kinesin-5 *cut7-22^{ts}* strain. We are examining additional molecular features of the Kinesin-14/γ-tubulin binding site and determining its relationship to Klp/β-tubulin interactions. By site-directed mutagenesis we have altered similar residues in β-tubulin helix 11 and are generating chimeric β- and γ-tubulins with switched helix 11 domains. Analysis of the tubulin derivatives in vivo and in vitro will allow us to determine affects on Klp/tubulin interactions of both plus- and minus-end directed Klps and on microtubule dynamics and organization.

2624-Pos Board B594

Structure-activity Relationships In Synthetic Systems Of Coupled Motor Proteins

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Motor proteins play an essential role in regulating the internal organization of the cytoplasm by maneuvering a wide variety of vesicles and organelles. Many of these transport processes depend on the ability of motors to function collectively, in groups that contain more than one motor molecule. Utilizing protein engineering and DNA-self-assembly techniques, we have created experimental models of these multi-motor systems. Our synthetic assemblies afford precise control over the number of coupled motors in a construct, as well as their relative positions and the mechanical compliance of the linkage between each motor and the solid support to which it is anchored. Furthermore, the molecular architecture of these assemblies has been characterized using both bulk methods and single-molecule microscopy techniques. Our initial optical trapping experiments revealed rich behavior in a system of two coupled kinesin motors; the force-velocity relationship for individual constructs reveals that coupled kinesin motors can move at higher-than-predicted velocities when under high loads. Additionally, the system's stepping dynamics appears to vary significantly with applied load. Here, we discuss our efforts to characterize the effect of mechanical compliance and inter-motor separation upon these behaviors. Understanding these structure-activity relationships is critical to our broader goal of a comprehensive, mechanistic model of collective motor protein transport. Such a model would provide important insight into the process by which a cell controls its internal order.

2625-Pos Board B595

Visualizing Collective Dynamics of Nonprocessive Motors in Membrane Tube Formation

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The emergent collective behavior of motor proteins plays an important role in intracellular transport. For example, processive motors (kinesins) work in concert to extract membrane tubes from membrane compartments. In this case, it has been shown that motor proteins form dynamic clusters that can collectively generate enough force to extract membrane tubes (Koster *et al*, *PNAS* 2003, Leduc *et al*, *PNAS* 2004). Recent *in vitro* experiments have shown that nonprocessive motors (ncds) can also extract membrane tubes: here, tubes show distinct phases of persistent growth, retraction, and an intermediate regime characterized by dynamic switching between the two (Shaklee *et al*, *PNAS* 2008). The physical mechanism by which nonprocessive motors collectively mediate membrane tube formation has, however, not yet been experimentally investigated.

We use a minimal *in vitro* model system where motors are specifically attached to a fluorescently labeled lipid on Giant Unilamellar Vesicles (GUVs) to examine motor behavior during membrane tube formation. Motors collectively extract membrane tubes from the GUV as they walk on underlying microtubules. FCS and FRAP experiments reveal a directed flow as processive motors walk at typical speeds (<500nm/s) along the underlying microtubule and accumulate at the tip of the growing membrane tube. However, fluorescence correlations in time show that nonprocessive motors exhibit purely diffusive behavior, decorating the entire length of the microtubule lattice with diffusion constants at least 10 times smaller than that of a lipid-motor complex freely diffusing in a lipid bilayer (1um^2/s); FRAP experiments confirm this longer timescale for exchange of motors in the tube. These results suggest that membrane-bound motor proteins interacting with a microtubule are restricted in their diffusive motion, potentially due to fast local binding/unbinding to the microtubule lattice. This restriction likely promotes dynamic motor accumulation needed for membrane tube regulation.

2626-Pos Board B596

Experimental Realization of a Feedback Controlled Flashing Ratchet

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A flashing ratchet transports diffusive particles by on and off switching of an asymmetric periodic, ratchet shaped spatial potential. Recent theory work has predicted that the use of a feedback algorithm based on particle positions can increase velocity by up to an order of magnitude compared to periodic flashing. Experimental implementation of feedback control is also predicted to show current reversals and synchronization effects. Feedback control could be used to model and understand the gating mechanisms in linear, dimeric molecular motors that lead to processivity.

We have successfully implemented feedback control of a flashing ratchet system and observed the predicted increase in velocity. We compare two different feedback algorithms for small particle numbers. The maximum instantaneous velocity method (MIV) considers the force on all the particles when the ratchet is on, and the maximum net displacement (MND) method considers the distance of the particles compared to a certain reference point. We also find that through manipulation of this reference point, the algorithm can be further improved to be more tolerant of feedback delay times. We find good agreement with Langevin simulations that take into account the feedback delay time and spatial sampling of the potential by the finite-sized microspheres.

We use an optical line trap to realize a flashing ratchet, and through real-time image analysis we achieve fast feedback with implementation delay time of 5 ms. We use an acousto-optic deflector to create the line trap by scanning an optical tweezer fast enough that a trapped silica microsphere feels a time-averaged potential.