**858-Pos**

**The Effects of Removal of C-termini of Tubulin for Mitotic Kinesin CENP-E Microtubule Interactions**

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The carboxyl-terminus of both a- and b- tubulin consists of helices H11 and H12 and are located on the outer surface of the microtubule (MT). H12 extends into a disordered region that is characterized by a highly negatively charged motif of approximately 10-18 residues (CTT). These MT CTTs have been shown to affect the MT interactions with kinesin molecular motors for function. The research presented here tests the hypothesis that the MT CTTs modulate the behavior of the kinesin motor CENP-E with the MT and affect ATP-promoted motility. Subtilisin was used to remove the CTTs of a,b-tubulin, and the impact of the loss of the CTTs was evaluated by MT-CENP-E cosedimentation and CENP-E promoted MT gliding assays. The cosedimentation results show a dramatic decrease in MT affinity to subtilisin-MTs in the presence of 1 mM MgAMPPNP although 100% CENP-E binding is achieved. In contrast, the 1 mM MgADP results demonstrate an increase in CENP-E binding to subtilisin-MTs and an increase in MT affinity in comparison to native MTs. The in vitro motility assays reveal an increase in the rate of CENP-E promoted MT gliding on subtilisin MTs at 7.2 nm/s relative to 5.1 nm/s on native MTs. However, after 15 min, most subtilisin-MTs detached from the coverslip suggesting almost complete dissociation of the subtilisin-MT-CENP-E complex. In contrast, CENP-E promoted native MT gliding for >60 min. This study provides further evidence for the importance of the electrostatic interactions mediated by the MT CTTs for CENP-E mitotic function.

**859-Pos**

**Dimeric Centromere Protein E (CENP-E) Promotes Microtubule-Elongation at the Plus-Ends Of Microtubules**

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Centromere protein CENP-E is a dimeric kinesin (Kinesin-7 family) with critical roles in mitosis including establishment of microtubule (MT)-chromosome linkage and processive movement of monooriented chromosomes on MTs for proper alignment at metaphase. Fluorescence microscopy studies were performed to test the hypothesis that CENP-E promotes MT-elongation at the MT plus-ends. CENP-E constructs were engineered, expressed, and purified which yielded dimeric and monomeric motor proteins. The results show that dimeric CENP-E promotes plus-end directed MT gliding at 11 $\pm$ 0.005 nm/sec (n=173 MTs). Real-time microscopy assays were performed to image CENP-E promoted elongation of GMPcPP-stabilized polarity marked FITC MTs. The results revealed that out of the 270 polarity marked MTs examined, 164 MTs (60%) exhibited CENP-E promoted MT plus-end extension by GTP-tubulin (1.48 $\pm$ 0.37 μm/30 min; n=200 MTs) in the presence of MgATP. In contrast, dimeric Kinesin-1, dimeric Eg5, and CENP-E in the presence of AMPPNP did not show this pronounced MT elongation. These results suggest that CENP-E as part of its function for chromosome kinetochore attachment to MTs plays a direct role in kinetochore MT extension during congression. Supported by NIH GM54141 to Susan P. Gilbert.

**860-Pos**

**The Molecular Mechanism of the Multi-Tasking Kinesin-8 Motor**

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The mitotic spindle is organised by many microtubule-binding proteins including kinesin molecular motors. Depletion of kinesin-8 motors causes mitotic defects and long spindle microtubules, suggesting that kinesin-8s play a role in microtubule length regulation. Kinesin-8 motors have the remarkable ability to both walk towards microtubule plus-ends and to depolymerise these ends on arrival, which results in length-dependent depolymerisation.

To understand how kinesin-8s achieve such multi-tasking, we studied the structure and function of the human kinesin-8 motor domain. We determined its crystal structure which is similar to that of the well-characterised depolymerising motors, the kinesin-13s. Both families of depolymerisers have an extended loop2, which is disordered in the kinesin-8 structure, and consistent with this, our kinesin-8 construct had depolymerising activity. However, its ATPase was stimulated by both tubulin - typical of a depolymeriser - and microtubule polymers - expected for a motile kinesin - demonstrating that the ability of kinesin-8s to both walk along microtubules and to depolymerise them is intrinsic to their motor domain. To help understand this functional paradox, we used cryo-electron microscopy to image microtubules bound by the kinesin-8 motor domain in nucleotide-free and ATP-like states and calculated the structures of these complexes at ~10Å resolution. Docking of the kinesin-8 crystal structure in the reconstructions revealed microtubule-dependent conformations of the motor. Strikingly, our reconstructions show that kinesin-8 loop2 provides an additional point of contact to the microtubule surface and likely contributes to the kinesin-8 processivity that is an essential aspect of their length regulatory activity. From our functional and structural studies we conclude that the kinesin-8s represents a unique family of microtubule depolymerizers and we can begin to dissect the molecular mechanism by which they act to control microtubule length and dynamics.

**861-Pos**

**Disintegration of Microtubules into Protofilaments and Ring-Shaped Structure Formation Induced by Kif2C Neck Region Peptide**

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Members of the kinesin-13 subfamily, including KIF2C, are known to depolymerize microtubules from both ends. The positive charge-rich region extending from *N*-side of the catalytic head, called the ''neck'' region, is considered to be important in the depolymerization activity. To obtain clues to functions of the KIF2C neck region, we synthesized several peptide segments derived from the neck region and examined their properties. When a three time molar equivalent of a 37 amino-acid residue peptide, KF01 (around E200), or another peptide consisting of the *N*-terminal half of KF01, namely, KF11, was mixed with microtubules, the turbidity of the mixture increased steeply. We confirmed the binding of KF01 to microtubules by coprecipitation experiments. Circular dichroism spectroscopy showed that the neck region peptides formed a characteristic secondary structure when they were mixed with microtubules. Electron microscopy revealed that KF01 had the ability to bundle microtubules. Furthermore, at a higher peptide concentration, we detected the presence of thin filaments, considered to be free protofilaments disintegrated from microtubules, and ring-shaped structures surrounding the microtubules, considered to consist of protofilaments. Although a similar ring formation has previously been reported to be induced by the head domain of KIF2C or KLP10A, a member of the kinesin-13 subfamily, the present study has shown for the first time that the peptide derived from the neck region can induce the formation of the rings even without a catalytic head. The KF01-induced disintegration of microtubules into protofilaments would reflect the importance of the neck region to microtubule depolymerization mechanism by the intact KIF2C protein.

**862-Pos**

**MCAK (Kinesin-13) has an Unconventional ATP Hydrolysis Cycle Adapted for Microtubule Depolymerization**

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Unlike members of the kinesin-1 subfamily, the microtubule-depolymerising kinesin-13, MCAK, has no translocation activity. Rather it diffuses on the microtubule lattice to accelerate targeting to both ends, where it carries out ATP-dependent catalytic depolymerisation. The ATP hydrolysis cycle of MCAK has been largely overlooked. However, it may hold the key to the strikingly different behavior of kinesin-13 proteins compared to the conventional translocating kinesins that move directionally on the lattice. We have elucidated the ATP hydrolysis cycle of MCAK in solution and in the presence of both free tubulin dimers and microtubules. In contrast to most other kinesins and also myosins, for which product release is rate-limiting, ATP cleavage limits the hydrolysis cycle of MCAK in solution. Therefore MCAK meets the microtubule from solution in the ATP-containing state which binds tightly. Lattice-stimulated ATP cleavage drives MCAK into a weakly-bound nucleotide state, which diffuses on the lattice to target the microtubule end. An end-specific feature of the microtubule acts as a nucleotide exchange factor, promoting exchange of ADP for ATP by increasing the rate constant for ADP dissociation by more than 20-fold over the equivalent process in solution. Nucleotide exchange triggers tight binding of ATP-MCAK at the microtubule end, deforming the bound tubulin dimer causing lattice destabilization, leading to depolymerization. Tubulin-stimulated ATP hydrolysis is required to allow dissociation of tubulin-MCAK complexes released from the MT end, thereby allowing catalytic depolymerization. The altered ATP hydrolysis cycle of MCAK, relative to kinesin-1, tailors its affinity for tubulin to produce the characteristic weakly-bound diffusive interaction with the microtubule lattice and the strong microtubule end-dependent binding that promotes depolymerization.