**847-Pos**
**Mechanistic Analysis of Kar3Cik1 for Mitotic Function**
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Kar3Cik1 is a S. cerevisae Kinesin-14 motor protein that functions to shorten cytoplasmic microtubule (MT) during yeast mating for nuclear fusion, yet cross-links interpolar MTs (ipMTs) during anaphase. The Kar3 head contains both an ATP and MT binding site, yet the Cik1 head lacks an ATP catalytic site. Pre-steady-state and steady state experiments were conducted to define the mechanochemical pathway by which Kar3Cik1 stabilizes anti-parallel ipMTs for its mitotic function. To initiate the cycle, we used a high ADP strategy to promote MT-binding by the Cik1 head at 4.9 ∈ 1/4M-1s-1. The initial association is then followed by a 4-5 s-1 conformational change to induce Kar3 head binding to the MT with rapid ADP release from the active site at 109 s-1. MantATP binding to the nucleotide free MT•Kar3Cik1 is fast at 2.1 ∈ 1/4M-1s-1 with koff = 16.6 s-1. Pulse-chase methodology further reveals that MgATP binding to MT•Kar3Cik1 follows a two step process, formation of a collision complex followed by a 64 s-1 isomerization step. ATP hydrolysis occurs at 26 s-1 followed by motor detachment from microtubule at 11.5 s-1. The rate-limiting step for steady-state ATP turnover at 5 s-1 is hypothesized to be the conformational change leading to Kar3 head binding to MT. These initial results suggest a model in which Kar3-Cik1 interacts with the MT through an alternating cycle of Cik1 binding followed by Kar3 binding. Because Cik1 does not have a nucleotide binding site, we propose that head-head communication is mediated by a strain-dependent mechanism. Supported by NIH GM54141.

**848-Pos**
**Probing the Regulatory Mechanisms of KCBP using EPR Spectroscopy**
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KCBP, a plant Kinesin-14, is involved in cell division and trichome morphogenesis by structuring and organizing bundles of microtubules. Our published crystal structure of KCBP in complex with its regulator KIC suggests that bound KIC would inhibit KCBP function by physically blocking binding of the motor to microtubules. The neck mimic of KCBP, a linker found at the C-terminus of motor domain, homologous to the neck linker of Kinesin-1, appears immobilized upon KIC binding. The capture of this structural module would disrupt the nucleotide-controlled conformational cycling necessary for generating force and normal function.
Our new experiments demonstrate that tail and motor of KCBP interact directly. Binding of tail has an inhibitory regulatory effect on KCBP motor. Interestingly, binding of KIC to motor tears the motor-tail complex apart suggesting that tail and KIC may share the binding site on the motor, including the neck mimic.
To test the proposed regulatory mechanisms, we assessed the conformational freedom of the neck mimic in KCBP (a.a. 876-1261, no neck) in the presence and the absence of KIC or tail (a.a. 12-503) using EPR spectroscopy. Spectra of spin probes attached to single Cys (S1220C or S1215C) on the neck mimic showed a mobile and an immobilized component. There was a significantly higher content of the immobile component in the spectrum of the Cys1220 mutant. The addition of KIC resulted in a shift of the spectrum into more mobile region in the Cys1220 mutant but not in the Cys1215 mutant. When the tail was added to the labeled motor, the EPR spectra did not change in either mutant. The spectra clearly resolve 2 conformations for each probe and binding of KIC leads to a less structured conformation of a portion of the neck mimic under conditions studied.

**849-Pos**
**ATPase Cycle of the Nonmotile Kinesin NOD Allows Microtubule End Tracking and Drives Chromosome Movement**
**Jared C. Cochran**[1], Charles V. Sindelar[2], Natasha K. Mulko[1], Kimberly A. Collins[3], Stephanie E. Kong[3], R. Scott Hawley[3], F. Jon Kull[1].
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Segregation of nonexchange chromosomes during Drosophila melanogaster meiosis requires the proper function of NOD, a nonmotile kinesin-10. We have determined the X-ray crystal structure of the NOD catalytic domain in the ADP- and AMPPNP-bound states. These structures reveal an alternate conformation of the microtubule binding region as well as a nucleotide-sensitive relay of hydrogen bonds at the active site. Additionally, a cryo-electron microscopy reconstruction of the nucleotide-free microtubule-NOD complex shows an atypical binding orientation. Thermodynamic studies show that NOD binds tightly to microtubules in the nucleotide-free state, yet other nucleotide states, including AMPPNP, are weakened. Our pre-steady-state kinetic analysis demonstrates that NOD interaction with microtubules occurs slowly with weak activation of ADP product release. Upon rapid substrate binding, NOD detaches from the microtubule prior to the rate-limiting step of ATP hydrolysis, which is also atypical for a kinesin. We propose a model for NOD's microtubule plus-end tracking that drives chromosome movement.

**850-Pos**
**Analysis of the Role of Unique Loop L5 in Rice Kinesin K16 Motor Domain**
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L5 is one of the unique loops locates in the vicinity of ATP binding site of kinesin. We have previously demonstrated that the point mutation at the L5 dramatically alters ATPase activity and interaction with microtubules. Therefore, the loop may be functional key region. The novel rice plant specific kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain is very stable, contrast to conventional kinesin that is very labile in ADP-free state. Recently, we have determined crystal structure of the novel rice kinesin K16 motor domain. The crystal structure revealed that the length of the loop L5 was much shorter than that of conventional kinesin. In the present study, we have tried to clarify how the shorter L5 relate to the function of K16. The K16 mutants that have elongated different length L5 were prepared. Microtubule dependent ATPase activity of K16 mutant that has same size of L5 to that of Eg5 was almost abolished. And the monastrol that is known as Eg5 specific inhibitor did not bind to the mutant. On the other hand the K16 mutant that has same size of L5 to that of conventional kinesin showed normal range of ATPase activity. We have also prepared the K16 mutant Q101C that has a single reactive cysteine reside in the L5. Photochromic molecule of azobenzene derivative PAM was incorporated into the cysteine residue to induce conformational change of L5 by ultraviolet and visible light irradiation. However, the kinesin modified by PAM did not alter the ATPase activity by light irradiations.

**851-Pos**
**Interaction of the Eg5 Loop 5 with the Nucleotide Binding Site**
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Loop 5 (L5) is a conserved loop that projects from the α2-helix adjacent to the P-loop at the nucleotide site of all kinesin super-family motors. In kinesin-1 and kinesin-3 motors, L5 is 6-8 amino acids in length. Kinesin-5 motors such as Eg5 have longer L5 loops, ~17 a.a. in length. X-ray structures show that L5 is the binding site for small molecules that inhibit microtubule-stimulated ADP release by Eg5. However, crystallography has failed to identify the function of L5 because all Eg5 structures, both with and without bound inhibitors, show similar conformations for L5. It is bent away from the nucleotide site with an unusual loop W127 residue interacting with hydrophobic surface patches and the inhibitor. The proximity of the Eg5 L5 to the nucleotide site suggests it could interact with a bound nucleotide, modulating function. Larson (this meeting) presents EPR spectroscopy data supporting this conclusion. We have used molecular modeling and molecular dynamics (MD) simulations to investigate the potential interaction of L5 and the nucleotide. The L5 domain of the Eg5•ADP x-ray structure was manually deformed via phi-psi backbone rotations. L5 was sufficiently lengthy that W127 could be located in proximity to the adenine ring of ADP. The modified structure was solvated in a box of explicit waters and energy minimized. After 1000 ps of MD simulation, a stable structure was obtained. The structure shows L5 interacting with the adenine ring of ADP via W127 in a pocket formed by the hydrophobic portions of the side chains of E129, D130, and by P27. The structure shows significant impingement on the ribose hydroxyls, consistent with the experimental results of Larson. Thus the simulations provide support for the hypothesis that L5 modulates Eg5 function via interaction with the nucleotide-binding site.

**852-Pos**
**Multivariate Data Analyses for Classifying Allosteric Inhibition in Human Eg5 Kinesin**
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Like other motor proteins, the human Eg5 kinesin couples ATP hydrolysis to large conformational changes distal from the nucleotide site, thereby driving movement along microtubules. The Eg5 motor domain uniquely possesses an allosteric L5 loop, responsible for sensitivity to small-molecule inhibitors. Prior studies support a common kinetic mode of inhibition by monastrol, S-trityl-L-cysteine, and ispinesib. However, missing is the role of individual residues