We have here investigated the effect of varying neck-linker length on the motile properties of Eg5Kin. As truncated versions of Eg5 contain the native 18 amino acids of the neck linker, we generated six Eg5Kin constructs comprising of 13 to up to the 18 amino acids of the native Eg5 neck linker, possibly providing a physiological context.
Using single-molecule fluorescence, we found that all six constructs are active motor molecules capable of processive motility. In a first set of experiments, we found that the neck-linker length influences the run length, but not the velocity of the motor. We thus confirm the findings of Shastry and Hancock (2010, Curr. Biol. 20:939) with a different motor. In addition we used optical-trap assays to investigate the change in the average force the motor constructs generated and found only a small variation. Our data thus suggest that the neck-linker length of Eg5 is at least not the sole determinant for speed and force generation.

665-Pos Board B465
Modulation of the Kinesin ATPase Cycle by Neck Linker Docking and Microtubule Binding
Jared C. Cochran, Yu Cheng Zhao, F. Jon Kull.
Kinesin motor proteins use an ATP hydrolysis cycle to perform various functions in eukaryotic cells. Many questions remain about how the kinesin mechanochemical ATPase cycle is fine-tuned for specific work outputs. In this study, we use isothermal titration calorimetry and stopped-flow fluorometry to determine and analyze the thermodynamics of the human kinesin-5 (Eg5/KSP) ATPase cycle. In the absence of microtubules, the binding interactions of kinesin-5 with both ADP product and ATP substrate involve significant enthalpic gains coupled to smaller entropic penalties. However, when the wild-type enzyme is titrated with a non-hydrolyzable ATP analog or the enzyme is mutated such that it is able to bind but not hydrolyze ATP, substrate binding is 10-fold weaker than ADP binding because of a greater entropic penalty due to the structural rearrangements of switch 1, switch 2, and loop L5 on ATP binding. We propose that these rearrangements are reversed upon ATP hydrolysis and phosphate release. In addition, experiments on a truncated kinesin-5 construct reveal that upon nucleotide binding, both the N-terminal cover strand and the neck linker interact to modulate kinesin-5 nucleotide affinity. Moreover, interactions with microtubules significantly weaken the affinity of kinesin-5 for ADP without altering the affinity of the enzyme for ATP in the absence of ATP hydrolysis. Together, these results define the energy landscape of a kinesin ATPase cycle in the absence and presence of microtubules and shed light on the role of molecular motor mechanochemistry in cellular microtubule dynamics.

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Chimeric Kinesin I/Eg5 Constructs Reveal Important Elements to Motor Activity
William R. Hesse, Miriam Steiner, Matthew Wohlever, Roger D. Kamm, Wonmuk Hwang, Matthew J. Lang.
Kinesin I (KHC) and kinesin V (Eg5) have very similar structure, yet very different roles. Eg5 is generally though of as having a low stall force (~1.5pN), slow (unloaded velocity ~100nm s^{-1}), and having limited processivity, while KHC has a stall force of 5-7pN, a velocity of 500-700nm s^{-1}, and having run lengths in the micron range.
We have recently shown that the formation of the cover neck bundle (CNB), which is the formation of a beta sheet between B0 (the coverstrand) and B9 (the first half of the necklinker), is necessary for the motor to generate significant amounts of force. CNB formation along with docking of the necklinker to the motor head (B7) creates the kinesin's power stroke. Loop 13 (L13), which has previously been shown to affect motor velocity with the mutation of highly conserved glycines to alanine, also forms contacts with B9, and has been shown to make a triple beta sheet structure consisting of B0, B9, and L13.
To investigate the relative roles of the coverstrand, B9, and L13 in motor behavior we have created chimeric KHC/Eg5 constructs that incorporate the sequences for these elements from Eg5 into the KHC motorhead. We have found that stall force and unloaded run length are greatly affected by the substitution of Eg5 structural elements into KHC. These results suggest that the motors operate best with a matched CNB and that L13 strongly affects the mechanical strength of the motor. While a match CNB appears to make the relative motor function more robust, B9 has a larger impact on motor function than B0. Furthermore, these structural elements cause the motor to stall at lower forces, be slower, and less processive, but they alone do not turn KHC into Eg5.

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Intra-Motor Domain Coupling is a Strong Driver of Eg5 Motor Activity
Joshua S. Waitzman, Adam G. Larson, Nariman Naber, Eric Landahl, Sarah E. Rice.
The tetrameric kinesin family member Eg5 walks a pair of motor domains along each of two antiparallel microtubules to help set up the mitotic spindle. Previous work has identified a strong coupling between the conformations of two structurally distant elements of the Eg5 motor domain: loop 5 (L5) and the motility-generating neck-linker. However, this work was performed in isolated monomers and how applicable these results are to higher-order assemblies of Eg5 remains unknown. Using electron paramagnetic resonance (EPR) spectroscopy, we have determined the structural relationships between the neck-linkers, L5s and nucleotide binding pockets of isolated Eg5 dimers. Intra-motor domain coupling appears to be a much stronger driver of conformation than inter-motor domain coordination_as was observed in the monomer, the major determinant of neck-linker conformation is the nucleotide state. The docking of the neck-linker upon ejection of ADP is conserved from the monomer to the dimer, and this conformational change is dependent upon the presence of an intact L5. We are currently investigating the effects of disrupting neck-linker-motor domain coupling on dimer motility. Our work forms a basis for future studies of motor domain coordination and provides context for the impact of external regulators on Eg5 activity.

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Coiled-Coil Stalk of Active Kinesin-Like Protein CENP-E is Stably Folded
Nikita Gudimchuk, Yumi Kim, Don W. Cleveland, Fazly I. Ataullakhanov, Ekaterina L. Grishchuk.
CENP-E is a plus-end directed kinesin-like motor, which resides at the kinetochores of mitotic chromosomes. Its activity contributes to chromosome congression in metaphase, and to the capture and stabilization of kinetochore microtubules. Full length CENP-E from Xenopus laevis has a flexible 230nm-long coiled coil stalk, whose function is not known. The stalk is thought to be extended when the motor is active, but it becomes folded in a phosphorylation-dependent manner when the motor domain is inhibited via intramolecular binding to the tail-domain. Here, we investigate the role of this stalk in vitro by studying the motility of microbeads coated with purified recombinant full length CENP-E and a truncated construct, which lacks the stalk and the tail. We show that when conjugated to the beads, both constructs exhibit similar velocities and run lengths even in the absence of in vitro phosphorylation, indicating that the full length CENP-E is entirely active. However, when we used tethered particle motion analysis of the beads carried along microtubules by a single full length motor, we found that CENP-E was not fully extended and behaved as a 40-70nm tether. Phosphorylation of CENP-E with CDK1, Aurora A or MPS1 kinases did not change this length, so this conformation does not depend on phosphorylation. To probe the elastic properties of this compact conformation we applied periodic sideways force to a bead moving along a microtubule. Our measurements demonstrate that the full length CENP-E can be stretched up to 100nm, but it cannot be fully unfolded even by the forces up to 5pN. Together, these data suggest that the compact conformation of CENP-E is stable and phosphorylation-independent, and it does not interfere with normal motor activity.

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Klp Navigation of Spindle Assembly from Poles
Leilani Cruz, Lan Seo, Laura Patrick, **Janet L. Paluh**.
Spindle assembly is the crucial initiating step in the mitotic mechanism. Its formation utilizes coordinated actions of Kinesin-like proteins (Klps) at poles, on microtubules and at chromosomes. We previously identified a novel conserved Kinesin-14 pole-based mechanism for spindle assembly by Klp binding to the γ-tubulin small complex (γ-TuSC) microtubule organizing center, (MTOC). Changes to the γ-TuSC MTOC in fission yeast alter microtubule dynamics to regulate bipolarity and both human and fission yeast Kinesin-14 Klps can regulate this mechanism. The Kinesin-14 Pkl1 motor domain associates with residues in helix 11 of γ-tubulin at a novel Klp binding site. The Kinesin-14 Pkl1 Tail domain is distinct from that of the well-characterized *Drosophila* Ncd, replacing microtubule association sites with specialized elements for spindle pole targeting and regulation of the γ-TuSC. Thus unlike *Drosophila* Ncd that crosslinks microtubules to stabilize mitotic spindle assembly, Kinesin-14 Pkl1 regulates assembly through the γ-TuSC. Klps can be classified into 14 families. Members of two ubiquitous families, Kinesin-14 and opposing Kinesin-5 Klps, each exhibit distinctions at multiple levels amongst members, including domain elements, functional mechanisms and spindle localization.