under hindering loads, and also to rapidly rebind and continue stepping following detachment. To test the hypothesis that kinesin-2 motors are specifically tuned for bidirectional transport, we carried out stopped-flow and steady-state biochemical studies of monomeric and dimeric kinesin-1 and kinesin-2. In solution, kinesin-2 motors were found to have a 30-fold higher affinity for mantADP than unmodified ADP, presumably due to the hydrophobic nature of the mant moiety. Extrapolated ADP on-rates in the presence of microtubules indicated that for unlabeled nucleotide, ADP dissociation is not rate limiting for either kinesin-1 or kinesin-2. Microtubule pelleting experiments indicated that in the ADP state, monomeric kinesin-2 motor domains have a nearly 10-fold higher microtubule affinity than kinesin-1 motor domains. However, this increased microtubule affinity does not translate into enhanced processivity of dimeric kinesin-2 when compared to kinesin-1. This result suggests that kinesin-2 spends a larger fraction of its hydrolysis cycle in the ADP state and thus is more prone to detaching under hindering loads, but following detachment it readily rebinds to the microtubule. This behavior results in a more dynamic competition with dynein that avoids the cargo coming to a complete standstill due to motor stalling.

1668-Pos Board B560
Increased Mechanical Output by a Kinesin Mutant
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Mechanical output by the kinesin motors depends on conformational changes of the motor that transduce and amplify force, coupled to specific nucleotide states. Large conformational changes, detected in previous structural studies, have led to the proposal that distortion of the central beta-sheet play an essential role in force transduction by the motor. We now find that mutation of an invariant residue in the central beta-sheet produces unexpected effects on force transduction by the motor, enhancing the velocity of motor movement on microtubules in vitro and the ability of the motor to crosslink and slide microtubules in the oocyte spindle. The mutated residue is in a hairpin loop that can undergo transition into a beta-strand, becoming part of the central beta-sheet. The most severe mutant shows ~2-fold increased motor velocity in microtubule gliding assays and strikingly elongated spindles in vivo. Simulations of spindle assembly indicate that the elongated spindles arise because of the tighter binding by the motor to microtubules and its faster velocity of movement. The mutated residue thus plays a central role in kinesin motor mechanism transduction. Its effects in greatly increasing both ADP release and microtubule binding by the motor indicate a role for the central beta-sheet in coupling the nucleotide- and microtubule-binding sites, and promoting ADP release. We propose that interactions of the mutated residue distort the central beta-sheet, inducing ADP loss and triggering a force-producing stroke. The increased rate of ADP loss by the mutant increases its rate of ATP hydrolysis, accounting for the increased mechanical output by the motor.

1669-Pos Board B561
The Mechanism of the Transition from Diffusive to Directed Movement in Mammalian Cytoplasmic Dynein
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Cytoplasmic dynein is a minus-end-directed molecular motor involving in various cellular functions. Mammalian cytoplasmic dynein has been reported to exhibit unidirectional movements for several micrometers in vivo (Kobayashi, 2009). However, some in vitro studies have reported that single molecules of cytoplasmic dynein/dynactin complex showed biased diffusive movements along microtubules (Ross, 2006; Miura, 2010), and a recent study has demonstrated that an mRNP complex bound by a few dyneins displayed bidirectional diffusive movements (Anrute-Nayak, 2012). To reveal the mechanism of the transition from diffusive to directed movement in cytoplasmic dynein, we designed recombinant dyneins using HEK293 cell expressing system. An artificially dimerized, tail-truncated human cytoplasmic dynein 1 (DHC380) showed unidirectional movement, whereas full-length dyneins purified from the same system displayed biased diffusion towards the microtubule minus end. Furthermore, using several other recombinant dyneins that have different head-to-head distances between two motor heads, we discovered that the dynein with longer head-to-head distance contained larger diffusive component. We also observed that multiple full-length dyneins bound to Qdot moved unidirectionally to the proximal side of the microtubules, while single dyneins carrying Qdot exhibited diffusive movements. These observations imply that the directed movement of dynein is influenced strongly by the head-to-head distances and the motor number, possibly leading to the regulation of dynein by these factors in the cell.

1670-Pos Board B562
Src Kinase regulates the Human Kinesin-5, Eg5, by Phosphorylating Tyrosines in the Eg5 Motor Domain
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The human kinesin-5, Eg5, is required to establish and maintain the mitotic spindle, using in silico, in vitro and cell culture methods, we show that Src kinase phosphorylates specific tyrosine residues in Eg5. These residues are located near the nucleotide pocket and the functionally critical L5 loop. Phosphomimetic and non-phosphorylatable Eg5 mutant proteins have diminished ATPase activity and microtubule sliding relative to wild-type Eg5. We also report that phosphomimetic proteins have greatly reduced affinity for the Eg5 inhibitor S-trityl-L-cysteine. This finding suggests that Src phosphorylation of Eg5 may provide cells a non-mutagensis-dependent strategy to evolve resistance to antimitic Eg5 inhibitors. In this case, combination treatment targeting both SFKs and Eg5 may inhibit mitosis more effectively than anti-Eg5 treatment alone. Ultimately, Src phosphorylation of Eg5 represents a novel regulatory mechanism for a human kinesin, and links the chemical and physical processes that cause mitosis.

1671-Pos Board B563
Effect of Phosphorylation on Motile Properties of the Mitotic Kinesin-5 Cin8
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The mitotic spindle is a microtubule-based bipolar structure which undergoes a well-defined set of morphological changes while mediating the segregation of duplicated chromosomes. It has been established that spindle morphogenesis is governed, in part, by the activity of molecular motors from the kinesin-5 family of bipolar motors with two pairs of catalytic domains located at the opposite sides of the active complex. Kinesin-5 motors are believed to perform their function by crosslinking and sliding apart antiparallel microtubules originating from opposite spindle poles. Sarcacromyces cerevisiae cells express two kinesin-5 homologues; Cin8 and Kip1 that overlap in essential mitotic functions such as spindle assembly and anaphase spindle elongation. Previous work from our laboratory indicated that Cin8 is differentially phosphorylated during mid-late anaphase at three cyclin-dependent kinase 1 (Cdk1) specific sites located in its motor domain. Phosphorylation of Cin8 at anaphase, causes its detachment from the spindles, reduces spindle elongation rate and aids in maintaining spindle morphology (Avunie-Masala et al., 2011). To understand how phosphorylation regulates the functions of Cin8, we examined its motor properties by a single-molecule fluorescence motility assay, in which movements of single Cin8-3GFP molecules were observed on fixed microtubules. Since Cin8 was shown to be phosphorylated only during anaphase, we first examined its motor properties in crude extracts of yeast cells arrested in the different points of the cells-cycle. We also compared the motile properties of phosphorylation-deficient and phosphorylation-mimic mutants of Cin8 to the motile properties of the wild type Cin8. We found differences in characteristics of Cin8 motility under the different conditions. Results will be presented.

1672-Pos Board B564
Kinetochore Kinesin CENP-E Tracks the Tips of Dynamic Microtubules via the ‘Tethered Motor’ Mechanism
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Member of kinesin-7 family CENP-E is a kinetochore-associated plus-end-directed motor, which is important for faithful chromosome segregation in mitosis. CENP-E assists chromosome transport to the spindle midzone, where the microtubule plus ends are located. Here we report that once reaching the dynamic microtubule ends in vitro, CENP-E converts from a lateral transporter into a microtubule tip-tracker, stably associating with the tips of both assembling and disassembling microtubules. We show that the binding of kinetochores and dynamic microtubule ends is destabilized in live cells when CENP-E function is perturbed via an inhibition or RNAi depletion, implying