dependent self-regulation, which allows kinesin to efficiently utilize ATP for cargo transport.

1917-Pos
Kinesin's Light Chains Inhibit the Head- and Microtubule-Binding Activity of its Tail
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Kinesin-1 comprises two heavy chains (KHCs) and two light chains (KLCs). The KHC tail inhibits ATPase activity by interacting directly with the enzymatic KHC heads, and the inhibitory segment of the tail also binds to microtubules. We have discovered a novel role for the KLCs in regulating the head- and microtubule-binding activities of the kinesin-1 tail. We show that KLCs reduce the affinity of the head-tail interaction over ten-fold. Functional assays confirm that the KLCs attenuate tail-mediated inhibition of kinesin-1 activity. We also show that KLCs block tail-microtubule binding. Inhibition of head-tail binding requires both steric and electrostatic factors. Inhibition of tail-microtubule binding is largely electrostatic and is more pronounced at physiological pH (pH 7.4) than under acidic conditions (pH 6.6). Full inhibition requires a negatively-charged linker region in the KLCs, between its KHC-interacting and cargo-binding domains. Our data support a model wherein KLCs promote activation of kinesin-1 for cargo transport by suppressing both the head-tail and tail-microtubule interactions. Additionally, KLC-mediated inhibition of tail-microtubule binding may influence kinesin-1's emerging role in microtubule sliding and cross-linking.

1918-Pos
The Kinesin-1 Tail Binds to Microtubules in a Manner Similar to Tau
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The kinesin-1 molecular motor contains two microtubule binding sites: an ATP-dependent site in the head domain and an ATP-independent site in the tail domain. In this work we show that the tail binds to microtubules with a sub-micromolar affinity, and that binding is mediated largely by electrostatic interactions. The tail binds to a site on microtubules that is distinct from the head domain binding-site but overlaps with the binding-site of the microtubule associated protein (MAP) tau. Tail binding also stimulates the assembly and promotes the stability of microtubule filaments in a manner similar but not identical to tau. The tail's microtubule binding-site is in close proximity to its regulatory and cargo-binding regions, which suggests that the tail-microtubule interaction described in this work may prove to play an important role in the activity and regulation of the kinesin-1 motor in the cell.

1919-Pos
To Block or not to Block: Isoform Specific Regulation of Kinesin Mediated Transport by the Microtuble Associated Protein Tau
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The microtubule associated protein (MAP) tau is known for its role in modulating microtubule (MT) dynamics in the neuron and has also been implicated in the regulation of kinesin-mediated axonal transport. Previous work has demonstrated that tau has a large inhibitory effect on kinesin's processive run length and binding frequency on MTs that is both concentration and isoform dependent, with the 3 repeat form (3RS) having a much larger inhibitory effect than the four repeat isoform (4RL). In the current study we have used stopped-flow kinetics to elucidate the mechanism by which tau inhibits kinesin-mediated transport in an isoform specific manner. We demonstrate that, in the presence of 3RS-tau, MTs are segregated into two populations, one in which kinesin can bind normally and one in which kinesin can still bind, but with a reduction of its on-rate. The observed on-rates do not vary with increasing tau concentration, but the relative amplitudes of each population do, with the population of MTs with a lower affinity for kinesin increasing at the expense of the population of MTs that kinesin can bind normally. Thus, our data suggests inhibition of kinesin by 3RS- tau is primarily of a non-competitive nature, ruling out a strictly steric blocking mechanism. On the other hand, a single population of MTs is observed in the presence of 4RL-tau, in which kinesin's binding rate is reduced in a linear fashion with increasing tau concentration, suggesting this isoform competitively blocks kinesin binding through a steric blocking mechanism. Taken together, our findings demonstrate a fundamental difference in the manner by which different isoforms of tau inhibit kinesin motility and provide new insight into the potential role of these MAPs in regulating axonal transport.

1920-Pos
Key Residues on Microtubules Responsible for Activation of Kinesin ATPase
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The enzymatic activity of molecular motors such as myosin, kinesin, and dynein is enhanced when they bind to cytoskeletal filaments. In the kinesin-microtubule (MT) system, MT binding accelerates ADP release from kinesin, thereby increasing the overall rate of ATP hydrolysis. This ADP release is coupled to kinesin transition from a weak-binding to a strong-binding state; therefore, it is essential for kinesin stepping.
We aimed to identify the critical residues on MTs involved in the weak- and strong-binding states by conducting a mutational analysis of tubulin using a yeast expression system. A comprehensive set of charged-to-alanine mutations in the area of MT spanning helix H11 to H12 in both α- and β-tubulin was expressed in yeast cells (36 mutations); the substitution of 8 residues resulted in a haploid lethal mutant, whereas the substitution of the other 4 residues led to slow cell growth. These findings indicated that the 12 residues probably play a vital role in the in vivo MT functions. Single molecule motility assay of kinesin with these mutant MTs revealed that 2 independent regions on the MT, the H11-12 loop/H12 of α-tubulin and H12 of β-tubulin, are essential for kinesin motility. Measurement of unbinding force showed that in the ADP state, kinesin-MT interaction is mediated via α-tubulin, whereas in the nucleotide-free and 5′-adenylylimidodiphosphate (AMP-PNP) states, this interaction is mediated via both α- and β-tubulin. Furthermore, mutations in the binding site in α-tubulin result in a reduction of the rate of ATP hydrolysis (k_{cat}), while mutations in the binding site in β-tubulin lower affinity for MTs (K_mMT). Thus, these findings suggest that kinesin releases ADP upon initial contact with α-tubulin, and is further locked on the MT via α- and β-tubulin.

1921-Pos
Open-Source Stochastic Simulation for Modeling Kinesin-1 Kinetics
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Kinesin-1 (conventional kinesin) is a homodimeric motor protein important for axonal transport. It has been well studied through ensemble and single-molecule assays. However, the enzymatic stepping cycle is complex, with many rate constants that are modulated by interaction of the two motor domains. This makes it difficult to predict how changes in a given rate constant may affect observable properties such as processivity, velocity, or stall force. We have written a simulation of kinesin walking using a Stochastic Simulation Algorithm. The model allows for interactions between the heads, and includes states that are not considered part of the normal cycle. This adds to the complexity of the model but also allows for probing rare events, such as those that lead to a finite processivity. Also included are rate constant dependencies on force and concentrations of ATP, ADP, and Pi, which may provide insight into other processes under investigation, such as kinesin backstepping. We intend to use the simulation to aid in interpreting our own gliding motility assay results and to place upper and lower limits on values for rate constants. Our source and executable codes will be freely available.
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1922-Pos
Structural Basis for the Mechanochemical Coupling of Kinesin-1 Revealed by Crystal Structural and Biochemical Analyses
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Kinesin-1 is a dimeric motor protein that moves along microtubules in a hand-over-hand manner. To move in such a coordinated manner, two motor domains have to coordinate their ATP hydrolysis reactions. Recent studies showed that ATP hydrolysis cycle of kinesin motor domain can be affected by either microtubule-binding or external strain posed to the neck linker, but the exact mechanisms are still unknown. At the last annual meeting, we reported the first crystal structure of nucleotide-free kinesin-1 and that the structure explains how kinesin's two motor domain coordinate to move processively. Here, to understand the mechanochemical coupling mechanism, we carried out detailed analysis of the kinesin crystal structure along with biochemical characterizations of alanine-mutant at key residues. First we modeled nucleotide-free kinesin-microtubule complex by docking to the 9Å cryo-EM density map by Sindelar et al (2007) and identified several possible salt bridge pairs between kinesin