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.
Acknowledgements: This work was supported by the DTRA CB Basic Research Program under Grant No. HDTRA1-09-1-008.

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

and tubulin surface, two of which are involved in stabilizing the extra turns of switch II helix (α4) formed toward the nucleotide-binding pocket. In contrast, only few salt bridge formations are possible in ADP state, explaining why ADP release causes specific and tight binding to microtubule. The structural change of α4 promotes hydrogen bond and hydrophobic interactions of highly conserved residues in α4 with switch II loop, pulling switch II loop away and promoting ADP release from nucleotide pocket. ADP release and ATP binding cause rotational movements of α4 and also rotational movements of nucleotide-binding P-loop and its surrounding elements. These nucleotide-dependent domain motions alter the mobility of the neck linker, providing structural basis for how kinesin's two motor domains coordinate to move processively.

1923-Pos

Probing the Mechanism of Kinesin-1 Motion in Three Dimensions Using the Photonic Force Microscope

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Kinesin-1 is a molecular motor essential for cellular function. It transports components around the cell by a processive movement along microtubules while hydrolysing ATP. Although extensively studied by a variety of techniques, the mechanism used by these single-molecule motors to produce this efficient motion on the nanometer scale is not fully understood.

In our investigations we use the Photonic Force Microscope (PFM) to trap and track a 500nm bead attached to a kinesin motor as it interacts with a microtubule *in vitro.* The PFM is an optical trap capable of recording a trapped dielectric particle's motion in three dimensions with nanometre spatial and microsecond temporal resolutions. Using the data recorded we can infer information about the molecular motor's position and its mechanical properties. By characterising different conformational states of the kinesin molecule from its changing mechanical properties as it processes, we expect to learn more about the cycle of events that make kinesin movement possible.

An understanding of how nature achieves this motion on the nanoscale will help combat diseases related to kinesin's malfunction and will allow production of similar artificial nanomachines in the future.

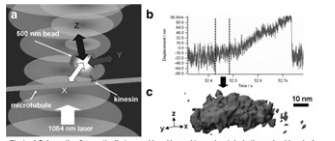

Fig.1: a) Schematic of an optically trapped bead bound to a microtubule through a kinesin-1 molecule. b) Position trace of a bead attached to a kinesin motor travelling along a microtubule orientated in the Y-axis. c) 3D iso-energy profile of bead centre positions in the bound state highlighted on graph.

1924-Pos

Multiple Interacting Kinesin-1 Motors Cooperate Negatively

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Many sub-cellular commodities are transported by more than one motor, and it is well-known that the combined function of motors can lead to unique transport behaviors. Yet, little is known about how grouping multiple motor proteins influences the motile properties of cargos, and in particular, relationships between the structural / compositional organization of motor complexes and key collective transport parameters (run lengths, detachment forces) have not been established.We have taken important steps towards solving this problem by synthesizing the first set of structurally-defined complexes of interacting kinesin-1 motors. Furthermore, we have developed 'single-molecule' assays that can examine new and important aspects of collective motor dynamics; namely, whether multiple motors cooperate in a positive or negative fashion and if these behaviors influence ensemble transport properties of multiple motor systems. Herein, we demonstrate that interactions among two elastically-coupled kinesin molecules lead to negative motor cooperativity, and that this behavior influences collective motor force production. We also describe how such effects can reconcile differences between measurements of cargo motions *in vitro* and in living cells.

1925-Pos

Kinesin-1's Behavior at Obstacles

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Using single molecule stepping assays, we were able to show that kinesin-1 stops when it encounters an obstacle in its path on the microtubule lattice. Based on the stepping mechanism of kinesin-1, we propose the following model to explain why the molecule stops at obstacles:

Kinesin-1's processivity requires the rear head to stay bound until the leading head is firmly attached to the next tubulin dimer. The fact that kinesin-1 follows a single protofilament limits the choice of forward binding to the next tubulin dimer along the same protofilament. Therefore, if a large molecule is blocking the next tubulin dimer, the leading head cannot bind and the rear head cannot detach. This situation effectively stalls the kinesin-1 molecule until it detaches from the microtubule or a forward binding site becomes free.

Based on this model, we were able to calculate the dissociation rate of kinesin-1 in the stopped state. This calculated value agreed very well with a direct measurement, indicating that the model accurately describes kinesin-1's behavior at obstacles. A very similar dissociation rate has been measured previously for single-headed kinesin-1 mutants, suggesting that kinesin-1 waits at obstacles in a one-head bound state.

Interestingly, in about 50 % of the observed stopping events, kinesin-1 did not detach at the end of the stopping phase, but overcame the obstacle and continued to walk. The rate with which kinesin-1 exited the stopped phase by overcoming the obstacle was almost identical to the dissociation rate measured for stopping events. Therefore, it is likely that kinesin-1 overcomes a roadblock by detaching from the microtubule and then, instead of leaving into solution, reattaching next to, or behind the obstacle.

1926-Pos

***In Vitro* Analysis of the Effect of Microtubule Acetylation on Kinesin Motility**

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Plus end-directed intracellular transport by kinesins on microtubules in eukaryotic cells directs cargo to the cell's periphery, but to carry out polarized transport, additional signals from microtubules must be recognized by cargo-carrying kinesins. One emerging hypothesis, supported by *in vivo* observations of preferential kinesin-1 transport along acetylated microtubules, suggests that post-translational modifications (PTMs) of tubulin subunits in subsets of microtubules serve as markers for intracellular transport. Here we are examining if and how acetylation of microtubules directly regulates kinesin motility. As a source of acetylated and unacetylated microtubules, we have used *Tetrahymena* doublets extracted from a wild type strain and a mutant strain wherein the otherwise acetylated Lysine-40 is mutated to an Arginine. For obtaining fluorescently-labeled kinesin, lysates were extracted from COS cells transfected with Kinesin-1 genetically labeled with three-tandem monomeric citrines (3xmCit-KHC). To evaluate the effect of acetylation on Kinesin-1 motility, we used TIRF (Total Internal Reflection Fluorescence) microscopy to perform single molecule *in vitro* motility assays and measure the velocity and run length of 3xmCit-KHC on acetylated and unacetylated doublet microtubules. Our observations show that while the *in vitro* velocity remains unaltered, twice as many binding events can be observed for 3xmCit-KHC on wild-type doublets than on unacetylated doublets. We conclude that the motor domain of Kinesin-1 directly recognizes acetylation of microtubules and has a greater tendency to bind acetylated microtubules than unacetylated microtubules. We suggest that acetylation of microtubules enhances the binding affinity of Kinesin-1, which in turn allows preferential transport by Kinesin-1 along acetylated microtubules. To exclude differences between motility assays as source for the observed preferential binding of Kinesin-1 to acetylated microtubules, we are now comparing the binding and motility of Kinesin-1 for acetylated and unacetylated microtubules in the same motility assay.

1927-Pos

Surface Passivation for Molecular Motor Protein Assays

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In kinesin motiliy assays, it has been shown that the surfaces with which kinesin interacts must be passivated in order to prevent kinesin from denaturing on them. The most popular surface blocker is the casein family of milk proteins. Casein is usually purified to various degrees from bovine milk and has many unknowns associated with it when reconstituted and used in motor protein assays. In order to obtain a clearer picture of how kinesin and microtubules interact, a cleaner surface passivation needs to be found. The interaction of kinesin with microtubules has been studied extensively, however, there are fewer studies that investigate how the interaction of kinesin and microtubules changes due to surface passivation. One recent study has shown that the differing components of casein (termed alpha, beta, and kappa) can significantly affect microtubules in gliding motility assays [1]. Gliding motility assays are assays where a glass cover slip is passivated and kinesin is prevented from interacting directly with the substrate. Microtubules are then propelled by the motor activity of a bed of immobilized kinesin molecules. Lipid molecules are fatty acids that can be purified to a much greater extent than casein can. Also, lipid molecules exhibit the same amphiphilic behavior as casein, they adhere to glass easily, and can be easily functionalized. Lipids are thus an attractive alternative