with this, when the Kinesin-2 neck linker was matched to the effective length of Kinesin-1 by deleting three residues and substituting an alanine for a proline, the Kinesin-2 run length nearly matched that of Kinesin-1. These results demonstrate that run length scales with neck linker length for both Kinesin-1 and Kinesin-2 and is sufficient to account for differences in processivity. In addition, we find that adding positive charge to neck linker inserts enhances processivity, providing a possible explanation for the lack of dependence of run length on neck-linker length observed by others. Our data is consistent with the hypothesis that increasing neck linker compliance reduces processivity by disrupting front head gating and potentially provides a unifying principle across kinesin families - longer neck-linkers lead to less processive motors.

1912-Pos

Optimal Size of the Neck Linker is Important for the Coordinated Processive Movement of Kinesin-1

Hiroshi Isojima, Teppei Mori, Michio Tomishige.
Department of Applied Physics, The University of Tokyo, Tokyo, Japan.

Kinesin-1 is a dimeric motor protein that walks along microtubules by alternately moving two motor 'heads'. Several recently published papers including ours provided evidences that kinesin dimer takes one-head-bound state while waiting for ATP and ATP-binding triggers the tethered head to bind to the forward tubulin-binding site. However, it is still not clear why rebinding of the tethered head, which is freely diffusing, to microtubule is prohibited during the ATP-waiting state. To explain this mechanism, we proposed a model based on the crystal structural analysis (Makino et al, this meeting) that ADP release of the tethered head is prohibited because the neck linker would be stretched out if both heads become nucleotide-free due to a steric hindrance posed on the neck linker. This model predicts that if the neck linker is artificially extended, the tethered head can easily rebind to the microtubule. To test this prediction, we engineered neck linker extended mutants by inserting poly-Gly residues and observed their conformational states using single-molecule FRET technique. We found that 5 amino acid extension of the neck linker allows the tethered head to rapidly rebind to the microtubule even in the absence of ATP, and that in this state both neck linkers adopt backward-pointing conformation. The neck linker extended mutants showed processive motility with reduced velocities compare to the wild-type, although the microtubule-activated ATPase rate was not changed, which are consistent with our previous results using poly-Pro insertion (Yildiz et al 2008). There results suggest that optimal size of the neck linker is important to prevent rebinding of the tethered head while waiting for ATP and to efficiently couple ATP hydrolysis energy with forward step.

1913-Pos

The Neck Linker of Kinesin-1 Functions as a Regulator of ATP Hydrolysis Reaction

Xiao Ling[1], Masahide Kikkawa[2], Michio Tomishige[1].
[1]Department of Applied Physics, University of Tokyo, Tokyo, Japan,
[2]Department of Cell Biology & Anatomy, University of Tokyo, Tokyo, Japan.

Kinesin-1 is a highly processive motor that moves along microtubule in a hand-over-hand manner. The neck linker that connects two motor domains has pivotal role in the head-head coordination but its exact role is still controversial. It have been widely believed that the neck linker acts as a mechanical element to propel the tethered head forward, however, we recently proposed an alternative model (biased-capturing model) based on crystallographic and cryo-EM analyses, in which the neck linker docking is not required for the forward stepping. We hypothesized that the neck linker docking rather functions to activate rate-limiting ATP hydrolysis reaction.

To test this hypothesis, we engineered a series of monomeric kinesin mutants whose neck linker was truncated and carried out biochemical and structural analyses. As the neck linker was deleted further from the C-terminus, microtubule-activated ATPase rate of the mutant kinesin decreased and it becomes almost undetectable when whole neck linker was removed. Single molecule fluorescent imaging showed that the neck linker-less monomer stably bound to the microtubule even in the presence of 1 mM ATP. Cryo-EM observation of the neck linker-less mutant on the microtubule in the presence of saturating AMP-PNP displayed a structure similar to that of nucleotide-free wild-type kinesin.

These results indicate that kinesin without the neck linker can bind to the microtubule but is incapable of proceeding ATP hydrolysis reaction, which is consistent with the idea that the neck linker acts as an activator of ATP hydrolysis reaction. This mechanism can explain the front head gating mechanism for head-head coordination: the neck linker of the leading head is pulled backward and the head cannot proceed ATP hydrolysis so that the head cannot detach until the trailing head detaches from microtubule.

1914-Pos

Coupling of Kinesin-1 Neck Linker Docking to the Nucleotide Binding Site

David D. Hackney.
Carnegie Mellon Univ., Pittsburgh, PA, USA.

Coupling of nucleotide binding to docking of the neck linker of kinsin-1 is important for generation of directional motility. One approach towards determining the magnitude of this coupling is to use isotopic exchange reactions to evaluate the free energy differences between states. Kinesin-1 monomer head domains catalyze the slow MT-dependent synthesis of bound ATP from bound ADP and free Pi (MT•E•ADP + Pi → MT•E•ATP + HOH) that results in oxygen isotopic exchange of $^{18}O/^{16}O$ between water and Pi. The tethered head domain of a kinesin dimer bound to MTs, however, catalyzes ATP synthesis at a 20-fold faster rate (Proc.Natl.Acad.Sci.USA 102, 18228 (2005)). This more rapid rate of ATP synthesis with a dimer suggests that the tethered head can bind to the microtubule behind the strongly attached head, because this positions the neck linker of the tethered head toward the plus end of the microtubule and would facilitate its docking on synthesis of ATP.

Isotopic exchange analysis of other constructs with alterations in the neck linker is in progress. One approach is to delete part of the neck linker and therefore prevent reversible docking. DKH335 has lost the C-terminal part of the neck linker that makes extensive contacts with the core. During net ATP hydrolysis, the full length head domain DKH346 resynthesizes ATP on average once in 40 turnovers. In contrast, DKH335 is reported here to hydrolyze ATP with no detectable ATP resynthesis (ATP resynthesis occurs only once in >500 turnovers). This is consistent with more rapid Pi release in the absence of a requirement for coupled neck linker undocking or with destabilization of bound ATP in the absence of neck linker docking.

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1915-Pos

Activity Scales and ATP Hydrolysis: Understanding the Thermodynamics of Molecular Motor Kinesin

Neha Awasthi, Volker Knecht, Reinhard Lipowsky.
MPI for Colloids and Interfaces, Golm, Germany.

Kinesin is a molecular motor that transports cargo along microtubule tracks and like most other molecular motors, is powered by ATP hydrolysis. The chemical energy derived from the ATP reaction cycle is converted into mechanical work. Understanding the thermodynamics of ATP hydrolysis coupled with the motor (an enzyme), can offer insights into the mechanism and energy landscape of the system [1]. Activity scales were introduced [1] as thermodynamic parameters with this motivation.

We present a scheme to estimate activity scales for ATP hydrolysis by relating them to the free energies of formations.

Extending the concept, we show that these activity scales are well-defined for chemical species in *any* equilibrium reaction. Hence, a complex equilibrium reaction can be *decomposed* in terms of the activity scales of the respective species. The equilibrium constant for the reaction can also be calculated if the activity scales are known. A quantum mechanical simulation scheme is used to calculate activity scales. Results are presented for some gas phase equilibrium reactions involving small molecules. The accuracy of the calculated activity scales is related to the level of theory used for the quantum mechanical simulations. We discuss the implications and challenges of such simulations in solvent environments for large molecules in biochemical reactions.

[1] R. Lipowsky and S. Liepelt, J. Stat. Phys. 130, 39, 2008.
[2] Neha Awasthi, V. Knecht, and R. Lipowsky, in preparation.

1916-Pos

Single Molecule Visualization of Self-Regulated Kinesin Motility

Tomonobu M. Watanabe, Toshio Yanagida, **Atsuko H. Iwane**.
Osaka Univ., Suita, Japan.

Kinesin-1 is an ATP-driven molecular motor that transports various cargoes in cells by binding its motor domain to microtubules. Its tail domain is thought to self regulate this binding. Here we inhibited kinesin ATPase activity and motility by interacting the heavy chain C-terminal tail region with the N-terminal motor domain. Ionic strength was found to heavily influence this self-regulation as both tail domain binding to the motor domain and ATPase activity were dependent on KCl concentration in in vitro experiments. Single molecule imaging experiments showed that the tail domain did not affect motility velocity but did lower the binding affinity of the motor domain to the microtubule. The decrease in binding was coupled to ATPase inhibition. Tail domain transfected into living cells failed to bind to microtubules, but did inhibit the interaction between the motor domain and microtubule, in agreement with the in vitro investigations. From these results, we propose a mechanism to describe this ion strength

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
Yao Liang Wong, Sarah E. Rice.
Northwestern University, Chicago, IL, USA.
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
Mark Seeger, Sarah Rice.
Northwestern University, Chicago, IL, USA.
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
Derrick P. McVicker, Andrew R. Thompson, Christopher L. Berger.
University of Vermont, Burlington, VT, USA.
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
Seiichi Uchimura[1], Yusuke Oguchi[2], You Hachikubo[1], Shin'ichi Ishiwata[2], Etsuko Muto[1].
[1]RIKEN, Saitama, Japan, [2]Waseda University, Tokyo, Japan.
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
Lawrence J. Herskowitz, Andy R. Maloney, Brigette D. Black, Brian P. Josey, Anthony L. Salvagno, Steven J. Koch.
University of New Mexico, Albuquerque, NM, USA.
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
Tsukasa Makino[1], Masaru Tanokura[2], Michio Tomishige[1].
[1]Department of Applied Physics, the University of Tokyo, Tokyo, Japan, [2]Department of Applied Biological Chemistry, the University of Tokyo, Tokyo, Japan.
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