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modified procedure based on the resin embedding technique. Confocal microscopy is used to study structural properties of actin filament networks on larger length-scales linked by the α-actinin protein. We vary the concentrations of actin and linking agents to study their effects on the properties of the actin filament bundles and networks. In the case where counter-ions are used to induce bundling, the concentrations of actin filaments and counter-ions in solution determine bundle size, as measured from TEM images. On the other hand, in the presence of the cross-linker, α-actinin, the molar ratio of α-actinin to actin plays an important role in the properties of the network structure, determining the branching frequency of the bundles. An experimentally guided simulation based on the α-actinin/actin filament system was carried out using CHARMM to attempt to replicate the features of the real system and therefore to study the physics behind the actin filament assembly process in these different regimes.

Kinesin & Dynein-family Proteins

2326-Pos The Kinesin-1 Motor Protein is Regulated by a Direct Interaction of its Head and Tail

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Board B441

Kinesin-1 is a molecular motor that transports cargo inside cells. Generally, more than 90% of kinesin-1 exists in a regulated conformation to conserve ATP and to ensure proper intracellular distribution and coordination of motors. In this regulated conformation, the coiled-coil stalk connecting the enzymatically active heads to the regulatory tails folds in half, bringing them in proximity. How this “folding” regulates the kinesin head has remained a mystery. Here we present biochemical and structural data, including a 9Å cryo-EM reconstruction, demonstrating that the tail interacts directly with the enzymatically critical Switch I region of the head. These data suggest mechanisms by which the tail may both regulate kinesin heads in solution and hold kinesin-1 in an idle state on microtubules. The interaction of Switch I with the tail is strikingly similar to the interaction of small GTPases with their regulators. Other motors may share similar regulatory mechanisms.

2327-Pos Strongly and Weakly Bound Kinesin Heads Are Positioned Differently on the Microtubule Lattice during Steady State

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Board B442

When dimeric kinesin-1 motor enzyme is waiting for ATP during steady state movement along a microtubule, one head has ADP bound at its active site and the other head is empty. It is unclear whether the two heads are positioned identically with respect to the microtubule lattice, and there is even controversy about whether the ADP head interacts directly with the microtubule surface at all. Using a kinesin construct bearing a novel, highly photostable DNA-Cy3 tag on one head and a fluorescent bead on the stalk, we employed two-color single molecule fluorescence tracking to make precise, signal-averaged measurements of the head movements accompanying stepping at limiting ATP concentrations. Head translation both parallel and perpendicular to the microtubule axis was measured. Changes in head orientation were also detected by comparing data from constructs with Cy3 at two different positions along the DNA ‘lever arm’. The observed movements are consistent with models in which both nucleotide free and ADP-bound heads interact directly with the microtubule. Significantly, we find that the two heads bind with a different position and orientation with respect to the lattice, demonstrating that the different functional properties of the two head configurations are associated with significant, nanometer-scale structural differences in the head-microtubule complexes.

2328-Pos

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WITHDRAWN

2329-Pos Essential Characteristics Of Kinesin-1’s Processive Motion Obtained With Single-molecule Fluorescence Microscopy

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Board B444

Kinesin-1 is a dimeric motor protein that uses the free energy obtained from the hydrolysis of ATP to transport a variety of cargo within eukaryotic cells. This highly processive motor takes on average more than a hundred steps of 8 nm along a microtubule before releasing. It is thought that early detachment of kinesin is prevented by keeping the catalytic cycles of both motor domains out of phase. In order to understand the details of the processive mechanism of kinesin’s motion it is necessary to obtain detailed knowledge about the processivity of the motor and the number of rate-limiting steps governing the chemomechanical cycle under different experimental conditions. Here, we use total internal reflection fluorescence microscopy to visualize single fluorescently labeled kinesins walking along microtubules and determine the run length and the randomness at different ATP concentrations at zero

load. We introduce a novel and efficient method to measure the length of processive runs, based on the motor density on the microtubule. In this approach the observation of individual motors is not required and consequently complications due to photobleaching are avoided.

Detailed analysis of the time dependence of kinesin's displacement and its variance is used to determine the randomness of the motion, which depends on the number of rate-limiting steps in the chemomechanical cycle. In line with earlier optical trapping studies (in which kinesin is subjected to a load), we found that two steps are rate limiting at saturating ATP concentrations and that a third, ATP-concentration dependent step takes over at lower ATP concentrations.

2330-Pos Interaction of ATP Driven Motor Proteins with ATP Analogue Having Syn Conformation with Respect to the Adenine-ribose Bond

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Board B445

It is known that, in solution, ATP analogues such as 8-Br-ATP with bulky substitution at the eight position of the adenine ring predominantly assume the *syn* conformation with respect to the adenine-ribose bond. Previously we have demonstrated that 8-Br-ATP induces intrinsic trp fluorescence enhancement of smooth muscle myosin that reflect the formation of the M**ADP Pi state. Moreover, the phosphorylated smooth muscle myosin supported actin translocation using 8-Br-ATP. Contrary, for skeletal muscle myosin, 8-Br-ATP induced neither trp fluorescence enhancement nor actin translocation. Kinesin is also ATP driven motor protein that has strikingly similar structure of motor domain to myosin. In the present study, interaction of kinesin with 8-Br-ATP has been examined. Interestingly, conventional kinesin supported microtubules translocation using 8-Br-ATP. This suggests conventional kinesin adopts the 8-Br-ATP in the normal conformation. Currently, we are studying the interaction of unconventional myosins, and kinesins derived from plant with 8-Br-ATP.

2331-Pos Microtubule Sliding and Rotation Driven by Single-Headed Kinesins

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Board B446

Some versions of the kinesin neck linker hypothesis propose that docking of the neck linker against the main part of the head generates the force for stepping. To test this proposal, we have engineered single-headed kinesins that can be surface-linked via their N-termini, leaving the C-terminal neck-linker hanging free. These N-linked kinesin heads drive microtubule sliding at similar rates to C-linked heads, thereby ruling out models that require connectivity between the neck linker and the load, including models in which the neck linker is the only element able to exert or transmit force. Instead, our findings indicate a force-generating mechanism that shifts both the N & C termini of the head towards the microtubule plus end, so that force can almost equally well be exerted by either the N or C terminus of the head. Whilst the neck linker is not required to exert or transmit force, a functionally-intact neck linker is nonetheless indispensable: in both N-linked and C-linked geometries, mutations that disrupt neck linker docking inhibit microtubule sliding, super-activate the kinesin ATPase and cause futile cycling. We propose that the key role of the neck linker is not to generate force, but to drive prompt unbinding of the head from the microtubule. We also find that kinesin heads drive anti-clockwise rotation of each sliding microtubule around its axis. We can account for the observation using a simple binding rule model in which the head selects the closest tubulin heterodimer in the lattice perhaps using a diffusional search, but only provided that the binding event produces a tilt of the core of the newly-attached head and biases its subsequent detachment position towards the plus end and the left.

2332-Pos Stepping dynamics of Kinesin Motors

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Among the multiple steps constituting the kinesin's mechanochemical cycle, one of the most interesting events is observed when kinesins move an 8-nm step from one microtubule (MT)-binding site to another. The stepping motion that occurs within a relatively short time scale (~ 100 microsec) is, however, beyond the resolution of current experiments, therefore a basic understanding to the real-time dynamics within the 8-nm step is still lacking. For instance, the rate of power stroke (or conformational change), that leads to the undocked-to-docked transition of neck-linker, is not known, and the existence of a substep during the 8-nm step still remains a controversial issue in the kinesin community. By using explicit structures of the kinesin dimer and the MT consisting of 13 protofilaments (PFs), we study the stepping dynamics with varying rates of power stroke (kp). We estimate that $1/kp <\sim 20$ microsec to avoid a substep in an averaged time trace. For a slow power stroke with $1/kp > 20$ microsec, the averaged time trace shows a substep that implies the existence of a transient intermediate, which is reminiscent of a recent single molecule experiment at high resolution. We identify the intermediate as a conformation in which the tethered head is trapped in the sideway binding site of the neighboring PF. We also find a partial unfolding (cracking) of the binding motifs occurring at the transition state ensemble along the pathways prior to binding between the kinesin and MT.

2333-Pos Single Molecule Imaging Of Hindered Movement Of Drosophila Kinesin On Crowded Microtubules

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Board B448

Recent reports regarding the behavior of kinesin when encountering an obstacle, such as another motor protein, describe apparently conflicting results. Crevel et al. (2004, *23*(1), EMBO J) used a monomeric rigor mutant of rat kinesin as an obstacle and showed, by means of stopped-flow or flash-photolysis, that the average residence time of wild-type kinesin decreases linearly with increasing obstacle concentration. A similar residence time was estimated for the crowded situation with only wild-type kinesin, suggesting that kinesin dissociates earlier on average when encountering a roadblock. In contrast to this, Seitz & Surrey (2006, *25*(2), EMBO J) suggested that kinesin waits until the obstacle unbinds and then proceeds. We clarify the situation with improved, single molecule measurements with recombinant, fluorescently tagged, short-tail kinesins interacting with immobilized microtubules in vitro. This approach overcomes potential weaknesses in the past work by directly visualizing the motility of the motor and eliminating potential confounding interactions caused by the long-tail rat kinesin and quantum dots. We expressed three constructs of the dimeric Drosophila kinesin DmKin401; a wild-type construct, a rigor mutant (T99N) and a mutant with impaired ATP hydrolysis (E164A). Additionally, each construct was fused with fluorescent protein. Studying the motility of DmKin401 in the presence of the different roadblocks, we find that the landing rate and the average run-length decrease substantially and occasional pauses become more frequent, resulting in a moderately lower average velocity.

2334-Pos Intramolecular Strain Governs Kinesin Stepping Behavior Along Microtubules

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Board B449

Kinesin 1 advances 8 nm along a microtubule per ATP hydrolyzed with the coordinated hand-over-hand movement of its two motor domains. The neck linker region has been proposed to function as a mechanical element that drives this movement. However, the structural principles that enable such precise and highly processive stepping tightly coupled with ATP hydrolysis remain uncertain. By inserting artificial peptides to the neck linker, we have created “extended” kinesin motors that can now take large (16 nm) and multi-directional (forward, sideway, and occasionally backwards) steps. However, the velocity of these extended motors is decreased, most likely due to decreased tension between the motors domains which results in futile ATP hydrolysis cycles. Consistent with this idea, the velocity of extended motors, but not wildtype, increases with an assistive force. Our results show that neck linker’s length and stiffness dictates the size and direction of the step and contributes to communication between kinesin motor domains during processive motion.

2335-Pos The Mechanism By Which Casein Enhances The Activity Of Surface-Immobilized Kinesin Motor Proteins

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Board B450

For functional assays of motor proteins, it is important to be able to tightly bind motors to surfaces while retaining their functional activity. In the standard microtubule gliding assay, kinesin motor proteins are adsorbed to glass surfaces. It has been empirically determined that pre-treating the surfaces with the milk protein casein and including casein in both the motor and microtubule solutions maximizes motor activity, but the precise function of casein is not clear, and substituting casein with BSA leads to a 50-fold decrease in motor activity. To understand the mechanism by which casein enhances kinesin activity, we used quartz crystal microbalance (QCM) measurements and kinesin functional assays to study the interaction of casein and kinesin with glass surfaces.

From dynamic light scattering, casein had a hydrodynamic radius centered around 16 nm, consistent with aggregates of ~70 casein subunits with average subunit MW 24kD. QCM experiments of casein binding to SiO2 surfaces are consistent with a tightly bound layer whose mass corresponds to a ~3 nm protein monolayer and a weakly bound casein layer with a Kd of 500 nM. In the absence of soluble casein, tail-less kinesin bound tightly to casein-treated surfaces, but soluble casein blocked the kinesin heads from binding. In contrast, headless kinesin bound to surfaces only in the presence of soluble casein. Finally, in a gliding assay removing soluble casein from the microtubule solution led to a reversible decrease in the motor activity. These data suggest a model in which a reversibly-bound casein layer prevents kinesin heads from interacting with the surface or with the tightly bound casein monolayer. Understanding the role of casein in gliding assays is a prerequisite for engineering novel surfaces for immobilizing and patterning functional motor proteins.

2336-Pos Direct Observation of Individual Head Motions in Kinesin Dimers

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Board B451

Optical-trapping assays for single kinesin motors typically involve attaching a small bead to the common stalk of the molecule. Forces applied to this bead tend to load both kinesin heads simultaneously, and displacements of the bead tend to report the average position of the entire molecule. As a consequence, the separate motions executed by the two heads during the stepping cycle remain uncertain. Here, we report a novel single-molecule assay for loading and tracking an individual head of Kinesin-1, by attaching an optically-trapped bead to the head domain via a short DNA tether. Under force-clamped conditions, we observed distinct steps of ~16 nm, exactly as anticipated for heads moving in a hand-over-hand walk. Interestingly, over a specific range of loads, the variance of the position signal during a step dwell interval often increased abruptly prior to the subsequent step, suggesting that the head binds to the microtubule more weakly immediately before executing a 16-nm forward motion. In addition, under moderate assisting loads, we observed a large jump (>16-nm) in motion at the start of step dwells as the applied load pulled the rear head forward, beyond its partner head and past the next microtubule binding site, before returning to the site to bind tightly. Analysis of this 'overstep' feature as a function of ATP concentration revealed that ATP binding to the new rear head, which is known to trigger neck-linker docking, was responsible for pulling the overextended head back to its normal position, yielding a net advance of 16 nm. Our results imply that both kinesin heads remain in contact with the microtubule during the majority of the catalytic cycle, and suggest that internal strain, communicated when both heads bind the microtubule, gates the cycle to ensure kinesin's remarkable processivity.

2337-Pos Single Molecule FRET Observations of Nucleotide-dependent Configuration Changes of Kinesin Dimer

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Board B452

Kinesin, a dimeric motor protein with two motor domains (heads), transports various cellular cargoes by moving processively along microtubules. Recent studies demonstrated that kinesin walks in a hand-over-hand manner by alternatively moving two motor heads. However, it is still not clear how ATP hydrolysis cycle is coupled to these structural changes, and especially it remained controversial whether kinesin takes one- or two-head-bound intermediate while it waits for binding of new ATP. To distinguish these two possibilities, we measured the distance between two kinesin heads using single molecule fluorescence resonance energy transfer (FRET) technique. Two reactive cysteines were introduced into the front of one head and the rear of another head in a heterodimeric kinesin, and were labelled with Cy3/Cy5 dyes. In the presence of AMP-PNP, FRET efficiency showed bimodal distribution with peaks at 10% and 90%, which is consistent with two-head-bound state. In the absence of ATP (with small amount of ADP) and also for mutant heterodimer that can bind to microtubule only with one head (one-head-bound state), FRET efficiency distribution showed distinct peak around 30%. Interestingly, FRET efficiency for the mutant heterodimer changed dramatically upon addition of AMP-PNP, which can be interpreted that the tethered head is displaced from rearward to forward position upon ATP-binding to the bound head. Then we observed FRET efficiency changes for kinesin moving processively under low ATP concentrations, where ATP binding is the rate limiting, and found that kinesin spent most of the time in 30% FRET state and briefly in 10 and 90% FRET states. These results support the idea that kinesin waits ATP-binding in a one-head-bound state and also explains how ATP-hydrolysis cycle is coupled to the configuration changes of two kinesin heads.

2338-Pos Photo-induced Generation Of The Atpase Activity Of Caged

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Board B453

Photo-responsive caged compounds have high potency in the application concerning functional biological molecules as photo-switching device. Kinesin is a motor protein that moves along microtubule by the energy generated from ATP hydrolysis. Recent crystallographic studies showed that kinesin has several unique functional loops in its motor domain. It has been suggested that L11 changes its length in a nucleotide dependent manner during ATP hydrolysis. In the present study, the photo-regulation of the catalytic activity of kinesin was investigated by treating with 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBB), an electrophilic caging reagent. Among the mouse brain kinesin mutants which have a single cysteine at A244 and A252 in L11, around 70–80% activity inhibition have been observed after reacted with 5 times concentration of DMNBB for 16 hours; The mutant A252C recovered more than 80% of its activity by irradiating at 366nm. The velocity of microtubule motility of caged A252C dropped from 0.52 ± 0.08 μm/s to 0.15 ± 0.05 μm/s and recovered to 0.33 ± 0.12 μm/s with UV light for 30 seconds. Present study successfully caged the mutant kinesin and altered its activity. Wild type of Kinesin Unc 116 motor domain derived from *C.elegans* which has a single reactive cysteine residue showed also significant inhibition of ATPase activity by modification with DMNBB and photo-induced generation of the activity.

2339-Pos Torsional properties of kinesin

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Board B454

Conventional kinesin is a molecular motor composed of two identical polypeptide chains that dimerize through a common stalk, whose peptide sequence suggests that it is primarily composed of an alpha-helical coiled coil with occasional interruptions. Although the

crystal structure of the head domain has been solved, the form taken by the stalk and head-linking regions remains largely conjectural. Models for kinesin structure, regulation, and molecular mechanics often incorporate additional flexible domains (termed “hinges” and “swivels”), based on sequence analysis and cryoEM data. Using a single-molecule assay responsive to the rotational motions of the stalk, we studied the torsional properties of kinesin as a function of stalk length and composition. We manipulated the composition of the stalk by (1) progressively truncating the stalk sequence and (2) mutating regions predicted to form gaps in the coiled-coil structure into a continuous coiled-coil form. In our assay, we simultaneously measured, by video tracking, the position and torsional angle of fluorescently-tagged beads attached to the C-terminus of microtubule-bound kinesin constructs. We found that putative coiled-coil domains have torsional stiffnesses as much as 50 times greater than non-coiled-coil segments. Using non-hydrolyzable nucleotide analogs and kinesin/microtubule amide cross-linking, we observed both unbounded and bounded diffusional rotations in our assay, which likely reflect one and two-heads-bound states of the dimer, respectively. The unbounded diffusion of the one-head-bound state suggests that each head of kinesin can swivel with respect to the stalk. Observations of kinesin motion at low nucleotide concentrations suggest that motors occasionally undergo random, ±180° rotations, with a probability of ~2 percent for every 8-nm step taken. This result is consistent with current models of kinesin walking by an asymmetric hand-over-hand mechanism, but punctuated by rare events where the kinesin molecule may rotate as it takes a step.

2340-Pos Interaction of AMP-PNP with Kinesin

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In order to better define the interactions between AMP-PNP, kinesin and microtubules (MTs), binding rates were determined for fluorescent mant-AMP-PNP and a monomeric human kinesin motor domain (H359). Mant-AMP-PNP binds to nucleotide-free kinesin at approximately 2.5 $\mu M^{-1}s^{-1}$ and dissociates at 0.4 s^{-1} yielding a calculated K_d of 0.16 μM. Unmodified AMP-PNP is similar with a dissociation rate of 0.5 s^{-1}. Mant-AMP-PNP binds to the kinesin-MT complex at approximately 0.06 $\mu M^{-1}s^{-1}$ and dissociates at 0.14 s^{-1} yielding a calculated K_d of 2.3 μM. Thus MTs actually inhibit the dissociation of AMP-PNP, in marked contrast to the large stimulation by MTs of the dissociation of ADP. Additionally, binding of kinesin to a MT reduces the affinity for AMP-PNP by 15-fold. This weak affinity of AMP-PNP for the kinesin-MT complex is due to a very low rate of binding by AMP-PNP (0.06 $\mu M^{-1}s^{-1}$).

The binding of AMP-PNP to free kinesin or a kinesin-MT complex forms a closed thermodynamic cycle with binding of the free and AMP-PNP forms of kinesin to MTs. The observed 15 fold weaker affinity of AMP-PNP for kinesin when bound to microtubules requires that nucleotide-free kinesin must bind 15 fold more strongly to MTs than does the AMP-PNP complex of kinesin. Thus binding of AMP-PNP significantly weakens the affinity of a kinesin motor domain for MTs relative to the nucleotide free-state, although even this weakened binding is likely to still be tight enough to be classified as a tight binding state. This result with AMP-PNP does not necessarily mean that the complex of kinesin with ATP itself has weaker affinity of a MT than does the nucleotide free form. The rapid hydrolysis of ATP, however, will require the use of other approaches to determine its affinity.

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2341-Pos Measuring the distance of cargo from the microtubule for Kinesin

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Board B456

Kinesin follows the microtubule protofilament axis.[1] A microtubule can have different numbers of protofilaments (ranging from 10 to 19) in vitro depending on the buffer condition during polymerization. The protofilaments run parallel to the microtubule axis for a 13-protofilament; whereas they take a right-handed supertwist with a pitch of 3.4 micrometers for a 12-protofilament microtubule and a left-handed supertwist with a pitch of 6.8 micrometers for a 14-protofilament microtubule. We observed the motility of single Kinesin molecules on non-13 protofilament microtubules. Using the FIONA technique we were able to localize Kinesin molecules with 1 nm accuracy. We have also developed a similar technique to localize the microtubules with 3 nm accuracy. We measured the distance of the cargo (QDot 655) at the tail domain to the microtubule to be roughly 50 nm for a Kinesin construct with 560 residues. By tracking the distance of the tail domain of the Kinesin molecules from the microtubule, we confirmed the helical motion that a single Kinesin molecule undergoes on a non-13 protofilament microtubule.

References

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2342-Pos Regulation of the Kinesin-1 Holoenzyme by the Tail and Kinesin Light Chains

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Board B457

In cells, kinesin-1 is regulated to prevent futile ATP hydrolysis. Inhibition of motor function occurs via a folding-over of the molecule, such that the kinesin heavy chain (KHC) tail domain can interact with and inhibit the microtubule (MT)-stimulated ATPase activity of the head. The kinesin light chains (KLCs) have also been shown to inhibit MT binding in the context of the heterotetrameric complex. Here, we have used complexed full-

length KHCs and KLCs expressed in vitro to investigate the contributions of the tail and the KLCs to regulation of MT binding. One hypothesis was that the negatively-charged heptad repeat domain of the KLCs may inhibit kinesin-1 MT binding by a charge clash with the acidic C-terminal tail of tubulin. To test this, we compared the MT binding activity of kinesin-1 in the presence of wild-type and subtilisin-treated (to remove the acidic tail) tubulin. We observed that MT binding was reduced in the presence of subtilisin-treated tubulin, indicating that an acidic charge clash was not the method of inhibition by the KLCs. To investigate the effects of tail-mediated inhibition, we made a K944A mutation in the conserved QIAK region of the tail domain and compared this mutant to wild-type kinesin-1 in MT binding assays. The mutant demonstrated significantly greater MT binding than wild-type, indicating that the point mutation was sufficient to suppress the inhibitory effect of the tail. Interestingly, a triple mutant (E184K/D185K/E188K) made in a region of the head (helix $\alpha3$) which is thought to interact with the QIAK tail region did not have an effect on regulation. Work is in progress to generate head mutants at other potential head-tail interaction sites and to test these constructs in MT binding experiments.

2343-Pos A First Look at the Structure of the Cargo-binding TPR Domain of Kinesin Light Chain 2 from Human

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Board B458

Kinesin-1 motor protein transports cargoes such as mitochondria and vesicles that contain APP, APOER2 or RNA. Kinesin-1 consists of two heavy chains (KHCs) and two light chains (KLCs). Each KHC contains three domains, the N-terminal motor domain, the neck/stalk domain and the tail domain, whereas each KLC has two domains, the N-terminal coiled-coil domain and the C-terminal TPR-containing domain. The tale domain of KHC and the TRP domain of KLC are involved in cargo binding. Much of structural works have been focused on the motor/neck domains of KHC. However, almost nothing is known about the structures of the cargo binding domains. Here we report the crystal structure of the TPR domain of the human KLC2. The structure shows that helical hairpins are folded into a double-layered, right-handed superhelix. We propose the inner layer as the bind site for the regions of cargo proteins. The inner layer is lined with positively charged residues suitable for binding the cargo peptide regions with negative charges.

2344-Pos Analysis of Conformational Change of Novel Rice Kinesin K16 Using Small Angle X-ray Solution Scattering

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We have previously succeeded to express a novel rice kinesin K16 motor domain in E. coli. Biochemical studies revealed that the kinesin has several unique enzymatic characteristics comparing with conventional kinesin.The most interesting property is that the ADP-free K16 motor domain is very stable, contrast to conventional kinesin that is very labile in ADP-free state. Recently, we have successfully dissolved the crystal structure of ADP bound K16 motor domain. The overall structure of the K16MD is similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequence (43.2 %). However, neck-linker region showed an ordered conformation in a position quite different from that observed in conventional kinesin previously. In this study, we designed the K16 motor domain chimera protein fused with GFP at the neck-linker in order to monitor the conformational change of the neck-linker during ATP hydrolysis by small angle X-ray solution scattering and FRET. The K16-GFP was successfully expressed by E. coli.. The K16-GFP chimera protein showed almost similar enzymatic properties to intact K16.We determined the Radius gyration (Rg) values of K16-GFP in the presence or absence of nucleotides by X-ray solution scattering. The Rg of nucleotide-free K16-GFP was about 42Å. In the presence of ADP and ATP, the Rg values were 38Åand 39Å, respectively. And AMP-PNP bound K16-GFP showed almost similar Rg value to that in the presence of ADP. These results may suggest that the neck-linker of nucleotide free K16 is in the undocked conformation, on the other hand, the neck-linker of ADP bound state is in the novel conformation as observed in the crystal structure.

2345-Pos Investigating the Force Generated by Multiple Kinesin Motors in vitro with a Novel Magnetic Technique

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Board B460

Is it possible for multiple motors to act on a single cargo so that the velocity increases as the number of motors increases? Although force generated by single kinesin motors in vitro has been extensively studied, the effect of multiple kinesin motors under load has been the subject of debate. Work in vivo suggests that multiple motors increase the force acting on vesicles and their respective velocities increase at higher motor densities. Employing a biotin-streptavidin linkage we have bound superparamagnetic beads to microtubules. Using an electromagnet in an upside-down motility assay, we apply a known opposing force onto a superparamagnetic bead. This force is uniform across a large region of interest and can be in excess of 10pN. This opposing force is sufficient to stall some microtubules in a motility assay with full length Drosophila kinesin-1. We determine the changes in velocities for varying number of motors pulling on a microtubule bound to a single bead.

2346-Pos Simulation Study on Structural fluctuations of molecular motor KIF1A in different nucleotide states

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Board B461

KIF1A (Kinesin-3) is a single-headed molecular motor that moves processively and unidirectionally along a microtubule using the chemical energy produced by ATP hydrolysis. KIF1A has the "strong" and "weak" binding modes for the microtubule (Nitta et al., Nature, 2004):

(i) In the strong binding mode, the equilibrium dissociation constants for microtubule is small (the affinity to microtubule of KIF1A is high). Therefore, KIF1A hardly detaches from microtubule. This binding mode is realized in AMP-PCP, AMP-PNP, and ADP-AlFx nucleotide states.
(ii) In the weak binding mode, the equilibrium dissociation constants for microtubule is high (the affinity to microtubule of KIF1A is low). Therefore, KIF1A easily detaches from microtubule. This binding mode is realized in ADP-Vi and ADP nucleotide states.

The mechanism of switching between these two modes is still unknown. We investigated which part of KIF1A plays an important role in switching. For this purpose, we calculated the structural fluctuations of KIF1A in all nucleotide states by means of the molecular dynamics simulation using Go-like model. It is found that the structural fluctuations of α4 helix correlates strongly to the binding strength to microtubule. Therefore, we suggest that α4 helix plays a crucial role in switching the binding strength that depends on the nucleotide state.

2347-Pos Probing The Dissociation Of Single Molecules Of Eg5 From Microtubules By Optical Trapping

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Eg5 is a member of the kinesin family of motor proteins. In cells, Eg5 tetramers are essential for the assembly and maintenance of the bipolar mitotic spindle. *In vitro*, Eg5 dimers formed by the recombinant human construct, Eg5-513-5His, step processively along microtubules, with an average run length of ~8 steps. This run length is relatively short compared to that of conventional kinesin (>100 steps), raising questions about what governs the extent of processivity in the kinesin superfamily. To begin to understand the molecular origins of Eg5 processivity, we directly visualized the individual steps of single dimers of Eg5-513-5His as these move along a microtubule substrate. We compared the kinetics of the final step prior to dissociation with all previous steps in a single run, as a function of both the nucleotide concentration and the applied force. We find that dissociation occurs after ATP binding and the force-dependent translocation step, even at near-maximal loads. This result suggests that load-induced unbinding is not the dominant pathway to microtubule release, and that dissociation occurs at some later step in the biochemical cycle, for example with ADP or phosphate release.

2348-Pos Monastrol Inhibition of an Eg5-head/DmKHC-Stalk Chimera

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Proper function and control of the mitotic spindle relies on the regulated activity of microtubule-based motors, as dyneins and kinesins. Particularly, Kinesin-5 has received considerable attention as immuno-depletion or specific inhibition of the motor by Monastrol results in spindle collapse. Kinesin-5 is a slow, plus-end directed motor, consisting of four identical heavy chains, which form an 80nm-long, four-stranded anti-parallel coiled-coil, with two motor-domains at each end of the bipolar tetramer. Confirming a long standing hypothesis, Kinesin-5 motors were shown to drive relative sliding of the microtubules which it cross-links. Single tetramers interacted for several seconds with a single microtubule, displaying directed and diffusive components, while Monastrol increased the observeable diffusive component.

Recent reports of processive stepping of tetramers and truncated dimeric constructs, and the finding that cross-linking triggers a dramatic increase in the directional component of individual Kinesin-5 tetramers, indicate that each dimeric end of the biolar tetramer is capable of mildly processive stepping.

To test whether the motor domain of XlEg5 is in deed capable of processive motility given a well-characterized, stably dimeric background, we exchanged the DmKHC motor and neck-linker with that of XlEg5. The chimeric motor produces similar speeds and force-characteristics as the truncated dimeric HsEg5-513, but displays a 40x increase in processivity. The increased run length allows for readily testing Monastrol-inhibition of processivity. Increasing the Monastrol-concentrations, we neither observed a reduction in speed nor in association-rate, but a dose-dependent and cooperative decrease in the run-length of the motor (IC50=6,4 µM, Hill coefficient = 1.95). In high-density surface gliding assays of both tetrameric motors and chimeric constructs, we find an IC50=6.2 µM for tetramer-gliding, and a ~40-fold increased IC50 for chimeric motors. These results strongly indicate that Monastrol exerts its inhibitry effect by terminating processive episodes in the wt-tetrameric Kinesin-5s.

2349-Pos A SINGLE-HEADED MODE OF EG5

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Teams of tetrameric kinesin-5 motors are essential to drive sliding apart of the half-spindles in mitosis and meiosis. Although truncated kinesin-5 dimers were recently shown to be able to walk an average of 8 steps along microtubules, it is unclear to what extent a walking mechanism applies in large teams of Eg5 molecules, as are found in the spindle.

To address this question, we engineered single-headed Eg5 heterodimers, in which one heavy chain contains an Eg5 motor domain and its neck linker followed by a truncated tail of kinesin-1, and the other chain consists of only the tail. If Eg5 dimers move in 8-nm steps by an alternate-heads walking mechanism, then deletion of one head of each head-pair should abrogate molecular walking and substantially reduce the stepsize, along with the microtubule sliding velocity. Contrary to this expectation, we find that lawns of single-headed Eg5 dimers drive microtubule sliding at very similar velocities to wild type 2-headed dimers, indicating that Eg5 molecules in teams can slide microtubules by using a hopping mechanism. In order to estimate the proportion of walking events to hopping events in a lawn of wild type Eg5 head-pairs, we compared the microtubule activated ATPase rates of one-headed and 2-headed constructs. The maximum per-molecule ATPase activity of single-headed Eg5 was almost the same as that of 2-headed molecules, indicating a mechanism in which microtubule-activation of one head tends to inhibit that of the other head. Taken together with our finding that single-headed Eg5 moves at similar velocity to double-headed wild type, these data argue for a “microtubule muscle” mechanism, in which members of a team of Eg5 dimers move by hopping, taking steps that are typically shorter, and possibly more forceful, than the 8-nm steps required by a deterministic alternate-heads walking mechanism.

2350-Pos Single molecule studies of the mitotic kinesin Eg5

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Board B465

In eukaryotic cells, formation of a bipolar mitotic spindle is essential for stable propagation of the genome. In vertebrates, proper spindle formation requires the activity of Eg5, a member of the kinesin-5 motor protein family; loss of Eg5 function leads to monopolar spindles and mitotic arrest. Eg5’s homotetrameric configuration, in which two pairs of motor domains are connected by a central stalk containing predicted coiled coil regions, allows it to crosslink and slide microtubules. Single molecule fluorescence studies of Eg5 have shown that its motion along single microtubules includes a directional component that requires ATP hydrolysis, as well as an ATP-independent diffusive component. Such modes of motion have been observed in a number of motor enzymes, but their structural and mechanistic bases are poorly understood. To probe the roles of Eg5’s non-motor domains and to establish structure-function relationships for these domains, we have generated a series of Eg5 constructs with deletions in the C-terminal region or with engineered modifications to the coiled-coil pattern within the protein’s stalk region. A dimeric, GFP-labeled version of Eg5 was generated and exhibits a velocity of 40 nm/s in a multiple motor motility assay. The motility of this construct has also been investigated using single molecule fluorescence microscopy, which allows observation of individual motors moving along microtubules. Under low salt conditions, the dimeric construct exhibited processive motion with a significant diffusive component, suggesting that the deleted domains may be required for modulating Eg5 activity. Observation of the additional constructs will yield further insight into the mechanisms of Eg5 motility and allosteric regulation.

2351-Pos Theory Of Microtubule Depolymerization By Kinesin Motor Proteins

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Kinesins from the kinesin-8 and -13 families promote microtubule depolymerization, a process thought to be important for the control of microtubule length in cell division. Although proteins from the kinesin-13 family appear to move without directional bias on microtubules (MTs), it has recently been shown that kinesin-8 family motors show biased motion. Here we describe a simple model for MT depolymerization by motors of the kinesin 8-family, including both directional motion and MT depolymerization. We calculate the dependence of the depolymerization velocity on the MT length, motor concentration, motor processivity, and motor depolymerization parameters. We compare results from analytic calculations and numerical simulations to published experimental data.

2352-Pos Structural Features of the Microtubule Important for MCAK Activity

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MCAK is a Kinesin-13 that depolymerizes microtubules (MTs) and regulates MT dynamics. MCAK binds specifically to the end of MT where it induces or stabilizes a curvature to depolymerize MTs into tubulin heterodimers. However, it is unclear what features of the MT are important for MCAK activity, and what MCAK releases from the MT. We used subtilisin-treated MTs (MTs lacking the C-termini of α- and β-tubulin) as well as alternative tubulin substrates to study

which structural and geometrical features of the MT are critical for MCAK activity. We found that removal of the C-termini significantly decreased the efficiency of MCAK-induced depolymerization. Removal of the C-termini slightly affected the ability of MCAK to bind to the lattice and ends of MTs, but did not alter MCAK localization to the ends of the MT. We also found that depolymerization of SMTs led to the release of curved tubulin oligomers rather than tubulin heterodimers. Our results indicate that the ability of MCAK to depolymerize SMTs is reduced, most likely due to a decrease in the efficiency of product formation. Using alternative tubulin substrates with varying C-terminal and geometric statuses to study the depolymerization cycle, we found that MCAK did not depolymerize tubulin rings regardless of the C-termini. However, MCAK could depolymerize parallel tubulin sheets lacking the C-termini, generating curved tubulin oligomers similar to the products resulting from depolymerization of SMTs. We propose that localization of MCAK to the ends of MTs is independent of the C-termini, and that MCAK stabilizes a curved conformation at the end of the MT. MCAK and curved tubulin oligomers are released from the end of the MT, and efficient release of this complex is dependent on the presence of the C-termini of tubulin.

2353-Pos The Effect Of Dimerisation On The Depolymerising Action Of MCAK

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The kinesin-13 family differs from the majority of kinesins in that, instead of the walking action normally associated with motor proteins, they act to depolymerise microtubules in an ATP-dependent manner. Rather than binding and moving along the microtubule lattice, kinesin-13 proteins associate with the microtubule ends and destabilise its structure causing it to depolymerise, possibly by bending the terminal tubulin subunits out of the plane of the microtubule wall.

MCAK (Mitotic Centromere-Associated Kinesin or kif2c) is a member of the kinesin-13 family and is an efficient depolymeriser of microtubules in vitro (Helenius, *et al*. 2006, *Nature* ***441***:115–119). It is a dimer that weakly associates with and diffuses along the microtubule lattice over short distances (~0.8 μm). This 1-dimensional diffusive search enhances the probability of MCAK finding the end of a microtubule because the microtubule end acts as an "antenna". Over short distances, the diffusive search mechanism is actually faster than ATP driven kinesin walking.

We have studied a monomeric construct, mMCAK, using total internal reflection fluorescence microscopy (TIRF). Using fluorescently-labelled microtubules we measured the depolymerisation rate of mMCAK as a function of protein and ATP concentrations. We found that the maximum depolymerisation rate, 2.7 $\mu m\ s^{-1}$, is significantly slower than that found for dimeric MCAK (4.0 $\mu m\ s^{-1}$, Helenius *et al.*) indicating that full activity requires the action of both heads. We also found that the apparent K_m, 106 nM, is about 10 times higher than for the dimeric molecule (4 nM). We are using two-colour TIRF experiments to visualise individual mMCAK molecules on the microtubule lattice to investigate whether so-called "antenna" distance is significantly shorter for mMCAK vs MCAK.

2354-Pos Fluorophore Quenching as a Sensor for Conformational Changes of Kinesin At the Single Molecule Level

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Kinesin-1 is a processive motor protein which moves along microtubules. We used single molecule fluorescence quenching to study the timing of the neck linker conformational change that occurs as kinesin walks along the microtubule. A dimeric, cysteine light kinesin construct (K560) was labeled at position 333, in the neck linker, with tetramethylrhodamine maleimide. In the microscope, we observed repetitive, alternating cycles of fluorescence and quenching, as individual kinesin motors walked along microtubules. This is entirely consistent with alternating formation and disruption of rhodamine dimers, as we demonstrated in a previous solution kinetic study (Rosenfeld, *et al*., J. Biol. Chem). By monitoring the position of the motor during these fluorescence cycles, we were able to conclude that one cycle of quenching/unquenching occurs with each 8 nm step, and that each step corresponds to an ATP-induced separation of neck linkers. These results, in conjunction with earlier work by several investigators, support a model in which the kinesin can dwell on the microtubule with its leading head strongly bound, with an empty catalytic site, and with its trailing weakly bound, with ADP in its catalytic site. The binding of ATP causes a conformational change on the leading motor domain which swings the trailing head forward and allows an 8 nm step to take place. Our experiment demonstrates the feasibility of using state-sensitive fluorescent probes to monitor conformational changes in real time at the single molecule level.

2355-Pos Tryptophan Analog Provides New Insights into Kinesin's Conformational Changes

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Conventional kinesin is a biomolecular motor that transports cargo along microtubule filaments. To perform its primary task, this dimeric protein must carry out three fundamental functions. First, each monomer must bind and hydrolyze ATP. Second, each mono-

mer must alter its affinity for microtubules; binding and releasing the filament as dictated by events in the ATP-binding site. Finally, each monomer must coordinate its microtubule- binding and release with the other so that the dimer can step processively along the filament. All of these fundamental tasks are achieved in the motor domain, where a series of small conformational changes in the active site is expected to initiate other conformational changes in the microtubule interaction site and the neck linker, allowing the choreographed stepping of the dimer along its microtubule track.

Though some of these conformational changes have been characterized, many of them remain elusive. One such conformational change is that of Loop 11 (L11). Located within the switch II cluster, adjacent to the active site, L11 is thought to undergo an important conformational change in the presence of microtubules that is critical to kinesin's interaction with the polymer. To date this conformational change has eluded detection. We have generated a L11 mutant (V238W) of human conventional kinesin that can be expressed with a tryptophan analog, 5-hydroxytryptophan, which can be selectively excited in the presence of tubulin's native tryptophan residues. Thus we can now monitor the conformational changes undergone by L11 as it carries out its important role in the interface between the kinesin motor domain and microtubules.

2356-Pos Studies on the Instability of the Ncd Coiled-coil Stalk Domain using FRET

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Ncd (kinesin-14) is a minus end-directed motor protein that is involved in spindle assembly during mitosis and meiosis. Ncd dimerizes through α-helical coiled-coil in the central stalk domain, and the coiled-coil formation has shown to be essential to produce efficient motility of microtubule by ncd motors (Endres et al. 2006). We recently studied the stability of the ncd coiled-coil stalk using CD spectroscopy and found that the coiled-coil is relatively unstable (melts reversibly around 25 °C) (Makino et al. 2007). To further investigate the role of the thermal instability of the stalk domain, we monitored the coiled-coil formation and unfolding using FRET. Structural states of the coiled-coil were detected by observing FRET between donor and acceptor fluorescent proteins (CFP and YFP) fused at the N-terminus of stalk domain of each polypeptide. When the temperature was increased from 25 to 37 °C, a sharp decline in the FRET efficiency was observed at various nucleotide conditions in the presence of microtubule, presumably indicating the thermal unfolding of the stalk coiled-coil. The transition temperature slightly differed depending on the nucleotide condition, and was higher in the presence of AMP-PNP (30 °C) compared to the nucleotide-free state (27.5 °C). Such nucleotide-dependent stability changes may be important to efficiently transmit power strokes by multiple ncd motors to a microtubule.

2357-Pos Single-Molecule Behavior of Minus End-Directed Motor Ncd

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Drosophila nonclaret disjunctional (Ncd) is a kinesin-14A family member that is involved in spindle assembly and maintenance during both meiosis and mitosis. Despite the high homology in the head domains, kinesin-1 and Ncd have different motility; kinesin-1 is processive, whereas Ncd is nonprocessive. It has been shown that truncated Ncd constructs exhibited no directional movement on microtubules, but it is not known how individual Ncd dimers containing the N-terminal region behave on microtubules. Here we characterize the motile properties of various lengths of truncated Ncd fragments fused with GFP in single-molecule fluorescence assays. We found that a near full-length construct containing the tail domain moved processively along microtubules.

Furthermore, a construct lacking the tail domain but containing the whole stalk domain also moved processively, while shorter constructs showed only a small or no bias toward microtubule minus end. Notably, the processive movement included a diffusive element, and was sensitive to the ionic strength, suggesting that it is retained by electrostatic interactions. Mutational analysis and motility assay using subtilisin-digested microtubules supported this idea. We next examined whether diffusive movement of Ncd requires ATP hydrolysis. In the presence of saturating ADP, an Ncd dimer construct which only contains the head and neck domains showed diffusive movement along microtubules without obvious directionality. From these observations, we speculate that the Ncd head could diffuse along microtubules in the weak binding state, and the Ncd tail and/or the charged part of the coiled-coil stalk would ensure the processive movement by serving as a tether to microtubules.

2358-Pos *Drosophila* Nod Has A Kinesin-Like Structure Yet A Myosin-Like ATPase Cycle

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Nod, a nonmotile Kinesin-10, localizes along chromosome arms and functions in the segregation of achiasmate chromosomes during meiosis. We performed a thermodynamic and kinetic analysis of the Nod motor domain and determined its 1.9 Å resolution crystal structure in the presence of MgADP. The structure reveals an overall fold that is most similar to Kinesin-1, however the microtubule binding region displays a unique conformation compared to all kinesin structures to date. These differences include an extension of α6, a new orientation of the relay helix (α4), and loss of the β5- L8 lobe. We performed cosedimentation experiments to measure the

affinity of Nod for the microtubule lattice under various nucleotide conditions. Nod binds very tightly to microtubules in the nucleotide-free state (0.07 μM), yet other nucleotide states (ATP, AMPPNP, and ADP) are significantly weakened (7–10 μM). We used presteady-state kinetics to determine the rate constants of key steps in the Nod ATPase pathway. We observe Nod interaction with the microtubule lattice occurring in two steps:

1. formation of the Mt-Nod-ADP collision complex, and
2. isomerization of the collision complex to form the tightly-bound Mt-Nod intermediate.

The rate of this microtubule-dependent isomerization correlates with the observed rate of ADP product release. In addition, ATP and ADP binding both occur via a two step process with a very rapid conformational change (>1000 sec^{-1}) to tighten nucleotide binding. Upon tight ATP binding, Nod actively detaches from the microtubule, analogous to the ATPase mechanism of myosin. Therefore, the overall Nod ATPase mechanism is fine-tuned to prevent rigor linkage of chromosome arms to the microtubule lattice in the spindle.

2359-Pos Cargo Transport and Force Generation by Single CENP-E Dimers

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Microtubule binding molecular motors, kinesins and dyneins, have specific functions in important biological processes including cell division. Centromeric protein E (CENP-E), a kinesin-7 family member, is an essential component of the kinetochore complex and is necessary for the proper attachment and positioning of chromosomes to the mitotic spindle during mitosis. Using total internal reflection microscopy to track quantum dot labeled proteins we show here that individual dimers of a Xenopus CENP-E construct can move processively, in discrete 8-nm steps, along microtubules by ATP hydrolysis. Measured velocities of motors at varying ATP concentrations follow Michaelis-Menten kinetics. Microtubule gliding assays with polarity marked microtubules indicate that CENP-E is a plus end-directed motor. In addition, we used optical trap measurements to demonstrate that single CENP-E dimers can produce force. Our results show that CENP-E dynamics is very similar to that of conventional kinesin and thus provide a mechanism for microtubule plus end-directed movement of chromosomes towards the midzone during mitosis.

2360-Pos Force-Velocity Curves of Motor Proteins Cooperating In Vivo

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The velocities of molecular motors as a function of opposing loads (i.e. their force-velocity curves) have been previously determined *in vitro* for single motors. Here we report force-velocity curves for single and multiple motors measured *in vivo*. These curves were obtained from four parameters–the intracellular viscosity, the size of cargo transported by the molecular motors, the cargo velocity, and the normalized cargo velocity distribution. These parameters were extracted from motion enhanced differential interference contrast (MEDIC) movies of live NT2 (neuron-committed teratocarcinoma) cells at 37°C and processed in the following five steps. First, the mean squared displacements and radii of intracellular particles undergoing Brownian motion were measured and used in a generalized Stokes-Einstein equation to determine the intracellular viscosity (η = 1.35 Pa s). Second, the radius of each directionally transported cargo was determined. Third, the position of each cargo was tracked at 8.3 frame/s and constant velocity segments of these trajectories were fit with straight lines. Fourth, the constant velocity segments, normalized by trajectory, were combined, and their histogram displayed 5 evenly spaced peaks, which were used to indicate the numbers of actively transporting motors. Finally, these four parameters–viscosity (η), radius (r), velocity (v), and number of active motors (N)–were combined in Stokes' equation, $F_N(v) = 6\pi r \eta v$, where $F_N(v)$ is the calculated force of N motors at velocity, v. The resulting *in vivo* force-velocity curve for one actively transporting motor lies between two previously reported *in vitro* kinesin force velocity curves. However, the curves for 2 and 3 actively transporting motors are significantly higher in both force and velocity, suggesting that each motor in a group contributes to the total pulling force and that motors can work cooperatively to attain higher transport velocities.

2361-Pos Dynein Arm Arrangement In Sea-urchin Sperm Flagellar Axonemes Revealed By Small-angle X-ray Diffraction Analysis

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Eukaryote cilia and flagella have in common the specific configuration of microtubule bundle, the 9+2 structure of axonemes. To know the dynamic features of axonemal structure and its regulatory mechanism of bending motion, X-ray diffraction analysis is one of the most powerful tools. In the present study, we developed a new method to make axonemes align in a buffer medium within an angular deviation of <5 degrees and used for X-ray fiber diffraction analysis. Using sea-urchin sperm axonemes, small-angle X-ray diffractions were observed at the BL45XU beam line of SPring8 (wavelength, 0.09 or 0.15 nm, camera length, 2–3 m). We observed clear layer line signals representing the longitudinal regularity (meridional diffractions) of axonemal structures. Equatorial signals reflecting structural regularity in the radial direction were also

observed. From the KCl-extraction experiments, we concluded that 24 nm layer-line signals were from outer dynein arms (ODAs). From the detailed analysis of 24 nm signals, we found that ODAs in a rigor state were arranged in parallel planes perpendicular to the axonemal axis, but in a relaxed state they were converted into another stable arrangement, *i.e.,* arranged along helical lines around axonemes (3.3±1.8-start helices) or in parallel planes oblique (19±2.7 degrees) to the axonemal axis. It implies that there are two stable specific dispositions of peripheral doublet microtubules (MTs) that are converted into one another depending on the cross-bridging states between ODAs and MTs. In the case of oblique or helical arrangements of ODAs, interactions between the central pair MTs and spoke heads would be dominant. We also determined the diameter of ODA center positions in the axonemes to be 135 nm that showed no obvious changes between in rigor and relaxed states.

2362-Pos Cross Linking Of Kinesin Using Bifunctional Photochromic Compounds Results In Photo-regulation Of Atpase Activity

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We have previously demonstrated the possible application of an azobenzene derivative in regulating a conformational change in skeletal muscle myosin. The bifunctional SH reactive azobenzene derivative, 4,4 -azobenzene-dimaleimide (ABDM), was incorporated into the SH1-SH2 region of skeletal muscle myosin subfragment-1 (S1), which is a potential energy-transducing site. Myosin S1 was modified with ABDM, and the global conformational change of S1 induced by the cis-trans isomerization of the cross-linked ABDM in response to UV/VIS light was investigated. It was shown that the cis-trans isomerization of ABDM promotes a swing in the lever arm of S1 in a direction opposite to that induced by ATP binding. In the present study, we have cross-linked kinesin using 4,4-azobenzene-dimaleimide (ABDM) in order to photo-control the ATPase activity. The mutants of kinesin motor domain A247C, T242C, A244C, L249C, A252C, G272C, and S275C, which have a single cysteine residue in L11 or L12, have been prepared. The mutants were cross-linked intermolecularly by ABDM at high efficiency 70–90%.The microtubules dependent ATPase activity of the cross-linked kinesin mutants A247C, T242C, A244C, L249C, G272C, S275C did not change on alternate UV-VIS light irradiation. On the other hand, A252C cross-linked by ABDM showed significant alteration of ATPase activity between UV and VIS light irradiation.

2363-Pos Impact Of C-terminal Truncation On Cytoplasmic Dynein

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Cytoplasmic dynein is a motor complex responsible for diverse cellular processes within eukaryotic cells, including mitosis, Golgi dynamics and the retrograde transport of various vesicles and organelles along the microtubule network. The motor unit of cytoplasmic dynein consists of three structurally distinct domains: a catalytic head, a cargo-carrying tail, and a microtubule-binding stalk. The head comprises six concatenated AAA+ (ATPases associated with diverse cellular activities) modules arranged in a ring-like structure, and a C-terminal sequence with unknown structural fold. While the ATP binding/hydrolysis mechanism of the multiple AAA+ modules has been well characterized, how the C-terminal sequence contributes to the head structure and the activity is poorly understood. In this study, we attempted to reveal the functional role of the C-terminal sequence, using a C-terminally truncated motor derived from Dictyostelium cytoplasmic dynein.

We show that the complete removal of the C-terminal sequence (406 residues (46kDa)) caused loss of all the motor activities (microtubule-dependent ATPase, ATP-sensitive microtubule binding, and microtubule sliding activities), despite maintaining the ring-like appearance as revealed by EM. On the contrary, partial removal of the C-terminal sequence (283 residues (30kDa)), the sequence missing in Saccharomyces Cerevisiae and other fungi, had little influence on the activity. We are now characterizing the motile activity of this construct at a single molecule level.

Microtubules & Microtubule-associated Proteins

2364-Pos The Structure of Kinesin-13 Rings and Its Functional Implications

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Board B479

Kinesin-13 proteins are key players during mitosis in that they effectively regulate microtubule dynamics by inducing microtubule depolymerization. This function of kinesin-13s is distinct from conventional kinesins, which are motile proteins that transport cargoes along the microtubule tracks. We are interested in understanding the structural basis for the unusual behavior of the kinesin-13s. In our previous studies, we have observed kinesin-13s form a novel structure of rings/spirals around microtubules in the presence of AMP-PNP, a non-hydrolysable analog of ATP. Three-dimensional structural analysis on negative-stain micrographs suggested the rings/spirals consist of a curved protofilament ring on the outside and two circumferences of kinesin-13 motors in the inside. One of these circumferences interacts with the curved protofilament and the other interacts with the microtubule surface. The structural model also predicts the existence of the motor-motor interaction to maintain the radial integrity of the ring complex. However, our recent data obtained by cryo-electron microscopy and helical reconstruction techniques suggests a different structural model of the ring complex.

The primary reconstruction suggests that only one kinesin-13 motor circumference exist inside the ring, which is responsible for interacting with both the microtubule lattice and the outermost curved protofilament. On the other hand, our live cell imaging