are good substrate for skeletal myosin and induce actin gliding in vitro motility assay. The synthesis of the two ATP analogues have been confirmed by FAB-MS. Excitation and emission maximums in the fluorescence spectrum of the ATP analogues were 474nm and 533nm for NBDTP, and 374nm and 430nm for MANTTP, respectively. NBDTP showed microtubules dependent ATP hydrolysis for conventional kinesin at the almost same level to that of regular ATP. The fluorescence changes of the ATP analogues during ATP hydrolysis have been also studied.

687-Pos Board B566

Photo-activation Of Atpase Activity Of Caged-kinesin

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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. The structure of conventional kinesin has been well studied and the key regions related to the function were clarfied. In the present study, the photo-regulation of the catalytic activity of mouse brain and C. elegans kinesins were investigated by treating with a caging reagent, 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBB). The mouse brain kinesin mutants that have a single reactive cysteine at 96C were prepared and modified by DMNBB in the presence and absence of ATP. For the kinesin modified in the absence of ADP, the ATPase activity was increased by 300% within 10 minutes. In the presence of ADP, the change of the ATPase activity was slower than that in the absence of ATP. Upon UV irradiation, the ATPase activity of the kinesin modified by DMNBB recovered to the level before modification. Wild type of C. elegans unc-116 kinesin motor domain derived from C. elegans, which has a single reactive cysteine residue. Modification with DMNBB and photo-irradiation on the wild type of C.elegans kinesin unc-116 showed also significant reduction and restoration of activity. We have identified the amino acid residue of kinesin unc-116, which affects activity by the modification with DMNBB as Cys16.

688-Pos Board B567

Interaction of Processive Motor Proteins with ATP analogue Having *Syn* Conformation with respect to the Adenine-ribose bond

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It is known that 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. However, the sliding velocity was approximately one-fifth of regular ATP. Moreover single molecular measurment using optical tweezers revealed that for kinesin, 8-Br-ATP induced nearly similar force generation with that of ATP. Myosin V is also processive motor protein like kinesin. Interaction of unconventional myosin V with 8-Br-ATP was also analyzed. Myosin V supported actin translocation using 8-Br-ATP. Currently, we are examining single molecular measurment of myosin V in the presence of 8-Br-ATP.

689-Pos Board B568

Photo-regulation of Kinesin ATPase Activity using Photochromic Molecule

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Azobenzene is a photochromic molecule that undergoes rapid and reversible isomerization between the cis- and trans-forms in response to ultraviolet (UV) and visible (VIS) light irradiation, respectively. Previously, we have introduced the sulfhydryl-reactive azobenzene derivative 4-phenylazophenyl maleimide (PAM) into the functional region of kinesin to reversibly regulate the ATPase activity of kinesin by photoirradiation. The five kinesin motor domain mutants, A247C, L249C, A252C, G272C and S275C, which contained a single reactive cysteine residue in loops L11 and L12 were prepared. The PAM-modified S275C and L249C mutants exhibited reversible alterations in ATPase activity accompanied by cis-trans isomerization upon UV and VIS light irradiation. In the present study, we prepared the six new mutants (A21C, G26C, S66C, R16C, R25C, M96C) that have single cysteine at near the ATP binding site in order to regulate kinesin activity more effectively than previous mutants. PAM was stoichiometrically incorporated into the cysteine residues in A21C, resulting in reduction of ATPase activity. However, PAM-modified A21C mutant did not exhibit reversible alterations in ATPase activity on UV-VIS light irradiation. PAM-modified G26C mutant also did no show the change in the ATPase activity upon UV-VIS light irradiation. On the other hand, PAM-modified S66C mutant exhibit reversible alterations in ATPase activity on UV-VIS light irradiation in a preliminary experiment.

690-Pos Board B569

Photo-control Of Atpase Activity Of The Kinesin Motor Domain Intermolecularly Cross-linked By Bifunctional Photochromic Compound

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Kinesin is an ATP driven dimeric motor protein carries cellular cargoes along microtubules. Azobenzene-dimaleimide (ABDM) is a bifunctional SH reactive photochromic compounds. We have previously demonstrated that ABDM was incorporated into the functional site of skeletal muscle myosin head (S1), and the global conformational change of S1 was induced by cis-trans isomerization of the cross-linked ABDM upon UV/VIS light irradiation, which may mimic the conformational change accompanied by energy transduction. We have also cross-linked kinesin using ABDM in order to photo-control the ATPase activity of kinesin. We have prepared the mutants of kinesin motor domain T242C, A244C, A247C, L249C, A252C, G272C, and S275C, which have a single cysteine residue in L11 or L12. Only A252C cross-linked by ABDM showed significant alteration of ATPase activity upon UV and VIS light irradiation. In the present study, we prepared novel mutants of kinesin motor domain Q21C, L25C, R26C, in L1, binding site, S66C, Q104C in ATP binding site, T196C in L9, K273C, K274C, Y276C in L12 to regulate ATPase activity efficiently. The mutants were cross-linked intermolecularly by ABDM at high efficiency 70–90%. These mutants cross-linked by ABDM showed alteration of ATPase activity between UV and VIS light irradiation. Especially, the kinesin mutant A252C was regulated most effectively. Additionally, We also prepared the mutant of dimmer kinesin A252C or T353C in order to photo-regulate motility of kinesin with ABDM.

691-Pos Board B570

Processive Motility of Heterodimeric Kinesin That Has Defect in the Neck Linker Docking

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Kinesin-1 is a motor protein that moves processively along microtubules in a hand-over-hand manner. The neck linker, a short stretch that connects two motor heads, has been shown to undergo ATP-dependent conformational changes, although its role on the processive motility is still controversial. To address this question, we employed a kinesin mutant at switch I (R203K) that can normally bind ATP but is unable to hydrolyse ATP (Klumpp et al. JBC 2003). First we observed the neck linker structural state of monomeric R203K bound to the microtubule using single molecule FRET. We found that the neck linker remained undocked conformation even in the presence of saturating ATP, suggesting that ATP-binding is not sufficient to stabilize neck linker docked state. Next we constructed heterodimeric kinesin that is composed of a wild-type head and a mutant R203K head. As recently been shown by Thoresen and Gelles (Biochemistry 2008), this heterodimer showed slow processive movement along microtubules. Then we observed the conformational changes of this heterodimer using a single molecule FRET sensor as previously developed to distinguish one-head-bound and two-head-bound states (Mori et al. Nature 2007), and found that the heterodimer showed hand-over-hand movement. Unexpectedly, they spent most of the time in the two-head-bound state where wild-type head is in the front and the mutant head is in the rear, indicating that the displacement of rear wild-type head to the forward binding site is not the rate-limiting. These results suggest that the neck linker docking in the microtubule-bound head is not essential for the tethered head to translate and bind to the forward tubulin-binding site and rather is required for promoting ATP-hydrolysis and subsequent detachment of the trailing head from microtubule.

692-Pos Board B571

Configuration Of The Kinesin1 Motor Domains In The ATP-waiting State As Revealed By Fluorescence Polarization Microscopy

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The molecular mechanism of coordination between kinesin1 heads during processive walking remains unclear, partly due to the lack of structural information on critical intermediates of the kinesin1 mechano-chemical cycle. To address this issue here we used ensemble and single molecule fluorescence

polarization microscopy to determine the mobility and orientation of the kinesin motor domains. We first investigated conditions mimicking a state when only one head is bound to the microtubule and the other one is tethered. For this we made heterodimeric constructs with impaired microtubule binding in one head. Our results indicate that the tethered head is very mobile. We then investigated the orientation of the head domains in homodimeric constructs moving processively at saturating or limiting [ATP]. At saturating [ATP] both motor domains are well oriented relative to the microtubule but at limiting [ATP] there is an increase in mobility. This result indicates that before ATP-binding one motor domain is mobile.

693-Pos Board B572

Cooperative Movement Of Wild-type Kinesin And Velocity-deficient Mutants

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In the classic sliding filament assay, the molecular motor kinesin exhibits processive movement on microtubules with a velocity that is invariant over a large range of motor concentrations. This indicates that kinesin motors move synchronously at high density, but studies examining the motility of 2–3 kinesin motors have shown a surprising lack of synchronization. These results together led us to believe that kinesin motors under high density conditions can pull one another off the microtubule track, accelerating dissociation. Using a computational model, we can demonstrate that this would enable synchronization of MT movement without complete motor to motor synchronization. To test this experimentally, we combined kinesin dimers containing a mutation in the neck-linker (termed VKN) that elicits a 3-fold reduction in velocity when compared to wild-type motor (5.4μm/min vs. 16.2 μm/min) with wild type motors in the sliding filament assay. No significant amount of microtubule buckling was observed for any mixture of wild-type and mutant motors; even at limiting dilutions, and speckled microtubules moved at the same velocities throughout their length, indicating that the motors behave cooperatively, coordinating their movement through a shared interaction with the microtubule. We plan to examine whether this cooperativity is positive (WT motors accelerating VKN mutant movement) or negative (VKN slowing down WT).

694-Pos Board B573

Kinesin Chimera Protein Fused with Calmodulin as a Molecular Shuttle

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Recently attention is focused on the application of molecular shuttles based on the motor protein kinesin and microtubule to drug delivery system (DDS) and lab-on-a-chip. In vivo, kinesin carries cargoes of biomolecular e.g., organelle which attach to the tail domain of kinesin. However, the molecular mechanism of the attaching and detaching of cargo is still obscure. Therefore, artificial binding systems have to be introduced on the molecular shuttle. Previously biotin-avidin and antigen-antibody reaction system have been used to attach kinesin to target cargoes. Although the systems are highly specific and tight, these are flawed as irreversible binding. In this study, we employed reversible cargo loading system using calmodulin (CaM) and M13 peptide for the molecular shuttle. We have designed kinesin K560 chimera protein fused with CaM at the C-terminal tail region of kinesin (K560-CaM). K560-CaM was successfully expressed by *E. coli*. expression system and purified. And M13 peptide fused with yellow fluorescent protein (M13-YFP) was also prepared as a target cargo. The ATPase activity and the microtubules gliding activity of K560-CaM were almost in the normal range of the kinesin wild type. The Ca^{2+} dependent reversible binding of K560-CaM and M13-YFP was observed with HPLC using size-exclusion column.

695-Pos Board B574

Evidence For Kinesin-1 Passing Obstacles On The Microtubule

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We have performed single molecule imaging with automated particle tracking and extensive statistical analysis of kinesin-1 motility in the presence of obstacles on microtubules in vitro. Minimal GFP-labeled wildtype kinesin predominantly detached immediately from the microtubule track in the presence of either motile or static (kinesin) roadblocks. Moreover, automated analysis allowed us to detect short pauses (<200 ms) within a processive run. For the case of motile obstacles we reasoned that the encountered obstacle unbinds quickly and allows further movement. But, surprisingly, also in the presence of static obstacles short pauses were detected, suggesting that kinesin is indeed able to 'pass' the obstacle. We propose that while processive kinesin passes an obstacle it may change protofilaments.

696-Pos Board B575

Unique Conformation of Kinesin-1′s Neck Linker in the Nucleotide-free State

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Kinesin-1 is a motor protein that moves along microtubule in a hand-over-hand manner. The neck linker-docking model has been proposed to explain processive motility but has been questioned because its free energy change is too small to account for the force production. An alternative model proposes that the tethered head freely diffuses and is captured preferentially at the forward binding site, although the structural basis is not clear. To explain this mechanism, we recently proposed that the neck linker has to adapt a backward-pointing conformation to promote ADP release (Mori et al. Nature 2007). Previous cryo-electron microscopy (cryo-EM) studies (Rice et al. Nature 1999) showed distinct densities from the gold cluster attached to the distal end of the neck linker in the nucleotide-free state, but the direction of the neck linker extension was uncertain. To identify the conformation of the neck linker in the nucleotide-free state, we attached gold cluster to the middle of the neck linker and observed the gold-labeled kinesin motor heads on the microtubule using cryo-EM at <15 Å resolution. The gold-density showed ellipsoidal shape extended along the protofilament and these densities were located rearward to the beginning of the neck linker. This density distribution indicates that the mobility of the neck linker is restricted toward the minus-end of the microtubule presumably due to steric constraints, which is consistent with the recently solved nucleotide-free kinesin crystal structure (Makino et al. this meeting). This conformational preference of the neck linker after ADP release provides structural basis for the preferential binding of the tethered head: ADP release and tight microtubule-binding is prohibited at the trailing position because the neck linker is pulled forward and is permitted only at the leading position.

697-Pos Board B576

Examination Of The Kinesin-1 Tail Interaction With Microtubules

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It is well known that the kinesin-1 tail domain contains a second microtubule binding-site independent of the binding-site located in the head domain (1,2), but the affinity and location of the tail-microtubule interaction on tubulin is not known. We have used fluorescence anisotropy to measure a Kd of kinesin-1 tail for microtubules in the submicromolar range, and we are currently performing experiments to determine the specific tubulin residues involved in forming this interaction. We hypothesize that the tail binding-site will include the extreme C-terminus of tubulin, which we will test by measuring the affinity of tail for tubulin with its C-terminal residues cleaved. Any effects that kinesin head domains or select microtubule-associated proteins may have on the affinity of the tail for microtubules will also be analyzed by fluorescence anisotropy, and the possibility for the tail to inhibit the ATPase activity of the head while bound to microtubules will be tested with an enzyme-coupled ATPase assay. These studies will test the hypothesis that the kinesin tail can fold over and simultaneously contact both the head domain and microtubules, producing a state in which both the ATPase activity of the head domain is inhibited and the kinesin molecule is anchored to the microtubule via its tail domain, as proposed by Dietrich et al (2).

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698-Pos Board B577

Location of Tethered Head of Kinesin-1 When Bound to a Microtubule

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A dimer of kinesin-1 motor domains (heads), each with a bound ADP, looses only one of its 2 ADP molecules on binding to a microtubule (MT). The first equivalent of one kinesin dimer per two tubulin heterodimers (one head per tubulin heterodimer) binds tightly (K_1), but additional kinesin binding can occur at higher kinesin concentration (K_2). Whether the heads are arranged as in L1,2 or D1,2 is controversial. These cases can be distinguished by two additional criteria as indicated in the figure. One is that binding of a second outer layer of kinesin on going from L1 to L2 should not result in additional release of ADP, whereas additional ADP will be released on going from D1 to D2. Initial