Support from the NIH NS053493 and the Dreyfus Foundation is gratefully acknowledged.

[1] Howard, Hudspeth, and Vale, Nature 342, 154–158 (1989).
[2] The Brownian motion of 2 μm beads in dilute PolymerX solutions and of 0.2–2 μm unattached vesicles in live cells, when analyzed by the Generalized Stokes-Einstein method, show similar G′ and G″ in the two environments.

704-Pos Board B583
Quantum-dot Assisted Characterization Of Helical Motor Paths On Microtubules
Bert Nitzsche, Felix Ruhnow, Stefan Diez.
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.
Advanced techniques in single-molecule optical microscopy have contributed greatly to our current view on the dynamics of motor proteins. While so far most studies have been limited to the 2-D imaging on a CCD-camera chip, a complete understanding of motor protein function requires insight in how motor proteins move in 3-D on the lattice of cytoskeletal filaments.
Here, we report a novel and versatile method to study the interactions of motor proteins with cytoskeletal filaments in 3-D with nanometer accuracy. We sparsely label reconstituted microtubules with quantum dots and use fluorescence microscopy to image their longitudinal and rotational movement over reflective silicon surfaces coated with motor proteins. We determine the 2-D xy-positions of the QDs with sub-pixel accuracy by nanometer tracking and combine this data with simultaneous height measurements based on fluorescence-interference contrast microscopy. We use this technique (i) to investigate the stability of the paths of cooperating processive kinesin-1 motors and (ii) to study the asymmetry in the powerstrokes of non-processive microtubule motors.

705-Pos Board B584
Diffusive Movement Of A Processive Kinesin On Microtubules
Hailong Lu, Yusuf M. Ali, Carol S. Bookwalter, David M. Warshaw, Kathleen M. Trybus.
University of Vermont, Burlington, VT, USA.
Conventional kinesin-1 is a processive motor protein that moves unidirectionally on microtubules. We found that when full-length kinesin containing a HIS tag at its C-terminus is bound to an anti-HIS Quantum dot (Qdot), it shows diffusive movement on microtubules in the presence of either ATP or ADP. Diffusive behavior was first described for the depolymerizing kinesin-13, MCAK (Helenius et al., 2006). When bound to a carboxylated Qdot, the same kinesin construct moves processively in the presence of ATP, but does not interact with microtubules in ADP. Further investigation with a truncated construct lacking the last 75 amino acids (kinesin-ΔC) showed both unidirectional and diffusive movement on microtubules in solutions containing a mixture of ADP and ATP. The diffusion constant depends on the concentration of ADP/ATP. When tested in solutions containing only ADP, kinesin-ΔC shows purely diffusive movement. We interpret these data to imply that kinesin-1 diffuses on microtubules when it is in the inactive, folded conformation, and it moves processively when in its active, extended conformation. We speculate that in the folded state, kinesin with bound ADP retains a relatively high binding affinity for microtubules compared to extended kinesin, thus allowing it to diffuse.

706-Pos Board B585
Alternating Site Mechanism Of Kinesin-1 Characterized By Single-molecule FRET Of Fluorescent ATP Analogues
Guenther Woehlke[1], Sander Verbrugge[2], Bettina Ebbing[1], Erwin Peterman[2].
[1]Technical University, Dept. Physics E22 (Biophysics), Munich, Germany,
[2]Vrije Universiteit Amsterdam, Amsterdam, Netherlands.
Kinesin-1 motor proteins move along microtubules in repetitive steps of 8 nm at the expense of ATP. To determine nucleotide dwell times during these processive runs, we are using here a FRET method at the single-molecule level that detects nucleotide binding to kinesin motor heads. We show that the fluorescent ATP analogue used produces processive motility with kinetic parameters altered less than two and a half-fold compared to normal ATP. Using our confocal fluorescence kinesin motility assay, we obtain fluorescence intensity time traces that are analyzed using autocorrelation techniques, yielding a time resolution of about a millisecond for the intensity fluctuations due to fluorescent ATP binding and release. To compare these experimental autocorrelation curves to kinetic models, we use Monte-Carlo simulations. We find that the experimental data can only be described satisfactory on the basis of models assuming an alternating site mechanism, thus supporting the view that kinesin's two motor domains hydrolyze ATP and step in a sequential way.

707-Pos Board B586
Expression and Characterization of Novel Rice Kinesin E15
Masako Tsuchida[1], Masafumi D. Yamada[2], Kazunori Kondo[2], Shinsaku Maruta[1].
[1]Department of Bioinformatics, Graduate school of Engineering, Soka University, Hachioji, Japan, [2]Department of Bioinformatics, Faculty of Engineering, Soka University, Hachioji, Japan.
Kinesin is an ATP driven motor protein that moves along microtubules. Kinesin plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics, and signal transduction. Kinesin species derived from vertebrates have been well characterized. In contrast, only a few kinesis have been characterized in plants. E15 is one of the kinesin encoded on rice genome. E15 has COOH-terminal motor domain and exhibits a high homology with the kinesin-14 family in *Arabidopsis thaliana*. However, this kinesin is not similar to other kinesin -14 family kinesins derived from animal, e.g., DmNcd, ScKar3, and CeKlp. Consequently, kinesin E15 may be plant-specific kinesin. In this study, we expressed the motor domain of a novel rice plant-specific kinesin, E15, in *Escherichia coli*. and studied its enzymatic characteristics and compared with other related kinesins. Molecular weight of the E15 motor domain was 37.6 kD. The MT-dependent ATPase activity was higher and the affinity for MT was weaker than rice kinesin K16 that we have previously reported. The optimum pH was pH 6.0–6.5, which is similar to K16. Interaction of E15 with fluorescent ATP analogues was also studied for the kinetic characterization. E15 showed weaker affinity for nucleotide than other kinesins. Currently, we are preparing E15 dimer for the motility assay.

708-Pos Board B587
Kinetic characterization of the Rice Kinesins using Fluorescent-ATP Analogue
Nozomi Umezu[1], Yuko Kubo[1], Kazunori Kondo[2], Toshiaki Mitsui[3], Shinsaku Maruta[1].
[1]Division of Bioinformatics, Graduate School of Engineering, Soka University, Tokyo, Japan, [2]Depertment of Bioinformatics, Faculty of Engineering, Soka University, Tokyo, Japan, [3]Graduate School of Science and Technology, Niigata University, Niigata, Japan.
Kinesin is an ATP driven motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesins derived from vertebrate have been well studied on their characterization. However, not so many studies for kinesins of plants have been done yet. Previously, we have expressed the novel rice kinesin K16 by *E.coli*. Biochemical and crystallographic studies of the K16 motor domain demonstrated that K16 has very unique properties and conformation, which may reflect the plant specific physiological role. We have also succeeded to express other several rice kinesins. In this study, we focused on rice specific kinesins D04, L05, N14 and O12. The kinesin motor domains of D04 and L05 are found at the N-terminal. In our preliminary study, D04 and L05 belong to kinesin-4 sub family and kinesin-7 (CENP-E) sub family, respectively. On the other hand, N14 and O12 are the C-terminal motor domain. N14 and O12 belong to kinesin-14 family. Kinetic characterizations of these kinesin motor domains were studied using fluorescent ATP analogue, NBD-ATP. The binding of NBD-ATP to the ATPase site and release from the site were monitored by the change of fluorescence intensity. The kinetic parameters of rice kinesins were compared with other related kinesins. The kinetic parameters of rice kinesins were apparently different from that of conventional kinesin.

709-Pos Board B588
Analysis of Crystal Structure and Solution Structure of the Motor Domain of Rice Kinesin K16
Shinsaku Maruta[1], Keiko Tanaka[1], Yuko Kubo[1], Toshiaki Mitsui[2], Zui Fujimoto[3].
[1]Graduate School of Engineering, Soka University, Hachioji, Tokyo, Japan,
[2]Niigata University, Graduate School of Science and Technology, Niigata, Japan, [3]Department of Biochemistry, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.
The novel rice plant specific kinesin K16 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 determined crystal structure of the novel rice kinesin K16 motor domain (K16MD) in complex with MgADP at 2.5A resolution. 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, the neck-linker of the ADP bound K16 motor domain showed an ordered conformation in a position quite different from that observed in conventional kinesin, which may reflect the unique enzymatic characteristics of rice kinesin K16. In the present study, we analyzed the inner structure of the K16 motor domain in detail and compared the structure with Eg5 and other related kinesins. It has been revealed that K16MD does not have interaction of amino acids side chains, which stabilizes the docking conformation of neck-linker. We have also analyzed the conformation of neck-linker in the solution using the K16 by FRET. Motor domain

mutant G220C chimera protein fused with GFP at the neck-linker has been prepared and labeled IAE-DANS. The FRET efficiencies between the fluorescent probe DANS at C220 and GFP in the presence and absence of nucleotides were analyzed.

710-Pos Board B589

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

Yuko Kubo[1], Satoshi Yasuda[2], Yasunobu Sugimoto[3], Masafumi D. Yamada[1], Toshiaki Arata[2], Katsuzo Wakabayashi[3], Shinsaku Maruta[1].
[1]Division of Bioinformatics, Graduate School of Engineering, Soka University, Tokyo, Japan, [2]Department of Biology, Graduate School of Science, Osaka University, Osaka, Japan, [3]Division of Biophysical Engineering, Graduate School of Engineering Science, Osaka University, Osaka, Japan.

We have previously revealed that rice kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain(MD) 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 MD, as expected from the high similarity of amino acid sequence. However, neck-linker region showed an ordered conformation in a position quite different from conventional kinesin. In this study, we designed the K16MD 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 EPR. 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 38Å and 39Å, respectively. These results may suggest that the neck-linker of nucleotide free K16 is in the docked conformation, on the other hand, the neck-linker of nucleotide bound state is in the novel conformation observed in crystal structure. We also analyzed conformational change of K16 in the solution by EPR. We constructed K16 mutants which have single cysteine at 331, 335 or 340 and labeled with 4-maleimido-2,2,6,6-tetramethyl-1-piperidinyloxy. But we could not observe notable change of mobility during ATP hydrolysis in the absence of microtubule for the three mutants. Currently, we are analyzing the distance between kinesin core reagion at 47 and neck linker at 328 using the dipolar EPR method.

711-Pos Board B590

The Effect Of Loads on the Collective Behavior of Neurospora Kinesin

Adam G. Hendricks, Bogdan I. Epureanu, Edgar Meyhofer.
University of Michigan, Ann Arbor, MI, USA.

The motor protein kinesin converts the energy from ATP hydrolysis and Brownian motion into directed movement. There is increasing evidence suggesting that several kinesin motors cooperate to transport cargoes. Recent experiments also suggest that the collective behavior of kinesin differs significantly from single-molecule behavior. We study the collective behavior of Neurospora kinesin (Nkin) in vitro. By laser trapping latex beads attached to microtubules through biotin-streptavidin linkages, we are able to apply forces to microtubules being transported by several kinesins attached to the coverslip. The density of motors on the coverslip is related to the average number of motors involved in the transport. We experimentally characterize the transport for a range of loads and motor densities.

Unconventional Myosins

712-Pos Board B591

Photo-Control of Myosin Va using Photoresponsive Calmodulin

Hideki Shishido[1], Nobuhisa Umeki[2], Osamu Sato[2], Mitsuo Ikebe[2], Shinsaku Maruta[1].
[1]Soka Univ., Tokyo, Japan, [2]Univ. of Masachusetts, Worcester, MA, USA.

Myosin Va is a processive motor that has a role as an organelle transporter in various cells. Myosin Va consists of motor domain, neck domain, coiled-coil region, and globular tail domain (GTD). The neck domain carries six IQ motifs, which act as the binding site for calmodulin (CaM) or CaM-like light chains. The GTD inhibits the Ca^{2+}/CaM dependent actin-activated ATPase activity of myosin Va. CaM is a physiologically important Ca^{2+}-binding protein that participates in numerous cellular regulatory processes. CaM has a dumbbell-like shape in which two globular domains are connected by a short α-helix. Each of the globular domains has two Ca^{2+}-binding site called as EF-hand. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. N- (4-phenylazophenyl) maleimide (PAM) is a photochromic compound that undergoes *cis-trans* isomerization by ultraviolet (UV) - visible (VIS) light irradiation reversibly. Previously we have demonstrated that the binding of the CaM to the target peptide is controlled by the isomerization of PAM. PAM was incorporated into CaM mutants that have a single reactive cysteine residue. The binding of PAM-CaM (N60C), PAM-CaM (D64C) and PAM-CaM (M124C) to M13-YFP were apparently photo-controlled by UV-VIS light irradiation reversibly at the appropriate Ca^{2+} concentration. In the present study, we have tried to photo-control the function of myosin Va using the PAM-CaM by UV-VIS light irradiation reversibly. The part of endogenous CaM of myosin Va heavy meromyosin (M5aHMM) was substituted by exogenous PAM-CaM. The M5aHMM substituted by PAM-CaM (M5aHMM/PAM-CaM) showed normal range of actin-activated ATPase activity. Currently, we are examining to photo-control the actin-activated ATPase activity of M5aHMM/PAM-CaM in the presence of exogenous GTD.

713-Pos Board B592

Modification Of Loop 1 Affects The Nucleotide-Binding Properties Of Myo1c, The Adaptation Motor In The Inner Ear

Nancy Adamek[1], Alena Lieto-Trivedi[2], Sheffali Dash[2], Michael A. Geeves[1], Lynne M. Coluccio[2].
[1]University of Kent, Canterbury, United Kingdom, [2]Boston Biomedical Research Institute, Watertown, MA, USA.

Myo1c is a ubiquitously expressed mammalian class I myosin that serves as a component of the hair cell's adaptation-motor complex in the inner ear. We have recently shown that a truncated form of Myo1c consisting of the motor domain and a single IQ domain, $Myo1c^{1IQ}$, has kinetic properties similar to full-length Myo1c (Adamek et al, 2008). We also showed that the ATPase cycle of Myo1c shows a unique response to Ca^{2+}, inhibiting the ATP hydrolysis step 7-fold and accelerating ADP release by 10-fold. Here we probed the role of loop 1, a flexible loop near the nucleotide-binding region, in defining the properties of Myo1c by creating six chimeras. We found that replacement of the charged residues in loop 1 with alanines or the whole loop with a series of alanines did not alter the ATPase, transient kinetics properties and Ca^{2+}-sensitivity of $Myo1c^{1IQ}$. Substitution of loop 1 with that of the corresponding region from tonic smooth muscle myosin II ($Myo1c^{1IQ}$-tonic) or replacement with a single glycine ($Myo1c^{1IQ}$-G) accelerated ADP release 2-3-fold from A.M in Ca^{2+}, whereas substitution with loop 1 from phasic muscle myosin II ($Myo1c^{1IQ}$-phasic) accelerated ADP release 35-fold. $Myo1c^{1IQ}$-tonic translocated actin in vitro twice as fast as wild type and $Myo1c^{1IQ}$-G 3-fold faster. The changes induced in Myo1c showed no resemblance to the behaviour of the loop donor myosins or to the changes observed with similar Myo1b chimeras (Clark et al, 2005).

714-Pos Board B593

Mechanics of myosin V near stall

Claudia Veigel[1], James R. Sellers[2].
[1]National Institute for Medical Research, Mill Hill, London, United Kingdom, [2]Laboratory of Molecular Physiology, NHLBI, Bethesda, MD, USA.

Motor proteins of the myosin family are driving many types of cellular motility. Functions are diverse, ranging from muscle contraction to endocytosis, cell locomotion, intracellular transport or signal transduction in hearing. Recent structural, kinetic and single molecule mechanical studies however revealed that the basic mechanisms of chemo-mechanical energy transduction are shared amongst myosin motors. This includes a working stroke in two phases coupled to the release of Pi and ADP and strain dependence of ADP release. Many details of the basic mechanism still remain unclear, including the effect of stall forces on the mechanics of a single motor head. Here we have used a single-headed myosin V construct (6 IQ) to investigate whether the conformational change associated with the working stroke can be reversed at high loads. We used optical tweezers to apply forces near stall for the processively moving dimeric motor (>2pN). We observed backstrokes of ~15nm, consistent with a reversal of the main conformational change of a single myosin V motor head. The dwell times of backstrokes were dependent on load. Implications of these findings for processive movement of the native, dimeric motor are discussed. Supported by MRC and NIH.

715-Pos Board B594

Force Dependence of a Myo1b Truncation Mutant

Joseph M. Laakso, Henry Shuman, E. Michael Ostap.
University of Pennsylvania Sch Med, Philadephia, PA, USA.

Myosin-Is are the single-headed members of the myosin superfamily that associate directly with cell membranes and play roles in regulating membrane dynamics. We previously characterized the force dependence of the widely expressed myosin-I isoform, myo1b, using an optical trap and a novel isometric force clamp. This myo1b isoform, which contains five IQ-motifs, is highly strain sensitive, with forces of $<$ 2 pN decreasing the rate of actin detachment $>$ 75 fold. We estimated the distance parameter (distance to the