for exactly the same substrates. We measured unlinking rate of topoII and examined its dependence on the geometry and tension in DNA. Chiral preference for left- or right-handed DNA braid is also studied.

2P-167 アクチン分子内ダイナミクスの解明を目指した部位特異的 1 分子 FRET 計測

Site-directed single molecule FRET measurement to clarify the intra molecular dynamics of actin

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Actin forms a dynamic polymeric network on which motor protein myosin V transports vesicles. Do actin filaments act as a just passive rail for myosin movements or a positive rail for activating them? Recently it has been reported using single molecule FRET measurement that actin in the filament has dynamic conformational change between active and inactive states in the time scale of second. In this study, we tried to confirm this conclusion using a different pair of dyes after improvement of the microscopy. Glutamate 41 of actin is substituted to Cysteine residue transgenically for site-directed labeling by SH-group reactive fluorescent dyes (Alexa555, Alexa647) in addition to Cysteine 374. As a result, the distance between Cys41 and Cys374 was 5.4 nm(SD=0.4 nm) (N=335 molecules) in the absence of myosin from the histogram data of the single molecule FRET result. Next we focused on the relationship between the distance and the number of myosin V existing on the filament at 1mM ATP. At ~0.4/ 1 pitch (Myosin/ 72 nm) the distance was 5.4 nm(SD=0.38nm) (N=195 molecules), 5.4 nm(SD=0.37nm) at ~0.05/ 1 pitch (N=209 molecules). We did not detect dynamic conformational change because photo bleaching time of the acceptor was short (mean ~3 sec). We have further improved the observation time by using time-raps imaging system and scavenger. We will report our latest achievements at the time of the meeting.

2P-168 解離駆動機構に基づくキネシンのシミュレーションモデル

A simulational model of a conventional kinesin based on the Driven by Detachment mechanism

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Although the core structure of kinesins is similar to that of myosins, no unified mechanism for the workings of these molecular motors has been proposed. In particular, the lever-arm hypothesis, which is widely considered to explain the behavior of myosins, cannot be applied directly to kinesins. I have proposed a mechanochemical process named "Driven by Detachment (DbD)" mechanism to explain characteristic behaviors of myosins, which include the backward movement of the myosin VI and the loose coupling phenomenon of the myosin II. The DbD mechanism assumes that the energy of ATP is solely used to detach a myosin from an actin filament by temporarily reducing the affinity of a myosin against an actin. After the affinity is recovered, a myosin has potential energy originating from the attractive force between a myosin and an actin. During the docking process, the potential energy is converted into elastic energy within a myosin molecule, and the elastic energy is finally used to conduct mechanical work of power strokes. For the model of the conventional kinesin, the neck linker was assumed to be a structure that was passively detached from and attached to the catalytic core independent of the nucleotide state. Under this assumption, simple simulations showed that the model of a dimeric kinesin moved processively along the microtubule by controlling the affinity of a kinesin against a tubulin. Moreover, if external force was applied to the center of the kinesin dimer, the kinesin moved backward along the microtubule.

2P-169 蛍光共鳴エネルギー移動法を用いた pPDM 架橋骨格筋ミオシンの構造解析

Analysis of Conformation of the pPDM Crosslinked Skeletal Muscle Myosin Using FRET.

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Previously biochemical studies have demonstrated that the highly reactive cysteine residues SH1 and SH2 can be crosslinked by variety of bifunctional reagents with different spans (3-14 Å) in the presence of nucleotides, suggesting that the region is highly flexible. The SH1-SH2 region is believed to play a key role in the conformational changes that occur in the myosin head during the force generation coupled to ATP hydrolysis. We have previously shown that the HMM, which SH1-SH2 was crosslinked by p-Phenylene-dimaleimide (pPDM) in the presence of ADP, have a novel conformation using quick freeze deep etch electron microscopy (QFDE-EM). We have also demonstrated that conformational change of the myosin motor domain during ATP hydrolysis can be monitored by measuring the FRET using fluorescent ATP analogue NBD-ATP. In the present study, we analyzed the conformation of the myosin crosslinked by pPDM using FRET between the ATP binding site and the A1 essential light chain (ELC). We prepared skeletal muscle myosin subfragment-1 (S1), which ELC was labeled by 6-bromoacetyl-2-dimethylaminonaphthalene (BD) at the Cys 177. And fluorescent ADP analogue NBD-ADP was trapped in the ATPase site of BD labeled S1. The FRET efficiency was estimated by measuring the change of fluorescence intensity of BD comparing with control BD-S1. FRET efficiency of pPDM-S1-NBD-ADP was lower than S1-NBD-ADP state. This suggests that the pPDM-S1-ADP forms more straight conformation an S1-ADP state in the ATP hydrolysis cycle.

2P-170 新規イネキネシン L05, D04 の NBD-ATP による速度論的解析

Kinetic characterization of the novel rice kinesin L05 and D04 using fluorescent ATP analogue.

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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 characterization 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 kinesin D04 and L05. The kinesin motor domains of D04 and L05 are found at the N-terminal. In our preliminary search, D04 and L05 belonged to kinesin-4 sub family and kinesin-7 (CENP-E) sub family each. Kinetic characterizations of the kinesin D04 and L05 motor domains were studied using fluorescent ATP analogue, NBD-ATP. The binding of the NBD-ATP to the ATPase site and release from the site were monitored by the change of fluorescence intensity. The kinetic parameters of D04 and L05 were compared with other related kinesins. The kinetic parameters of D04 and L05 were apparently different form that of conventional kinesin. These kinesin may be classified into different subfamily from kinesin 1.

2P-171 F₁-ATPase 回転モーターの角度依存的ヌクレオチドアフィニティ

Angle Dependence of the Affinity for Nucleotides in Rotary Motor F₁-ATPase.

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F₁-ATPase is a rotary molecular motor in which a central γ subunit rotates against hexagonally arranged subunits $\alpha_3\beta_3$. Three β -subunits, each hosting a catalytic site, hydrolyze ATP sequentially to power the rotation of γ , whereas they synthesize ATP from ADP and inorganic phosphate when γ rotates in reverse under an external force. To see how the catalytic sites change their properties upon rotation, we have measured the angle dependence of nucleotide affinity at single-molecule level. The angle of γ was controlled by attaching magnetic beads to the γ subunit and applying a rotary magnetic field. Simultaneously, binding/release of a fluorescent nucleotide (Cy3-ATP or -ADP) in a single molecule of F₁ was observed with TIRF microscopy. With Cy3-ATP and unlabeled ATP, the angle between binding and release of Cy3-ATP during continuous hydrolysis rotation was ~240° on average. The probability of binding was maximal ~30° past an ATP-waiting angle (all angles are measured in the hydrolysis direction). During synthesis rotation, binding of Cy3-ATP was most frequent ~40° past an ATP-waiting angle, and release was observed after about -70° of rotation. Cy3-ADP, in contrast, was bound mostly 60°~90° past an ATP-waiting angle both in hydrolysis and synthesis rotations in the absence of phosphate. The release occurred after $\pm 210^\circ$ of rotation in either direction. In the presence of phosphate, Cy3-ADP was bound at 30°~10°, and the angle between binding and release was $\pm 240^\circ$.

2P-172 ミオシン5の高速1分子イメージング

Single Myosin V tracking with improved time resolution

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Myosin V moves along the actin filament in a hand-over-hand manner with a step-size of 37 nm. A rapid stepping transition includes quick release of a rear head from the actin molecule and a 74 nm long forward movement before a strong rebinding occurs again. Recent measurements using scanning probe microscopy have exhibited 5.5 nm sub-steps in the moving dynamics, suggesting that the released head may indeed interact with each actin monomer and track on one protofilament until reaching its true binding site (T.Okada et al 2007). However, little is known on the details of this rapid stepping transition because of short dwell times.

Here we report the direct observation of the rear head dynamics with microsecond time resolution using a dark-field imaging microscopy. We used 40 nm gold nanoparticles, which have a strong signal to provide enough photons and response time within the given observation time. We observed large 49nm and 25 nm intermediate steps which is similar to the value previously reported (A.R. Dunn 2007), but so far did not detect 5.5 nm steps in spite of our microscopy performance (spatial resolution of ~2 nm). In order to detect the 5.5 nm sub-steps clearly(e.g. abolition of measurements near the limit of the instrument performance, and by-eye data selection), we are currently working on the further improvements of the stiffness between Myosin and gold probes and will report our latest achievements at the time of the meeting.

2P-173 荷電粒子の1次元ブラウン運動は微小管の電荷分布にどのように影響されるか?

How does the charge distribution of microtubules affect the one-dimensional Brownian motion of charged particles?

Itsushi Minoura, Seiichi Uchimura, Etsuko Muto (RIKEN Brain Science Institute)

The repetitive weak and strong binding cycle of motor proteins with tubulin is