simultaneous observation at the video rate (30 frames per seconds) showed that Cy3-ATP bindings and steps in rotation occur concomitantly. We will discuss about the reaction schemes that can explain these results.

3P-152 F_0 部位により減速された F_0F_1 -ATP 合成酵素の ATP 加水分解駆動の回転

ATP-driven rotation of F_0F_1 -ATP synthase rate-limited by F_0 sector

Ryota Iino, Tham Khék Chian, Kazuhito V. Tabata, Hiroshi Ueno, Hiroyuki Noji (Institute of Scientific and Industrial Research, Osaka University)

F_0F_1 -ATP synthase (F_0F_1) is a complex of two rotary motors, and ATP hydrolysis/synthesis reaction catalyzed by water-soluble F_1 motor is coupled with proton translocation through membrane-embedded F_0 motor. In this study, using F_0F_1 from *Escherichia coli*, mutation was introduced into putative proton channel in the a-subunit of F_0 (Angevine CM, et. al., PNAS 2003, JBC 2007) and its properties were investigated. The mutant F_0F_1 showed much lower ATP hydrolysis, proton transport, and ATP synthesis activities as compared with those of wild type in ensemble-molecule assay. Next, ATP-driven rotation of single molecules reconstituted into supported membrane was observed at time resolution up to 0.5 ms using 220-nm bead as a probe. At ATP concentration of 3 mM, rotation rate of mutant was much lower than that of wild type. Furthermore, although not always visible, steps in rotation were observed. Analysis of the pair-wise distance indicated minimal step-size of $\sim 36^\circ$, consistent with number of the c-subunit (c_{10}) in a rotor ring of F_0 motor. These results indicate that proton translocation through the a-subunit of F_0 sector can be rate-limiting factor of ATP-driven rotation of F_0F_1 .

3P-153 生体分子モーターのナノメートル計測を可能にする高圧力顕微鏡の開発

Development of a high-pressure microscope for measuring nanometer-displacements of single molecular motors.

Manabu Hasumi (1), Yoshifumi Kimura (1), Masahide Terazima (1) and Masayoshi Nishiyama (1,2). (1: Dept Chem, Graduate School of Science, Kyoto Univ; 2: PRESTO, JST)

Molecular motors are biogenic force generators acting in the nanometer range. The motor function is strongly dependent on the load, such as applied force and pressure. Here, we report novel microscopy for enabling to measure nanometer-displacements of single molecular motors under pressurized conditions. We have developed a high-pressure chamber (hastelloy C276, $60 \times 50 \times 20$ mm), which could be available up to 200 MPa. The numerical apertures on the side of the objective and condenser lenses were 0.60 and 0.55, respectively. The chamber was mounted on a custom-made microscope on the vibration free table. Microscopic observation was carried out by using a long-working distance objective lens (NA=0.6, WD $\sim$ 3 mm, OLYMPUS). The microscope apparatus enabled to observe the bright-field, phase-contrast and dark-field images with a CCD camera. To perform a bead assay for measuring nanometer-displacements of single molecular motors, a quadrant photodiode was installed to the microscope. Beads, 0.5 μ m in diameter, were attached on the observation window of the high-pressure chamber. Nanometer-measurements of the bead was performed by projecting the bright-field image of the bead on the quadrant photodiode. The resolution was enough to perform a bead assay for measuring bacterial flagellar rotation with sub-millisecond resolution. The present technique would provide a powerful tool to reveal the detailed mechanism how application of pressure affects on the motility of molecular motors.

3P-154 アゾポリマ表面に固定した微小管上のダイニン 1 分子運動

Motility assay of single dynein molecules on immobilized microtubules on azopolymer

Keitaro Shibata (1), Ken'ya Furuta (1), Masaki, Edamatsu (1), Taiji Ikawa (2), Masahito Shiozawa (2), Mamiko Narita (2), Osamu Watanabe (2) and Yoko Y. Toyoshima (1). (1: Dept Life Sciences, Graduate School of Arts and Sciences, The Univ. of Tokyo; 2: Toyota Central R&D Labs)

Cytoplasmic dynein is a microtubule-based motor protein that uses the energy derived from ATP hydrolysis to drive a power stroke. An important unsolved question is how the dynein molecule undergoes conformational change during the movement. To address this, we aim to observe the dynamic conformational change of cytoplasmic dynein using a high-speed atomic force microscope (AFM). For this purpose, we used recombinant yeast cytoplasmic dynein walking processively on a microtubule that fixed on a substratum. However, 25 nm of the microtubule diameter is too large to get a good contrast image, and microtubules are destabilized when the cantilever fast scans. To overcome these problems, we first tried to reduce the steric hindrance of microtubules by burying the microtubule to the substratum. Microtubules was absorbed and then immobilized on a film of a photoresponsive polymer containing azobenzene (azopolymer) by exposure to light from blue-LEDs. We observed that GFP-tagged yeast cytoplasmic dynein moved processively on the immobilized microtubules on the film of the polymer by TIRFM. Second, we stabilized microtubules by crosslinking with glutaraldehyde. We successfully observed that the glutaraldehyde-stabilized microtubules were able to glide on the dynein coated surface and single molecules of GFP-tagged yeast cytoplasmic dynein moved processively on the glutaraldehyde-stabilized microtubules.

3P-155 電子顕微鏡—三次元再構成法を用いた車軸藻ミオシンの構造

Chara corallina myosin structure using EM-3D reconstruction

Hiroshi Mine(1), Kohji Itoh(2), Keiichi Yamamoto(2), Takuo Yasunaga(1)(1: Kyusyu Institute of Technology(2: Chiba University)

Chara corallina myosin is a motor protein, which belongs to Class XI of myosin and is involved in cytoplasmic streaming of Chara corallina. This myosin has the interesting and mysterious features as the below : the fastest sliding velocity (~ 70 micron/sec) among known myosin classes, high ATPase activity and a relatively high duty ratio(~ 0.3). The sequence analysis of Chara myosin indicated class XI like head with six tandem IQ motifs and a coiled-coil region for dimerization, and a globular tail to bind to cargo. So far although there have been many biochemical studies about Chara myosin, its detailed structure feature is still unclear. Thus, we observed the Chara myosin's structure by electron microscopy. As a next step, we are trying to observe actin-binding acto-S1 without nucleotides or with ATP to reconstruct their 3D structure. We will here report the recent progress. These structures will clarify the relationship between structure and kinetic mechanism of this mysterious myosin, which can produce the fastest sliding velocity.

3P-156 F_1 -ATPase に対するヌクレオチド結合の温度依存性

Temperature dependency of the nucleotide binding to F_1 -ATPase

Hideimitsu Osakabe(1), Shoichi Toyabe(1), Tetuaki Okamoto(1), Eiro Munezaki(1). (Dept Phys, Chuo Univ)

F_1 -ATPase is a molecular motor in which γ -subunit rotates in $\alpha_3\beta_3$ subunit complex by hydrolyzing ATP. The catalytic site for the ATP hydrolysis resides in each of the three β -subunits. One ATP hydrolysis causes 120 $^\circ$ step rotation and this step rotation is further divided into 80 and 40 $^\circ$ substeps. It is known that ATP binding causes the 80 $^\circ$ step and analysis of ATP binding step is important.

In the present study, we investigated the nucleotide binding to F_1 at various temperature and tried to get knowledge about its thermodynamic properties.

Using a mutant F_1 in which a tryptophan residue was introduced in the catalytic site ($\beta Y341W$), we have measured the nucleotide binding from the decrease in tryptophan fluorescence at 4 $^\circ$ C and 25 $^\circ$ C. We will further carry out the measurement at various temperatures and evaluate the temperature dependency of the nucleotide binding. We expect that the experimental data will provide us an insight into the coupling of the nucleotide binding and step rotation.

3P-157 光反応性ケージド化合物を用いたキネシンの光制御

Photo-regulation of kinesin using photo-responsible caged compound

Kazuya Aritomi(1), Masafumi D. Yamada(2) and Shinsaku Maruta(2). (1:Dept Bioinfo, Faculty of Engineering, Soka Univ; 2: Div Bioinfo, Graduate school of engineering, Soka Univ)

Kinesin is one of the motor proteins, which moves along the microtubules (MTs) by the energy generated from ATP hydrolysis. Kinesin plays many roles in intracellular transport, mitosis and meiosis, and axonal flow in the nervous system along microtubules. Recently the kinesin-microtubules motility system has been a focus of attention as a molecular shuttle. Caged compounds are widely used to start biological reactions by the photolytic release of an effector molecule from a biologically inactive precursor. DMNBB is one of the effector molecules that chemically modify the thiol group of the functional bio-molecules. The incorporated DMNBB is released rapidly by ultraviolet (UV) light irradiation. In the present study, we have tried to introduce photo-switching system in to kinesin using the DMNBB. The kinesin mutants that have a single cysteine at 96C were prepared and modified by 4,5-dimethoxy-2-nitrobenzyl bromide (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.

3P-158 クラミドモナス内腕ダイニン c 及び g 尾部構造の詳細

Structure Details of *Chlamydomonas* Inner-Arm Dynein-c and -g Tail Domain.

Hitoshi Sakakibara. (Biological ICT Group, Nat. Inst. Info. Commun. Tech.)

There are seven inner-arm dyneins named a-g found in *Chlamydomonas* flagella. Each dynein except f is composed of a heavy chain and light chains (actin and P28 or centrin). These light chains and N-terminus of heavy chains locate on tails of the dyneins and should play important role for in vivo localizations. Now, I focus on the tail domain structure of single headed inner-arm dyneins and examined structure detail of dynein tail by using negative staining electron microscopy and following single-particle image processing. Dynein-c, which has P28 and dynein-g, which has centrin, were chosen for the first study. About 5,000 particles of dynein-c image and 7,000 particles of dynein-g image were used for analyses. The tail domain was settled at the center of particles. These particles were aligned, classified with the tail domain images and averaged. The three tail subdomains, slender neck, shaft, and base, were observed in both dynein-c and dynein-g. I found that a blob, 5 nm in major axis, was observed at the end of the base in both dyneins. The morphology of the blob was similar to that of actin monomer. On the other hand, the shaft of dynein-g was distinct from that of dynein-c in morphology. Dynein-g had the single winding structure at its shaft, whereas dynein-c had two parallel straight structures. From these observations, I suppose that P28 and centrin are on the shafts and that the actin is at the end of base. The localizations of actin, P28 and centrin will be determined by using antibodies or actin-binding proteins.