stator that conducts protons, and the C ring, which functions as the rotor. The mechanism of energy coupling between proton influx and torque generation remains unknown. To understand the energy coupling mechanism, the proton influx coupled with flagellar motor rotation should be measured with high spatial and temporal resolution. We have developed an intracellular pH imaging system that can measure local pH near the motor using a pH-sensitive green fluorescent protein, pHluorin, as a ratiometric fluorescent probe (Miesenböck et al., 1998). This system can measure the intracellular pH distribution at a pH resolution of 0.02 at every 3 ms. We carried out single-cell pH imaging upon shifting external pH from 7.0 to 6.0 in the presence of a membrane-permeant weak acid, benzoate, and observed an intracellular pH shift from 7.3 to 6.0 within 3 sec. We then measured intracellular pH and flagellar motor rotation simultaneously. Upon a downward pH shift, the direction of motor rotation switched from counterclockwise to clockwise within 10 ms. We also fused pHluorin to flagellar motor proteins to measure the pH in the cytoplasm around the motor. Simultaneous measurements of local pH and motor rotation are under way.

3P-124 自己調節するキネシン運動の可視化

Visualization of self-regulated kinesin motility.

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Kinesin is a motor protein that transports various cargoes and organelle along microtubules in cells. It is composed of a motor domain, which hydrolyzes ATP; a tail domain binding, which binds to the cargo; and a long coiled-coil region, which connects the motor and tail domains.

Because its primary responsibility is transport, it is important to understand the mechanism for kinesin motility regulation. We therefore observed the self-regulated behavior of reconstituted kinesin at the single molecule level using a recombinant motor domain and a recombinant tail domain *in vitro*. Furthermore, we directly observed the role of the tail domain in living cells.

We found that the tail domain did not affect motility velocity but did affect the binding of the motor domain to the microtubule both *in vitro* and in the cell. We propose a simple model for the mechanism of the self-regulated kinesin motility in accordance with these results.

3P-125 アクチンへの G146V 変異は骨格筋ミオシンとの相互作用中の力発生を阻害する。

G146V mutation in actin inhibits force generation during interaction with skeletal myosin.

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To unveil the possible roles of structural changes between the large and small domains of actin in myosin movements, we have been characterizing mutant actin in which Gly146 located between the two domains was replaced by Val. Last year, we reported that gliding velocity of G146V filaments on skeletal (sk) HMM surfaces was greatly decreased (by 80%). Force production in multiple motors assay was also inhibited to a similar extent, but parallel decrease of actin activated sk S1 ATPase activity was not observed. In contrast, those filaments moved and produced force normally on myosin V.

Thus, we are currently performing experiments to understand the molecular mechanism of this myosin class-specific inhibition of movement by G146V actin. Stopped-flow analyses showed that ATP induced dissociation rates of acto-sk S1 with or without bound ADP did not differ between G146V and WT filaments, indicating that prolonged strongly-bound state is not the cause of the slow movement of G146V filaments on sk HMM. Taken together, it was suggested that force generation itself is impaired. We are developing a FRET-based assay to probe the structural changes of actin involving G146. We prepared control actin carrying two engineered Cys residues at Thr41 and Ala235 in the small and large domain, respectively, but lacking the endogenous reactive Cys374, and labeled the two Cys residues with two fluorophores for intramolecular FRET. Single molecular FRET analysis of this actin in filaments showed that actin subunits take multiple states. We plan to analyze this FRET signal with and without the G146V mutation in the presence or absence of myosin. Also, to understand why movement of G146V filaments on myosin V was normal, we are characterizing mutant myosin II carrying a chimeric substitution with a myosin V sequence.

3P-126 2mM ATP における DNSO 下での F1-ATPase のステップ運動 Stepping rotation of F1-ATPase at 2 mM ATP in the presence of DMSO (dimethylsulfoxide).

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F1-ATPase is the catalytic moiety of FoF1-ATP synthase which catalyzes ATP synthesis from ADP and inorganic phosphate using the electrochemical potential difference of proton across the mitochondrial inner membranes, chloroplast thylakoid membranes and bacterial plasma membranes. Previously, Feldman and Sigman reported that enzyme-bound ATP was synthesized by chloroplast F1-ATPase from medium Pi and enzyme-bound ADP. Yohda and Yoshida also reported that TF1 synthesizes enzyme-bound ATP in the presence of DMSO, suggesting that DMSO increases the affinity for Pi. In this report we examined the effect of DMSO on the hydrolysis of ATP and ATP-driven rotation by $\alpha 3 \beta 3 \gamma$ subcomplex of thermophilic F1-ATPase. We found that the ATPase activity was severely attenuated and clear stepwise rotation was observed at 2mM ATP in the presence of 30% DMSO. We are now trying to identify the position of DMSO induced pause and investigating which catalytic step is slowed down by DMSO.

3P-127 F1-ATPase の β -サブユニット(Y341W)単体へのヌクレオチド結合の温度依存性

Temperature dependence of nucleotide binding to β -subunit of F1-ATPase revealed by fluorescence measurement.

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In the $\alpha 3 \beta 3 \gamma$ sub-complex of F1-ATPase, the central γ subunit rotates 120 degree against $\alpha 3 \beta 3$ ring while hydrolyzing one ATP molecule; thus it is called as a molecular motor. Recently, the rotary mechanism of this molecular motor has been elucidated in detail. But thermodynamic aspect of the process is still unclear. As a first step of investigation, we examined the temperature dependence of nucleotide binding to the $\alpha 3 \beta 3 \gamma$ complex by use of the genetically engineered Trp at the nucleotide binding site (β Y341W). The fluorescence intensity of the Trp decreases upon nucleotide binding and we could monitor the nucleotide binding at a temperature range of 277 to 323 K. The van't Hoff plot of ADP binding gave a straight line and ΔH_0 and ΔS_0 were estimated to be some -20 kJ/mol and 20 J/mol/K, respectively. We are now trying to carry out a similar analysis for the isolated β subunit. By comparing the thermodynamic data for $\alpha 3 \beta 3 \gamma$ complex and isolated β subunit, we will discuss the effect of complex formation, subunit-subunit interaction, and conformational change induced by nucleotide binding.

3P-128 レーザー照射による繊維状タンパク質のバイアスブラウン運動

Biased brownian motion of fibrous proteins under laser irradiation.

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Most types of biological motion were driven by motor proteins moving along fibrous rail proteins using the energy of ATP. The rail proteins have a unique structural polarity which determines the direction of moving. In connection with the mechanism of mechano-chemical energy transduction, one of the potent models is a thermal ratchet model which illustrates that the temperature differences between motor and rail proteins convert the isotropic Brownian motion into directional movement. Recently, we have demonstrated that the infrared laser irradiation to the nanogold-attached actin filament could modulate the one-dimensional Brownian motion from isotropic to be anisotropic. In order to obtain the further evidence that the temperature differences between filamentous proteins with structural polarity and their surrounding molecules could drive the filament into the biased Brownian motion, we have tried to examine two biological filaments; microtubules as another rail protein and bacterial flagella as a non-rail protein with structural polarity.

For the purpose to obtain higher yield for thermal energy from laser light, we have introduced the semi-conductor probe with fluorescent emission, "quantum-dot": One of the famous tools for the field of cell biology. In addition with the improvement in thermal probe, the equipment for the filament observation was also improved. Inverted dark field microscope with visible laser light was contrived in order to obtain a clear image of filaments and to alleviate the spiritual stress of experimenter.

3P-129 アクチンフィラメントへのミオシンの協同的結合はアクチン-ミオシン-ADP-Pi 状態で起こる

Cooperative binding of myosin to actin filaments occurs in the actin-myosin-ADP-Pi state

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Many reports have been made about cooperative interaction between myosin and actin filaments. These reports suggest that the cooperative binding involves with force generation of the actomyosin motor, but the mechanism remains unknown. In this study, we examined the cooperative interaction in more detail using fluorescence microscopy. To analyze the cooperative binding, we prepared GFP-