nism, in which multiple components such as tmRNA, SmpB and EF-Tu are facilitated in the process. Our previous results show that GTP hydrolysis stimulated by SmpB is necessary for tmRNA A-site entry and subsequent tagging of specific amino acids. In these experiments C-terminus truncation of SmpB has negative effect on GTP hydrolysis of EF-Tu and tagging ability of tmRNA, suggesting that SmpB coordinates with each process. To elucidate the mechanism precisely, it is important to understand the interaction of each component, especially the order of binding and unbinding or the dwell time of each event. Here we apply single molecule fluorescence imaging *in vitro* to elucidate the trans-translation mechanism.

Ribosomes were specially immobilized onto glass surfaces and the GTP hydrolysis reaction in trans-translation occurs in adjustable PURE system using recombinant factors. The interaction of tmRNA and ribosome was monitored by fluorescence dye labeled tmRNA or TLD, tRNA-like domain of tmRNA. Immobilized ribosome subunits were also labeled by fluorescence dye. The association of tmRNA and ribosome was measured by colocalization of tmRNA and ribosome. With EF-Tu, interaction of tmRNA and ribosome was observed. And with the loss-of-function mutations EF-Tu (G222D), tmRNA did not interact with ribosome.

1P174 新規偏光顕微鏡による単一分子の3次元配向測定 Three-dimensional orientation measurements of single molecules by newly developed polarized fluorescence microscopy

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Single molecule fluorescence techniques have become increasingly useful for observing the dynamic properties of single molecules. One such property is a protein's orientation, which is necessary to measure the protein's three-dimensional motility. We have developed new microscopy for determining such orientations based on the principals of polarization analysis proposed by Fourkas. This method requires only that one collect fluorescence counts from a single molecule at three different polarizations followed by a simple mathematical calculation to yield the three-dimensional orientation. In this method, a relatively small number of photons are sufficient for reliable orientation measurements. This fact should also decrease the time scale needed to determine the orientation of a given fluorophore. Here, we demonstrate the axial rotation of actin filaments sliding over myosin molecules fixed onto a glass surface by measuring the polarization of individual rhodamine phalloidin fluorophores sparsely bound to the filaments. This new microscopy will have particular applicability for various biophysical studies interested in a wide range of dynamic processes that depend on single molecule orientation dynamics.

1P175 プリズムによる3次元光学トラッキング技術の応用 / 1分子を 1B1255 対象にした光ピンセット操作と立体視表示 Application of 3-D prismatic optical tracking: single-molecule optical tweezers approach and stereoscopic 3-D view

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We have developed a new method for tracking single particles in three dimensions under a conventional microscope by dividing light in two components using a wedge prism. This method, 'three-dimensional prismatic optical tracking' termed *tPOT*, is here combined with optical tweezers to quantify molecular scale 3-D force in motor proteins. Precise calibration of trapping force to bead displacement with sub nanometer scale enables us to determine the force exerted not only in *xy*-plane direction but also along optical axis (*z*-axis). With this experimental set-up, we applied force to rotary motor F₁-ATPase that showed rotation on a glass surface. Our research group previously used the procedure in which the objective was displaced to impose the load on single molecules held between the trapped bead and the glass, which frequently caused the measurement error because of drifting motions of the specimen. This method has been modified as follows; 1) the trap center is displaced with time by moving the focusing lens along *z*-axis; 2) spring constant of the trap is increased via the adjustment of the laser power. In either case positions of the stage and the objective were fixed and thus repetitive measurements were reliably performed. We also demonstrate an application of *tPOT* to a variety of microscopy techniques taking advantage of two components of light as a parallax view. Stereoscopic 3-D view on a monitor is reconstructed by two eyes as a real-time image captured under a conventional microscope with high-magnification.

1P176 新規蛍光性 ATP アナログと ATP 駆動型分子モーターとの相互作用

Interaction of novel fluorescent non-nucleotide ATP analogue with ATP-driven molecular motors

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Previously numerous ATP analogues have been synthesized for analysis of ATPases, such as myosins or kinesins. Ribose-modified ATP analogues are known to be 2' and 3' isomers mixture. To avoid mixture of isomers, we designed and synthesized non-nucleotide fluorescent ATP-analogue, *N*-methylanthraniloyl amino ethyl triphosphate (MANTTP) which similar structure to the non-nucleotide ATP analogue 2-[(4-azido-2-nitrophenyl) amino] ethyl triphosphate (NANTP). It is known that NANTP are good substrate for skeletal myosin and induces actin gliding *in vitro* motility assay. Excitation and emission maximums in the fluorescence spectrum MANTTP were 374nm and 430nm, respectively. MANTTP was hydrolyzed by conventional kinesin and skeletal myosin, and induced dissociation of acto-myosin. The MANTTPase of myosin was significantly activated by actin. The ADP form of MANTTP showed the formation of skeletal muscle myosin-MANTDP-BeFn complexes which mimic the transient state in ATPase cycle. The fluorescent intensity of MANTTP itself does not change on binding to myosin ATP binding site, however, MANTTP showed significant FRET between intrinsic tryptophan residue of skeletal muscle myosin and MANTTP. The binding of MANTTP to myosin can be observed as fluorescence increase on the stopped flow system. The second-order rate constant for MANTTP first binding to myosin is $0.30 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$. It was shown that the novel fluorescent ATP analogue is applicable to the kinetic studies on ATPases.

1P177 Observation of conformational change of β subunit in $\alpha_3\beta_3$ subcomplex of F₁-ATPase by high-speed AFM

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F₁-ATPase is a rotary motor enzyme driven by ATP hydrolysis. The rotational motion of the central shaft γ subunit is considered to be driven by conformational change of the three catalytic β subunits. The domain motion of the each β subunit with three steps, which synchronizes with the substep rotation of the γ subunit has been observed using advanced TIRF[1]. A recent study also showed that F₁ mutant with a largely truncated γ could rotate in the correct direction [2], implying the possibility that the cooperative conformational change of the β subunit occurs even in the $\alpha_3\beta_3$ subcomplex without γ . Here we applied high-speed AFM to directly observe the conformational change of the β subunit in $\alpha_3\beta_3$ complex.

High-speed AFM images clearly resolve each subunit of the $\alpha_3\beta_3$ complex fixed on a mica substrate. The subunits appear to be arranged in six-fold symmetry and the whole shape does not change without ATP. At lower ATP concentrations (1-3 μM), AFM movies shows the conformational change in the three alternate subunits in the $\alpha_3\beta_3$ complex which are likely to be the catalytic β subunits. Furthermore, the conformational change of the three subunits takes place in counterclockwise rotation. These results indicate that the conformational change of the three β subunits occurs cooperatively and unidirectionally even without the γ subunit.

[1] T. Masaie et al. (2009) *Nat. Struct. Mol. Biol.* **15**, 1326

[2] S. Furuike et al. (2008) *Science* **319**, 955.

1P178 高速原子間力顕微鏡でタンパク質の化学-力学変換を可視化するための PEG-ATP の合成

Synthesis of PEG-labeled ATP to directly visualize the mechanochemical coupling in proteins by high-speed atomic force microscopy

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We have developed and improved a high-speed atomic force microscopy (HS-AFM) that enables us to directly visualize activity of biological molecules in buffer conditions with sub-nanometer resolution at rate of several frames per second (Ando et al., PNAS, 2001; Ando *et al.*, *Pflugers Archiv. Eur. J. Physiol.*, 2008). Thus far, HS-AFM imaging has elucidated several dynamic processes performed by proteins including bacteriorhodopsin, myosin-V and chaperonin GroEL-GroES. However, because this microscopy cannot visualize small molecules such as nucleotides, the mechanochemical coupling in proteins has not been structurally studied yet. To realize this study, we here synthesized PEG-labeled ATP in which EDA-ATP was covalently modified by various lengths NHS-PEG. The purity of PEG-labeled ATP was more than 95%. To test the function of PEG-labeled ATP, we examined actin-gliding assay of myosin II with TIRF microscopy. The average translocation velocity of actin filaments obtained by PEG5000-labeled ATP was similar to that obtained