3A0912 F₁-ATPase と繊毛軸系の機能に関わる構造変化**Motions in F₁-ATPase and ciliary axonemes that drive functions**

Tomoko Masaike¹, Koji Ikegami², Rinako Nakayama¹, Mitsutoshi Setou², Takayuki Nishizaka¹ (¹*Department of Physics, Gakushuin University*, ²*Department of Cell Biology and Anatomy, Hamamatsu University School of Medicine*)

Our goal is to visualise how motions and functions of components are coupled in various hierarchy levels of biological systems under optical microscopes.

As a model of enzymes, we chose F₁-ATPase. Cooperative ATPase activity at three catalytic β subunits is known to be greatly accelerated by the presence of the central shaft subunit γ . To investigate involvement of physical rotation of γ in conformational states of β , orientation of the C-terminal helix of β was measured using advanced TIRF microscopy, while γ was forcibly rotated using magnetic tweezers. We found that conformational changes of β are dependent on both orientation of γ and direction of its rotation. Thus the interplay between β and γ indeed contributes to conformational states and thus chemical states of the enzyme during efficient rotary catalysis.

The next target is an organelle. A fluorescent bead was attached to the axonemal tip of a tracheal cilium of a mouse, and beating motions were recorded under a three-dimensional tracking microscopy. We obtained 3-D coordinates of trajectories and fitted them with spherical shells. We found multiple phases of velocities along the spherical shells during strokes. Moreover, counterclockwise rotational motions and a difference of height between effective and recovery strokes were quantitatively indicated. Cilia may follow different trajectories in synchronised effective and recovery strokes to avoid drag of the opposite stroke and maintain effective fluid flow for elimination of harmful materials.

3A0924 高速暗視野照明による金ナノロッドの方向検出システムを用いた F₁-ATPase の回転の検出**Detection of rotation of F₁-ATPase using high-speed orientational detection of gold nanorod**

Sawako Enoki^{1,2}, Ryota Iino^{1,2}, Hiroyuki Noji^{1,2} (¹*Grad. Sch. Engineering, Univ. Tokyo*, ²*CREST*)

The scattered light from single gold nanorod is strongly polarized along the axis of the rod and can be used for the detection of its orientation. For the orientational detection, the gold nanorod would be a better probe than a fluorescent dye, because its high scattering intensity made it possible the detection at high rate for a long time without photobleaching. Furthermore, gold nanorod would be better than gold nanoparticle, because the orientational change that causes only very small lateral displacement of the attached probe can be detected. At last annual meeting, we reported two dark-field imaging systems based on objective-type evanescent and backscattering illuminations to detect the orientation of nanorod by analyzing the scattering intensities. Here, we tried defocus imaging of scattering of gold nanorod based on objective-type backscattering illumination. We tested defocus imaging of nanorod by observing the rotation of molecular rotary motor, F₁-ATPase. By analyzing the scattering pattern from gold nanorod, we could detect the orientation of the gold nanorod quantitatively at high speed (10 μ s) and at high angle resolution. There are several advantages for defocus imaging compared to previously reported imaging systems. The angle can be directly determined from the image and angle range was wide (0-360 degree), while those of previously reported systems are narrow (0-90 degree). We will apply this imaging system to observation of domain motion of proteins.

3A0936 UTP を基質とした時の F₁-ATPase の回転運動**Characterization of UTP driven rotation of F₁-ATPase**

Hideonobu Arai, Rikiya Watanabe, Hiroyuki Noji (*Grad. applchem., Univ. Tokyo*)

F₁-ATPase (F₁) is a rotary motor protein driven by ATP hydrolysis. In our early work, we examined the competency of various nucleotides to drive the rotation of F₁. While ATP, GTP, and ITP have been shown to support the rotation, we have not found evidence of UTP-driven rotation despite of the hydrolyzability of UTP. In this study, we again attempted to verify the incompetency of UTP to drive the rotation by using very pure UTP that did not contain detectable ATP (<0.0025 %). At various UTP concentrations, we observed the anticlockwise rotation of F₁. From the Michaelis-Menten analysis, the rate constant of UTP binding was determined as 10⁴ M⁻¹s⁻¹, which is 1,000 times smaller than that of ATP. Thus, the rotation is not attributable to the possible ATP contamination,

showing that UTP drives the rotation of F₁. We measured the rotary torque of UTP rotation from the viscous coefficient (Γ) and the angular velocity of the rotation probe (ω); $N = \Gamma\omega$. The estimated torque was around 40 pNnm as same as the ATP-driven rotation. To confirm this, we also conducted a buffer exchange experiment from ATP to UTP to compare the angular velocity of individual F₁ molecules in the presence of ATP or UTP. No obvious difference was found. These results showed that F₁ efficiently converts the hydrolysis energy of UTP into mechanical work even though UTP is a very low affinity substrate. The present work suggests that the base moiety of nucleotides is dispensable for the chemomechanical coupling of F₁.

3A0948 Single Molecule Analysis of Inhibitory Pausing States of V₁-ATPase

Naciye Esma Tirtom¹, Yoshihiro Nishikawa², Daichi Okuno³, Masahiro Nakano⁴, Ken Yokoyama⁵, Hiroyuki Noji¹ (¹*Department of Applied Chemistry, School of Engineering, University of Tokyo*, ²*Dept. of Biotechnology, Osaka University*, ³*RIKEN, Osaka, Japan*, ⁴*The Institute of Scientific and Industrial Research*, ⁵*Dept. of Biomolecular Sciences, Kyoto Sangyo University*)

V₁-ATPase, the hydrophilic domain of V-ATPase, is a rotary motor fueled by ATP hydrolysis. Some biochemical studies indicated a strongly-inhibited state of V₁-ATPase, which we analyzed in detail at single molecule level, in this study. Here we showed that *Thermus thermophilus* V₁-ATPase had two types of inhibitory pauses interrupting continuous rotation: a short pause (SP, 4.2 s) that occurred frequently during rotation and a long inhibitory pause (LP, >30 min) that terminated all active rotation. Both pauses occurred at the same angle with ATP binding and hydrolysis. Kinetic analysis revealed that the time constants of inactivation into and activation from SP were too short to correspond to biochemically predicted ADP inhibition, suggesting that SP is a newly identified inhibitory state of V₁. The time constant of inactivation into LP was 17 min, consistent with one of the two time constants governing the inactivation process observed in bulk ATPase assay. When forcibly rotated in forward direction by using magnetic field, V₁ in LP resumed active rotation. Solution ADP suppressed the probability of mechanical activation, suggesting that mechanical rotation enhanced inhibitory ADP release. These features were highly consistent with mechanical activation of ADP-inhibited F₁, suggesting that LP represents the ADP-inhibited state of V₁. Mechanical activation largely depended on the direction and angular displacement of forced rotation, implying that V₁-ATPase rotation modulates the off rate of ADP.

**3A1010 F₁-ATPase のトルク伝達における DELSEED ループの役割
The role of DELSEED loop in torque-transmission of F₁-ATPase**

Kazuma Koyasu¹, Rikiya Watanabe¹, Mizue Tanigawara², Hiroyuki Noji¹ (¹*Department of Applied Chemistry, School of Engineering, The University of Tokyo*, ²*Graduate School of Frontier Biosciences, Osaka University*)

F₁-ATPase is an ATP-driven rotary motor that generates torque at the stator-rotor interface. The torque-generating unit, β , largely changes its conformation, by swinging the C-terminal domain inwardly upon ATP binding. The protruding loop of the C-terminal domain, so called DELSEED loop (D-loop) forms a large contact interface with the rotor subunit. From these observations, D-loop has been supposed to have a crucial role in the torque transmission of F₁. In our recent work, we analyzed the rotation behaviors of the mutant F₁ of which D-loop residues were all substituted to glycine in order to disrupt the specific interaction with the rotor and rigidity of the loop (Tanigawara *et al.* submitted). Surprisingly, the mutant has shown active rotation although it halved the torque. In the present study, we attempted to identify which reaction step is impaired by the glycine substitution. For this purpose, we measured the angular dependency of the equilibrium constant and reaction rate of ATP binding and hydrolysis steps by conducting the single-molecule stall-release experiment (Watanabe *et al.* NCB 2012). It was shown that the angular dependency of ATP binding was reduced to 60% while that of hydrolysis was not changed. These results suggest that D-loop has a crucial role in the force transmission from the catalytic reaction center to the rotor subunit in ATP-binding process but not in hydrolysis step.

3A1022 F₁-ATPase の回転子 γ サブユニットに有限のトルク発生に必要な残基はない**None of the rotor residues of F₁-ATPase are essential for torque generation**