data on MLC-regulated actomyosin behavior, the latter mechanism involving a decreased number of actin-myosin crossbridges is more likely to occur during quick cell shortening. In addition, the rapid disassembly we observed was independent of MLC. Thus, the present study indicates that cell shortening-induced reduction in actin-myosin interactions promotes rapid and orientation-dependent disassembly of SFs.

13:00~17:00 L会場 / Room L

分子モーター 1

Molecular motor 1

1L1312 F₀F₁の回転に伴うプロトン流観察のための担持脂質膜システムの開発

Development of a supported phospholipid bilayer system for the observation of proton flow coupled with the rotation in single F₀F₁

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F₀F₁ is a membrane protein that synthesizes ATP using proton flow through the F₀ portion embedded in the membrane. The ATP synthesis coupled with the rotation of the γ subunit against $\alpha_3\beta_3$ complex in the hydrophilic F₁ portion has been shown at the single molecule level. However, the proton flow that drives ATP synthesis in the whole ATP synthase by rotating the ring of c subunits in the F₀ portion has not been measured directly.

Here, we developed a supported phospholipid bilayer (SPB) system based on the vesicle fusion method. The aim was to form a small space between the membrane and the glass surface to visualize the proton flow as a change in pH. A quartz crystal microbalance confirmed smooth spread of small unilamellar vesicles of phosphatidylcholine into planar membranes on the glass regardless of the presence of F₀F₁. The membrane remained fluid in the presence of F₀F₁, as monitored by fluorescence recovery after photobleaching of the pH sensitive dye pHrodo incorporated in the SPB. Buffer exchanges indicated that the fluorescence of pHrodo responds to small changes in pH around the probe. The F₀F₁ in the SPB was active, showing ATP-driven rotation of a magnetic bead attached to the c-ring via avidin-biotin interactions. We are now trying to observe the proton flow accompanying the rotation of F₀F₁ under an optical microscope.

1L1324 Development of circular orientation fluorescence emitter imaging (COFEI) with the advanced TIRF microscopy

Mitsuhiro Sugawa¹, Fumihiko Fujii², Tomoko Masaike¹, Takayuki Nishizaka¹ (¹*Department of Physics, Gakushuin University*, ²*High Performance Bioimaging Research Facility, Osaka University*)

The advanced total internal reflection fluorescence microscopy (TIRFM), in which a polarized excitation light is rotated in image plane, enables us to detect an orientation of single fluorescent molecule in several degrees accuracy (Masaike et al., *Nat. Struct. Mol. Biol.* 2008). Here we developed a new polarization measurement system based on that advanced TIRFM. This system is equipped with a wedge prism setting in a rotary stepping motor just in front of CCD camera. Since photons emitted from a fluorescent probe come to CCD through the rotating wedge prism, a fluorescent spot makes a circular trajectory. Furthermore, the polarization-modulation of the excitation is synchronized with the rotation of the prism so that the intensity of the signal from single fluorescent probe becomes darker and brighter, two times, in one revolution and then the resultant profile of the fluorescent circular trajectory shows symmetric intensity gradient, from which the orientation of the probe and its change are determined. We developed a simple mathematical model of the bright-dark pattern of a fluorescent circle to measure an orientation of the fluorescent molecule. For demonstration, we performed the rotation assay of F₁-ATPase using as a marker Qrod, a rodlike quantum dot. Qrod has on average 5 nm of the minor diameter and 50 nm of the major diameter in size, which is optimal to detect the rotational step of F₁-ATPase by either movement of the fluorescent spot or rotation of the bright-dark pattern.

**1L1336 金ナノロッドの高速配向検出による F₁-ATPase の回転の検出
Detection of rotation of F₁-ATPase using high-speed orientational detection of gold nanorod**

Sawako Enoki, Ryota Iino, Hiroyuki Noji (*school of engineering, the university of Tokyo*)

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 2D and 3D 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. Here we applied two dark-field imaging systems based on objective-type evanescent and backscattering illuminations to detect the orientation of nanorod at a time resolution of 10 μ s. The scattered light from single nanorod was divided into two orthogonal directions using a beam displacer and the image was projected onto a high-speed CMOS camera. The orientation was determined quantitatively from the ratio of scattering intensities of two orthogonal components. To test each method, the rotation of F₁-ATPase (F₁), a ATP-driven rotary molecular motor in which the γ subunit rotates against the $\alpha_3\beta_3$ cylinder, was detected. When a nanorod was attached to the rotor γ subunit, the intensities of two orthogonal components showed anti-correlated periodical changes in the presence of ATP, strongly suggesting the rotation of F₁.

1L1348 表面プラズモン共鳴を引き起こす金微粒子を用いた蛍光ヌクレオチドと F₁-ATPase の回転の同時観察

Simultaneous observation of fluorescent-nucleotides and rotation of F₁-ATPase using plasmonic gold nanoparticle

Hiroshi Ueno¹, Shoichi Toyabe¹, Eiro Muneyuki¹ (¹*Dept. of Phys., Fac. of Sci. & Eng, Univ. Chuo*, ²*ISIR, Univ. Osaka*)

F₁-ATPase (F₁) is an ATP-driven molecular motor. So far chemomechanical coupling of F₁ has been directly visualized by simultaneous imaging of fluorescent-nucleotides and rotation. However, a strict synchronism between chemical events and rotation has not been fully understood yet because of the noise in single molecule detection and limited temporal resolution (33ms). To overcome these problems, we constructed the simultaneous observation system of dark-field and fluorescence images using gold nanoparticle as both an optical probe for clear step and a plasmonic particle for fluorescence enhancement. Metal nanoparticles interact strongly with specific wavelength of light, which causes a localized surface plasmon resonance. The plasmonic particle generates large electric field near its surface which enhances the fluorescence of dyes around a particle. We expected this effect to improve the S/N ratios in fluorescence images. Imaging was achieved by objective-type TIRFM with a special mirror. Simultaneous observations were carried out at 30Hz or 100Hz recording rate. As expected, we found the enhancement of Cy3-ATP fluorescence (~ 2 times higher than that of the non-plasmonic particles) with little change in the noise. Furthermore, at 10ms resolution, fluorescence change (Cy3-ATP binding/release) was almost in synchronism with rotation. Interestingly, a small change in fluorescence was sometimes observed at 120° or at 200° when the long catalytic dwell could be found. Currently, we are determining the cause of these phenomena.

1L1412 回転モーター F₁-ATPase の強制回転によるヌクレオチドアフィニティの操作

Manipulating the Nucleotides Affinity in Rotary Motor of F₁-ATPase by Forced Rotation

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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, bind ATP sequentially and hydrolyze it to power the rotation of γ , whereas they synthesize ATP from ADP and inorganic phosphate (Pi) when γ rotates in reverse under an external force. To see how the catalytic sites select their binding nucleotide according to the γ angle, we measured the angle dependence of nucleotide affinity in single molecules of F₁.

The angle was controlled by attaching magnetic beads to the γ and applying a rotary magnetic field. Simultaneously, binding/release of a fluorescent nucleotide (Cy3-ATP or -ADP) to/from F₁ was observed under TIRF microscopy. Experiments were performed in the presence and absence of Pi, ATP, ADP, or ADP+Pi. The rate constants of binding and release of Cy3-nucleotides were estimated as the function of the γ angle, and finally the angle