binding angle. In this study, we observed the rotating F_1 in the presence of ATP, ADP, and Thio-Pi and synthesized ATP γ S by forcible manipulation with the magnetic tweezers. From this experiment, we determined that Pi was released at $+320^\circ$.

see 2TA4-05

2P-131 ミオシン 6 の SHREC 計測

SHREC measurement of myosin-VI stepping motion

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Myosin-VI is a motor protein that plays an important role in a large variety of cellular events such as vesicle transport and anchoring actin bundles to the plasma membrane. Myosin-VI is thought to move processively as a dimer along an actin filament in a hand-over-hand fashion with large steps similar to myosin-V. However, unlike myosin-V, its step size is largely variable. Recently, we showed using FIONA method that myosin-VI does not have widely distributed step but rather has two step types, a regular large step (72nm) and short step (44nm). The large steps are consistent with the hand-over-hand model. The short steps, however, are not explained by canonical stepping model. We also showed that the fraction of short steps largely increases in the presence of ADP. We speculate that ADP regulates which mechanism, myosin-VI employs in its subsequent step. To test this hypothesis, we performed an advanced multi-color FIONA technique called single-molecule high-resolution colocalization (SHREC) which involves labeling the two heads with differently-colored Q-dots, finding direct evidence for gait disorder. Now we are analyzing what is the condition that myosin-VI switches between two types of stepping manner. Furthermore, to clarify how myosin-VI switches between short and long steps, we are measuring myosin-VI movement under several ADP concentrations.

2P-132 高温におけるキメラベン毛モーターの回転トルク応答

Torque response of bacterial flagellar motor induced by transient heating.

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see 2TA4-02

2P-133 ミトコンドリアマトリックスのインポートモータ mtHsp70 の作動機構の解析

Step-size analyses of the mitochondrial Hsp70 import motor reveal the Brownian ratchet in operation

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Newly synthesized mitochondrial precursor proteins have to become unfolded by the mitochondrial Hsp70 (mtHsp70) import motor to cross the mitochondrial membranes. To assess the mechanism of unfolding of precursor proteins by mtHsp70, we designed a system to measure step sizes of the mtHsp70 import motor, which are distances at which the motor system moves along polypeptide chains during a single turnover of ATP. We made a series of fusion proteins consisting of a mitochondrial presequence containing the first mtHsp70 binding site, a spacer sequence containing an Hsp70 avoidance segment followed by the second mtHsp70 binding site, and different folded mature domains. Analyses of the dependence of the import rates of those fusion proteins on the lengths of Hsp70 avoidance segments allowed us to estimate the step sizes, which differ for different mature domains and different lengths of the spacers. These results suggest that the mtHsp70 import motor functions as a molecular Brownian ratchet rather than a power stroke generator to unfold mitochondrial precursor proteins.

2P-134 チューブリン C 末端の伸長した構造が弱結合に果たす役割

The role of extended C-terminal tail of tubulin on weak-binding interaction between motor proteins and microtubules

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see 2TA4-04

2P-135 KIF1A の 1 次元ブラウン運動にチューブリン C 末端は必要か？

Is the tubulin C-terminus tail necessary for the 1D Brownian motion of KIF1A?

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2P-136 *Gluconobacter* 属酢酸菌の酸性条件下でのべん毛運動

Flagellar motility of *Gluconobacter* species under acidic conditions

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Gluconobacter oxydans, a member of acetic acid bacteria, produces acetic acid from ethanol via its ethanol oxidative respiratory chain. Through this incomplete oxidation process, acetic acid bacteria can produce energy as the form of proton motive force. Thus, we hypothesize that energy metabolism is crucial in the oxidative fermentation, such as acetic acid production. Flagellar motility is a major locomotive mean of bacteria and can be expected to consume energy, the proton motive force, at work. The *G. oxydans* 621H strain has a set of flagellar genes and swims presumably by its flagella. Although the pH value of the medium dropped to approx. 3.5 by acetic acid production, *G. oxydans* swam well. Here, we examined the flagellar motility under acidic conditions.

Flagellar motility of *G. oxydans* was observed with high levels of swimming fraction, when the cells were grown on ethanol or glucose which is converted to acetic acid or gluconic acid, respectively, making culture media acidic. Flagellar filament was purified to reveal a single flagellin molecule of a 52-kDa protein of which 5 N-terminal amino acid sequence was identical to that of flagellin B in the genome database. The flagellar filaments were dissociated to the flagellin subunits by 50 °C treatment at pH8.0, whereas remained intact by 80 °C treatment at pH5.0. These data suggest that *Gluconobacter* flagella and its motility are more robust under acidic conditions than under neutral to alkaline conditions.

2P-137 基質の周期的伸張・収縮による粘菌アメーバの運動極性形成の機構

A possible mechanism of directional migration in *Dictyostelium* cells induced by the cyclic stretch and shrinkage of the substratum

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Living cells are constantly subjected to various mechanical stimulations. They must sense the mechanical aspects of their environment and respond appropriately for proper cell function. In general, cells adhere to substrata. Thus, the cells must receive mechanical stimuli mainly from the substrata and respond to them. For example, migrating cells can create their own polarity and migrate in a certain direction even in the absence of any chemoattractant. In order to generate such polarity, cells must sense mechanical stimuli from the substrata and transduce them into intracellular signals.

To investigate the relationship between signals derived from mechanical stimuli and related cell functions, one of the most appropriate techniques is the application of mechanical stimuli via stretching of elastic substrata. Under the cyclic stretching stimuli, *Dictyostelium* cells migrate perpendicular to the stretching direction. It is generally believed that the mechanism of cell migration is based on actin polymerization at the front of the migration cells and actomyosin-dependent contraction at the rear. To understand whether the cyclic stretching stimuli induce polarized cell migration via actomyosin-dependent contraction, we analyzed the migrations of several kinds of myosin II mutant cells under the cyclic stretching stimuli. We will discuss the relationship between the directional cell migration induced by the cyclic stretching stimuli and the actomyosin-dependent contraction.

2P-138 マクロファージによる逐次貪食過程の観察

Single event observation of sequential phagocytosis by macrophage

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Phagocytosis is a general function that is observed at various cell systems from protozoa to higher animals. We have observed the sequential phagocytosis by a mouse peritoneal macrophage through time lapse observation of a single macrophage which was fed two zymosans using with optical tweezers. When the interval was longer than a threshold time, the macrophage engulfed the particles sequentially, but when the interval was shorter than the time, the engulfment was simultaneous. In this report, we tried to investigate the effect of the interval, position and number of particle contact on the manner of phagocytosis. At first, we investigated the effect of the interval of contact timing between two particles using mouse peritoneal macrophages and zymosans. Some population of macrophages did not show phagocytosis, and some zymosans did not be engulfed by active macrophages. The inconsistency was probably due to the ununiformity of macrophages and zymosans. Therefore, we tried to prepare the uniform macrophages and foreign particles to obtain the detailed information about phagocytosis at a single event level. We are investigating if the sequential