investigated by a swarming assay and measurement of the swimming speed. These results suggest that the flagellar motor of *Bacillus subtilis* presumably generates torque by the electrostatic interaction between the rotor and the stator.

3P-141 *in vitro* motility assay における微小管の進行方向性

The turning tendency of the microtubule driven by the axonemal dynein subpecies c

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We examined the turning tendency of the microtubule driven by the axonemal inner-arm dynein subpecies c in *in vitro* motility assay. We found that the microtubule tend to turn to the right with the angular velocity of 0.016 rad/s in the high dynein density condition (2000 molecule/ μm^2). This tendency is decreased by lowering the dynein surface density. These observations suggest that the distance between the dynein molecule at the leading tip of the microtubule and the dynein molecule capturing the microtubule next is crucial for the turning motion. At high solution viscosity (0.5% methyl-cellulose in solution) or using the microtubule with the high flexural rigidity (polymerized with GMPCPP), the turning tendencies were slightly decreased. These results indicate that the suppression of the Brownian motion of the microtubule tip affects its turning behavior. By exploring the origin of this turning motion, we are going to discuss about the molecular mechanism of dynein subpecies c motility and also utilize this character for constructing the nano-bio device.

3P-142 ミオシン線維極極トラックに沿ったアクチン滑り運動速度の温度依存性

Temperature dependence of sliding velocity of actin filaments along bipolar tracks of myosin filament.

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Actin filaments move at a fast speed towards the central bare zone and at a slower speed away from the bare zone of myosin filament (Yamada et al., 1990; Sellers & Kachar, 1990; Yamada & Wakabayashi, 1993). We previously reported that the apparent activation energy of the movement in the forward direction, towards the bare zone, was lower than that in the backward direction. In our previous experiment, however, myosin filaments were not imaged together with the moving fluorescent actin filament. Actin filaments sliding in the forward and backward direction were taken from those sliding fast and slowly, respectively. In order to improve the protocol, we made a new flow chamber with which the solution was exchanged without moving the chamber on the microscope stage. Myosin filaments were firstly imaged by a solution containing 5nM Cy3ATP, and the motility assay solution containing fluorescent actin filament and ATP was introduced into the chamber. The image of the fluorescent myosin filament was superimposed on the video record of the actin filament movement when analyzing the velocity. The apparent activation energy was confirmed to be similar to that reported previously. It has generally been observed that a less flexible enzyme has a higher energy barrier for the reaction processes (Varley & Pain, 1991; Chaen et al., 1997). We discuss the cause for the higher energy barrier for the movement in the backward direction in relation to the flexibility of the myosin molecules.

3P-143 べん毛モーター高速回転時のステップ

Steps in fast flagellar rotation

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The bacterial flagellar motor is a rotary molecular machine driven by ion flux across the cytoplasmic membrane. The motor spins at several hundreds Hz. Coupling ion is either protons or sodium ions, depending on the species. The free energy gained from passing ions through the ion channel of the stator is converted to the mechanical torque by interaction between a stator and a rotor.

We reported the stepping motion of the flagellar motor of chimeric PomAPotB, works as a sodium-driven flagellar motor in *Escherichia coli*, by lowering sodium-motive force (1). To detect flagellar motor steps under physiological conditions, i.e. in very fast spinning, we have developed a back-scattering laser dark-field microscopy. This system has better than 0.6 nm resolution at more than 100 kHz sampling rate by imaging scattering light from gold nanoparticles as a marker of motor rotation (2, 3). We have found our equipment is enough to detect motor steps in faster than 100 Hz rotation and have acquired many flagellar steps in a wide range of sodium-motive force. Currently we are analysing their statistical properties, dependence on free energy, and the possible existence of sub-steps. (1) Sowa, Y., Rowe, R. D., Leake, M. C. Yakushi, T., Homma, M., Ishijima, A. and Berry, R. M. *Nature* 437 916-919 (2005) (2) Yasuda, R., Noji, H., Yoshida, M., Kinoshita, K. Jr and Itoh, H. *Nature* 410 898-904 (2001) (3) Yuan, J. and Berg, H. C. *PNAS* 105 1182-1185 (2008)

3P-144 滑り運動を可能にするアクチン・ミオシン間相互作用の自由エネルギーランドスケープ

Free energy landscape of the interaction between actin and myosin underlying the sliding movement of the myosin head

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A single molecule measurement has shown that myosin moves along actin filament with successive two to five mechanical steps of about 5.3 nanometers, which correspond to hydrolysis of a single ATP molecule (Kitamura et al., *Nature* 397, 129, (1999)). This phenomenon cannot be explained by the conventional "lever-arm model". Using a coarse-grained model of proteins with Go-like interactions, we have generated the structural ensemble of actin-myosin complex by the hybrid of Monte Carlo and molecular dynamics and obtained the free energy landscape. With the restraint by the probe attached on the myosin tail, the free energy of myosin binding sites adjacent on the actin filament had a slope which can bias the myosin movement. Moreover, the two dimensional free energy landscape analysis have shown that the conformational change within the myosin head and the movement of the myosin head along the actin filament are correlated with each other. These results are consistent with our previous postulate (Terada et al., *PNAS* 99, 9202, (2002)) that the sliding movement of the myosin head is coupled to the conformational changes toward the lowest free energy state of actomyosin complex.

3P-145 ツリガネムシ収縮過程の高速ビデオカメラによる研究

Contraction process of *Vorticella* stalk measured by high-speed camera

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A protozoan *Vorticella* consists of a slender stalk and a zooid of which shape looks like a bell. The stalk is attached to a substrate and spontaneously contracts in milliseconds and re-extends in a few seconds. The contraction of the stalk is due to the spasmoneme, the organelle placed helically in the stalk. The spasmoneme contracts on the addition of Ca^{2+} and re-extends when Ca^{2+} removed. Such a contraction without ATP is fundamentally different from many other types of biological movements. The motion of a zooid has been considered as the behavior of a damped harmonic oscillator with Hookean spring force of a stalk and a friction force subject to a zooid. In this report, a stretched exponential function is newly proposed to fit the temporal change of the contraction length and its adequacy is discussed in comparison with the conventional damped spring model. In general, a stretched exponential function consists of many single exponential functions with various characteristic life times. In our case, the power component is bigger than 1. This is the case that the stretched exponential function is described as a superposition of a step function. That is why it is assumed that each bundle of filaments contracts like a step function in stalk of *Vorticella*. On the other hand, a stretched exponential is deduced by Avrami to explain the crystallization kinetics that the crystal particles appear as nuclei and grow with the particle impingement. From this point of view, the contraction process of *Vorticella* undergoes like that some bundles of filaments are selected randomly to contract (It corresponds to the nucleation), and that the bundles next to the contracted ones contracts one after another. The space dimension of the nucleation is smaller than 1, which is consistent with one-dimensional contraction for *Vorticella*.

3P-146 アメーバ運動における温度効果の動画像解析

Study of Temperature Effect on Amoeboid Locomotion by Motion Image Analysis

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Amoebas are unicellular protozoans that live in the soil and water. Their movement, called amoeboid locomotion, is considered as the most primitive form of animal locomotion. There are some morphotypes of the shape and movement depending on species, however, the main mechanisms which drive the movement are thought to be same, such as cytoplasmic streaming, actin poly/depolymerization near cell cortex (sol-gel transition) and the extension/contraction of pseudopodium. The power of this movement is thought to be generated by the slipping movement between the actin filament and the myosin using ATP as energy source. Amoeboid locomotion is the integrated phenomena, which is generated by many interactions of mesoscopic scale. It is, conversely, thought to be affected and changed by the mesoscopic interactions with the extracellular environment. In this study, we aim to detect the mesoscopic weak interactions between amoebas and environment by quantifying the change of amoeboid