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LIS1 is a product of causal gene for human lissencephaly and known as a regulator of cytoplasmic dynein. We have previously shown that this dimeric protein binds to cytoplasmic dynein directly and regulates the dynein motor activity in vitro; the binding of LIS1 to cytoplasmic dynein inhibited its motor activity and the inhibition can be recovered by the N-terminal fragment of NDEL1, which is also known as a regulator of cytoplasmic dynein. To investigate the molecular mechanism of the regulation by LIS1, we generated the GST fusion protein of N-terminal domain deleted LIS1 (dl-LIS1-GST) and carried out in vitro motility assay. In the presence of dl-LIS1-GST, the motor activity of cytoplasmic dynein is inhibited as in the presence of full length molecule of LIS1 and the inhibition recovered by the N-terminal fragment of NDEL1. This indicates that the N-terminal domain of LIS1 is not necessary for the regulator activity of LIS1. Then we removed GST from dl-LIS1-GST and examined dl-LIS1 was a monomeric protein. We conduct in vitro motility assay with this monomeric dl-LIS1. In contrast to the case of dl-LIS1-GST, dl-LIS1 had lower effect on the dynein motility than dl-LIS1-GST, and the weak inhibition could not be recovered by the N-terminal fragment of NDEL1. This result indicates that the dimerization of LIS1 molecule may be effective for the regulation activity of LIS1 and the interaction between LIS1 and NDEL1.

3P-136 同一細胞におけるべん毛モーター回転運動の同時計測

Simultaneous measurement of rotational movement of multiple flagellar motor in a single cell

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E. coli swims in aqueous environment using several peritrichous flagella. The flagellum has a rotary motor driven by ion flux, and the motor rotates clockwise/counter clockwise in a response to external stimuli. The stimuli sensed by chemoreceptor on the cell are transmitted to the motor via response regulator, CheY. The interaction of phosphorylated CheY to the motor causes the directional change of the motor rotation (switching). To understand the process of signal transduction from receptor to the motor, we simultaneously measured the rotational movement of multiple flagellar motor in a single cell.

Micro-beads (diameter 0.5 μm) on the flagellar filament are observed by phase contrast microscopy and those images were recorded by high speed camera. Angular velocities and change of rotational directions of motors are calculated from the position of beads in sequential images.

The positive correlation was shown for changing rotational direction between two motors in the same cell, whereas no correlation in different cells was shown. This result implies the switching of two motor in an identical cell is coordinated. Moreover, lag (about 100 ms) was appeared in the correlation of directional change in different motors on identical cell. These results suggest that the chemotactic signal not only affects to the probability of rotational direction, but also directly affects to the change of rotational direction. To clarify the cause of lag in correlation, we are now investigating the relationship between the lag in correlation and the location of chemoreceptors.

3P-137 PFG-SE H-NMR によって明らかにしたアクチン水溶液中のプロトン拡散係数の増大：重合の寄与

Accelerated proton diffusion coefficient in actin aqueous solutions revealed by pulse field gradient spin echo proton NMR: Effect of actin polymerization

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Previously, we have found a layer of water molecules showing higher rotational mobility around actin filaments than bulk water. Proton diffusion coefficient in pure water is known to be almost the same value with that of water molecule. In this study, we investigated the translational diffusion of water molecules through the proton diffusion coefficient of actin aqueous solution by pulse field gradient spin echo proton NMR (PFG-SE H-NMR). A pair of field gradient pulses reduces the spin echo intensity of the proton of water molecules in actin solution. The echo intensity was measured as a function of the intensity of the field gradient pulses (10–300 mT/m). Applying Stejskal and Tanner's equation to the echo attenuation plot, the diffusion coefficient of water molecule was derived. Actin was extracted from acetone-treated powder of chicken skeletal muscle and dissolved in a solution containing 2mM Hepes-KOH (pH 8.0), 0.1mM CaCl_2 , 0.1mM ATP and 1mM DTT (G-buffer). For actin polymerization, the actin solution was incubated at 25°C for 3 hours, and the degree of polymerization was measured by fluorescence spectroscopy using unlabeled actin mixed with actin labeled with N-(1-pyrenyl)iodoacetamide. PFG-SE H-NMR measurement of actin solution showed that the proton diffusion coefficient increased with actin concentration and was higher than that of bulk water above 3 mg/mL F-actin. This result indicates that the polymerization of actin accelerates the diffusion of water molecules in actin solution. The increased diffusion coefficient of water in the F-

actin solution is most likely to be attributed to the hyper-mobile water around F-actin, which we previously revealed by dielectric relaxation spectroscopy.

3P-138 二つの頭部が非対称な役割を演じる細胞質ダイニンの理論モデル Kinetic model of cytoplasmic dynein with asymmetric roles of the leading and trailing heads

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Cytoplasmic dynein is a two-headed molecular motor that moves processively along a microtubule (MT) toward its minus end. The movement is stepwise, and the step size of 8 nm coincides with the periodicity of MT (S. Toba et al., PNAS, 2006). Based on structural and kinetic data, D. Tsygankov et al. (J. Chem. Phys., 2009) proposed a detailed kinetic model to explain the processive motion of dynein. In this model, each head goes through six states in a cycle of ATP hydrolysis in the following sequence: ADP-bound states in prepower-stroke (pre-PS) and post-PS conformations, a nucleotide-free state in a post-PS conformation, ATP-bound states in a post-PS and pre-PS conformations, and a state with ADP and Pi bound in a pre-PS conformation. In the first three states, the head is bound to the MT, while it is detached from the MT in the last three states. Tsygankov et al. assumed that the two heads in the same state are equivalent (symmetric model), and proposed a scenario for coordination of two heads in which the transition rates for the power stroke and for the ADP release depend on whether the other head is bound to MT. Here we propose another scenario of coordination, and modify the kinetic model of Tsygankov et al. in such a way that the transition rates depend on whether a head is in front or back of the other if the both heads are bound to the MT (asymmetric model). The transition rates are estimated from statistical-mechanical point of view. We will study the average step size and the dwell time based on our model. The results will be compared with those by Tsygankov et al. and with experiments.

3P-139 誘電緩和と分光によるアクトミオシンの水と解析:ADP 型とAMP.PNP 型の比較

Hydration analysis of actomyosin: comparison between ADP type and AMP-PNP type

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The hydration properties of acto-S1 solution were studied by microwave dielectric spectroscopy in the frequency range from 0.2 to 26 GHz using a vector network analyzer (Agilent 8364B) and 85070E software for dielectric spectroscopy. Actin and myosin were extracted from chicken pectoral muscle. From the solution, Myosin-S1 was prepared with Mg-papain, after ultracentrifugation the sup was used. G-actin was prepared from acetone powder and dissolved in G-buffer containing 0.1mM CaCl_2 , 1mM ATP, 1mM DTT, and 4mM Hepes pH8.0. G-actin was polymerized with KCl (final 50mM) and MgCl_2 (final 2mM) to upon mixing F-actin (10mg/ml) solution and S1 (4.36mg/ml) solution, ATP (final 1mM) was added to enable through mixing. Then the solution was incubated in room temperature for 15 minutes to consume ATP to get acto-S1.ADP solution. The solution was then concentrated to 14–15 mg/ml using aquacide II. The dielectric relaxation properties of the acto-S1.ADP solution were analyzed by means of Wagner mixture theory and double-debye fitting after removing the buffer spectral component. We obtained as a preliminary result; $\text{fch}=20.3\text{GHz}$, $\text{dh}=40.0$, $\text{fcl}=5.1\text{GHz}$ and $\text{dl}=11.4$. Then MgAMPPNP solution was added to the acto-S1.ADP solution. The result was; $\text{fch}=22.2\text{GHz}$, $\text{dh}=28.1$, $\text{fcl}=4.2\text{GHz}$ and $\text{dl}=12.2\text{GHz}$. It suggests that hyper-mobile component dh decreased by about half. Now we are carrying out more precise experiments.

3P-140 枯草菌べん毛モーター固定子タンパク MotA、MotP の保存された荷電アミノ酸残基の解析

Analysis of the conserved charged residues in flagellar stator proteins MotA and MotP of *Bacillus subtilis*.

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Many species of bacteria can swim by rotating the flagella, filamentous organelles that extend from the cell surface. Energy coupling is mediated by inward ion flux through channels in membrane-embedded flagellar stator complexes. In *Escherichia coli*, it has been proposed that electrostatic interactions between the rotor (FliG) and stator (MotA) are critical for torque generation.

However, the mechanism of torque generation of the Na^+ driven flagellar motor is unidentified. In this study, we elucidated the mechanism of torque generation both H^+ the Na^+ driven flagellar motor of MotAB and MotPS in *Bacillus subtilis*.

We selected the conserved charged amino acid residues from the alignment of MotA and MotP, and introduced neutral amino acid residues to the charged residues that existed in the cytoplasm loop between the 2nd and 3rd transmembrane segments, and also in some charged residues that were assumed to be important for the torque generation. The motility of each mutant was