Torque-speed relationship of the flagellar motor consisting of two distinct stators

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The bacterial flagellum is an organelle for cell locomotion and consists of a helical propeller and a rotary motor at its base. The rotary motor is powered by electrochemical potential difference of ions across the cytoplasmic membrane. Rotational energy is mediated by inward ion flux through the channel of the stator complexes. Most bacterial flagellar motors are driven by the electrochemical gradient of H⁺, and their stator complexes consist of MotA and MotB. It has been found recently that the Na⁺-dependent stator required for the motility of alkaliphilic *Bacillus* is expressed from a pair of genes, *motPS*. Furthermore, *Bacillus subtilis*, which belongs to neutrophile, has not only *motAB* but also *motPS* on its genome. We have shown that *B. subtilis* MotPS plays a major role at an elevated pH, Na⁺ concentration and viscosity, and that both MotAB and MotPS contribute in motility of *B. subtilis*. However, there has been no direct evidence to date that these two stator complexes coexist in the flagellar motor. We therefore measured the rotation speed of single motors by bead assay under various conditions to see whether these two stators actually work together in a motor. A spherical bead was attached to the sheared flagellar filament, and the rotation of the bead was followed by a photo-detector. Motor torque was calculated from the rotational speed, bead size and radius of the bead rotation. The details of the analyses will be reported at the conference.

2PS007 べん毛特異的分子シャペロン FlgN から紐解くⅢ型蛋白質輸送制御 Reading of the flagellar type III protein export regulation from a flagellar specific chaperone FlgN

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The bacterial flagellum is a rotary motor for motility. It builds up with basal body, hook and filament. Most of flagellar component proteins are transported to outside the cell from inside by the flagellar type III protein export apparatus. It consists of six integral membrane protein (FlhA, FlhB, FlhC, FlhD, FlhE, FlhF) and three soluble proteins (FlhG, FlhH, FlhI). In addition, FlgN, FlhS, FlhT and FlhA act as flagellar specific chaperones. The export apparatus is considered to regulate the protein export.

FlgN binds to the hook-filament junction protein, FlgK and FlgL. FlhS binds to the filament protein FlhC. FlhT binds to the filament cap protein FlhD. These chaperones act as bodyguard to prevent their premature aggregation. Not only that, FlgN interacts with FlhI, FlhJ and FlhAC. Through FlgN interacting with FlhI and FlhAC, FlgN facilitates the export of FlgK and FlgL. Thus, FlgN seems to have many functions for the protein export regulation. In the same way, FlhS and FlhT too. However, most of the regulatory mechanism and chaperone's functions remains unknown in detail.

In previous time, to investigate the regulatory mechanism, we carried out genetic analysis of a *Salmonella* $\Delta flhH-flhI flhB$ (P28T) $\Delta flgN$ mutant and its suppressor mutants which have FlhA(D456V, T490M) and FlhS(I7V, L114stop, W122stop). We are continuing its analysis. As a result, We thought that FlgN is the most important key protein to clarify the regulatory mechanism.

2PS008 Controlling Bacterial Flagellar Motor Rotation by Optical Spanner

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Bacterial Flagellar Motor (BFM) is a powerful molecular machine that can propel bacterium to swim in low Reynolds number environment. Many studies have focused on the mechanical properties of the BFM using electro-rotation and optical tweezers. However, due to the rotational nature of the motor motion, it's not easy to control the rotation by a symmetry external potential such as optical tweezer. The external torque can be applied to a micron size birefringent particle through circular-polarized light. We synthesize uniform vaterite micro particles with surface coating that can be attached to the bacterial flagella. While the motor rotates, we can apply external torque to accelerate/decelerate the motor. We will show our latest result of the torque speed relationship of single bacterial flagellar motor.

2PS009 Insights into myosin V stepping mechanism using point mutations in the converter

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Dimeric myosin V is an intracellular transporter, which moves cargoes towards the barbed end of actin filaments in cells. Its ability to perform as individual molecules implies that the ATPase cycles in two head domains must be coordinated to ensure the efficient unidirectional motility. The kinetics of nucleotide binding to myosin V is directionally modulated by load, suggesting that the head-head communication may be achieved via intramolecular load generated when both heads are bound to actin. Here we used point mutations in the converter domain to directly test the effect of the intramolecular load on the processive stepping of myosin V. The converter is a compact structure, which transmits tiny conformational changes at the nucleotide-binding site to the lever arm. To disturb the transmission mechanism, we replaced with alanines, one at a time, two phenylalanine residues that form a hydrophobic cluster with the C-terminus of the relay helix. These mutations are inferred to reduce intramolecular load but affect neither the nucleotide binding nor actin affinity. We found that the F697A mutation, which completely eliminates intramolecular load, abolishes the motility of myosin V motors. At the same time, the F749A mutation, which only partly reduces intramolecular load, significantly increases the proportion of backward steps, providing strong experimental evidence that the efficient unidirectional processive stepping of myosin V is ensured by the head-head communication based on the intramolecular load.

2PS010 負荷存在下におけるアクチン-ミオシン VI 相互作用の自由エネルギーランドスケープ解析

Free energy landscape analysis of the actin-myosin VI interaction under the applied force

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Myosin VI moves toward the minus end of an actin filament, oppositely to the motions of myosin II and myosin V. Besides, the large step size (~36nm) of myosin VI, which has the short lever arms, suggests that the Brownian motion in the weakly bound state plays an important role in the search for the strong binding position on the actin filament.

A single molecule measurement using the single headed myosin VI (Iwaki *et al.*, *Nat. Chem. Biol.* **5**, 403 (2009)) has shown that the probability of strong binding of myosin VI to the actin filament is dependent on the direction of the applied force. We here simulate this experimental situation by extending the methods which we previously used for simulating the Brownian movement of myosin II in the weakly bound state along the actin filament (Takano *et al.*, *PNAS* **107**, 7769 (2010)). Using the coarse-grained model of the actin-myosin VI system, we performed Langevin molecular dynamics simulations to obtain the effective free energy landscape in the presence of the different forces applied to myosin VI. We examine the difference of actin-myosin interface between forward and backward pulling conditions and correlate this difference with the obtained effective free energy landscape. Finally, we also discuss on the importance of this difference in the context of reaction cycle of the myosin VI.

2PS011 ミオシン 6 の双機能性とレバーアーム構造の関係

Relation between myosin VI's dual functionality and its lever arm structures

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Myosin VI is a molecular motor which walks along actin filaments using the energy of ATP hydrolysis in a dimmer state. Myosin VI has unique aspects differ from other myosin families. As one of the functional aspects, myosin VI has two different rolls, a transporter and an anchor, in a cell. Recently, we performed simultaneous observations of myosin VI during steps which revealed that myosin VI has unique stepping mechanism taking both distant and adjacent binding states to the actin filament. We assume that this stepping mechanism is important for enabling its dual function. We also assume that myosin VI's lever arms, which has only two light chains, decrease a steric hindrance between them and enable to take the adjacent binding state for its dual function. Currently, to confirm this point, we are investigating the stepping behaviors of recombinant myosins which have modified lever arms.