to the surface via their C-terminus, leaving the N-terminal coiled-coil structure hanging free. These C-linked Ncds drove microtubule sliding at similar rates to N-linked Ncds, thereby ruling out models in which the rigid coiled-coil structure is the only element able to exert or transmit force. Instead, our findings indicate another force-generating mechanism that shifts both the N & C terminus of the head, so that force can almost equally well be exerted by either the N or C terminus of the head. It was previously reported that a chimera Ncd whose C-terminus was replaced with kinesin-1 neck was able to move when it was attached to the glass at its C-terminus, so the kinesin-1 neck was regarded as a critical part. But our result shows that the kinesin-1 neck is not necessary.

1A1522 細菌べん毛モーター固定子複合体 MotA/B チャンネルの選択的透過メカニズム

Selective permeation mechanism through the channel of the stator complex MotA/B in the flagellar motor

Yasutaka Nishihara, Akio Kitao (IMCB, Univ. of Tokyo)

Bacterial flagellar motor is composed of a rotor and stators. *Escherichia coli* have proton-driven motor embedded in the membrane and the stator consists of MotA and MotB proteins. Experimental studies have suggested that the stator is a torque-generating unit and that binding of protons across the membrane to Asp32 of MotB induces changes of the stator structure and the interaction of MotA with the rotor. However, atomic-level structure of the transmembrane (TM) regions of the stator has not been determined yet and the detailed mechanism of torque generation is still unclear. In this study, we constructed the structure of TM regions of the stator using molecular modeling and calculated free energy profiles for ion permeation through the channel of the stator. For the modeling, the distance restraints derived from disulfide cross-linking experimental data were employed in simulated annealing and molecular dynamics (MD) calculations. The free energy profiles for the permeation of water molecule, hydronium ion and sodium ion were calculated with the steered MD calculations. These profiles show that water molecule should overcome a high free energy barrier (< 7 kcal/mol) whereas no high free energy barrier was found in the hydronium ion permeation. We identified that difference of interaction between the permeated molecules and main chain is the origin of the selectivity.

1A1534 Sodium Dynamics of the Bacterial Flagellar Motor

Chien-Jung Lo¹, Yoshiyuki Sowa², Teuta Pilizota², Richard Berry² (¹Department of Physics, National Central University, Taiwan, ²Clarendon Laboratory, Department of Physics, University of Oxford)

Many species of bacteria swim by means of flagella, the long helical filaments connected to rotary molecular motors embedded in the cell membrane. Motors can run up to several hundred hertz. The motor is powered by the transmembrane ion-motive force: a combination of electrical voltage (Vm) and chemical potential. To explore the fundamental mechanism of this powerful molecular machine, we measured the torque-speed curves of sodium-driven chimeric flagellar motor in *Escherichia coli*. We test the motor speed in 25 different sodium-motive-force conditions. Similar to other report, the general shape of torque-speed curve is concave down with negative slope. The shape is less sensitive to the membrane voltage but very sensitive to the sodium concentration. The knee and the zero-torque speed depended on sodium concentration and the stall torque depended on the total sodium-motive-force. In high load conditions, the motor operates close to thermodynamic equilibrium with high efficiency. By energy balance, the ion number required to drive the motor in high load in different smf are 37 ± 2 ion/rev. We also build a 4-state kinetic model to fit the data. Using Monte Carlo Optimization, we found the model parameters to fit the experimental data. We found the ion binding in low sodium concentration and the ion transition through stators are the rate limiting steps. The fitted parameters indicate that the motor mechanism is 'power-stroke' and ion transit is channel-like rather than carrier-like.

1A1546 Engineered disulfide crosslink in the the periplasmic region of PomB impaired function of the Na⁺-driven flagellar stator complex

Shiwei Zhu, Na Li, Seiji Kojima, Michio Homma (Division of Biology Science, Graduate School of Science, Nagoya University)

In the functioning bacterial flagellar motor, multiple stator units composed of PomA and PomB must be incorporated into the motor at the appropriate places around the rotor. When assembled, stator units are anchored at the peptidoglycan layer through the periplasmic region of the B subunit. Crystal

structures of this region have been recently solved, and it was proposed that the large conformational changes in this region are required for activation and assembly of the stator complex. To test this model, here we employed the disulfide crosslinking approach. If the large conformational changes are induced during the assembly of stator, then an intramolecular disulfide bridge that is engineered to covalently link between two movable elements may inhibit stator function. Based on structural information of the periplasmic region of PomB (PomBC), cysteine replacements were introduced into the cysteine-less PomB protein. We found that mutant strains with single cysteine replacement remain motile, but when we introduced several pairs of cysteine, one in the α 1 helix (154-169) and another in the core domain (C-terminal region from residue 170) of PomBC, pairs of M157C-I186C, L160C-I186C, I164C-L217C and L168C-Q213C inhibited the function of the Cysteine-less PomB. However, after treating with DTT, all of them restored the motility less than 1 hour. Thus, we speculated that the crosslink due to the disulfide bridge formed by one pair of cysteine inhibits the conformational change in the periplasmic region of PomB. (1529/1560)

1A1558 高圧力が引き起こすべん毛繊維のダイナミック構造変化

Dynamic conformational changes of flagellar filament observed by high-pressure microscopy

Masayoshi Nishiyama¹, Yoshiyuki Sowa² (¹The Hakubi Center, Kyoto Univ., ²Hosei Univ.)

The bacterial flagellar motor is a molecular machine that rotates a flagellum in both directions. CCW rotation allows the left-handed helical filaments to form a bundle that propels the cell smoothly, whereas CW rotation of a filament leads to change the shape of filament in right-handed helix and break the bundle, and inhibits smooth swimming of the cell, called a tumble. The switching in the helical structure is thought to be caused by directional mechanical actions arising from abrupt change of exerted torque by the motor rotation. Here, we show that application of pressure can also change the helical structure of flagellar filaments. The flagellar filaments in *E. coli* cells were fluorescently labeled, and then the images were acquired by using an improved high-pressure microscope. We measured the diameter and pitch of the individual filaments and then classified them into 11 possible waveforms which are predicted from structural data. At 0.1MPa (ambient pressure), all flagellar filaments form left-handed helical structure (normal form). At 40 MPa, we found not only left-, but also right-handed structures such as coiled-coil and curly I (or II). At 80 MPa, only the right-handed structures existed. After the pressure was released, most filaments returned to the initial left-handed structures. The application of pressure is thought to enhance the structural fluctuation and/or association of water molecules with the exposed regions of flagellin molecules, and results in switching the helical from left- to right-handed structure.

1A1610 c-di-GMP 結合タンパク質 YcgR のホモログ PlzD の大量発現による *Vibrio alginolyticus* でのべん毛運動阻害

Flagellar motility inhibition by overexpression of PlzD, a YcgR homolog of c-di-GMP binding protein, in *Vibrio alginolyticus*

Takuro Yoneda, Wakako Morimoto, Seiji Kojima, Michio Homma (Grad. Sci., Univ. Nagoya)

Bacterial flagellum is a locomotive apparatus which is needed to swim or to swarm. At the base of each flagellum, there exists a membrane-embedded motor. The ion flux through the stator couples to the rotor-stator interaction that generates torques. Recently it was found that YcgR inhibits motility in the presence of cyclic diguanylate (c-di-GMP) in *E. coli*. Its N-terminal YcgR/PilZ domain is considered to be required to interact with flagellar basal protein(s) and C-terminal PilZ domain is a receptor for c-di-GMP. It was reported that PlzD of *Vibrio cholerae*, a homolog of YcgR, affected the flagellar motility under a poor nutrient condition. In this study, we cloned PlzD from *Vibrio alginolyticus*. *V. alginolyticus* PlzD conserves all 5 residues required for binding to c-di-GMP for PlzD of *Vibrio cholerae*. PlzD inhibited motility in nutrient-poor plate and EGFP-PlzD was localized at cell pole. N-terminally-truncated PlzD shows weaker motility inhibition than wild type PlzD, and EGFP-PlzD_{Δ1-35} exhibits no fluorescent localization. Based on these results, we speculate that N-terminal region of PlzD is important for interact flagellar basal protein(s) and that PlzD can assemble around flagellar basal body. PlzD as well as YcgR repress motility significantly in *E. coli* MS1467 strain (ΔyhjHΔycgR). This indicates that PlzD can function in *E. coli* and may suggest a conformational similarity between N-terminal region of PlzD and YcgR though the sequence homology is very low.