2PS001 タンデム PomA を固定子とする Na⁺駆動型キメラべん毛モーターの回転計測**Rotation Measurement of Na⁺-driven Chimeric Flagellar Motor with Tandem PomA**

Yong-Suk Che, Yoshiyuki Sowa (Department of Frontier Bioscience, Hosei University)

Four PomA and two PomB, homologs of MotA and MotB in the H⁺-driven flagellar motor of *Escherichia coli*, form a stator complex in the Na⁺-driven flagellar motor of *Vibrio alginolyticus*. The motor torque is generated by the interaction between the cytoplasmic domain of PomA and the C-terminal domain of FliG, a component of the rotor. It was shown that a tandem fused PomA is functional as a torque generator in *V. alginolyticus* (Sato and Homma, JBC 2000). Furthermore, a chimeric stator (which consists of PomA and the chimeric fusion protein PotB) works as a Na⁺-driven flagellar motor in *E. coli* (Asai et al., JMB 2003). In the last annual meeting, we demonstrated that tandem PomA dimer was expressed as a single polypeptide in *E. coli* and swimming speed of *E. coli* cells with tandem PomA was about half of that with the monomeric PomA, suggesting that tandem PomA functions as a torque generator in *E. coli*.

Here, in order to characterize the details of tandem PomA, we carried out rotation measurements of single flagellar motors in *E. coli* cells expressing tandem PomA using 1 μm beads attached to truncated flagellar filaments. Maximum motor speed with tandem PomA was 80 Hz, similar to that with monomeric PomA. However, the motor speed with tandem PomA distributed broadly compared to that with monomeric PomA. We also measured motor speeds with tandem PomA with several combinations of mutants in the cytoplasmic charged residues, which are thought to be crucial for torque generation. Details of these results will be discussed.

2PS002 枯草菌べん毛モーターの異なる 2 種類の固定子 MotA サブユニットと MotP サブユニットの細胞質内ループの荷電アミノ酸残基の機能的役割**The functional role of the charged residues of two different stators: MotA subunit and MotP subunit in *B. subtilis* flagellar motor**

Yuka Takahashi, Masahiro Ito (Grad. Sch. Life., Univ. Toyo)

The flagellar motor is energized by either H⁺ or Na⁺ motive force. MotAB-type stators use H⁺, while MotPS-type and PomAB-type stators use Na⁺ as coupling ions. The MotAB-type stator flagellar motor torque in *E. coli* is considered to be generated by electrostatic interactions at the interface between rotor and stator as indicated by previous studies. There are conserved charged residues in the cytoplasmic loop of MotA, which probably interact with the conserved charged residues of the C-terminal domain of rotor FliG. However, it is not clear whether the electrostatic interaction between the rotor and the Na⁺-type stator PomAB is required. Here we studied a flagellar motor that consists of two different stators, MotAB and MotPS, in *B. subtilis* and tried to identify critical charged residues for torque generation in each MotA and MotP subunit. We identified the conserved charged residues in the cytoplasmic loop of MotA and MotP. *B. subtilis* with mutations in conserved charged residues and several other charged residues were measured for swarming and stator subunit protein expression levels. These charged residues in the cytoplasmic loop of stators can be divided into two types: those important to torque generation by interaction between rotor and stators, and those involved in stabilization of the structure of stators. Thus, important charged residues for motility in the H⁺-driven MotAB and Na⁺-driven MotPS were identified in this study.

2PS003 べん毛モーターの回転と GFP 標識固定子の同時観察**Simultaneous observation of the rotation of flagellar motor and GFP-labeled stator unit in a functioning flagellar motor**Mizuki Nakajima¹, Hajime Fukuoka², Yuichi Inoue², Hiroto Takahashi², Akihiko Ishijima² (¹Grad.Sch.Life.Sci., Tohoku Univ., ²IMRAM, Tohoku Univ.)

The bacterial flagellar motor is driven by the influx of coupling ions, and the interaction between stators and a rotor is thought to generate the motor rotation. It have been estimated the number of stators in a motor from the stepwise increment of rotational speed or the bleaching steps derived from GFP-labeled stator units. However, it is not quantified how many stator units are required for each step of speed-increment, and the timing of stator assembly in the step of

speed-increment is not measured. To clarify these questions, the rotational speed of motor and stator assembly should be measured simultaneously. To observe stator assembly, we newly designed GFP-fused stator units based on PomAPotB, which functions as the Na⁺-driven stator units in an *Escherichia coli* cell. When we observe the tethered cell expressing GFP-PomAPotB, the stepwise increment of rotational speed was detected when the motility medium without Na⁺ was replaced to the medium containing 85 mM of Na⁺. Next, we developed the microscopic system to simultaneously obtain the bright field image (wavelength more than 600 nm, taking by high speed camera) for the measurement of rotational speed and the fluorescence image (wavelength 500 - 560 nm, taking by EMCCD camera) for the observation of stator assembly. We would like to discuss this simultaneous measuring result in the annual meeting.

2PS004 蛍光観察による細菌鞭毛モーターの解析**Imaging of fluorescently-tagged motor components of bacterial flagella**

Yoshiyuki Sowa, Yong-Suk Che (Department of Frontier Bioscience, Hosei University)

Bacteria, such as *Escherichia coli*, can swim by rotating their helical flagella, protruding from the cell body. Rapid rotation of each filament (up to several hundreds hertz) is driven by a motor located in the cell envelope at the base of the filament. This motor is powered by the flux of ions across the cytoplasmic membrane. We have measured its rotation by a bead assay, in which we attach a small s bead onto the flagellar filament or hook of the motor and detect its motion. This assay is very useful for measuring the basic properties of the motor and detecting elementary steps in flagellar rotation. However, we can detect motor rotation only via the filament or hook, although the rotational torque is generated at the cytoplasmic side of the motor components, FliG, FliM, FliN, MotA and MotB. To understand how the motor works, direct observation of motor movements at the site of torque generation is required. In this study, we aim at developing the assay to detect motor dynamics by fluorescent microscopy at single-molecule level. To track movements of single fluorescent dyes with nanometer accuracy, we have built total internal reflection fluorescence (TIRF) microscope and are currently optimizing it. We also have succeeded in labeling MotB with rhodamine dyes, which are more suitable for single-molecule tracking than fluorescent proteins such as GFPs. We confirmed the rhodamine labeled MotBs function in a motor and currently observe them by TIRF microscopy.

2PS005 べん毛輸送装置構成タンパク質 FliP ペリプラズム領域の構造機能解析**Structural and functional analyses of a periplasm region of FliP, a component of the flagellar protein export apparatus**Takuma Fukumura¹, Yukio Furukawa¹, Yumiko Saijo-Hamano¹, Katsumi Imada², Keiichi Namba^{1,3}, Tohru Minamino¹ (¹Grad. Sch, Frontier Biosci., Osaka Univ., ²Grad. Sch. Sci. Osaka Univ., ³QBiC, RIKEN)

Most of flagellar proteins are transported by a specific export apparatus from the cytoplasm to the growing distal end of the flagellum where they self-assemble. The export apparatus consists of a water-soluble ATPase complex and a proton-driven export gate made of six membrane proteins FlhA, FlhB, FliO, FliP, FliQ, and FliR. The ATPase complex escorts flagellar proteins from the cytoplasm to the export gate. It has been reported that FlhA plays a role in energy transduction along with the ATPase complex. However, the structure and function of other gate proteins still remain unknown. FliP is the only protein that has a signal peptide in its N terminus. FliP has a molecular weight of about 26 kDa and is predicted to have four transmembrane helices (TM). FliP has a relative large periplasmic domain (FliPp) with ca. 8.5 kDa between TM-2 and TM-3. A deletion of FliPp results in a loss-of-function phenotype. We focused on FliPp to elucidate the role of FliP in flagellar protein export. Alanine-substitution of highly conserved residues of FliPp indicated that Glu-178 is critical for the export function. We constructed an *E. coli* expression system of FliPp from *Thermotoga maritima* for structural analysis. The results of size exclusion chromatography, static light scattering and analytical ultracentrifugation indicated that purified FliPp forms homo-tetramer. Far-UV CD spectra showed that the secondary structure is mostly α-helix. Crystallization for X-ray structural analysis is now in progress.

2PS006 2 種類の異なる固定子から構成されるべん毛モーターのトルク特性の解析