of intact flagellar basal body. Comparison with the structure of a purified basal body revealed the density of the export platform and the ring structure derived from FliI within and below the C ring. The flagellar basal body of the $\Delta fliH/fliI$ -bypass mutant, which maintains flagellar protein export and motility in the absence of FliH and FliI showed that the two densities within and below the C ring were completely lost, suggesting that the platform structure is not essential for flagellar protein export.

2PS029 細菌べん毛モーターの固定子組込み機構に対する MotB(D33N)点変異の影響

Effect of the D33N mutation in MotB on stator assembly of the bacterial flagellar motor

Shuichi Nakamura¹, Tohru Minamino², Nobunori Kami-ike², Seishi Kudo¹, Keiichi Namba^{2,3} (¹Department of Applied Physics, Tohoku University, ²Graduate School of Frontier Bioscience, Osaka University, ³Riken Quantitative Biology Center)

The bacterial flagellar motor converts the energy of proton translocation across the membrane through the proton-channel stator complex into torque. The MotA/B proton channel complex is firmly anchored to the peptidoglycan (PG) layer through the PG-binding domain of MotB to act as the stator of the motor. The stator units dynamically associate with and dissociate from the motor during flagellar motor rotation, and an electrostatic interaction between MotA and a rotor protein FliG is required for efficient stator assembly. However, the association and dissociation mechanism of the stator units still remains unclear. In this study, we analyzed the speed fluctuation of the flagellar motor of *Salmonella enterica* wild-type cells carrying a plasmid encoding a nonfunctional stator unit, MotA/MotB(D33N), which lost the proton conductivity. The wild-type motor rotated stably but the motor speed fluctuated considerably when the expression level of the MotA/B(D33N) stator was increased. Rapid accelerations and decelerations were also observed. These results suggest that the MotA/B(D33N) stator retains the ability to associate with the motor but rapidly dissociates from it as well. We propose that the stator dissociation process depends on the proton translocation through the proton channel.

2PS030 MS リングに突然変異が生じたサルモネラ菌べん毛モータの構造安定性と出力特性

Structural stability and rotational characteristics of the flagellar motor of *Salmonella* MS-ring mutant

Shun Taga¹, Akira Asaumi¹, Shuichi Nakamura¹, Fumio Hayashi², Kenji Oosawa², Seishi Kudo¹ (¹Department of Applied Physics Tohoku University, ²Department of Chemistry and Chemical Biology Gunma University)

The bacterial flagellar motor is a rotary nanomachine fueled by the proton motive force. Two ring structures, MS ring and C ring, together act as a rotor, and responsible for torque generation by interacting with a stator complex consisting of MotA and MotB. It has been known that the flagella of a *Salmonella* FliF mutant are fragile as compared with those of wild-type strain, suggesting that the MS ring plays an important role in keeping stability of the motor structure. In this study, the mechanical strength and the rotational characteristics of the FliF mutant motor were analyzed to understand how MS ring is involved in the structural stability. Quantitative measurement of the strength revealed that the mechanical strength of the FliF mutant motor decreases up to a half of wild-type motor. However, both the swimming speed and the motor speed of the FliF mutant were almost same with those of wild-type strain. These suggest that the defect of MS ring can decrease the structural stability of flagellar motor without impairment of torque generation.

2PS031 The role of the flagellar C ring in hook-length control by FliK

Kaoru Uchida, Tatsuya Yamasaki, Shin-Ichi Aizawa (Grad. Sch. Pref. Univ. Hiroshima)

The flagellar hook has an average length of 55 nm, which is controlled mainly by FliK. We previously demonstrated that mutations of any one of the fliG, fliM, and fliN genes gave rise to short hooks in *Salmonella enterica* serovar Typhimurium. FliG, FliM, and FliN form the cytoplasmic ring (C ring) at the flagellar base, which is a multifunctional structure necessary for flagellar protein secretion, torque generation, and switching of the motor rotation. We also showed that hook length of the C-ring deficient mutants broadly distributes around 55 nm, suggesting a tight length-control by the C ring. In order to elucidate the role of the C ring in hook-length control, we introduced two

modified fliK constructs into C-ring mutants. A recombinant FliK with the size of 373 a.a. produced short hooks and the other recombinant FliK with the size of 570 a.a. produced long hooks, suggesting that FliK is dominant in hook length control even in the C ring mutants. However, the hook length in C-ring mutants varied in wider range than that of the wild type. We will be discussing the interaction between the C ring and FliK.

2PS032 べん毛モーターのトルク発生器における帯電残基の性質

Characterization of the electrically-charged residues in the torque-generating unit of the flagellar motor

Satoshi Inaba, Shin-Ichi Aizawa (Prefectural University of Hiroshima)

FliG is one of the rotor components of the flagellar motor in *Salmonella enterica* serovar Typhimurium. It is said that five conserved charged residues (K264, R281, D288, D289, R297) in the C-terminal domain of FliG are directly involved in torque generation. These five charged residues formed a ridge on the surface of the domain. Among these residues, R281, D288 and D289 are belonging to the same α -helix. R281X and D288X/D289X gave rise to motility-defective mutants by substitution with either Thr or Ala. In order to examine the functional roles in detail of the residues R281 and R288/R289, we substituted these residues with all 20 amino acids.

Among variants of R281X, four (R281D, E, G, and P) were Mot⁺, and the rest swam at various speeds. Distribution of their rotational speeds continuously dispersed in a wide range, indicating that torque generated in the motor may change according to changes in conformation near the R281 site. Among D288X/D289X pair variants, three pairs (D288E/D289E, S/S, Y/Y) swam, while the rest were Mot⁺. To fill the gap between R281 and D289, we chose D284 and I285 and substituted them with 20 a.a. The variants I285D, R, T swarmed. The variants D284E, F, R, and V swarmed. These results indicate that D284 and I285 at the middle of the α -helix are not directly involved in torque generation. In summary, a pair of D288/D289 residues might be the major element of the torque generator, and that R281 residue might play a subsidiary role in the torque generator.

2PS033 Meaning of HAP1 in the length measurement of the hook

Kohei Dono, Kaoru Uchida, Shin-Ichi Aizawa (Grad. Sch., Pref. Univ. Hiroshima)

In the bacterial flagella, the hook occupies a region between the rod and HAP1 in an average length of 55 nm to connect the filament to the basal body. The hook is composed of FlgE. We analyzed the hook in a temperature-sensitive flgE mutant of *Salmonella enterica* serovar Typhimurium. The mutant cells produced wild-type flagella when grown at 30 °C but short hooks without filaments at 37°C. We found by electron microscopy that the mutant hooks produced at 30°C had wild-type length (average length: 55 nm) in intact flagella, whereas they were shorter (45 nm) in the hook basal body (HBB) obtained by acid treatment of intact flagella. SDS-PAGE showed that the HBB preparation did not contain HAP1 that is supposedly attached on the distal end of wild-type hook, indicating that interaction between the mutant hook and HAP1 might become weaker by a mutation of the hook protein. As a consequence, we learned that the hook length that we have so far measured was length of the hook region including HAP1, and that authentic hook length was about 45 nm and HAP1 length was about 10 nm in the wild-type "hook". The HAP region (HAP1 and HAP3) in intact flagella is distinguishable from the hook and filament by its appearance and thin width. However, surface appearance of the HAP region resembles that of the hook than the filament, which led us to measure hook length including HAP1. We will show length distribution of HAP region in wild type and mutants.

2PS034 コフィリンのアクチン線維への協同的結合の直接観察

Direct observation of cooperative binding of cofilin to actin filaments

Kimihide Hayakawa¹, Hitoshi Tatsumi², Takafumi Goto², Masahiro Sokabe^{1,2} (¹FIRST Research Center for Innovative Nanobiodevice, Nagoya Univ., ²Dept. of Physiol., Nagoya Univ. Grad. Sch. of Med.)

ADF/cofilin proteins are ubiquitously distributed actin severing proteins in eukaryotic cells. Several biochemical and electron microscopic studies have suggested that cofilin binds to the actin filament in a cooperative manner. We directly observed the binding of single Alexa-labeled cofilin molecules to actin filaments in solution by a single molecule imaging technique. We analyzed the non-contiguous (isolated, non-cooperative) cofilin binding to an actin filament