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Mycoplasma mobile, a pathogenic bacterium forms membrane protrusion, the gliding machinery, and glides on solid surface smoothly up to 4.5 $\mu\text{m/s}$ and 27 pN in the direction of protrusion. *M. mobile* is thought to glide by repeated catch-pull-release of sialylated oligosaccharide fixed on solid surface by hundreds of 50 nm flexible legs sticking out from the gliding machinery. However, the directed displacement of cell is difficult to explain, because the legs are flexible. To elucidate this mystery, we examined a starved cell binding to sialylated oligosaccharide, by pulling forward and backward at a speed of 160 nm/s, using a bead manipulated by a laser trap. Tension was generated at similar rate for forward and backward, 0.09 ± 0.03 pN/nm. In these processes, the tension occasionally shifted down, suggesting temporal detachment of legs from sialylated oligosaccharide fixed on solid surface, namely "slippage". The slippage occurred at tension of averages, 22.5 ± 13.9 pN and 48.4 ± 28.0 pN for forward and backward, respectively, whereas no significant difference was found in the occasional decrease in tension between both directions. The tension at slippage did not differ significantly between directions, when two types of non binding mutants lacking valid legs were artificially bound to glass. These results suggest that *M. mobile* binds to solid surface in a direction dependent manner, and this directed binding may cause the directed gliding.

2PS024 Leg movements suggested from inhibition of mycoplasma gliding by free sialylated oligosaccharid

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Mycoplasmas, known as a pathogen of human pneumonia, bind to solid surfaces and glide to one direction. In gliding, mycoplasmas catch sialylated oligosaccharides, the common target of many pathogens. In previous studies, we analyzed the inhibitory effects of synthesized sialylated oligosaccharide on binding of gliding mycoplasmas and concluded the affinity, the cooperativity, and the recognized structure. In the present study, we analyzed the effects of those compounds to the gliding of *M. mobile* cells, to know the relationship between sialylated oligosaccharide binding and the gliding mechanism. Gliding speed was reduced by the addition of sialylated oligosaccharide with time, and the decrease appeared accelerated with time after the decrease became obvious. The decrease in gliding speed should be caused by the decrease in the working legs in the addition of free sialylated compound. The acceleration in decrease may suggest that the binding inhibition of a leg affect the affinity of neighboring legs. The cells showed pivoting around the gliding machinery before the detach. The shortage of propelling force caused by the free sialylated oligosaccharide may induce remaining of legs after stroke on glass, some of which form the center of cell pivoting. All of these results support our assumption that the leg after stroke should be removed by the continuous cell movement caused by other legs in the gliding mechanism.

2PS025 Swarm Dynamics of *Vibrio alginolyticus*

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Swarming is an important step for bacterial colonization and spreading. We investigate the swarming dynamics of the dual flagellated bacterium, *Vibrio alginolyticus*, in different growing stages and external conditions. While growing on agar surface, *Vibrio alginolyticus* elongate and synthesize lateral flagella that can move rapidly in the thin liquid layer above agar. The cell motion is constrained by the cell to cell interaction and hydro-dynamical interaction with the liquid boundary conditions. We measured the colony spreading speed and the single cell motility in different growing stages and agar concentrations. The cells lengths are widely distributed. The cell density is higher in the colony edge. Edge-cells show parallel alignments while interior-cells show swirling motion with different time and length scale. We found the edge-cells shows waving while the interior-cells shows turbulent like collective motion. Far behind the spreading edge, cell density reduced down to single-cell layer. We exam the collective motion and cluster size distribution in this condition. *Vibrio alginolyticus* shows different motility dynamics in different growing stage. We aim to build up a simply model to explain the collective motion of *Vibrio alginolyticus*.

2PS026 表面タンパク質のらせん流で動くバクテリア

Helical Flow of Surface Protein Required for Bacterial Locomotion

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A large group of bacteria belonging to the phylum *Bacteroidetes* show a characteristic rapid surface movement known as gliding motility. *Bacteroidetes* gliding motility is different from other motility systems, but its mechanism is unknown. Here, we visualized the dynamics of cell adhesin SprB, and proposed "helical loop track" to explain the motility.

A soil bacterium, *Flavobacterium johnsoniae* has a 669 kDa cell-surface adhesin, SprB, which is responsible for the gliding motility and cell adhesion. Electron microscopic experiments revealed that SprB formed 150 nm long filaments, and dynamic movements of SprB were observed by fluorescence microscopy. SprB was located dispersedly on a long pitch of helical loop along the membrane, and moved on the cell surface along an apparent right-handed helical closed loop. When the apparent velocities of SprB signals were determined with respect to the glass substratum, about half of the SprB signals moved with velocities of 3.4 $\mu\text{m/s}$ and the other half moved with velocities of -0.5 $\mu\text{m/s}$. Taking the velocity of cells (1.9 $\mu\text{m/s}$) into consideration, SprB signals migrating in opposite directions appeared to move at the similar speeds with respect to the cell. The results suggest a model for *Flavobacterium* gliding, supported by mathematical analyses, in which adhesins always moved on an endless right-handed helical track at a constant speed, while some SprB tightly bound to the solid surface, generating rotation and translation of the cell.

2PS027 Gene manipulation of gliding bacterium, *Mycoplasma mobile*

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Mycoplasma mobile, a pathogenic bacterium, glides on solid surfaces with a unique mechanism, not related to known bacterial motility systems or conventional motor proteins. The gliding machinery is composed of three huge surface proteins and more than ten internal proteins. To elucidate the gliding mechanism and the roles of component proteins, the genetic manipulation including homologous recombination should be a powerful tool. However, this organism has not been transformed so far by standard procedures used for other *Mycoplasma* species. In this study, we examined and improved the steps of transformation as follows. (i) The electroporation conditions have been adjusted to fit to the *M. mobile* cells which are much tougher than the other species. (ii) The recovery time after electroporation was elongated from 2 h to 12 h. (iii) The growth medium was modified to get clearer colony shapes. Then, we achieved the transformation of *M. mobile* with efficiencies ranging 10^{-5} to 10^{-7} per competent cells by 10 μg of vector DNA, and also fluorescence labeling of cells by using enhanced yellow fluorescent protein (EYFP). We are constructing special vectors allowing us gene expression and homologous recombination.

2PS028 低温電子線トモグラフィーによって明らかとなった細菌べん毛蛋白質輸送装置の構造と機能

Structure and function of bacterial flagellar protein export apparatus revealed by electron cryotomography of *Salmonella* minicells

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The bacterial flagellum is a motility organelle composed of a basal body that functions as a rotary motor and a long helical filament that works as a propeller. Most of flagellar axial proteins are translocated into the central channel of the growing flagellum for self-assembly at the distal end by the flagellar protein export apparatus. A structural study on many different types of intact flagellar basal bodies by electron cryotomography (ECT) has suggested that the export apparatus is highly conserved in their structures across species. However, soluble components of the export apparatus have not yet been clearly observed in the basal body of *Salmonella enterica* because the wild-type cell is about 1 μm in diameter, which is too thick to visualize the intracellular structures by ECT in detail. To visualize the structure of the export apparatus in the cell, we constructed *Salmonella* minicell by overproducing FtsZ to make the cell small enough for a high-resolution ECT analysis and carried out the structural analysis

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

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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

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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

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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

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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