2P151 マイコプラズマ滑走タンパク質分子の可視化による構造解析
Structure of Proteins Involved in *Mycoplasma mobile* Gliding Revealed by Visualization

Yuhei Tahara¹, Noriyuki Kodera², Toshio Ando², Makoto Miyata¹ (¹*Grad. Sch. Sci., Univ. Osaka City.*, ²*Bio-AFM Frontier Research Center, Univ. Kanazawa.*)

Mycoplasma mobile, a fish pathogen, glides on solid surfaces with a unique mechanism. In this mechanism, Gli521 (521kDa) transmits movements from motor to leg as "crank", and Gli349 (349kDa) attaches to solid surface and pull cell body as "leg". Here, we analyzed molecular shapes of these proteins by negative-staining electron microscopy and high speed AFM. Gli521 molecule is 120 nm long and shaped "ω", featured with a central hinge, at which the molecule bends only to one direction. Gli349, known as 100 nm "8th note", shaped molecule by rotary shadowing TEM was divided into small and large domains connected by a thin, elastic filament. We will discuss the movements of molecules in the gliding mechanism.

2P152 ヒト肺炎 *Mycoplasma pneumonia* の滑走運動装置と構成タンパク質の結晶化
Crystallization of gliding machinery and component proteins of *Mycoplasma pneumoniae*

Yoshito Kawakita¹, Lisa Matsuo¹, Tsuyoshi Kenri³, Miki Kinoshita¹, Katsumi Imada², Makoto Miyata¹ (¹*Grad. Sch. Sci., Univ. Osaka City*, ²*Grad. Sch. Sci., Univ. Osaka*, ³*National Institute of Infectious Diseases*)

Mycoplasma pneumoniae, a human pathogenic bacterium glides with repeated catch and release of sialylated oligosaccharides on host cell surfaces through a membrane protrusion at a pole. The rod structure on the attachment organelle can be divided into three parts, Terminal button, Paired plates, and Wheel complex from the tip. To study the functions and structures, we expressed and purified four Wheel complex proteins coded by MPN311, MPN312, MPN387 and MPN526, a Terminal button protein coded by MPN309, and P1 adhesin coded by MPN141. To date, MPN311 and MPN387, responsible for the connection of tip to cell body and motility, respectively, are successful for crystallization after modifying some protein constructs.

2P153 マイコプラズマ・モービレの滑走にかかわるチューブリンホモログの構造解析
Structural analysis of tubulin homolog involved in *Mycoplasma mobile* gliding

Masaru Yabe, Miki Kinoshita, Makoto Miyata (*Graduate school of science, Osaka city university*)

Mycoplasma mobile glides with a unique mechanism. We focus on P42 protein composed of 356 amino acids, a component of the gliding machinery. Its amino acid sequence showed 23% identity in C-terminal 86 amino acids residues with a bacterial tubulin, FtsZ of *Thermus* sp. RL. N-terminal 235 amino acids, however, did not show similarity with FtsZ, suggesting that P42 evolved from tubulin and achieved a role specific for *Mycoplasma* gliding. To study its functions and structure, we expressed P42 and purified. As the full length protein easily formed clumps, we removed a P42 specific coil-coil near the C terminus. The protein did not show GTPase activity, consistent with the sequence analysis.

2P154 Investigating stators assembly of flagellar motors in *Escherichia coli*

Lin Tsai-Shun, Lo Chien-Jung (*National Central University Taiwan*)

Stators of bacterial flagella motor are the drivers to prompt motor rotation for swimming at low Reynolds number environment. Motor basal part is about 45 nm in diameter and surrounded by ~11 stators which work independently. The whole motor can rotate at few hundred hertz. Here we develop a super-resolution microscopy to study the stator binding position in the motor. We use photo-active green fluorescent protein (PAGFP) fused stator protein (PomA) in *Escherichia coli*. Under low expression level, motor rotate at low speed (~ few Hz) with few stators only. By measuring the relative position of these stators, we investigate the number of stators and the assembly process of these stators in these nanometer powerful molecular machines.

2P155 Tracking of bacterial flagellar motor rotation by fluorescent microscopy

Yoshiyuki Sowa^{1,2}, Yong-Suk Che¹ (¹*Dept. Frontier Bioscience, Hosei Univ.*, ²*Reserch center for Micro-Nano Tech., Hosei Univ.*)

The bacterial flagellar motor is a reversible rotary nano-machine, about 45 nm in diameter, embedded in the bacterial cell envelope. It is powered by the flux of coupling ions across the cytoplasmic membrane driven by an electrochemical gradient. The torque is thought to be generated at the cytoplasmic side of the motor components, FliG, FliM, FliN, MotA and MotB. To understand how the motor works, we have developed the assay to track functional motor components labeled with fluorophores by total-internal reflection fluorescent microscopy. Single fluorescent molecules in *E. coli* cells were detectable using our system. We have been trying to observe behaviors of each component during motor rotation at single molecule level.

2P156 タンデム PomA 変異体を固定子とする Na⁺駆動型キメラベンモーターの回転計測
Rotation Measurement of Na⁺-driven Chimeric Flagellar Motor with Tandem PomA Mutants

Yong-Suk Che¹, Yoshiyuki Sowa^{1,2} (¹*Dept. Frontier Bioscience, Hosei Univ.*, ²*Reserch center for Micro-Nano Tech., Hosei Univ.*)

Bacterial flagellar motor with chimeric stators (PomA/PotB) works as a Na⁺-driven motor in *E. coli*, which has H⁺-driven one in nature. The motor is driven by independent stator complexes consist of two sets of A₂B sub-complexes (A₄B₂ in total). To study the functional roles of individual A subunits, we carried out rotation measurements of single flagellar motors with tandem fused PomA dimmer, which expressed as a single peptide. The motor with tandem PomA can rotate smoothly, but its speed was ~30% lower than that with monomeric PomA. Furthermore, single mutation of crucial charged residue in either side of the tandem PomA still allowed motor rotation, indicating that four A subunits do not work equivalently in the stators for torque generation.