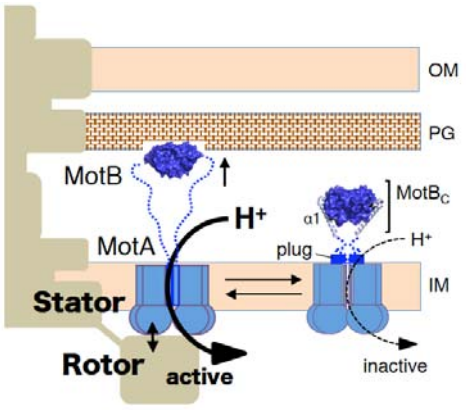
How to anchor and activate flagellar stator units: assembly-coupled stator activation mechanism revealed by the crystal and solution structures of the stator fragment

Seiji Kojima
Division of Biological Science, Graduate School of Science, Nagoya University
E-mail: z47616a@cc.nagoya-u.ac.jp

Bacterial flagellar motor consists of a rotary part (the rotor) and up to a dozen stator units that surround each rotor, and generates torque by the rotor-stator interaction that couples with the ion flow through the channel in the stator. Each stator unit is activated only when they are anchored around a rotor via the periplasmic region of the stator B subunit. Previously we determined the crystal structure of this region from *Salmonella* MotB (MotB$_C$) and proposed a large conformational change during the stator incorporation into the motor. The mutation L119P the in helix α1 of MotB$_C$ increased proton-conducting activity of the stator and its incorporation into the motor, suggesting that this mutant stator mimics the active conformation. To investigate the activation mechanism of this mutant, we solved the crystal structure of MotB$_C$ that carries the L119P mutation, and analyzed its solution structure by NMR.

The crystal structure was determined at 2.0Å resolution and it forms a dimer as is wild-type MotB$_C$. Overall structure was almost identical to that of wild-type MotB$_C$, except for the helix α1 of each subunit was disordered by the L119P mutation. To investigate the solution structure of MotB$_C$-L119P, we labeled it with ^{15}NH-Lys and performed the NMR measurement. Results were consistent with the crystal structure, showing the significant structural change localized on the N-terminal half of α1 of L119P mutant. These results raise a possibility that this change by L119P mutation alters the peptidoglycan binding of MotB, so we then assessed the peptidoglycan (PG)-binding ability of MotB$_C$ by the co-sedimentation with the purified PG sacculus. Surprisingly, not wild type but L119P mutant MotB$_C$ was co-sedimented with PG, suggesting that the mutation changes a MotB$_C$ conformation to be competent with PG binding. This study links the functional and structural changes of stator units by the mutation L119P in MotB, and supports our model for assembly-coupled stator activation, in which the conformational change in the helix α1 of MotB$_C$ is required for both stator activation and anchoring at the PG layer.



Working model for the flagellar stator incorporation and activation.