別紙 4

報告番号	※　甲　第　　号

主　論　文　の　要　旨

論文題目　Structural and functional analysis of the periplasmic region of B subunit in the sodium-driven flagellar stator
(ナトリウム駆動型べん毛モーター固定子 B サブユニットの
ペリプラズム領域に関する構造機能解析)

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論　文　内　容　の　要　旨

Bacterial flagellar motor is a miraculous rotary molecular machine and propels bacteria to swim toward favorable environment. The torque is generated by the rotor-stator interaction coupled with the ion (H^+ or Na^+) flow passing through the channel in the stator. The stator is dynamically assembled around the rotor. Anchoring the stator unit to the peptidoglycan layer with proper orientation around the rotor is believed to be essential for smooth rotation of the motor.

The stator unit of the sodium-driven flagellar motor in *Vibrio* is composed of PomA and PomB, and is thought to be fixed to the peptidoglycan layer and the T-ring by the C-terminal periplasmic region of PomB. A hybrid stator complex that is composed of a *Vibrio* PomA subunit and a chimeric B subunit (PotB) consisted of an N-terminal region of *Vibrio* PomB and a C-terminal region of *E. coli* MotB, has been convinced to work as the sodium-driven type in *E. coli* whose original motor is driven by proton motive force. We constructed a deletion variant of PotB (with a deletion of residues 41 to 91 [Δ41-91], called PotBΔL), which lacks the periplasmic linker region including the segment that works as a "plug" to inhibit premature ion influx. This variant did not confer motile ability, but we isolated a sodium-driven, spontaneous suppressor mutant, which has a point mutation

(R109P) in the MotB/PomB-specific α-helix that connects the transmembrane and peptidoglycan binding domains of PotBΔL in the region of MotB. Overproduction of the PomA/PotBΔL(R109P) stator inhibited the growth of *E. coli* cells, suggesting that this stator has the high sodium-conducting activity. Mutational analyses of Arg109 and nearby residues suggest that the structural alteration in this α-helix optimizes PotBΔL conformation and restores the proper arrangement of transmembrane helices to form a functional channel pore. It is speculated that this α-helix plays a key role in assembly-coupled stator activation.

To compare *E. coli* MotB, we determined the crystal structure of a C-terminal fragment of PomB ($PomB_C$) at 2.1 Å resolution, and the structural information suggests a conformational change in the N-terminal region of $PomB_C$ for anchoring the stator. On the basis of the structure, we designed double Cys-replaced mutants of PomB for *in vivo* disulfide crosslinking experiments and examined their motility. The motility can be inhibited reversibly by reducing reagent. The results of these experiments revealed that the N-terminal two thirds of α1 (154-164) changes its conformation to form a functional stator around the rotor. The crosslinking did not affect the localization of the stator nor the ion conductivity, suggesting that the conformational change between the α1 helix and core domain of $PomB_C$ occurs in the final step of the stator assembly around the rotor.

In this study, it was clearly demonstrated that the structural change of the C terminal α1 helix and core domain of the B subunit is important for the activation of stator function as well as for anchoring the stator unit. Dynamic structural change probably induces the channel opening of stator and subsequently the ion influx through the stator complex. However, it is still mysterious how the stator is associated and disassociated with the rotor.