Insights into the Stator Assembly of the Na^+-Driven *Vibrio* Flagellar Motor from the Crystal Structure of MotY

Motile bacteria swim by rotating a helical filamentous organelle called flagellum. Each flagellum is driven by a reversible rotary motor embedded in the cell envelope. The flagellar motor is about 45 nm in diameter and divided into a rotating part (rotor) and a stationary part (stator) (Fig. 1). The energy source of the motor is a membrane gradient of a specific ion (H^+ or Na^+), and its flux through the stator channel is coupled with rotor-stator interactions to generate torque [1]. Marine bacterium *Vibrio alginolyticus* swims using a single polar flagellum in liquid environment. Its Na^+-driven motor rotates surprisingly fast, up to 1700 rps at 300 mM NaCl, and requires unique proteins, MotX and MotY, in addition to the PomA/PomB stator proteins for torque generation (Fig. 1, right). MotX and MotY form a complex in the periplasm, and MotX affects the membrane localization of PomB, suggesting that MotX interacts with PomB. Recently, MotX and MotY have been found to form a ring-like structure (T-ring) beneath the P-ring of the flagellar basal body in *V. alginolyticus*. Furthermore, in the absence of MotX or MotY, the PomA/PomB stator does not localize to the flagellated cell pole, suggesting that these two proteins are involved in the incorporation and/or stabilization of the PomA/PomB complex within the motor [2]. To understand the role of MotY in the assembly and function of the Na^+-driven motor, we determined the structure of MotY from *V. alginolyticus* at 2.9 Å resolution using diffraction data collected at beamline **BL41XU** [3].

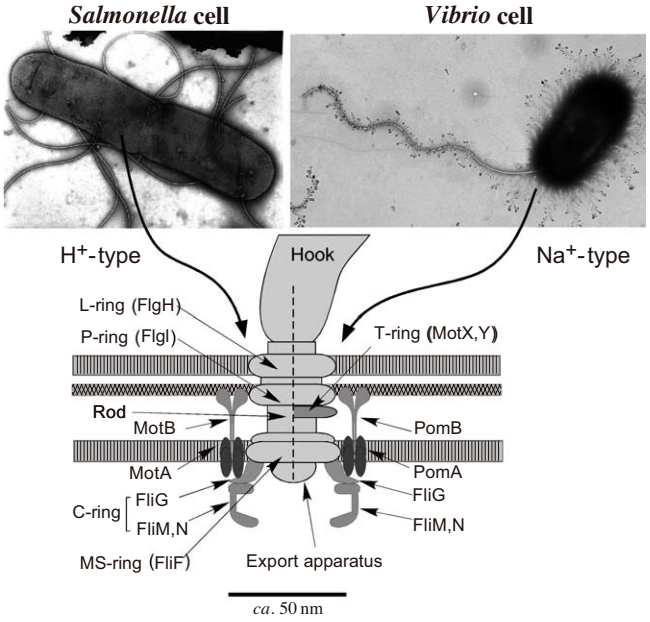

Fig. 1. Cell body and flagellar basal body structure of the H^+- and Na^+-driven types. The stator consists of MotA and MotB for the H^+-driven motor, and of PomA and PomB for the Na^+-driven type. For *Vibrio*'s Na^+-driven polar flagellar motor, MotX and MotY, which form the T-ring in the basal body, are also essential for rotation.

The structure shows two distinct domains: an N-terminal domain (MotY-N) and a C-terminal domain (MotY-C) (Fig. 2(a)). MotY-N has a unique structure, composed of two β-sheets and two α-helices. The C-terminal end of helix α2, which is followed by MotY-C, is anchored to sheet-B through a disulfide bridge between Cys-25 in β3 and Cys-147 in α2 (Fig. 2(b)). The two Cys residues are conserved in MotY from various bacteria with polar flagella. Therefore, MotY proteins containing the C25S, C147S, and C25S/C147S substitutions were susceptible to proteolysis and resulted in cells with reduced or lost motility, indicating that the disulfide stabilizes MotY-N [4]. MotY-C that contains a putative peptidoglycan-binding motif forms an α/β sandwich structure showing remarkable similarity to other OmpA/MotB-like proteins, such as the core domain of Pal and the OmpA-like domain of RmpM. The structural similarity suggests that MotY interacts with the peptidoglycan layer like those proteins. In the crystal structure, however, the putative peptidoglycan-binding site of MotY-C was more widely open than those of Pal and RmpM, and most of the loops forming the peptidoglycan-binding pocket were disordered (Fig. 2(c,d)). A disordered peptidoglycan-binding pocket may be an important property for free MotY since, after secretion into the periplasm, MotX and MotY presumably must diffuse around until they collide with the basal-body and form the T-ring structure. Therefore, MotY should not bind tightly to the peptidoglycan layer before it encounters the basal-body. Interaction with the basal-body may induce folding of the disordered chains into a functional peptidoglycan-binding pocket.

On the basis of the structure and lines of evidence obtained from biochemical experiments using MotY fragments, we propose a model for stator assembly (Fig. 3). MotY may form a complex with MotX and diffuse around the periplasm with little or no affinity for the peptidoglycan layer (Fig. 3(a)). When the complex associates with the basal-body via MotY-N, conformational changes are induced that greatly increase the affinity of MotY-C for peptidoglycan. The

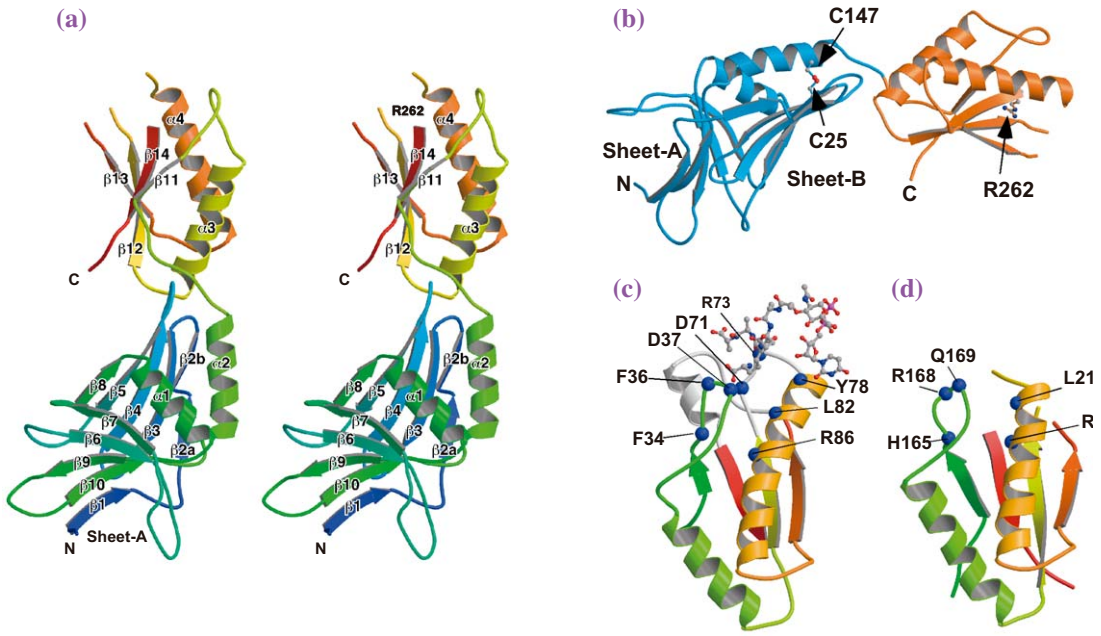

Fig. 2. Structure of MotY. **(a)** Stereo view of the Cα ribbon drawing of MotY. **(b)** The N-terminal and C-terminal domains are shown in blue and orange, respectively. The disulfide bond between Cys-25 and Cys-147 and the Arg-262 residue are displayed in a ball-and-stick format. **(c)** **(d)** Structure of Pal in complex with the peptidoglycan precursor **(c)** [5] and putative peptidoglycan-binding regions of MotY **(d)**. The residues contributing to the binding of the peptidoglycan precursor and the corresponding residues in MotY are indicated by blue balls. The peptidoglycan precursor is shown in a ball-and-stick representation.

PomA/PomB complex also forms before associating with the motor and diffuses around in the cytoplasmic membrane (Fig. 3(b)). When the PomA/PomB complex encounters the basal-body, the association of the periplasmic C-terminal domain of PomB with MotX positions PomB to bind to the peptidoglycan layer in the proper orientation to interact productively with the rotor (Fig. 3(c)).

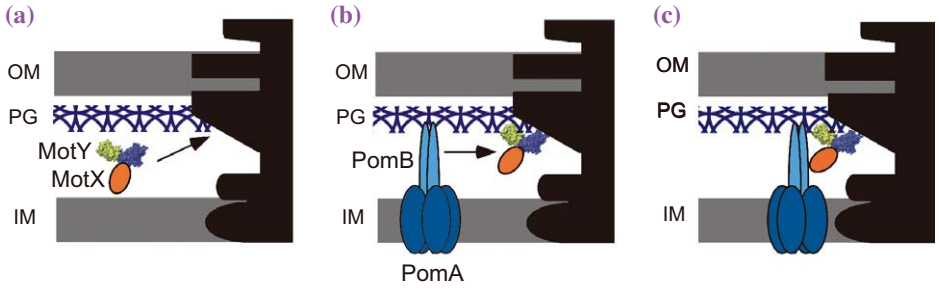

Fig. 3. Model of stator assembly in the Na^+-driven polar flagellar motor of *Vibrio*. MotY is shown as a space-filling model colored blue for MotY-N and yellow for MotY-C. MotX, PomA, and PomB are shown in orange, dark blue, and cyan, respectively. Details of the model are described in the text.

Seiji Kojima[a], Michio Homma[a] and Katsumi Imada[b,*]

[a] Division of Biological Science, Nagoya University
[b] Department of Frontier Biosciences, Osaka University

*E-mail: kimada@fbs.osaka-u.ac.jp

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