Crystal structure of SecDF, a Sec translocon-associated membrane protein

Most proteins synthesized in the cytoplasm are secreted across membranes. The bacterial Sec Translocon, consisting of three membrane proteins, SecY, SecE, and SecG, provides a channel-like pathway for protein translocation. SecA ATPase drives the protein movement across the membrane (Fig. 1(a)), which is facilitated by the proton motive force (PMF) and a membrane-integrated Sec component, SecDF [1,2]. However, the structure and function of SecDF have remained unclear. We reported the first crystal structure of SecDF and elucidated the mechanism of SecDF-driven protein translocation through a structure-based functional analysis [3,4].

The functional importance of SecDF was previously shown *in vivo* and *in vitro*. For instance, a SecDF-deficient *Escherichia coli* strain is severely defective in protein export, exhibiting cold sensitivity for growth. Despite the vital importance of SecDF *in vivo*, the mechanism of protein transport enhancement by SecDF has not been defined. To clarify how SecDF is involved in protein translocation in detail, we started structural biological analysis of SecDF in 2004. In 2006, we obtained an initial electron density map of SecDF at ~4.0 Å resolution by the single-wavelength anomalous dispersion method using selenomethionine-labelled crystals. However, the low quality of the initial map hampered the model building. To address this problem, we separately determined the higher resolution structures of periplasmic domains, P1 and P4, by X-ray crystallography and NMR, respectively. These periplasmic structures enabled us to correctly build the SecDF model. In 2010, we finally refined the structure of full-length *T. thermophilus* SecDF, consisting of 735 residues, at 3.3 Å resolution (Fig. 1(b)). The X-ray diffraction data sets for full-length SecDF were collected at beamline **BL41XU**.

As shown in Fig. 1(b), transmembranes (TM) 1-6 and TM7-12 are assembled in a pseudo-symmetrical manner. The periplasmic surface of the TM regions is covered by the P1 and P4 domains. The P1 domain is composed of head and base subdomains. The head domain protrudes into the periplasmic space, while the two connecting loops between the head and base subdomains form a constricted region.

A comparison of the crystal structure of full-length SecDF (called the F form; Fig. 2(a)) with the model of the full-length I form, which was built by docking the base subdomain of the isolated P1 structure onto that of the full-length SecDF (Fig. 2(b)), revealed a significant difference in the head-to-base orientation of the P1 domain. This conformational transition is due to the ~120° rigid-body rotation of the head domain. *In vivo* and *in vitro* analyses using *E. coli* SecDF suggested that conformational flexibility of the essential P1 domain is required for effective protein translocation. In addition, biochemical experiments revealed that the flexible P1 domain directly interacts with unfolded proteins, such as preproteins.

The TM arrangement of SecDF is similar to that of multidrug efflux transporter AcrB [5]. Homotrimeric AcrB extrudes a variety of drugs by utilizing the proton gradient across the membrane, whereas SecDF functions as a monomer associated with the Sec translocon. We propose that the conserved Asp and Arg residues at the transmembrane interface between the TM1-6 and TM7-12 bundles (Fig. 1(b)), in a similar manner to the conserved charged residues of AcrB, play essential roles in the movements of protons and preproteins. The mutants in which the

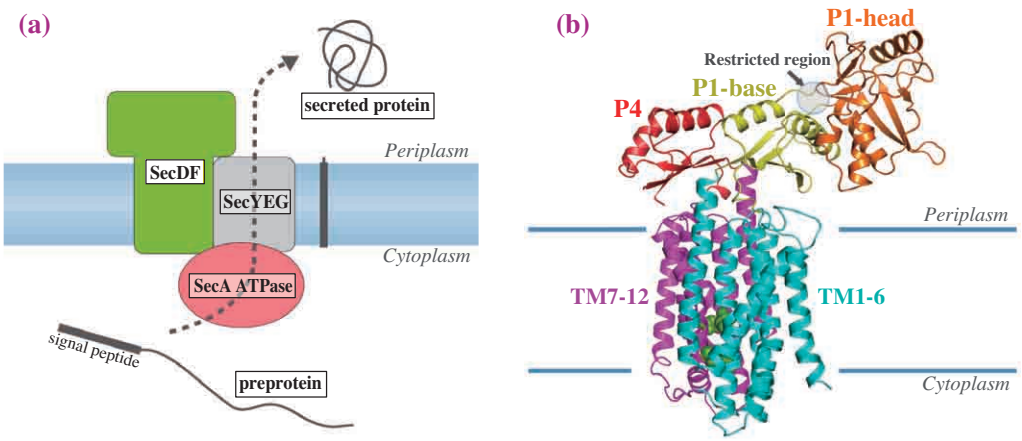

Fig. 1. **(a)** Protein translocation *via* Sec machinery. **(b)** Crystal structure of SecDF.

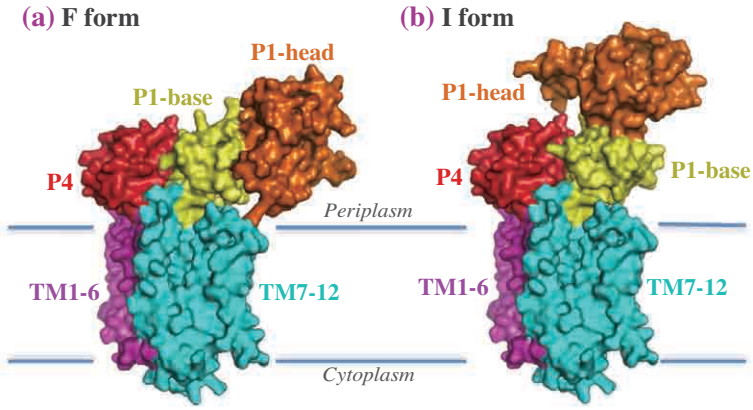

Fig. 2. Conformational transition of SecDF. **(a)** F form, **(b)** I form.

charged residues in *E. coli* SecDF were replaced by uncharged residues lacked the SecDF activity. This observation is consistent with the hypothesis that the charged residues in the TM region of SecDF participate in proton transport. The highly conserved SecDF regions are clustered from the center of the TM to the periplasmic base region underneath the head. The hinge motion of the P1 domain is likely to be functionally related to the proton flow through the TM region of SecDF.

Taken all together, we proposed the working model of SecDF-enhanced protein translocation (Fig. 3). The Sec translocon could be just underneath the protruding P1 head domain, enabling it to interact with a translocating preprotein *via* the Sec translocon. The preprotein-capturing F form could transform to the I form, preventing the backward movement of the preprotein, which leads to acceleration of protein transport. Then, the preprotein-releasing I form could convert to the F form. By repeating this conformational transition cycle coupled with proton flow, SecDF can facilitate protein transport. Here, we did not describe functional analysis of Vibrio SecDF or the electrophysiological analyses of SecDF, as mentioned in the original paper [3], owing to space limitations. Indeed, all of our data is consistent with the idea that SecDF utilizes the proton gradient across the membrane for protein translocation. We finally propose that SecDF functions as a dynamic, membrane-integrated chaperone, which is possibly powered by the proton-motive force, to actively complete Sec translocon-mediated protein translocation. Although the crystal structural information for all of the Sec proteins, Sec translocon, SecA ATPase, and SecDF is now available, it is unclear how the Sec proteins interact with each other and form the Sec translocon complex. To fully understand the SecA-dependent protein translocation, we are still attempting to obtain much more information about the Sec translocon machinery.

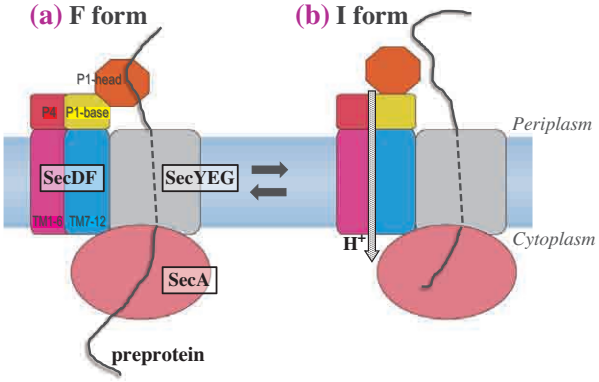

Fig. 3. Working model of PMF-driven protein translocation by SecDF. **(a)** F form, preprotein-capturing state. **(b)** I form, preprotein-releasing state.

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