



## The mechanism of protein export enhancement by the SecDF membrane component

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**Protein transport across membranes is a fundamental and essential cellular activity in all organisms. In bacteria, protein export across the cytoplasmic membrane, driven by dynamic interplays between the protein-conducting SecYEG channel (Sec translocon) and the SecA ATPase, is enhanced by the proton motive force (PMF) and a membrane-integrated Sec component, SecDF. However, the structure and function of SecDF have remained unclear. We solved the first crystal structure of SecDF, consisting of a pseudo-symmetrical 12-helix transmembrane domain and two protruding periplasmic domains. Based on the structural features, we proposed that SecDF functions as a membrane-integrated chaperone, which drives protein movement without using the major energetic currency, ATP, but with remarkable cycles of conformational changes, powered by the proton gradient across the membrane. By a series of biochemical and biophysical approaches, several functionally important residues in the transmembrane region have been identified and our model of the SecDF function has been verified.**

**Key words:** Sec translocon, SecDF, protein transport

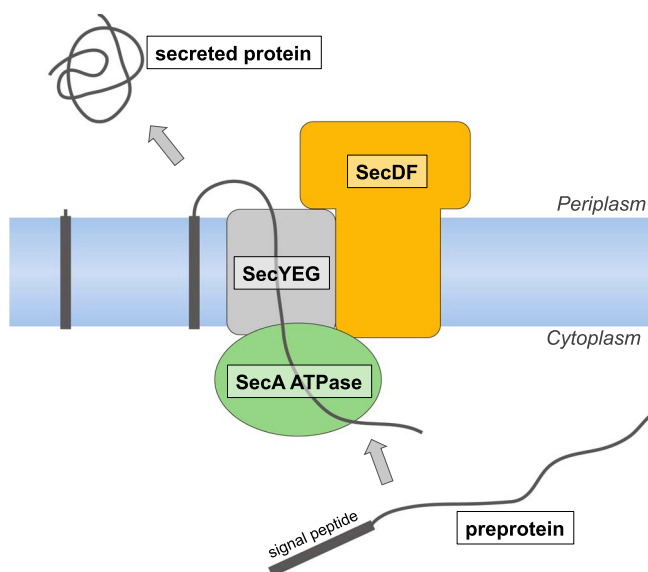
Newly synthesized preproteins that contain a N-terminal cleavable signal peptide for secretion are targeted to the membrane and then translocated across it<sup>1,2</sup>. The Sec translocon provides a channel-like pathway for protein translocation across membranes, which normally prevent even the

permeation of small ions. The bacterial Sec translocon, consisting of three membrane proteins, SecY, SecE and SecG, is embedded within the cytoplasmic membrane and forms a protein-conducting channel to export proteins from the cytosol to the periplasmic space. In eukaryotic cells, the corresponding process across the ER membrane is achieved by the SecYEG-homolog Sec61 $\alpha\gamma\beta$  machinery. To elucidate the molecular mechanism of the Sec translocon machinery, structural and functional studies of Sec proteins have been performed. Although there is an hourglass-shaped pathway in the center of SecY for protein transport<sup>3,4</sup>, the Sec translocon does not function by itself. SecYEG transiently interacts with SecA ATPase and SecDF (Fig. 1). All of the Sec proteins are considered to function through large conformational changes during protein translocation *via* the translocon. Synthesized preproteins, which are prevented from folding in the cytosol by a chaperone such as SecB, are directed to the membrane-bound SecA. Conformational changes of SecA, accompanied by ATP hydrolysis cycles, then achieve the insertion of the preprotein into the Sec translocon<sup>5,6</sup>. Using repeated ATP-induced conformational changes, SecA drives the stepwise protein secretion<sup>7</sup>. Although the protein translocation is enhanced by the PMF<sup>8–10</sup> and SecDF<sup>11–17</sup>, the structure and role of SecDF have remained unclear. In this review, we describe the first 3D structure of SecDF and propose a new working model of the PMF-driven protein translocation by SecDF, based on its structural features and subsequent functional analyses<sup>18–20</sup>.

### Role of SecDF

The functional importance of SecDF in protein translocation as well as membrane protein biogenesis was previously shown *in vivo*. A SecDF-deficient *Escherichia coli* strain is

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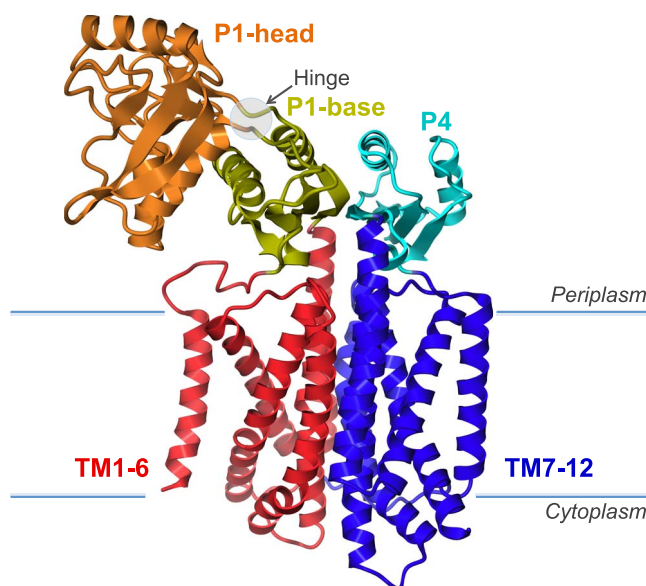
**Figure 1** Protein translocation *via* the Sec translocon complex.

severely defective in protein export at any temperature and cold sensitive for growth. SecDF is proposed to facilitate the preprotein release to the periplasmic side of the membrane in the later stage of translocation. Since the exact role of SecDF in protein translocation has not yet been defined, we examined whether SecDF functions in the steps subsequent to the initiation of translocation, using an *in vitro* reaction system<sup>21</sup>. As a result, the initiation of translocation required SecA ATPase, whereas the later steps occurred even in the absence of ATP, and were dependent on the presence of both SecDF and PMF. These results show that SecDF is involved in the PMF-driven progression and completion of protein translocation.

### Architecture of SecDF (F form and I form)

The crystal structure of full-length SecDF, consisting of a 12-helix transmembrane domain and two protruding periplasmic domains, the P1 and P4 domains, was determined at 3.3 Å resolution by a single-wavelength anomalous dispersion analysis (Fig. 2; PDB ID 3AQP). TM1–6 and TM7–12 are assembled pseudo-symmetrically. The periplasmic surface of the TM regions is covered by an anti-parallel  $\beta$ -sheet, which is formed by eight-strands of the P1 and P4 domains. The P1 domain is divided into head and base subdomains. The P4 domain is structurally homologous to the P1 base subdomain, and both are assembled in a pseudo-symmetrical manner similar to that of the TM region. The characteristic P1 head domain protrudes into the periplasmic space, while the two loops connecting the head and base subdomains form a constricted region.

A comparison of the full-length crystal structure (called the F form; Fig. 3a) with the model of the SecDF structure, which was built by superimposing the base subdomain of



**Figure 2** Crystal structure of SecDF. SecDF consists of a pseudo-symmetrical 12-helix transmembrane domain (TM) and two protruding periplasmic domains (P1 and P4).

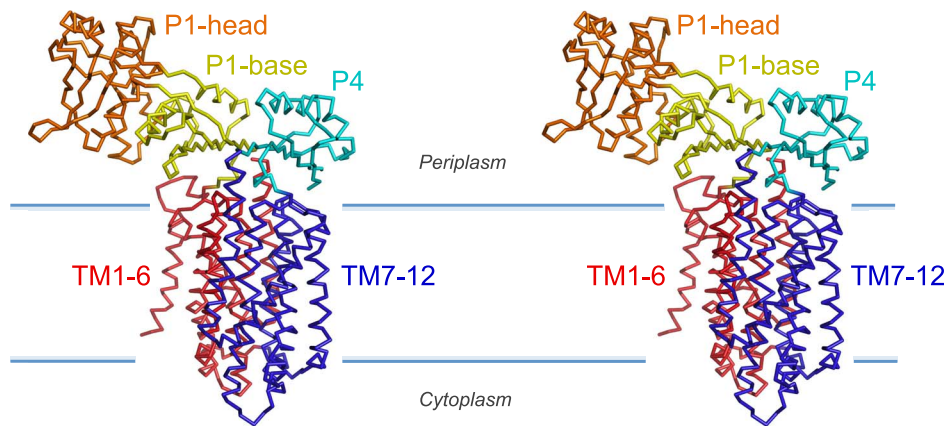
the isolated P1 structure (PDB ID 3AQO) onto that of the full-length SecDF (called the I form; Fig. 3b), revealed a significant difference in the head-to-base orientation of the P1 domain. This conformational change is due to the  $\sim 120^\circ$  rigid-body rotation of the head domain, which is attributed to a hinge motion at the connecting loops between the head and base domains.

*In vivo* and *in vitro* analyses using *E. coli* SecDF revealed that the head domain is functionally essential and that the conformational flexibility of the P1 domain is required for effective protein translocation. In addition, biochemical experiments suggested that the flexible P1 domain directly interacts with unfolded proteins, such as preproteins. Taken together, the conformational changes induced by preprotein-binding to the P1 domain could contribute to the enhancement of protein translocation.

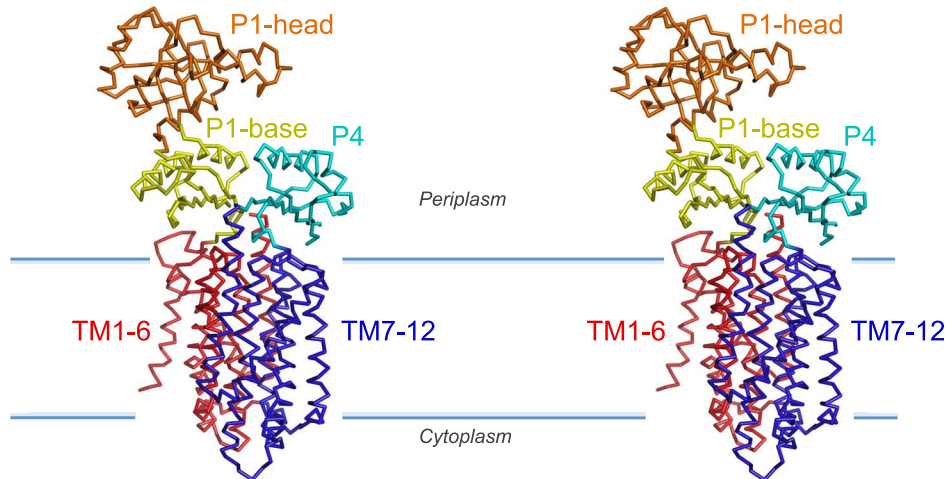
### SecDF driven by proton flow

The TM arrangement of SecDF is similar to that of a member of the RND superfamily<sup>22</sup>, Multi-Drug Efflux Transporter AcrB<sup>23</sup>. Homotrimeric AcrB extrudes a variety of drugs by utilizing the proton gradient across the membrane, whereas SecDF functions as a monomer associated with SecYEG. We propose that the conserved Asp and Arg residues at the transmembrane interface between the TM1-6 and TM7-12 bundles (Fig. 4), in a similar manner to the conserved charged residues of AcrB<sup>24</sup>, play essential roles in the movements of protons and preproteins. The mutants in which the charged residues in *E. coli* SecDF were replaced by uncharged residues lacked the SecDF activity. This

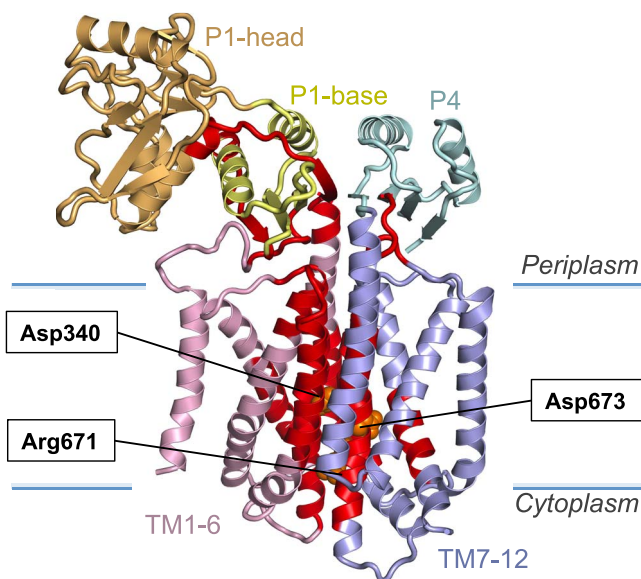
## (a) F form



## (b) I form



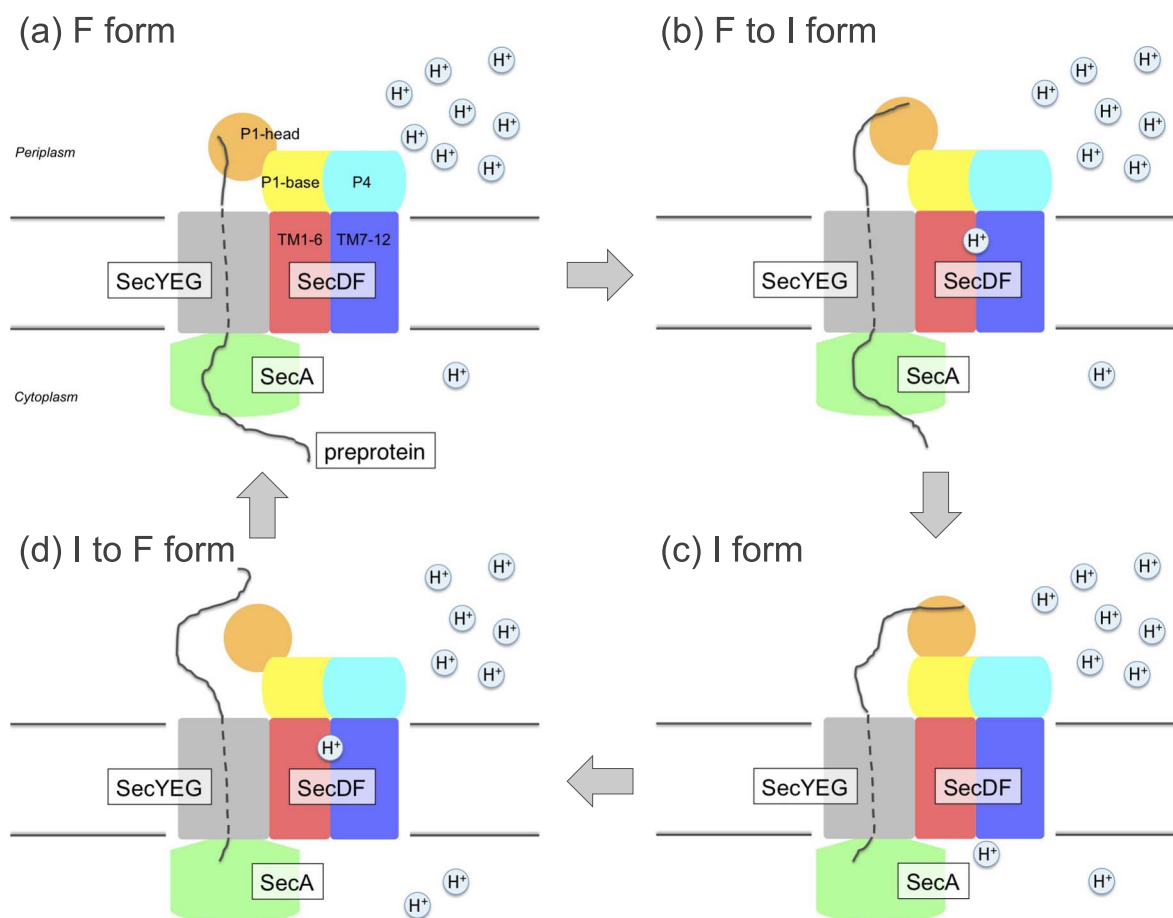
**Figure 3** Conformational transition of SecDF. Stereo-views of the F form (a) and the I form (b).



observation is consistent with the hypothesis that the charged residues in the TM region of SecDF participate in proton transport. Furthermore, function analysis of *Vibrio alginolyticus* SecDF demonstrated that a cation gradient across the membrane was required for the SecDF activity.

To verify that SecDF conducts protons, we performed biophysical analyses. The SecDF-dependent channel activities were detected by patch clamp experiments. The ion-conduction was regulated by a proton gradient and by a binding of an unfolded protein to SecDF. We also confirmed SecDF-dependent proton import using a proton-specific fluorescence probe. The highly conserved SecDF regions<sup>25</sup> are clustered from the center of the TM to the periplasmic base region underneath the head (Fig. 4). SecDF mutants

**Figure 4** Conserved residues of SecDF. The conserved regions of SecDF are shown in red. Functionally important, conserved residues are colored orange in a sphere representation.



**Figure 5** Working model of PMF-driven protein translocation by SecDF. (a), F form, preprotein-capturing state. (b), F form to I form, preprotein-holding state (c), I form, preprotein-releasing state, (d) I form returned to F form.

lacking the flexible P1-head domain were defective in not only the enhancement of protein translocation but also the ion-conductance signals in the patch clamp assay. The hinge motion of the P1 domain is likely to be functionally related to the proton flow through the TM region of SecDF. Consequently, we conclude that the proton gradient across the membrane is the driving force for SecDF function.

### A working model of SecDF-enhanced protein translocation

Based on its structural features and subsequent functional analyses, we verified the following SecDF functions: (1) The driving force for the achievement of ATP-independent protein translocation by SecDF is the PMF. (2) The TM region of SecDF participates in proton transport and contains several essential hydrophilic residues. (3) The P1 domain of SecDF, which interacts with an unfolded protein, undergoes functionally important structural alterations. Taken together, we propose a working model of SecDF-mediated enhancement of protein translocation (Fig. 5). SecYEG could reside just beneath the protruding head domain of

SecDF (Fig. 5a), enabling it to capture a translocating preprotein *via* the translocon channel. The protein-capturing F form could then change to the I state, preventing the backward movement<sup>26</sup> of the substrate and driving the forward movement (Fig. 5b). Thus, SecDF can actively assist with protein translocation. The conversion from the I to F form could occur after the bound protein is released from the I form (Fig. 5c, d). The reformed F form can bind to the more C-terminal part of the substrate. By repeating this conformational transition cycle coupled with proton flow, SecDF can enhance protein transport.

### Concluding remarks

In this study, we elucidated the mechanism of protein translocation enhancement by SecDF. However, further studies will be needed to define the exact proton pathway in SecDF and to reveal the mechanism of energy transduction, by which the proton flow-induced conformational transition in the TM region is converted into the motion of the P1 head, which presumably occurs within the conserved region of SecDF. 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. Visualization of the dynamic Sec machinery will require further structural-based mechanistic studies as well as the full complex structure of the Sec machinery.

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