Conformational Transition of Sec Machinery Inferred from Bacterial SecYE Structures

Most proteins synthesized in the cytoplasm are secreted across or integrated into membranes. The protein-conducting channel, called Sec translocon, provides a channel-like pathway for protein translocation [1]. In bacteria, the core component of the translocon complex is composed of SecY, SecE and SecA ATPase that drives the protein movement across the membrane (Fig. 1(a)). In 2008, we determined the first crystal structure of the bacterial Sec translocon at 3.2 Å resolution and proposed the initiation mechanism of the SecA-driven protein translocation from structural-based functional analysis [2].

Although the three-dimensional structure of the Sec translocon had not been solved for a long time, the first structure of the SecY complex, archaeal SecYEβ, reported in 2004 provided a lot of findings about the Sec machinery [3]. As shown in Fig. 1(b), the transmembrane (TM) 1-5 and TM6-10 of SecY are arranged like a 'clam shell' and SecE backs up the architecture. There is an hourglass-shaped pathway in the center of SecY for protein transport, which is blocked by a 'plug helix'. During protein translocation, conformational changes have been suggested, such as removal of the plug and expansion of the inner space of SecY. Because the archaeal SecYEβ structure was in a closed state with the plug blocking the channel and archea lacks SecA, the structure determination of different states of the Sec translocon, as well as that of the bacterial Sec translocon, is crucial to fully understand the SecA-dependent pathway. We collected the X-ray diffraction data of SecYE from a SecA-containing organism, *Thermus thermophilus*, at 3.2 Å resolution at beamline **BL41XU** and determined its crystal structure after seven years of trial and error. The structure, solved as a complex with an anti-SecY Fab fragment, is similar to that of the archaeal SecYEβ, except that it has a novel crevasse on the cytoplasmic side (Fig. (2)). The Fab interacts with the protruded cytoplasmic parts to which SecA binds, which raises a possibility that Fab mimics SecA to interact with SecY. Structural comparison with the closed form (archaeal SecYEβ) revealed that expanding the TM2-TM8 distance creates the crevasse that is composed of highly conserved hydrophobic residues. Interestingly, this region just corresponds to a potential signal peptide-binding site. Here, we named the Fab-bound form of SecYE as 'pre-open' state. Molecular dynamics and disulphide mapping analysis suggested that SecA binding induces the conformational changes from the stably closed state to the pre-open state.

From site-specific cross-linking analysis based on the structures of *T. thermophilus* SecYE and SecA, a normally embedded conserved region of SecA, Motif IV, interacts with the protruded region of SecY (Fig. 3(a) dashed line). The observation that the interaction physically separates NBF1 (ATPase domain) and IRAa (regulatory domain of ATPase activity) of SecA excellently explains the Sec translocon-mediated stimulation of the ATPase activity. Taken all together, we proposed the model of SecY and SecA interactions in the first stage of protein translocation (Fig. 3(a)); first, SecY and SecA specifically interact with each other; next, the binding simultaneously induces their cooperative conformational changes, transformation of SecY from the closed to pre-open form and exposure of Motif IV of SecA; finally, after the transfer of the pre-protein with its signal sequence to the hydrophobic crevasse of SecY, the protein translocation starts. We are currently performing experiments to verify whether the model is reasonable.

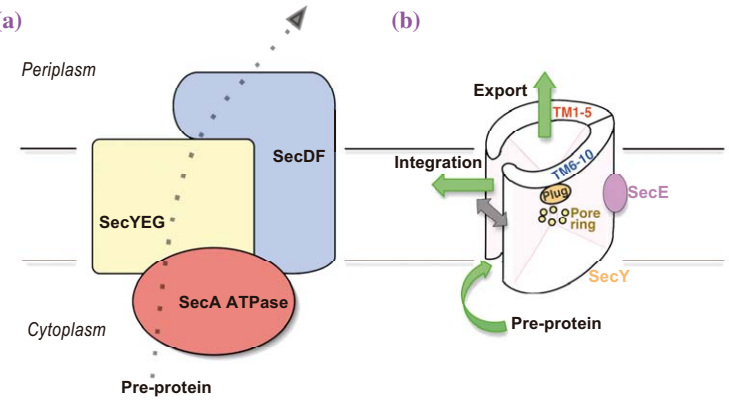

Fig. 1. Protein translocation *via* Sec machinery. **(a)** Sec translocon membrane protein complex, **(b)** Architecture of hourglass-shaped SecY complex.

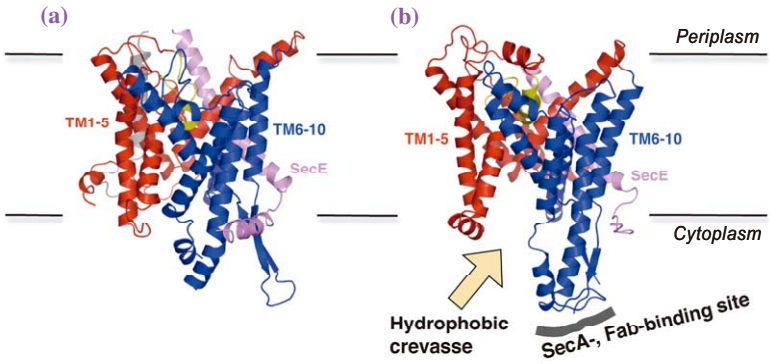

Fig. 2. Crystal structures of Sec translocon. **(a)** Closed form (Archae SecYEβ), **(b)** Pre-open form (*T. thermophilus* SecYE).

In the same issue as our original paper [2], the structure of the SecYEG-SecA complex (4.5 Å) was reported [4]. In that article, conformational changes of SecY and SecA were also observed. The pre-protein binding domain of SecA formed the 'clamp' that was located just above the channel pore of SecY. On the other hand, the clam shell architecture of SecY was slightly opened more than the pre-open form. This structural analysis suggested that the IRA1 of SecA repeatedly pushes the pre-protein into the pore of SecY, which drives protein transport [Fig. 3(b) red arrow]. Our proposed model in Fig. 3(a) may represent the conformational changes of Sec translocon prior to the stage shown in Fig. 3(b).

In Fig. 3(a), we represented the model that dimeric SecY complexed with monomeric SecA functions. Here, we did not discuss the oligomeric state of Sec proteins owing to space limitations, whereas it still remains unclear. To fully understand the SecA-dependent protein translocaiton, we are attempting to obtain much more information about the Sec translocon machinery. It is endlessly interesting [5].

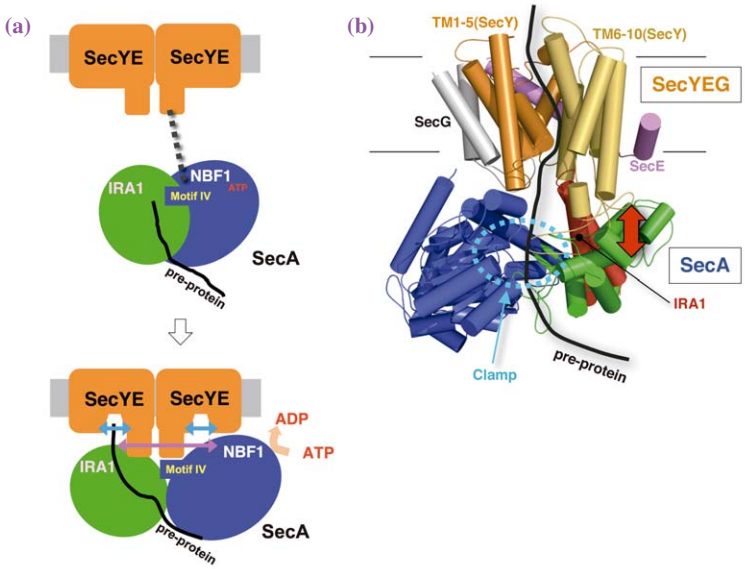

Fig. 3. SecA-SecY interactions. **(a)** Our proposed model of the protein translosation. Both SecA and SecY components undergo conformational changes of their interactions. **(b)** SecA-driven protein translocation model inferred from the crystal structure of SecA-SecYEG complex. SecA is shown in red, green and blue. SecY, SecE and SecG are shown in orange, pink and gray, respectively.

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References

[1] T.A. Rapoport: Nature **450** (2007) 663.
[2] *T. Tsukazaki, H. Mori, S. Fukai, R. Ishitani, T. Mori, N. Dohmae, A. Perederina, Y. Sugita, D.G. Vassylyev, K. Ito and O. Nureki: Nature **455** (2008) 988.*
[3] B. van den Berg *et al.*: Nature **427** (2004) 36.
[4] J. Zimmer *et al.*: Nature **455** (2008) 936.
[5] *H. Mori and T. Tsukazaki: Tanpakushitu Kakusan Koso **54** (2009) 685 (in Japanese).*