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Molecular mechanisms of the enhancement of protein export by the membrane protein complex SecDF

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Research aims

The SecYEG translocon and the SecA ATPase cooperate to facilitate protein export across the bacterial cytoplasmic membrane. In addition to these essential core components, SecDF, a complex containing two membrane-integrated Sec factors, plays important roles in efficient protein export *in vivo*. We determined the crystal structure of SecDF from *Thermus thermophilus* at 3.3 Å resolution and proposed a working hypothesis based on biochemical and biophysical studies that were designed using the protein structure (See Figure 1).[1] According to the model, SecDF forms a complex with the SecYEG translocon, captures a substrate polypeptide emerging from the translocon by its P1 (the first periplasmic) domain and undergoes conformational changes by using the proton motive force (PMF) to facilitate forward movement of the polypeptide. However, modes of interaction between SecDF and Sec-related factors (including SecYEG) and substrate polypeptides remain largely unknown. To elucidate these mechanisms, we performed systematic site-directed *in vivo* photo-cross-linking analysis targeted to *Escherichia coli* SecD.

Methods

The site-specific *in vivo* photo-cross-linking procedure developed by Schultz's group is an innovative method that allows for analysis of transient protein-protein interactions that occur in living *E. coli* cells.[2] In this system, a photoreactive amino acid, *p*-benzoyl phenylalanine (pBPA), can be incorporated at any position in a target protein using an engineered tRNA synthetase and mutated tRNA pair, and interactions can be detected as UV irradiation-dependent generation of cross-linked products. Previously, we have successfully applied this method to identify SecA neighboring sites in SecY.[3] Based on the *Thermus thermophilus* SecDF (TtSecDF) structure, we designed *E. coli* SecD mutations to introduce pBPA on the molecular surface of the protein. We constructed 55 SecD derivatives for cross-linking experiments. Cross-linked proteins were identified by immunoblotting analysis using specific antibodies against candidates including Sec related proteins.

Results and discussion

Our photo-crosslinking experiments gave the following results:

1) ***Cross-linking with SecF (colored in magenta in Fig. 2)***
When pBPA was introduced at several positions in the SecD transmembrane regions located at the interface with SecF on the TtSecDF crystal structure, cross-linked products between SecD and SecF were generated. This supports the idea that *E. coli* SecDF assumes a conformation similar to TtSecDF in the membrane.

2) ***Cross-linking with periplasmic chaperones DegP and Skp (in red, Fig. 2)***
SecD derivatives containing pBPA in an alpha helix in the P1 domain facing the periplasmic space generated several cross-linked bands. We identified partner proteins as Skp and DegP, periplasmic chaperones that prevent aggregation of newly translocated outer membrane proteins and facilitate their delivery to the outer membrane. An interest-

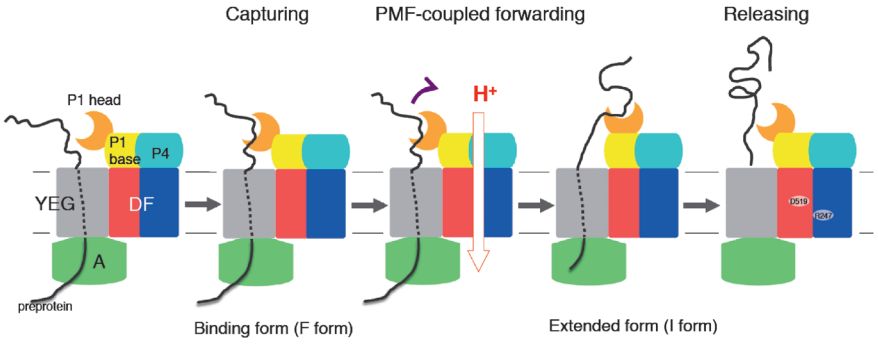

Fig. 1. Working model for SecDF-mediated facilitation of protein translocation

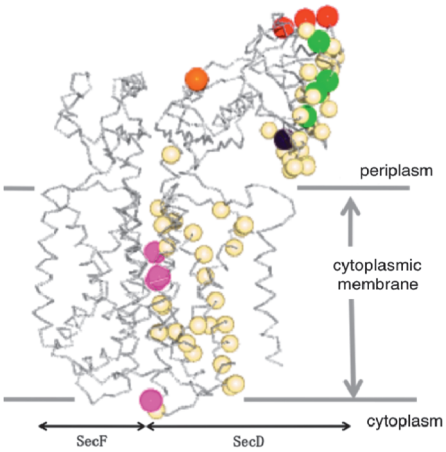

Fig. 2. Summary of site-directed *in vivo* photo-cross-linking experiments targeted to *E. coli* SecD. TtSecD amino acid residues corresponding to those in *E. coli* SecD that generated cross-linked products when substituted with pBPA are shown as colored spheres on the TtSecDF crystal structure (F form). Spheres colored in magenta, red, green, and orange indicate amino acid residues that are in close proximity to SecF, periplasmic chaperones (DegP and Skp), maltose binding protein and P1 head subdomain in I form, respectively. Spheres in white show amino acid resides that generated no cross-linked product when substituted with pBPA.

ing hypothesis is that the P1 domain could act not only as a binding site for a translocating substrate (see below), but also as a docking platform for the periplasmic chaperones to promote translocation and folding of the substrate polypeptide.

3) *Cross-linking with maltose binding protein (MBP) (in green, Fig. 2)*

When SecD derivatives having pBPA in a narrow crevasse in the P1 domain were used, SecD-MBP cross-linked products were formed. Although it remains to be shown whether this cross-linking represents interaction of translocating MBP with SecD, our results raise the possibility that the crevasse in the P1 domain functions as a binding site for translocating substrate polypeptide chains.

4) *Intramolecular cross-linking (in orange, Fig. 2)*

The P1 domain is composed of head and base sub-domains. Our previous biochemical studies showed that SecD could assume two different conformations, designated as I and F, by rigid body movement of the P1 head subdomain. Upon UV-irradiation, the SecD (Arg268pBPA) mutant generated a band that migrated slightly slower than SecD. This mutant protein was presumably formed by intra-molecular cross-linking that occurred when the P1 domain was in the form I conformational state. Interestingly, when cells expressing this SecD derivative were treated with CCCP (a proton ionophore) prior to UV-irradiation to collapse the PMF across the membrane, the amount of cross-linked product was dramatically reduced. Furthermore, the introduction of an additional SecD or SecF mutation that abolished both the proton conductance and the protein export-enhancing activities of SecDF resulted in almost complete disappearance of the cross-linked product. These results are in agreement with our model, which suggests that proton conductance of SecDF couples with the movement of the P1 domain.

Conclusion

We succeeded in identifying several SecD-neighbor factors by systematic, site-directed *in vivo* photo-cross-linking analysis targeted to *E.coli* SecD. These results strongly support our model of SecDF function. Further study will provide us with important insights into the molecular mechanisms of PMF-driven SecDF function.

References

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3) Mori, H. and Ito, K. (2006) Different modes of SecY-SecA interactions revealed by site-directed in vivo photo-cross-linking. *Proc. Natl. Acad. Sci. USA.* **103**: 16159–16164