

Break on through to the other side – the Sec translocon

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The transfer of newly synthesized polypeptides across the membrane occurs at the translocon. Although the central role of the translocon in protein translocation is understood, much regarding the manner by which these membrane protein complexes function remains to be learned. The recent solution of the crystal structure of a Sec complex, together with other biochemical, biophysical and structural findings, suggests that answers might be forthcoming.

Proteins designated to reside outside the cytoplasm must, at some point, traverse a lipid-based membrane. Throughout evolution, signal sequence-bearing secretory preproteins as well as integral proteins are transported across the prokaryal plasma membrane or eukaryal endoplasmic reticulum membrane at translocons, which are protein complexes based on SecYEG/Sec61 $\alpha\beta\gamma$ heterotrimers. Although great advances have been made in elucidating the manner by which translocating proteins are recognized, targeted to translocons and ultimately delivered across the membrane, outstanding questions remain. Of these, those of most pressing concern are the oligomeric status, regulated assembly and dynamic behaviour of translocons.

The Sec complex revealed

Based on numerous biochemical, biophysical and electrophysiological studies, it has long been predicted that the translocon serves as the channel through which protein cargo traverses the membrane [1–6]. However, with the solution of the 3D structure of the SecYE–Sec61 β complex from the archaea *Methanococcus jannaschii* by Rapoport and co-workers [7], structural support for these earlier claims has finally arrived.

The organization of the complex is such that SecY is bundled into two halves that are held together by the SecE subunit, with the Sec61 β subunit on the periphery of the SecYE core. Together, these components give rise to a structure best described as an ‘hour glass’. When viewed from the cytoplasmic face, the complex presents a funnel-like space that tapers and eventually constricts towards the middle of the molecule (Figure 1). A similar funnel shape is assumed by the second half of the complex, but here a small SecY transmembrane domain provides a loop that is thought to act as a plug, restricting passage through the core of the complex. Movement of the plug would, therefore, yield a continuous aqueous channel through which a translocating preprotein could pass. By shifting the position of the SecY transmembrane helices that come

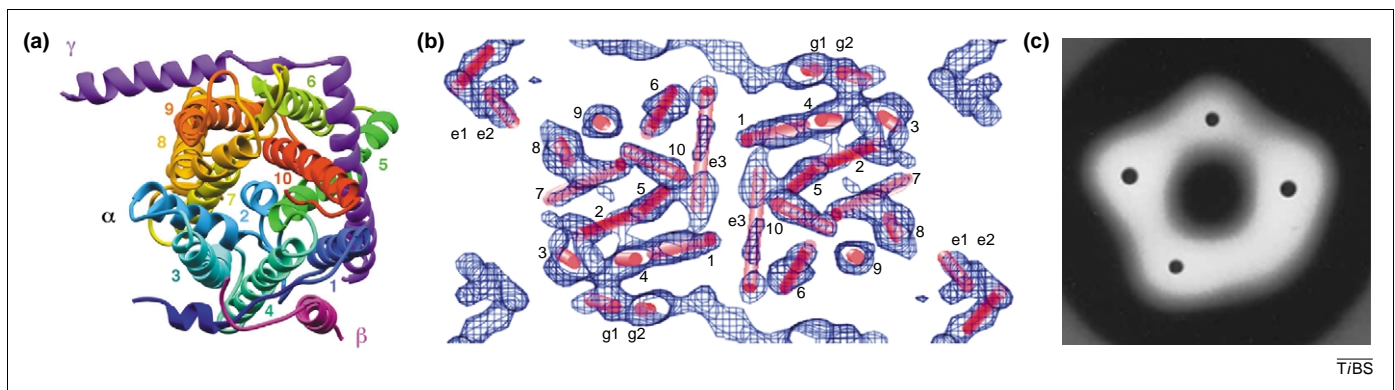


Figure 1. Different views of the oligomeric state of the Sec translocon. **(a)** The 3D structure of the *Methanococcus jannaschii* SecYE β complex, as viewed from the top, with SecY helices numbered and differentially coloured. In the crystal, portions of transmembrane domain 2, shown in blue, serve as a plug in the protein-conducting channel. **(b)** 8-Å 3D structure derived from tilted 2D crystals of purified *Escherichia coli* SecYEG, viewed from the top. The twofold symmetry of the Sec translocon is presented. The helices within the monomers are shown in red and numbered as reported in Ref. [17]. The membrane-spanning domains of SecY are denoted by numbers alone, whereas transmembrane domains of SecE and SecG are denoted by e1–3 and g1–g2, respectively. The assignment of SecE and SecG membrane-spanning domains 1 and 2 is arbitrary. **(c)** Low-resolution averaged projection map of the purified canine Sec61p complex. The ring structure of the Sec translocon shown is thought to comprise four Sec protomers, denoted by black dots on the ring. Part (a) reproduced, with permission, from Ref. [7] (www.nature.com). Part (c) reproduced, with permission, from Ref. [11].

together at the narrowest portion of the channel, various degrees of secondary structure in a transiting preprotein could be tolerated. A groove situated between transmembrane domains lying at the edges of the two SecY halves is postulated to provide the site for the signal sequence and would also provide the exit route for transmembrane domains of translocating membrane proteins. Finally, although many questions related to archaeal protein translocation remain unanswered [8], the evolutionarily conserved nature of the Sec complex suggests the *M. jannaschii* structure to be representative.

How many Sec complexes does it take to cross a membrane?

The structure-based prediction that a single copy of the SecYE β complex forms the protein-conducting pathway is in agreement with previous biochemical work. Relying on analysis of isolated translocation-arrested preprotein >intermediates, the translocation channel has been reported to be formed from a single SecYEG complex [9,10]. Hence, it is paradoxical that earlier results showed the translocon to be an oligomer of Sec complexes. Low-resolution electron microscopy images of purified mammalian, yeast and bacterial Sec complexes have all revealed ring-like structures made up of three or four Sec61 $\alpha\beta\gamma$ /SecYEG heterotrimers surrounding a ~20-Å indentation that is postulated to represent the translocation pore [11–13]. Similarly, the multimeric, ring-like organization of the translocon was also suggested by 3D reconstructions of the ribosome–translocon complex [14–16]. However, a lower-order oligomeric status of the translocon has been observed. Blue-native polyacrylamide gel electrophoresis (PAGE), cysteine-scanning analysis, and electron-density maps generated from tilted 2D crystals all suggest a dimeric organization of the bacterial SecYEG complex [17–19]. In the 8-Å projection structure [17], the dimeric translocon does not present a ring-like structure but it does contain a central cavity that is closed on its periplasmic face by two highly tilted SecE transmembrane helices from adjacent protomers. Thus, it has been proposed that either the pore-like structure formed by the tetramer or the cavity observed within the dimer, in fact, reflect a depression at the interface of the protomers rather than a true translocation channel [7].

Whereas the SecYE β structure predicts the monomer to comprise the translocon, it still remains to be shown that an active monomeric form of the Sec complex even exists in the membrane. Indeed, recent fluorescence resonance energy transfer (FRET) analysis has revealed the strong tendency of the Sec complex to oligomerize within the lipid bilayer [20]. However, if a single Sec protomer within the oligomer proves to be sufficient for formation of the protein-conducting channel, one can then ask what function the oligomer would serve. This question is pertinent not only to

protein translocation, but also to other membrane transport systems in which a similar paradox exists [21].

The dynamic translocon

The availability of a 3D structure of the Sec complex not only provides fuel for further investigations into the quaternary structure of the translocon, but also offers insight into the dynamic behaviour of the protein-translocating channel. Indeed, the structural results, together with several recent biochemical and biophysical investigations, suggest that the Sec complex undergoes a variety of transitions during the translocation event both at the level of the channel and in terms of its oligomeric status.

At the protomeric level, mechanistic predictions based on the SecYE β structure suggest the complex pore to be of variable diameter. It is thought that by shifting the positions of helices lining the pore, a wider passage could be obtained. This concept is supported by analysis of *prl* mutants, which are a series of mutations that up-regulate the activity of the translocon and are localized to residues lining the pore to possibly maintain the complex in its wider open state. Moreover, movement of SecE, which acts as a clamp around SecY, could modify the size of the translocation pore. The ability of molecules of different sizes to gain access into the aqueous translocon pore during translocation has also led to the conclusion that the pore does not retain a fixed diameter [6].

Dynamic considerations also come into play when considering the quaternary structure of the translocon. It has been shown that SecYEG dimers reversibly dissociate into monomers in a detergent-dependent manner [18] and that an equilibrium between Sec tetramers and monomers exists in detergent solution, as detected by analytical centrifugation [22]. Although this effect takes place outside the membrane environment, it illustrates the potentially dynamic association of Sec protomers. By contrast, FRET experiments have failed to observe any exchange of the protomeric components within the membranous oligomer [20]. These results argue that the translocon might not experience rearrangements in its oligomeric status during the translocation event *per se*, yet, the seemingly loose association between monomers comprising the oligomer suggests that translocation-related dynamic changes could occur.

Such changes could lie behind the observed translocation-dependent assembly of higher-order Sec complex multimers. Analysis of proteoliposome-reconstituted, detergent-solubilized, dimeric SecYEG revealed that binding of SecA – the ATPase that drives bacterial translocation – could lead to the recruitment of two SecYEG dimers to form the circular tetrameric SecYEG complex [13]. Similarly, reconstitution of the mammalian translocon ring structure required the presence of ribosomes, apparently for recruitment of individual Sec61 complexes [11]. The ability of SecA or ribosomes to orchestrate translocon assembly recalls the signal hypothesis postulated by Blobel and Dobberstein [23], who predicted that a transient tunnel for protein passage

across the membrane would form in response to the presence of a signal peptide.

Meshing the past with the present

The availability of an atomic structure of the Sec complex has greatly challenged our thinking of the translocon and requires that many previously held views be revisited. For instance, in agreement with EM-derived models of the ribosome-translocon junction [14–16], the cytoplasmic SecY loops thought to form the ribosome-binding site in the *M. jannaschii* SecYE β structure seemingly fail to present a surface that could form a tight seal with a bound ribosome. This is inconsistent with the results of protease accessibility and fluorescence-based studies, which predict the existence of a tight seal between the ribosome and the translocon [5,24]. Such differences could, however, be explained by the participation of additional proteins at the ribosome–translocon contact site. Given the tapered structure and dimensions of the SecYE β complex, it is difficult to imagine how SecA could deeply penetrate into and across the plane of the membrane during bacterial translocation [25]. Still, it should be stressed that the *M. jannaschii* structure is apparently in the closed state. It is conceivable that the structure of the open, translocation-active and/or oligomeric translocon will be consistent with these earlier observations.

What's next?

With the availability of advanced biochemical tools for the analysis of translocon function together with high-resolution structural approaches, it should be possible to catch the translocon 'in the act' of receiving, translocating and releasing its preprotein cargo, thereby, enabling detailed examination of the changes experienced by the translocon at each step of the translocation event. Such studies will also provide insight into the dynamic interactions of the translocon with other components of the translocation machinery, such as the ribosome, elements of the signal recognition particle pathway and SecA. Indeed, the first view of the translocon structure not only represents a milestone in the translocation field, but also serves as a starting point for future studies that are designed to overcome present barriers in our understanding of protein translocation.

Acknowledgements

Support comes from the Israel Science Foundation (grant 433/03 to J.E.) and from the CNRS (F.D.).

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