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A snapshot of membrane protein insertion

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The cytoplasm is the main place for protein translation from where nascent proteins are transported to their working areas, including the inside, outside, and membrane of the cell. The majority of newly synthesized membrane proteins is co-translationally inserted into the membrane by the evolutionary conserved Sec translocon. In this issue of *EMBO Reports*, Kater *et al* [1] use single-particle cryo-electron microscopy to visualize a high-resolution structure of the *E. coli* SecYEG translocon:ribosome-nascent chain complex in a lipid environment constituted by nanodiscs. This snapshot represents an early intermediate state in membrane protein insertion and provides important information for understanding the molecular mechanism of membrane protein biogenesis.

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See also: **L Kater *et al***

The Sec translocon represents the major route for co-translational membrane protein insertion and integration. The translocating channel is constituted by the SecYEG complex in the membrane of bacteria and by the evolutionary conserved Sec61 complex in the endoplasmic reticulum membrane of eukaryotic cells. Ribosome-nascent chain complexes (RNCs) that synthesize proteins with signal sequences or N-terminal transmembrane regions are directed to and complexed with the Sec translocon, and the nascent proteins are co-translationally incorporated into the membrane. Although a variety of studies have been performed to elucidate this mechanism, the detailed architecture of the translocating complex has not been fully clarified. Of note, the Sec translocon mediates not only protein integration into membranes, but also protein translocation across membranes and is therefore referred to as the protein-conducting channel [2].

SecY, the core component of the Sec translocon, is composed of ten α-helical transmembrane (TM) regions, of which pseudosymmetrical TM1-5 and TM6-10 are assembled like a clam shell (Fig 1, lower part) [3,4]. In the quiescent state, the hourglass-shaped space located in the center of SecY is completely sealed from both sides by a plug helix of SecY itself and a loop connecting the TM1 and TM2 regions of SecG. SecY possesses a protruded loop consisting of two β strands, providing an interaction site with the ribosome. Secreted proteins penetrate the membrane perpendicularly via the Sec translocon. Proteins that need to be integrated into the lipid bilayer exit the channel through a shell border region called lateral gate. The lateral gate expands during transfer, involving large conformational changes of SecY, which are probably stabilized by the single membrane-spanning protein SecE. In *Escherichia coli*, SecE has two additional TMs at the N-terminal region with unknown functions.

The architecture of the RNC-Sec translocon complex has been investigated by cryo-electron microscopy (cryo-EM) over the past two decades [2]. The recent development of single-particle cryo-EM using a direct detector enables us to separately visualize each TM region of the Sec translocon in complex with the ribosome. Several cryo-EM structures of detergent-solubilized RNC-Sec translocon complexes were reported, but this non-physiological setup failed in many cases to accurately reflect the native SecY embedded in the membrane. In 2011, Frauenfeld *et al* [5] resolved the structure of the SecYEG complex embedded in a lipid nanodisc bound to a translating ribosome, which revealed the arrangement of TM regions within the lipid bilayer. The structure appeared to represent a later stage of membrane protein translocation, but since the resolution was not sufficient at that time to discuss the detailed molecular mechanism, higher resolution structures of the RNC-Sec translocon were awaited.

An important step in the structure determination of membrane proteins in a native-like lipid bilayer was adapting nanodiscs. Nanodisc particles formed by lipids and membrane scaffold proteins (MSPs) can keep membrane proteins in a native-like condition, enabling lipid-embedded membrane proteins to stably exist in solution without detergent (Fig 1A, lower part). Nanodisc technology utilizing MSPs was first adapted by Alami *et al* [6] for the analysis of SecYEG and also used by Kedrov *et al* to analyze the function and oligomeric state of Sec proteins [7][8]. In the current issue of *EMBO Reports*, Kater *et al* reconstituted SecYEG into ~12-nm nanodiscs formed by POPG/POPC lipids and MSP1E3D1. The nanodiscs provide a wide lipid region around SecYEG, which does not lose the interactions with the ribosome and can accommodate transmembrane regions of the substrate. Using this setup, the authors obtain the structure of lipid-embedded SecYEG bound to RNCs by single-particle cryo-EM (Fig 1B) [1].

Kater and co-workers used the N-terminal region of a single membrane-spanning protein followed by the stalling motif of TnaC as nascent chain. The TnaC peptide induces an arrest of protein translation to expose a short hydrophobic nascent chain composed of the first 48 amino acids of FtsQ (see Fig 1A upper part). The short nature of the nascent chain was sufficient to start protein insertion and could potentially trap an intermediate state of the lipid-embedded SecYEG:RNC complex. A schematic image of the structure of the SecYEG-RNC complex is shown in Fig 1A and B. The nascent chain (substrate) could be traced in the ribosomal exit tunnel and near the Sec translocon. Although the substrate density near the translocon is not as clear, each TM region of SecY could be assigned. Hence, the authors succeeded in determining an intermediate

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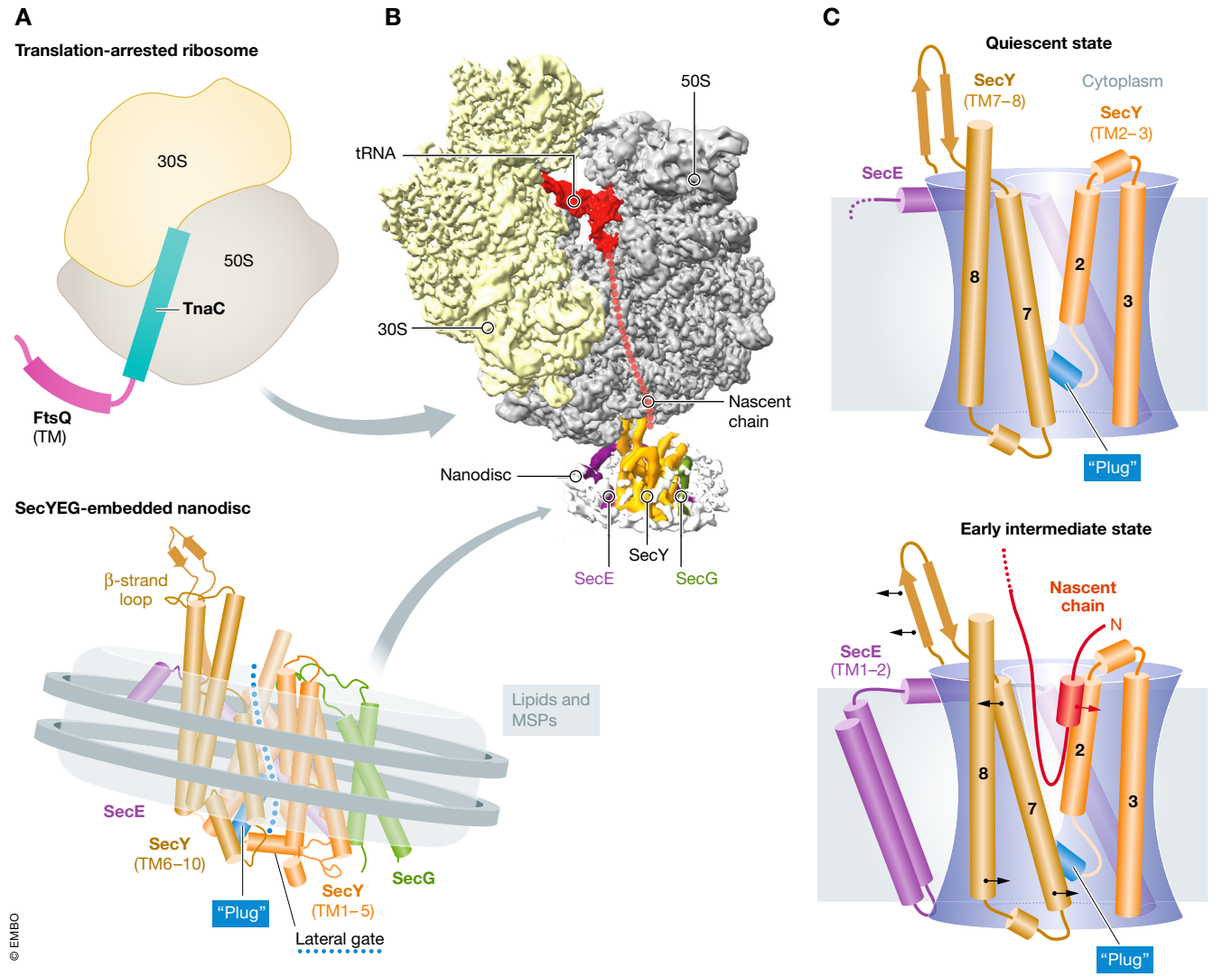

Figure 1. Cryo-electron microscopy structure of the ribosome–Sec translocon complex in an early intermediate state.
(A) Preparation of ribosome and Sec translocon to obtain an intermediate state of membrane protein insertion into the lipid bilayer. (Top) Translation of the fusion protein composed of the N-terminal FtsQ TM region and TnaC, including an arrest sequence, is paused. (Bottom) SecYEG-embedded nanodisc retains native-like lipid environment. MSPs, membrane scaffold proteins. (B) The structure of SecYEG-embedded nanodisc and ribosome-nascent chain (RNC) complex using cryo-electron microscopy. SecYEG represents an early intermediate state. The substrate map in the ribosome tunnel is identified. The substrate part outside of the ribosome is supposed to be located at the cytoplasmic side of the lateral gate. (C) Schematic representation of the quiescent state and the early intermediate state of SecYEG. Panels (B) and (C) are modified from Kater *et al* [1].

structure of membrane protein insertion. As mentioned above, some density near the Sec translocon were not clearly assigned, but is likely part of the substrate as a complementing cross-linking experiment indicated that the substrate protein is located in the cytoplasmic cavity of SecY. The substrate seems to be rather flexible during partitioning to the lipid bilayer. The map of SecG was also indistinct, suggesting that the interaction between SecG and SecY is weak. Since SecG is considered to function as a cap [4], we are looking forward to further high-resolution analysis elucidating its accrete function. The flexibility of the substrate represents an obstacle in structure determination. This can be resolved by fixing the substrate onto the Sec translocon, e.g., by disulfide bond formation, a strategy that recently allowed the determination of an intermediate step of protein translocation across the membrane by cryo-EM [9]. Ma *et al* [9] reported the structure of lipid-embedded SecYEG in complex with SecA ATPase, and the substrate. In this context, the SecYEG complex interacts with the motor protein SecA ATPase, instead of the ribosome, through the same cytoplasmic loops.

In the RNC-SecYEG structure from Kater *et al*, the cytoplasmic side of the lateral gate of SecY, which interacts with substrates, is in a pre-open state. The authors applied successive molecular dynamics simulations without the ribosome to support the notion that the interaction of ribosomes with the β-strand cytoplasmic loop of SecY induces structural changes in SecY. Interestingly, *E. coli* SecE has two

additional non-conserved transmembrane α-helices at the N-terminal region. Though this region is considered to be flexible and unnecessary, in this structure, the periplasmic region of the two helices interacts with that of SecY, which might be a clue to understanding the unknown functions of SecE. It is possible that SecE promotes the efficiency of membrane insertion and integration by interacting with the substrate protein emerging from the lateral gate. The two α-helices of the N-terminus of SecE are also more flexible in molecular dynamics simulations without ribosomes. This can be interpreted as a specific conformational change of SecE when complexed with the ribosome during membrane protein biogenesis. Moreover, the authors compare their structure of the Sec translocon in nanodiscs together with the RNC to previously reported structures of Sec translocons obtained with either X-ray crystallography or electron microscopy to get insight into the conformational transitions that occur during protein insertion. Based on this analysis, the authors propose that their structure of the nanodisc-embedded RNC-SecYEG complex represents an early intermediate state in membrane protein insertion (Fig 1C). In later steps, SecYEG would undergo large structural changes, including further expansion of the lateral gate.

In the recent years, the combination of single-particle cryo-EM and the nanodisc system became a powerful tool to study membrane proteins. For example, eight cryo-EM structures of an ATP-binding cassette exporter in a lipid environment have been reported recently, revealing the fundamental turn-over steps [10]. In the coming future, further snapshots of membrane protein integration using RNC-Sec translocon particles with substrates of different lengths will allow refining our knowledge on the dynamics of protein translocation. The continuous improvement of resolution through the development of technical and computational methods for cryo-EM will enable the field to uncover the structures of various protein translocations at various states.

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