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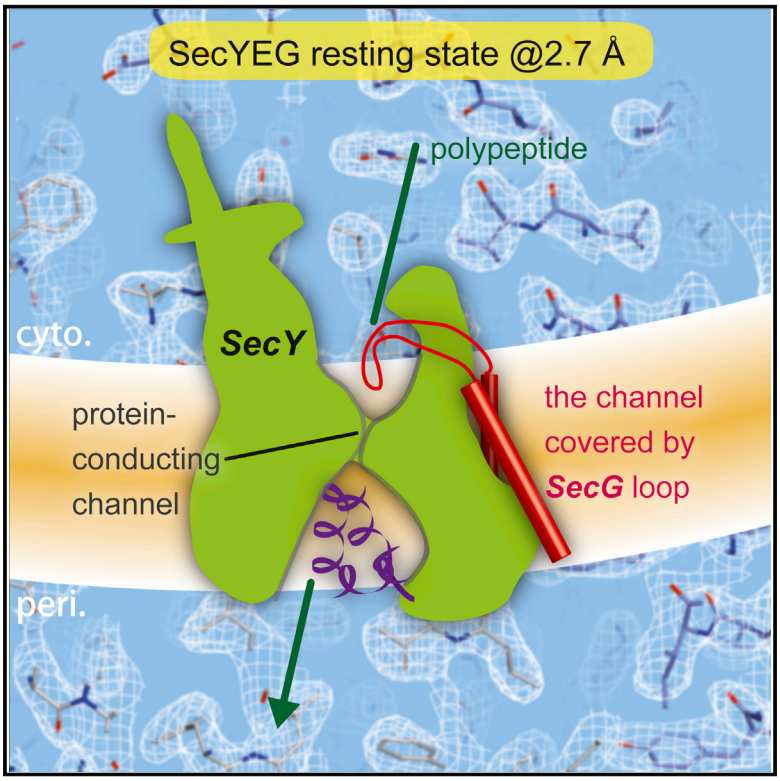
Report

Crystal Structures of SecYEG in Lipidic Cubic Phase Elucidate a Precise Resting and a Peptide-Bound State

Graphical Abstract



Authors

Yoshiki Tanaka, Yasunori Sugano, Mizuki Takemoto, ..., Yuji Sugita, Osamu Nureki, Tomoya Tsukazaki

Correspondence

nureki@bs.s.u-tokyo.ac.jp (O.N.), ttsukaza@bs.naist.jp (T.T.)

In Brief

The Sec translocon is an essential protein-conducting channel composed of SecY/E/G in bacteria and Sec61α/γ/β in eukaryotes. Tanaka et al. solve high-resolution and peptide-bound SecYEG structures in a lipid environment, providing notable insights into the cytoplasmic side of the Sec translocon.

Highlights

- Crystal structures of full-length and peptide-bound SecYEG were determined
- The cytoplasmic loop of SecG covers the protein-conducting channel
- The cytoplasmic loop of SecG blocks protein translocation

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Crystal Structures of SecYEG in Lipidic Cubic Phase Elucidate a Precise Resting and a Peptide-Bound State

Yoshiki Tanaka,[1,6] Yasunori Sugano,[1,6] Mizuki Takemoto,[2,3] Takaharu Mori,[4] Arata Furukawa,[1] Tsukasa Kusakizako,[2,3] Kaoru Kumazaki,[2,3] Ayako Kashima,[2] Ryuichiro Ishitani,[2,3] Yuji Sugita,[4] Osamu Nureki,[2,3,*] and Tomoya Tsukazaki[1,5,*]
[1]Department of Systems Biology, Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5, Takayama-cho, Ikoma, Nara 630-0192, Japan
[2]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[3]Global Research Cluster, RIKEN, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan
[4]Theoretical Molecular Science Laboratory, RIKEN, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan
[5]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[6]Co-first author
*Correspondence: nureki@bs.s.u-tokyo.ac.jp (O.N.), ttsukaza@bs.naist.jp (T.T.)
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SUMMARY

The bacterial SecYEG translocon functions as a conserved protein-conducting channel. Conformational transitions of SecYEG allow protein translocation across the membrane without perturbation of membrane permeability. Here, we report the crystal structures of intact SecYEG at 2.7-Å resolution and of peptide-bound SecYEG at 3.6-Å resolution. The higher-resolution structure revealed that the cytoplasmic loop of SecG covers the hourglass-shaped channel, which was confirmed to also occur in the membrane by disulfide bond formation analysis and molecular dynamics simulation. The cytoplasmic loop may be involved in protein translocation. In addition, the previously unknown peptide-bound crystal structure of SecYEG implies that interactions between the cytoplasmic side of SecY and signal peptides are related to lateral gate opening at the first step of protein translocation. These SecYEG structures therefore provide a number of structural insights into the Sec machinery for further study.

INTRODUCTION

More than 30% of newly synthesized proteins in the cell are transported via the Sec translocon, a process that is mediated by Sec factors (Deshaies et al., 1991; Gardel et al., 1990; Ito, 1984; Nishiyama et al., 1994; Oliver and Beckwith, 1981; Rapoport, 2007; Riggs et al., 1988). In the Sec pathway, N-terminal signal peptides of secretory and membrane preproteins are recognized and targeted to the plasma membrane for bacteria and the ER membrane for eukaryotes, and the mature polypeptides are then either translocated across or integrated into the membrane (Blobel and Dobberstein, 1975a, 1975b; Denks et al., 2014). Simultaneously, a Sec translocon, namely the bacterial SecYEG complex or the eukaryotic Sec61 complex, forms the pathway for the preproteins. Protein localization via SecYEG is either driven post-translationally by the cytosolic motor SecA ATPase or accomplished in combination with nascent chain synthesis. The localization is enhanced by the proton-driven membrane protein SecDF (Tsukazaki et al., 2011). Structural studies of the Sec translocon have revealed that the transmembrane region of SecY forms an hourglass-shaped channel that possesses a lateral gate that opens to the membrane interior (Egea and Stroud, 2010; Tsukazaki et al., 2008; van den Berg et al., 2004; Zimmer et al., 2008). In the resting state, the center of the channel, called the pore ring, is too narrow to conduct preproteins and is sealed with a flexible plug from the periplasmic side, maintaining the membrane-impermeability barrier (Park and Rapoport, 2011). During protein translocation and insertion, conformational changes in SecYEG are induced by interactions between preproteins and their cytosolic partners, such as SecA and the ribosome (Bischoff et al., 2014; Frauenfeld et al., 2011; Gogala et al., 2014; Park et al., 2014; Zimmer et al., 2008). Although a signal peptide is also proposed to bind to the cytoplasmic side of the lateral gate at the first step, which triggers the opening of the lateral gate, a detailed view of this interaction remains unclear. In addition, higher-resolution structures of the Sec translocon, comprising its three components SecY, SecE, and SecG, are required for more precise analyses of the Sec translocon machinery.

RESULTS AND DISCUSSION

SecYEG Structures in the Lipidic Cubic Phase

Two different types of *Thermus thermophilus* SecYEG (TtSecYEG) crystals were obtained in the lipidic cubic phase (LCP). The crystals belonged to the space groups *I*222 and $C222_1$, which diffracted X-rays to 2.7- and 3.6-Å resolution, respectively. The diffraction data enabled us to refine the full-length