Structural basis of the Sec translocon and YidC revealed through X-ray crystallography

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Abstract

Protein translocation and membrane integration are fundamental, conserved processes. After or during ribosomal protein synthesis, precursor proteins containing an N-terminal signal sequence are directed to a conserved membrane protein complex called the Sec translocon (also known as the Sec translocase) in the endoplasmic reticulum membrane in eukaryotic cells, or the cytoplasmic membrane in bacteria. The Sec translocon comprises the Sec61 complex in eukaryotic cells, or the SecY complex in bacteria, and mediates translocation of substrate proteins across/into the membrane. Several membrane proteins are associated with the Sec translocon. In *Escherichia coli*, the membrane protein YidC functions not only as a chaperone for membrane protein biogenesis along with the Sec translocon, but also as an independent membrane protein insertase. To understand the molecular mechanism underlying these dynamic processes at the membrane, high-resolution structural models of these proteins are needed. This review focuses on X-ray crystallographic analyses of the Sec translocon and YidC and discusses the structural basis for protein translocation and integration.

Key words

protein translocation

protein insertion

X-ray crystallography

membrane protein

Sec translocon

Introduction

Membrane or secretory proteins are synthesized by cytoplasmic ribosomes, and their nascent polypeptides possess a specific membrane-targeted signal sequence for transport across or integration into the membrane. The transported polypeptides then fold into mature proteins and function at the appropriate locations. The membrane restricts the passive diffusion of small molecules and ions across the membrane. To overcome the membrane permeability barrier and environmental factors such as the pH or salt concentration, sophisticated machineries present in the membrane enable protein translocation across and integration into the membrane. The Sec translocon is an evolutionarily conserved protein-conducting channel at the endoplasmic reticulum (ER) membrane of eukaryotic cells, or the cytoplasmic membrane of bacteria and archaea (Fig. 1A)[1,2]. The Sec translocon is an essential hetero-ternary protein complex, comprising membrane proteins Sec61α/γ/β in eukaryotic cells or SecY/E/G in bacteria (Fig. 1B), and is involved in translocation and integration of nascent ribosomally synthesized, unfolded proteins, in a signal sequence-dependent manner. In the case of soluble proteins, the N-terminal membrane-targeting signal sequences of precursor proteins are cleaved during translocation, thus decreasing the size of the mature proteins[3]. In many membrane proteins, the first transmembrane region contains the targeting signal and is not cleaved. As shown in Figure 1, protein translocation via the Sec translocon is classified into co-translational and post-translational translocations. The basic mechanism of co-translational translocation in bacteria and eukaryotic cells is the same. During co-translational translocation, the highly hydrophobic signal sequence emerging from the ribosomal exit tunnel is recognized by the signal-recognition particle (SRP) and is targeted to the membrane co-translationally, along with the ribosome, owing to interactions between the SRP and the SRP receptor present in the membrane[4]. Thereafter, the ribosome directly interacts with the protein-conducting channel (i.e., the Sec translocon), which is present in the membrane. Subsequently, protein translocation occurs simultaneously with protein translation. During post-translational translocation, unfolded proteins are targeted to the membranes. The Sec62/63 complex and BiP protein are involved in this translocation in eukaryotic cells. BiP proteins drive protein translocation via a ratchet mechanism, which can move in only one direction owing to substrate interactions and conformational transitions of BiP by ATP hydrolysis[5]. In *Escherichia coli*,

precursor proteins, maintained in an unfolded state by chaperones such as SecB, are directed to the membrane where the SecA ATPase drives protein translocation[6,7], although many chaperones including SecB are not essential for the viability of *E. coli*. SecA repeatedly undergoes conformational changes to move the precursor protein into the Sec translocon, using energy from ATP hydrolysis[8]. Because the Sec translocon itself is a passive protein channel, other factors, including Sec62/63, SecA, and BiP, play indispensable roles in protein translocation, as described above. Data from an electron microscopy study demonstrated that the eukaryotic Sec translocon is associated with translocon-associated proteins (TRAPs) and the oligosaccharyl-transferase (OST)[9,10]. The cryo-electron microscopic density map of a TRAP showed it protruding into the ER space, probably interacting with the substrate protein during protein translocation. In bacteria and archaea, the SecD–SecF complex (SecDF), which interacts with Sec YEG, promotes protein translocation[11,12]. SecDF repeatedly undergoes drastic conformational changes with the substrate at the trans side of the plasma membrane using proton motive force, which promotes substrate release into the trans-side space[13-15]. SecDF can drive protein translocation at the trans side of the plasma membrane independently of SecA[13]. Hence, SecDF is considered a second protein-translocation motor. The bacterial Sec translocon is involved in membrane protein sorting during co-translational translocation in collaboration with YidC, a membrane protein[16,17]. YidC functions as a chaperone, facilitating co-translational membrane protein folding. Moreover, YidC directly binds to the ribosome and is responsible for membrane insertion of certain single- and double-spanning membrane proteins. Additionally, MPIase (membrane protein integrase), a glycolipozyme, also plays an essential role in membrane protein insertion, before YidC inserts proteins into the membrane[18,19]. YidC corresponds to plant Alb3 in the thylakoid membrane and eukaryote Oxa1 in the inner mitochondrial membrane (Fig. 1C). The YidC/Oxa1/Alb3 family proteins, containing five conserved transmembrane α-helices, are involved in membrane protein insertion and assembly of the respiratory chain-related complex[20]. YidC of gram-negative bacteria possesses additional transmembrane and periplasmic regions (P1) at its N-terminus. This review is focused on studies of the crystal structures of the Sec translocon and YidC, aimed at elucidating the mechanisms underlying protein transport across/into the membrane at the atomic level, and provides a detailed and comprehensive description of these membrane proteins.

Architecture of Sec translocon

Molecular modeling based on the first reported crystal structure of the Sec translocon in 2004 (Fig. 2A left, B)[21] provided many insights into the mechanisms of protein translocation across and integration into the membrane, via the Sec translocon. Subsequently, different types of functional analyses were performed to elucidate the molecular mechanism underlying the action of Sec translocon[1,2,22]. The available crystal structure models of Sec translocons in the Protein Data Bank (PDB)[21,23-28] are summarized in Table 1. The first reported structure of the Sec translocon is from an archaeon, *Methanocaldococcus jannaschii*[21], and is designated as SecYEβ in this review. As of March 20, 2019, the highest-resolution structure of the Sec translocon was reported in 2014 for *Thermus thermophilus* SecYEG (2.7-Å resolution; Fig. 2A, right)[27]. Some reports do not include SecG/β, a non-essential subunit[29-32], because it easily dissociates from the essential SecYE complex and does not influence its stability. Purified *T. thermophilus* SecYEG, generated by overexpressing SecG with an additional plasmid in SecYEG-overexpressing cells, was crystallized in the lipidic cubic phase, facilitating determination of a high-resolution structure of the Sec translocon[27]. Both the *M. jannaschii* SecYEβ and *T. thermophilus* SecYEG crystal structures represent the resting states of the Sec translocon (Fig. 2A, B). Ten transmembrane α-helices of SecY compose the core of the Sec translocon, which is stabilized by the cytoplasmic α-helix parallel to the membrane and the tilted transmembrane α-helix of single-membrane-spanning SecE (triple membrane-spanning, in the case of *E. coli*). SecG, containing two transmembrane α-helices, is peripherally located adjacent to transmembrane region 1 (TM1) and TM4 of SecY. The single-membrane-spanning protein, Secβ, an alternative component of SecG, is located in a position similar to that of SecG. The N-terminal and C-terminal halves, TM1–5 and TM6–10, respectively, are arranged in a pseudosymmetrical manner and linked by a cytoplasmic loop, called a hinge. The protruding cytoplasmic region 4 (C4) between the TM6–7 regions and C5 between the TM8–9 regions provide major interaction sites for cytosolic factors, including SecA and ribosomes. The interior channel of SecY is hourglass-shaped, its center containing a constricted region called the pore ring (Fig. 2C). The narrow point is formed by six hydrophobic amino acid residues, primarily including Ile, at the middle regions of TM2, 5, 7,

and 10, and does not permit secretion of substrate proteins via the Sec channel, based on the crystal structures. Furthermore, the trans-side funnel of the hourglass-shaped space is occupied at the exterior side by a short α-helix, called a plug, between TM1 and TM2, resulting in a completely sealed SecY channel. Although previous structural studies on the Sec translocon revealed that the cytoplasmic funnel of SecY is not occupied, Tanaka et al. reported a high-resolution structure of SecYEG wherein the cytoplasmic loop of SecG covers the cytoplasmic side of the channel[27], thus restricting membrane permeability in a manner similar to that of the plug domain (Fig. 2D). Therefore, the SecG loop can function as a cytoplasmic cap for the SecY channel. The cytoplasmic N-terminal region of Secβ is disordered in structural models; however, it could be located near the cytoplasmic funnel in the resting state, similar to the SecG loop. The mechanism underlying this covering process from each side of the pore ring may be universally conserved. The boundary area between TM1–5 and TM6–10 of SecY on the opposite side of the tilted SecE transmembrane α-helix is called a lateral gate, comprising TM2, 3, 7, and 8, which are binding sites for the signal sequences[33]. The Sec translocon in the resting states is not wide enough for protein transport. Therefore, the pore ring, plug, cap, and lateral gate regions have been predicted to undergo conformational changes and/or are dislocated, thereby enabling protein translocation across and integration into the membrane via the Sec translocon. The variable models of the Sec translocon have been experimentally verified[27,34-37]. Recent structural X-ray crystallographic[21,23-28], electron microscopic[38-44], and functional[45,46] analyses strongly suggested that the oligomeric state of the Sec translocon is one heterotrimeric unit, although an efficient functional state comprising two or more units cannot be excluded[47,48]. Several crystal structures of the SecY complex imply that interactions between SecA, Fab, or a peptide mimicking a part of the signal peptide and the protruded cytoplasmic regions of SecY (which are intrinsically flexible) induce conformational changes in the lateral gate (Fig. 2E). Similar to these crystal structures, binding of cytosolic factors to the Sec translocon would trigger structural changes to easily interact with precursor proteins initially during protein translocation. In the SecA-bound conformation of SecY, the plug domain is dislocated outwards, thereby expanding the inner space of SecY. This structural change may lower the energy barrier to protein translocation via SecY.

Sec translocon in the protein translocation state

An outstanding report shows the crystal structure of SecYE and precursor segment-inserted SecA at 3.7-Å resolution (Fig. 3A, B)[26]. For the structural analysis, the polypeptide was artificially introduced into a loop of SecA as a fusion protein, accompanied by the generation of an intermolecular disulfide bond between the peptide and SecY at the trans side of the plasma membrane to stabilize the protein-translocation intermediate. The lateral gate is the most widely open in the available crystal structures. The signal peptide of the substrate is located at the expanded lateral gate, surrounded by TM2, 3, and 7, presumably oriented toward the hydrophobic regions of the lipid bilayer. During insertion, the signal peptide can be laterally released from the expanded gate to the membrane via hydrophobic interactions. The part of the substrate being transported is located along the center of the Sec translocon, and the pore ring is larger than that in other crystal structures. Four of six residues of the pore were found to tightly interact with the transported peptide, simultaneously blocking membrane permeability like a gasket. Hence, even during protein translocation, SecY can maintain the membrane barrier simultaneously.

Recent structural studies of Sec translocon

Recent structural studies on the ribosome–Sec translocon complex by electron microscopy at medium resolution revealed densities corresponding to the α-helices and conformational changes in transmembrane regions and the localization of precursor proteins, providing insights into protein translocation across and integration into the membrane[38-44]. Furthermore, samples can be directly observed by electron microscopy without crystallization steps, which are needed for X-ray crystallography. Electron microscopic images of the ribosome–nascent chain complex (RNC) probably include various intermediate states of SecY complexes, thus providing several snapshots of co-translational translocation. The cryo-electron microscopic structure of Sec61 and RNC, including a signal peptide, elucidated that the signal peptide is observed in a manner similar to that of the SecYE–SecA–signal peptide complex (Fig. 3C, left)[42]. A different cryo-electron microscopic imaging analysis of SecY and RNC, including two newly synthesized transmembrane α-helices, revealed that two transmembrane regions are peripherally located near the lateral gate of SecY (Fig. 3C, right)[38]. This

structure is considered to represent the intermediate state after the substrate is sorted into the membrane via the Sec translocon. Although the electron density of the translocating peptide was unclear upon electron microscopic analyses, probably owing to its unfolded conformation, polypeptides are thought to traverse the central pore of the Sec translocon. Cryo-electron microscopic analysis has revealed several snapshots of the active Sec translocon during protein translocation. Because the Sec translocon contains highly motile regions, including the plug, cap, and cytoplasmic regions, we cannot accurately refine the structural models of the Sec translocon at atomic resolution using the current electron density data at limited resolution, thus preventing an accurate understanding of the transition states of the Sec translocon. Future structural analyses are required at a higher resolution. Highly flexible regions may not be visible even in high-resolution structures determined by X-ray crystallography, but cryo-electron microscopic analysis may elucidate several forms of such flexible regions because recently developed programs can analyze several states separately. Structural studies of the Sec translocon have indicated that the passive Sec translocon has a flexible structure, which appropriately changes to direct the transportation of proteins to the trans side of the plasma membrane or into the membrane, in response to interactions with cytosolic factors and precursor proteins. The fundamental concepts underlying transitions of the Sec translocon (including changes in pore size, opening and closing the lateral gate, and plug dislocation) were reported with the first crystal structure of the Sec translocon[21] and have been supported by structure-based functional studies for more than a decade[1].

Overall structures of YidC

The YidC core comprises five conserved transmembrane α-helices (cTM1–5) (Fig. 1C). The arrangements of the transmembrane α-helices of YidC were predicted based on an evolutionary co-variation analysis[49]; however, the detailed interactions, arrangements, and tilting angles of the transmembrane regions remain unknown. Crystal structures of YidC derived from three species were published (Table 1) in the past five years[50-53]. All reported crystal structures for YidC displayed monomeric states (Fig. 4A–C), concurrent with recent functional and structural reports that YidC functions as a monomer[54-56], although functional dimeric states of YidC have been previously proposed[57,58]. The first transmembrane α-helix (1st TM) of *E.*

coli YidC, which functions as a signal sequence, was disordered even in the recent higher-resolution crystal structure (Fig. 4A)[50]. Furthermore, the 1st TM was reported to interact with SecY and SecG[59] and to be involved in substrate binding[60,61]; however, the significance of this interaction is not yet clear. The first periplasmic regions (P1) of *E. coli* and *T. maritima* (Fig. 1C) do not share the same architecture, suggesting that the P1 region is not essential in *E. coli*[62]. However, a part of the P1 region of *E. coli* interacts with Sec components and YidC[63,64], potentially contributing to the formation of the Sec holo-translocon complex[65]. The N-terminal extension of cTM1, called EH1, is a conserved amphiphilic helix parallel to the membrane surface. The hydrophobic half of EH1 is embedded in the membrane. EH1 may function as a float to stabilize YidC localization in the membrane. The five conserved transmembrane α-helices create a hydrophilic cavity (Fig. 4B). A comparison of the transmembrane regions of the reported crystal structures of YidC revealed that hydrophilic cavities of the same size are evolutionarily conserved among YidC family proteins (Fig. 4D).

Detailed structures and functions of YidC

The cavity is positively charged and exposed to the cytoplasm and the membrane, whereas the trans side of the plasma membrane is entirely closed by tightly packed hydrophobic residues (Fig. 4B). At the center of the cavity, a conserved positively charged residue, Arg, in *B. halodurans*, *T. maritima*, and *E. coli*, primarily contributes to the characterized positive charge of the cavity (Fig. 4C). The positive charge in *B. halodurans* was shown to be essential for cell growth and insertion of MifM, a substrate of YidC[51]. In contrast, the positive charge in *E. coli* is important, but replaceable[52,66]. The difference in positive charge requirements may be related to the importance of the functions of substrate proteins of YidC in each species. Systematic mutational analysis revealed that the hydrophilicity of this region is also an important factor influencing YidC activity[67]. Short, rigid loops of the trans side, showing lower B-factors upon crystallographic analysis, structurally support the closed extracellular side of the transmembrane region. On the opposite side, the C1 loop forms a hairpin loop comprising two hydrophilic α-helices, which protrude from the transmembrane region to the cytoplasm. It has been suggested that the C1 region contains sites for interaction with substrates[59]. The arrangements of the C1 regions in the crystal structures are not the same, i.e.,

they are flexible, concurrent with higher B-factors in the C1 region compared to other core regions (Fig. 4E). In the case of *T. maritima* YidC, the cytoplasmic loops, including the C1 loop, were disordered. Although the C2 loop was disordered in reported crystal structure models (except for the structure reported most recently), the 2.8-Å resolution data from *E. coli* YidC helped characterize the C2 loop (Fig. 4C, E). The C2 loop is located near the entrance of the hydrophilic cavity. The B-factors of the C2 loop are even higher than those of the C1 loop, implying that the C2 region is most flexible in the core of YidC. The C2 loop at the cavity entrance may function as a cover to prevent excessive exposure of hydrophilic regions in the membrane. The fundamental role of C2 may be similar to that of the SecG loop[27]. The crystal structure of *B. halodurans* YidC (Form II) (Fig. 4C, right) only shows the C-terminal region, which interacts with the C1 region. Because the C-terminal, C1, and C2 regions were reported to interact with the ribosome[54,68,69], the cytoplasmic regions may bind the ribosome cooperatively. Functional analysis using deletion mutations supported the importance of the loops of the core region[51]. Because YidC contains a hydrophilic cavity facing the membrane interior, YidC may preferably not exist stably in the hydrophobic membrane. Three molecular dynamics simulations of YidC revealed that YidC can stably exist in the membrane with some cytoplasmic fluctuations[50,51,70]. During the simulations, the cavity of YidC was filled with approximately 20 water molecules. One of the important functions of the transmembrane region of YidC is to generate a pool of water molecules at the membrane. Together, the structure derived from all conserved regions from EH1 to cTM5 seem crucial for YidC activity. The conserved, positively charged cavity of YidC could reflect the importance of electrostatic interactions. A certain type of YidC substrate is negatively charged. In the case of the single-membrane-spanning substrate MifM, three positively charged residues are positioned at the N-terminal region. When the negatively charged residues were mutated, the MifM-insertion activity of YidC decreased, thus increasing the possibility that the interaction between the positive charge in the hydrophilic cavity and the negative charges of substrates is important for YidC-dependent membrane protein insertion[51]. Subsequently, a site-specific photo-crosslinking analysis elucidated direct interactions between the cavity and MifM[51]. Hence, a membrane-insertion model of a simple membrane protein such as a once-spanning or twice-spanning transmembrane protein resulting from YidC activity has been proposed (Fig. 5A). Initially

during protein insertion by YidC, the flexible C1 and C2 regions may recognize and interact with the substrate, and then the substrate is temporally captured by the YidC cavity, which is mediated by electrostatic and hydrophilic interactions between the substrate and YidC. Thereafter, the captured substrate protein is sorted from the cavity into the membrane via hydrophobic interactions with membrane lipids. The non-uniform distribution of electrostatic charges resulting from the membrane potential derived from the proton motive force further influences protein sorting.

Conserved mechanism of membrane protein insertion

Cryo-electron microscopic analysis of the RNC and YidC complex showed the transmembrane segments of a substrate in the front of the entrance of the cavity exposed to the membrane[49]. This state is assumed to be adopted immediately after the substrate is released from the YidC cavity. A molecular-dynamics simulation suggested that the thickness of the membrane surrounding YidC was reduced by the existence of YidC, thus decreasing the local energy barrier of protein translation across the membrane[70]. A similar reduction in thickness was previously reported based on a molecular-dynamics simulation of the outer-membrane protein BamA[71], which functions as a membrane protein insertase for the outer membrane. Owing to the lack of energy sources such as ATP at the outer membrane, protein insertion is achieved via a delicate balance involving molecular interactions, collision frequency, and concentration. YidC-like proteins identified in Archaea and in the ER membrane of eukaryotes contain three transmembrane α-helices, corresponding to cTM1, 2, and 5 in YidC, which are proposed to form a hydrophilic surface similar to that of YidC[72,73]. The functional roles of YidC family proteins and the YidC-like proteins may be conserved in each membrane as primitive machinery.

Collaborative functional model of SecYEG and YidC for membrane protein insertion

YidC functions not only as an insertase, but also as a membrane chaperone for integrating certain types of multi-membrane-spanning proteins into the membrane in collaboration with SecYEG[74,75]. Cross-linking experiments revealed that cTM3 and cTM5 mainly interact with substrates[60,76], and the lateral

gate of SecY interacts with YidC[64]. Therefore, the entrance for the lipid bilayer of YidC should face the lateral gate of SecYEG. To elucidate the molecular mechanism underlying collaborative protein integration, the detailed three-dimensional structure of the complex of YidC and SecYEG warrants elucidation. Based on the cryo-electron microscopic structure of the Sec holo-translocon complex at ~10-Å resolution[77], it is difficult to discuss the detailed interactions and conformational transitions of the components. Further high-resolution structural analysis studies are required. The cryo-electron microscopic structures of RNC in complex with SecYEG or YidC have been reported previously, as described above. Therefore, future studies may potentially reveal the structure of the RNC–YidC–SecYEG complex at improved resolution. As the hydrophilic cavity of YidC faces the membrane, YidC can shelter the hydrophilic region of the newly synthesized membrane protein being sorted from the lateral gate of the Sec translocon. The number of YidC molecules in the cell would be greater than that of SecYEG[78], presumably enabling several YidCs to function simultaneously as chaperones for Sec-dependent membrane integration/maturation, which is important for membrane protein biogenesis. The mechanism underlying substrate recognition by YidC as a chaperone during membrane protein folding is in complete contrast with that of soluble chaperones, which typically provide hydrophobic surfaces to prevent misfolding of soluble proteins (Fig. 4B). The positively charged YidC cavity preferentially interacts with and transports negatively charged regions of substrates to the opposite side, such that YidC is more likely to be involved in the positive inside rule of membrane proteins[79]. Several YidC substrates have been identified[17]; however, identification of other YidC substrates is necessary to further clarify the details regarding YidC-mediated capture and release of substrates into the membrane. Unidentified substrates with high stability even in an aqueous buffer are preferable for functional analysis, especially for in vitro experiments with purified proteins.

Concluding remarks

Considering recent developments in electron microscopy, crystallization procedures, and data-collection systems, determination of high-resolution structures as snapshots during protein translocation is promising. In high-resolution cryo-electron microscopic structural analyses of the Sec translocon and YidC in

co-translational translocation studies, the samples contained ribosomes, the size of which allowed the construction of molecular models. In contrast, X-ray crystallography is considered desirable for structural analysis in post-translational translocation studies, because the Sec translocon complex lacks a ribosome. However, the latest structural studies of the post-translational *Saccharomyces cerevisiae* Sec61 complex (consisting of Sec61α/γ/β, Sec62/63, and Sec71/72) by cryo-electron microscopy and single-particle analysis was reported at a maximum resolution of 3.4 Å[80] and 4.1 Å[81]. It is noteworthy that the authors built molecular models of Sec translocon without a ribosome structure. Similar to that of SecY in Fig. 2E, the lateral gate of Sec61 is opened by cytosolic interactions. In addition, a Sec61β–Sec63 fusion protein was used for the former[80] study to stabilize the post-translational Sec translocon complex. A bacterial SecY–SecA fusion protein, possessing protein-translocation activity, can be embedded into nanodisc particles[82]. Therefore, further structural analysis of the Sec translocon in the post-translational pathway, without a ribosome, can be performed by electron microscopy in combination with some fusion proteins to uncover several detailed intermediate architectures. In the near future, electron microscopy will be considered one of the more powerful tools for structural analysis at atomic resolution, even for post-translational protein translocation studies. Moreover, time-dependent structural analyses are also required to further the current understanding of protein transport. Single-molecule analysis helps resolve the underlying mechanism[83,84], and high-speed atomic force microscopic observations of one unit may provide an overall view of structural changes occurring during protein translocation in real time[85]. Numerous interesting questions regarding a comprehensive understanding of protein transport remain to be answered.

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316 **Table 1 | Crystal structures of the Sec translocon and YidC**

Species	Resolution	States	Notes	PDB ID	Reference
Sec translocon					
Methanocaldococcus jannaschii SecYEβ	3.2 Å	Resting state	Stable mutant	1RH5	[21]
	3.5 Å	Resting state		1RHZ	[21]
	3.5 Å		Plug mutant	2YXQ	[28]
	3.6 Å		Plug mutant	2YXR	[28]
Thermus thermophilus SecYE + Fab	3.2 Å	Fab-bound		2ZJS	[23]
Thermotoga maritima SecYEG + SecA	4.5 Å	SecA-bound		3DIN	[25]
Pyrococcus furiosus SecYE	2.9 Å	C-terminal interacting		3MP7	[24]
Thermus thermophilus SecYEG	2.7 Å	Resting state	Best resolution	5AWW	[27]
	3.6 Å	Peptide-bound		5CH4	[27]
Geobacillus thermodenitrificans SecYE + SecA + nanobody	3.7 Å	Precursor-bound	SecA-precursor fusion	5EUL	[26]
YidC					
Bacillus halodurans YidC	2.4 Å	Form I		3WO6	[51]
	3.2 Å	Form II		3WO7	[51]
Escherichia coli YidC	3.2 Å			3WVF	[52]
	2.8 Å		All YidC cores modeled	6AL2	[50]
Thermotoga maritima YidC	3.8 Å			5Y83	[53]

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Figure legends

Figure 1 | Bacterial protein translocation and integration via the Sec translocon and YidC.

A, Protein translocation across and integration into the membrane. During post-translational translocation, precursor proteins with an N-terminal signal sequence are targeted to and translocated across the membrane, which is driven by Sec62/63 complex and BiP in eukaryotes and SecA ATPase and SecDF in bacteria. During co-translational translocation, the ribosome–nascent chain complex (RNC) is directed to the membrane by the interaction between signal recognition particle (SRP) and its receptor, and interacts with SecYEG or YidC. Subsequent membrane protein integration via the interior of the Sec translocon and/or YidC occurs co-translationally. B, Schematic representation of Sec translocon components. C, Schematic representation of the YidC/Oxa1/Alb3 protein family.

Figure 2 | Crystal structures of the Sec translocon.

A, The Sec translocon in the resting state. Crystal structures of SecYEβ from *Methanocaldococcus jannaschii* (PDB ID 1RH5) (left) and SecYEG from *Thermus thermophilus* (PDB ID 5AWW) (right). B, Schematic representation of the Sec translocon. C, Magnified views of the pore ring of the structures in A from the cytoplasm. D, Cut-away models of the surface representation of *T. thermophilus* SecYEG without the plug and cap regions. The plug and cap regions are represented by the ribbon model with a stick model for the side chains. E, Crystal structures of the Sec translocons in which the cytoplasmic region interacts with other molecules in the crystals (PDB ID 3MP7, 5CH4, 2ZJS, and 3DIN).

Figure 3 | Structures of the Sec translocon in the intermediate stages of protein transport.

A, Crystal structures of the SecYEG–SecA complex with part of the precursor protein expressed as a fusion protein (PDB ID 5EUL). B, Magnified views of the pore ring of the structure in A from the cytoplasmic side . C, Electron microscopic structures of the Sec translocon of the RNC complex during protein transport (PDB ID 3JC2 and 5ABB).

Figure 4 | Crystal structures of YidC.

A, Crystal structure of *E. coli* YidC at 2.8-Å resolution, elucidating all YidC core regions (PDB ID 6AL2). The cTM numbers are shown. B, Cut-away model of the *E. coli* YidC structure. C, Gallery of crystal structures of YidC (PDB ID 6AL2, 5Y83, 3WO6, and 3WO7). D, Superimposition of the core region of *B. halodurans*, *T. maritima*, and *E. coli* YidCs. E, Magnified view of the C1 loop region.

Figure 5 | Functional model of YidC.

A, Membrane-insertion model of a single-membrane-spanning protein via YidC, independently of SecYEG. YidC temporally captures the precursor protein at the positively charged cavity. Thereafter, the substrate protein is sorted primarily via hydrophobic interactions. B, Chaperone activity model of YidC. YidC protects a hydrophilic region sorted from the lateral gate of the Sec translocon until its interacting region emerges from the gate, promoting correct folding of substrate proteins.

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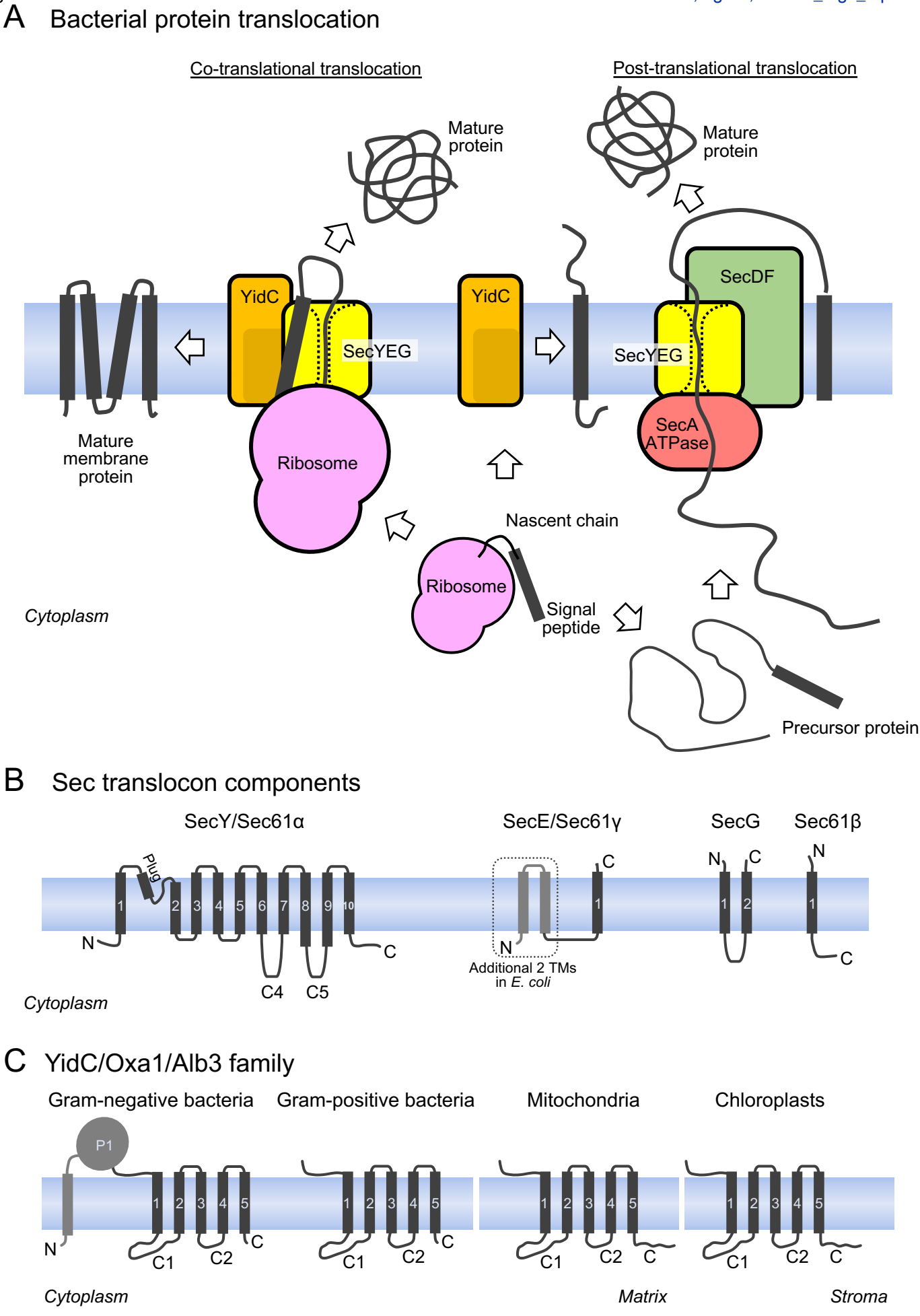
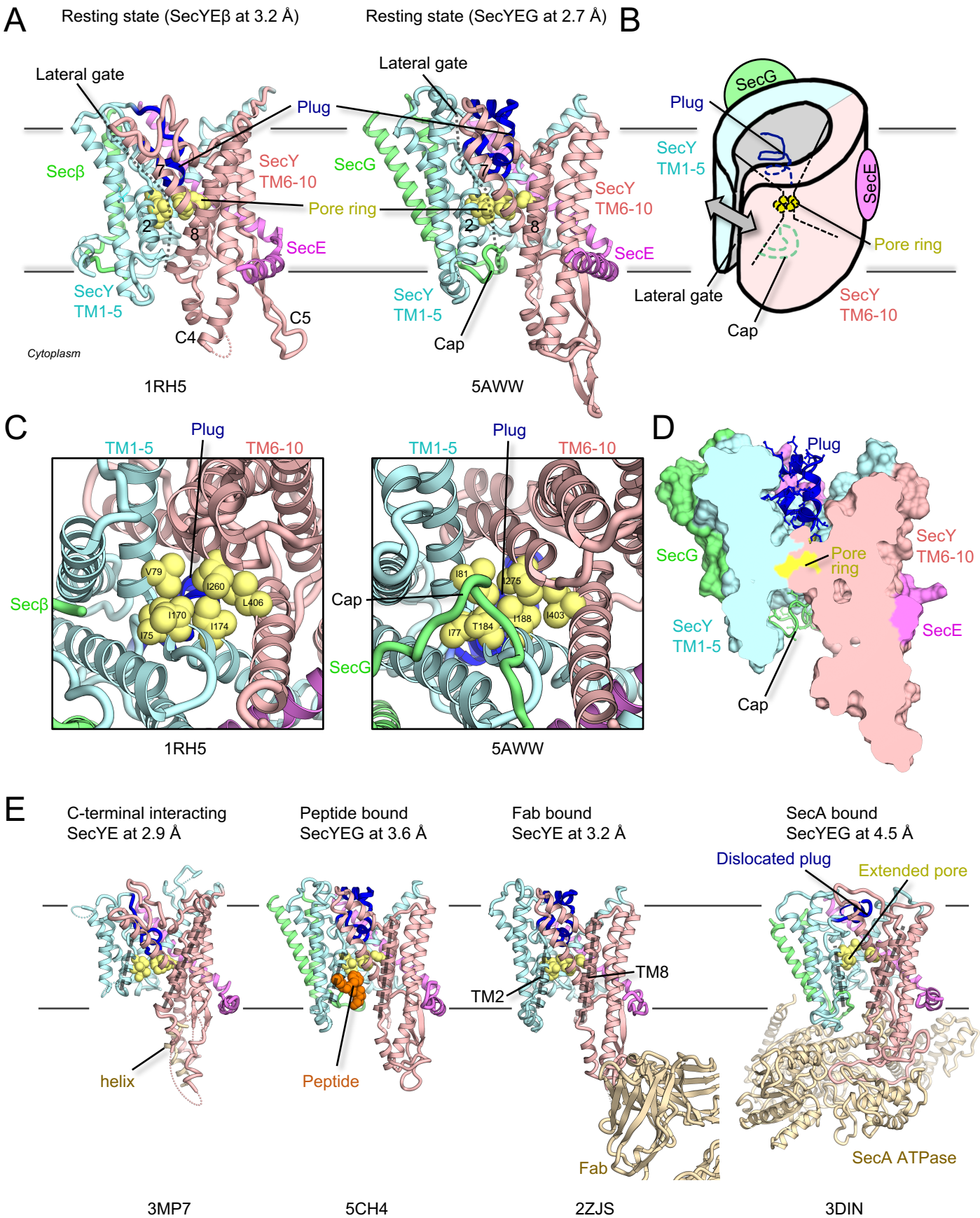
Figure



Tsukazaki Fig. 1



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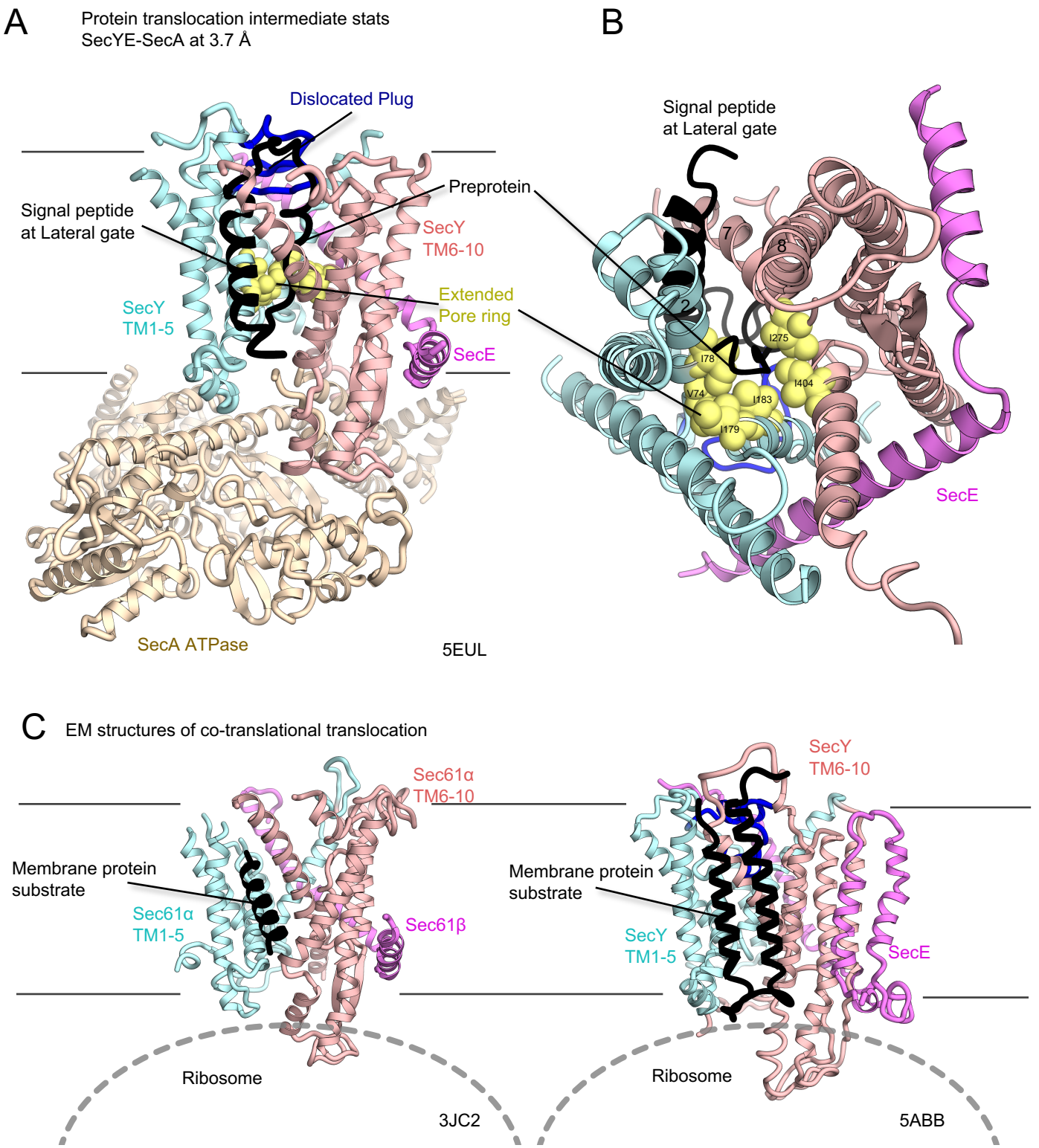

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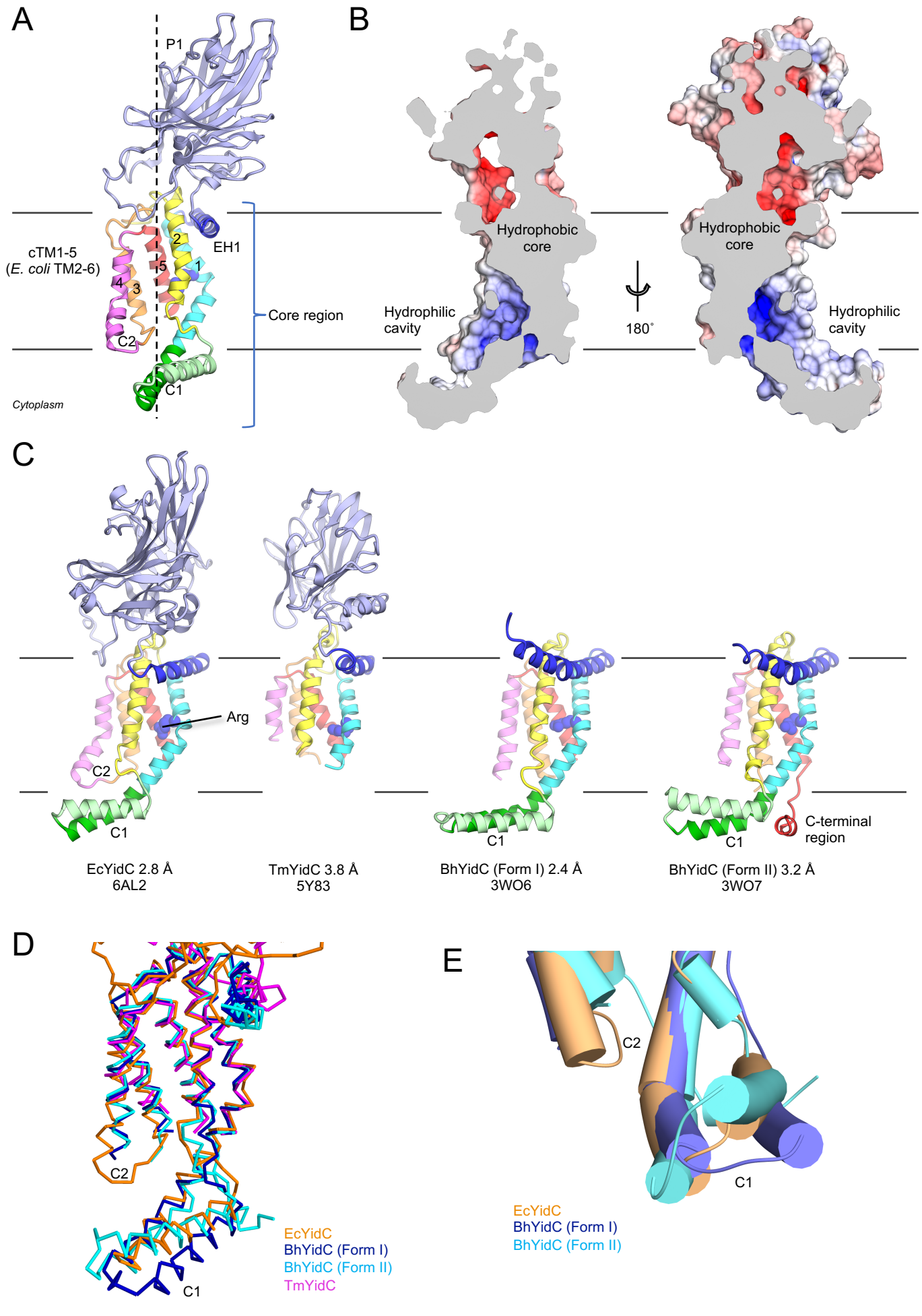
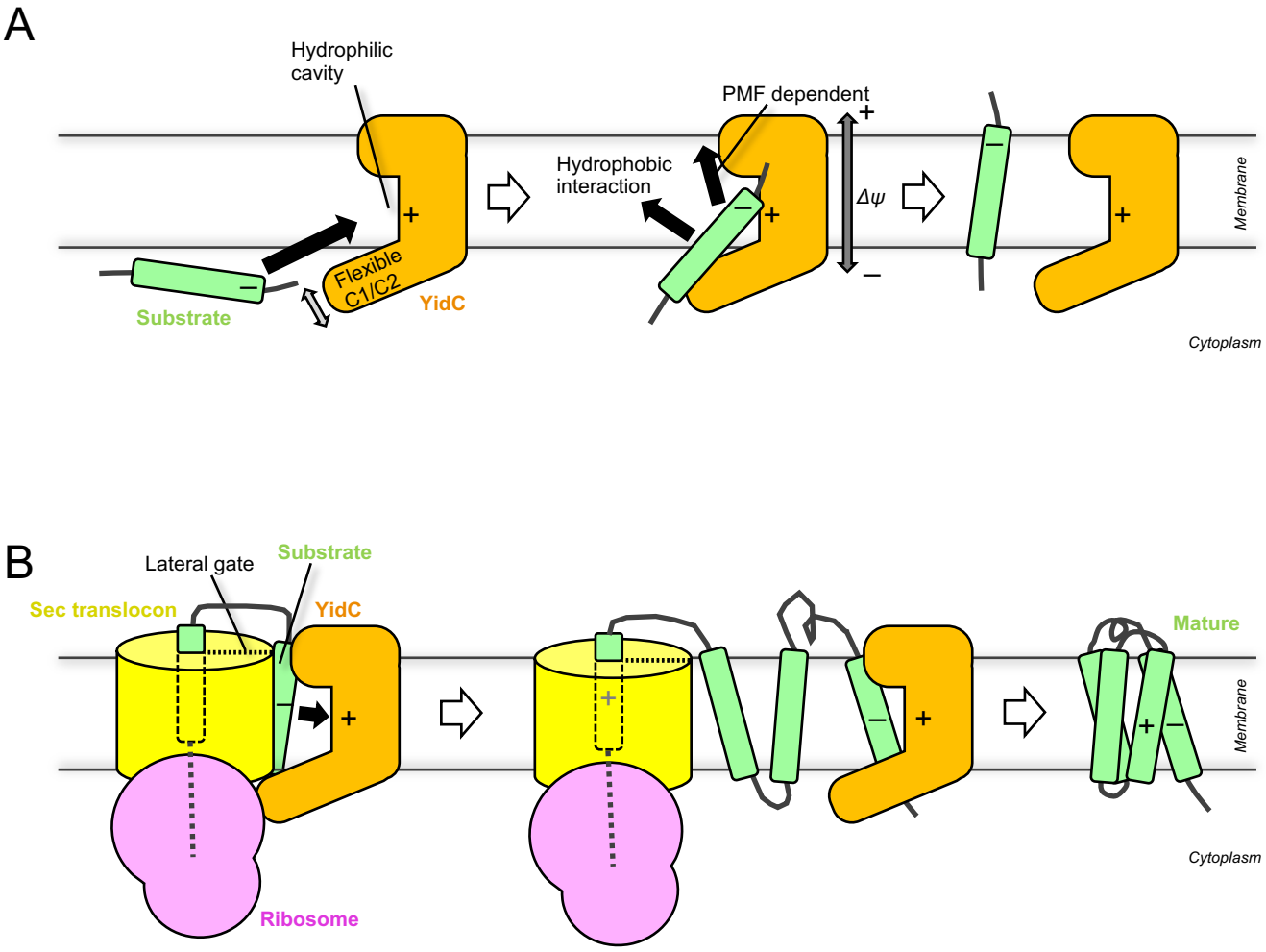


Tsukazaki Fig. 4



Tsukazaki Fig. 5