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Translocation of proteins through the Sec61 and SecYEG channels

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Summary

The Sec61 and SecYEG translocation channels mediate the selective transport of proteins across the endoplasmic reticulum and bacterial inner membrane respectively. These channels are also responsible for integration of membrane proteins. To accomplish these two critical events in protein expression, the transport channels undergo conformational changes to permit export of luminal domains and integration of transmembrane spans. Novel insight into how these channels open during protein translocation has been provided by a combination of the analysis of new channel structures, biochemical characterization of translocation intermediates, molecular dynamics simulations, and in vivo and vitro analysis of structure-based Sec61 and SecY mutants.

Introduction

Protein translocation across the endoplasmic reticulum and export of most proteins from the prokaryotic cytosol occurs through an evolutionarily conserved protein translocation channel that is called the SecYEG complex in prokaryotes and the Sec61 complex in eukaryotes. These channels are composed of one large subunit (SecY or Sec61) that has 10 transmembrane (TM) spans and smaller β (Sec β , SecG, Sbh1/Sbh2 or Sec61 β) and γ (SecE, Sss1p or Sec61 γ) subunits. Protein translocation occurs by posttranslational and cotranslational modes in both eukaryotes and prokaryotes. This review will focus on recent progress concerning the mechanism of precursor transport through protein translocation channels, and consider how integration of membrane proteins adds an additional level of complexity to the translocation reaction.

Structural domains of protein translocation channels

The first structures of eukaryotic translocation channels were obtained by three-dimensional reconstruction of cryoelectron microscope images of ribosome-channel complexes [1,2]. Due to the low resolution ($\sim 20\text{\AA}$) of these structures and the presence of protein-bound phospholipid and detergent, active translocation channels were thought to be composed of three to four copies of the Sec61 complex [3,4]. The large ($\sim 20\text{\AA}$) central opening visualized in several 3-D reconstructions [1,2,4] led to the hypothesis that the transport channel was

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formed by an interface between Sec61 heterotrimers. Contemporary biophysical studies suggested that an active Sec61 complex forms a large diameter (40–60Å) aqueous pore in the membrane [5]. A large diameter SecYEG channel was suggested by evidence that segments of the SecA ATPase penetrate through the SecYEG channel during precursor export [6].

Analysis of a high resolution X-ray crystal structure of the *Methanococcus jannaschii* SecYE β complex led to the hypothesis that a single heterotrimer, rather than an oligomeric interface, forms an active translocation channel [7]. The ten TM spans of SecY are arranged in a clamshell-like structure with TM spans 1–5 and TM spans 6–10 forming the two sides of the channel (Fig. 1a). The tilt of the TM spans yields an hourglass shaped pore with a narrow central constriction (pore ring) consisting of six hydrophobic residues (Fig. 1a). The exofacial side of the pore is occluded by an α -helical segment termed the plug-domain. The narrow pore ring and the plug are structural features of the closed conformation of translocation channels. Point mutations in *E. coli* SecY that reduce the fidelity of signal sequence recognition (prlA mutants) map to the pore ring and the plug domain [7]. Site-specific disulfide crosslinking between a transport substrate and SecY residues that line the interior of the channel provide evidence that precursors pass through the pore ring during transport [8].

The signal sequence of a precursor can be crosslinked to TM2 and TM7 of Sec61p [9] thereby defining a signal sequence-binding (SSB) site (Fig. 1a, red and blue α -helices). The interface between TM spans 2, 3, 7 and 8 forms the lateral gate of the channel (Fig. 1b) through which the TM spans of integral membrane proteins must pass during biosynthesis and membrane integration [10]. Signal sequence insertion into the SSB site to allow expansion of the pore ring would require a partial separation of the lateral gate interface, while lateral exit of a TM span in an α -helical conformation would require a 10–12Å gap between TM spans 2/3 and TM spans 7/8. Since three of six pore ring residues reside on TM2 and TM7 [7] lateral gate separation induces pore ring expansion. After pore expansion, the translocation channel can accommodate an α -helical segment in the central pore and a signal sequence in the SSB site, but not a folded protein domain. A flexible hinge to allow clamshell-like opening of SecY is located between TM5 and TM6 (Fig. 1c). Molecular dynamics simulations suggest that the short loop labeled HL-1 is the flexible link in the *M. jannaschii* SecY hinge [11].

The ribosome-binding site on mammalian Sec61 α was mapped to L6 and L8 [12], two large cytoplasmically exposed loops that are visible in the SecYE β structure (Fig. 1b and 1c). Mutagenesis of yeast Sec61p identified two highly conserved basic residues in each loop that are critical for the cotranslational translocation pathway; a charge reversal mutation in L8 (R406E) eliminates ribosome-binding activity [13]. Remarkably, loop 8 in the *M. jannaschii* crystal structure is L shaped, with the tip of the loop parallel to the membrane surface, and located closer to the membrane bilayer than the tip of loop 6 (Fig. 1b and 1c). A recent 3-D reconstruction of an *E. coli* SecYEG-70S ribosome complex suggests that L8 of SecYEG adopts an extended conformation upon binding to ribosomes with the loop tip contacting the polypeptide exit site on the large ribosomal subunit [14]. Interestingly, point mutations in L6 and L8 (e.g. R357E) of *E. coli* SecY abolish ribosome binding activity [14], as observed previously for the homologous residues in yeast Sec61p [13], indicating that the ribosome binding sites are evolutionarily conserved. Arginine 357 in *E. coli* SecY is also a critical contact site for the SecA ATPase [15,16]. Point mutations (e.g. R357E) at this site in *E. coli* SecY cause a severe defect in SecA-dependent protein translocation [17,18].

Opening the translocon lateral and luminal gates

Protein translocation channels must undergo a conformational change to allow signal sequence insertion into the SSB site. Two recent X-ray crystal structures provide the first views of partially open protein translocation channels [19,20]. The structure of the closed conformation of *Thermus thermophilus* SecYE resembles the *M. jannaschii* SecYE β structure. Binding of an Fab antibody fragment to the tip of loop 8 induced a separation of the cytosolic end of the lateral gate of *T. thermophilus* SecYE. The extent of this structural transition is best appreciated by comparing the interface between TM2/3 and TM7/8. In the closed conformation (*M. jannaschii* SecYE β), extensive side chain contacts link TM2 to both TM7 and TM8 (Fig. 2a). A change in the tilt angle of the *T. thermophilus* TM8 with respect to TM spans 1–5 eliminated side chain contacts between TM8 and TM2 and reduced contacts between TM7 and TM2 (Fig. 2b). For this reason, Tsukazaki and colleagues proposed that the SecYE-Fab complex provides a model for a “pre-open” conformation of the translocon [20].

A *Thermatoga maritima* SecYEG-SecA cocrystal provided a second structure of a partially open translocation channel [19]. Binding of SecA to SecYEG in the presence of a nonhydrolyzable ATP analogue induces a conformational change in SecYEG [21]. Since the SecA ATPase is activated by SecYEG [22], and might undergo a conformational change in the presence of SecYEG, the authors locked SecA in the ATP-bound conformation by obtaining crystals in the presence of ADP-BeF₃. Examination of the SecY lateral gate (Fig. 2c) shows that binding of SecA (ADP-BeF₃) to SecYEG caused a separation of both the cytosolic and exofacial portions of the lateral gate. Further expansion of the lateral gate would be necessary before a signal sequence could be inserted in an α -helical conformation [19].

The conformational change of the SecYEG lateral gate appears to be initiated by a rigid-body movement of TM spans 6–10 to accommodate insertion of a two-helix finger of SecA adjacent to cytosolic loops 6 and 8 of SecY [19]. Protein-crosslinking experiments show that the two-helix finger of SecA contacts a translocation substrate as it enters the cytoplasmic funnel of the transport pore [23], and provides an ATP hydrolysis dependent pushing force on the substrate [24]. The precise binding site on SecA for signal sequences remains a matter of debate. While it is clear that the signal sequence contacts the precursor-binding domain (PBD; also known as the polypeptide-crosslinking domain (PPXD)) of SecA, alternative binding grooves on the surface of the PBD have been identified by different experimental approaches [19,25–27]. The two more recent studies [19,26] indicate that the precursor binds in a cleft between the PBD and nucleotide-binding domain 2 (NBD2), that is closed into a precursor-binding clamp in the SecA-SecYEG cocrystal structure [19].

Twenty residues between TM1 and the plug helix are disordered in the *T. maritima* structure. Nonetheless, the C-terminal tip of the plug helix (F67) is 12.7 Å from a pore ring residue (I191) in the partially open conformation. The corresponding residues (I62 and I174) in the closed conformation of *M. jannaschii* SecYE β are only 3.9 Å apart. The plug domain of the *T. thermophilus* SecY-IgG complex remains deep within the luminal face of the pore, and the corresponding residues (F62 and I188) are 3.9 Å apart.

The location of the plug domain in a fully open SecYEG channel is a matter of current investigation. A disulfide bond can form between a cysteine residue in the plug-helix (SecY-F67C) and a cysteine residue (SecE-S120C) in the periplasmic tail of SecE [28]. Therefore, the plug helix is thought to move roughly 20 Å when the channel opens, and to occupy a peripheral binding site near SecE. A synthetic peptide that corresponds to the *E. coli* plug helix will bind to *E. coli* SecYEG, and can be photocrosslinked to SecY sequences that

precede and follow the plug helix [29]. The plug helix does not occupy the peripheral binding site in the SecA-SecYEG cocrystal, but is instead close to TM7 [19]. Although previous biophysical studies indicated that the transport channel has a polar character consistent with an aqueous pore [30], a recent study using an environmentally sensitive fluorophore incorporated into a nascent polypeptide revealed that the channel interior has an apolar patch in the vicinity of the plug domain [31].

Site specific photocrosslinking experiments established that several SecY cytosolic loops (L2, L6 and L8) and the SecY C-terminus form a binding site for the SecA ATPase [32]. The SecYEG-SecA cocrystal structure provides conformation for many of these contact sites [19]. Remarkably, R346 in loop 8 of *T. maritima* SecY (equivalent to R357 in *E. coli* SecY) does not contact SecA in the cocrystal structure, suggesting that the critical role for R357 may precede ATP-binding to SecA. Disulfide bond formation has been used to identify residues of the SecA ATPase that contact SecY [20,33]. The majority of SecA residues identified by this approach are surprisingly far from SecY in the cocrystal. A possible explanation for this paradox is that the SecYEG-SecA (ADP-BeF₃) structure may represent one of several possible binding modes for SecA on SecYEG.

Physiological role of the plug domain

The role of the plug domain has been tested by determining whether a plug domain deletion causes a lethal phenotype either due to inactivation of the translocation channel or elimination of the membrane permeability barrier. Unexpectedly, *S. cerevisiae* or *E. coli* cells that express a complete plug domain deletion (*sec61Δ52–74* or *SecYΔ42–74*) are viable and do not have a significant growth defect [34,35]. Although the X-ray crystal structure of a partial plug domain deletion (*M. jannaschii* SecYΔ57–67) revealed that adjoining peptide segments of SecY can form a minimal plug domain [36], this possibility seems unlikely when much larger segments are removed (e.g. SecYΔ42–74). Functional analysis of the plug domain deletion mutants revealed the following: (a) the plug domain stabilizes the closed conformation of the channel [35] as suggested by molecular dynamics simulations [11], (b) deletion of the plug domain reduces the fidelity of signal sequence recognition (*prlA* phenotype [35–37]), and diminishes the topological fidelity of membrane protein integration [37] and (c) deletion of the plug domain allows ion-leakage through SecYEG [38].

Roles of translocon β and γ subunits

The membrane embedded portions of SecE (Sss1 in yeast, Sec61 γ in mammals) and Sec β (SecG in eubacteria, Sbh1/Sbh2 in *S. cerevisiae*) are on the exterior surface of the channel (Fig. 1a), so these segments do not contact translocation substrates. The translocon γ -subunits (SecE and orthologues) are encoded by essential genes, so at a minimum are essential for the structural stability of the translocation channel [39,40]. Molecular dynamics simulations indicate that SecE is unlikely to act as a clamp to promote channel closing [11]. The TM span of SecE may be important for the formation of back to back SecYEG dimers [7,41]. Although SecYEG dimers can be readily detected by native gel electrophoresis [42], the functional importance of SecYEG dimer during translocation remains a matter of debate [33,43].

The β -subunits of translocation channels are nonessential in *S. cerevisiae* and *E. coli* [44,45]. However, cells that lack SecG or Sbh1/Sbh2 have conditional growth defects, and reduced translocation activity [44–46]. Diverse roles have been proposed for the eukaryotic translocon β -subunits [46], some of which are unrelated to protein translocation (as discussed in [46–48]). Sbh1p and Sbh2p are important for cotranslational translocation in yeast and appear to form part of an interaction surface for the SRP receptor on the Sec61

complex [46]. Several recent papers have shown that the more severe phenotypes caused by deletion of *S. cerevisiae* or *S. pombe* translocon β -subunits can be suppressed by expressing the TM spanning segments of Sbh1p, Sbh2p or human Sec61 β [46–48]. Taken together, it would appear that the TM span of the β -subunit is important for channel stability, while the cytosolic domain provides binding sites for interacting proteins.

Integration of membrane proteins

Integration of a simple type II ($N_{\text{cyt}}\text{-}C_{\text{lum}}$) membrane protein is conceptually similar to translocation of a secreted protein. The N-terminal TM span of the type II protein inserts into the SSB site to gate the channel. Based upon the results of photocrosslinking experiments, a TM span inserts into the SSB site in an α -helical conformation and is in contact with phospholipids and Sec61 [49]. Biophysical studies suggest that transmembrane spans adopt an α -helical conformation prior to emergence from the polypeptide exit site on the large ribosomal subunit [50]. Exit of the TM span from the SSB site and entry into the membrane bilayer is governed by the hydrophobicity of the residues in the TM span [51,52]. Integration of multi-spanning membrane proteins adds additional levels of complexity to channel function [53]. During integration of a multi-spanning membrane protein, subsequent TM spans that are integrated in the $N_{\text{lum}}\text{-}C_{\text{cyt}}$ orientation do not pass through the translocon pore but instead move laterally to enter a vacant SSB site, while subsequent TM spans that are integrated in the $N_{\text{cyt}}\text{-}C_{\text{lum}}$ orientation after synthesis of a cytosolic loop must insert into the SSB site to re-gate the translocon. The *in vivo* kinetics of membrane protein integration in *S. cerevisiae* has been investigated using ubiquitin translocation assay reporters [54]. These experiments indicate that the structural transitions of the translocon that allow lateral exit of TM spans into the lipid bilayer occur less rapidly than elongation of the nascent polypeptide, and this results in cytosolic exposure of transmembrane spans and luminal domains [54]. Photo-crosslinking studies using elongation-arrested membrane protein integration intermediates have been extensively used to monitor successive events in the integration of complex integral membrane proteins (e.g. [55]). In our opinion, artificially arrested integration intermediates monitor equilibrium conformations of the nascent membrane protein rather than bona fide kinetic intermediates in the integration pathway. A challenge for the future will be to develop methods that provide real-time biophysical information concerning the mechanism of membrane protein integration.

Conclusions and perspective

Research in the past several years has revealed new information concerning the mechanism of protein transport through the SecYEG and Sec61 translocation channels. Additional intermediates in the SecYEG-SecA ATPase cycle, perhaps with a model precursor, need to be prepared and characterized at a structural level. A major question that remains unresolved is how a ribosome-nascent chain complex initiates opening of prokaryotic and eukaryotic translocation channels. The SecA ATPase is required for cotranslational integration of inner membrane proteins with large periplasmic domains [56]. How can SecYEG provide simultaneous binding sites for both SecA and the ribosome, given that the binding sites overlap? The Sec62/Sec63 complex serves as a signal receptor for yeast posttranslational translocation, and in concert with the luminal Hsp70 protein Kar2p, mediates translocation through the Sec61 complex [57,58]. As such, this represents a second translocon gating mechanism that is not understood at a structural or mechanistic level. Structure-based models for the translocation reaction will need to be tested in multiple experimental systems using genetic, biochemical and biophysical approaches.

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Figure 1.

M. jannaschii SecYE β (PDB 1RHZ). **(a)** Cytosolic face. TM spans are color coded as follows: TMs 1, 3–5, (cyan); TMs 6, 8–10 (magenta); TM2 (blue); TM7 (red). The plug domain (residues 52–74) is shown as yellow spheres. Pore ring residues (I175, V79, I170, I174, I260 and L406) are shown as black sticks. **(b)** Lateral gate of SecYE β viewed from the plane of the membrane. TM spans are color coded as follows: TM2, blue; TM3, cyan; TM7, red and TM8, magenta. Loops 6 and 8 are pink and olive respectively. All other SecY segments are sky blue. Residues that make significant contacts across the lateral gate are shown as spheres. **(c)** The hinge domain of SecYE β . The hinge helix is cyan. Hinge loops (HL-1 and HL-2) are red. Loops 6 and 8 are colored as in panel **b**. The arrow labeled M.B. is the estimated location of the membrane bilayer. All structure views were generated using Pymol.

Figure 2.

Comparison of SecY lateral gate domains. Transmembrane spans 2, 3, 7 and 8 are colored blue, cyan, red and magenta, respectively. Loop 2 is slate and loop 7 is violet. The plug domain is shown as yellow spheres. **(a)** The *M. jannaschii* plug corresponds to residues 52–74. Lateral gate residues (shown as spheres) are as follows: TM2 (V79, T80, I83, L87 and S91); TM3 (E122, L125, F126 and A129); TM7 (L261, A264, L265, N268, L271, W272 and R278); TM8 (F330, F333, W334 and T337). **(b)** The *T. thermophilus* plug domain corresponds to residues 50–76 (PDB 2ZJS). Residues that align with those in panel **a** are shown as spheres. **(c)** The segment of the *T. maritima* plug domain shown here corresponds to residues 62–81 (PDB 3DIN). Residues that are shown as spheres include TM2 and TM3 residues that align with those shown in panel **a**, as well as the following: TM7 (F275, A278, I279, I282, A285, I286, I279); TM8 (F317, Y321). All structure views were generated using Pymol.