Protein Translocation by the Sec61/SecY Channel

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Key Words

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Abstract

The conserved protein-conducting channel, referred to as the Sec61 channel in eukaryotes or the SecY channel in eubacteria and archaea, translocates proteins across cellular membranes and integrates proteins containing hydrophobic transmembrane segments into lipid bilayers. Structural studies illustrate how the protein-conducting channel accomplishes these tasks. Three different mechanisms, each requiring a different set of channel binding partners, are employed to move polypeptide substrates: The ribosome feeds the polypeptide chain directly into the channel, a ratcheting mechanism is used by the eukaryotic endoplasmic reticulum chaperone BiP, and a pushing mechanism is utilized by the bacterial ATPase SecA. We review these translocation mechanisms, relating biochemical and genetic observations to the structures of the protein-conducting channel and its binding partners.

Contents

INTRODUCTION

Protein transport across the ER membrane in eukaryotes is an early and decisive step in the biosynthesis of many proteins (for earlier reviews, see Hegde & Lingappa 1997, Johnson & van Waes 1999, Matlack et al. 1998). These proteins can be divided into two groups: soluble proteins, such as those ultimately secreted from the cell or localized to the ER lumen, and membrane proteins, such as those in the plasma membrane or in other organelles of the secretory pathway. In eubacteria and archaea, protein transport occurs directly through the plasma membrane and is also an important step in the biosynthesis of secreted and membrane proteins. Soluble proteins cross the membrane completely and usually have N-terminal cleavable signal sequences, whose major feature is a short hydrophobic segment (typically 7–12 amino acid residues). Membrane proteins have different topologies, with one or more TM segments, each containing about 20 hydrophobic residues. Membrane proteins have soluble domains that are translocated through the membrane as well as soluble domains that remain in the cytosol. Both types of proteins use the same machinery for translocation across the membrane: a protein-conducting channel with a hydrophilic interior (Crowley et al. 1993, Simon & Blobel 1991). This channel, in contrast to those channels that transport ions and small molecules, has the unusual property of being able to open in two directions: perpendicular to the plane of the membrane to allow a polypeptide segment across and within the membrane to allow a hydrophobic TM segment of a membrane protein to exit laterally into the lipid phase. The protein-conducting channel is formed by an evolutionarily conserved heterotrimeric membrane protein complex termed the Sec61 complex in eukaryotes and the SecY complex in eubacteria and archaea. In this review, we summarize our current understanding of how the channel functions in protein translocation, with special reference to its recently determined X-ray structure (van den Berg et al. 2004).

ER: endoplasmic reticulum

TM: transmembrane

THE Sec61/SecY COMPLEX

The largest subunit of the heterotrimeric Sec61/SecY complex is the α-subunit, termed Sec61α in mammals, Sec61p in *Saccharomyces cerevisiae*, and SecY in eubacteria and archaea (for a review, see Rapoport et al. 1996). This subunit spans the membrane ten times, with both the N- and C termini in the cytosol. The β-subunit is termed Sec61β in mammals, Sbh1p in *S. cerevisiae*, SecG in eubacteria, and Secβ in archaea. In eukaryotes and archaea, this subunit spans the membrane once with the N- terminus in the cytosol. SecG in eubacteria spans the membrane twice. The γ-subunit is termed Sec61γ in mammals, Sss1p in *S. cerevisiae*, and SecE in eubacteria and archaea. In most species, this subunit is a single-spanning protein with its N terminus in the cytosol. In some eubacteria, e.g., *Escherichia coli*, the γ-subunit has two additional N-terminal TM segments that are not essential for its function. The α- and γ-subunits of the

Sec61/SecY complex are found in all organisms and show low, but significant, sequence conservation. The β-subunits are homologous among eukaryotes and archaea but have no obvious sequence similarity to the eubacterial SecG. The α- and γ-subunits are essential for viability of yeast and eubacteria, whereas the β-subunit is not. Together, these observations indicate that the α- and γ-subunits constitute the core of the channel-forming complex. Several organisms have two copies of Sec61 or SecY (Bensing & Sullam 2002, Rapoport et al. 1996), and in some cases the second copy may transport specific substrates (Bensing & Sullam 2002). In *S. cerevisiae*, there is a second Sec61 complex (Ssh1 complex), which is not essential for viability and seems to function exclusively in cotranslational translocation (Finke et al. 1996).

The initial evidence that the Sec61/SecY complex forms a protein-conducting channel came from systematic cross-linking experiments in which photoreactive probes were placed at different positions of a polypeptide substrate (Mothes et al. 1994). Substrates with probes at positions predicted to be within the membrane could be cross-linked to the α-subunit of the Sec61 complex, but not to other membrane proteins. These data indicated that the α-subunit surrounds the polypeptide chain as the chain passes through the membrane. Strong support for the notion that the Sec61/SecY complex forms a channel came from experiments in which the purified complex was reconstituted into proteoliposomes and shown to be the essential membrane component for protein translocation (Akimaru et al. 1991, Brundage et al. 1990, Gorlich & Rapoport 1993).

THREE DIFFERENT MODES OF TRANSLOCATION

The protein-conducting channel formed by the Sec61/SecY complex is a passive pore that allows a polypeptide chain to slide back and forth. The channel therefore needs to associate with partners that provide a driving force for translocation. Depending on the partner, the channel can function in three different translocation modes.

The first mode, cotranslational translocation, involves the ribosome as the major channel partner (**Figure 1**). This is a general translocation mechanism found in all organisms and cells, and it is responsible for the integration of most membrane proteins. Cotranslational translocation begins with a targeting phase during which a ribosome-nascent chain complex is directed to the membrane by the signal recognition particle (SRP) and its membrane receptor (SRP receptor) (for review, see Halic & Beckmann 2005, Luirink & Sinning 2004). Once the ribosome is bound to the protein-conducting channel, the elongating polypeptide chain is moved from the ribosome to the membrane channel; GTP hydrolysis during translation provides the energy for translocation (**Figure 1**). When the ribosome synthesizes a cytosolic domain of a membrane protein, the polypeptide chain emerges from the ribosome-channel junction sideways into the cytosol (Mothes et al. 1997). In a later section we discuss cotranslational translocation in more detail.

In eukaryotes, there is a second mode of translocation by which proteins are transported after completion of their synthesis (posttranslational translocation). Proteins that use this mode have a less hydrophobic signal sequence and may therefore escape interaction with SRP during their synthesis (Ng et al. 1996). The mechanism of posttranslational translocation has been determined in *S. cerevisiae* (Matlack et al. 1999), and it is likely to be the same in higher eukaryotes. In this mode of translocation, the channel partners are another membrane protein complex (the Sec62/63 complex) and the lumenal protein BiP, a member of the Hsp70 family of ATPases. In yeast, the Sec62/63 complex is a tetramer that, together with the Sec61 complex, forms a seven-component Sec complex (Deshaies et al. 1991, Panzner et al. 1995). In addition to the essential proteins Sec62p and Sec63p, this complex contains the
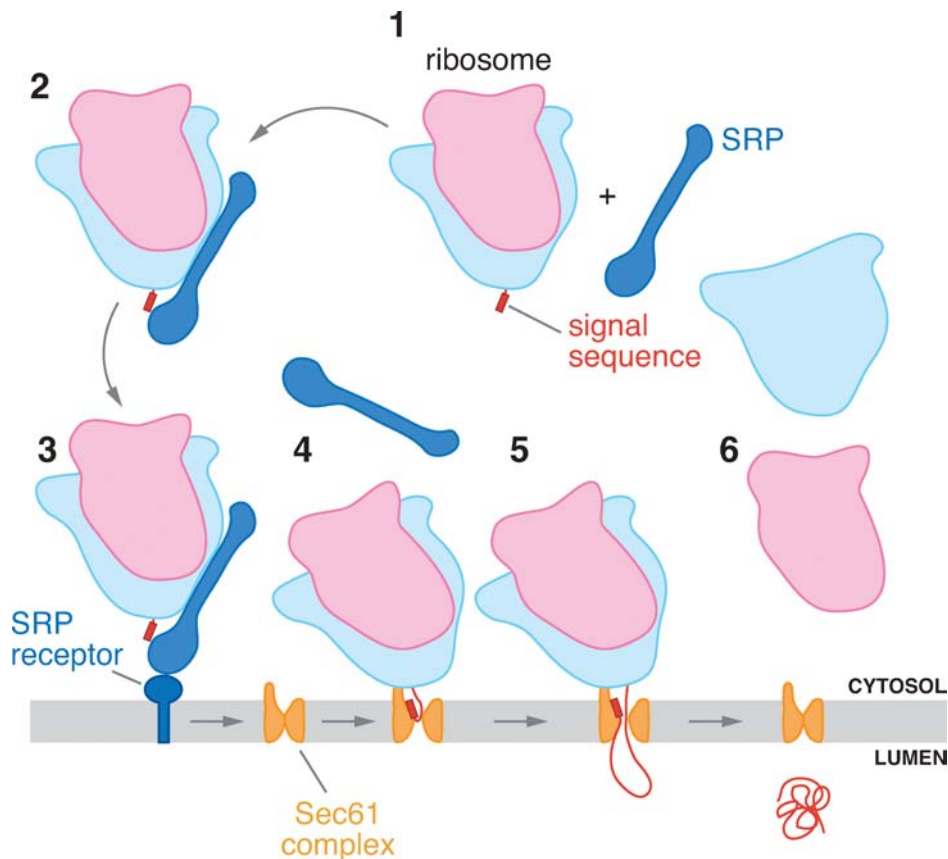
Figure 1

Cotranslational translocation of a secretory protein. The scheme shows different steps in the translocation of a eukaryotic secretory protein. (*1* and *2*) The signal recognition particle (SRP) binds to the signal sequence in a growing polypeptide chain as well as to the ribosome (large subunit, *light blue*; small subunit, *pink*). (*3*) The entire complex is targeted to the membrane by an interaction of the SRP with the SRP receptor. (*4* and *5*) The SRP is released, and the ribosome binds to the protein-conducting channel formed by the Sec61 complex. The polypeptide inserts into the channel as a loop, with the N and C termini in the cytosol. The signal sequence is intercalated into the wall of the channel, and the following polypeptide segment is located in the pore proper. (*6*) The remainder of the polypeptide chain moves from the ribosome tunnel, through the channel, and to the other side of the membrane. The signal sequence is cleaved at some point during translocation.

nonessential components Sec71p and Sec72p. Mammalian cells have Sec62p and Sec63p but lack the other two proteins (Meyer et al. 2000, Tyedmers et al. 2000).

The driving force for posttranslational translocation is generated by a ratcheting mechanism (**Figure 2**) (Matlack et al. 1999). A polypeptide in the channel can slide in either direction, but its binding to BiP inside the ER lumen prevents movement back into the cytosol, resulting in net forward translocation. ATP-bound BiP, with an open peptide-binding pocket, interacts with a lumenal domain of Sec63p, termed the J domain. This interaction stimulates rapid ATP hydrolysis and closure of the peptide-binding pocket around the incoming polypeptide chain. When the polypeptide has moved a sufficient distance in the forward direction, another BiP molecule can bind to it; this process
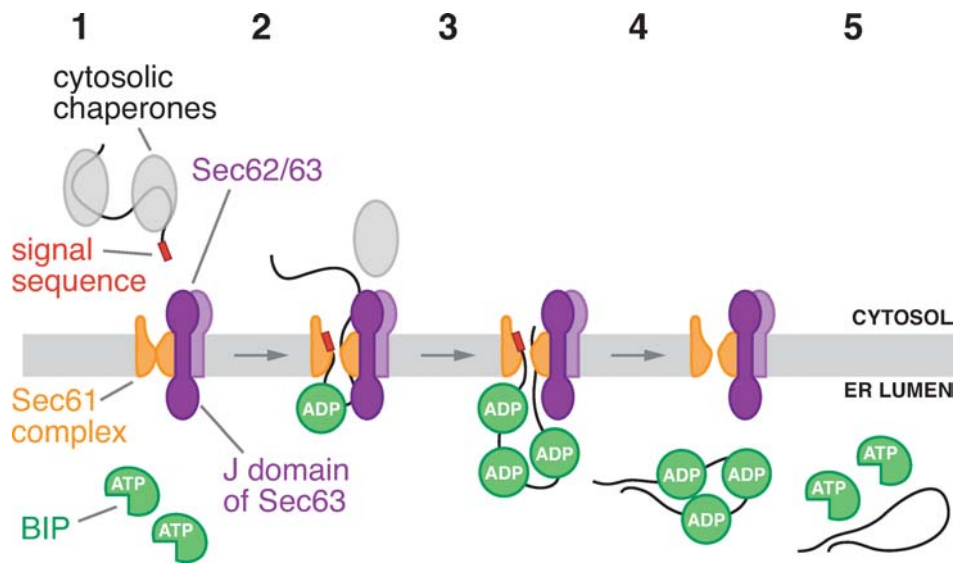
Figure 2

Posttranslational translocation in eukaryotes. (*1*) After it is synthesized in the cytosol, an unfolded polypeptide is kept in solution by cytosolic chaperones. (*2*) It is targeted by its signal sequence to the translocation channel, comprised of the Sec61 complex and the Sec62/63 complex, and the cytosolic chaperones are released. The J domain of Sec63 stimulates ATP hydrolysis by BiP, and ADP bound BiP binds to the polypeptide chain emerging into the ER lumen. (*3*) When the polypeptide has moved a sufficient distance into the ER lumen, another BiP molecule can bind to it. (*4*) This process is repeated until the polypeptide chain has completely traversed the channel. (*5*) BiP is released upon exchange of ADP for ATP; this exchange opens the peptide-binding pocket.

is repeated until the polypeptide chain has completely traversed the channel. When ADP is exchanged for ATP, the peptide-binding pocket opens, and BiP is released.

Several aspects of the ratcheting mechanism deserve comment. First, prior to translocation, a polypeptide substrate loses all bound cytosolic chaperones, facilitating its passive forward movement. Cross-linking experiments show that several different chaperones bind to the completed polypeptide and probably cycle on and off (Plath & Rapoport 2000). However, once a polypeptide chain has bound to the Sec complex through its N-terminal signal sequence, even chaperones that interact with the C terminus are released. The Sec complex does not stimulate the dissociation of chaperones, but rather prevents their rebinding, perhaps through the sizable cytosolic domains of Sec62p and Sec63p. A specific targeting molecule, similar to SRP in cotransla-

tional translocation, has not been found. Second, BiP binds to a diverse set of substrates and, within each polypeptide, to different segments. Although BiP preferentially binds hydrophobic peptides under equilibrium conditions, it shows little sequence specificity when activated by the J domain of Sec63p (Misselwitz et al. 1998). Under such nonequilibrium conditions, even segments that do not fit perfectly into the peptide-binding pocket can bind. Third, the location of the J domain ensures that BiP activation only occurs close to the channel, where BiP binding to the polypeptide chain is most effective in preventing its backsliding (**Figure 2**). Once a polypeptide segment has moved away from the channel, new BiP molecules do not bind, whereas those that bound previously can dissociate. Fourth, forward movement of the polypeptide chain is likely by Brownian motion. This is supported by the observation that

in proteoliposomes containing the Sec complex, ATP-independent translocation occurs if BiP is replaced by antibodies to the substrate (Matlack et al. 1999). Mathematical modeling also shows that a Brownian ratcheting mechanism is sufficient to explain the kinetics of translocation (Liebermeister et al. 2001).

A third mode of translocation, found only in eubacteria, also occurs posttranslationally; it is used by most secretory proteins (for review, see Mori & Ito 2001). In this case, the channel partner is a cytosolic ATPase, termed SecA. SecA likely undergoes conformational changes coupled to its ATPase cycle and pushes polypeptides through the SecY channel in a stepwise manner (**Figure 3**) (Economou & Wickner 1994). We discuss the mechanism of SecA-mediated translocation in more detail later.

Archaea probably have both co- and posttranslational translocation (Irihimovitch & Eichler 2003, Ortenberg & Mevarech 2000), but it is unclear how they perform the latter, as they lack both SecA and the Sec62/63 complex.

THE X-RAY STRUCTURE OF THE SecY COMPLEX AND ITS IMPLICATIONS

Significant insight into the function of the protein-conducting channel is provided by the 3.2 Å resolution X-ray structure of the detergent-solubilized SecY complex from the archaebacterium *Methanococcus jannaschii* (van den Berg et al. 2004). Given the sequence similarities mentioned above, it is likely that the structure is representative of all species. In addition, the structure of the *E. coli* SecY complex, determined by EM of two-dimensional crystals (Breyton et al. 2002), shows that all TM segments are virtually superimposable onto those of the archaeal complex (van den Berg et al. 2004). This observation also means that the structure of the SecY complex in detergent is very similar to that in a lipid bilayer.

In the X-ray structure, the SecY complex contains one copy of each of the three subunits (**Figure 4**). Viewed from the cytosol, the complex has an approximately square shape. The two small subunits (SecE and Secβ) are
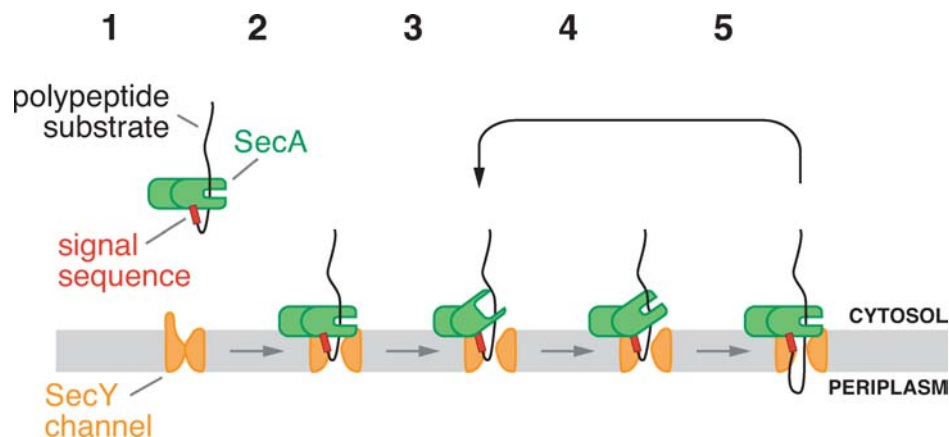

Figure 3

SecA-mediated posttranslational translocation in eubacteria. The scheme shows a model for the different steps in translocation. (*1*) SecA binds to a polypeptide substrate bearing an N-terminal signal sequence. (*2*) The complex binds to SecY, and the polypeptide substrate inserts as a loop into the channel. (*3*) The SecA polypeptide–binding groove opens and moves away from the channel, leaving a polypeptide segment in the channel. (*4*) The binding groove grabs the next section of the polypeptide chain and then closes. (*5*) The polypeptide-binding domains move toward the channel, pushing the polypeptide segment into the channel. Steps (*3*)–(*5*) are repeated until the polypeptide chain is fully translocated (not shown).
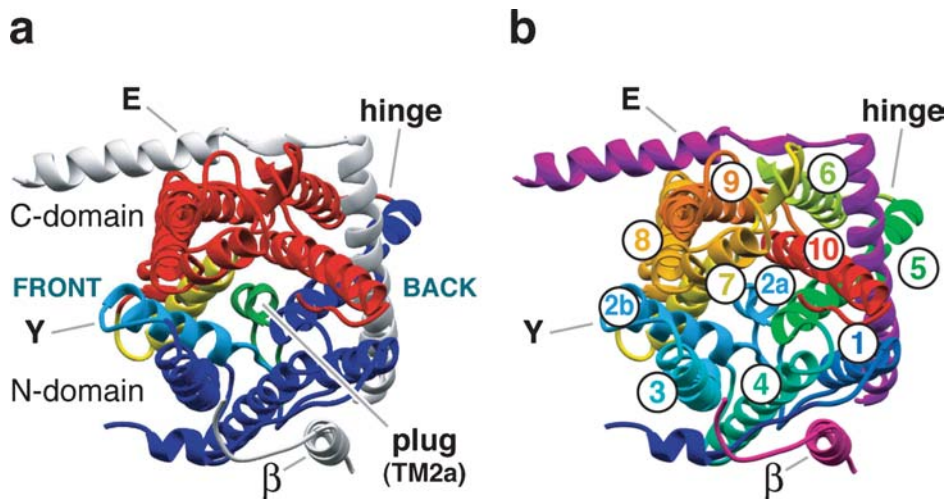
Figure 4

(*a*) The structure of the *M. jannaschii* SecY complex viewed from the cytoplasm. The N-terminal domain of SecY (TM1–5) is shown in dark blue, with the exception of TM2b (*bright blue*). The C-terminal domain (TM6–10) is shown in red, with the exception of TM7 (*yellow*). The signal sequence intercalates at the front, between TM2b and TM7. The plug (TM2a), which blocks the pore of the closed channel, is shown in green. The proposed hinge region between TM segments 5 and 6 is labeled. (*b*) A cytoplasmic view of the *M. janaschii* SecY complex with individual helices colored and labeled.

located at the periphery of the complex. The SecE subunit contacts SecY extensively, occupying two sides of the square. The SecY subunit contains ten TM segments, organized into N- and C-terminal domains, comprising TM1–5 and TM6–10, respectively (**Figure 4**). The two domains are connected at the back of the complex by the loop between TM5 and TM6. SecY displays pseudosymmetry such that its C-terminal domain is essentially an upside-down version of its N-terminal domain. The domain organization of SecY and the locations of the two small subunits at the periphery leave the front of the complex as the only site that could open laterally toward the lipid phase. Such a lateral gate is necessary for the function of the SecY complex. The complex can therefore be likened to a clamshell that can open at the front toward the lipid, with the hinge located at the back of the complex between TM5 and TM6. The SecE subunit may serve as a brace that prevents the two domains from separating completely.

The X-ray structure suggests that the channel pore is located at the center of a single copy of the SecY complex (van den Berg et al. 2004) rather than at the interface of three or four complexes (Beckmann et al. 1997, Breyton et al. 2002, Manting et al. 2000, Morgan et al. 2002). Disulfide bridge formation between cysteines in a translocation substrate and cysteines in SecY support the notion that the polypeptide chain moves through the center of a single SecY molecule (Cannon et al. 2005). In addition, almost all of the conserved residues in the SecY complex are located not at the periphery but in the center of the complex (van den Berg et al. 2004). Mutations that allow proteins with defective or missing signal sequences to be transported (prl mutations; Bieker et al. 1990, Derman et al. 1993) are also located in the center of the SecY complex. Moreover, the interface of laterally associated complexes cannot form a hydrophilic pore; similar to all other membrane proteins, a single SecY complex has an entirely hydrophobic belt of ∼25 Å width around it.

In a membrane, this belt would be exposed to the hydrophobic interior of the lipid bilayer. Together, these observations suggest that the pore is contained within a single SecY complex. Indeed, the structure shows a cytoplasmic funnel that may mark the channel entrance. The funnel tapers to a close in the middle of the membrane and is blocked on the extracytoplasmic side by the presence of a small helical segment (TM2a) dubbed the "plug" (**Figure 4a**) (van den Berg et al. 2004). The crystal structure of the archaebacterial SecY complex therefore corresponds to that of a closed channel; this is as expected, given that the complex was crystallized in the absence of translocation partners and substrate.

Opening of the channel appears to require movement of the plug (**Figure 5**). Cysteines introduced into the plug and into the TM segment of SecE of the *E. coli* SecY complex form a disulfide bridge in vivo (Harris & Silhavy 1999), suggesting that the plug moves toward the back of the complex, into a cavity at the extracellular side. Disulfide bridge formation cannot be explained by the structure of the closed channel, in which the cysteines would be too far apart (>20 Å). As expected, locking the channel into a permanently open state by inducing disulfide bridge formation is lethal to cells (Harris & Silhavy 1999).

The channel is probably in a dynamic equilibrium, with the plug moving between the closed and open positions. In the unoccupied channel, the equilibrium is on the side of the closed state, but it can be shifted toward the open state by the binding of a signal sequence or, in the case of many membrane proteins, a TM segment. Cross-linking experiments have shown that the hydrophobic core of a signal sequence forms a short helix containing about two turns. This helix intercalates between TM2b and TM7 of Sec61/SecY at the front of the molecule and contacts phospholipids (Plath et al. 1998). The translocation substrate is inserted as a loop; the signal sequence is intercalated into the channel wall, and the following polypeptide segment is located in the pore proper. Signal sequence intercalation requires a hinge motion at the back of Sec61/SecY to open the "mouth of
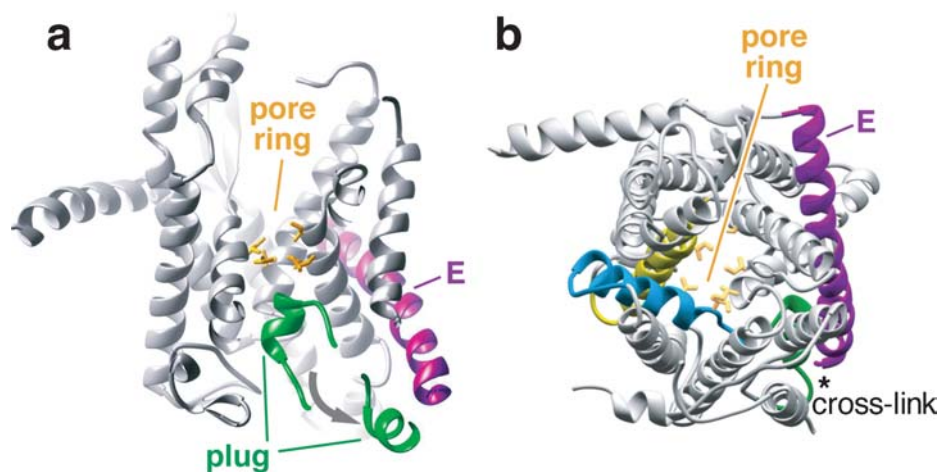

Figure 5

Plug movement leads to opening of the SecY channel. (*a*) View from the side of the channel with the front half of the model cut away. The modeled movement of the plug toward the SecE subunit is indicated by an arrow. The side chains of residues in the pore ring are colored in gold. (*b*) Cytosolic view, with the plug modeled in its open position. TM2b and TM7 located at the front of the complex are shown in blue and yellow, respectively. The asterisk indicates the region where introduced cysteines result in cross-links between the plug and the TM segment of SecE (Harris & Silhavy 1999).

the clamshell." The separation of the two halves of the molecule may destabilize interactions that keep the plug in the center of the molecule, thus promoting channel opening. In support of this model, many signal sequence suppressor mutations in SecY appear to destabilize the structure of the closed channel (van den Berg et al. 2004). Once the signal sequence is inserted into the channel walls, the polypeptide segment distal to the signal sequence may move through the pore and prevent the plug from returning to its closed-state position (**Figure 1**).

The binding of a channel partner (SecA or the ribosome) may also regulate channel opening. Support for the notion that ribosomes destabilize the closed state of the channel comes from electrophysiological experiments, in which increased ion conductance is observed when a nontranslating ribosome is bound to the channel (Simon & Blobel 1991). The ribosome binds exclusively to the cytosolic loops located in the C-terminal half of Sec61/SecY (Raden et al. 2000) and therefore does not prevent the separation of the two halves of the molecule.

The open channel may be shaped like an hourglass, with hydrophilic funnels on both sides of a constriction in the center of the membrane. This is consistent with the observation that a translocating polypeptide chain moves through the membrane in an aqueous environment (Crowley et al. 1993, Simon & Blobel 1991). During translocation, a substrate may primarily make contact with residues at the channel constriction, minimizing substrate-channel interactions. Restriction of contacts between the translocating chain and the channel to a narrow region is supported by recent experiments (Cannon et al. 2005).

The constriction point of the channel, or pore ring, consists of six hydrophobic amino acid residues, which in many species are isoleucines (**Figure 5**) (van den Berg et al. 2004). The pore ring may fit like a gasket around the translocating polypeptide chain, thereby providing a seal that restricts the passage of ions and other small molecules during protein translocation. In this model, the membrane barrier can be maintained in all modes of translocation. In an alternative model, the seal for small molecules is provided by the binding of a ribosome to the cytosolic side of the channel or by the binding of BiP to the ER lumenal side (Crowley et al. 1994, Hamman et al. 1998). This model is at odds with the available structural data (for further discussion see Rapoport et al. 2004). In addition, it does not explain how the membrane barrier is maintained in the absence of a ribosome (during posttranslational translocation) or in the absence of BiP (in prokaryotes).

In addition to plug movement, widening of the pore is likely required to allow polypeptide chain translocation. The diameter of the pore ring, as observed in the crystal structure, is too small to allow passage of even an unfolded, extended polypeptide chain. Widening of the channel may occur by movement of the helices to which the pore residues are attached. Flexible glycine-rich sequences in the cytosolic loops between TM4 and TM5 and between TM9 and TM10 may allow the channel to accommodate movement of these helices. Pore widening is required to explain the experimentally observed translocation of α-helices, a 13-residue disulfide-bonded polypeptide loop (Tani et al. 1990), or of amino acid side chains modified with bulky groups (De Keyzer et al. 2002, Kurzchalia et al. 1988). The flexibility of the pore region is supported by molecular dynamics simulations, which show that a ball of 10–12 Å or a helix with a diameter of 10 Å may move through the pore (P. Tian & I. Andricioaei, J. Gumbart & K. Schulten, personal communications). The intercalation of a signal sequence at the front of Sec61/SecY (opening of the clamshell) may cause additional widening of the pore, as is required for loop insertion of a polypeptide chain.

The estimated maximum dimensions of the pore based on the X-ray structure are $\sim 15 \times 20$ Å. This is much smaller than the estimate of a pore diameter of at least 40 Å; this

latter estimate derives from the observation that large reagents can pass through the membrane channel to quench fluorescent probes in a nascent polypeptide chain (Hamman et al. 1997). Such a large hydrophilic channel can be generated only if several Sec61/SecY molecules associate with their front surfaces and open to fuse their pores. However, at least in eubacteria, SecY molecules appear to associate back-to-back. This is the arrangement in the dimer seen by EM in two-dimensional crystals of the *E. coli* SecY complex (**Figure 6**) (Breyton et al. 2002), and the functional importance of this orientation in posttranslational translocation is supported by cross-linking experiments (Kaufmann et al. 1999). The fluorescence quenching data are also at odds with the X-ray structure of the large ribosomal subunit, because the same reagents quench probes inside the ribosomal tunnel, which has a diameter much narrower than 40 Å (Hamman et al. 1997). A relatively narrow pore is also consistent with the fact that even a small polypeptide domain can-

Figure 6

Back-to-back arrangement of *E.coli* SecY complexes in the dimer as seen in the EM structure derived from two-dimensional crystals (Breyton et al. 2002). TM2b and TM7 at the front of the complexes are colored in blue and yellow, respectively. The plug is shown in dark green. Cysteines introduced at the positions indicated by the red spheres result in efficient disulfide formation (X) between two SecE subunits (Kaufmann et al. 1999).

not fold inside the channel (Kowarik et al. 2002).

MECHANISM OF COTRANSLATIONAL TRANSLOCATION

Ribosome-Channel Interaction

The eukaryotic ribosome-channel complex has been visualized by single-particle EM (Beckmann et al. 1997, 2001, Menetret et al. 2000, Morgan et al. 2002). The ribosome likely is associated with four copies of the Sec61 complex. A low-density area in the center of the assembly was initially interpreted as a central pore, but in the most recent reconstructions at ~15–17 Å resolution, with an improved contour level, a pore is no longer visible (Beckmann et al. 2001, Morgan et al. 2002). Although the resolution of the EM data is insufficient to unambiguously dock the X-ray structure of the SecY complex, a plausible arrangement of the four Sec61 molecules consists of two side-by-side associated dimers, which in turn are formed by back-to-back assembled monomers (**Figure 7**) (Menetret et al. 2005). Such a side-by-side packing of dimers is seen in the two-dimensional crystals of the *E. coli* SecY complex (Breyton et al. 2002). This arrangement generates a low-density central region, but this region is entirely hydrophobic and may be filled with lipid or, after solubilization, with detergent. The β-subunits contribute significantly to the interface between the dimers (Bessonneau et al. 2002, Breyton et al. 2002), but are not essential, suggesting that ribosome binding may play a significant role in assembling the tetramer.

The linkage between the ribosome and the four copies of the Sec61 complex consists of approximately four to seven connections (Beckmann et al. 2001, Menetret et al. 2005, Morgan et al. 2002). Several ribosomal proteins and regions of ribosomal RNA, which may be involved in the interaction, have been identified (Beckmann et al. 2001, Morgan
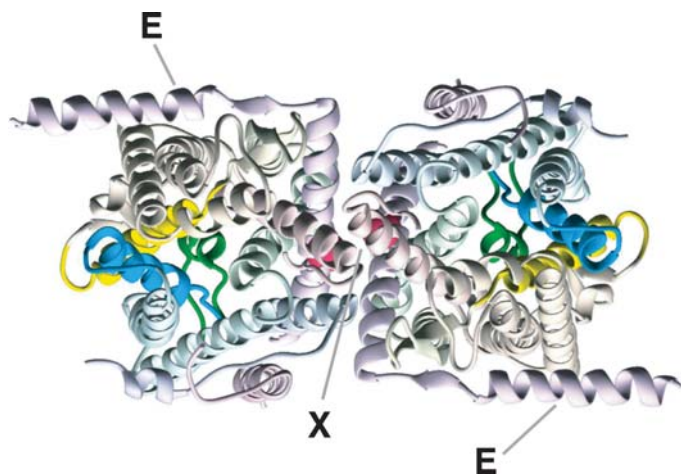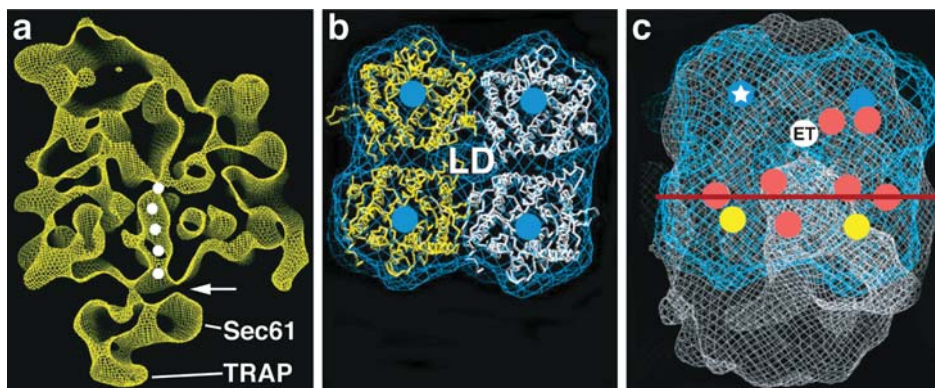
Figure 7

EM structure of ribosome-Sec61 channel complexes and possible oligomeric arrangement of Sec61 complexes (Menetret et al. 2005). (*a*) Ribosome-channel complexes derived from native ER membranes were analyzed by single-particle EM. A cross section through the ribosome-channel complex, viewed from the side, is shown. The ribosomal exit tunnel, from which a growing polypeptide chain would emerge, is marked by white dots. The gap between the ribosome and channel is labeled with an arrow. A lumenal protrusion is formed by the TRAP (translocon-associated protein) complex. (*b*) A tetrameric assembly of SecY complexes, as seen in the EM structure of two-dimensional crystals of the *E. coli* SecY complex (Breyton et al. 2002). The position of the pores within each SecY molecule is indicated by a blue dot. The expected position of a low-density central region is labeled LD. A mask generated to encompass the whole volume of the tetramer is shown as a blue mesh. (*c*) A lumenal view of the Sec61 channel bound to the ribosome. The blue mask enveloping the tetrameric SecY assembly shown in (*b*) is docked into the density. The ribosomal exit tunnel is marked by a white dot and labeled ET. Connections between the ribosome and the channel are marked by red dots. The prominent line of connections is indicated by a red line. The pores of the two copies of Sec61 that are separated from the ribosome exit site by the line of connections are labeled with yellow dots. One of the other two pores (in *blue*) may translocate a polypeptide chain. The Sec61 complex with only weak or no connections to the ribosome is additionally labeled with a white asterisk. The region of density not occupied by the blue meshwork is assigned to TRAP.

et al. 2002). Biochemical data suggest that RNA provides the major contacts with the channel (Prinz et al. 2000). As expected from the asymmetry of the ribosome, the four copies of Sec61 complex bind differently; one of them has no or only weak connections (**Figure 7***c*), whereas the others have multiple linkages. Two of the Sec61 molecules are on one side of a line of connections, which separates them from the exit site where the nascent chain emerges from the ribosome, leaving one of the other two copies to form the active pore. The ribosome-channel junction is open and thus provides a path for polypeptides from the ribosomal exit site into the cytosol, as is required when the ribosome synthesizes cytosolic domains of membrane proteins. The gap of 12–15 Å width between the ri-

bosome and channel is consistent with the size of the cytosolic loops in the C-terminal half of Sec61/SecY (van den Berg et al. 2004), which provide the major ribosome-binding sites (Raden et al. 2000). The size of the gap may prevent many large cytosolic molecules from reaching the pore and passing through it, but the pore ring inside the channel is likely the main device that maintains the membrane barrier.

If the channel is formed from a single copy of the Sec61 complex, what is the role of oligomerization? The answer is not yet known, but one possibility is that oligomerization serves to create binding sites for the recruitment of other components. In eukaryotes, these other components may include signal peptidase, which cleaves signal sequences

TRAM:
translocating
chain–associating
membrane protein

TRAP: translocon-
associated
protein

from translocating polypeptides; oligosaccharyl transferase, which attaches carbohydrate chains to them; and TRAM, a multispanning membrane protein that may serve as a membrane chaperone (see below). All of these proteins are close to the channel, but have no strong affinity for either the isolated Sec61 complex or the ribosome (Gorlich & Rapoport 1993). Oligomerization may also be the trigger for the recruitment of the TRAP complex, a tetrameric membrane protein complex of unknown function. EM analysis of ribosome-channel complexes derived from native ER membranes shows that TRAP is bound to the two Sec61 complexes that are inaccessible to the nascent chain (**Figure 7c**) (Menetret et al. 2005). This suggests that the function of these Sec61 complexes is to recruit TRAP rather than to translocate a nascent chain. Oligomerization of the Sec61 complex may also regulate ribosome binding. Tetramers may provide a larger number of linkages, resulting in strong ribosome binding during translocation, whereas dissociation of the tetramers may weaken the interaction and facilitate ribosome release upon termination of translocation.

The ribosome-channel interaction in eubacteria and archaea has not been studied extensively. It is unclear whether it is as tight as in eukaryotes or whether tetramers of the SecY complex are involved. Several bacterial membrane proteins require SecA for translocation of their extracellular domains (Neumann-Haefelin et al. 2000). For steric reasons, the ribosome and SecA cannot bind simultaneously to the channel. This suggests that, in contrast with the situation in eukaryotes, the ribosome in eubacteria and archaea may dissociate during translocation.
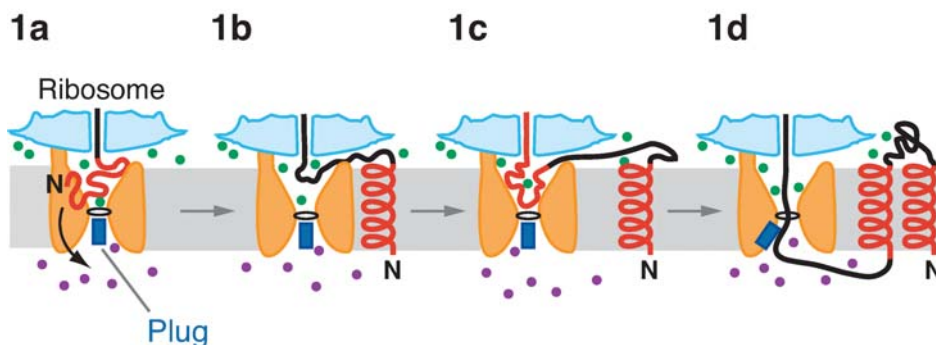
Membrane Protein Integration

The integration of membrane proteins is more complicated than the translocation of soluble proteins, and many issues are still unresolved. In the following section, we briefly summarize our current understanding (for

a more extensive discussion of controversial points, see Rapoport et al. 2004).

In contrast to a signal sequence, which always has its N terminus in the cytosol, the first TM segment of a nascent membrane protein can have its N terminus on either side of the membrane, depending on the amino acid sequence of the protein. In a multispanning protein, the first TM segment often determines the orientation of the subsequent ones, which alternate correspondingly. A model for how the orientation of the first TM segment may be determined is depicted in **Figure 8**. A passive orientation of downstream TM segments is suggested by the fact that many membrane proteins seem to have evolved by the fusion of two halves that have opposite orientations. In this respect, it is interesting to note that the transporter EmrE is proposed to be a dimer of identical subunits with opposite topologies (Ma & Chang 2004), similar to the postulated evolutionary predecessors of current membrane proteins possessing pseudo twofold symmetry. There are, however, exceptions in which internal TM segments have a preferred orientation regardless of the behavior of preceding TM segments (Gafvelin & von Heijne 1994, Goder et al. 1999, Locker et al. 1992, McGovern et al. 1991, Nilsson et al. 2000, Sato et al. 1998).

During the synthesis of a membrane protein, TM segments must move from the aqueous interior of the channel through its lateral gate into the lipid phase. The lateral gate is formed by relatively short segments of TM8, TM7, TM2b, and TM3 (van den Berg et al. 2004). Because TM2b and TM3 are located in the N-terminal half of SecY and TM7 and TM8 are located in the C-terminal half, the gate may undergo "breathing," i.e., continuous opening and closure. This may be facilitated in the open channel when the plug has moved toward the back of the channel and no longer contacts the gate's TM segments. Breathing of the lateral gate would occasionally expose segments of a polypeptide chain located in the aqueous channel to the hydrophobic interior of the lipid bilayer,

N-terminus of first TM segment is translocated
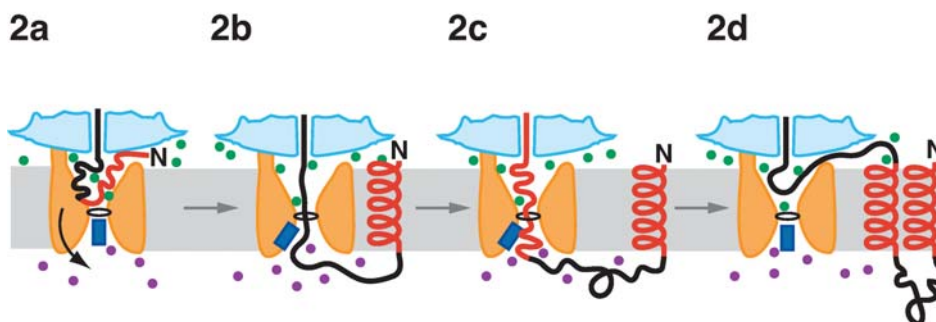

C-terminus of first TM segment is translocated

Figure 8

Model of membrane protein integration. (*1a*, *1b*) When the first TM segment (*red*) of a membrane
protein has fully emerged from the ribosome, the N-terminus can flip across the membrane (*arrow*) if the
TM segment is long and hydrophobic and the preceding polypeptide segment is not positively charged
or folded (N terminus translocated; *upper panel*) (Wahlberg & Spiess 1997). The N terminus may be
translocated through the channel after a brief displacement of the plug, and the TM segment partitions
into the lipid. (*1c*, *1d*) The following hydrophilic polypeptide segment emerges into the cytosol through
the gap between the ribosome and the channel. The next TM segment (*red*) inserts into the channel as a
loop, destabilizing its closed state. The channel opens by movement of the plug, and the second TM
segment of the polypeptide partitions into the lipid. As this occurs, the next hydrophilic segment enters
the channel and will ultimately be translocated to the other side of the membrane. (*2a*, *2b*) In the cases of
other proteins with a short first TM segment or a preceding region that is either folded or positively
charged, the N terminus may stay in the cytosol. The TM segment inserts into the channel as a loop,
destabilizing its closed state. Upon chain elongation, the C-terminal end flips across the membrane
(*arrow*), allowing the TM segment to partition into the lipid and leaving the channel occupied by the
following hydrophilic region of the polypeptide chain. (*2c*) The N terminus of the second TM segment
enters the open channel. (*2d*) When sufficient hydrophobic residues have emerged from the ribosome,
they will exit laterally into the lipid, allowing the plug to return to its closed state position. The following
hydrophilic segment will emerge into the cytosol through the gap between the ribosome and the channel.
During translocation and membrane integration of a polypeptide, either the plug or the nascent chain
hinders the passage of small molecules (*green* and *purple*) through the channel.

enabling them to equilibrate between the two phases. If sufficiently long and hydrophobic, a segment exits into the lipid phase (Duong & Wickner 1998, Heinrich et al. 2000). Because TM segments differ widely in sequence, they are unlikely to play an active role in opening of the lateral gate. A passive partitioning model is also supported by the observation that a hydrophobicity scale, derived from peptide interactions with an organic solvent, can be used to predict the tendency for a TM segment to integrate into the membrane (Hessa et al. 2005).

The open channel is most likely too small to allow "storage" of several TMs; during the synthesis of a multispanning membrane protein, the TMs leave the channel one by one, or perhaps in pairs. After moving through the lateral gate, some hydrophobic TMs are immediately surrounded by lipid, while other TMs that contain charges remain in proximity of the channel (Heinrich et al. 2000), sometimes until termination of translation (Do et al. 1996). Factors other than hydrophobicity of a TM, perhaps properties of the flanking region, may also influence how long a TM remains close to the channel (McCormick et al. 2003, Meacock et al. 2002). TMs that remain close to the channel for prolonged periods of time appear to be associated with TRAM, a protein located at the front of the Sec61 channel (Mothes et al. 1998). TRAM can be cross-linked to the signal sequences of secretory proteins and to charged TMs of nascent membrane proteins (Do et al. 1996, Görlich et al. 1992, Heinrich et al. 2000), and it is required for the translocation of secretory proteins with weakly hydrophobic signal sequences (Voigt et al. 1996). It may act as a membrane chaperone to stabilize TMs with hydrophilic residues and facilitate the association of these TMs until they can be released as a hydrophobic assembly into bulk lipid. The bacterial YidC protein, which has a similar topology as TRAM and is required for the integration and folding of some membrane proteins, may have an analogous function (Dalbey & Kuhn 2004).

During synthesis of a cytosolic domain of a membrane protein, the ribosome remains bound to the channel (Mothes et al. 1997). The nascent chain must therefore emerge between the ribosome and channel into the cytosol (**Figure 8**). Such a lateral path may be provided by the gap of 12–15 Å between the two partners, as seen in EM reconstructions. In contrast to models in which the ribosome-channel junction opens and closes (Johnson & van Waes 1999), the junction in the model proposed in **Figure 8** is always open, allowing a nascent chain to move sideways into the cytosol.

SecA-MEDIATED POSTTRANSLATIONAL TRANSLOCATION IN EUBACTERIA

The mechanism by which the cytoplasmic ATPase SecA moves polypeptide chains through the SecY channel is still poorly understood, but some new insights are provided by structural studies. SecA consists of five domains: two RecA-like folds, referred to as nucleotide-binding folds 1 and 2 (NBF1 and NBF2); the preprotein cross-linking domain (PPXD); the helical scaffold domain (HSD); and the helical wing domain (HWD) (**Figure 9a** and **b**) (Hunt et al. 2002, Osborne et al. 2004, Sharma et al. 2003). The ATPase site of SecA is similar to that in superfamily 1 and 2 helicases, with the nucleotide bound at the interface between NBF1 and NBF2. Nucleotide-dependent domain movements in SecA may therefore be similar to those seen in the helicase PcrA (Velankar et al. 1999). Contrary to earlier assumptions, there is only one nucleotide-binding site in SecA, with both NBF domains providing residues critical for ATP hydrolysis (Mitchell & Oliver 1993, Or et al. 2002, Papanikou et al. 2004, Schmidt et al. 2001, Sianidis et al. 2001). A conserved arginine residue in NBF2 likely senses the presence or absence of the γ-phosphate and triggers the appropriate domain movements (Or et al. 2002). These changes may be transmitted to
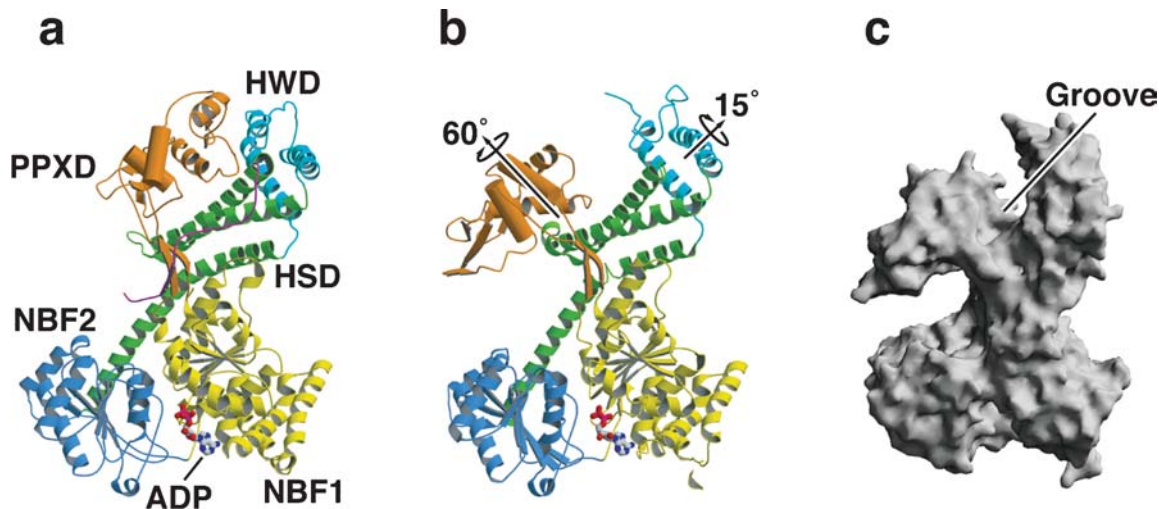
Figure 9

The structure of SecA. (*a*) Dimeric *Bacillus subtilis* SecA in a closed conformation (Hunt et al. 2002). A single subunit is shown. Nucleotide-binding fold 1 (NBF1) is shown in yellow, nucleotide-binding fold 2 (NBF2) in blue, the preprotein cross-linking domain (PPXD) in orange, the helical scaffold domain (HSD) in green, and the helical wing domain (HWD) in cyan. ADP is shown in a ball and stick representation. (*b*) Monomeric *B. subtilis* SecA in an open conformation (Osborne et al. 2004), colored as in (*a*). The arrows indicate movements required to convert the open conformation to the closed conformation. (*c*) A surface representation of SecA in the open conformation. The groove is proposed to close around the translocating polypeptide.

and amplified by the other domains that bind the polypeptide substrate and push it into the SecY channel.

SecA exists in equilibrium between monomeric and dimeric states (Benach et al. 2003, Ding et al. 2003, Or et al. 2002, Woodbury et al. 2002). When isolated, it is mostly a dimer. The X-ray structure by Hunt et al. (2002) may correspond to the physiological dimer, although other dimeric forms have been postulated (Sharma et al. 2003). Dissociation into monomers is stimulated upon interaction with ligands such as lipids (Benach et al. 2003, Bu et al. 2003, Or et al. 2002) or synthetic signal peptides (although the latter is controversial), thereby suggesting that the monomer is the active species in translocation. This is supported by the observation that cross-linked products corresponding to SecA dimers are lost upon interaction with the SecY complex (Or et al. 2002). A monomeric mutant of SecA retains some activity at least

under some conditions (Or et al. 2004), although the same mutant was found to be inactive in other studies (Jilaveanu et al. 2005, Randall et al. 2005). Upon solubilization, a single copy of SecA is found in a complex containing an arrested translocation substrate and SecY (Duong 2003). However, the exact nature of the complex during protein translocation is unclear, as it has been claimed that two SecAs may associate with two or four SecY complexes (Duong 2003, Manting et al. 2000, Tziatzios et al. 2004). SecA-induced tetramers of SecY complexes, observed by EM, may be arranged in a similar way as ribosome-associated Sec61/SecY complexes (Veenendaal et al. 2004).

Compared to the structure of the *Bacillus subtilis* SecA dimer, monomeric SecA is in an open conformation, in which the PPXD, HSD, and HWD have undergone dramatic movements, while the NBF domains have remained at the same position

(**Figure 9a** and **b**). In the open conformation, the HSD/HWD and the PPXD form a large groove (**Figure 9c**) (Osborne et al. 2004) that is likely the polypeptide-binding site, as indicated by cross-linking and mutagenesis studies (Kimura et al. 1991, Kourtz & Oliver 2000). The groove is similar in dimensions to those seen in other proteins—such as OppA, DnaK, and SecB—that interact with a wide range of peptide substrates (Sleigh et al. 1999, Xu et al. 2000, Zhu et al. 1996). In all of these proteins, a deep binding groove appears to wrap around the peptide, allowing binding to substrates that differ in sequence. Whether SecA binds signal sequences in a more specific way is unclear, but a potential binding site is a hydrophobic groove located at the interface of NBF1, the HSD, and the PPXD (Hunt et al. 2002).

It is likely that, as originally proposed, SecA pushes the polypeptide substrate through the SecY channel (Economou & Wickner 1994), but it is unclear exactly how this happens. A pushing mechanism implies that there are two polypeptide-binding sites that alternate in their affinities for the polypeptide substrate and that can move relative to each other. One possibility is that both sites are located in SecA, similar to helicases. However, as only one peptide-binding groove is apparent from the SecA structures, it seems more likely that SecY provides the second binding site.

It has been proposed that SecA inserts deeply into the SecY channel, reaching the other side of the membrane (Economou & Wickner 1994, Eichler & Wickner 1997, Kim et al. 1994, Ramamurthy & Oliver 1997, van der Does et al. 1996). This mechanism has been inferred from the fact that SecA is accessible to proteases and labeling reagents added from the outside of the cell. However, the structural data indicate that SecA is too big to insert into the channel. Thus, the previous data may be better explained if we assume that SecA adopts a protease-resistant conformation upon SecY binding (van der Does et al. 1998) and is accessible to labeling reagents

through the open SecY channel. The modification sites are indeed spread out over the entire SecA molecule (Hunt et al. 2002).

Taking into account the recent structural data, it seems likely that SecA pushes the polypeptide into the SecY channel without itself inserting deeply into the channel (**Figure 3**). In this model, SecA binds to a polypeptide segment, pushes it into the channel, and then releases it. Backsliding of the polypeptide substrate is reduced by its interactions with the SecY channel. Next, SecA releases the substrate and undergoes a conformational change, moving the peptide-binding site away from the channel to bind the next polypeptide segment. This cycle continues until the entire polypeptide is translocated. Although the current data suggest that SecA-mediated translocation is processive [i.e., a single SecA translocates each polypeptide substrate entirely (Joly & Wickner 1993, Schiebel et al. 1991)], the occasional disengagement of SecA, or even a nonprocessive mode of translocation, cannot be completely ruled out. In addition, although early experiments suggested that during each cycle SecA pushes 20–30 residues through the channel (Schiebel et al. 1991, Uchida et al. 1995, van der Wolk et al. 1997), this step size seems very large (it corresponds to ~100 Å of extended polypeptide). It is clear that further studies are required to resolve these issues.

PERSPECTIVES

Structural studies of ribosome/Sec61 complexes, of SecA, and particularly of the SecY channel have significantly advanced our understanding of the mechanism of protein translocation. Interpretation of these structures has been made possible by equally important genetic and biochemical data accumulated in many laboratories over the years. The recent data have led to new hypotheses that need to be tested experimentally. In addition, these data highlight a number of unresolved issues. For example, how exactly does SecA move polypeptides through the SecY

channel? What is the role of the oligomerization of the Sec61/SecY channel? How do interacting partners of the Sec61/SecY channel regulate its function? How are membrane proteins integrated and folded? Progress will depend on a combination of different approaches, with the structure of an active channel being a major goal for the future.

SUMMARY POINTS

1. The protein-conducting channel, formed by the Sec61/SecY complex, is required for both the translocation of polypeptides across cellular membranes and for the integration of these polypeptides into lipid bilayers.

2. The X-ray structure of the SecY complex provides new insight into how the protein-conducting channel functions.

3. Polypeptide translocation may occur posttranslationally or cotranslationally.

4. Each different mode of translocation requires different channel partners.

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This paper describes the first high-resolution structure of the SecY protein translocation channel, which is a central and essential component of protein secretion and membrane protein integration in all cells.

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