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Translocation of proteins through a distorted lipid bilayer

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Abstract

Membranes surrounding cells or organelles represent barriers to proteins and other molecules. However, specific proteins can cross membranes by different translocation systems, the best studied being the Sec61/SecY channel. This channel forms a hydrophilic, hourglass-shaped membrane channel, with a lateral gate towards the surrounding lipid. However, recent studies show that an aqueous pore is not required in other cases of protein translocation. The Hrd1 complex, mediating the retro-translocation of misfolded proteins from the endoplasmic reticulum (ER) lumen into the cytosol, contains multi-spanning proteins with aqueous luminal and cytosolic cavities and lateral gates juxtaposed in a thinned membrane region. A locally thinned, distorted lipid bilayer also allows protein translocation in other systems, suggesting a new paradigm to overcome the membrane barrier.

Keywords

Protein translocation; membrane distortion; lipid bilayer; structure; endoplasmic reticulum

Proteins move through membranes by different mechanisms

The generation of cells and cellular compartments relies on membranes providing barriers to even small molecules. Transport systems are therefore required to allow the translocation of molecules between the two aqueous compartments on either side of a membrane. Protein translocation systems exist in prokaryotes in the plasma membrane [1,2] and the outer membrane of gram-negative bacteria [3], and in eukaryotes in the endoplasmic reticulum (ER) [1], the inner and outer membranes of mitochondria [4], all three membranes of chloroplasts (i.e. the outer and inner membranes and the thylakoid membrane) [5], peroxisomes [6], and probably other membranes [7]. In all these cases, integral membrane proteins act as translocases that facilitate the membrane crossing of their polypeptide substrates. Some translocation systems allow an entire polypeptide chain to cross the membrane; they do so by providing an environment inside the membrane that lowers the

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energy barrier for moving hydrophilic amino acid residues and amide bonds through the hydrophobic interior of a phospholipid bilayer. Translocation systems can also insert or extract membrane proteins. The insertion of membrane proteins requires some hydrophilic polypeptide segments to cross the membrane, while others stay behind as hydrophobic transmembrane (TM) segments are embedded into the lipid phase. Although the energy cost for translocating a hydrophilic segment across the membrane is offset by the energy gained from partitioning hydrophobic TMs into lipid, most proteins do not insert spontaneously. During the extraction of membrane proteins, some hydrophilic polypeptide segments have to cross the membrane and hydrophobic TM segments need to move from the lipid bilayer into the cytosol, both thermodynamically unfavorable processes. In this review, we will discuss how the barrier of a membrane is overcome in different protein translocation systems. We begin with the classic Sec system in which translocation occurs through an entirely hydrophilic channel and then emphasize a new paradigm, according to which proteins cross the membrane through a locally thinned phospholipid bilayer. This membrane distortion is caused by the translocase possessing short TM helices and clustered hydrophilic residues that are exposed to the lipid environment, which lead to a reduction of the distance between the lipid head groups of the two opposing bilayer leaflets and distort the bilayer.

Protein translocation through the hydrophilic SecY/Sec61 channel

Protein translocation is best understood for the Sec system [1]. This system is responsible for the secretion of most proteins from prokaryotic and eukaryotic cells, as well as for the insertion of most membrane proteins. Translocation occurs through a conserved protein-conducting channel, the SecY channel in the plasma membrane of prokaryotes or the Sec61 channel in the ER membrane of eukaryotes. The SecY/Sec61 channel has an hourglass shape, with hydrophilic cavities on its cytosolic and extra-cellular sides (Figure 1A) [8,9]. The cavities are separated by a pore ring of hydrophobic amino acids that project their side chains inwards. The extra-cellular cavity is filled with a plug domain that is displaced during translocation. The channel also has a lateral gate that can open towards the surrounding lipid.

Translocation of a secretory protein begins with loop insertion of the polypeptide chain (Figure 1B) [10–12]. The hydrophobic core of a signal sequence forms the N-terminal part of the hairpin structure; it moves through the lateral gate into the lipid phase and ultimately forms a helix at the outside of the channel. The C-terminal part of the polypeptide loop is located in the actual pore as an extended polypeptide segment from the extra-cellular cavity through the pore ring to the cytosolic cavity. This polypeptide segment is therefore in an aqueous environment. During subsequent translocation, the signal sequence remains in place, while the C-terminal part of the loop is moved through the hydrophilic channel. Eventually, the signal sequence is cleaved off.

The initial insertion of a polypeptide loop into the SecY/Sec61 channel does not require energy input in the form of ATP or GTP; loop insertion occurs spontaneously, driven by the partitioning of a hydrophobic polypeptide segment into the lipid phase and by the accomodation of the following hydrophilic polypeptide segment inside the aqueous channel. This hydrophilic segment can passively diffuse in either direction through the channel, but in

general, directional movement is powered by one of three mechanisms [1]. In cotranslational translocation, the ribosome feeds the polypeptide chain directly into the channel; in posttranslational translocation in bacteria, the cytosolic ATPase SecA uses a power-stroke mechanism to push the polypeptide through the channel [13,14]; in posttranslational translocation in eukaryotes, the chaperone BiP binds to the translocating polypeptide when it emerges in the ER lumen and prevents its back-sliding into the cytosol, thus acting as an ATP-dependent ratcheting molecule [15]. Membrane proteins are generally inserted into the membrane co-translationally [1], and their hydrophobic TMs exit sequentially the lateral gate of the channel into the surrounding lipid.

The hydrophilic SecY/Sec61 channel is an ancient and general solution to the problem of moving a large variety of proteins across membranes. Accordingly, translocation is triggered by primitive features of the substrate, hydrophobic amino acid stretches, and the channel is conserved in all organisms. Hydrophilic channels have also been described for mitochondrial and chloroplast protein import [4,5]. However, the paradigm is difficult to apply to the secretion of bacterial proteins by the Tat system [2] or protein import into peroxisomes [6], in which folded proteins completely cross the membrane. The general requirement of an entirely aqueous channel is also challenged by recent work on ER-associated protein degradation (ERAD) [16]. In addition, hydrophilic channels are not always required for membrane protein insertion. For example, certain peptides can insert across phospholipid bilayers [17], and a long hydrophilic segment following the TM segment of cytochrome b_5 can translocate into the lumen of protein-free liposomes *in vitro* [18]. Toxin proteins can also insert spontaneously into lipid bilayers, translocating some residues to the other side [19]. Nevertheless, the translocation of membrane protein segments is generally facilitated by translocases. Examples include membrane protein insertion by YidC in bacteria and its homologs in mitochondria and chloroplasts [20], the insertion of tail-anchored membrane proteins by the Get1/2 and EMC complexes [21,22], and the insertion of proteins into the inner mitochondrial membrane by the Tim22 complex [4]. As discussed below, in all these cases, translocation is not facilitated by hydrophilic channels, but rather by the local thinning of the membrane.

Retro-translocation of misfolded ER-luminal proteins by the Hrd1 complex

Misfolded luminal ER proteins undergo ER-associated degradation (ERAD-L). They are retro-translocated into the cytosol, poly-ubiquitinated, and eventually degraded by the proteasome. In yeast, ERAD-L is mediated by the Hrd1 complex, composed of the multi-spanning ubiquitin ligase Hrd1, and four additional proteins (Hrd3, Der1, Usa1, and Yos9) [23]. Several other ubiquitin ligases in the ER membrane mediate the degradation of misfolded membrane proteins, each acting on a specific set of substrates, but the Hrd1 complex is unique because it allows the complete translocation of polypeptides from the ER lumen to the cytosol, i.e. the reversal of the path of secretory proteins.

The function of the Hrd1 complex is best understood for misfolded glycoproteins. When such a protein lingers in the ER lumen for too long, its N-linked glycan is trimmed by glycosidases to generate a terminal α1,6-mannose residue [24]. Previous data in conjunction with a recent cryo-EM structure indicate that the α1,6-mannose residue binds to the

mannose 6-phosphate receptor homology (MRH) domain of the Yos9 protein, and an adjacent, downstream polypeptide segment interacts in an extended conformation with a groove in Hrd3 (Figure 2A) [23,25]. Dual recognition ensures that only terminally misfolded proteins are targeted to ERAD-L, whereas intermediates of protein folding are spared.

In the next step, a loop of the polypeptide substrate is inserted into and moved through the ER membrane by a mechanism that was elucidated by a single-particle cryo-EM structure and photo-crosslinking experiments (Figure 2A) [16]. Hrd1 contains eight TM segments and has a deep aqueous cavity on the cytosolic side of the membrane, with a lateral gate towards the interior of the membrane. Hrd1 is linked through the Usa1 protein with Der1, an enzymatically inactive member of the rhomboid protease family [26]. Der1 has six TM segments that form a cavity on the luminal side and a lateral gate towards the surrounding lipid phase. The lateral gates of Hrd1 (between TMs 3 and 8) and Der1 (between TMs 2 and 5) face one another in a region of locally thinned membrane (Figures 2B; C): the EM map shows a pronounced depression on its cytosolic side in which the detergent micelle thickness is reduced from ~40Å to ~24Å [16]. The local thinning of an actual membrane was confirmed by all-atom molecular dynamics (MD) simulations of a Hrd1/Der1 complex [16]. One reason for the thinning is the laterally open cytosolic cavity of Hrd1, which contains numerous hydrophilic residues. In fact, Hrd1 alone can cause local membrane thinning, as shown by cryo-EM and MD simulations [16]. Der1 also contributes to local membrane thinning, as it has a cluster of several hydrophilic residues on the cytosolic end of the lateral gate helix 2, which are located within the boundaries of a normal lipid bilayer; mutation of these amino acids to hydrophobic residues greatly impeded ERAD-L [16]. In addition, Der1's lateral gate helix 5 is tilted with respect to the membrane normal and consists of only a short stretch of hydrophobic amino acids. Membrane thinning by Der1 is again supported by MD simulations [16]. Der1's relative, the actual rhomboid protease, also distorts the membrane, as demonstrated by its abnormally fast diffusion rate in the plane of the membrane [27].

An ERAD-L substrate inserts into the Hrd1/Der1 complex as a loop, as shown by photo-crosslinking experiments: one side of the substrate loop interacts with Der1 and the other with Hrd1 [16,28,29] (Figure 2A, D). Both strands of the polypeptide hairpin are relatively hydrophilic and must therefore be stabilized inside the membrane. During loop insertion, the polypeptide would encounter not just protein, but also the lipid phase between the lateral gates of Der1 and Hrd1. This thinned and distorted membrane region would lower the energy barrier to accommodate a polypeptide chain. Based on crosslinking experiments[29], it seems that 5–7 residues of the substrate transiently encounter a lipid environment during loop insertion.

In the proposed model, polypeptides would not cross the ER membrane through an entirely hydrophilic channel, as in the case of the SecY/Sec61 channel. Although translocation in both directions is initiated by loop insertion of the substrate, in the case of Sec61/SecY, one side of the substrate hairpin is hydrophobic and exits the lateral gate into lipid, and only the other part of the hairpin is translocated through the channel. In the case of the Hrd1 retro-translocon, both hairpin segments of the substrate are hydrophilic and translocated by the

two “half-channels” formed by the luminal and cytosolic cavities of Der1 and Hrd1, respectively, juxtaposed in a thinned membrane region.

Once the substrate is inserted as a loop into the Der1-Hrd1 complex, one or both parts of the loop could slide back and forth until a suitable lysine residue emerges in the cytosol and is poly-ubiquitinated by the Hrd1 ligase. The bulky poly-ubiquitin chain would then prevent back-sliding of the polypeptide into the ER lumen and serve as a recognition signal for the Cdc48 ATPase complex (p97 or VCP complex in mammals), which would subsequently pull the polypeptide into the cytosol, where it can be degraded by the proteasome (Figure 2D) [23].

Misfolded ER membrane proteins use different pathways for their translocation into the cytosol. In *S. cerevisiae*, some membrane proteins are retro-translocated by the Hrd1 complex, although Der1 is not required and Usa1 only in some cases [30,31], while others are handled by the multi-spanning ubiquitin ligase Doa10 [32]. Recent reconstitution experiments show that the TM segment of a single-spanning substrate interacts with Doa10 and can then passively diffuse out of the membrane, provided that the luminal substrate segment is in an unfolded state; otherwise pulling by the Cdc48 ATPase is required to extract the protein [33]. Yet other membrane proteins, including proteins mistargeted to the inner nuclear membrane and orphan components of protein complexes, are retro-translocated by the Asi complex, a multi-spanning ubiquitin ligase complex containing three proteins [34,35]. Finally, the Der1-homolog Dfm1, which again belongs to the rhomboid family, can also facilitate the movement of many membrane proteins into the cytosol [36–38]. Dfm1 probably functions on its own on substrates that are poly-ubiquitinated by different ubiquitin ligases; it delivers these substrates directly to the associated Cdc48 ATPase [39]. In higher organisms, such as mammals, several other multi-spanning ubiquitin ligases can mediate the degradation of certain membrane proteins [40]. How all these proteins recognize specific membrane protein substrates remains a mystery, but one may assume that they all possess lateral gates, cause local thinning of the membrane, and employ the Cdc48 ATPase to pull proteins out of the membrane.

Non-enveloped viruses also cross membranes without a hydrophilic channel. Polyoma and SV-40 viruses move from the plasma membrane to the ER by retrograde vesicular trafficking. In the ER, the virions undergo reduction of disulfide bridges, which causes a conformational change that exposes hydrophobic surfaces [41]. Translocation of these hydrophobic particles into the cytosol requires Derlin-2 [42], a homolog of yeast Der1, suggesting that translocation may occur through a locally distorted membrane.

Membrane protein insertion by YidC and related proteins

YidC in bacteria and its homologs in mitochondria (Oxa1) and chloroplasts (Alb3) mediate the insertion of membrane proteins [20]. Five TMs of these translocases form a structure with a deep cytosolic cavity and an opening towards the lipid (Figure 3A) [43]. YidC functions as a monomer either together with the SecY channel or on its own [44]. MD simulations and Cys-based alkylation scanning in intact cells show that the short length of TMs 3–5 cause local membrane thinning. The cytosolic cavity contains water molecules that

penetrate deep into the membrane up to the position of a conserved Arg residue [45]. These data suggest that TM segments of substrates enter the cavity and that the thinned membrane region facilitates the flipping of hydrophilic segments to the other side of the membrane.

A similar mechanism is probably used by the Get1/Get2 complex in yeast and the homologous WRB/CAML complex in mammals to insert tail-anchored membrane proteins into the ER membrane [21]. Tail-anchored proteins have a single TM segment close to the C-terminus, with a short hydrophilic segment translocated across the membrane; they are inserted into the membrane post-translationally [21,46]. Get1 and WRB contain three TMs that form a structure similar to the core formed by three of the six TMs in YidC [47]. Get2/CAML contributes TMs that are positioned in a roughly similar way as the additional TMs of YidC. Importantly, the Get1/Get2 (WRB/CAML) complex does not form a hydrophilic channel. Similar to YidC, these proteins have a hydrophilic groove that likely allows the lateral exit of the TM segment into lipid and facilitates the flipping of the C-terminal hydrophilic segment across the membrane (Figure 3B) [47]. Surprisingly, two Get1/Get2 complexes seem to form the functional unit together with a dimer of Get3, the chaperone that delivers a single molecule of the substrate [47]. In this model, a substrate molecule would thus have a choice between two insertase molecules.

Another ER membrane protein complex, the EMC, has also been proposed to serve as an insertase for tail-anchored membrane proteins [22]. Recent structures of the EMC indeed confirm that the triple-spanning EMC3 subunit has a similar structure as Get1 and the core of YidC [48–51]. The arrangement of the TMs of EMC6 is reminiscent of that of the other TMs in YidC. As in the other insertases, there is a hydrophilic cavity that would locally thin the membrane (Figure 3C). Indeed, an EM map determined in nanodiscs shows a thinned lipid phase next to the cavity [48]. The EMC has also been proposed to function in the biosynthesis of multi-spanning membrane proteins [52,53]. However, its bulky cytosolic domain would prevent its close association with a ribosome-bound Sec61 channel [49]. Given the presence of a large luminal domain, it seems possible that the EMC has additional roles in post-translational membrane protein biogenesis, perhaps as a membrane chaperone [51].

Protein insertion into the inner mitochondrial membrane by Tim22

Mitochondrial solute transporters, also called carrier proteins, are synthesized in the cytosol, transported across the outer mitochondrial membrane, and inserted into the inner membrane by the Tim22 complex [4]. The complex contains multiple subunits, but ultimate membrane insertion is mediated by Tim22 itself. Initial negative-stain EM and voltage-conductance data suggested that Tim22 forms two associated pores [54]. However, recent single-particle cryo-EM structures indicate the absence of a continuous hydrophilic channel [55,56]. In these structures, Tim22 has four TMs that form a curved surface inside the membrane (Figure 4A). The concave side has invaginations on both sides of the membrane, which contain charged residues located within the normal membrane boundaries. The mutation of these amino acids to uncharged residues abolishes the function of Tim22 [55]. As in the other cases discussed, the lipid-interacting surface of the protein is much shorter than the thickness of a normal bilayer. The six TMs of mitochondrial carrier proteins arose during

evolution from the triplication of a double-spanning membrane protein [57], so perhaps the thinned membrane caused by Tim22 facilitates loop insertion of each repeat.

Protein secretion by the Tat system

Many proteins are secreted from bacterial or archaebacterial cells by the twin-arginine (TAT) system [2]. In contrast to translocation through the SecY/Sec61 channel, which requires substrates to be in an unfolded state, the Tat system translocates folded proteins. Substrates for the Tat pathway include redox enzymes that require cofactor insertion in the cytosol and multimeric protein complexes that are difficult to assemble in the extracellular space. Three Tat proteins are involved (TatA, B, C), but only TatA is thought to provide the conduit through the membrane [58]. TatA is a small protein with an N-terminal TM and a following amphipathic helix. It forms oligomeric rings by interaction of the TMs of a variable number of TatA molecules. The short TM helices are only hydrophobic for three turns of an α-helix, which is approximately half the length of the hydrophobic region of a typical lipid bilayer (Figure 4B) [59]. In addition, an essential and conserved Gln residue is located inside the membrane. MD simulations confirm that the lipids in the center of the ring are distorted compared with lipids in bulk membrane [59]. The thinned and distorted membrane within the TatA oligomer likely provides the pathway for translocation of substrate proteins. The variable diameter of the ring would allow the translocation of folded proteins of different sizes.

The multi-spanning TatC protein, involved in the recognition of the twin-arginine signal sequence and the recruitment of TatA, also has an unusual structure, displaying a cavity towards the cytosol with a conserved Glu/Gln residue inside the membrane [60,61]. MD simulations show that this residue disturbs the lipid bilayer and allows water molecules to enter the membrane. Thus, TatC also seems to contribute to the distortion of the lipid bilayer.

Another system in which proteins are likely translocated in a folded state is protein import into peroxisomes [6]. The mechanism of translocation is not well understood, but it involves the Pex5 protein that recognizes substrates with a C-terminal SKL sequence in the cytosol and a membrane protein docking complex (Pex13/14) on peroxisomes. Although there is no evidence, it is tempting to speculate that these components cause thinning of the membrane as well.

β-barrel membrane protein insertion by the BAM and SAM systems

The outer membrane in gram-negative bacteria and the outer mitochondrial membrane contain β-barrel proteins that are inserted by the related BAM and SAM systems, respectively. The heart of the insertion machinery is a β-barrel protein, BamA or Sam50 [62]. These proteins have a seam between the first and last β-strands, which is significantly shorter than the normal membrane thickness [63,64]. During membrane insertion of a new β-barrel protein, the seam opens and hydrogen-bonded anti-parallel β-strands of the substrate bud from BamA/Sam50. Budding is completed when the first and last strand of the new barrel form a β-sheet [65,66]. The short β-strands at the seam of BamA and Sam50, as well as a kink of the last β-strand at a conserved glycine residue [67], could simply facilitate

the lateral opening of the barrel. However, the membrane is also distorted at this site, as shown by MD simulations [68], which may facilitate membrane insertion of the substrates.

Membrane thinning can serve other purposes than protein translocation

Local membrane thinning has been observed with proteins that are involved in lipid movement across the bilayer. For example, in one nucleotide-state of a P4 type ATPase, the lipid bilayer is locally thinned to about half the normal membrane thickness [69]. The distortion of the lipid bilayer, together with specific binding sites whose affinity changes during the ATPase cycle, likely facilitates the flipping of certain phospholipids. In the case of the TMEM16F protein, a Ca^{2+}-activated scramblase that passively moves phospholipids between the leaflets of a bilayer, a cavity closed towards the membrane is converted by Ca^{2+}-binding into a polar furrow across the membrane, which is accessible to the phospholipid headgroups [70–72]. The membrane is distorted at both entrances of the furrow, which decreases the energy barrier for moving the polar head group of a phospholipid from one leaflet to the opposite leaflet of the membrane. It is thus clear that local membrane thinning is used for processes other than protein translocation.

Concluding remarks

Historically, two opposing protein translocation models were hotly debated. In one model, which became the dominant school of thought, proteins were postulated to move through the membrane in an aqueous channel [73]. In the alternative model, formulated in the “direct transfer model” [74], the “helical hairpin hypothesis” [75], and the “membrane trigger hypothesis” [76], translocation would occur through the lipid phase without the help of a membrane protein. As first suggested by systematic photo-crosslinking experiments [77] and later confirmed by a crystal structure [8], the SecY/Sec61 complex indeed forms a hydrophilic channel that allows many proteins to move completely or partially through the membrane. Nevertheless, as discussed in this review, some elements of the alternative models also turn out to be correct: in many systems, protein translocation occurs through the lipid phase. However, the lipid phase corresponds to a locally thinned membrane with a distorted lipid bilayer, in which the distance between the phospholipid head groups of the two leaflets is reduced by 30–40% in all instances discussed in this review. The thinned membrane region is not generated spontaneously, but rather induced by membrane proteins. One interface encountered by the translocating polypeptide is still contributed by protein, but a hydrophilic channel that extends all the way through the membrane is not required, even when proteins move completely across the membrane, such as during the retro-translocation by the Hrd1 complex or protein secretion by the Tat system. The new paradigm by which the membrane barrier is overcome seems to be wide-spread, particularly among systems that insert or extract membrane proteins. However, so far the new paradigm is based on structures of the translocases obtained in the absence of substrates and on MD simulations of translocases placed in a lipid environment. Although the membrane path of polypeptides has been elucidated by crosslinking experiments, structures are needed, in which translocation substrates are caught in the act of moving through the membrane, to test whether proteins actually move through the thinned membrane regions (see outstanding question box). The composition and arrangement of the phospholipids in the thinned membrane region also

remain to be investigated, preferably not just by MD simulations, but also by experimental techniques. Finally, the mechanism by which folded proteins cross membranes, particularly in the case of peroxisomal protein import, remains one of the greatest mysteries.

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Glossary

Twin-arginine translocation (Tat) system
Substrates contain a cleavable twin-arginine signal sequence with the conserved motif S/TRRXFLK at their N-terminus. The Tat system exists in bacteria, archaea, and plants, and exports folded proteins.

Thylakoid membrane
Thylakoids are internal membrane-bound compartments in chloroplasts and cyanobacteria. They are the sites of the light-dependent reactions of photosynthesis.

Rhomboid proteases
These are intramembrane serine proteases. Their substrates are cleaved within TMs.

Cdc48/p97 (VCP) ATPase
This ATPase, called Cdc48 in yeast and p97 or valosin-containing protein (VCP) in mammals, plays an essential role in many cellular processes by segregating poly-ubiquitinated proteins from complexes or membranes. Cdc48/p97 consists of an N-terminal domain and two ATPase domains. Six Cdc48 monomers form a double-ring structure surrounding a central pore. Cdc48/p97 cooperates with a number of different cofactors, including a dimeric complex consisting of Ufd1 and Npl4.

Ubiquitin ligases
Also called E3 ubiquitin ligases. These proteins recruit an E2 ubiquitin-conjugating enzyme that has been loaded with ubiquitin, recognize a protein substrate, and assist or directly catalyze the transfer of ubiquitin from the E2 to the protein substrate.

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Outstanding questions box:

- What is the structure of a translocase/substrate complex? There are structures of the SecY/Sec61 complex and the BAM complex with translocating substrates, but in other cases, the exact path of the polypeptide remains speculative. Ideally, these structures should be determined in nanodiscs or even native membranes.
- What exactly is the arrangement and composition of phospholipids in the thinned membrane region where protein translocation is postulated to occur? Is the locally thinned membrane enriched in certain lipid species, such as those with shorter acyl chains? Are acyl chains tilted or compressed? How do lipids rearrange during protein translocation? Can MD simulations be supplemented by experimental evidence?
- How do folded proteins move across membranes and how is the driving force provided in these cases?
- How are proteins imported into peroxisomes?
- Are hydrophilic channels used for protein import into the matrix of mitochondria or the stroma of chloroplasts?

Highlights

- In all cells, proteins are translocated completely or partially across membranes. Translocases are generally required to overcome the energy barrier of a membrane.
- An entirely hydrophilic conduit is formed by the evolutionarily conserved SecY/Sec61 channel that is responsible for the secretion of proteins and the insertion of most membrane proteins.
- A growing number of translocase structures indicate that entirely hydrophilic channels are not always required. An example is the Hrd1 complex that mediates the retro-translocation of misfolded proteins from the ER lumen into the cytosol. In this case, translocation occurs through luminal and cytosolic hydrophilic cavities and a locally thinned, distorted membrane region.
- In several other systems, translocation also occurs through a protein-induced distorted lipid bilayer, indicating a new paradigm for lowering the energy barrier.

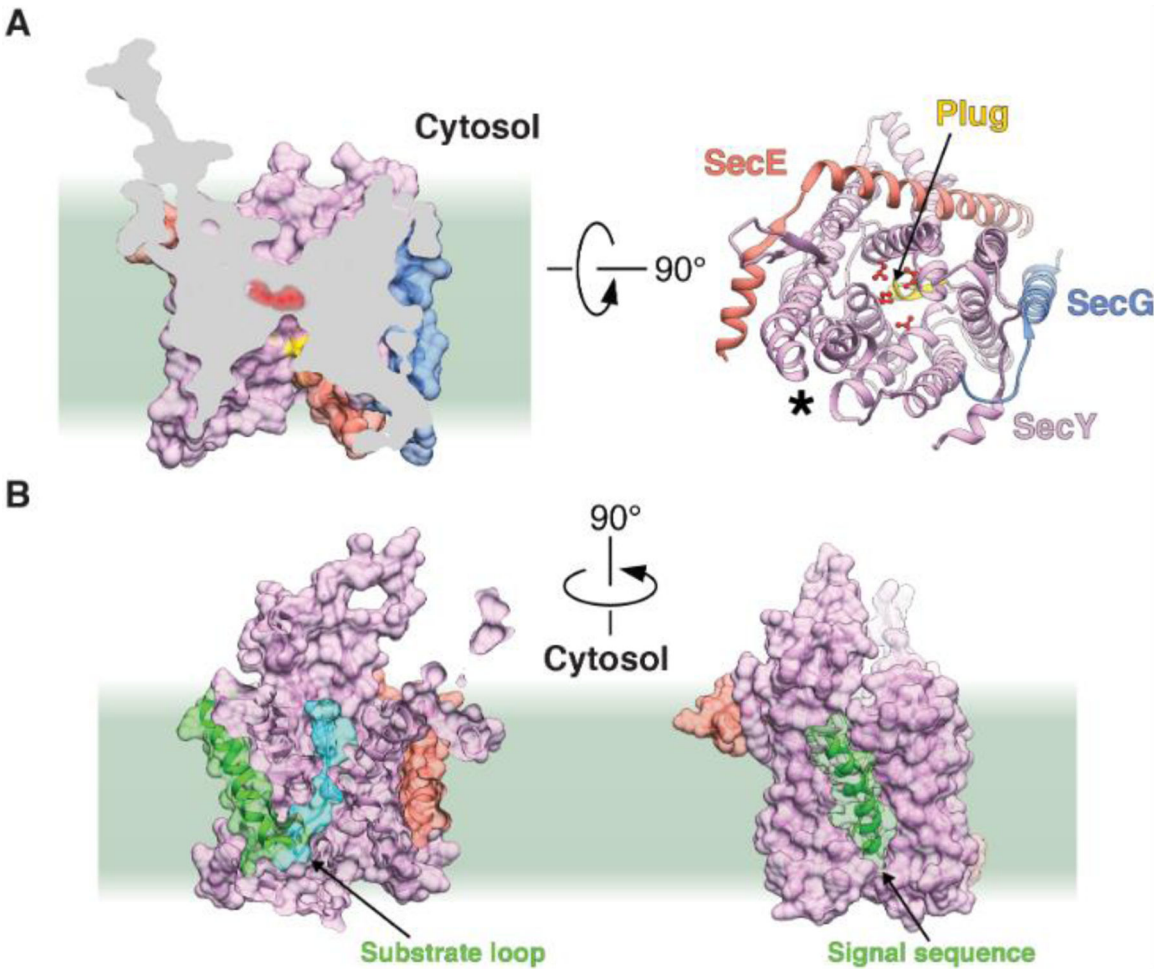

Figure 1. Protein translocation through the hydrophilic SecY channel.

(A) The left panel shows a cut through a space-filling model of the crystal structure of the SecY complex from *M. jannaschii* (PDB code 1RH5), viewed from the side. The pore ring is shown in red. The right panel shows a cartoon model of the channel viewed from the cytosol. The three subunits of the SecY complex (SecY, SecG, and SecE) are shown in different colors. The plug domain is in yellow and the pore ring residues are shown as red sticks and balls. The star indicates the position of the lateral gate. The eukaryotic Sec61 channel has a similar structure [9]. (B) Position of a translocating polypeptide chain in the *G. thermodenitrificans* SecY channel (PDB code 6ITC). The signal sequence constitutes one part of a hairpin structure (left panel; in green) and forms an α-helix outside the lateral gate (right panel). The following unfolded polypeptide segment forms the other part of the hairpin (left panel; in cyan).

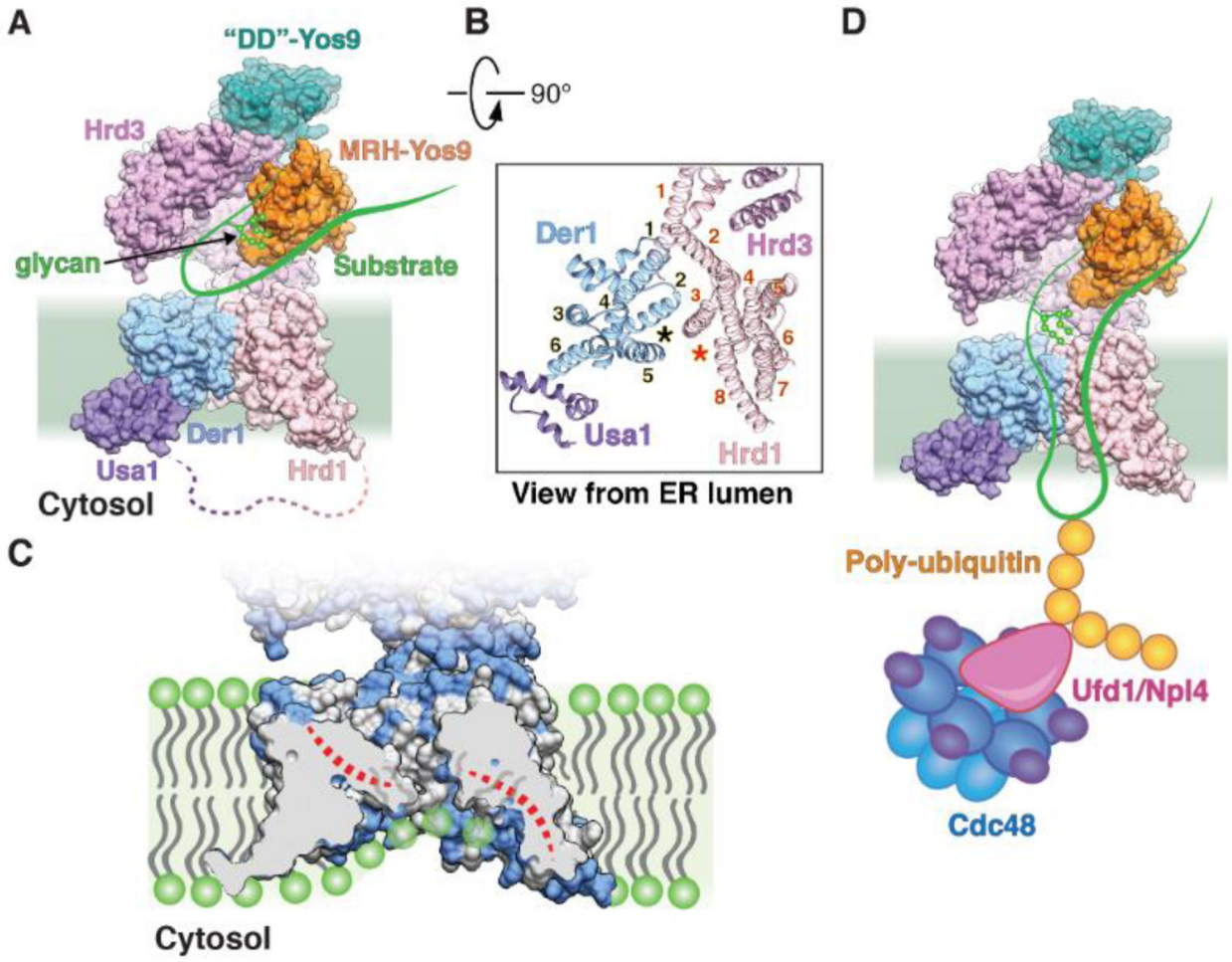

Figure 2. Retro-translocation of a misfolded ER luminal protein by the Hrd1 complex.

(A) Space-filling model of a cryo-EM structure of the Hrd1 complex (PDB code 6VJZ), with the subunits shown as in different colors. A segment of Usa1 that is invisible in the EM map, is shown as a broken line. The misfolded substrate, shown schematically in green, is recognized in the ER lumen by an interaction of the attached glycan with the MRH domain of Yos9 and accomodation of an adjacent extended polypeptide segment into a groove of Hrd3. (B) Cartoon model of the Hrd1 complex viewed from the ER lumen. Hrd3 was omitted for clarity. The TM segments of Der1 and Hrd1 are numbered. The stars indicate the lateral gates of Hrd1 and Der1. (C) Membrane thinning by the Hrd1 complex. Shown is a cut through a space-filling model, viewed from the side. Hydrophilic residues are colored in blue. The cytosolic and luminal cavities of Hrd1 and Der1 are indicated by red dashed arcs. Phospholipid molecules are shown schematically with head groups in green and acyl chains in grey. Substrates will encounter lipid molecules in the gap between two indicated cavities. (D) Loop insertion of the polypeptide into the Hrd1 complex and extraction of the polypeptide from the membrane by the Cdc48 ATPase complex. During loop insertion, one side of the hairpin interacts with Der1 and the other with Hrd1. Once subtrate emerges in the cytosol, the Hrd1 ligase catalyzes the attachment of ubiquitin molecules, which are subsequently recognized by the Ufd1/Npl4 cofactor of the Cdc48 ATPase. The polypeptide

is then moved through the central pore of the double-ring ATPase and thereby pulled out of the membrane.

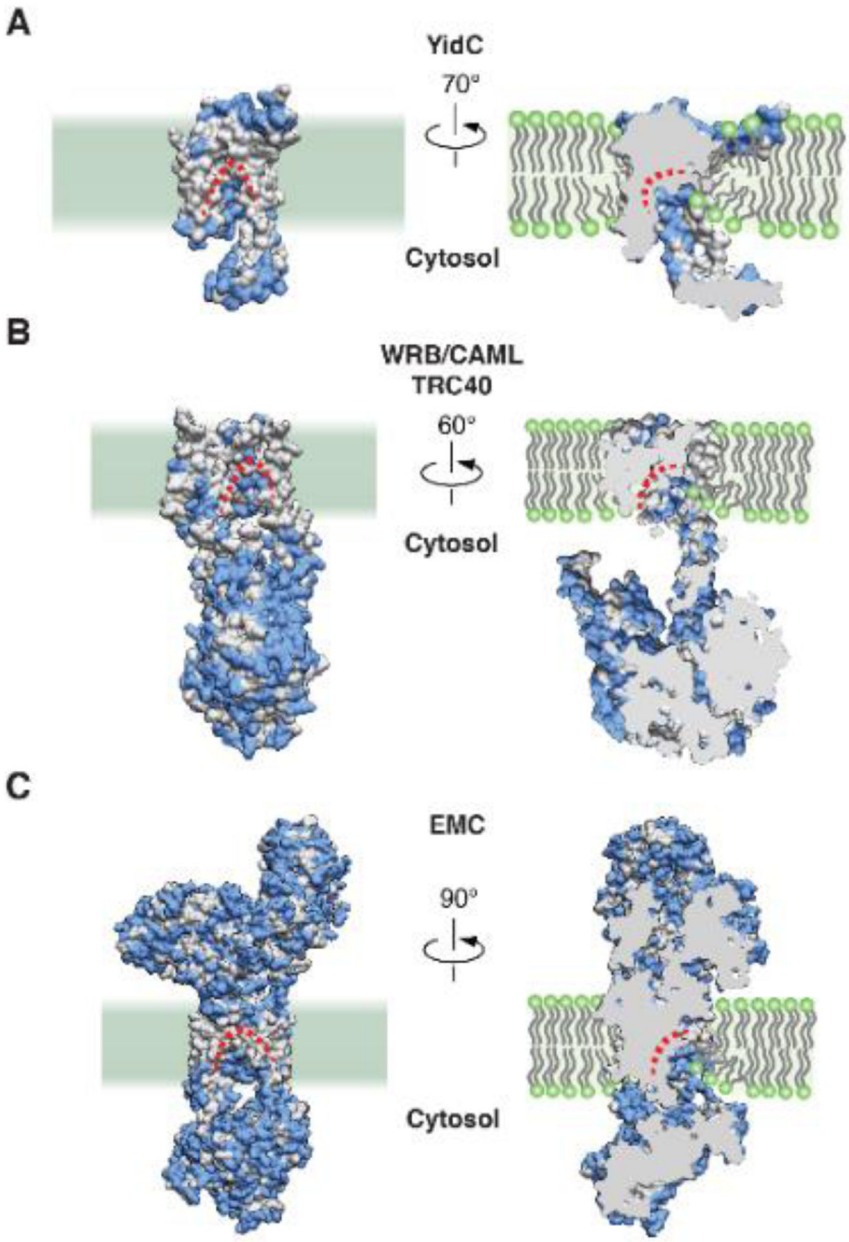

Figure 3. Membrane protein insertion by YidC and related proteins.

(A) The left panel shows a space-filling model of YidC from *B. halodurans*, viewed from the side (PDB code 3WO6). Hydrophilic residues are colored in blue. A cytosolic groove, indicated by a red dashed arc, causes local membrane thinnning. The lipid bilayer is indicated in grey. The right panel shows a cut through a space-filling model. Phospholipid molecules are shown schematically with head groups in green and acyl chains in grey. (B) As in (A), but for the human WRB/CAML/TRC40 complex (PDB code 6SO5). Only one copy of WRB/CAML is shown for clarity. Surprisingly, some hydrophilic residues are located outside the groove. The right panel shows the hypothetical position of phospholipid molecules, which needs to be confirmed by MD simulations. (C) As in (B), but for the EMC from humans (PDB code 6WW7).

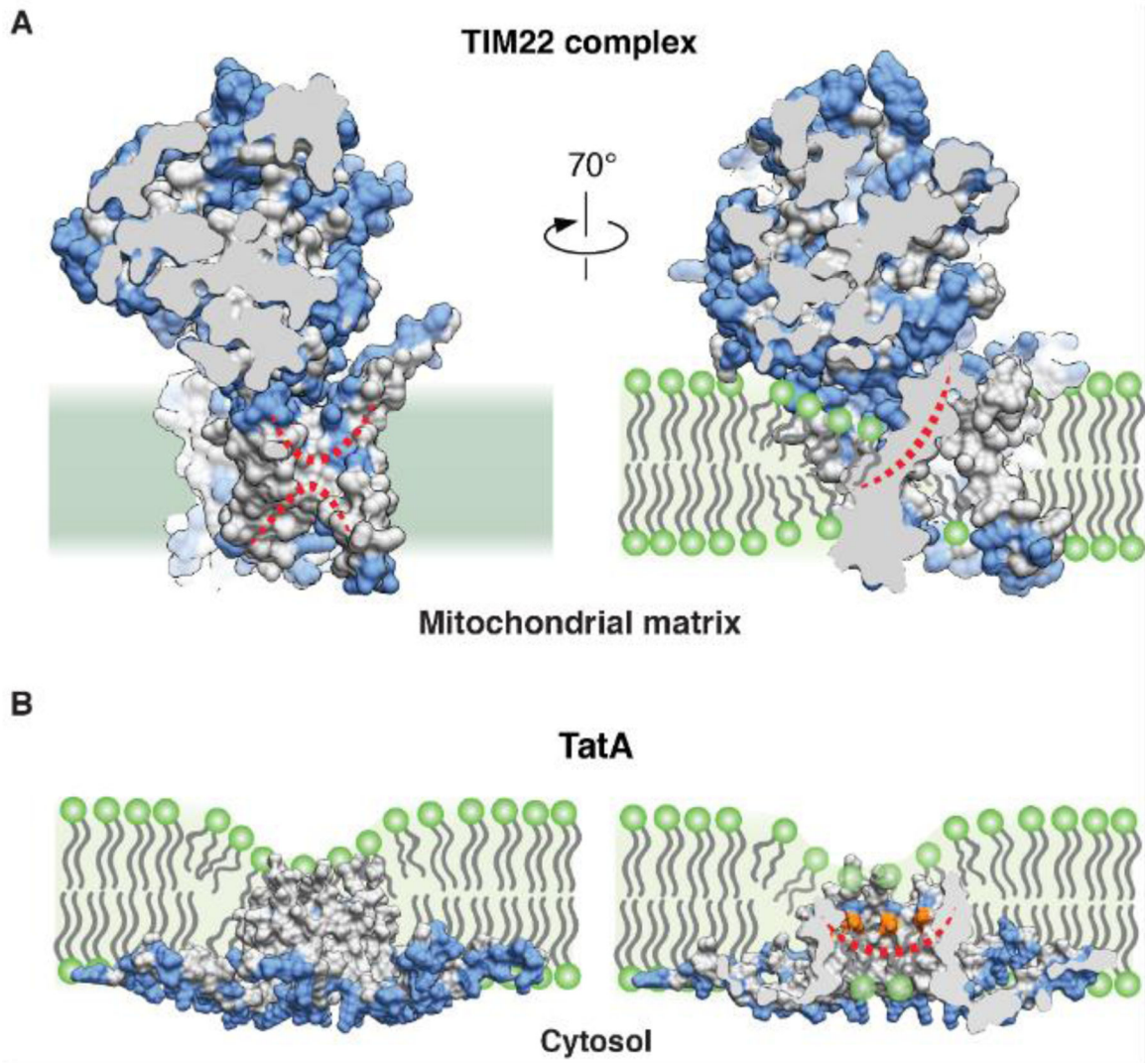

Figure 4. Membrane thinning by the Tim22 complex and the TatA protein.

(A) The left panel shows a space-filling model of a cryo-EM structure of the Tim22 complex from *S. cerevisiae* (PDB code 6LO8). Hydrophilic residues are colored in blue. Cavities, located on both sides of the membrane and indicated by red dashed arcs, may cause local membrane thinning. The lipid bilayer is indicated in grey. The right panel shows a cut through a space-filling model. In this view, the matrix cavity is in the back. Phospholipid molecules are shown schematically with head groups in green and acyl chains in grey. (B) The left panel shows a space-filling model of an NMR structure of a TatA oligomer from *E. coli* (PDB code 2LZS). Hydrophilic residues are colored in blue. Phospholipid molecules are shown schematically with head groups in green and acyl chains in grey. The right panel shows a cut through a space-filling model. Short TM segments and the clustering of a conserved Gln residue (in orange) in the oligomer generate thinning of the membrane at the extra-cellular side (indicated by a red dashed arc).