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REVIEWS

Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes

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A decisive step in the biosynthesis of many proteins is their partial or complete translocation across the eukaryotic endoplasmic reticulum membrane or the prokaryotic plasma membrane. Most of these proteins are translocated through a protein-conducting channel that is formed by a conserved, heterotrimeric membrane-protein complex, the Sec61 or SecY complex. Depending on channel binding partners, polypeptides are moved by different mechanisms: the polypeptide chain is transferred directly into the channel by the translating ribosome, a ratcheting mechanism is used by the endoplasmic reticulum chaperone BiP, and a pushing mechanism is used by the bacterial ATPase SecA. Structural, genetic and biochemical data show how the channel opens across the membrane, releases hydrophobic segments of membrane proteins laterally into lipid, and maintains the membrane barrier for small molecules.

For almost 40 years, researchers have been fascinated by the question of how proteins are transported across or are integrated into membranes. Pioneering work by G. Palade[1] demonstrated that in eukaryotic cells secretory proteins cross the endoplasmic reticulum membrane before being transported in vesicles to the plasma membrane. The laboratories of G. Blobel and C. Milstein then discovered that these proteins are directed to the endoplasmic reticulum membrane by signal sequences[2,3]. A little later, signal sequences were also found to direct the translocation of proteins across the bacterial plasma membrane[4,5]. Genetic experiments identified components required for translocation, initially in bacteria and later in yeast[6–8], and the establishment of an *in vitro* system initiated biochemical studies[9]. All of these achievements set the stage for investigations into the molecular mechanism of translocation, which will be the focus of this review.

Proteins transported across the eukaryotic endoplasmic reticulum membrane or the prokaryotic plasma membrane include soluble proteins, such as those ultimately secreted from the cell or localized to the endoplasmic reticulum lumen, and membrane proteins, such as those in the plasma membrane or in other organelles of the secretory pathway. Soluble proteins cross the membrane completely and usually have amino-terminal, cleavable signal sequences, the major feature of which is a segment of 7–12 hydrophobic amino acids. Membrane proteins have different topologies in the lipid bilayer, with one or more transmembrane segments composed of about 20 hydrophobic amino acids; the hydrophilic regions of these proteins either cross the membrane or remain in the cytosol. Both types of proteins are handled by the same machinery within the membrane: a protein-conducting channel. The channel allows soluble polypeptides to cross the membrane and hydrophobic transmembrane segments of membrane proteins to exit laterally into the lipid phase.

Structure of the translocation channel

The translocation channel is formed from a conserved heterotrimeric membrane protein complex, called the Sec61 complex in eukaryotes and the SecY complex in bacteria and archaea (for more details, see refs 10 and 11). The α- and γ-subunits show significant sequence conservation, and both subunits are essential for the function of the channel and for cell viability. The β-subunits are not essential; they are similar in eukaryotes and archaea, but show no obvious homology to the corresponding subunit in bacteria.

The α-subunit forms the pore of the channel, as initially shown by experiments in which photoreactive probes were systematically placed at different positions of a stalled translocating polypeptide[12]; all positions predicted to be within the membrane cross-linked only to the α-subunit of the Sec61 complex, indicating that this subunit surrounds the polypeptide chain during its passage across the membrane. In addition, experiments in which the purified Sec61/SecY complex was reconstituted into proteoliposomes showed that it is the essential membrane component for protein translocation[13–15]. The channel has an aqueous interior, as demonstrated by electrophysiology experiments[16] and by measurements of the fluorescence lifetime of probes incorporated into a translocating polypeptide chain[17,18].

The crystal structure of an archaeal SecY complex provided important insight into how the α-subunit forms the channel[10]. The structure is probably representative of complexes from all species, as indicated by sequence conservation and by the similarity to a lower-resolution structure of the *Escherichia coli* SecY complex, determined by electron microscopy from two-dimensional crystals[19,20]. Viewed from the cytosol, the channel has a square shape (Fig. 1a). The α-subunit is divided into two halves, transmembrane segments 1–5 and 6–10. The loop between transmembrane segments 5 and 6 at the back of the α-subunit serves as a hinge, allowing the α-subunit to open at the front—the 'lateral gate'. The γ-subunit links the two halves of the α-subunit at the back by extending one transmembrane segment diagonally across their interface. The β-subunit makes contact only with the periphery of the α-subunit, probably explaining why it is dispensable for the function of the complex.

The ten helices of the α-subunit form an hourglass-shaped pore that consists of cytoplasmic and external funnels, the tips of which meet about half way across the membrane (Fig. 1b). Whereas the

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cytoplasmic funnel is empty, the external funnel is plugged by a short helix. The crystal structure therefore represents a closed channel but, as will be discussed later, biochemical data indicate how it can open and translocate proteins. The constriction of the hourglass-shaped channel is formed by a ring of six hydrophobic residues that project their side chains radially inward. The residues forming this 'pore ring' are amino acids with bulky, hydrophobic side chains.

Different modes of translocation

The channel alone is a passive pore; it must associate with partners that provide a driving force for translocation. Depending on the partner, there are three known ways in which the channel can function.

In co-translational translocation, the main partner is the ribosome. This mode of translocation is found in all cells and is used for the translocation of secretory proteins as well as for the integration of most membrane proteins. Co-translational translocation begins with a targeting phase. The signal or transmembrane sequence of a growing polypeptide chain is recognized by the signal-recognition particle (SRP); after this, the ribosome–nascent-chain–SRP complex binds to the membrane, first by an interaction between SRP and its membrane receptor, and then by an interaction between the ribosome and the translocation channel (Fig. 2; for review of the targeting phase, see refs 21 and 22). The elongating polypeptide chain subsequently moves directly from the tunnel inside the ribosome into the associated membrane channel. GTP hydrolysis is required for chain elongation by the ribosome, but polypeptide movement through the channel is independent of nucleotide hydrolysis[23]. In the case of membrane proteins, certain polypeptide segments do not enter the channel, but instead emerge from the ribosome–channel junction into the cytosol[24], generating a cytosolic domain.

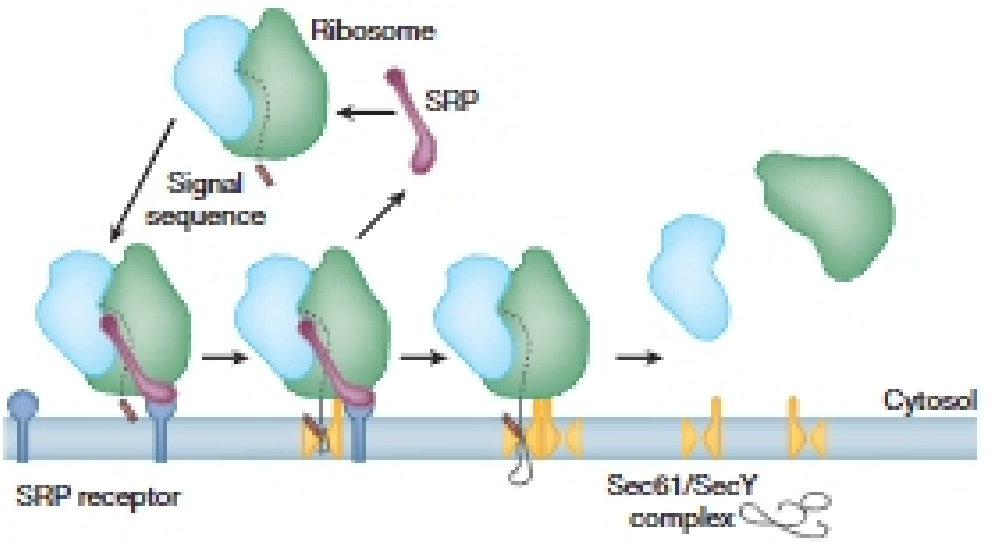

Figure 2 | Model of co-translational translocation. The scheme is mostly based on experiments with the eukaryotic system, but is probably similar for all organisms.

In most if not all cells, some proteins are transported after completion of their synthesis, that is, post-translationally. This pathway seems to be used by a larger fraction of proteins in simpler organisms, such as bacteria and yeast, perhaps because in these fast-growing cells translocation does not always keep pace with translation. This pathway is used mostly by soluble proteins, such as secretory proteins, which possess only moderately hydrophobic signal sequences that cause them to escape recognition by the SRP during their synthesis[25,26]. These proteins need to remain unfolded or loosely folded after their release from the ribosome[27]. Post-translational translocation occurs by different mechanisms in eukaryotes and bacteria.

During post-translational translocation in yeast, and probably in all eukaryotes, the channel partners with another membrane-protein complex, the tetrameric Sec62/Sec63 complex, and with the luminal chaperone BiP, a member of the Hsp70 family of ATPases[28,29]. In *Saccharomyces cerevisiae*, the Sec62/Sec63 complex consists of the essential Sec62 and Sec63 proteins as well as the dispensable Sec71 (also known as Sec66) and Sec72 proteins. Mammalian cells only have Sec62 and Sec63 (refs 30 and 31). Translocation begins with the binding of a translocation substrate to the channel (Fig. 3). During this step, all cytosolic chaperones are released from the substrate[32]. Once the polypeptide is inserted into the channel, its translocation occurs by a ratcheting mechanism[33]. The polypeptide chain in the channel can slide in either direction by brownian motion, but

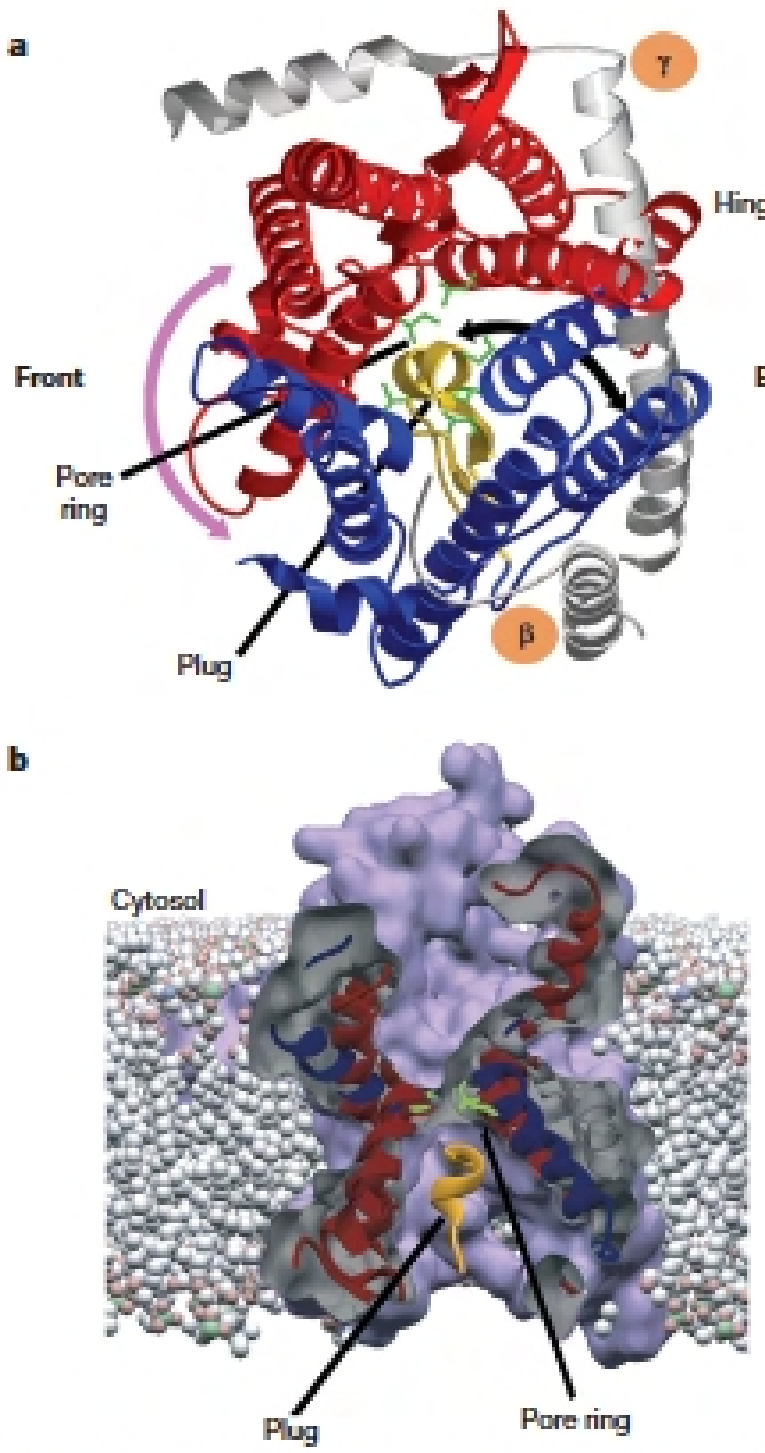

Figure 1 | The translocation channel. **a**, View from the cytosol of the crystal structure of the SecY complex from *Methanococcus jannaschii*. The α-subunit consists of two halves, transmembrane segments 1–5 and 6–10 (in blue and red, respectively), which can open the lateral gate at the front (purple double-headed arrow). The β- and γ-subunits are shown in grey. In the closed channel, the plug (in yellow) is in the centre of the α-subunit. Plug movement towards the back (black double-headed arrow) opens the channel across the membrane. The pore-ring residues are indicated in green. **b**, Cross-sectional view of the channel from the side.

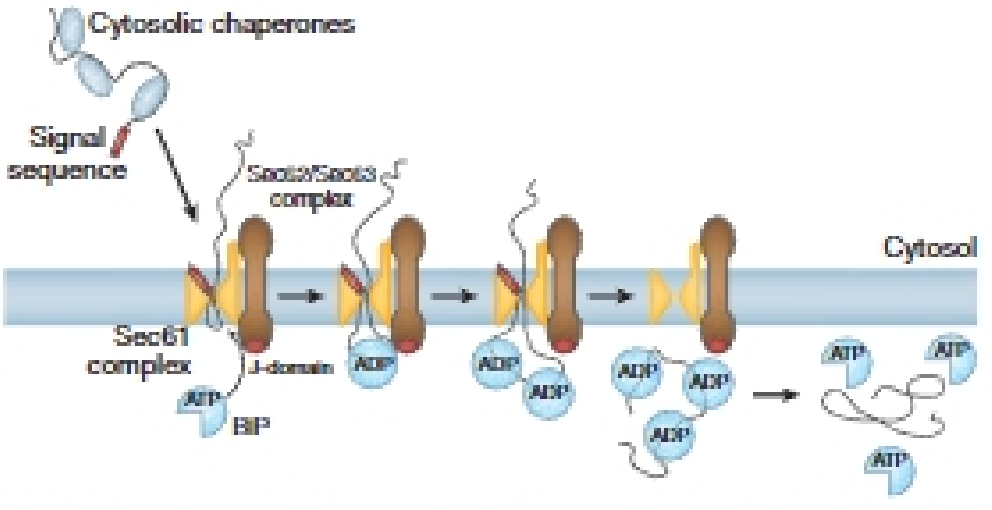

Figure 3 | Model of post-translational translocation in eukaryotes. It is possible that oligomers of the Sec61 complex mediate translocation, similar to the situation with the other modes of translocation (Figs 2 and 4).

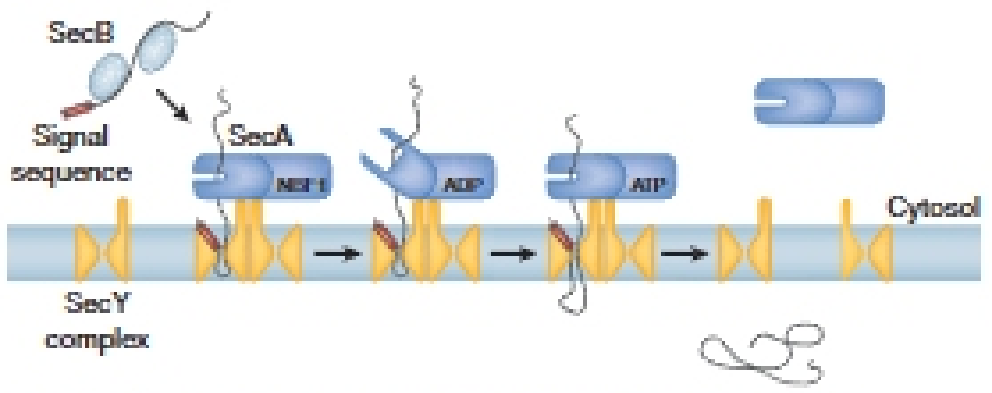

Figure 4 | Model of post-translational translocation in bacteria.

its binding to BiP inside the lumen of the endoplasmic reticulum prevents movement back into the cytosol, resulting in net forward translocation. ATP-bound BiP with an open peptide-binding pocket interacts with the J-domain of Sec63, which causes rapid ATP hydrolysis and closure of the peptide-binding pocket around the translocation substrate. J-domain-activated BiP has a low binding specificity[34], allowing it to interact with essentially any polypeptide segment that emerges from the channel into the lumen of the endoplasmic reticulum. When the polypeptide has moved sufficiently in the forward direction, the next BiP molecule can bind. This process is repeated until the polypeptide chain has completely traversed the channel. Finally, exchange of ADP for ATP opens the peptide-binding pocket and releases BiP.

In bacterial post-translational translocation, the channel partners with the cytosolic ATPase SecA. SecA has several domains, including two nucleotide-binding folds (NBF1 and NBF2) that bind the nucleotide between them and move relative to one another during the ATP hydrolysis cycle. The other domains also move, perhaps allowing SecA to alternate between the closed and open conformations that are observed in crystal structures[35,36]. A large groove in the open state might close around the polypeptide chain, because it is similar in dimensions to those seen in other proteins that interact with a wide range of substrates. Several experiments indicate that SecA functions as a monomer during translocation[37–40], but the issue is still controversial[41–43]. The translocation of many substrates begins with their binding to SecB, a cytosolic chaperone[44] (Fig. 4). Next, SecA interacts with SecB and accepts the polypeptide, probably binding both the signal sequence and the segment following it[45–47]. The subsequent transfer of the polypeptide into the channel requires a full cycle of ATP hydrolysis by SecA[48]. Once inserted into the channel, the substrate is translocated by a 'pushing' mechanism[49]. Although the details are not yet clear, a plausible mechanism assumes that the polypeptide-binding groove of SecA closes around the polypeptide chain and moves towards the channel, pushing the polypeptide into it (Fig. 4). The size of SecA means that it is unlikely that it inserts deeply into the SecY channel, as proposed earlier[49,50]. On nucleotide hydrolysis, the groove opens, releases the peptide, and moves away to 'grab' the next segment of the substrate. This cycle continues until the entire polypeptide is translocated. An electrochemical gradient across the membrane stimulates translocation *in vitro* and is required *in vivo*[51], but it is unclear how the gradient is used.

Archaea probably have both co- and post-translational translocation[52,53], but it is unknown how post-translational translocation occurs because these organisms lack SecA, the Sec62/Sec63 complex and BiP.

Opening the channel across the membrane

In all modes, the translocation of a secretory protein begins with its insertion into the channel. The polypeptide inserts as a loop (Fig. 5a), with the signal sequence intercalated into the walls of the channel and the segment distal to it located in the pore proper[54]. Opening of the channel for loop insertion probably occurs in two steps. The first is the binding of a channel partner—the ribosome, the Sec62/Sec63 complex or SecA. This event probably destabilizes interactions that keep the plug in the centre of the Sec61/SecY molecule. The ribosome and SecA interact with cytosolic loops in the carboxy-terminal half of Sec61/SecY[55], and these might transmit conformational changes to other parts of the molecule, resulting in transient displacement of the plug and continuous opening and closing of the lateral gate. This is supported by the observation of increased ion conductance when non-translating ribosomes are bound to the channel[16].

The second step is the intercalation of the hydrophobic segment of a signal sequence into the lateral gate. Photocrosslinking experiments with a yeast *in vitro* system show that the hydrophobic region of a bound signal sequence forms a helix of about two turns, which is intercalated between transmembrane segments 2b and 7 (ref. 56). The signal sequence can also be crosslinked to phospholipid molecules, indicating that it sits at the interface between channel and lipid. The binding of the signal sequence would separate transmembrane segments 2b and 7 and further destabilize plug interactions, causing the plug to move from the centre of Sec61/SecY into a cavity at the back of the molecule. Disulphide-bridge crosslinking shows that the plug indeed comes close to the transmembrane segment of the γ-subunit during translocation[57,58]. This model is also consistent with the observation that many mutations that allow the translocation of proteins with defective signal sequences (signal-suppressor mutations) would be expected to destabilize the closed channel[10,59].

Finally, the open state of the channel would be fixed by the insertion of the polypeptide segment distal to the signal sequence into the pore proper. During subsequent translocation, the signal sequence stays put, whereas the rest of the polypeptide moves through the pore. The plug could only return to the centre of Sec61/SecY when the polypeptide chain has left the pore. At some point, the signal sequence is cleaved by signal peptidase and is then further degraded by the signal peptide peptidase, a presenilin-like enzyme that cleaves the hydrophobic segment within the membrane[60].

The pore

The crystal structure indicates that a single copy of the Sec61/SecY complex forms the pore; according to this model, a polypeptide

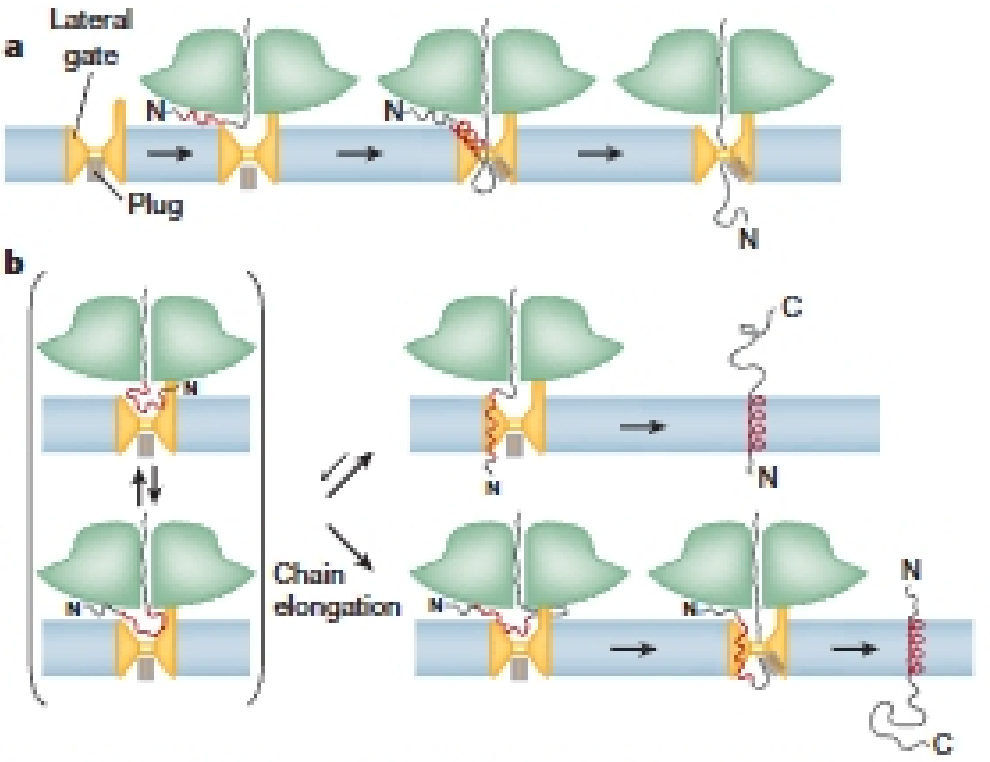

Figure 5 | Different stages of translocation. **a**, Translocation of a secretory protein. The red line indicates the hydrophobic region of a signal sequence. Depicted is the co-translational mode of translocation, but similar schemes can be envisioned for the other modes. For simplicity, only the translocating Sec61/SecY copy is shown. **b**, Translocation of membrane proteins. When a hydrophobic transmembrane sequence (in red) has emerged from the ribosome, it can bind reversibly in several conformations. If the hydrophobic sequence is long and the N terminus is not retained in the cytosol, it can flip across the membrane (upper panel). If the N terminus is retained in the cytosol and the polypeptide chain is further elongated by the translating ribosome (indicated by the loop between the ribosome and channel), the C terminus can translocate across the membrane (lower panel).