The expanding role of the ER translocon in membrane protein folding

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Eukaryotic polytopic membrane proteins are cotranslationally inserted into the ER membrane by a multisubunit protein-conducting channel called the Sec61 translocon. Although most major translocon components have been identified and reconstituted, their stoichiometry and functional organization remain unknown. This has led to speculative and sometimes conflicting models describing how multiple transmembrane (TM) segments might be oriented and integrated during nascent polytopic protein biogenesis. Kida et al. (see p. 1441 of this issue) shed new insight into this area by demonstrating that functional translocons exhibit a remarkable flexibility by simultaneously accommodating at least two hydrophilic translocating peptides that are separated by multiple hydrophobic TMs. These surprising findings support an expanded role for the translocon in membrane protein biogenesis and require reassessment of current views based on a single small functional pore.

Approximately 30% of proteins encoded by the human genome enter the secretory pathway through the Sec61 translocon at the endoplasmic reticulum (ER) membrane. Among these is a diverse group of substrates (ion channels, transporters, etc.) that contain multiple transmembrane (TM) hydrophobic helices and therefore require precise localization of peptide domains in three cellular environments, the cytosol, ER lumen, and lipid bilayer. Understanding how the translocon accomplishes this task is a major challenge in modern cell biology (Alder and Johnson, 2004; Rapoport et al., 2004; Pitonzo and Skach, 2006). The eukaryotic translocon contains multiple copies of the core Sec61αβγ heterotrimer and several associated components: TRAM, TRAPαβγδ, oligosaccharyltransferase (OST), and signal peptidase complex. Crystal structure of an archaebacterial Sec61 homologue, SecYβγ, revealed a 40 Å × 40 Å protein containing a small (~8 Å) pore-like central cavity with a constricting ring of hydrophobic residues, a short extracytosolic helical plug, and a single potential lateral opening (van den Berg et al., 2004). This structure immediately suggested that the pore of the long sought-after eukaryotic protein-conducting channel might be formed within a single sec61 heterotrimer (Rapoport et al., 2004). Nascent chain cross-linking to putative pore residues and molecular simulations predicting that the pore could potentially expand to accommodate a translocating polypeptide (~16 Å) have supported this notion (Cannon et al., 2005). Attempts to fit bacterial SecYβγ into eukaryotic translocon electron density maps further predict that four Sec61 molecules are configured with outward facing lateral exits such that only one channel is available for nascent chain translocation (Menetret et al., 2005). New findings by Kida et al. (p. 1441 of this issue) now support an alternate model in which the translocon can simultaneously accommodate at least two membrane-spanning peptides and multiple intervening TM segments.

A particularly useful approach in defining how functional translocons accommodate the needs of diverse secretory and transmembrane substrates has been to incorporate probes into truncated biogenesis intermediates during synthesis and thereby interrogate the molecular environment of the nascent polypeptide (Johnson, 2005). By examining the accessibility of incorporated fluorescent probes to membrane-impermeant quenching agents, Johnson et al. (2005) showed that engaged translocons contain a relatively large aqueous pathway (40–60 Å) that is opened upon insertion of a signal sequence and remains open during translocation of secretory protein domains. In the case of single-spanning membrane proteins, the translocation pathway is closed at its lumenal end by the action of BiP, and then opened to the cytosol shortly after synthesis and compaction of the hydrophobic TM segment in the ribosome exit tunnel (Johnson, 2005). This coordinated behavior stops peptide translocation into the ER lumen and provides the nascent elongating polypeptide access to the cytosol. Thus, simple membrane protein topology appears to be directed by distinct translocon gating events that control the direction of peptide movement through the translocation and integration pathway.

How then does the translocon coordinate axial translocation of peptide loops and lateral integration of multiple TMs during synthesis of native polytopic proteins? One possibility is that Sec61 receives the nascent chain in a linear manner and carries out each translocation and integration event independently and in sequential progression (Pitonzo and Skach, 2006). Insertion of a signal or signal anchor sequence into Sec61 would

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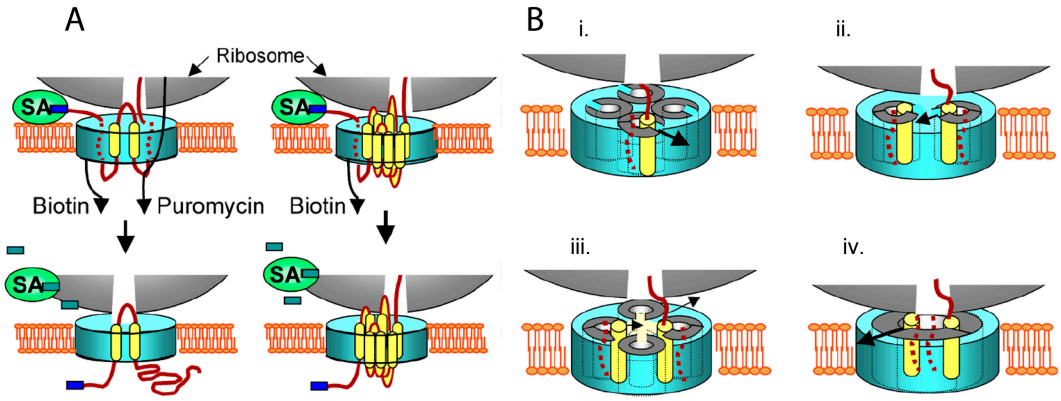

Figure 1. **Potential orientation of multiple peptide segments in the translocon.** (A) Truncated integration intermediates of Kida et al. (2007) showing N- and C-terminal hydrophilic peptides (dashed lines) and hydrophobic TMs (yellow ovals) retained in a TM orientation within the translocon (blue disc) by cytosolic streptavidin (SA)-avidin binding peptide (ABP) and peptidyl tRNA. In both intermediates, release of SA with biotin results in N terminus translocation. (B) Potential configurations of Sec61αβγ heterotrimer (gray cylinder) within an assembled translocon. Four subunits with lateral exit sites (arrows) accommodate only one TM, whereas (ii) two front-to-front subunits, (iii) four front-to-front subunits, or (iv) a single large pore could potentially accommodate multiple peptide regions.

initiate translocation, while synthesis of a TM (stop transfer) segment in the ribosome would terminate translocation and redirect peptide movement beneath the ribosome into the cytosol. If, however, the pore were comprised of a single Sec61αβγ heterotrimer, then size constraints would allow translocation of only a single strand of peptide and require that each TM segment be released from Sec61 before the next TM could enter. Although such a model presents an appealing simplicity, it was shown more than a decade ago that many polytopic protein TMs integrate into the bilayer as pairs or even groups (Skach and Lingappa, 1993; Borel and Simon, 1996). Chemical and photocrosslinking studies have also shown that multiple TMs, up to four in aquaporin 4, can simultaneously cross-link to translocon components including Sec61α (Meacock et al., 2002; McCormick et al., 2003; Sadlish et al., 2005).

To examine the nature of the translocon pore during synthesis of multiple TMs, Kida et al. (2007) fused an avidin-binding peptide to the N terminus of a type-I signal anchor and translated truncated nascent chains in vitro to generate uniform cohorts of ribosome-bound integration intermediates. In the presence of added streptavidin (SA), the N-terminal hydrophilic peptide remains trapped in the cytosol but can be subsequently translocated into the ER lumen when SA is displaced by biotin (Fig. 1 A). Two intermediate translocation states were identified. In the first, the signal anchor presumably engages the translocon but does not initiate translocation. In the second, the signal anchor initiates translocation, but SA binding to the N terminus forces the hydrophilic connecting peptide to adopt a TM orientation. Remarkably, both conformations remain in a translocation-competent state even after synthesis of up to seven additional TMs. Analysis of one intermediate revealed that two hydrophilic peptide regions, one connected to SA and the other connected to the ribosome, simultaneously spanned the membrane and could be translocated into the ER lumen by addition of biotin or puromycin, respectively (Fig. 1 A).

A strength of the Kida et al. (2007) study is the careful development of the avidin–biotin binding system and rigorous analysis of N-linked glycosylation that convincingly defines the disposition of multiple TMs in topologically complex intermediates. It is therefore difficult to reconcile the translocation competence of two peptide domains with a single functional translocon pore that can accommodate only one translocating nascent chain at a time. If ribosome-bound hydrophilic peptide domains translocate exclusively through an aqueous pore, as is generally believed, then the translocon must contain a minimum of two functional small pores or alternatively, a larger pore that can accommodate at least two peptide strands. Although it is conceivable that translocation of one peptide strand might occur outside the translocon pore, as has been observed for posttranslational insertion of tail-anchored proteins (Brambillasca et al. 2006), both membrane-spanning regions cross-link to Sec61α, indicating they reside either within or closely adjacent to a translocation pore. To address these issues, the authors provide two potential models. Adjacent Sec61αβγ subunits could either form a face-to-face dimer with opposed exit sites, or a side-on dimer with an expanded pore. Although the en-face model is consistent with a recent structure of ribosome-bound SecY (Mitra et al., 2005), the authors acknowledge that archaebacterial SecY provides little guidance as to how the second configuration might be stabilized.

An additional question raised by this study is where intervening TMs might reside while the N and C termini are retained within the translocation pore(s). If the Sec61 lateral exit site faced outward, then TMs would likely accumulate at the translocon periphery (Fig. 1 B), either in the lipid bilayer or possibly in association with translocon accessory proteins such as TRAM or TRAP. However, purified Sec61 and solubilized translocons form oligomeric ring-like structures that contain up to four Sec61αβγ heterotrimers, raising an alternate possibility that some or all Sec61 subunits might face inward (Fig. 1 B). If the center of the translocon contains lipid molecules, as has been proposed, then such a configuration could explain why TMs rapidly cross-link to lipids and yet reside within the translocon in nonrandom orientations for prolonged periods of time (Meacock et al., 2002; McCormick et al., 2003; Sadlish et al., 2005). If indeed, nascent TMs initially exited Sec61 into a central location or were

accommodated by non-Sec61 components before frank integration, then fully assembled translocons could potentially provide a unique environment for facilitating early membrane protein folding (Pitonzo and Skach, 2006). Although further work is clearly needed, Kida et al. (2007) have added an important piece to the fascinating puzzle of how an expanded translocon might accommodate the diverse needs of its substrates.

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