Protein Translocation across the Rough Endoplasmic Reticulum

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The rough endoplasmic reticulum is a major site of protein biosynthesis in all eukaryotic cells, serving as the entry point for the secretory pathway and as the initial integration site for the majority of cellular integral membrane proteins. The core components of the protein translocation machinery have been identified, and high-resolution structures of the targeting components and the transport channel have been obtained. Research in this area is now focused on obtaining a better understanding of the molecular mechanism of protein translocation and membrane protein integration.

Protein translocation across the rough endoplasmic reticulum (RER) is an ancient and evolutionarily conserved process that is analogous to protein export across the cytoplasmic membranes of eubacterial and archaebacterial cells both with respect to the mechanism and core components. The RER membrane of eukaryotic cells is contiguous with the nuclear envelope and is morphologically composed of interconnected cisternae and tubules. Electron microscope images of mammalian cells and tissues revealed that the cisternal regions of the cytoplasmic surface of the endoplasmic reticulum are densely studded by membrane-bound ribosomes (Palade 1955a,b), giving rise to the term "rough ER." The RER-bound ribosomes in *en face* images are often arranged in spirals or hairpins (Palade 1955a; Christensen and Bourne 1999), indicative of polyribosomes that are actively engaged in protein translation.

Consistent with this high density of membrane-bound ribosomes, the RER is a major site of protein biosynthesis in eukaryotic cells. The nuclear envelope, the Golgi, lysosome, peroxisome, plasma membrane, and endosomes are biosynthetically derived from the rough ER. The three major groups of proteins that are synthesized by RER-bound ribosomes include secretory proteins, integral membrane proteins destined for ER-derived membranes, and the lumenal-resident proteins of the ER, Golgi, nuclear envelope, and lysosome. For those membranes that are not physically linked to the ER (e.g., the lysosome), integral membrane and lumenal proteins are delivered to their destination by vesicular transport pathways. Bioinformatics analysis of fully sequenced eukaryotic

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genomes indicates that roughly 30% of open reading frames encode integral membrane proteins (Wallin and von Heijne 1998); hence, a major role of the RER is the biosynthesis of membrane proteins. An important class of membrane proteins that are integrated into the RER has single carboxy-terminal TM spans and are known as tail-anchored (TA) membrane proteins. The posttranslational integration pathway for TA proteins has been a subject of several recent reviews (Borgese and Fasana 2011; Shao and Hegde 2011), thus we will not address the TA pathway in this article.

THE SIGNAL HYPOTHESIS

Biochemical experiments to address the role of membrane-bound ribosomes in secretory protein biosynthesis began in earnest in the 1960s (Redman and Sabatini 1966; Redman et al. 1966). In 1971, Gunter Blobel proposed that secretory protein mRNAs encode a signal that promotes targeting of ribosomes to the RER. The following year, Cesar Milstein and colleagues discovered that a secretory protein (IgG light chain) is synthesized as a higher-molecular-weight precursor (Milstein et al. 1972). In a landmark paper formally presenting the signal hypothesis, Blobel and Dobberstein showed that the IgG light chain could be synthesized in vitro, cotranslationally translocated across canine pancreas microsomal membranes, and proteolytically processed into the mature polypeptide (Blobel and Dobberstein 1975). During the next several years, protein sequence analysis showed that secretory proteins are synthesized as precursors that have an amino-terminal hydrophobic signal sequence and a processing site for an RER-localized signal peptidase (von Heijne 1983). The TM spans of integral membrane proteins, which are also hydrophobic, function as signal sequences when located near the amino terminus of membrane proteins (Friedlander and Blobel 1985) or when inserted into a signal sequence-deficient reporter protein (Mize et al. 1986). Thus, ribosomes synthesizing secretory or membrane proteins are targeted to the RER by nonidentical segments of hydrophobic amino acids.

TARGETING OF mRNAS AND TRANSLATING RIBOSOMES TO THE ROUGH ENDOPLASMIC RETICULUM

Although signal sequences provide a protein-based address code for the ER, what are the cellular proteins or lipids that decode a sequence composed of a string of hydrophobic amino acids? The identification of the signal recognition particle (SRP) by Walter and Blobel resolved this question (Walter and Blobel 1980). The SRP selectively binds to ribosomes translating mRNAs encoding presecretory proteins (Walter et al. 1981), reduces the protein synthesis elongation rate (Walter and Blobel 1981b), and mediates selective targeting of the ribosome–nascent chain complex (RNC) to the RER (Fig. 1) (Walter and Blobel 1981a).

In mammalian cells, the SRP is composed of six protein subunits and the 7S RNA (Walter and Blobel 1982). An elongated architecture of the SRP was determined by mapping protein-binding sites onto the folded structure of the 7S RNA (Siegel and Walter 1988; Strub and Walter 1990), and by electron microscopy (Andrews et al. 1987). Of the protein subunits, SRP54 has received the most attention because it contains a methionine-rich domain that binds directly to the signal sequence and to the 7S RNA (Zopf et al. 1990). Cryoelectron microscopy of SRP–ribosome–nascent chain complexes (SRP–RNCs) revealed that SRP54 is positioned near the polypeptide exit site on the large ribosomal subunit, whereas the *Alu* subdomain of the SRP particle is positioned near the elongation factor-binding site (Halic et al. 2004). Recently, several groups have reported that SRP or the eubacterial homolog Ffh (see below) is recruited to the ribosome before the amino terminus of the protein emerges from the polypeptide exit tunnel (Bornemann et al. 2008; Berndt et al. 2009), thereby increasing the probability that SRP will discriminate between an authentic signal sequence and a nonsignal sequence shortly after the amino terminus of the protein emerges from the large ribosomal subunit. Recognition of the signal sequence by the SRP allows cotranslational delivery of RNCs to the RER. Cotranslational integration may be particularly important

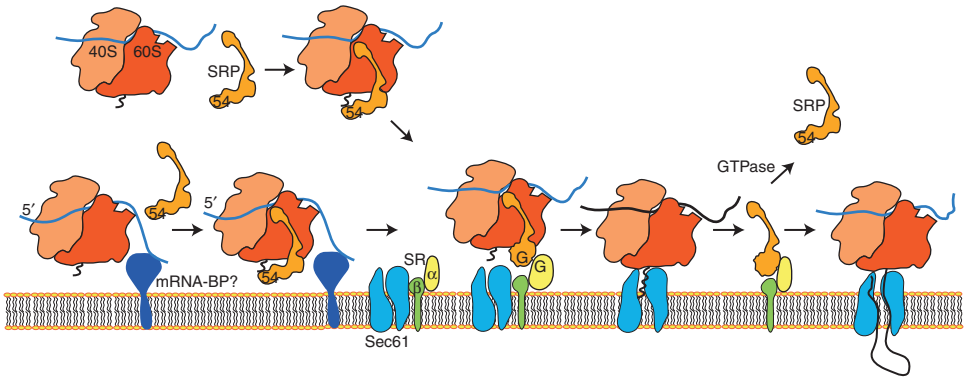

Figure 1. Targeting of RNCs to the Sec61 complex. The mRNAs encoding proteins with ER signal sequences may be targeted to the vicinity of the RER by a translation-independent mechanism and bind to a currently unidentified mRNA-binding protein (mRNA-BP). The SRP particle binds to the 80S ribosome and mediates targeting to the ER via interaction with SRα. Cooperative GTP binding to SRP54 and SRα leads to dissociation of SRP from the RNC and attachment of the RNC to the Sec61 complex. Signal sequence insertion into the SSB site gates the translocation channel.

for the biosynthesis of multispanning membrane proteins because TM spans are prone to aggregation in aqueous environments.

Are certain classes of mRNAs targeted to the RER by translation-independent pathways? It has long been recognized that membrane-bound and free polysome fractions isolated from tissues synthesize different classes of proteins (Ramsey and Steele 1976). The use of high-throughput methods to analyze the partitioning of mRNAs between membrane-bound and free polysome fractions showed that nucleocytoplasmic proteins are primarily synthesized by free polysomes and that membrane-bound polysomes are enriched in mRNAs encoding endomembrane resident proteins (Diehn et al. 2000). Unexpectedly, mRNAs encoding certain nucleocytoplasmic proteins (e.g., Hsp90 and calcinuerin) are strongly enriched in the membrane-bound polysome fraction (Diehn et al. 2000; Lerner et al. 2003). Secondly, mRNAs encoding secretory proteins were not as enriched in the membrane-bound polysome fraction as mRNAs encoding endomembrane resident proteins (Chen et al. 2011). Evidence for translation-independent binding of mRNAs to the RER has also been obtained, suggesting that mRNA targeting to the vicinity of the RER may precede SRP-dependent targeting of RNCs to the protein translocation channel (Fig. 1) (Pyhtila et al. 2008).

THE SRP54 AND SRα GTPase REGULATE THE DELIVERY OF RNCs TO THE TRANSLOCATION CHANNEL

The discovery that SRP delivers RNCs to the RER provided the foundation for identifying an RER-localized SRP receptor (SR) for the SRP–RNC complex (Gilmore et al. 1982a,b). The heterodimeric SR (SRα + SRβ) is localized to the ER by integration of the β-subunit (Lauffer et al. 1985). Dissociation of the SRP–SR complex from the signal sequence precedes RNC binding to the protein translocation channel (Gilmore and Blobel 1983).

SRα, SRP54, and the 7S RNA are evolutionarily conserved; FtsY and the Ffh–4.5S RNA complex are the eubacterial equivalents of the SR and SRP (Poritz et al. 1988; Poritz et al. 1990; Miller et al. 1994). In the eubacterial organism *Escherichia coli*, the SRP–SR targeting pathway is primarily involved in the biosynthesis of inner membrane proteins (Ulbrandt et al. 1997; Koch et al. 1999). Most periplasmic proteins and β-barrel outer membrane proteins are translocated by a posttranslational SecA–SecYEG-dependent pathway (for a recent review, see Park and

Rapoport 2012). Disruption of the budding yeast (*Saccharomyces cerevisiae*) genes encoding SR or SRP subunits or the SRP RNA yielded slow-growing strains that have severe, yet transient, defects in protein translocation of a subset of proteins with RER signal sequences (Hann and Walter 1991; Ogg et al. 1992). In contrast to budding yeast, the SRP54 and SRP RNA genes are essential in *Schizosaccharomyces pombe* (Brennwald et al. 1988; Althoff et al. 1994).

The interaction between the SRP receptor and the SRP–RNC complex is regulated by a GTPase cycle (Connolly and Gilmore 1986, 1989) that results in dissociation of SRP54 from the signal sequence (Connolly et al. 1991) and attachment of the RNC to the protein translocation channel (Fig. 1). Protein sequence analysis and GTPase assays using purified SRP and SR led to the conclusion that SRα, SRβ, and SRP54 are members of the GTPase superfamily (Connolly and Gilmore 1993; Miller et al. 1993). The minimal components for the SR–SRP GTPase cycle are SRα, SRP54, and the 7S RNA (Miller et al. 1993). The most thorough kinetic analysis of the SRP–SR GTPase cycle has been achieved using bacterially expressed derivatives of Ffh, FtsY, and 4.5S RNA (Peluso et al. 2000; Shan and Walter 2003).

Unlike many GTPases, the hydrolysis cycle of the SRP–SR complex is not regulated by conventional guanine-nucleotide exchange factors (GEFs) or GTPase-activating proteins (GAPs). The requirement for the SRP RNA in the GTPase cycle is explained by the finding that the 4.5S RNA increases the rate of Ffh–FtsY complex formation and disassembly following hydrolysis (Peluso et al. 2000). The SRP and SR GTPases have a low affinity for guanine ribonucleotides compared with many other GTPases, and a lower affinity for GTP than for GDP (Connolly and Gilmore 1993; Rapiejko and Gilmore 1997). Indeed, before formation of the SRP–SR complex, GTP binding to Ffh and FtsY is reversible, and the binding specificity (GTP vs. ATP) is surprisingly weak for FtsY (Shan and Walter 2003). GTP hydrolysis by SRP and SR are catalytically linked and dependent on nucleotide occupancy of both sites (Powers and Walter 1995; Rapiejko and Gilmore 1997).

Several roles have been proposed for the SRP–SR GTPase cycle. One role is to control the assembly and disassembly of the SRP–SR complex; nonhydrolyzable GTP analogs stabilize the SRP–SR complex, whereas GTP hydrolysis promotes complex dissociation (Fig. 1) (Connolly et al. 1991). Recent evidence indicates that additional proofreading steps occur after signal sequence recognition by SRP to increase the fidelity of the protein translocation reaction (Zhang et al. 2010). When reconstituted into proteoliposomes, signal sequence dissociation from SRP54 and GTP hydrolysis by the SRP–SR complex is blocked unless an active Sec61 complex is present to serve as a receptor for the RNC complex (Song et al. 2000). Thus, the GTPase cycle regulates multiple steps in the delivery of an RNC to the protein translocation channel.

STRUCTURAL BIOLOGY OF THE SRP–SR TARGETING PATHWAY

SRP54 and Ffh are composed of an amino-terminal domain (N-domain), the central GTPase (G-domain), and the carboxy-terminal methionine-rich M-domain (Bernstein et al. 1989). Homologous N- and G-domains are also present in SRα and FtsY. The simpler composition of the eubacterial SR and SRP facilitated structural analysis of the SRP family of GTPases. High-resolution structures of the nucleotide-free forms of Ffh_{NG} and $FtsY_{NG}$ (Freymann et al. 1997; Montoya et al. 1997) and the GDP-Mg^{2+}-bound form of Ffh_{NG} (Freymann et al. 1999) highlighted the homologous architecture of these GTPases and helped explain their low affinity for ribonucleotides. The structure of the Ffh_{NG}–$FtsY_{NG}$ complex obtained in the presence of a nonhydrolyzable GTP analog revealed a composite active site formed upon heterodimerization (Egea et al. 2004; Focia et al. 2004) that helped explain why Ffh and FtsY act as reciprocal GAPS (Powers and Walter 1995). A co-crystal structure of Ffh_{NGM} bound to domain IV of the 4.5S RNA revealed that the hydrophobic signal sequence-binding groove in the M-domain of Ffh terminates at the RNA-binding interface (Batey et al. 2000) and suggests that signal

sequence binding to the M-domain may be communicated directly to the G-domain via the 4.5S RNA (Rosendal et al. 2003).

TRANSLOCATION CHANNELS AND RIBOSOME-BINDING SITES

After the discovery of SRP and the SR, several laboratories focused their efforts on the identification of the protein translocation channel. A yeast screen for gene products that were required for translocation of a secretory protein led to the identification of the essential *SEC61*, *SEC62*, and *SEC63* genes (Deshaies and Schekman 1987; Rothblatt et al. 1989). Subsequent analysis showed that all three genes encode ER-localized integral membrane proteins that assemble into the SEC complex (Deshaies and Schekman 1989; Deshaies et al. 1991; Feldheim et al. 1992). Mutations in Sec61p inhibited translocation of secreted proteins and integration of membrane proteins, thereby providing the first evidence that Sec61 was the core subunit of the protein translocation channel (Stirling et al. 1992).

The mammalian translocation channel was initially detected by cross-linking an in vitro-assembled translocation intermediate to several different ER membrane proteins in the 30- to 40-kDa range (Wiedmann et al. 1989; Kellaris et al. 1991). Purification of the cross-linking targets resulted in identification of the TRAM protein (Görlich et al. 1992a) and the Sec61α protein (Görlich et al. 1992b). Mammalian Sec61α is homologous to yeast Sec61 and to the *E. coli* SecY protein (Görlich et al. 1992b), showing that protein translocation channels are conserved between eukaryotic and eubacterial organisms. Fungal genomes (e.g., *S. cerevisiae*) do not encode an obvious TRAM homolog.

Proteoliposomes containing the mammalian Sec61 complex (Sec61α, Sec61β, and Sec61γ) and the SR are active in translocation of a subset of secretory proteins (Görlich and Rapoport 1993). Incorporation of TRAM into Sec61–SR proteoliposomes stimulates translocation of all substrates tested, consistent with an accessory role for TRAM at an early stage in protein translocation (Voigt et al. 1996). Unlike TRAM, which contacts only the amino-terminal regions of nascent secretory proteins, Sec61 can be cross-linked to a photoreactive amino acid analog incorporated at any site in the nascent polypeptide (Mothes et al. 1994). Thus, Sec61 forms the aqueous transport pore that had been detected by a variety of biophysical and biochemical approaches (Gilmore and Blobel 1985; Simon and Blobel 1991; Crowley et al. 1993,1994).

Long before the identification of Sec61, multiple proteins had been proposed to be ER-localized ribosome receptors. The Sec61 complex binds nontranslating ribosomes with an affinity comparable to ribosome-stripped ER membranes (Kalies et al. 1994). Proteolytic digestion of canine Sec61 in intact membranes inhibits ribosome-binding activity by severing the carboxyl terminus and two cytosolically exposed loops (L6 and L8) (Fig. 2B) (Raden et al. 2000). Charge reversal substitutions at conserved basic residues in L6 (e.g., R275E) and L8 (R406E) of *S. cerevisiae* Sec61p cause a cotranslational translocation defect by interfering with RNC attachment to Sec61 (Cheng et al. 2005).

TRANSFER OF RNCS FROM THE SRP–SR COMPLEX TO THE SEC61 COMPLEX

The ability of SRP to reduce the protein synthesis elongation rate is necessary in vivo both in budding yeast and in mammalian cells (Mason et al. 2000; Lakkaraju et al. 2008) to allow sufficient time for targeting of SRP–RNC to the SR. In addition to binding SRP54, SRα has a high affinity for the large ribosomal subunit, thereby facilitating formation of the SR–SRP–RNC complex (Mandon et al. 2003). Cryo-EM structures of the SRP–RNC complex and the Sec61–RNC complex have shown that the SRP and Sec61 have overlapping binding sites near the polypeptide exit tunnel (Halic et al. 2004). Upon formation of the SR–SRP–RNC complex, movement of the M-domain of SRP54 exposes the Sec61-binding site (Halic et al. 2004) for subsequent attachment of the RNC to the Sec61 complex.

The GTP-bound conformation of SRβ forms the membrane-binding site for SRα (Ogg et al. 1998; Legate et al. 2000; Schwartz and Blobel

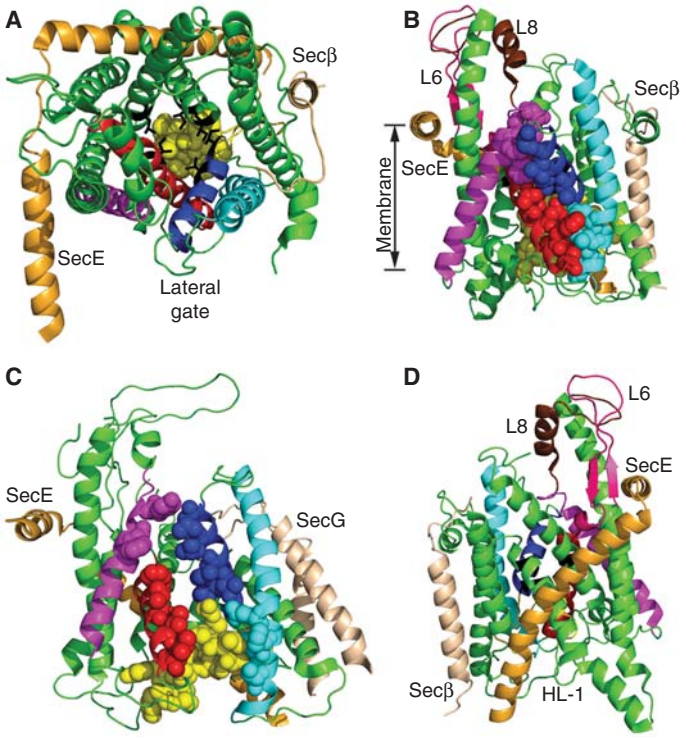

Figure 2. SecYEβ and SecYEG translocation channels. TM spans of SecY are color coded as follows: TMs 1, 4–6, 9–10 (green), TM2 (blue), TM3 (cyan), TM7 (red), and TM8 (magenta). (Yellow spheres) The plug domain. Cytosolic loops 6 and 8 are pink and chocolate, respectively, in panels *B* and *D*. (*A*) The cytosolic face of the *Methanocaldococcus jannaschii* SecYEβ complex in the closed conformation. (Black sticks) Pore ring residues. (*B*) Lateral gate of *M. jannaschii* SecYEβ viewed from the plane of the membrane. (Spheres) Lateral gate contact residues (LGCRs). (*C*) The partially open conformation of the *Thermotoga maritima* SecYEG complex. (*D*) The hinge domain of *M. jannaschii* SecYEβ. The HL-1 hinge loop is labeled. All structure views were generated using PyMOL and PDB files 1RHZ and 3DIN.

2003). Interestingly, deletion of the SRβ-TM span has a less severe impact on SR function than inactivation of the SRβ GTP-binding site (Ogg et al. 1998). Yeast genetic experiments using SRβ-ΔTM cells provided evidence that the SR interacts with translocation channels via Sec61β subunits (Jiang et al. 2008), thereby providing a mechanism to position the SRP–RNC adjacent to a vacant protein translocation channel (Fig. 1).

STRUCTURES OF PROTEIN TRANSLOCATION CHANNELS

The first cryo-EM structures of the yeast Sec61–RNC complex (Beckmann et al. 1997, 2001) and the mammalian Sec61–80S complex (Hanein et al. 1996; Menetret et al. 2000; Morgan et al. 2002) were thought to contain three to four Sec61 heterotrimers. An oligomeric interface was proposed to form a large-diameter (~25 Å) transport pore (Hanein et al. 1996; Beckmann et al. 1997; Morgan et al. 2002). Biophysical studies supported the concept of a large pore (~40 Å) (Hamman et al. 1997) that was sealed by the ribosome on the cytoplasmic face of the ER and by BiP in the ER lumen (Hamman et al. 1998).

The X-ray crystal structure of *Methanocaldococcus jannaschii* SecYEβ (Van den Berg et al. 2004) was obtained in the absence of a ribosome or a nascent polypeptide, hence the structure

is in a closed conformation. The membrane-exposed surface of SecYEβ complex lacks polar residues, arguing strongly against a transport pore formed by oligomer formation. SecE and Secβ, like their eukaryotic homologs Sec61β and Sec61γ, are C-tail-anchored membrane proteins with a single TM span (Fig. 2A). The 10 TM spans of SecY are arranged in two five-helix bundles (TM1-5 and TM6-10) to form an hourglass-shaped channel with a polar interior (Fig. 2A). A central constriction or pore ring is formed by side chains of hydrophobic residues projecting from TM spans closest to the channel center (Fig. 2A, side chains shown as black sticks). The exoplasmic face of the channel is blocked by a reentrant loop referred to as the plug domain (Fig. 2A, yellow spheres). Most point mutations in *E. coli* SecYEG that cause the prl phenotype, which corresponds to enhanced translocation of precursors with signal sequence mutations, map to the plug domain or the pore ring (Smith et al. 2005). Disulfides formed between a secretory protein precursor and a cysteine residue located near the pore ring provide evidence that secretory proteins are transported through the central pore (Cannon et al. 2005).

Budding yeast express an auxiliary protein translocation channel known as the Ssh1 complex (Finke et al. 1996) that is exclusively involved in the cotranslational translocation pathway (Wittke et al. 2002). Higher-resolution cryo-EM structures showed that single copies of mammalian Sec61, yeast Ssh1, or *E. coli* SecYEG form protein-conducting channels when bound to an RNC (Becker et al. 2009) or a 70S ribosome (Menetret et al. 2007). The two primary contact sites on the cytoplasmic surface of Sec61 or Ssh1 for the RNC are loops 6 and 8 (Becker et al. 2009), consistent with previous mutagenesis experiments (Cheng and Gilmore 2006). Cytosolic loop 8 undergoes a conformational change upon RNC binding to project into the polypeptide exit tunnel of the large ribosomal subunit.

TM2 and TM7 (blue and red α-helices in Fig. 2) of yeast Sec61 can be photocross-linked to the signal sequence of a nascent polypeptide (Plath et al. 1998), so this region has been termed the signal sequence-binding (SSB) site. Integration of a membrane protein necessitates lateral passage of the TM span from the central pore of SecY or Sec61 into the lipid bilayer. The lateral gate (TM2, TM3, TM7, and TM8) (Fig. 2A,B) is the only site where a TM span could exit the channel interior without crossing a cytosolic or lumenal loop joining two SecY TM spans (Fig. 2A,B).

The *Thermatoga maritima* SecYEG–SecA complex (Zimmer et al. 2008), the *Thermus thermophilus* SecYE–Fab complex (Tsukazaki et al. 2008), and the *Pyrococcus furiosus* SecYEβ complex (Egea and Stroud 2010) provided high-resolution structures of partially open protein conducting channels. SecA-dependent opening of SecYEG occurs by rigid-body movement of TMs 6–10 relative to TMs 1–5 (Zimmer et al. 2008) and is accompanied by movement of the plug domain away from the pore ring (Fig. 2C). In the fully open conformation, a translocation channel could accommodate a signal sequence in the SSB site and a nascent polypeptide in the central pore. The segment labeled HL-1 in loop 5 (Fig. 2D) is thought to be a hinge that allows the channel to open (Gumbart and Schulten 2007). Interestingly, the yeast *sec61-2* point mutations map to a conserved glycine in HL-1 (Nishikawa et al. 2001), highlighting the importance of the hinge in Sec61 function. Several yeast *sec61* alleles, including *sec61-3*, that cause a general defect in protein translocation can be suppressed by *prl* alleles, indicating that the transition between the closed and open conformations of the channel controls translocation efficiency and fidelity (Trueman et al. 2011).

How is the membrane permeability seal maintained when a translocation channel is in the open or closed conformation? Biophysical studies have suggested that BiP seals the lumenal face of the translocon during membrane protein integration (Hamman et al. 1998; Haigh and Johnson 2002). A second hypothesis was that the plug domain of SecY forms the membrane permeability seal (Van den Berg et al. 2004). Deletion of the plug domain in yeast Sec61p (Junne et al. 2006) or in *E. coli* SecY (Maillard et al. 2007) causes a minor growth defect and the prl phenotype (Junne et al. 2007). The membrane permeability barrier is reduced in *E. coli* cells expressing a SecY plug-deletion

mutant (Saparov et al. 2007; Park and Rapoport 2011) when the channel is in the closed state (Park and Rapoport 2011). Replacement of three or more pore ring residues in *E. coli* SecYEG with alanine or glycine residues caused a disruption of the membrane permeability seal and a severe growth defect (Park and Rapoport 2011). In contrast, replacement of all six pore residues in yeast Sec61p with alanine or glycine yields viable strains that display the prl phenotype (Junne et al. 2010).

POSTTRANSLATIONAL TRANSLOCATION—ROLE OF THE SEC62/SEC63 COMPLEX

Translocation assays conducted using microsomes and translation extracts prepared from budding yeast provided overwhelming evidence for an ATP-dependent posttranslational translocation pathway (Hansen et al. 1986; Rothblatt and Meyer 1986; Waters and Blobel 1986). ATP dependence is explained by the involvement of cytoplasmic (Chirico et al. 1988) and lumenal (Vogel et al. 1990) Hsc70 proteins. The Ssa family of cytosolic Hsc70s help maintain precursor proteins in a translocation-competent conformation (Deshaies et al. 1988).

Partitioning of yeast translocation substrates between the cotranslational and posttranslational translocation pathways is dependent on the hydrophobicity of the signal sequence (Ng et al. 1996). Most integral membrane proteins that lack cleavable signal sequences use the SRP-dependent pathway. Proteins with less hydrophobic cleavable signal sequences are translocated by the SEC complex. The heptameric SEC complex is composed of a heterotrimeric Sec61 complex (Sec61p, Sbh1p, and Sss1p) combined with a Sec62/Sec63 complex (Sec62p, Sec63p, Sec66p, and Sec72p) (Esnault et al. 1993,1994; Feldheim et al. 1993; Fang and Green 1994; Feldheim and Schekman 1994; Panzner et al. 1995). Mutations in yeast BiP (Kar2p), Sec62p, and Sec63p inhibit posttranslational translocation in vivo (Rothblatt et al. 1989) and in vitro (Deshaies and Schekman 1989; Sanders et al. 1992; Brodsky et al. 1995).

The Sec62/Sec63 complex has functionally important cytoplasmic and lumenal domains (Fig. 3A). A point mutation in the *sec63-1* allele (Rothblatt et al. 1989) alters a critical residue in the lumenal J-domain of Sec63, thereby inhibiting the interaction between Sec63p and Kar2p (Lyman and Schekman 1995). Binding of Kar2 to the precursor provides a driving force for posttranslational translocation (Matlack et al. 1999). Truncation of a carboxy-terminal 27-residue acidic segment of Sec63p, which interacts

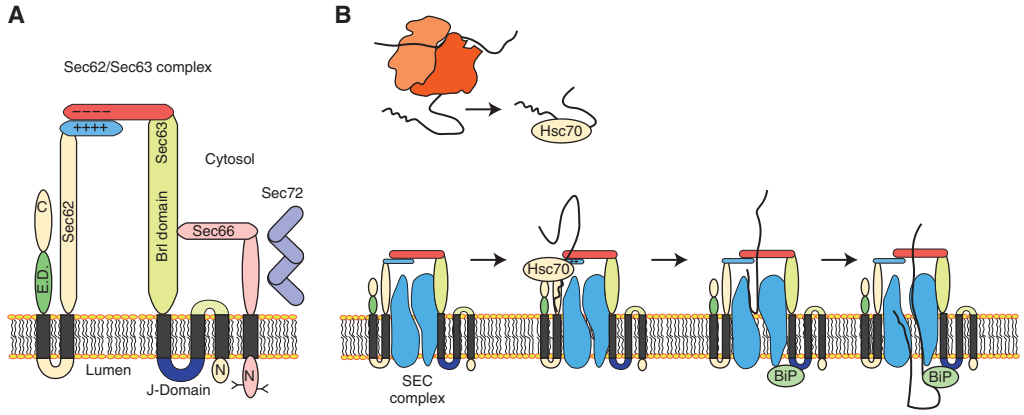

Figure 3. Posttranslational translocation pathway in yeast. (*A*) A diagram of the yeast Sec62/Sec63 complex. (*B*) Posttranslational translocation through the SEC complex. Fungi-specific subunits (Sec66 and Sec71) are not shown for clarity. Substrate delivery to the Sec62/Sec63 complex by Hsc70 precedes signal sequence insertion into the SSB site. BiP is recruited to the SEC complex by the lumenal J-domain of Sec63. BiP binding to substrates promotes posttranslational translocation.

with the basic amino terminus of Sec62 (Fig. 3A) (Wittke et al. 2000), promotes dissociation of Sec62p (Ng and Walter 1996) and causes a translocation defect (Ng et al. 1996). Carboxy-terminal deletions of Sec62 that remove a poorly understood effecter domain (Fig. 3B, segment labeled E.D.) are lethal (Wittke et al. 2000).

Photocross-linking experiments have shown that the signal sequence of a secretory protein can be inserted into the SSB site of Sec61 in the absence of ATP. The mature region of the precursor is then in contact with Sec62 (Müsch et al. 1992; Plath et al. 1998). Subsequent transport of the mature region of the protein through the Sec61 is ATP and Kar2p dependent. It is not known whether the Sec62/Sec63 complex simply serves as a targeting site for posttranslational substrates or, instead, promotes lateral gate separation of Sec61 to allow signal sequence insertion into the SSB site.

POTENTIAL ROLES FOR THE SEC62/SEC63 COMPLEX IN COTRANSLATIONAL TRANSLOCATION

Fully assembled SEC complexes as well as Sec61 heterotrimers are readily detected upon solubilization of yeast microsomes (Panzner et al. 1995). SEC complexes lack ribosome-binding activity (Prinz et al. 2000), suggesting that the cytosolic domains of the Sec62/Sec63 complex occlude the RNC-binding site on Sec61p (Harada et al. 2011). According to one viewpoint, Sec61 and Ssh1 heterotrimers mediate cotranslational translocation (Panzner et al. 1995; Cheng et al. 2005). An alternative hypothesis is that the Sec61 complex is assembled into either the heptameric SEC complex or a hexameric SEC′ complex (SEC complexes lacking Sec62) (Jermy et al. 2006). Formation of both the SEC and SEC′ complexes is proposed to depend on an interaction between the BRL domain of Sec63 (Jermy et al. 2006) and cytosolic loops of Sec61 (Fig. 3A) (Harada et al. 2011). The role of the Sec62/Sec63 complex in cotranslational integration of membrane proteins has not been resolved, despite an intriguing report that yeast Sec66 and Sec72 are involved (Green et al. 1992). Evidence has been presented that Sec63p (in cooperation with Kar2p) provides an essential driving force for all protein translocation reactions in yeast (Young et al. 2001; Willer et al. 2003; Jermy et al. 2006).

Homologs of Sec62 and Sec63 are encoded by the genomes of metazoan organisms, unlike Sec66 and Sec72, which are fungi-specific. Mammalian Sec62 and Sec63 are abundant ER proteins; biochemical experiments indicate that a portion of the ER pool of Sec62 and Sec63 will copurify with the Sec61 complex (Meyer et al. 2000; Tyedmers et al. 2000; Guth et al. 2004). The physiological role of mammalian Sec62 and Sec63 is unclear because protein translocation primarily occurs by a cotranslational pathway in mammalian cells. Evidence that the mammalian Sec62/Sec63 complex is dispensable for cotranslational translocation of several standard secretory proteins has been provided by in vitro translocation assays using SR–Sec61 proteoliposomes supplemented with the TRAM or TRAP complexes (Görlich and Rapoport 1993; Voigt et al. 1996; Hegde et al. 1998; Fons et al. 2003).

Small secretory proteins (less than 75 residues) are translocated by a posttranslational pathway in mammalian cells (Muller and Zimmermann 1987; Schlenstedt and Zimmermann 1987; Shao and Hegde 2012). Mammalian cells treated with siRNAs specific for Sec62 or Sec63 show reduced translocation or integration of several proteins including preprocecropin A, a small secretory protein (Lang et al. 2012). Thus, one documented role for mammalian Sec62 and Sec63 is posttranslational translocation of proteins that are too small to be targeted by the SRP pathway.

CONCLUDING REMARKS

Mid- to high-resolution structures of most of the core components of the protein translocation machinery have now been obtained both in isolation and as part of larger complexes. A noteworthy exception is the lack of mid- to high-resolution structural information regarding the SEC complex. Although we have a reasonable understanding of secretory protein translocation, there remain important knowledge gaps in terms of molecular mechanism.

The targeting mechanism for small secretory and membrane proteins is not well understood, nor is it known whether small integral membrane proteins are integrated by the Sec61 complex or by the SEC complex. The mechanism of integration of multispanning membrane proteins, because of its greater complexity, is an area of considerable interest. Interactions between the Sec61–RNC complex and adjacent membrane-embedded proteins like TRAM, TRAP, the signal peptidase, and the oligosaccharyltransferase have been detected and are likely to be important. These larger assemblies are often referred to as translocons to reflect the coordination and temporal links between protein translocation, nascent chain modification, and protein folding.

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