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Topical Review

Protein Translocation Across the Membrane of the Endoplasmic Reticulum

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Abstract. Eukaryotic cells are characterized by the existence of membrane-bound subcellular compartments which perform a variety of specialized functions. Proteins destined for these compartments begin their synthesis in the cytosol and must be subsequently targeted to their functional compartment by specific signal sequences present in the newly synthesized polypeptide chain. The translocation of preproteins across biological membranes is a fundamental process of intracellular trafficking and organelle biogenesis. Entry into the secretory pathway occurs by translocation of proteins into or across the membrane of the endoplasmic reticulum (ER). This process involves two distinct steps which are dependent on the orchestrated action of several proteins. The initial step of targeting involves recognition of the signal sequence and delivery of the protein precursor to the ER in a translocation competent conformation. The subsequent translocation event is characterized by interaction of the preprotein with the translocation channel followed by unidirectional movement across the lipid bilayer of the ER membrane into the lumenal space. The study of the mechanism of the translocation process is one of the most intriguing and rapidly advancing areas in cell biology. Here we review recent findings in both the yeast *Saccharomyces cerevisiae* and mammals concerning the mechanisms of the translocation step and discuss the roles of the proteins implicated in this process.

Key words: Endoplasmic reticulum — Protein translocation — Translocon—Sec61 — Membrane proteins

Targeting to the ER Occurs by Several Pathways

Proteins entering the secretory pathway contain an ER targeting signal, commonly referred to as the signal sequence. This signal is usually located towards the amino-terminus and consists of a continuous stretch of hydrophobic residues (6–20) flanked by one or more basic residues to the N-terminal side of the hydrophobic core [101]. In most cases, the signal sequence is cleaved during the translocation of the nascent chain and the remainder of the polypeptide is translocated into the lumen of the ER. Integral membrane proteins are inserted into the lipid bilayer of the ER by use of either a stop-transfer sequence or a signal-anchor sequence [for review *see* 50].

The capacity of mammalian microsomes to translocate presecretory proteins across the lipid bilayer has provided a wealth of information about the targeting of precursors to the ER membrane [for review *see* 102]. A cotranslational mechanism involving a cytosolic ribonucleoprotein complex, Signal Recognition Particle (SRP), and its cognate SRP receptor (SR) in the ER membrane has been described. SRP binds to the nascent polypeptide signal sequence as it emerges from the translating ribosome. Subsequent interaction of SRP with the ribosome results in an inhibition of translation elongation. This inhibition is relieved when SRP dissociates from the nascent polypeptide chain and the ribosome upon interaction with SR. The ribosome then interacts directly with the ER membrane and components of the translocation machinery effectively coupling translation and translocation (Fig. 1). A small number of polypeptides have been shown to be capable of SRP-independent, post-translational translocation in vitro across mammalian ER membranes. Typically precursors

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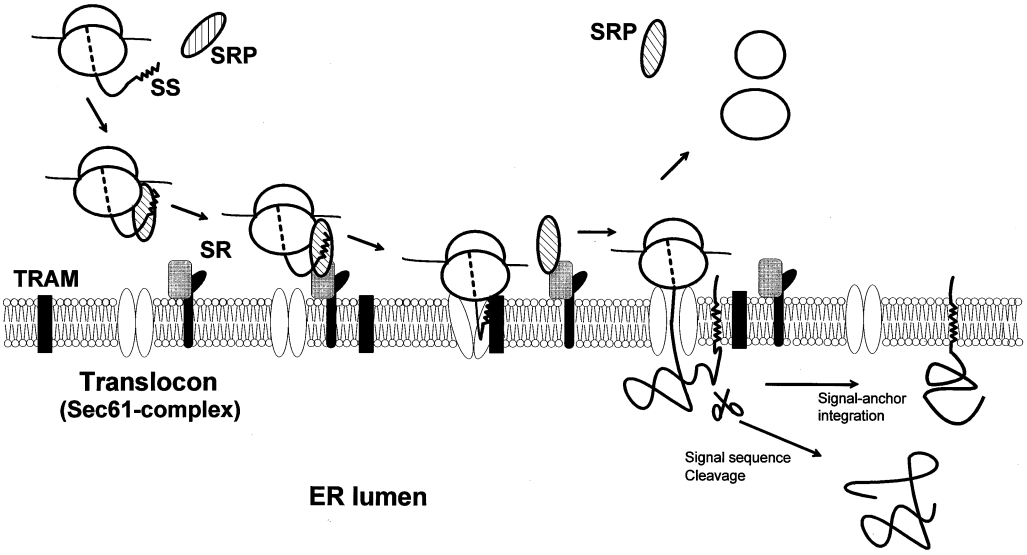

Fig. 1. SRP-dependent targeting and translocation. SRP binds to the signal sequence (SS) of a nascent polypeptide emerging from the ribosome and also a site on the ribosome itself to form a targeting complex in which polypeptide elongation is delayed. Upon targeting, SRP binds to its cognate receptor (SR) in the ER membrane. This interaction promotes the release of SRP from the signal sequence and the ribosome leading to the formation of a ribosome-translocon junction which seals off the signal sequence from the cytosol and the ER lumen in an aqueous compartment [for review *see* 102]. Translation resumes and as the nascent polypeptide extends, the translocon opens to form a pore allowing the passage across the membrane. In mammals, the TRAM protein appears to be recruited to the translocation site at an early stage of the process to facilitate translocation of nascent polypeptides. Release of the signal sequence from SRP and subsequent dissociation of SRP from SR is dependent on a GTPase cascade, permitting SRP and SR to engage in another round of targeting. In the case of soluble proteins, the signal sequence is cleaved concomitantly with translocation by signal peptidase (scissors symbol). Most membrane proteins have an uncleaved signal sequence, which exits the translocon laterally into the lipid bilayer to function as a signal-anchor sequence. Proteins with more complex topologies are generated by a succession of signal-anchor and stop-transfer sequences which form transmembrane domains [for review *see* 50].

in this category are no more than 70 amino acid residues [90, 105], and may be poor substrates for interaction with SRP. For the vast majority of presecretory proteins, translocation across the ER membrane in mammals has a strict requirement for SRP and therefore occurs cotranslationally. However, the exact role of SRP in precursor targeting has been complicated by the discovery of the nascent-polypeptide-associated complex [NAC; 106]. In the absence of additional factors, eukaryotic ribosomes have an intrinsic ability to bind the ER membrane [55, 62]. NAC appears to block the ER membrane binding site on the ribosome and also prevents binding of SRP to non-signal sequences [for review *see* 103]. Some functions attributed solely to SRP now appear to depend on the interplay between SRP and NAC.

SRP homologs have been identified amongst distantly related organisms such as the yeast *Saccharomyces cerevisiae* suggesting that the SRP dependent pathway is ubiquitous amongst eukaryotes. Surprisingly, mutant yeast cells lacking SRP are viable albeit with a severe growth defect and defects in the translocation of some proteins [12, 44, 96]. This observation implies that targeting to the ER can occur by redundant pathways in vivo. The nature of the SRP independent mechanism is at present unclear. One possibility is that it represents a post-translational mechanism which has been demonstrated in vitro for precursors as large as 250 residues [45]. In this case precursors that are released from the ribosome after translation termination are maintained in a ''translocation competent'' state by interaction with Hsp70 molecules (Fig. 2). Evidence indicates that the targeting of the vacuolar protease Carboxypeptidase Y (CPY) occurs independently of SRP and Hsp70s, suggesting the existence of an alternative pathway which may involve novel cytosolic targeting components [14, 44, 45].

ER Translocation Requires an Evolutionary Conserved Core Complex

Since the primary function of a biological membrane is to maintain a permeability barrier, the protein translocation process raises an interesting mechanistic question: how do large hydrophilic polypeptides pass through the membrane without compromising its integrity? Several lines of evidence indicate that polypeptides are translocated *via* an aqueous channel formed, at least in part, by integral membrane proteins of the ER [for reviews *see* 48 and 64]. A combination of genetic and biochemical ap-

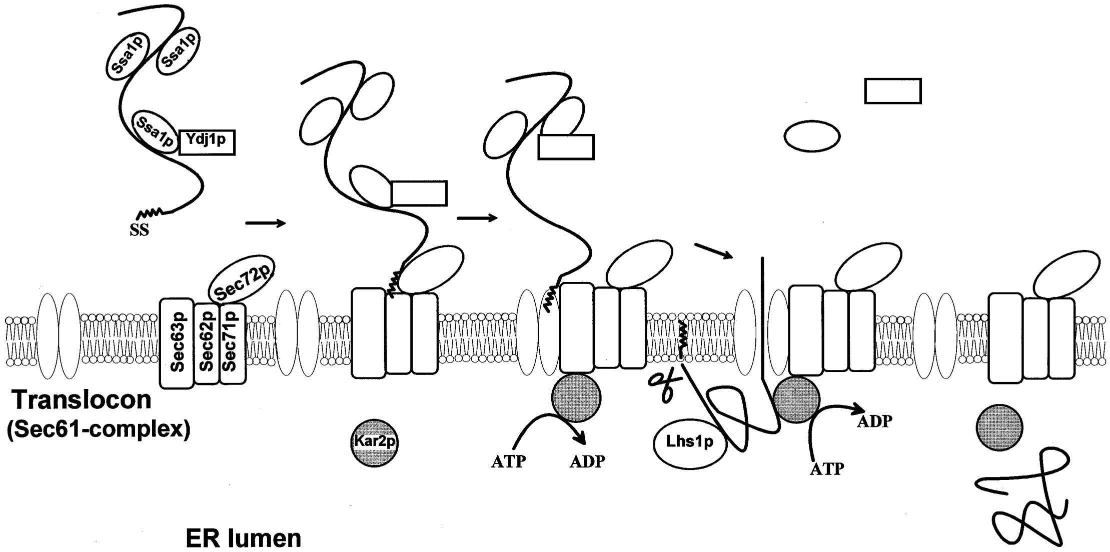

Fig. 2. Post-translational targeting and translocation in yeast. A fully synthesized preprotein is preserved in a translocation competent conformation principally by interaction with Ssa1p, a cytosolic chaperone of the Hsp70 class. The ATPase activity of Ssa1p requires interaction with the DnaJ homolog Ydj1p in vitro and this interaction may be required to release Ssa1p molecules from the preprotein at the ER membrane prior to translocation. The role of Ssa1p is topologically distinct from that of the lumenal Hsp70 molecules Kar2p and Lhs1p [10, 18]. However, the mechanism of signal sequence recognition in the cytosol and subsequent ER targeting remains unknown. At the ER membrane, the targeted preprotein interacts with the Sec62p/Sec63p tetrameric protein complex (consisting of Sec62p, Sec63p, Sec71p and Sec72p) which may function as a signal sequence receptor. This targeted complex interacts with the translocon in an ATP-dependent step in which Kar2p interaction with Sec63p results in the transfer of the signal sequence to the translocon (*see text*). Conformational change in the translocon may open the pore through which the nascent polypeptide is transported thereby initiating translocation. Signal sequence cleavage occurs concomitantly with translocation (scissors symbol). Propagation and completion of transfer to the ER lumen may require further interaction between Kar2p and Sec63p. A more elaborate version of this model has been proposed [60]. The exact role of Lhs1p in this process is currently unknown, although yeast cells lacking this protein show significant defects in the translocation of precursors which are transported post-translationally [18].

proaches have converged to identify components of this translocation complex, or ''translocon.''

Genetic studies in the budding yeast *Saccharomyces cerevisiae* led to the identification of the Sec61p, a multi-spanning integral membrane protein of the ER required for the translocation of both secretory and membrane proteins [26, 72, 97, 107]. Crosslinking experiments have shown Sec61p to be in intimate contact with prepro-α-factor (ppαF) during translocation across the bilayer suggesting that this protein corresponds to a component of the translocon *per se* [70, 85]. A mammalian homolog of Sec61p (Sec61α) sharing 56% identity with the yeast protein has been identified (Table), which can also be crosslinked to translocating chains [41, 49, 51, 65, 69, 75] and is in tight association with membrane-bound ribosomes during translocation [41, 57].

Sec61α has been isolated from dog pancreas microsomes as a complex with two other integral membrane proteins, Sec61β and Sec61γ both belonging to the C-terminal anchor class [42, 47]. A similar Sec61-complex is found in yeast where Sec61p interacts directly with Sss1p (homologous to Sec61γ) [33, 34], and a Sec61β homolog, Sbh1p [79]. Significantly, mammalian Sec61γ can complement a yeast *sss1* mutant [47] emphasizing the extent of functional conservation of the Sec61-complex. The tripartite nature of the Sec61-complex also draws comparison with the molecular nature of the SecY/E/G translocase of *E. coli* [13, 30]. Bacterial SecY appears to be related to Sec61p/Sec61α [41, 91], whilst SecE shares a lesser degree of sequence identity with Sss1p/Sec61γ [47]. Interestingly, bacterial SecG and yeast Sbh1p show no convincing sequence similarity but, perhaps significantly, neither of these components are essential for viability in their respective organisms [38, 76].

The Sec61-Complex Performs Multiple Roles in the Translocation Process

Substantive evidence that Sec61p/Sec61α is a core component of ER translocon has been provided by the ability of the purified proteins to promote translocation reactions when reconstituted into liposomes. The cotranslational translocation of proteins into mammalian-derived proteoliposomes requires only SRP, SR and the Sec61-complex [42]. This observation, coupled with crosslinking studies [for review *see* 64], provided compelling evidence that the Sec61-complex is directly involved in the translocation reaction. Whilst SRP, SR and Sec61-

Table. Components implicated in ER membrane translocation

Component	*S. cerevisiae*	Mammals	Function
Cytosol			
Ssa1p	√	√(Hsp70)	Molecular chaperone
Ydj1p	√	—	DnaJ partner of Ssa1p
SRP	√	√	Protein targeting to the ER membrane
ER membrane			
SR	√	√	SRP receptor
RR	—	√[a]	Putative ribosome binding
TRAM	—	√	Required for the translocation of some precursors
Sec61p	√	√(Sec61α)	Essential translocon subunit associated with Sbh1p and Sss1p
Sbh1p	√	√(Sec61β)	Non-essential translocon subunit
Sss1p	√	√(Sec61γ)	Essential translocon subunit
Sec62p	√	—[b]	Subunit of the Sec62p-Sec63p complex which is involved in posttranslational targeting
Sec71p	√	—	Subunit of Sec62p-Sec63p complex
Sec72p	√	—	Subunit of Sec62p-Sec63p complex/signal sequence recognition
Sec63p	√	—[c]	Subunit of Sec62p/Sec63p complex also interacts with Kar2p
Ssh1p	√[d]	—	Subunit of a second trimeric complex together with Sbh1p and Sss1p
Sbh2p	√[d]	—	Homolog of Sbh1p
ER lumen			
Kar2p	√	√(BiP)[e]	Required during several phases of translocation
Lhs1p	√	—[f]	Required for the efficient translocation of several preproteins

√: Present and role in translocation demonstrated or implied. —: No homolog or correlate discovered to date. [a] The p180 putative ribosome receptor (RR) has been implicated in translocation [85], but is not required in the minimal biochemical reconstitution [41]. [b] Mammalian sequences with significant similarity to yeast Sec62p have been revealed upon inspection of the Expressed Sequence Tags (ESTs) database (*unpublished results*). A *Drosophila* homolog has been characterized and can functionally replace the yeast protein [75]. [c] A potential mammalian homolog of Sec63p has been identified [6] but it has not been implicated in translocation. [d] Components of a trimeric complex implicated in translocation by virtue of its similarity to the Sec61-complex (*see text*). [e] BiP is present but there is no direct evidence that it is required for translocation (*see text*). [f] Grp170, a potential homolog of Lhs1p has been identified [15].

complex are sufficient for the translocation of some precursors, the addition of the TRAM protein stimulates the translocation of others and is essential for translocation of ppαF [42]. TRAM is an integral membrane protein originally identified as the principal crosslinking partner of short translocating chains [40]. However, the exact role of TRAM remains obscure, especially since it can be crosslinked to precursors which do not require its activity in the reconstituted reaction. It does not function in the topogenesis of single-spanning membrane proteins [78], but has been suggested to function in the formation of an early translocation intermediate. Evidence suggests that its requirement is dependent on the structure of the signal sequence [100]. Although not a core component of the translocon, it may be recruited to the translocon interacting with the nascent polypeptide at an early phase of translocation (Fig. 1), perhaps disengaging after signal sequence processing. Crosslinking studies have implicated several other membrane proteins in the translocation process [64], but reconstitution of translocation in their absence suggests that any role performed by these proteins in translocation may be peripheral, or of greater significance in vivo.

It has long been suggested that cotranslational translocation would involve a ribosome receptor (RR) in the ER membrane, plus a signal sequence receptor (SSR) activity to promote the delivery of precursors to the translocation site [4, 5, 17, 39, 80]. Indeed, candidates for such factors have been reported including the putative ribosome receptor, p180 [87], and the TRAP complex (translocon-associated protein) [46]. Molecular reconstitution suggests that these factors are not required for translocation in vitro [42]. Current evidence suggests that the Sec61-complex itself may be directly involved in both ribosome binding and signal-sequence recognition.

The Sec61-Complex Interacts Directly with Ribosomes and is a Key Component of the Translocon

Sec61-complexes copurify with ribosomes in a detergent-solubilized, ribosome-associated membrane protein fraction, and the ribosomes can be removed by a puromycin and high salt treatment [41, 57]. The same treatment had been previously demonstrated to remove ribosomes from rough microsomal membranes [1]. This is consistent with a direct high affinity interaction between the Sec61-complex and ribosomes thereby providing a molecular basis for coupling between translation and translocation. The absence of additional putative ribosome receptors for the minimal biochemical reconstitution of translocation is also consistent with a direct interaction between translating ribosomes and the Sec61-complex [42].

Although it remains to be formally proven, an increasing body of evidence strongly supports the possibility that the nascent chain-ribosome-regulated pores identified by Simon and Blobel [93] are formed at least in part by the Sec61-complex. These pores identified by electrophysiological techniques opened when partially synthesized proteins trapped across the ER membrane were released with puromycin under conditions where the ribosome remained membrane-bound. Removal of the ribosome by increasing salt concentration led to pore closure observed as a loss of conductance [93]. This strongly indicated that the ribosome is able to maintain the pore in an open conformation. Data obtained from probing the physico-chemical properties of the translocation site are consistent with a model of a proteinaceous, aqueous pore which is isolated from the cytosol by its tight junction with the ribosome, and which shields the translocating polypeptide from the lipid bilayer [for review *see* 48]. The trimeric Sec61-complex in both yeast and mammals has been widely implicated as a component of the pore as a result of crosslinking of proteins at various stages of translocation [41, 49, 51, 65, 69, 70, 75, 85]. Fluorophore quenching experiments have demonstrated that nascent chains of up to 70 residues trapped across the membrane are sealed off from the ER lumen in a tight ribosome-membrane junction [19, 20]. Longer chains are however accessible to the lumen indicating that a lumenal gate may be opened as a result of chain elongation [20]. This is also consistent with the proteolytic protection of 70 residues portions of translocating chains by the ribosome and the translocation apparatus [66].

Post-Targeting Signal Recognition is Essential for Efficient Translocation

Signal sequence recognition at the membrane is crucial in regulating the translocation efficiency of targeted precursors [3, 55]. Truncated pre-prolactin (pPL) nascent chains were crosslinked efficiently to the 54kDa subunit of SRP, Sec61α and TRAM in the presence of microsomes [55]. Signal sequence mutations were constructed to examine the significance of these interactions. Remarkably the reduction of the hydrophobicity of the pPL signal sequence did not significantly perturb crosslinking. However, there was a dramatic reduction in translocation efficiency compared to the non-mutant control, implying a post-targeting signal discrimination event at the membrane [55]. The reconstitution of SRP/NAC-independent translocation of ribosome-coupled nascent chains was exploited to analyze signal sequence recognition at the ER membrane at the molecular level. It was found that pPL nascent chains containing mutant signal sequence, were not efficiently translocated, even though they were bound to the membrane with comparable affinity. Liposomes containing only the Sec61-complex were able to discriminate functional signal sequence, suggesting that the Sec61-complex performs the recognition event [55]. Microsomal membranes were able to bind and translocate pPL more efficiently than membranes reconstituted with only the Sec61-complex suggesting that additional factors are involved in increasing the efficiency and/or stability of the ribosome-nascent chain-membrane interaction in vivo. Interestingly, in *E. coli*, secretory proteins bearing signal sequence mutations defective in translocation are suppressed by mutations that map to the *SecY* and *SecE* genes encoding the SecY and SecE components of SecY/E/G translocase [23, 32] suggestive of a signal recognition role. By analogy, the Sec61-complex appears to perform the same recognition function in eukaryotes by integrating ribosome-binding, signal sequence recognition and polypeptide conducting properties to function as a translocon.

Lipids may also play a role in the signal sequence discrimination function, especially since there is a strong correlation between the hydrophobicity of a signal sequence, its ability to interact with lipid, and its ability to function as a signal sequence [54]. Moreover, recent studies have demonstrated the efficient crosslinking of lipid to functional signal sequences [65]. In *E. coli* requirements for acidic phospholipids [for reviews *see* 31 and 81] and nonbilayer phospholipids [82] for efficient translocation have been demonstrated. A model in which the translocation site is open laterally to the bilayer would provide an attractive explanation for the role of Sec61p in the insertion of integral membrane proteins.

Post-Translational Translocation in Yeast

Components of the SRP/SR targeting pathway and of the Sec61-complex have been identified in yeast and are clearly ubiquitous throughout eukaryotes [29, 52, 56, 103, 108]. However, genetic and biochemical studies in yeast have identified several additional ER components required for a post-translational translocation mechanism. These include the integral membrane proteins Sec62p, Sec63p, and Sec71p, the peripheral membrane protein, Sec72p, and the ER lumenal proteins Kar2p and Lhs1p [18, 25–28, 35–37, 43, 61, 84, 98, 99]. Of these Sec62p, Sec63p, Sec71p, and Sec72p form a stable tetrameric complex [11, 25, 35, 37] which can also be found in association with the trimeric Sec61-complex [25, 79, 83]. This heptameric complex is required for the molecular reconstitution of a post-translational translocation reaction in yeast which is stimulated by ATP and Kar2p [79]. There was no requirement for a TRAM protein, a homolog of which has not been identified in yeast (Table). The requirement for the Sec61-complex indicates that it may function in a modular manner, interacting with the Sec62/Sec63p-complex during a post-translational reaction and with a translating ribosome during a cotranslational reaction. The mechanism by which post-translationally translocated polypeptides are initially targeted to the ER remains uncertain. Evidence suggests that the translocation of some precursors may depend upon the activities of the cytosolic chaperones Ssa1p and Ydj1p, which are believed to maintain precursors in a loosely-folded conformation required for their productive interaction with the membrane translocon (Fig. 2) [2, 14, 16, 21, 22, 24].

Crosslinking studies have provided considerable insight into the molecular events occurring at the yeast membrane during translocation in vitro. Sec61p was the major ATP-dependent crosslinking partner of a ribosome-coupled ppαF trapped across the bilayer, whilst crosslinking to Sec62p occurred in the absence of ATP [70]. This suggested that interaction with Sec62p preceded a normally ATP-dependent transfer of the nascent chain to Sec61p, consistent with ATP-independent and dependent phases of translocation previously identified [86]. Sec62p is present in ER membranes at only about 10% the amount of Sec61p which may be consistent with a transient role for Sec62p at the Sec61-complex [25]. Similar experiments demonstrated that ppαF chains trapped in a translocation complex could be efficiently crosslinked to Sec61p only in the presence of ATP [85]. The efficiency of crosslinking to Sec61p is greatly diminished in microsomes prepared from either *sec62* or *sec63* mutant strains, suggesting that Sec62p and Sec63p may be involved in the presentation of ppαF to the Sec61-complex. Sec61p crosslinking was also deficient in two *kar*2 mutants alleles, while a third mutant exhibited nearly wild type levels of crosslinking, but remained translocation defective, consistent with a failure to complete translocation. Furthermore, trapped ppαF chains could be crosslinked to Kar2p indicative of a direct interaction between the translocating protein and Kar2p [85]. These observations strongly suggest that Kar2p acts at two phases of translocation. First, at an early stage involving interaction of the nascent polypeptide with the Sec61-complex, and second by direct interaction with the nascent chain as it emerges into the lumen.

Genetic and biochemical data indicate that Kar2p and Sec63p physically interact [11, 79, 92]. Furthermore, a number of observations indicate that the interaction between Kar2p and Sec63p provides a critical link between different phases of translocation. Hsp70s appear to function in translocation in concert with DnaJ-related partner proteins [8]. Kar2p interacts with the ‘‘DnaJ box’’ of Sec63p which is located on the lumenal side of the membrane [11, 36], suggesting that Kar2p and Sec63p comprise an Hsp70/DnaJ pair of the ER lumen. A mutation in the conserved DNAJ box of Sec63p [71] exhibited the same stalling phenotype as a *kar2* mutant which resulted in ppαF interaction with Sec61p [63, 84]. This suggested that Sec63p may function similarly to Kar2p both early in precursor engagement and at the later stage in precursor transfer to the lumen. Genetic and biochemical criteria demonstrated that the stalling phenotype was due to a defective interaction between Kar2p and Sec63p, leading to a model for the concerted action of Kar2p and Sec63p in post-translational translocation [63]. In the first step ATP-independent binding of precursor to a putative signal sequence receptor complex consisting of Sec62p, Sec71p, Sec72p and possibly Sec63p occurs (Fig. 2). In the second step the ATP-dependent transfer of precursor from this complex to the pore is mediated by Sec63p and Kar2p. In the final stage, propagation and completion of translocation is also mediated by Sec63p and Kar2p by an ATP-dependent mechanism. Kar2p directly interacts with the translocating chain and uses the energy of ATP hydrolysis to pull the chain into the lumen acting as a translocation motor in a manner similar to that proposed for the action of Hsp70/TIM44 in mitochondrial protein translocation [for reviews *see* 8 and 88]. The proposed role for Kar2p in contributing to the energetics of translocation would be consistent with the observed requirement for lumenal ATP in the translocation of ppαF [67, 68]. Sec63p may function by recruiting, and hence concentrating, Kar2p at the translocation site although it remains to be determined whether Sec63p might also stimulate the ATPase activity of Kar2p. In addition, it may transduce the influence of Kar2p on the translocon possibly by promoting conformational changes resulting in translocon opening and closure. Such a mechanism might explain how the translocon may be gated in the absence of a ribosome.

Other Lumenal Proteins Function in ER Translocation

The Kar2 protein is clearly implicated in post-translational translocation. However, mutants in the essential *KAR2* gene are defective in the translocation of a range of protein precursors including some which are translocated in a cotranslational manner [73, 85, 99]. It is conceivable that a defect in post-translational translocation in vivo might indirectly lead to a block in cotranslational translocation due to partially translocated precursors ‘‘jamming’’ the Sec61 translocon. However, a more direct role for Sec63p and Kar2p in cotranslational translocation is suggested by recent studies on the translocation of pre-invertase into yeast microsomes in vitro [9].

The role of Hsp70 molecules in translocation has been further complicated by the identification of Lhs1p, a novel Hsp70 of the yeast ER lumen, which shares limited sequence identity with Kar2p. An *lhs1* null mutant is viable, but exhibits a severe defect in the translocation of a number of secretory preproteins in vivo [18]. Moreover, this mutation displayed a genetic interaction with *kar2* mutants, strongly suggesting that Kar2p and Lhs1p perform overlapping functions. It has been proposed that Lhs1p is a chaperone of the yeast ER lumen involved in both polypeptide translocation and subsequent protein folding [18]. Two DnaJ homologues have been identified in the yeast ER, namely Sec63p and Scj1p [84, 91]. Evidence suggests that Kar2p interacts with both Sec63p and Scj1p, but there is currently no data implicating Scj1p in protein translocation [91]. However, it has been suggested that Scj1p might function in translocation by regulating the interaction of Kar2p with Sec63p [8]. It remains to be seen whether or not Lhs1p interacts with either Sec63p or Scj1p.

The role of ER lumenal proteins in the SRP-dependent cotranslational translocation of proteins across mammalian ER membranes remains controversial. Depletion of the lumenal proteins from rough microsomes by alkaline extraction at pH 9.5–10, releases a class of proteins necessary for efficient in vitro cotranslational protein translocation [74]. In these studies, only an incomplete translocation reaction was achieved in the absence of lumenal proteins. Re-additional of lumenal proteins to washed microsomes resulted in a partial restoration of translocation activity. In contrast, the in vitro reconstitution of cotranslational protein translocation into proteoliposomes using purified membrane components does not depend on lumenal proteins [42].

Further support for a role of lumenal proteins in cotranslational translocation has come from genetic studies in the yeast *Yarrowia lipolytica* which have led to the identification of *SLS1* which encodes a novel protein of the ER lumen [6]. *SLS1* was identified by virtue of a synthetic lethal interaction with a mutation in a component of *Y. lipolytica* SRP. An *sls1* null mutation, although viable, displays temperature sensitive growth and is defective in the translocation of an SRP-dependent preprotein. In addition, crosslinking experiments demonstrated that Sls1p is located in close proximity to the Sec61p translocon [6]. This suggests that Sls1p may play a role in the propagation and/or completion of translocation on the lumenal face of the ER membrane.

The Requirements for Mammalian Post-translational Translocation may be Similar to Yeast

A category of very small secretory proteins and larger chimeric derivatives have been demonstrated to translocate post-translationally across mammalian microsomes [89, 90, 105]. An ATP-dependent cytosolic machinery comprising an Hsp70 and at least one additional cytosolic protein is involved in stimulating the translocation of these precursors [109]. This may be analogous to the interaction between Ssa1p and Ydj1p necessary for the translocation of some preproteins in yeast [2, 14] (Fig. 2). The Sec61-complex and TRAM have been implicated in the post-translational translocation by chemical crosslinking experiments [59]. The inhibition of both co- and post-translational import by photoaffinity inactivation of microsomes with azido-ATP suggests that a novel protein is involved in both modes of translocation [58]. It will be of interest to find whether or not this protein is related to yeast Lhs1p. The possibility that a Sec62p/Sec63p-related complex may exist in higher eukaryotes has been strengthened by the discovery of a *Drosophila* Sec62p homolog which is able to complement a yeast *sec62* null mutation and correct the translocation defect of a temperature sensitive *sec62* mutation [77], and by the appearance of a potential human homolog in sequence databases (*unpublished results*). In addition a potential mouse homolog of Sec63p has also been reported [7]. Whether or not these Sec62/Sec63 ‘‘homologs’’ correspond to components of a conserved translocation mechanism, present throughout eukaryotes, remains a crucial question in the field.

A Second Translocon Functions in Yeast

The yeast genome sequencing project has revealed a gene (*SSH1*) displaying significant (32%) amino acid identity with yeast Sec61p [53] with a possible membrane topology similar to that determined for Sec61p [107]. Ssh1p the product of this gene exists in a heterotrimeric ER membrane complex (the Ssh1p-complex) consisting of Ssh1p, Sss1p and an Sbh1p homolog Sbh2p [38]. Neither Ssh1p nor Sbh2p are essential for growth although cells lacking Ssh1p display a mild growth defect [38]. Whilst Sbh1p and Sbh2p individually are not essential, cells lacking both proteins are inviable at higher temperature and also display a translocation defect suggesting that these subunits have a peripheral role in the translocation process. This indicates that Sec61p and Sss1p can form a dimeric complex which probably functions as the core unit of the translocation machinery [38]. The Ssh1p complex forms a tight interaction with membrane-bound ribosomes, but unlike the Sec61p-complex it cannot be copurified with the Sec62/Sec63p-complex. It appears that the Sec61-complex functions as a modular translocon with a role in both co- and post-translational translocation pathways as a trimeric complex and as a constituent of the heptameric complex respectively. Correspondingly, the Ssh1p complex may function only in the cotranslational pathway, suggesting that the essential nature of Sec61p may be related to its role in post-translational translocation [38]. The existence of this second nonessential translocon is however an enigma. If the two trimeric complexes translocate distinct subsets of substrates, then the Ssh1p complex either translocates proteins that are not essential for cell viability or the subsets overlap substantially. This would also necessitate different targeting pathways leading to either the Sec61p or the Ssh1p complex. Direct evidence for a function of Ssh1p in cotranslational translocation may have to await the reconstitution of SRP-dependent translocation into yeast proteoliposomes.

Concluding Remarks and Perspectives

Considerable advances have been made in uncovering the identity of protein components implicated in ER translocation. Reconstitution with purified components and an extensive genetic analysis in yeast are providing continuing insight into the mechanism of translocation. It is becoming increasingly possible to address key questions at a detailed molecular level. What is the mechanism of post-targeting signal recognition and how does the Sec61-complex carry out this function? It will be of interest to determine whether mutants generated in the genes encoding Sec61p and Sss1p are defective in this function. How is the gating of the pore regulated? The role that the ribosome and the Sec62/Sec63p-complex may play in translocon gating awaits further examination. The component corresponding to the lumenal gate identified by Crowley et al. [19] also remains to be determined. The process whereby membrane proteins integrate into the bilayer and adopt the correct topology remains largely unknown. Furthermore, the potential role of molecular motors in providing the directionality and driving force for translocation also awaits a more detailed analysis. Accounting for the involvement of an increasingly large number of gene products implicated in ER translocation particularly in yeast will provide a considerable challenge. In addition, it will be intriguing to learn if higher eukaryotes share the burden of translocation between more than one complex as in yeast. Other interesting points include the relative contribution of co- and post-modes in yeast and mammals and an investigation of any tissue and developmental specificity of these pathways in higher eukaryotes. Finally, similarities between some of the components required for translocation into the ER and translocation across the mitochondrial membrane, the cytoplasmic membrane of *E. coli* and the thylakoid membrane [29, 52, 56, 60, 88, 108] suggest that protein translocation may occur via common mechanisms. Continued comparison between different systems may yield important information about the salient features of the translocation process.

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