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Review

Protein translocation across the ER membrane ☆

Richard Zimmermann [a,*], Susanne Eyrisch [b], Mazen Ahmad [b], Volkhard Helms [b]

[a] Medical Biochemistry & Molecular Biology, Saarland University, D-66041 Homburg, Germany
[b] Computational Biology, Saarland University, D-66421 Saarbrücken, Germany

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ABSTRACT

Protein translocation into the endoplasmic reticulum (ER) is the first and decisive step in the biogenesis of most extracellular and many soluble organelle proteins in eukaryotic cells. It is mechanistically related to protein export from eubacteria and archaea and to the integration of newly synthesized membrane proteins into the ER membrane and the plasma membranes of eubacteria and archaea (with the exception of tail anchored membrane proteins). Typically, protein translocation into the ER involves cleavable amino terminal signal peptides in precursor proteins and sophisticated transport machinery components in the cytosol, the ER membrane, and the ER lumen. Depending on the hydrophobicity and/or overall amino acid content of the precursor protein, transport can occur co- or posttranslationally. The respective mechanism determines the requirements for certain cytosolic transport components. The two mechanisms merge at the level of the ER membrane, specifically, at the heterotrimeric Sec61 complex present in the membrane. The Sec61 complex provides a signal peptide recognition site and forms a polypeptide conducting channel. Apparently, the Sec61 complex is gated by various ligands, such as signal peptides of the transport substrates, ribosomes (in cotranslational transport), and the ER lumenal molecular chaperone, BiP. Binding of BiP to the incoming polypeptide contributes to efficiency and unidirectionality of transport. Recent insights into the structure of the Sec61 complex and the comparison of the transport mechanisms and machineries in the yeast *Saccharomyces cerevisiae*, the human parasite *Trypanosoma brucei*, and mammals have various important mechanistic as well as potential medical implications. This article is part of a Special Issue entitled Protein translocation across or insertion into membranes.

Contents

Abbreviations: BiP, immunoglobulin heavy chain binding protein; EM, electron microscopy; ER, endoplasmic reticulum; ERj, ER resident J-domain protein; GPI, glycosylphosphatidylinositol; Grp, glucose regulated protein; Hsp, heat shock protein; NAC, nascent chain associated complex; NEF, nucleotide exchange factor; OST, oligisaccharyl transferase; RAMP, ribosome associated membrane protein; Sec, protein or complex that is involved in protein secretion; SPC, signal peptidase complex; SRP, signal recognition particle; SR, SRP receptor

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* Corresponding author. Tel.: +49 6841 1626510; fax: +49 6841 1626288.
E-mail address: bcrzim@uks.eu (R. Zimmermann).

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1. Introduction

Protein translocation into the endoplasmic reticulum (ER) is the first step in the biogenesis of most extracellular and many soluble organelle proteins of eukaryotic cells (such as resident proteins of the ER, ER-Golgi intermediate compartment/ERGIC, Golgi, endosome, and lysosome) [1–3]. Typically, protein translocation into the ER involves cleavable amino terminal signal peptides in precursor proteins and sophisticated transport machinery. The signal peptides for ER targeting are 15 to 30 amino acid residues in length and have a tripartite organization, comprised of a core of hydrophobic residues flanked by a positively charged aminoterminal and a polar, but uncharged carboxyterminal region [4–8]. Two mechanisms can be distinguished that differ in their relationship to translation (termed co- and posttranslational mechanisms) and with respect to the relevant cytosolic components. The two mechanisms merge at the ER membrane, specifically at the heterotrimeric Sec61 complex that comprises α-, β-, and γ-subunits. In addition, they involve further components, most notably the ER-lumenal chaperone BiP and its co-chaperones and nucleotide exchange factors (NEFs). During or immediately after translocation, the precursors are typically processed on the lumenal face of the ER membrane by the signal peptidase complex (SPC) [9–15], oligosaccharyl transferase (OST) [15–21], and/or GPI transamidase [22–29]. However, processing by these enzymes is not a prerequisite for transport into the ER.

In general, protein translocation into the ER is followed by folding and assembly of the newly-imported polypeptides. Folding and assembly of proteins involve some of the above-mentioned components, such as BiP and its co-chaperones and nucleotide exchange factors [30–39]. After folding and assembly, the native proteins are delivered to their functional location by vesicular transport (with the exception of resident ER proteins). In the case of mis-folding or mis-assembly, the polypeptides are exported to the cytosol and delivered to the proteasome for degradation (termed ERAD) [Sommer, this issue]. The export of some mis-folded polypeptides from the ER lumen to the cytosol also involves some of the above-mentioned components, such as the Sec61 complex and BiP [40–49].

2. Co- and posttranslational transport mechanisms

Protein transport into the ER can occur co- or posttranslationally. The cytosolic transport components are dedicated either to cotranslational (signal recognition particle, SRP) or posttranslational (heat shock proteins, Hsp) transport (Table 1). In posttranslational transport, fully synthesized precursor polypeptides are transported with the help of cytosolic molecular chaperones, belonging to the Hsp70 and Hsp40 chaperone families [50–54]. By cycling on and off, the chaperones keep the precursor polypeptides soluble and competent for interaction with the transport components in the ER-membrane. In cotranslational transport, nascent precursor polypeptides are transported with the help of SRP and its receptor on the ER-surface (SRP-receptor, SR) [55–65]. SRP binds to nascent precursor polypeptides as soon as their signal peptides emerge from the translating ribosomes. This interaction slows down protein synthesis, thereby allowing the complex of ribosome, nascent polypeptide chain, and SRP to reach the SRP-receptor (SR) at the ER-membrane. Thus, SRP is involved in ER-targeting, in addition to being a molecular chaperone for the nascent polypeptide. Furthermore, the synthesis of many polypeptides is initiated on ribosomes that are continuously attached to the ER-membrane [66–70]. In this case, SRP and cytosolic chaperones may not be required for translocation. Here, polypeptides that lack a signal peptide for ER-targeting may be recognized by the nascent chain associated complex (NAC). This interaction may lead to release of the translating ribosomes from the membrane and completion of protein synthesis in the cytosol [71–76].

In *Saccharomyces cerevisiae*, SRP-dependent (cotranslational) and Hsp70-dependent (posttranslational) pathways are equally important. The cotranslational pathway is predominantly used by precursors with more hydrophobic signal peptides [77]. In *Trypanosoma brucei*, there appears to be a less-stringent selectivity between co- and post-translational pathways compared to yeast. SRP and SR are essential only for cotranslational membrane insertion of polytopic membrane proteins [78]. There is a significant amount of posttranslational protein transport into the trypanosomal ER, and this mechanism can be employed by many different precursor polypeptides in a parallel manner to the SRP-dependent pathway [79]. However, it seems this SRP-independent pathway is the only choice for precursors of GPI-anchored membrane proteins, such as variant surface glycoprotein (VSG) [79]. After analyzing the hydrophobicity of signal peptides of GPI-anchored versus non-GPI-anchored proteins, it was suggested that GPI-anchored membrane proteins are routed to the SRP-independent pathway due to their less hydrophobic signal peptides [8,79]. This is reminiscent of the situation in yeast [77]. This specialization may be related to the abundance and specific role in survival of trypanosomal GPI-anchored membrane proteins within its two hosts (mammals and insects). In mammalian cells, the cotranslational pathway appears to be the predominant one; however, the mammalian ER has the capacity for posttranslational protein transport [80–82]. The unifying feature of the posttranslationally transported precursor polypeptides is that they contain less than 75 amino acid residues, i.e. they are below the minimal size of a nascent polypeptide chain to cotranslationally interact via its signal peptide with SRP on the ribosomal surface. However, posttranslational transport was also observed for an artificial precursor, a hybrid between one of the small precursors and the cytosolic protein, dihydrofolate reductase. Furthermore, the SRP-independent and cotranslational mechanism may be more common than originally expected since RNAi-mediated knock down of SRP subunits hardly affected protein secretion in trypanosomes and some mammalian cell types [83–85].

As mentioned above, protein export from bacteria and archaea is mechanistically related to protein transport into the ER [see Eichler, Tommassen, and Driessen, this issue]. There are several evolutionarily related transport components present in the plasma membrane of bacteria and archaea and the ER membrane, and the signal peptides are very similar in bacteria and eukaryotes (even inter-changeable in some cases). Furthermore, the SRP/SRP-receptor system is mechanistically conserved in bacteria and archaea, although it is dedicated to cotranslational transport of precursors to polytopic membrane proteins in bacteria [86].

3. Transport components

3.1. ER-resident protein translocases in yeast

In yeast cells, targeting or specific membrane association in cotranslational transport involves SRP and its receptor on the ER

Table 1
Proteins and complexes that are involved in protein transport into the endoplasmic reticulum.

Function	*Homo sapiens*	*Trypanosoma brucei*	*Saccharomyces cerevisiae*
Targeting machinery	SRP[a]	SRP	SRP
	7SL RNA	7SL RNA	scR1 RNA
	–	sRNA-76	–
	SRP72	SRP72	Srp72p
	SRP68	SRP68	Srp68p
	SRP54	SRP54	Srp54p
	–	–	Srp21p
	SRP19	SRP19	Sec65p
	SRP14	–	Srp14p
	SRP9	–	Srp7p
	SRP-receptor (SR)	SRP-receptor (SR)	SRP-receptor (SR)
	SRα (docking protein)	SRα	SRα
	SRβ	SRβ	SRβ
Cytosolic or *cis*-acting chaperones	Hsc70		Ssa1p
	Hdj2		Ydj1p
	Hsp40		
Translocons	Sec61-complex	Sec61-complex	Sec61-complex
	Sec61α	Sec61α	Sec61p
	Sec61β	Sec61β	Sbh1p
	Sec61γ	Sec61γ	Sss1p
	Alternative Sec61-complex	–	Alternative Sec61-complex
	Sec61α2		Ssh1p
	Sec61β		Sbh2p
	Sec61γ		Sss1p
Auxiliary components	TRAM protein	?	–
	TRAP-complex (SSR)	?	–
	TRAPα		
	TRAPβ		
	TRAPγ		
	TRAPδ		
	Sec62/63-complex	Sec62/63-complex	Sec62/63-complex
	Sec62 (HTP1, TLOC1)	–	Sec62p
	Sec63 (ERj2)	Sec63	Sec63p (Ptl1p, Npl1p)
	–	Sec71	Sec66p (Sec71p)
	–	–	Sec67p (Sec72p)
	trans-acting chaperone	*trans*-acting chaperone	*trans*-acting chaperone
	BiP (Grp78, HspA5)	BiP	Kar2p (BiP)
	NEFs	NEFs	NEFs
	Grp170 (Orp150, HYOU1)	?	Lhs1p (Ssi1p, Cer1p)
	Sil1 (BAP)	?	Sil1p
	Alternative Sec62/63-complex	–	–
	ERj1 (Htj1, DNAJC1)		
	RAMP4 (SERP1)	?	–
	PAT-10	?	–
Modifying enzymes	Signal peptidase complex (SPC)	Signal peptidase complex (SPC)	Signal peptidase complex (SPC)
	Oligosaccharyl transferase (OST)	Oligosaccharyl transferase (OST)	Oligosaccharyl transferase (OST)
	GPI-transamidase	GPI-transamidase	GPI-transamidase

[a] There is structural information from cryo-EM available for dimeric complexes of ribosomes and SRP [63], trimeric complexes of ribosomes, SRP, and SR [64], for dimeric complexes of ribosomes and the Sec61 complex [155–160], for dimeric complexes of ribosomes and ERj1 [143], and for dimeric complexes for ribosomes and OST [18]. Furthermore, there is structural information from X-ray analysis available for Hsp40- and Hsp70-proteins [120–125], SRP subunits [59–62], archaean SecY [161], for cytosolic Sil1 and Grp170-homologous nucleotide exchange factors [125–128], for the cytosolic domain of Sec63 [111,112], and for the bacterial ortholog of the catalytic subunit of eukaryotic SPC [14].

surface (SRP-receptor or SR) [87–90]. Targeting in post-translational transport of precursor proteins involves a heterotrimeric complex of membrane proteins, comprising Sec62p, Sec71p (alternative name: Sec66p), and Sec72p (also termed Sec67p), that serves as a signal peptide receptor (Table 1) [91–97]. In co- and posttranslational translocation, membrane insertion and completion of translocation occur at the level of the heterotrimeric Sec61 complex (comprising Sec61p, Sbh1p, and Sss1p) and involve additional components, such as the ER-lumenal chaperone, BiP (or Kar2p), its membrane receptor and co-chaperone, Sec63, and its NEFs (Sil1p and Lhs1p) [91–128]. BiP has roles in facilitating posttranslational insertion of precursor polypeptides into the Sec61 complex and as a molecular ratchet in the completion of translocation [115].

There is an additional heterotrimeric Sec61 complex present in the yeast ER that acts alternatively to the above-mentioned Sec61 complex in co- and posttranslational transport (termed here, alternative Sec61 complex; comprising Ssh1p, Sbh2p, and Sss1p) [103,104]. Like the Sec61 complex, the alternative Sec61 complex is also involved in ERAD [40–43,104].

The genes coding for Sec61p, Ss1p, Sec62p, Sec63p, and Kar2p are essential [91,98,106]. Non-lethal mutations in any one of these genes results in temperature-sensitive mutants that at the restrictive temperature accumulate precursor polypeptides in the cytosol and are defective in protein secretion. Furthermore, simultaneous deletion of both NEF genes results in synthetic lethality [120].

3.2. ER-resident protein translocases in trypanosomes

Recent work on the protein transport machinery located in the ER of *T. brucei* identified homologues of many, but not all, of the respective yeast proteins (Table 1) [78,79]. As in yeast, membrane insertion and completion of translocation occur at the level of the heterotrimeric Sec61 complex (comprising Sec61α1, Sec61ß, and Sec61γ). The key observation from a RNAi approach, that included analysis of secretion or membrane integration of different model proteins, was that trypanosomal Sec63 and, by our inference, trypanosomal BiP are involved in co- as well as posttranslational transport [79]. As in yeast, Sec63 was found to be essential. The effect

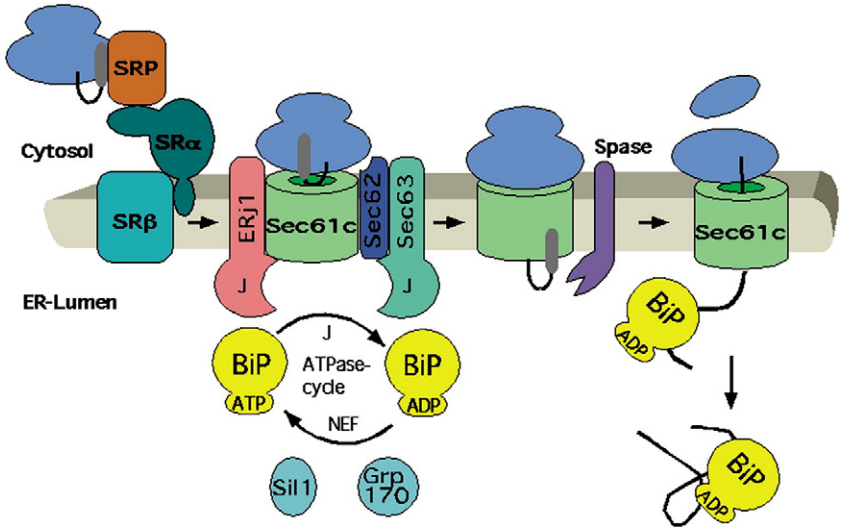

Fig. 1. Hypothetical model for cotranslational and SRP & SR dependent protein transport into the mammalian ER. Note that cleavable signal peptides within nascent precursor proteins insert into the Sec61 complex in a loop like fashion that orients the aminoterminus to the cytosol and the carboxyterminus plus the SPC cleavage site to the ER lumen.

of Sec63 silencing was as dramatic as the simultaneous silencing of components of the SRP-dependent (SRP68) plus the Hsp70-dependent (Sec71) pathways. Thus, this work provided the first in vivo support outside of yeast that these ER resident chaperones play a role in cotranslational transport of precursor polypeptides into the ER. Another important observation was that, of the signal peptide receptor subunits involved in posttranslational transport into the yeast ER [110], only the putative homologue of Sec71p is present in the trypanosomal ER membrane, and this trypanosomal Sec71, in contrast to the situation in yeast, is essential and relevant only for the posttranslational pathway [79]. Therefore, it was found that mainly the GPI-anchored membrane proteins, such as VSG, are affected by silencing of *SEC71*.

3.3. ER-resident protein translocases in mammals

In mammalian cells, targeting or specific membrane association in cotranslational transport involves SRP and its receptor on the ER surface (SRP-receptor or SR) [55–65]. Membrane insertion and completion of translocation occur at the level of the heterotrimeric Sec61 complex (comprising Sec61α1, Sec61ß, and Sec61γ) and involve additional components, most notably the ER-lumenal chaperone, BiP (Table 1) [129–138]. BiP's membrane receptors and co-chaperone(s), Sec63 (and ERj1), and its NEFs (Sil1 and Grp170) may be considered additional subunits of the ER-resident protein translocase in mammalian cells (Fig. 1) [38,39,139–143]. The same may be true for mammalian Sec62 that forms a stable complex with Sec63 [139,140,144]. Based on in vitro experiments, BiP has roles in gating the Sec61 channel [135,136,138], in facilitating insertion of precursor polypeptides into the Sec61 complex [134], and in serving as a molecular ratchet in the completion of translocation [133,137]. Furthermore, there are several additional proteins in the mammalian ER membrane considered as additional subunits of the ER-resident protein translocase in mammalian cells, such as TRAM protein, the TRAP complex (originally termed signal sequence receptor- or SSR-complex), and PAT-10 protein (Table 1) [145–152]. Precursors with long aminoterminal and long hydrophobic core regions showed a low TRAM dependence [146].

There is a second *SEC61*-gene present in mammals, termed *SEC61A2*. Thus, a second Sec61 complex may exist in the mammalian ER, termed here the alternative Sec61 complex (comprising Sec61α2, Sec61ß, and Sec61γ). Currently, however, there is no information available on this complex.

4. Structural information on ER-resident protein translocases

The first structural insights into the composition of ER-resident protein translocases came from analyses of the Sec61 complexes of membrane proteins derived from microsomal detergent extracts, first for yeast microsomes and, subsequently, for canine pancreatic microsomes. In yeast extracts, the Sec61-complex is found as a monomer, dimer, and in complex with either Sec62, Sec63, Sec71, and Sec72 (termed SEC complex) or Sec63, Sec71, and Sec72 (SEC prime complex). In canine pancreatic extracts, the Sec61-complex is found as an oligomer [153] and to some extent in complex with Sec62 and Sec63 [139,140].

More recent information on the composition of the protein transport machinery present in the ER of mammalian cells came from fluorescence resonance energy transfer (FRET) experiments that employed fluorescently labeled antibodies against translocase subunits, permeabilized MDCK-cells, and fluorescence microscopy [154]. Results from this approach supported the notion that heterotrimeric Sec61 complexes are present as oligomers in mammalian ER membranes. Furthermore, these data demonstrated that SR, TRAP, and TRAM are in close proximity with Sec61 complexes in the course and absence of protein translocation. An association of Sec61 complexes with TRAP complexes was also observed in the most recent 3D-reconstructions after cryo-EM (see below).

An additional criterion argues that certain mammalian ER membrane proteins participate in cotranslational protein translocation, i.e. ribosomal association in detergent extracts from mammalian microsomes. Originally, the term ribosome-associated membrane proteins or RAMPs was coined for this class of membrane proteins (after solubilization in the presence of 400 mM potassium chloride) [131]. The Sec61 complex is a RAMP, as is the protein RAMP4 [150,151]. More recently, ERj1 and Sec62 were characterized as RAMPs, although their ribosome association is seen only under more physiological salt concentrations (up to 200 mM potassium chloride) and, therefore, may be more dynamic as compared to the high-salt resistant RAMPs [142–144]. This view is supported by quantitative fluorescence microscopy [144]. When various permeabilized mammalian cells were incubated in the presence or absence of RNaseA and, subsequently, with fluorescently labeled antibodies, RAMPs were identified at the cellular level by differential fluorescence signals −/+ RNase. According to this cell based assay, Sec61α1, Sec61ß, Sec62, and ERj1 are RAMPs, i.e. are associated with ribosomes in the intact ER. Again, this association was observed in the course and absence of protein translocation.

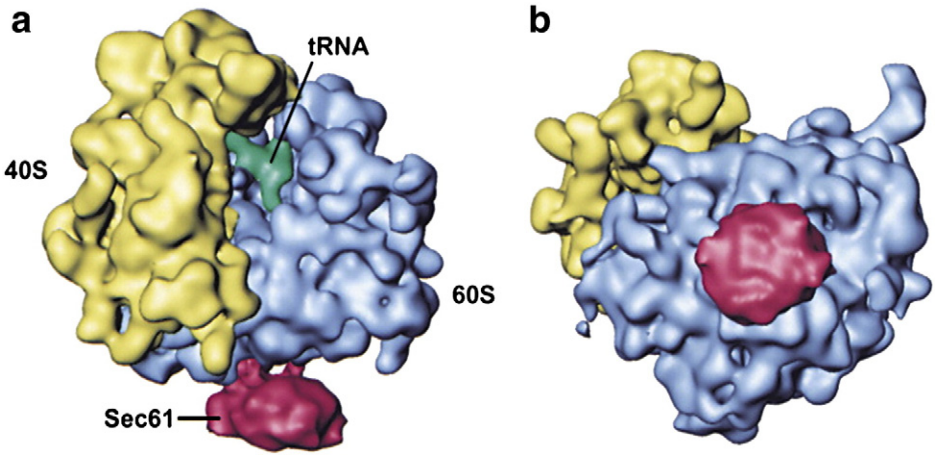

Fig. 2. Structural information for cotranslational transport into the mammalian ER. The 3D reconstructions after cryo-EM of translating ribosomes with the Sec61 complex (Sec61) were taken from reference [143]. Views from the plane of the membrane (a) and the ER lumen (b).

4.1. 3D-reconstructions after cryo-EM

Over the last 14 years, cryo-EM became an increasingly powerful tool for structural analysis of complexes between ribosomes and protein transport components [155–160]. With respect to yeast and mammalian Sec61 complexes, the original 3D-reconstructions gave rise to a model where two to four heterotrimeric Sec61 complexes with an overall diameter of about 10 nm were associated with the tunnel exit of a single ribosome in the presence or absence of a nascent precursor polypeptide chain (resolution: about 25 Angström) (Fig. 2) [155,156]. Current data from a higher resolution, reached with the most advanced microscopes and state of the art algorithms, indicates a single heterodimeric Sec61 complex is associated with a single ribosome and that the originally observed additional mass was due to lipids and detergent molecules (resolution: 9–11 Angström) [159,160]. The most recent reconstructions are consistent with a postulate, based on the X-ray analysis of an inactive archaean SecY complex (see below), suggesting polypeptide translocation occurs in the central pore of a heterotrimeric Sec61 complex. Furthermore, the more recent reconstructions also described complexes between ribosomes, the Sec61 complex, and the TRAP complex [159].

The 3D-reconstructions after cryo-EM also provided insights into the intimate contacts between the ribosomal tunnel exit and the Sec61 complex. At the level of the ribosomes, rpl23a (in yeast rpl25) and rpl35 were identified as the nearest neighbors of the Sec61 complex. At the level of the Sec61 complex, cytosolic loops 6 and 8, formed between transmembrane segments 6 and 7, and 8 and 9, respectively, were characterized as interaction sites with the ribosome. These results were consistent with previous biochemical experiments [65,153]. In addition, these recent structural data cast doubt on the view that the intimate contacts between ribosomes and the Sec61 complex are sufficient to prevent ion efflux from the ER lumen during cotranslational protein translocation. In fact, there is a large gap between the ribosomal surface near the tunnel exit and the cytosolic surface of the Sec61 complex.

4.2. Crystallization and X-ray analysis

In 2004 the first, and so far only, structure of a Sec61 complex family member was solved by crystallization and subsequent X-ray analysis (resolution: 3.2 Angström) [161] for the heterotrimeric SecY complex from the archae *Methanococcus jannaschii* (comprising α-, ß-, and γ-subunits). The structure was characterized as two covalently linked halves of the α-subunit that were clamped together by the γ-subunit and contained an hourglass shaped central pore and an overall diameter of about 4 nm. Due to the similarity between the subunits of the archaen SecY complex and the mammalian Sec61 complex, molecular modeling allowed us to create a homology model for the human heterotrimeric Sec61 complex that is more or less indistinguishable from the SecY structure (Fig. 3). Since the crystallized complex did not contain any precursor polypeptides in transit, it was proposed that (i) the central pore within the α-subunit is the site for protein translocation, (ii) the central pore can laterally open towards the side opposite to the clamp provided by the γ-subunit, to allow lateral exit of signal peptides from the pore [162], and (iii) constriction between the two funnels of the hourglass was plugged by a short helix [161]. Furthermore, it was proposed that the constriction can be widened by a conformational change that results in plug displacement and simultaneous generation of a pore for the polypeptide in transit that is lined at the constriction site by a ring of hydrophobic residues. It was suggested that the potential pore had a maximal internal diameter of up to 0.8 nm, i.e. dimensions that can accommodate an unfolded polypeptide chain or a single α-helix.

4.3. Electrophysiological data

In addition to direct structural methods, the structure of the mammalian Sec61 complex was also addressed by indirect methods, such as electrophysiological approaches (single channel recordings from planar lipid bilayers) [163–166]. This technique was first applied by Simon and Blobel in 1989 [163]. The Sec61 complex had not yet been identified and the canine pancreatic channel that was characterized correlated with protein translocation and was termed the protein-conducting channel (PCC) with an internal diameter of about 1 nm. More recently, this approach was applied to the purified and reconstituted canine pancreatic Sec61 complex. The complex was characterized as an aqueous pore that is transiently opened by signal peptides within precursor polypeptides at early and the final stages of protein translocation and closes either spontaneously or as triggered by BiP [165]. In the open state, two internal pore diameters were observed with the highest frequency for this highly dynamic channel, and corresponded to mean values of 1.2 and 2.2 nm, respectively (full range: 0.6 to 6 nm). Internal pore diameters of 4 to 6 nm had previously been observed in fluorescence quenching experiments, however, without directly linking these channels to the Sec61 complex [167,168]. Furthermore, these fluorescence quenching experiments identified BiP as a lumenal plug of the mammalian Sec61 complex and suggested that an unidentified Hsp40-type co-chaperone of BiP is involved in this reaction [135,136,138].

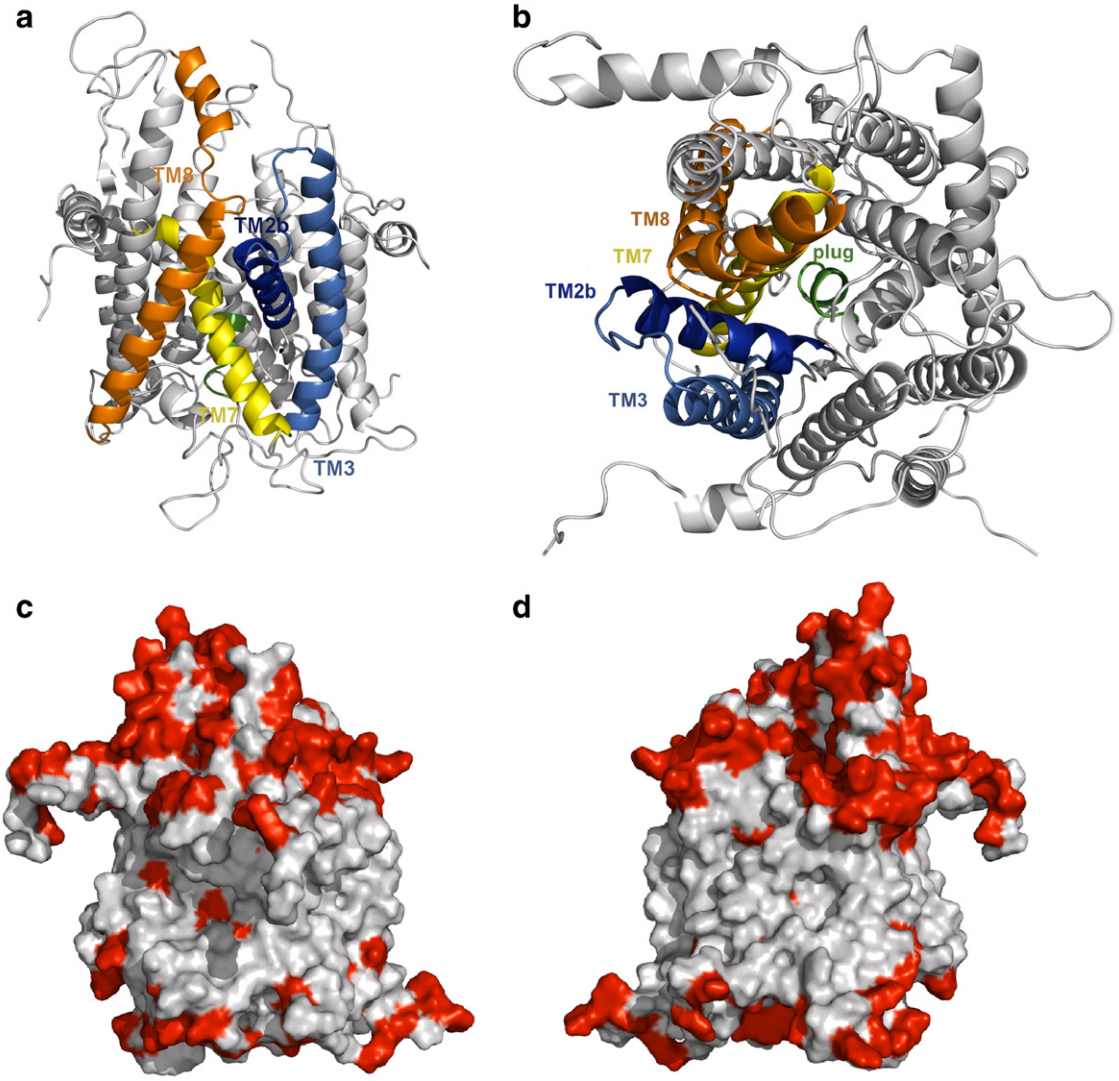

Fig. 3. Homology model for the human heterotrimeric Sec61 complex. (a, b) Views from the plane of the membrane (a, front) and the cytosol (b). Transmembrane helices (TM) forming the front of the lateral gate and the plug, respectively, are indicated in colour. (c, d) Distribution of polar residues (shown in red) on the external surface of the front (c) and the back (d). As no structural information is available for human Sec61, a homology model was generated using the X-ray structure of archaean SecY (PDB code: 1rhz [161]) as a template. The amino acid sequence of human Sec61 and its sequence alignment with bacterial SecY were taken from [161]. Ten homology models were generated by the program MODELLER 9.5 using an all-hydrogen model. Each model was optimized with the variable target function method, followed by a refinement step using molecular dynamics with simulated annealing. The whole optimization procedure was repeated three times and the best model was selected according to the molecular probability density function score. We note that according to this homology model, the constriction site of the human complex is lined by a ring of hydrophobic residues, too.

5. Driving forces

5.1. In cotranslational translocation

Various types of evidence demonstrate a strong interaction between ribosomes and Sec61 complexes. These range from biochemical assays that led to the definition of RAMPs to the 3D-reconstructions after cryo EM of ribosome/Sec61 complexes [131,155,156]. Therefore, it is safe to conclude that cotranslational translocation of polypeptides into the ER is driven by elongation of the nascent polypeptide chain, i.e. the hydrolysis of GTP, at least at certain stages.

However, when translation is terminated there are still about 70 amino acid residues of the nascent polypeptide chain outside of the ER, i.e. buried in the ribosomal tunnel and the polypeptide conducting channel within the heterotrimeric Sec61 complex. Since movement of polypeptides through the Sec61 complex is reversible [169], complete translocation of this carboxyterminal peptide may require help from an ER lumenal polypeptide binding protein (molecular ratchet, see below). In principle, any ER lumenal protein with a binding site for unfolded polypeptides may be able to fulfill this function, such as OST, SPC, GPI transamidase, protein disulfide isomerase (PDI), or peptidyl-cis/trans-isomerase (PPIase). Indeed, nascent polypeptides in transit through the Sec61 complex could be cross-linked to protein disulfide isomerases and peptidyl-cis/trans-isomerase immediately after their release from the translating ribosomes [170,171]. This point was driven home by the observation that the efficiency of cotranslational protein transport into mammalian microsomes can be artificially increased by incorporation of biotin into nascent precursor polypeptides and by trapping of avidin in the reconstituted microsomes (of note: avidin had no effect in the absence of biotinylation) [137]. However, there is no doubt that the ER lumenal chaperone, BiP, is ideally suited to fulfill this function, since it can be recruited to the ribosomal tunnel and the polypeptide conducting channel within the heterotrimeric Sec61 complex and simultaneously be activated on demand. Indeed, BiP had the same effect as avidin on import into reconstituted microsomes in the absence of biotinylation of nascent

polypeptide chains [137]. In this case, the driving force can be seen as the hydrolysis of ATP by the ATPase, BiP. This view is consistent with the Hsp40-type co-chaperone of BiP, Sec63, being involved in cotranslational protein transport of precursor proteins in yeast and trypanosomes [79,107,108]. Alternatively, three-dimensional folding of the aminoterminal peptide of a polypeptide chain in transit may drive translocation of the carboxyterminal peptide. Obviously, the relevance of this alternative driving force increases with the overall mass of the precursor protein. As a rule, small presecretory proteins may be better candidates for relying on BiP for the completion of cotranslational translocation.

5.2. In posttranslational translocation

Posttranslational translocation is very similar to completion of translocation of carboxyterminal peptides in cotranslational translocation, except that the precursor polypeptide has to first be inserted into the Sec61 complex. Posttranslational translocation of precursor polypeptides has most extensively been characterized in yeast. ATP and BiP were required for the insertion of precursor polypeptides into the Sec61 complex as well as for the completion of translocation into the ER [113–116]. Furthermore, these reactions involved the Hsp40-type co-chaperone of BiP, Sec63p, as well as the two NEFs, Sil1p and Lhs1p [109,117,118]. These results are consistent with recent findings for posttranslational protein transport into the trypanosomal ER [79].

Molecular chaperones of the Hsp70 type family reversibly bind to substrate polypeptides via their substrate binding domains (SBD) [172–174]. Hsp70 binds to hydrophobic stretches within essentially unfolded polypeptides, such as those emerging from ribosomal tunnels or aqueous protein conducting channels [175,176]. The binding and release of substrates by Hsp70 are modulated by communication between its SBD and its nucleotide binding-domain (NBD). Furthermore, the NBD is controlled by the ATPase cycle and different Hsp70 interaction partners [120–128]. The ATP-bound state of BiP has a low affinity for substrate polypeptides and the ADP-bound state has a high substrate affinity. Hsp40-type co-chaperones stimulate the ATPase activity, as they favor substrate binding. Nucleotide exchange factors stimulate the exchange of ADP for ATP and, therefore, induce substrate release. Hsp40 proteins are characterized by J-domains that allow interaction with Hsp70 [177–183].

Accordingly, a role for BiP in early and late stages of posttranslational translocation can be envisaged, where immediately after the reversible insertion of a precursor polypeptide into the Sec61 complex, it binds to the aminoterminal end and, thereby, prevents back-sliding into the cytosol (Fig. 1). Subsequently, the precursor becomes stepwise inserted into the Sec61 complex and, thereby, increasingly trapped in the lumen, as was first described in the molecular ratchet model for posttranslational import of precursor proteins into mitochondria. This point was driven home by the observation that the efficiency of posttranslational protein transport into yeast microsomes can be artificially increased by trapping precursor polypeptide-specific antibodies in the reconstituted microsomes [116]. In addition, BiP may be involved in opening of the Sec61 complex, i.e. in triggering the conformational changes in the heterotrimeric Sec61 complex that lead to plug displacement and allowing the insertion of a precursor polypeptide into the complex.

5.3. In special cases

The hepatitis B virus (HBV) L protein represents an interesting example of posttranslational translocation of a large soluble domain across the mammalian ER membrane [184]. Originally, it is integrated into the ER membrane in a typical cotranslational process that is determined by an internal signal peptide, located in the carboxy-terminal region of the aminoterminal domain. Its aminoterminal pre-S domain, with an overall size of 168 amino acid residues, remains cytosolic. In the course of maturation, about half of the L molecules posttranslationally translocate their preS domain into the ER, thereby creating a dual topology that is maintained in the secreted virion envelope and allows the L protein to fulfill a dual function. The interesting feature, in the context of this review, is that translocation of the preS domain is driven by BiP, i.e. the hydrolysis of ATP [185]. Furthermore, this process involves an ER membrane resident Hsp40-type co-chaperone, ERj4, and the NEF, Sil1. Biogenesis of the adenovirus E3-6.7 K protein, the hepatitis C virus (HCV) NS4B protein, and the non-viral aquaporin-1 appears to involve similar posttranslational processes [186–188].

6. Insertion of proteins into the ER membrane

The insertion of membrane proteins into the ER membrane is the first step in the biogenesis of most plasma membrane and organelle membrane proteins of eukaryotic cells (such as membrane proteins of the ER, ER-Golgi intermediate compartment/ERGIC, Golgi, endosome, lysosome, and peroxisome). In general, this process is seen as a variation of protein translocation across the ER membrane [189] that typically does not involve cleavable signal peptides. Exceptions of this are precursors of type I membrane proteins that contain cleavable signal peptides and are inserted into the Sec61 complex in analogy to presecretory proteins and processed by SPC to a mature form with an orientation in the membrane where the aminoterminus faces the ER lumen. Instead, the first potential membrane spanning peptide within the nascent precursor polypeptide chain that emerges at the ribosomal tunnel exit serves as the signal peptide. This non-cleavable signal peptide can insert into the Sec61 complex, like a cleavable signal peptide, in a loop like fashion and lead to an orientation in the membrane where the aminoterminus faces the cytosol (termed type II membrane protein). Alternatively, the non-cleavable signal peptide can insert into the Sec61 complex head-on and lead to an orientation in the membrane where the aminoterminus faces the ER lumen (termed type I membrane protein). The features that determine the particular mode of insertion are described by the “positive inside rule” [190]. Subsequently-emerging membrane spanning peptides in double-spanning and polytopic membrane proteins alternately arrest and re-initiate translocation [191–197]. The term stop-transfer sequence was coined to characterize the arrest function of every second membrane spanning peptide in the latter membrane proteins. In membrane insertion, future membrane spanning peptides leave the Sec61 complex through the lateral gate laterally into the lipid bilayer; this is in contrast to protein translocation.

The features that govern lateral release of a future membrane spanning peptide were recently characterized in detail [198–201]. In particular, extensive studies on the leader peptidase protein have characterized the membrane insertion efficiencies of a third engineered transmembrane segment, the so-called H-segment [199,201], where the 20 naturally occurring amino acids were inserted at different positions with respect to the bilayer center. The observed insertion efficiencies were converted into an apparent free energy scale for insertion, termed the “biological scale.” Some amino acids showed strong position dependence between the hydrophobic core and the polar interface area [201]. A computational model accounting for this position dependence could well reproduce the measured data suggesting that the insertion efficiency of a putative transmembrane segment simply depends on the sum of the insertion free energies of the individual amino acids [202–204]. Interestingly, an alternative statistical model for the membrane insertion free energies derived from the distribution of amino acids in the known three-dimensional structures of helical TM proteins agrees well with the model derived from the H-segment data [202]. This supports the transferability of the “biological scale” to a wide range of helical TM architectures.

Sofar, we have discussed the insertion of transmembrane helices one-by-one. In fact, experimental data suggested that up to four

transmembrane helices may be present in the translocation pore at the same time [205,206]. Obviously, many membrane transporters and channels need to allow passage of polar substrate molecules through a central pore lined by mostly polar residues. If all the pore-lining helices carrying these polar residues had to be released one-by-one from the translocon into the lipid bilayer, this would often be quite unfavorable. In fact, it was shown that the average insertion free energies for TM helices from polytopic membrane proteins are less favorable than for single TM-spanning helices in bitopic membrane proteins [202,203]. Moreover, cooperative effects may even occur between TM helices of different proteins. An example for this is the heterodimer formed by the E1- and E2-glycoproteins of hepatitis C virus. The genes coding for these two proteins are direct neighbors on the HCV genome and are translated as a continuous chain. Upon passage through the translocon, the two protein chains are cleaved and inserted into the membrane. The E1 proteins in the *flaviviridae* family contain a highly conserved positively charged Lys residue in their TM segment and the E2 proteins contain a highly conserved negative Asp residue in the TM part. According to the biological scale, insertion of the individual E1 and E2 helices would be very unfavorable. Mutagenesis results, however, support the formation of an inter-helical salt bridge between the E1 and E2 helices leading to a strong stabilization of the E1–E2 dimer [207,208]. Molecular modelling supported the important role of this ion-pair in the center of the membrane for the cooperative insertion by the translocon and for the stability of this TM dimer [209]. In summary, the data on H-segments show that the general decision whether a part of a protein chain is inserted into the lipid bilayer or translocated is primarily based on simple physico-chemical principles. On a second level, this apparent simplicity is modulated by cooperative effects between sequential helices that is subject of on-going experimental and computational studies. For example, molecular dynamics simulations of protein-loaded membranes showed that cooperative interactions with neighboring membrane proteins may significantly affect the measured values for individual TM segments [210].

Still, it remains unclear if the Sec61 complex alone or together with the lipid bilayer provides the read out. At the lateral release of future membrane spanning peptides, single peptides, pairs, or multimers of peptides may exit the Sec61 complex. Thus, in the two latter cases, the Sec61 complex also contributes to the folding of transmembrane proteins.

7. Medical aspects

7.1. Pharmacology

Recent work in several laboratories identified a number of small molecule inhibitors of co- and posttranslational protein transport in the yeast, trypanosomal, and mammalian ER. The inhibitors fall into two categories, inhibitors of the Sec61 complex and inhibitors of BiP. The first class of molecules to be discovered as Sec61 inhibitors was the group of cyclodexipeptides, such as Cotransin, CAM741, and Apratoxin A [211–213]. The pharmacological potential of cotransin and CAM741 is that these compounds selectively inhibit surface expression of vascular cell adhesion molecule 1 (VCAM1), i.e. may be used in fighting chronic inflammatory conditions. Apratoxin A is a potential anti-cancer drug. More recently, Eeyarestatin I, originally described as an inhibitor of protein export from the ER, was added to this growing list [214]. All four inhibitors seem to directly affect the Sec61 complex with respect to cotranslational transport in vitro as well as at the cellular level. It was suggested that Eeyarestatin I exerts its effect at a stage prior to the site of action of the cyclodexipeptides [214].

There are many different small molecules that affect Hsp70-type molecular chaperones at different stages of their functional cycle. Most of these represent variants of 15-deoxyspergualin and NSC 630668-R/1. Two of these compounds were found to interfere with the Hsp70/Hsp40 interaction and to inhibit posttranslational protein translocation into yeast microsomes (MAL3-39 and MAL3-101) and trypanosomal microsomes (MAL3-101), respectively [215,216].

Furthermore, it was observed that the inhibitor of bacterial protein SecA, CJ-21,058 and its variant equisetin, inhibit posttranslational protein translocation into trypanosomal microsomes [216]. However, in theses cases the drug target is currently unknown. The latter two inhibitors of posttranslational transport of precursor proteins, such as VSG, were also shown to kill bloodstream trypanosomes in vitro and, therefore, were characterized as potential lead compounds for anti-trypanosome drug development.

Findings on the composition of protein translocase in the trypanosomal ER are also of potential pharmacological relevance because of the apparent absence of a Sec71p homologue in human cells [79]. We note, however, that so far, only procyclic- and not bloodstream-trypanosomes have been investigated; therefore, the observed Sec71 dependence of GPI-anchored membrane protein biogenesis may not exist for bloodstream trypanosomes. However, if trypanosomal Sec71 could be specifically targeted by small molecules in a mammalian host, a related anti-trypanosomal drug would become feasible.

7.2. Diseases

Most recently, two diseases were linked to the mammalian Sec61 complex. A point mutation in the *SEC61A1* gene was found to cause diabetes and hepatosteatosis in mice, suggesting some human type 2 diabetes patients may suffer from mutations in the *SEC61A1* gene [217]. In this disease model, the mutation did not directly affect protein translocation but led to ER stress and subsequent ß-cell apoptosis.

The *SEC61γ* gene was among the most frequently amplified genes in the most prevalent type of adult brain tumors, glioblastoma multiforme [218]. Overexpression was observed for 77% of glioblastoma multiforme, but not for lower-grade gliomas. Previously, similar results were obtained for the *SEC62* gene in association with prostate cancer [219], while, the *SEC63* gene was found to be among the most frequently mutated genes in cancers with deficient DNA mismatch repair, such as hereditary nonpolyposis colorectal cancer (HNPCC)-associated small-bowel cancer, and in sporadic gastric and colorectal cancers with frequent microsatellite instability [220–222].

Furthermore, loss of function mutations were linked to polycystic liver disease (*SEC63* gene) and the neurodegenerative disease termed Marinesco–Sjögren syndrome (*SIL1* gene) [223–227]. Both diseases are thought to result from loss of function of both alleles, but apparently have opposite effects on the respective target cells, progressive cerebellar atrophy with ataxia due to Purkinje cell loss in Marinesco–Sjögren syndrome and progressive development of biliary epithelial cysts throughout the liver due to cell proliferation in polycystic liver disease.

Last but not least, Shiga toxigenic *Escherichia coli* strains can cause massive morbidity and mortality. Typically, these pathogens produce AB_5 toxin (also termed subtilase AB) and are responsible for gastrointestinal diseases, such as the life-threatening hemolytic uremic syndrome. The B-subunit pentamer binds to toxin receptors on the cell surface, while the A-subunit has subtilase-like serine protease activity [228]. During an infection, the bacterial cytotoxin enters human cells by endocytosis, followed by retrograde transport to the ER. In the ER, BiP is the major target of the catalytic A-subunit, which inactivates BiP by limited proteolysis. Eventually, all BiP functions are completely lost and the affected cells die.

8. Conclusions

The mechanism of protein translocation into the ER is reasonably well understood in general terms, but is far from being completely explained. It is clear that the Sec61 complex provides the aqueous path for the entry of soluble polypeptides into the ER lumen as well as the lateral entry of membrane proteins into the ER membrane (with

the exception of tail anchored membrane proteins) [189,229] [Borgese, this issue]. Based (i) on the effects of small molecule inhibitors on the mammalian Sec61 complex, (ii) on the presence of different SEC complexes in yeast, (iii) on differential effects of mutations or gene silencing in yeast and trypanosomes, respectively, and iv) on the differential effects of disease-causing mutations in humans, it has to be concluded that different precursor polypeptides pose different demands to the protein transport machinery of the ER in eukaryotic cells. This manifests itself at the atomic level of the Sec61 complex and additional transport components, such as BiP+Sec63, TRAM protein, etc. (Table 1). Therefore, the functions of all these auxiliary components will have to be elucidated to reach the next level of understanding. Yet another level of heterogeneity can undoubtedly be expected when questions related to the regulation of protein translocation into the ER in different cell types of the human body are seriously addressed. This will almost certainly also shed light on related processes, such as protein insertion into the ER membrane and ER protein export (ERAD).

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