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Review

Protein translocation across the ER membrane ☆

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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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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