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Minireview

Protein translocation: common themes from bacteria to man

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Abstract

Protein transport across the endoplasmic reticulum membrane in eukaryotes and across the cytoplasmic membrane in bacteria have turned out to be highly related. The core component of the translocation apparatus is the Sec61/SecYp complex; at least two of its subunits are conserved in evolution. The Sec61/SecYp complex is involved in both co- and post-translational transport pathways. The two modes require probably distinct additional components.

Key words: Protein transport; Endoplasmic reticulum; Secretion; Membrane biosynthesis

1. Introduction

The secretion of proteins from cells is a ubiquitous process. In prokaryotes, secretory proteins are transferred directly across the plasma membrane whereas in eukaryotes, they are first translocated in an analogous process across the endoplasmic reticulum (ER) membrane, but are subsequently transported in vesicles to the plasma membrane. Similar pathways are used by membrane proteins. In all organisms, the translocation of proteins across, and their integration into, the membrane are triggered by hydrophobic signal sequences which are interchangeable; prokaryotic signal sequences can function in eukaryotes and vice versa. Whereas signal sequences of secretory proteins are often cleavable, those of membrane proteins may serve as permanent membrane anchors. Membrane anchoring may also involve additional hydrophobic sequences.

The transport of a protein across the membrane may occur during its synthesis (co-translationally) or after its completion (post-translationally). In both cases, the process is initiated by a targeting phase (for review, see [1]). One mechanism of co-translational targeting involves the signal recognition particle (SRP) which only recognizes signal sequences of nascent chains that are bound to the ribosome. Other targeting pathways involve cytosolic chaperones such as SecB, groEL and hsp 70, which may function either co- or post-translationally and which keep polypeptides in a translocation-competent conformation.

The mechanism of the actual translocation process that follows the targeting phase also seems to differ depending on whether the polypeptide is transported co- or post-translationally. The co-translational mode requires the binding of the translating ribosome to the membrane and it is believed that the elongating nascent chain is transferred directly from the ribosome into the membrane [2]. Thus, the membrane binding of the ribosome may be instrumental for the translocation process. In contrast, since the ribosome does not play a role in the post-translational mode of translocation of proteins, other mechanisms of transport may be postulated. In both cases, however, it is thought that polypeptides are transferred across the membrane through a protein-conducting channel, formed at least in part from transmembrane proteins [2,3]. However, for some proteins the signal sequence may trigger their direct transport across the phospholid bilayer without the involvement of membrane proteins [4].

In this short review, we will concentrate on the actual process of protein translocation which has been elucidated only recently. We will discuss the membrane components involved in co- and post-translational translocation processes and speculate about mechanisms.

2. Co-translational translocation of proteins

Co-translational translocation occurs in all classes of organisms, although its importance may vary. Whereas it seems to be the predominant mode in mammals, in prokaryotes and in *S. cerevisiae* many proteins may be transported post-translationally. Evolutionary conservation of the co-translational pathway is indicated by the ubiquitous occurrence of the SRP-mediated targeting pathway, as well as of essential membrane components [5].

The mechanism of co-translational translocation has been best studied in the mammalian system. The recent

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reconstitution of the translocation apparatus into proteoliposomes, using purified membrane components from dog pancreas microsomes [6], indicates a surprising simplicity of the system. The basis machinery appears to consist of only three components: the SRP receptor, the Sec61p complex and the TRAM protein. These components are sufficient for the translocation of all secretory proteins as well as for the insertion of all membrane proteins thus tested.

The SRP receptor is most likely only required for the targeting process. It consists of two subunits that can both bind GTP [7–9]. The α-subunit makes contact with the ribosome/nascent chain/SRP complex and its GTP-binding site is rquired for the transfer of the nascent chain into the membrane [10]. The role of the β-subunit is still unknown.

The Sec61p complex is probably the core component of the translocation site. It consists of three subunits [6]. The large α-subunit was discovered to be a homolog of Sec61p of *S. cerevisiae* which was found earlier in genetic screens for mutants defective in translocation [11–13]. Sec61α is predicted to span the membrane ten times. The β- and γ-subunits of the mammalian Sec61p complex are small membrane proteins which are anchored by C-terminal hydrophobic tails. The γ-subunit is homologous to the SSS1 protein from *S. cerevisiae* and can functionally replace it in yeast cells [14]. Homologs of Sec61α, β and γ have also been found in a number of other eukaryotes, indicating that a Sec61p complex may be generally involved in protein translocation in these organisms [14].

Sec61α contacts polypeptide chains which are being transferred through the membrane [15–17]. Photocrosslinking experiments have demonstrated that Sec61α is the major neighbor of each of about 40 amino acids which follow the polypeptide segment located in the ribosome (W. Mothes, S. Prehn and T.A. Rapoport, unpublished results). Thus, Sec61α may be the major, if not the only, component of a putative protein-conducting channel that is linked to the ribosome.

In mammals, the Sec61p complex is likely to mediate the binding of the ribosome during co-translational translocation. The Sec61p complex is tightly associated with membrane-bound ribosomes after solubilization of ER membranes [16]. This interaction can be induced by the targeting of a nascent chain to the membrane and the isolated Sec61–ribosome complex is dissociated under conditions identical to those needed for the release of ribosomes from the membranes. Sec61α is shielded by the membrane-bound ribosome and it shows the same remarkable resistance to protease treatment as the ribosome–membrane link (K.-U. Kalies, D. Görlich and T.A. Rapoport, unpublished results). Under physiological salt conditions, the Sec61p complex accounts for the majority of binding sites for ribosomes lacking nascent chains. Finally, since some proteins, like preprolactin, require for their translocation only the presence of the SRP receptor and the Sec61p complex in proteoliposomes [6], it seems likely that the Sec61p complex is sufficient for the binding of the translating ribosome. All these data suggest that the Sec61p complex is the long-sought after receptor for ribosomes and make it unlikely that other proposed proteins, such as p34 [18] or p180 [19], play an essential role in the binding of ribosomes during translocation.

The ribosome seems to make a tight seal with the membrane, probably by forming numerous contacts with the cytosolic loops of Sec61α. The membrane-inserted nascent chain is not accessible to proteases or iodide ions added to the cytosolic compartment [20,21]. The tight association of the ribosome and the Sec61p complex suggests that the nascent chain is transferred directly from the channel in the ribosome into the protein-conducting channel in the membrane. Thus, the latter may simply be an extension of the ribosomal channel, and the nascent chain may pass through the membrane in a vectorial manner because there is only one exit from the extended channel. According to such a model, a pumping, pulling or pushing machinery would not be required. However, it is possible that the nascent chain emerging in the ER lumen is bound by chaperone factors which facilitate transport [22].

The function of the third component of the mammalian translocation apparatus, the TRAM protein, is not fully understood. The TRAM protein is a multi-spanning membrane protein that contacts a nascent chain during early phases of its transfer through the membrane [23]. In the case of preprolactin, it has been shown to interact predominantly with the hydrophilic part of the signal sequence preceding its hydrophobic core [24]. The presence of the TRAM protein is of variable importance for the translocation of different proteins into proteoliposomes [6]. It is absolutely required for some proteins, like prepro-α-factor, and only stimulatory for others, like preprolactin. The difference seems to depend on the structure of the signal sequence, although the precise features that determine the TRAM dependence have not yet been identified.

One may speculate that the TRAM protein is involved in the membrane insertion of a nascent chain. A loop insertion, with one part of the hairpin being the hydrophobic core of the signal sequence, is believed to be the first step in protein translocation. For secretory proteins and some membrane proteins, the N-terminal end of the loop stays in the cytosol and the C-terminal part moves through the membrane. For other membrane proteins, the N-terminal part of the loop is transferred across the membrane yielding the inverse orientation. TRAM could be involved in determining the orientation by interacting with one of the polypeptide regions flanking the hydrophobic core of a signal sequence. It could also function during the insertion of polypeptide loops into the membrane in the case of multi-spanning proteins.

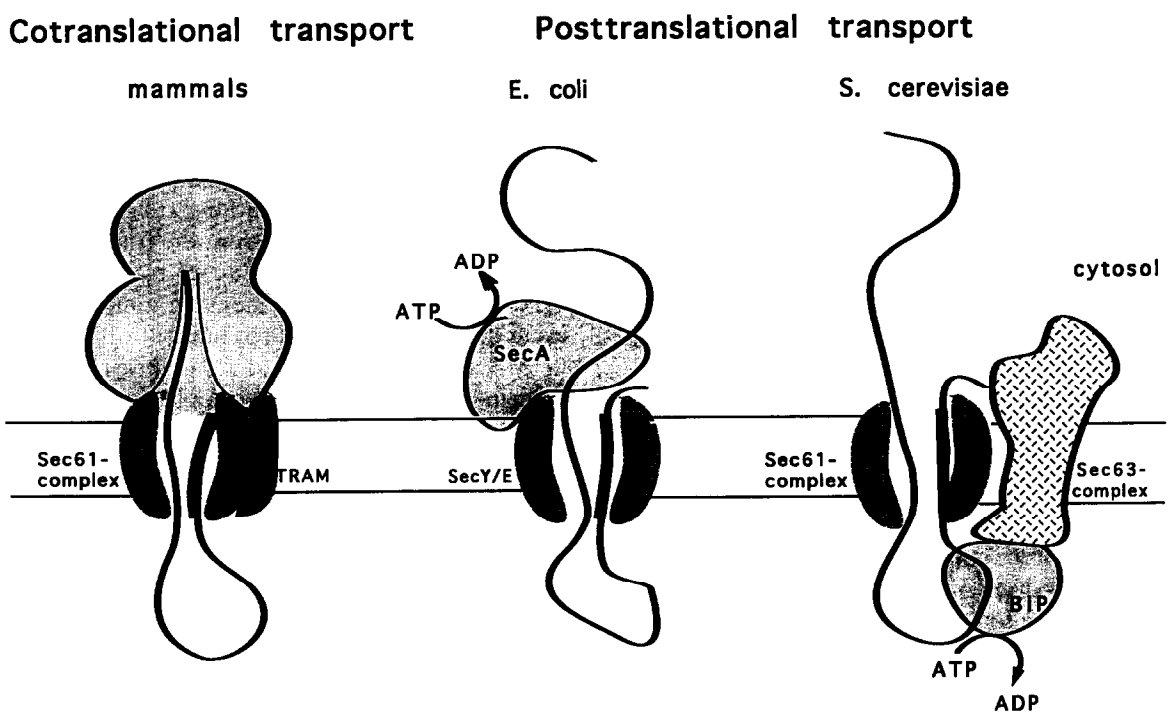

Fig. 1. A simplified scheme for co- and post-translational translocation pathways. Both modes of translocation in all classes of organisms require the Sec61/SecYp complex which presumably forms a protein-conducting channel. This core component interacts either with the ribosome during co-translational transport in mammals (and probably other organisms) or with other components during post-translational translocation in bacteria and yeast. In bacteria, the additional component, SecAp, is believed to push the polypeptide across the membrane in an ATP-dependent reaction. In yeast, the chaperone, BiP, in association with the Sec63p complex may consume ATP to pull the polypeptide across the membrane. The thick portion of the polypeptide chain indicates the hydrophobic core of the signal sequence.

In addition to the Sec61p complex and the TRAM protein, the mammalian translocation site contains other components. These include the signal peptidase and oligosaccharyltransferase, the two known enzymes involved in the modification of nascent polypeptide chains, as well as proteins of unknown function, such as the 'translocon-associated protein' (TRAP) complex (previously called SSR) (for review, see [1]).

3. Post-translational translocation of proteins

The post-translational mode of transport is best known for *E. coli*. Reconstitution experiments have shown that the machinery required for the translocation of proteins across the cytoplasmic membrane is related to the mammalian system mediating co-translational translocation [25,26]. Only two membrane components are essential: the peripheral membrane protein, SecAp, and the integral SecYp complex.

SecAp is an ATPase (translocation ATPase) that accepts the polypeptide chain from the cytosolic chaperone, SecBp [27]. It is thought to push the polypeptide chain into the membrane by interacting with polypeptide segments and with the SecYp complex. Multiple rounds of ATP-driven binding and release steps may move the polypeptide chain across the membrane [28]. After the initial SecAp-dependent membrane insertion of a polypeptide, an electrochemical potential can substitute, at least in part, for the SecAp ATPase.

The SecYp complex consists of SecYp, SecEp and the BandI protein [29]. It is structurally related to the Sec61p complex of eukaryotes. SecYp is homologous to the mammalian Sec61α and yeast Sec61p [16], and is also adjacent to polypeptide chains crossing the membrane [30]. SecEp is predicted to span the membrane three times but only the last anchor is essential for its function [31], exactly the region that is similar in structure to Sec61γ and SSS1p [14]. Band I seems to be identical with p12 (W. Wickner, personal communication), a protein that stimulates translocation in vitro but is not essential [32]; its structure is not related to that of Sec61β.

In addition to these components, genetic screens have identified SecDp and SecFp, two multi-spanning membrane proteins which are not essential for viability of *E. coli* cells [33]. These proteins may be involved in the maintainance of an electrochemical potential across the cytoplasmic membrane [34] or in the release of polypeptide chains from the translocation site into the periplasm [35].

Most likely, the translocation machinery in Gram-positive bacteria and in certain chloroplasts is very similar to that of *E. coli*, as indicated by the existence of homologs to SecAp, SecYp and SecEp [14,16,36].

Post-translational translocation in *S. cerevisiae* may require a core component, the Sec61p complex, similar to the SecYp complex in *E. coli*. Both Sec61p and SSS1p are essential for the translocation of all proteins tested [11,37]. In addition to the core component, however, the post-translational translocation of prepro-α-factor in vitro also depends on the Sec63p complex [38] that is located in proximity to the Sec61p complex [39]. The Sec63p complex consists of Sec63p, Sec71p, Sec72p, Kar2p, and possibly Sec62p [38]. the Sec63, Sec71 and Sec62 proteins span the membrane 3, 1 and 2 times, respectively, Sec72p is a peripheral membrane protein, and Kar2p (BIP) is a lumenal chaperone [40–43]. Sec62p contacts polypeptides early during their translocation [15]. Kar2p is thought to bind the polypeptide chain as it emerges in the ER lumen and to pull it across the membrane.

Some mutations in the components of the Sec63p complex perturb translocation in vivo, even of proteins which can be transported only in a co-translational manner in vitro. Whether the Sec63p complex is involved in both modes of translocation is not yet clear. It is conceivable that it only functions in post-translational transport and that its effects in vivo on the co-translational mode are caused by the sequestration of the Sec61p complex required for both pathways.

In conclusion, the Sec61/SecY complex seems to be the core component in all known translocation pathways (see Fig. 1). For the co-translational mode, it simply binds to the ribosome and no further component is needed for the directional transport of a protein. For the post-translational mode, however, other different proteins seem to associate with the Sec61/SecY core. One role of these additional components must be to provide a driving force for vectorial translocation, either by pulling the polypeptide chain across the membrane, as proposed for Kar2p in the yeast system, or by pushing it across, as suggested for SecAp in *E. coli*. The additional components may also function in place of the ribosome to prevent leakage of small molecules through the protein-conducting channel, and in place of the SRP in signal sequence recognition.

4. Perspectives

By now, most components of the translocation apparatus appear to have been identified. The basic machinery has turned out to be highly conserved in evolution and the existence of a protein-conducting channel is now likely, although its precise composition remains to be clarified. Despite the progress, the mechanism of translocation remains a mystery.

Among the most urgent questions are those concerning the initiation of the translocation process: how is a polypeptide chain inserted into the translocation site? Does the signal sequence first interact with lipid before the chain is threaded into the channel? Which components determine the orientation of a membrane-inserted signal sequence? How is the protein-conducting channel opened? Does this involve the association or dissociation of membrane proteins? Equally important is the problem of termination of translocation: are factors needed to release polypeptide chains from the translocation site to allow its recycling for subsequent rounds of transport? When do hydrophobic segments of a membrane protein leave the translocation site to interact with the phospholipid? Does this occur as soon as they emerge into the protein-conducting channel or only after termination of translocation? Is there a function for the small subunits of the Sec61/SecYp complex in gating the channel?

The establishment of reconstitution systems for *E. coli* and mammals has paved the way to answer these questions. Hopefully, similar reconstitution systems with purified components will soon be available for the yeast system as well, which would permit a comparative study of co- and post-translational translocation pathways by the powerful combination of genetic and biochemical approaches.

References

[1] Rapoport, T.A. (1992) Science 258, 931–936.
[2] Blobel, G. and Dobberstein, B. (1975) J. Cell Biol. 67, 835–851.
[3] Simon, S.M. and Blobel, G. (1991) Cell 65, 371–380.
[4] Wickner, W.T. and Lodish, H.F. (1985) Science 230, 400–407.
[5] Dobberstein, B. (1994) Nature 367, 599–600.
[6] Görlich, D. and Rapoport, T.A. (1993) Cell 75, 615–630.
[7] Tajima, S., Lauffer, L., Rath, V.L. and Walter, P. (1986) J. Cell Biol. 103, 1167–1178.
[8] Connolly, T. and Gilmore, R. (1989) Cell 57, 599–610.
[9] Miller, J.D., Wilhelm, H., Gierasch, L., Gilmore, R. and Walter, P. (1993) Nature 336, 351–354.
[10] Rapiejko, P.J. and Gilmore, R. (1992) J. Cell Biol. 117, 493–503.
[11] Deshaies, R.J. and Schekman, R. (1987) J. Cell Biol. 105, 633–645.
[12] Rothblatt, J.A., Deshaies, R.J., Sanders, S.L., Daum, G. and Schekman, R. (1989) J. Cell Biol. 109, 2641–2652.
[13] Stirling, C.J., Rothblatt, J., Hosobuchi, M., Deshaies, R. and Schekman, R. (1992) Mol. Biol. Cell 3, 129–142.
[14] Hartmann, E., Sommer, T., Prehn, S., Görlich, D., Jentsch, S. and Rapoport, T.A. (1994) Nature 367, 654–657.
[15] Müsch, A., Wiedmann, M. and Rapoport, T.A. (1992) Cell 69, 343–352.
[16] Görlich, D., Prehn, S., Hartmann, E., Kalies, K.U. and Rapoport, T.A. (1992) Cell 71, 489–503.
[17] Sanders, S.L., Whitfield, K.M., Vogel, J.P., Rose, M.D. and Schekman, R.W. (1992) Cell 69, 353–365.
[18] Tazawa, S., Unuma, M., Tondokoro, N., Asano, Y., Ohsumi, T., Ichimura, T. and Sugano, H. (1991) J. Biochem. (Tokyo) 109, 89–98.
[19] Savitz, A.J. and Meyer, D.I. (1990) Nature 346, 540–544.
[20] Connolly, T., Collins, P. and Gilmore, R. (1989) J. Cell Biol. 108, 299–307.
[21] Crowley, K.S., Reinhart, G.D. and Johnson, A.E. (1993) Cell 73, 1101–1115.
[22] Simon, S.M., Peskin, C.S. and Oster, G.F. (1992) Proc. Natl. Acad. Sci. USA 89, 3770–3774.

[23] Görlich, D., Hartmann, E., Prehn, S. and Rapoport, T.A. (1992) Nature 357, 47–52.
[24] High, S., Martoglio, B., Görlich, D., Andersen, S.L.S., Ashford, A.J., Giner, A., Hartmann, E., Prehn, S., Rapoport, T.A., Dobberstein, B. and Brunner, J. (1993) J. Biol. Chem. 268, 26745–26751.
[25] Brundage, L., Hendrick, J.P., Schiebel, E., Driessen, A.J.M. and Wickner, W. (1990) Cell 62, 649–657.
[26] Akimaru, J., Matsuyama, S.I., Tokuda, H. and Mizushima, S. (1991) Proc. Natl. Acad. Sci. USA 88, 6545–6549.
[27] Hartl, F.-U., Lecker, S., Schiebel, E., Hendrick, J.P. and Wickner, W. (1990) Cell 63, 269–279.
[28] Schiebel, E., Driessen, A.J.M., Hartl, F.-U. and Wickner, W. (1991) Cell 64, 927–939.
[29] Brundage, L., Fimmel, C.J., Mizushima, S. and Wickner, W. (1992) J. Biol Chem. 267, 4166–4170.
[30] Joly, J.C. and Wickner, W. (1993) EMBO J. 12, 255–263.
[31] Schatz, P.J., Bieker, K.L., Ottemann, K.M., Silhavy, T.J. and Beckwith, J. (1991) EMBO J. 10, 1749–1757.
[32] Nishiyama, K., Mizushima, S. and Tokuda, H. (1993) EMBO J. 12, 3409–3415.
[33] Pogliano, J.A. and Beckwith, J. (1994) EMBO J. 13, 554–561.
[34] Arkowitz, R.A. and Wickner, W. (1994) EMBO J. 13, 954–963.
[35] Matsuyama, S., Fujita, Y. and Mizushima, S. (1993) EMBO J. 12, 265–270.
[36] Valentin, K. (1993) Mol. Gen. Genet. 236, 245–250.
[37] Esnault, Y., Blondel, M.O., Deshaies, R.J., Schekman, R. and Kepes, F. (1993) EMBO J. 12, 4083–4094.
[38] Brodsky, J.L. and Schekman, R. (1993) J. Cell Biol. 123, 1355–1363.
[39] Deshaies, R.J., Sanders, S.L., Feldheim, D.A. and Schekman, R. (1991) Nature 349, 806–808.
[40] Deshaies, R.J. and Schekman, R. (1990) Mol. Cell Biol. 10, 6024–6035.
[41] Feldheim, D., Rothblatt, J. and Schekman, R. (1992) Mol. Cell Biol. 12, 3288–3296.
[42] Green, N., Fang, H. and Walter, P. (1992) J. Cell Biol. 116, 597–604.
[43] Vogel, J.P., Misra, L.M. and Rose, M.D. (1990) J. Cell Biol. 110, 1885–1895.