The EMBO Journal vol.14 no.22 pp.5485–5493, 1995

A complex of the signal sequence binding protein and the SRP RNA promotes translocation of nascent proteins

Stefanie Hauser, Gerald Bacher, Bernhard Dobberstein and Henrich Lütcke[1]

ZMBH, Zentrum für Molekulare Biologie der Universität Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

[1]Corresponding author

Translocation of proteins across the endoplasmic reticulum membrane is initiated by the signal recognition particle (SRP), a cytoplasmic ribonucleoprotein complex consisting of a 7S RNA and six polypeptides. To investigate the functions of the SRP components, we have tested the activities of several SRP subparticles. We show that the SRP GTPase (SRP54) alone binds a signal sequence and discriminates it from a non-signal sequence. Although SRP54 alone is unable to promote translocation, SRP54 in a complex with SRP RNA is both necessary and sufficient to promote translocation of an elongation-arrested nascent protein in a GTP-regulated manner. For co-translational translocation, additional SRP components are required. We discuss the implications of our results for the function of the *Escherichia coli* SRP which is homologous to the SRP54/SRP–RNA complex.

Keywords: endoplasmic reticulum/protein translocation/signal recognition particle (SRP)/signal sequence recognition/4.5S RNA

Introduction

Signal recognition particle (SRP) is a cytosolic ribonucleoprotein complex which promotes the membrane translocation of secretory and membrane proteins. The mammalian SRP is thought to fulfil its task by virtue of three activities: (i) it first binds a nascent polypeptide's hydrophobic signal sequence that has just emerged from the ribosome; (ii) it subsequently retards or arrests further elongation until (iii) it releases the signal sequence at the rough endoplasmic reticulum (RER) through a GTP-dependent interaction with the SRP receptor (SR or docking protein) there. The released signal sequence inserts into the RER membrane and thus the co-translational translocation of the nascent polypeptide is initiated (see Walter and Johnson, 1994; Lütcke, 1995 for recent reviews).

The mammalian SRP is composed of a 7S RNA and six polypeptides which are named according to their apparent molecular masses (in kDa) and are attached to the RNA either as heterodimers (SRP9/14 and SRP68/72) or as monomers (SRP19 and SRP54) (Walter and Blobel, 1980, 1982). SRP can be disassembled into these components and reassembled from them to constitute a functional particle (Walter and Blobel, 1983a). Thus it was possible to assemble partial or modified SRP-derived particles in order to test their functions and thereby identify SRP components in the various activities of SRP (Siegel and Walter, 1988; Bernstein *et al.*, 1993; Zopf *et al.*, 1993).

The binding of signal sequences was suggested to be performed by SRP54 as this was the only SRP component photo-cross-linked to the signal sequence of a nascent polypeptide (Krieg *et al.*, 1986; Kurzchalia *et al.*, 1986). The findings that the free SRP54 alone could similarly be cross-linked to signal sequences and compete with SRP in such an assay demonstrated that SRP54 alone is sufficient for signal sequence binding (Lütcke *et al.*, 1992; Zopf *et al.*, 1993). Recently it has been proposed that SRP is assisted in the discrimination of signal sequences by a cytosolic protein termed nascent polypeptide-associated complex (NAC, Wiedmann *et al.*, 1994). However, the mechanism by which signal sequences are discriminated is still unclear.

The retardation of elongation and efficient co-translational translocation require signal sequence binding and the entire SRP (Siegel and Walter, 1988). A SRP subparticle lacking the Alu domain [termed SRP(S)] only inefficiently promoted translocation *in vitro* (Siegel and Walter, 1986).

When the ribosome–SRP complex interacts with the SRP receptor (SR) at the RER membrane the signal sequence is released from SRP, the elongation resumes and translocation is initiated (Wiedmann *et al.*, 1987; Connolly and Gilmore, 1989). SR consists of an α-subunit and a β-subunit, both of which are GTPases; SRP54 is also a GTPase. To release the signal sequence, SRP54 of SRP probably interacts with the SRα-subunit (Bernstein *et al.*, 1993; Miller *et al.*, 1993, 1994; Zopf *et al.*, 1993). Evidence exists that both SRα and SRP54 must bind guanine nucleotide to allow this interaction (Rapiejko and Gilmore, 1992; G.Bacher, H.Lütcke, B.Jungnickel, T.A.Rapoport and B.Dobberstein, unpublished observations). 7S RNA and SRP68/72 have also been implicated in the interaction with the SR (Siegel and Walter, 1988; Miller *et al.*, 1993). However, the role these components play in the release of a signal sequence still needs to be elucidated.

Bacteria also have SRP-like particles and homologues of the SRα subunit (reviewed by Lütcke, 1995). As judged from their RNAs, bacterial SRPs are smaller than the mammalian SRP. The *Escherichia coli* SRP comprises a 4.5S RNA and a homologue of SRP54 (P48 or Ffh, for Fifty-four-homologue) (Bernstein *et al.*, 1989; Römisch *et al.*, 1989; Poritz *et al.*, 1990; Ribes *et al.*, 1990). It functions in protein translocation *in vivo* (Phillips and Silhavy, 1992), as does the SRα-homologue, FtsY (Luirink *et al.*, 1994). By virtue of P48, the *E.coli* SRP interacts with various signal sequences (Luirink *et al.*, 1992; J.Luirink, personal communication). Furthermore, a particle which consists of a 4.5S RNA and P48 can form a complex with a fusion protein comprising a large portion of FtsY (Miller

et al., 1994). It is likely that this particle is identical to the *E.coli* SRP. However, the mechanism by which such a small SRP may function in protein translocation is still unclear.

We are interested in elucidating the functions of individual components of the mammalian SRP. In this study, we have addressed the question of which SRP components enable SRP to promote the membrane translocation of nascent proteins. To approach this, we have functionally tested several different SRP subparticles for the ability (i) to bind specifically a hydrophobic signal sequence, and (ii) to promote translocation. We show that SRP54 attached to SRP RNA fulfils the principal requirements to promote membrane translocation of an elongation-arrested nascent polypeptide. Additional components are necessary to allow translocation to occur co-translationally.

Results

Preparation and characterization of SRP subparticles

To study the functions of SRP components, we prepared SRP subparticles by cleaving the canine SRP with RNase or by assembling isolated SRP proteins with *in vitro*-synthesized SRP RNAs. The mammalian SRP was isolated from dog pancreas (Walter and Blobel, 1983c). SRP was cleaved with micrococcal nuclease (Gundelfinger *et al.*, 1983; Siegel and Walter, 1986) into the Alu domain and SRP(S) which comprises SRP19, SRP54 and SRP68/72 attached to the central large S fragment of the 7S RNA (see Figure 1A, centre and left). The RNA of the SRP(S) used in this study is shown in Figure 1B. No intact 7S RNA can be seen, indicating that cleavage was complete (Figure 1B, compare lane 4 with lanes 3 and 2). All other subparticles were assembled from components expressed from the respective cDNAs. These components were the mammalian SRP54, SRP19 and 7S RNA and the *E.coli* P48 and 4.5S RNA.

The canine SRP54 (Römisch *et al.*, 1989) was synthesized in baculovirus-infected insect cells (Summers and Smith, 1987), enriched from the cell lysate and separated from residual insect cell SRP by centrifugation through a 5–20% sucrose gradient. The SRP54 fraction was either used directly (Figure 1C and D, lanes 2) or after assembly into SRP subparticles. The human SRP19 (Lingelbach *et al.*, 1988) was expressed in *E.coli* (S.Oertle and K.Strub, unpublished results) and partially purified (Figure 1C, lane 1). Further purification was achieved by assembling SRP19 with the 7S RNA and SRP54 and subsequent sucrose gradient sedimentation of the resulting RNP complex (Figure 1C, lane 4). The 7S RNA was synthesized by *in vitro* transcription of the human cDNA (Strub *et al.*, 1991) (Figure 1B, lane 1, compare with lane 2).

The *E.coli* 4.5S RNA was synthesized *in vitro* using a cDNA (Wood *et al.*, 1992). The *E.coli* P48 containing six histidine residues added to its C-terminus was synthesized in *E.coli* and purified as described previously (Lentzen *et al.*, 1994) (Figure 1C, lane 5).

SRP subparticles were assembled from SRP54, 7S RNA and SRP19 (Figure 1C and D, lanes 4), from SRP54 and 4.5S RNA (Figure 1C and D, lanes 3) and P48 and 4.5S RNA (Figure 1C, lane 5) and designated, according to their composition, 54/7S/19, 54/4.5S and 48/4.5S,

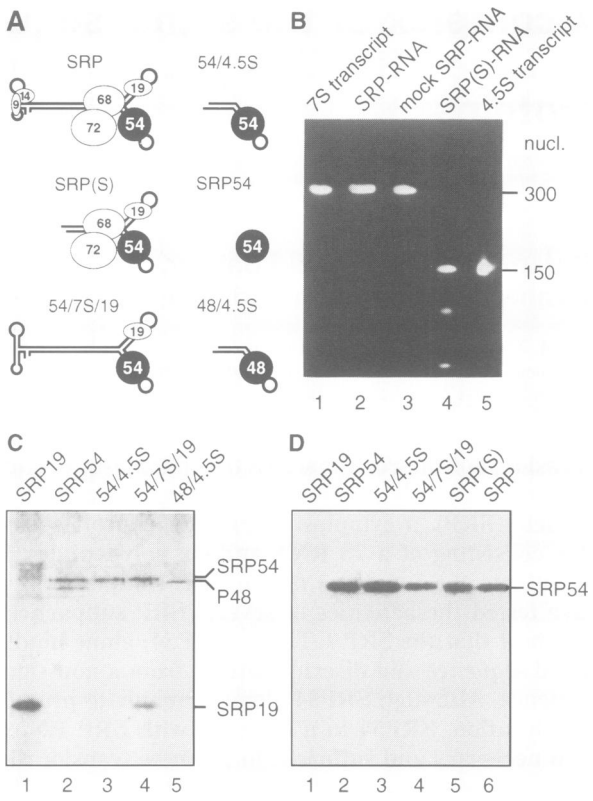

Fig. 1. Characterization of SRP and SRP subparticles. (**A**) Cartoons of canine SRP and the SRP subunits, SRP(S), 54/7S/19, 54/4.5S and 48/4.5S. (**B**) Denaturing RNA gel showing ethidium bromide-stained 7S RNA (lane 1) and 4.5S RNA (lane 5) transcripts in comparison with 7S RNA that had been extracted from canine SRP (lane 2), from mock-digested SRP (lane 3) or micrococcal nuclease-digested SRP (lane 4). (**C**) SDS–polyacrylamide gel showing the Coomassie-stained partly purified SRP54 (lane 2) and SRP19 (lane 1) used to assemble the subparticles. The subparticles 54/4.5S (lane 3), 54/7S/19 (lane 4), and 48/4.5S (lane 5) are shown after purification over 5–20% sucrose gradients. (**D**) Immunoblot of a gel similar to that in (C) showing comparable amounts of free SRP54 (lane 2) and of SRP54 contained in SRP (lane 6) and the various subparticles (lanes 3–5).

respectively (see Figure 1A). The subparticles were separated from unassembled protein by sucrose gradient centrifugation. The SRP subparticles and SRP54 are collectively referred to throughout this paper as the 'SRP subunits'.

Signal sequence discrimination by SRP and the SRP subunits

SRP is known to bind to nascent polypeptides. To characterize this binding and test whether the SRP subunits can discriminate between signal and non-signal sequences, we used photo-cross-linking. Stable ribosome-associated nascent chains (RNCs) comprising the N-terminal 86 amino acid residues of wild-type PPL (PPL86) were synthesized from a truncated mRNA in a wheat germ lysate (Gilmore *et al.*, 1991). Included during the synthesis were [^{35}S]methionine and lysyl-tRNA modified with the photoactivatable cross-linker 4-(3-trifluoromethyldiazarino)benzoic acid (N^{ε}-TDBA-Lys-tRNA) to allow the labelled nascent polypeptide to be cross-linked to interacting proteins upon irradiation with UV light (Wiedmann *et al.*, 1987). After synthesis, the RNCs were purified by centrifugation through a high-salt/sucrose cushion (High *et al.*, 1991a). Subsequently they were incubated with

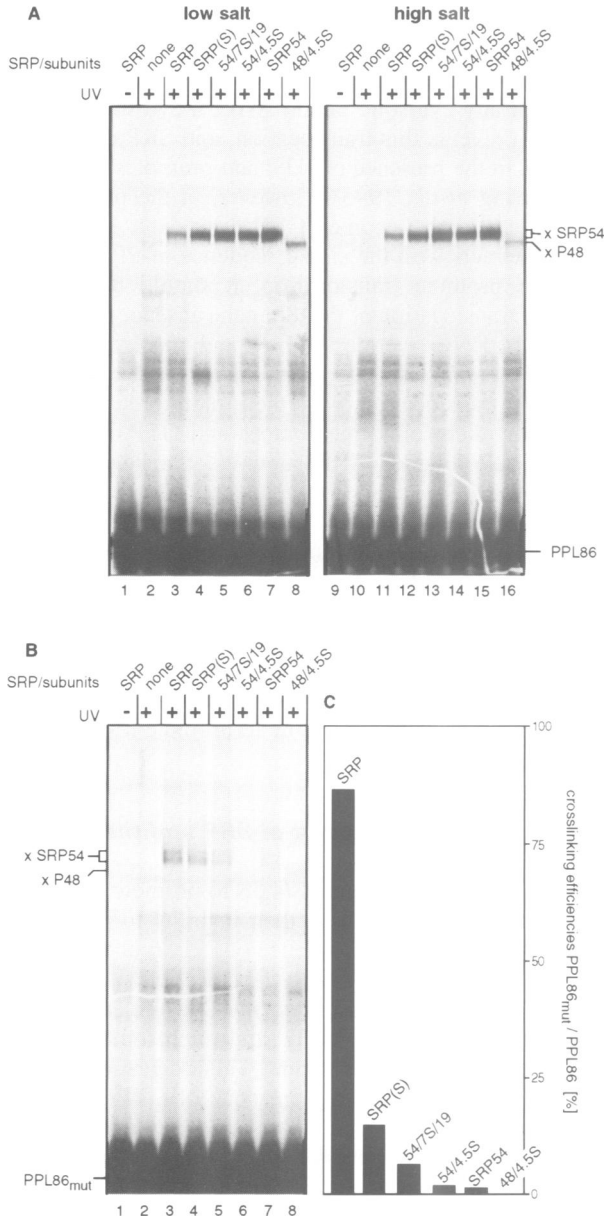

Fig. 2. Binding of SRP subunits to PPL86 nascent chains. Stable ribosome-associated nascent polypeptides comprising the N-terminal 86 amino acids of preprolactin (PPL86) were synthesized in a wheat germ lysate and purified. [^{35}S]methionine and N^{ε}-TDBA-Lys-tRNA were included to label PPL86 and to allow the cross-linking of its signal sequence which was either authentic (**A**) or mutated to be non-functional (**B**). SRP or the SRP subunits (as indicated) were allowed to bind to the RNCs and cross-linking was induced by UV-irradiation (+UV) directly (–high salt, lanes 1–8 in A and B) or after spinning the ribosomes through a high-salt-containing sucrose cushion (+ high salt, lanes 9–16). Samples were analysed by SDS–PAGE followed by phosphoimaging. (**C**) Cross-linked and free PPL86 and $PPL86_{mut}$ were quantified and the cross-linking efficiencies determined and compared (lanes 1–8 in B versus A) as described in Materials and methods.

SRP or SRP subunits and UV irradiated. Samples were analysed by SDS–PAGE and visualized by phosphoimaging. Photoadducts with PPL86 are shown in Figure 2A (lanes 3–8). Using specific antibodies they were shown to contain SRP54 and P48, respectively (data not shown); this confirms previous observations (Krieg *et al.*, 1986; Kurzchalia *et al.*, 1986; Luirink *et al.*, 1992).

As SRP is known to interact with signal sequences of nascent polypeptides in a high salt-resistant manner (Walter and Blobel, 1983d; High *et al.*, 1991a), we next asked whether the interaction between the RNCs and the SRP subunits likewise resisted high-salt extraction. The RNCs were incubated with the SRP subunits, centrifuged through a high-salt/sucrose cushion, and subsequently UV irradiated. As can be seen in Figure 2A, the SRP54-containing SRP subunits were cross-linked to the signal sequence of PPL86 with similar efficiencies after the centrifugation as before (Figure 2A, lanes 11–15, compare lanes 3–7). The cross-linking of P48 to PPL86 was less efficient before, and reduced by ~70% after, the high-salt centrifugation (Figure 2, compare lanes 8 and 16), indicating that the 48/4.5S particle had interacted differently with the RNC.

We next tested if the SRP subunits could discriminate between PPL and PPL with a mutant signal sequence. The mutant signal sequence contains in the hydrophobic core a proline and two charged amino acid residues and does not function in translocation (Luirink *et al.*, 1992). In the case of SRP, PPL86 and the mutant PPL86 were cross-linked to SRP54 with similar efficiencies (Figure 2B, lane 3; Figure 2C). However, the SRP54 of the SRP subunits was cross-linked much less efficiently to the mutant than to the authentic signal sequence (Figure 2B, lanes 4–8; Figure 2C). This suggests that components present in SRP and lacking in the SRP subunits increase the cross-linking efficiency between the mutant PPL86 and SRP54 (see Discussion).

Promotion of translocation by SRP and SRP subparticles

Protein translocation can be tested during ongoing translation (co-translationally) or after the elongation of the nascent polypeptide has been arrested. In the first case, a nascent polypeptide grows continuously, starts folding, and its signal sequence becomes decreasingly accessible to SRP. Finally, the polypeptide becomes incompetent for translocation (Siegel and Walter, 1986; Wiedmann *et al.*, 1987). Therefore, the time when SRP interacts with the signal sequence and subsequently with the membrane is critical in a co-translational assay (Rapoport *et al.*, 1987). In contrast, no such time limit exists when elongation cannot occur, e.g. because elongation-arrested RNCs are used in the translocation assay (Gilmore *et al.*, 1991).

To test co-translational translocation, full-length preprolactin (PPL) was synthesized in the presence of the SRP subunits and microsomal membranes. Co-translational translocation was assessed by the cleavage of the signal sequence from PPL. As judged by the appearance of the mature prolactin (PL), efficient translocation of PPL was only promoted by SRP (Figure 3, lanes 5 and 6). SRP(S) only inefficiently promoted translocation, consistent with its known inability to retard elongation (Figure 3, lanes 7 and 8; compare also Siegel and Walter, 1986). The other SRP subunits did not promote the translocation of PPL (Figure 3, lanes 9–16; compare also Siegel and Walter, 1988).

To test if the SRP subunits promoted translocation of elongation-arrested nascent polypeptides, we incubated the RNCs containing PPL86 (compare Figure 2) with the SRP subunits and microsomal membranes. Translocation was monitored by signal peptide cleavage from the puromycin-released PPL86 and the accumulation of the mature

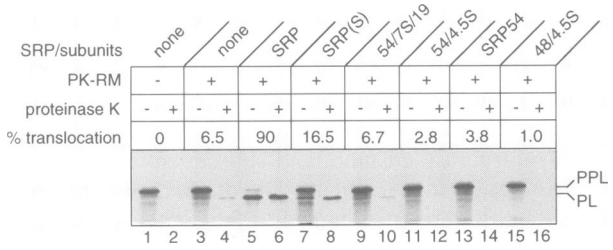

Fig. 3. Translocation of PPL in the presence of SRP subunits. Full-length PPL was synthesized in a wheat germ lysate in the absence (–PK-RM) or presence (+ PK-RM) of microsomal membranes and SRP or the SRP subunits to allow translocation to occur. One half of each sample was digested with proteinase K (+ proteinase K) or left untreated (–proteinase K). Radioactivity in PPL and PL bands of the undigested samples was quantified and translocation efficiencies were calculated as described in Materials and methods.

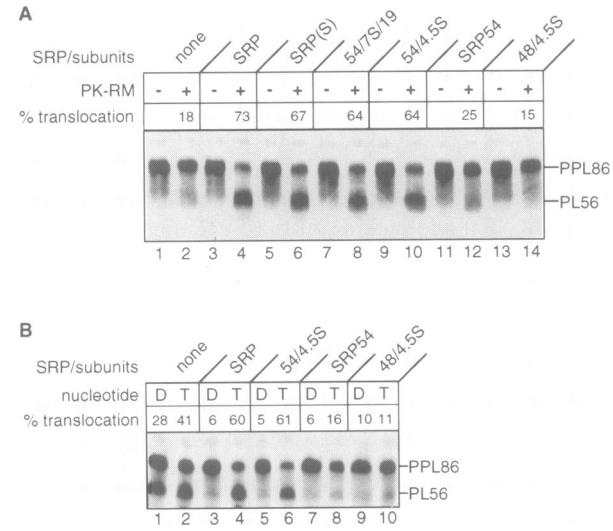

Fig. 4. Translocation of elongation-arrested nascent chains. PPL86 arrested on the ribosome (RNC) was synthesized as in Figure 2. (**A**) Wheat germ translation mixtures containing RNC were incubated in the presence of SRP or the indicated SRP subunits. One half of each sample was then incubated in the absence (–PK-RM) or presence (+ PK-RM) of PK-RMs. PPL86 was released from the ribosome after the addition of puromycin and further incubation. (**B**) Purified RNCs were first incubated with the indicated SRP subunits and subsequently with PK-RM in the presence of GDP or GTP, as indicated. Puromycin-release was as in (A). All samples were TCA-precipitated and analysed by SDS–PAGE and phosphoimaging. Translocation efficiencies were calculated as described in Materials and methods.

PL56 (Connolly and Gilmore, 1986). All SRP54-containing SRP subunits promoted the translocation of PPL86, as is indicated by the appearance of PL56 (Figure 4A, compare lanes 5–10 with lanes 3 and 4) which was protected against added protease (not shown). Translocation depended on GTP (not shown, but compare with Figures 4B and 5).

The translocation across the microsomal membrane promoted by the free SRP54 was very inefficient, being only slightly above background in repeated experiments (Figure 4A, lanes 11 and 12). Since part of the free SRP54 was found to associate with wheat germ SRP (unpublished observations), it is likely that free SRP54 does not promote translocation. Translocation was not promoted by 48/4.5S (Figure 4A, lanes 13 and 14).

Translocation in the absence of cytosol

Recently, it has been shown that ribosome-attached PPL can be translocated across ER membrane vesicles in the absence of any cytosolic factor. NAC, a cytosolic protein complex, prevents this translocation, and SRP overcomes this block in the presence of GTP and promotes translocation (Lauring *et al.*, 1995). However, in the presence of SRP, translocation becomes GTP-dependent. If the free SRP54 interacts with the signal sequence of PPL86 but is unable to promote translocation, it should block (like NAC) the translocation of PPL86 in the absence of cytosol. To test this, RNCs were assembled and purified as before (cf. Figure 2) and incubated with the SRP subunits. GTP-dependent translocation was then monitored after the addition of microsomal membranes and GTP or GDP. In the absence of added SRP subunits, translocation was observed which was independent of added GTP (Figure 4B, compare lanes 1 and 2). GTP-independent translocation did not occur in the presence of SRP or SRP subunits (Figure 4B, lanes 3, 5, 7 and 9). However, in the presence of GTP both SRP and 54/4.5S promoted translocation (Figure 4B, lanes 4 and 6). In contrast, the free SRP54 and 48/4.5S strongly impaired translocation (Figure 4B, lanes 8 and 10). Thus, the free SRP54 binds the signal sequence but is unable to promote translocation in the absence of SRP RNA.

GTP-dependent interaction of SRP and the SRP subunits with the SR

The inability of SRP54 and 48/4.5S to promote translocation could have resulted from their inability to bind to the SR, to release the signal sequence of PPL86 or to mediate the insertion of PPL86 into the translocation site. We first tested whether the signal sequence was released from SRP54 in the presence of GTP and microsomal vesicles and inserted into the membrane. This occurs when SRP interacts with the SR in the presence of GTP (Connolly and Gilmore, 1989; High *et al.*, 1991a). In the membrane, the signal sequence is known to contact components of the RER translocation site, Sec61α and TRAM (Görlich *et al.*, 1992a,b). To identify these interactions we used photo-cross-linking of the radiolabelled PPL86 (Krieg *et al.*, 1986; Kurzchalia *et al.*, 1986; High *et al.*, 1993). SRP and the SRP subunits were allowed to bind the signal sequence of PPL86 as described above. The complexes were incubated with microsomal membranes in the absence or presence of GTP.

When the complexes of RNC and SRP or the SRP54-containing subparticles were allowed to interact with PK-RMs in the presence of GTP, the signal sequence of PPL86 was released from SRP54 and was now found in contact with Sec61α (Figure 5, lanes 6, 8, 10 and 12). This is deduced from the reduced cross-linking between PPL86 and SRP54 (Figure 5, compare lanes 6, 8, 10 and 12 with lanes 5, 7, 9 and 11) and the appearance of photoadducts between PPL86 and Sec61α (Figure 5, lanes 6, 8, 10 and 12) which could be immunoprecipitated with Sec61α-specific antisera (not shown).

In contrast to the SRP54-containing subparticles, the free SRP54 and 48/4.5S were unable to release the signal sequence of PPL86 at the RER membrane: the photoadducts to SRP54 or P48 were not diminished,

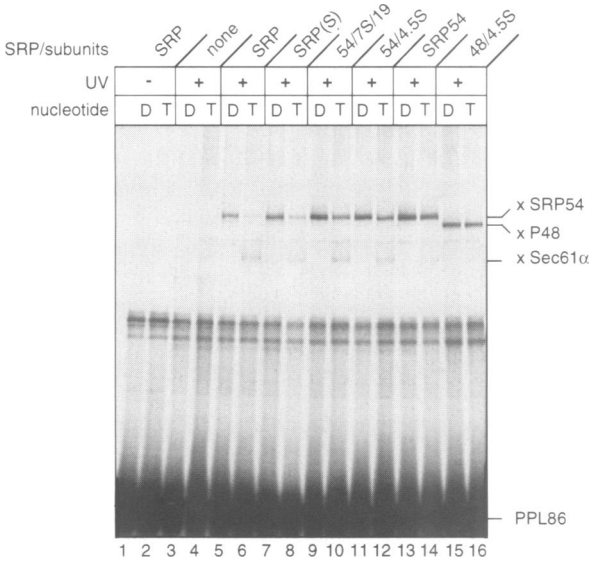

Fig. 5. GTP-dependent interaction of SRP subparticles with RER membrane vesicles and membrane insertion of the signal sequence. Purified RNCs were incubated in the presence of SRP or the SRP subunits and then incubated with PK-RMs in the presence of GTP or GDP. After chilling the samples on ice, cross-linking from the lysine residues was induced by irradiating with UV-light. To cross-link the membrane-inserted PPL86 to Sec61α, a PPL mutant (PPLΔK4ΔK9K46) was used in which cross-linking could occur only from one lysine residue in position 46 (High *et al.*, 1993). Samples were TCA-precipitated and analysed by SDS–PAGE and phosphoimaging.

despite the presence of microsomal vesicles and GTP (Figure 5, lanes 14 and 16).

Association of the SRP subunits with the SR

The release of the signal sequence from SRP requires the association of SRP with the SR in the RER membrane. In the presence of non-hydrolysable GTP, SRP associates with the SR in a high-salt-resistant manner (Connolly *et al.*, 1991; Rapiejko and Gilmore, 1992). To test for such an interaction, the SRP subunits were allowed to bind to microsomal membranes at low ionic strength in the presence of GTP or its non-hydrolysable analogue β-γ-imidoguanosine 5′-triphosphate (GMPPNP). Microsomes and associated components were then centrifuged through a high-salt/sucrose cushion and the pellet (Figure 6, 'P') fractions analysed for the presence of SRP54 or P48.

Like SRP, all subparticles containing SRP54 appeared in the pellet fractions after an incubation in the presence of GMPPNP (Figure 6, lanes 3–10, compare top and bottom). In contrast, only small amounts of the free SRP54 and P48/4.5S were found in the pellet fraction in the presence of GMPPNP. However, the same amounts were also found in the pellet fraction in the presence of GTP (Figure 6, lanes 11–14, compare top and bottom). This indicates that the free SRP54 and P48/4.5S were unable to form GMPPNP-stabilized complexes with the microsomes. This correlated with their inability to release the signal sequence for translocation (as described above).

Discussion

We have found that an SRP subparticle lacking five of the SRP proteins (SRP9/14, SRP19 and SRP68/72) failed

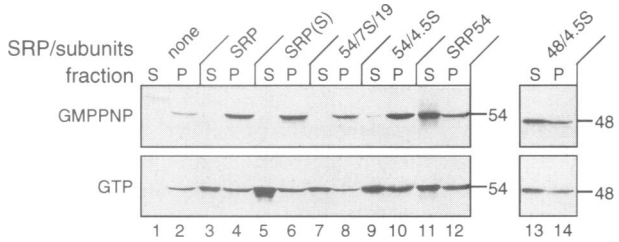

Fig. 6. Binding of SRP subunits to RER membrane vesicles in the presence of GMPPNP. SRP and the SRP subunits were allowed to bind to PK-RMs in the presence of GMPPNP (top) or GTP (bottom). Salt-resistant binding was assessed by adjusting the samples to 0.5 M KOAc, centrifuging the PK-RMs through a sucrose cushion of the same ionic strength and analysing the membrane-bound (P) and unbound (S) material by SDS–PAGE, Western blotting and immunodecorating for SRP54 (lanes 1–12) or P48 (lanes 13 and 14).

to promote co-translational translocation but was able to mediate the GTP-dependent translocation of elongation-arrested nascent PPL. This suggests that SRP54 and SRP RNA, together with the SR, are the basic machinery for promoting protein translocation and that the additional SRP components adapt the basic machinery to the co-translational mode of protein translocation.

Specific binding of signal sequences by SRP54

Free SRP54 as well as SRP54 in the SRP subparticles were found to contact the signal sequence of PPL. As judged by the extractability with high salt, the SRP subunits interact with signal sequences in a similar way as the intact SRP (High *et al.*, 1991a). No interaction was found with a mutant PPL signal sequence. This indicates that SRP54 alone (or attached to SRP RNA) can readily discriminate a signal sequence from a non-signal sequence. Since discrimination was found also in the absence of cytosol we conclude that this is an ability inherent to SRP54.

In contrast to the subparticles, the SRP54 of SRP contacted nascent chains with and without a signal sequence. This indicates that in the assay system used here, SRP apparently cannot discriminate between signal and non-signal sequences. However, this situation does not reflect the conditions in the cytosol where we have to assume that different chaperones with varying substrate specificities interact with nascent and not yet folded polypeptides. One such protein interacting with the nascent chain has actually recently been characterized. This protein, termed nascent polypeptide-associated complex (NAC) makes contact with nascent chains earlier than SRP. It was shown to prevent SRP from binding to nascent chains lacking a signal sequence (Wiedmann *et al.*, 1994). The ability of NAC to displace SRP from non-signal sequences but not from signal sequences indicates (i) that NAC has a different substrate specificity than SRP and (ii) that SRP binds differently to signal and non-signal sequences.

Both NAC and SRP interact with nascent chains. Such an interaction could be mediated by the nascent chain and/or by the binding of these components to the ribosome. Competition for a binding site on the ribosome could explain the ability of NAC to compete with SRP in case there is no signal sequence in the nascent chain.

That SRP54 of SRP interacts differently with signal

and non-signal sequences is also supported by our finding that SRP54 alone has a very low affinity for the mutant signal sequence (Figure 2). Furthermore, it was shown that non-signal sequences that interact with the SRP54 of SRP are released independently of GTP (Lauring *et al.*, 1995; and M.Wiedmann, personal communication). An explanation for the cross-linking of the mutated signal sequence may be that SRP, but not the free SRP54, binds to the ribosome such that SRP54 is close to the nascent polypeptide as it emerges from the ribosome. Any one of the additional SRP components may place SRP54 in that position on the ribosome. An affinity of SRP for the ribosome that is independent of a signal sequence has been detected previously (Walter *et al.*, 1981). Such an affinity would also explain why SRP competes better than SRP54 for the cross-linking to a signal sequence of a nascent polypeptide (Zopf *et al.*, 1993).

Co-translational translocation and translocation of elongation-arrested nascent polypeptides

SRP(S) which lacks SRP9/14 and part of the 7S RNA only inefficiently promoted the co-translational translocation of PPL. The other subparticles lacking in addition SRP68/72 were found to be unable to promote co-translational translocation. This is consistent with previous observations (Siegel and Walter, 1988). Both types of subparticles however efficiently promoted the translocation of the elongation-arrested PPL86. We therefore conclude that SRP9/14 and SRP68/72 and possibly SRP19 function in adapting SRP to the co-translational mode of protein translocation.

The adaptation of SRP to co-translational translocation could involve several functions. (i) Nascent polypeptides could efficiently be checked for the presence of a signal sequence. To achieve this SRP could associate with the ribosome close to the site where the polypeptide emerges from the ribosome. (ii) Once the signal sequence has been bound, the retardation of elongation could extend the time span in which the nascent polypeptide remains competent for translocation. (iii) As elongation appears to be only retarded in most cases (Prehn *et al.*, 1987; Campos *et al.*, 1988; Wolin and Walter, 1989), a mechanism that stimulates the attachment to the membrane could also contribute to efficient co-translational translocation.

Both SRP9/14 and SRP68/72 could be required for any one of the functions postulated above. Both heterodimers have already been shown to be required for the SRP-mediated retardation of elongation (Siegel and Walter, 1988). The lack of this function clearly reduced the efficiency by which PPL was co-translationally translocated. No co-translational translocation was observed with 54/7S/19 and the cross-linking of this subunit to the mutant PPL86 was strongly reduced. Our favoured explanation of these findings is that SRP subunits lacking SRP9/14 and SRP68/72 have a reduced affinity for the ribosome. This would reduce their chances of binding to the nascent polypeptide. However, we cannot rule out the possibility that the SRP subunits interact with the membrane less efficiently than SRP. It is possible that SRP68/72 is required for an efficient interaction with the membrane. This is suggested from the observation that the selective modification of SRP68/72 impaired SRP's interaction with an affinity matrix containing solubilized SR (Siegel and Walter, 1988).

SRP RNA and SRP54 are necessary and sufficient to promote translocation

We show that the core components of SRP that promote translocation are SRP54 and SRP RNA. Previously it had already been observed that translocation requires a domain of SRP54 which is not essential for signal sequence binding (Zopf *et al.*, 1993) and that both SRP54 and SRP RNA are necessary and sufficient for an interaction with the SR (Miller *et al.*, 1993). In the present study, a particle consisting of SRP54 and 4.5S RNA was indistinguishable from SRP with respect to the translocation of ribosome-associated nascent chains: (i) it interacted with the SR and formed a high-salt-stable complex with the membrane in the presence of non-hydrolysable GTP (Connolly *et al.*, 1991; Rapiejko and Gilmore, 1992); (ii) it released the signal sequence only in the presence of microsomal membranes and GTP; and (iii) the signal sequence then contacted proteins in the translocation site, and the nascent polypeptide was translocated after its release from the ribosome.

SRP54 alone does not promote translocation, whereas it does so in conjunction with the 7S RNA and SRP19. SRP19 is required for the binding of SRP54 to the 7S RNA but not for the binding to the 4.5S RNA of *E.coli* and to a 7S RNA fragment which lacks the stem–loop to which SRP19 binds (Römisch *et al.*, 1990; Zopf *et al.*, 1990). We have not obtained any hint for a function of SRP19 other than to facilitate the RNA binding of SRP54: the 54/4.5S subparticle promoted the translocation of PPL86 like the 54/7S/19 subparticle. SRP54 has to be complexed either with 7S RNA or with 4.5S RNA to function in protein translocation. A similar conclusion has been reached previously for the P48 of *E.coli* (Wood *et al.*, 1992). Because the inability of the free SRP54 to promote translocation correlates with its inability to release the signal sequence in the presence of microsomal membranes and to form a GMPPNP-stabilized complex with the SR, we suggest that the SRP RNA is required for the functional interaction with the SR. It remains unclear whether the SRP RNA directly contacts the SR or whether its contribution is indirect, e.g. via an effect on the SRP54. As the mammalian 7S RNA could be replaced in our experiments by the 4.5S RNA of *E.coli* without affecting function and, conversely, can replace the SRP RNAs of *E.coli* or *Bacillus subtilis in vivo* (Ribes *et al.*, 1990; Nakamura *et al.*, 1992), it appears that the nucleotide residues necessary for function are conserved in these SRP RNAs.

The function of an E.coli SRP

The *E.coli* SRP comprises P48 and 4.5S RNA and thus corresponds to the smallest SRP subparticle studied here, 54/4.5S. Based on these structural and on previously established functional similarities we suggest that the *E.coli* 48/4.5S particle has the same capacity as the hybrid 54/4.5S particle: (i) both can discriminate between signal and non-signal sequences (Figure 2 and Luirink *et al.*, 1992); and (ii) 54/4.5S can release the signal sequence in a GTP-dependent manner after contact with the SR in RER membrane vesicles. *E.coli* 48/4.5S is known to interact with the *E.coli* homologue of SRα, FtsY (Miller

et al., 1994). Although it is not known yet, we consider it likely that the signal sequence is released from P48 upon contact with FtsY. In contrast to SR—which is found membrane-bound— FtsY is found predominantly cytosolic (Luirink *et al.*, 1994). Thus, the release of the signal sequence from P48 is not necessarily coupled to its membrane insertion. This is consistent with the inability of 54/4.5S to promote co-translational translocation.

When nascent chains or preproteins are released from *E.coli* SRP into the cytosol then it is likely that they are taken over by chaperones. Some preproteins do not need to involve SRP for their translocation but only involve chaperones. The reason why *E.coli* SRP is only required for a subset of preproteins is not yet known. One possibility is that the *E.coli* SRP has a higher affinity for some signal sequences of nascent polypeptides than chaperones. This is supported by the finding that *E.coli* SRP does not bind to polypeptides released from the ribosome (Luirink *et al.*, 1992). A further possibility is suggested by the fact that both P48 and FtsY are GTPases. GTPases are regulators which are usually activated by the binding of GTP and inactivated by the hydrolysis of GTP. Furthermore, GTPases are known to be regulated by factors which trigger the binding and the hydrolysis of GTP. Thus, the translocation GTPases P48 and FtsY—like their eukaryotic homologues—would make the secretion of proteins requiring these components regulatable. In contrast, proteins which can be translocated without the interference of theses GTPases would be secreted constitutively. The main function of P48 and FtsY would then be to allow the regulated secretion of some of the *E.coli* proteins.

Materials and methods

Materials

General chemicals were from Merck, Darmstadt or Sigma, München, Germany. Restriction enzymes and yeast tRNA were from Boehringer Mannheim; baculovirus transfer vector pVL1392 and High Five™ cells were from Invitrogen (Leek, The Netherlands); Insect Express medium from Serva (Heidelberg, Germany); tissue culture equipment from Nunc (Roskilde, Denmark); protease inhibitors from Sigma and Boehringer; CM Sepharose, Resource S™ column and micrococcal nuclease from Pharmacia (Freiburg, Germany), SP6 and T7 RNA polymerases from New England Biolabs (Schwalbach, Germany); [^{35}S]methionine and ECL Western blotting reagents were from Amersham Buchler (Braunschweig, Germany); nitrocellulose transfer membranes and filters were from Schleicher & Schuell (Dassel, Germany); keyhole limpet haemocyanin was from Calbiochem (La Jolla, CA, USA). pE19 for SRP19 expression was a gift from Drs K.Strub and S.Oertle, Université de Genève, Switzerland; 4-(3-trifluoromethyldiazarino)benzoic acid (TDBA) was a gift from Dr J.Brunner, ETH, Zürich, Switzerland.

Expression and partial purification of SRP19, SRP54 and P48

Previously, recombinant SRP19 has been assembled with canine proteins and RNA into functional SRP (S.Oertle and K.Strub, unpublished results). The cDNA encoding the human SRP19 was expressed in *E.coli* using the pET-derived (Studier *et al.*, 1990) plasmid pE19. After 1 h of induction with 0.4 mM IPTG at 37°C, the bacteria were chilled to 4°C, harvested, resuspended in 1% of the original volume of column buffer [CB19, 50 mM triethanolamine, pH 7.5, 5 mM $Mg(OAc)_2$, 1 mM DTT, 20 μg/ml PMSF, 50 mM KOAc] and lysed in a French press. Cellular debris and insoluble material were removed by two sequential centrifugations (30 min at 37 000 *g;* 30 min at 150 000 *g*) and finally a passage through a 0.22 μM pore size filter. The material was directly loaded onto a Resource S™ column at a flow rate of 0.5 ml/min and subsequently washed at a rate of 5 ml/min with 2.5 column volumes of CB19 and another 4 volumes of CB containing 0.4 M KOAc. SRP19 was eluted by a step gradient of 0.65 M KOAc in CB19 and frozen directly in aliquots in liquid N_2. The SRP19 fraction was judged >80% pure by Coomassie staining (Figure 1C, lane 1).

The cDNA encoding the canine SRP54 (Römisch *et al.*, 1989) was inserted into the pVL1392 transfer vector after restriction with *Pst*I and *Not*I and integrated into the baculovirus genome using published procedures (Summers and Smith, 1987). To express SRP54, High Five™ cells were infected at 10 p.f.u./cell and grown for 2 days in Insect Express medium at 27°C. Approximately 10^8 cells were scraped off the tissue culture flasks, washed with PBS, and resuspended in 20 ml lysis buffer [50 mM HEPES/KOH, pH 7.5, 5 mM $Mg(OAc)_2$, 1 mM DTT, 2 mM EGTA, 0.01 mg/ml PMSF, 0.5 mg/ml Pefabloc, 0.1 mg/ml leupeptin, 0.05 mg/ml chymostatin, 0.01 mg/ml pepstatin, 0.01 mg/ml aprotinin]. After hypotonic lysis for 30 min at 0°C, the cells were homogenized by 30 strokes in a tightly fitting Dounce homogenizer. Nuclei and cellular debris were sedimented by centrifugation for 10 min at 1300 *g*, washed in 1 ml column buffer (CB54, 50 mM HEPES/KOH, pH 7.6, 5 mM $Mg(OAc)_2$, 1 mM DTT, 0.01 mg/ml PMSF) containing 0.5 M KOAc for 15 min at 0°C and centrifuged as before. The two supernatants were adjusted to 1 mM EDTA, combined and centrifuged at 186 000 *g* for 15 min at 0°C to remove insoluble material. The supernatant was loaded onto a CM Sepharose column equilibrated in CB54 at a flow rate of 0.2 ml/min and washed with 5 column volumes of CB54. Approximately 450 μg of SRP54 were eluted by a step gradient of CB54 containing 0.4 M KOAc. To remove any contaminating insect cell SRP, ~90 μg of the SRP54 eluate was loaded onto a sucrose gradient and centrifuged like the SRP subparticles (cf. below). Free SRP54 was recovered in the third fraction from the top. The isolated SRP54 was estimated by Coomassie staining to be >60% pure (Figure 1C, lane 2). A blot of this preparation (Figure 1D, lane 2) shows that the major contaminating band was unrelated to SRP54. SRP54 itself appeared to be intact and to co-migrate with the SRP54 derived from canine SRP (Figure 1D, lane 6).

The *E.coli* P48 with six histidine residues added to its C-terminus was synthesized in *E.coli* using plasmid pDS12-48His6 and purified by metal chelate chromatography as described previously (Lentzen *et al.*, 1994).

Transcription

Plasmids p7Swt1 (Strub *et al.*, 1991) and pT3/T7 4.5Swt (Wood *et al.*, 1992) were linearized with *Xba*I and *Bam*HI, respectively, and used to synthesize 7S RNA and 4.5S RNA by run-off transcription with T7 RNA polymerase. Messenger RNAs encoding PPL, PPL86, PPL86 containing a signal sequence mutant (Luirink *et al.*, 1992), and PPL86 ΔK4.ΔK9.K46 (High *et al.*, 1993) were synthesized by *in vitro* transcription of the respective cDNAs (linearized with *Eco*RI or *Pvu*II for PPL and PPL86, respectively) using SP6 RNA polymerase and dissolved in H_2O.

Preparation of SRP subparticles

To assemble 54/7S/19, 54/4.5S and 48/4.5S, ~1.3 nmol of the respective proteins were incubated with 2.5 nmol 7S RNA or 4.5S RNA in 50 mM HEPES/KOH, pH 7.6, 0.4 M KOAc, 4 mM $Mg(OAc)_2$, 1 mM DTT, 0.01 mg/ml PMSF, 0.1 mg/ml Pefabloc, 2 mM EGTA (assembly buffer) for 15 min at 37°C. The resulting subparticles were separated from unassembled material by centrifugation through a linear gradient (5–20% sucrose in assembly buffer containing 0.25 M KOAc) for 14 h at 45 000 r.p.m. (270 000 *g*) at 4°C in a SW60 rotor and recovered from the eighth of 11 or 10 fractions collected from the top. The subparticles were judged >95% pure as judged by Coomassie staining (Figure 1C, lanes 3–5).

To prepare SRP(S), 150 pmol of gradient-purified SRP (prepared as described; Walter and Blobel, 1983c) were digested with 600 U micrococcal nuclease for 1 h at 37°C in 16 mM HEPES/KOH, pH 7.6, 0.24 M KOAc, 1.5 mM $CaCl_2$, 2.2 mM $Mg(OAc)_2$, 1.3 mM DTT in a final volume of 62 μl. Mock digestion was in the presence of additional 4 mM EGTA. To control for complete digestion (Figure 1B), the RNA prepared from 23 pmol of (treated) SRP was analysed on a 8% sequencing-type urea–polyacrylamide gel (Maniatis *et al.*, 1982).

SRP and the SRP subunits (except 48/4.5S) were adjusted to similar concentrations of SRP54 based on Western blotting using the SRP54-specific antiserum 87 (cf. below) and the ECL system. The concentration of the 48/4.5S particle was adjusted to that of 54/4.5S by Coomassie staining (Figure 1C).

Removal of SRP from rough microsomes

Rough microsomes (RMs) were prepared from dog pancreas and salt-washed as described (K-RMs) (Walter and Blobel, 1983b). To largely

remove residual SRP, the K-RMs were treated with 1 mM puromycin and 0.65 M KOAc in RM-buffer [50 mM HEPES/KOH, pH 7.5, 50 mM KOAc, 2.5 mM $Mg(OAc)_2$, 1 mM DTT] for 15 min at 37°C and, after adjustment to 2 M sucrose, floated through a step of 1.5 M into 1 M and up to the interface of 0.25 M sucrose (all at 4°C in RM buffer containing 0.65 M KOAc). The resulting PK-RMs were diluted 5-fold in RM buffer, collected for 1 h at 160 000 *g*, resuspended to 2 eq/µl by douncing in RM buffer containing 0.25 M sucrose and frozen in small aliquots in liquid N_2.

Translation, translocation and photo-cross-linking

N^{ε}-TDBA-Lys-tRNA was prepared as described (High *et al.*, 1991b). Conditions for translation in wheat germ lysate in the presence of [^{35}S]methionine and the modified tRNA (where indicated) were as described (Stueber *et al.*, 1984; High *et al.*, 1991b, 1993).

Translocation of full-length PPL occurred during the translation in the presence of PK-RM (1 eq/12.5 µl) and SRP (0.25 pmol/12.5 µl) or equivalent concentrations of the SRP subunits, as indicated. Up to 10-fold higher concentrations of 54/7S/19 or SRP54 in this assay did not alter the results (not shown). Proteinase K digestion was at 0.75 mg/ml for 10 min at 25°C.

For assays in the absence of protein synthesis, RNCs containing PPL86 or mutant PPL86 were assembled for 15 min at 25°C and subsequently stabilized by adding 2 mM cycloheximide (CX). The complexes were used directly (Figure 4A) or after purification (Figures 2, 4B and 5). For purification, the translation mixtures were adjusted to 0.5 M KOAc, incubated for 10 min at 0°C, and centrifuged for 30 min at 400 000 *g* and 4°C in a TLA100.2 rotor through ≥4 volumes of a 0.5 M sucrose cushion in RM buffer containing, 0.5 M KOAc, 5 mM $Mg(OAc)_2$ and 2 mM CX, and the ribosomal pellets finally resuspended in the original volume RM buffer containing 2 mM CX. SRP or the SRP subunits were added at 20 nM to 25 µl RNC and allowed to bind at 125 mM (Figure 2) or 170 mM KOAc (Figures 4 and 5) for 30 min at 0°C, followed by 2 min at 25°C. To monitor the interaction with the wild-type and mutated signal sequence, the resulting complexes were UV-irradiated on ice for 2 min either directly or after adjustment to 0.5 M KOAc and centrifugation as before in the presence of 1 A_{260} unit of carrier ribosomes (Figure 2). Alternatively (Figures 4 and 5), the interaction with the membrane was allowed after adding 3 eq PK-RMs and 0.5 mM guanine nucleotides (as indicated) at 120 mM KOAc for 8 min at 25°C. Samples were either irradiated as before (Figure 5) or translocation was allowed by adding 1.5 mM puromycin for another 15 min at 37°C (Figure 4).

Samples were TCA-precipitated and analysed by SDS–PAGE [PPL on a 12.5% SDS–polyacrylamide gel, PPL86 on a 22% SDS–polyacrylamide/6 M urea gel (Haeuptle *et al.*, 1986), and cross-linking reactions on 10–16% SDS–polyacrylamide gels] and phosphoimaging using a Fuji Phosphoimager BAS1000. Radioactivity was quantified using the Fuji MacBAS V1.0 software. In Figure 2A, radioactivity representing cross-linked PPL86 was compared directly between corresponding lanes (Figure 2A, lanes 11–16 as % of lanes 3–8: ~85% in lanes 11–15, ~30% in lane 16). In Figure 2A and B, cross-linking efficiencies were calculated by expressing the radioactivity in cross-linked PPL86 or $PPL86_{mut}$ as percentage of the combined radioactivity in free and cross-linked PPL86 or $PPL86_{mut}$. Figure 2C shows cross-linking efficiencies in Figure 2B (lanes 3–8) expressed as percent of the cross-linking efficiencies in the corresponding lanes in Figure 2A. In Figures 3 and 4, translocation efficiencies were calculated for samples that had received membranes but no protease. For this, the radioactivity in PL (or PL56) was expressed as percent of the combined radioactivity in PL (or PL56) and PPL (or PPL86).

Re-binding of SRP and the SRP subunits to rough microsomes

SRP (2 pmol) or equivalent amounts of the SRP subunits were allowed to bind to 30 eq PK-RMs in the presence of 0.1 mM GMPPNP or GTP for 60 min at 25°C in RM buffer in a total volume of 40 µl. After adjustment to 0.5 M KOAc and an additional 10 min at 0°C, membrane-bound and unbound material was separated by centrifuging the membranes through a 75 µl cushion (0.5 M sucrose, 0.5 M KOAc in RM buffer) for 15 min at 70 000 r.p.m., 4°C in a TLA100 rotor. The membrane pellet was washed once in RM buffer and the unbound material contained in the supernatant was TCA precipitated, before both were dissolved in SDS sample buffer and analysed by SDS–PAGE, Western blotting, and immunodecoration using SRP54- and P48-specific antisera (cf. below).

Rabbit polyclonal antisera and immunoprecipitations

The SRP54-specific antiserum 87 was raised by immunizing a rabbit with a synthetic peptide coupled to keyhole limpet haemocyanin with glutaraldehyde. The peptide corresponds to amino acid positions 136–152 of the canine SRP54 (Römisch *et al.*, 1989) and is 100% conserved in the SRP54 homologues from tomato and the yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (in the latter except for the N-terminal Cys residue). It was used in immunoblots in combination with the ECL system at a dilution of 1:1000 (Figures 1 and 6) and was successful in immunoprecipitations under native and denaturing conditions (not shown). The *E.coli* P48 was detected using a rabbit antiserum (Poritz *et al.*, 1990) and ECL. Immunoprecipitations of TRAM and Sec61α were under denaturing conditions using rabbit antisera raised as described previously (Görlich *et al.*, 1992a,b).

Acknowledgements

We are indebted to E.Kretzschmar and H.D.Klenk, Philipps-Universität Marburg, for generously providing time, expertise and facilities in the introduction to the baculovirus expression system; to S.Oertle and K.Strub, Université de Genève, for the plasmid pE19; to J.Brunner, ETH, Zürich, for TDBA; to H.D.Bernstein and P.Walter, UCSF, for the P48-specific antiserum; and to K.Schröder, this laboratory, for the Sec61α-specific antiserum. We thank B.Martoglio, B.Jungnickel and H.Schröder for stimulating discussions and helpful suggestions, M.Wiedmann for sharing data before publication, and A.Lütcke, B.Martoglio and K.Strub for critical comments on the manuscript. H.L. is supported by a Habilitandenstipendium from the Deutsche Forschungsgemeinschaft (DFG, Lu 507-1/-3). S.H. and this work were supported by a grant from DFG to H.L. (Lu 507-2/-4).

References

Bernstein,H.D., Poritz,M.A., Strub,K., Hoben,P.J., Brenner,S. and Walter,P. (1989) Model for signal sequence recognition from amino-acid sequence of 54K subunit of signal recognition particle. *Nature*, **340**, 482–486.

Bernstein,H.D., Zopf,D., Freymann,D.M. and Walter,P. (1993) Functional substitution of the signal recognition particle 54-kDa subunit by its *Escherichia coli* homolog. *Proc. Natl Acad. Sci. USA*, **90**, 5229–5233.

Campos,N., Palau,J., Torrent,M. and Ludevid,D. (1988) Signal recognition-like particles are present in maize. *J. Biol. Chem.*, **263**, 9646–9650.

Connolly,T. and Gilmore,R. (1986) Formation of a functional ribosome-membrane junction during translocation requires the participation of a GTP-binding protein. *J. Cell Biol.*, **103**, 2253–2261.

Connolly,T. and Gilmore,R. (1989) The signal recognition particle receptor mediates the GTP-dependent displacement of SRP from the signal sequence of the nascent polypeptide. *Cell*, **57**, 599–610.

Connolly,T., Rapiejko,P.J. and Gilmore,R. (1991) Requirement of GTP hydrolysis for dissociation of the signal recognition particle from its receptor. *Science*, **252**, 1171–1173.

Gilmore,R., Collins,P., Johnson,J., Kellaris,K. and Rapiejko,P. (1991) Transcription of full-length and truncated mRNA transcripts to study protein translocation across the endoplasmic reticulum. *Methods Cell Biol.*, **34**, 223–239.

Görlich,D., Hartmann,E., Prehn,S. and Rapoport,T.A. (1992a) A protein of the endoplasmic reticulum involved early in polypeptide translocation. *Nature*, **357**, 47–52.

Görlich,D., Prehn,S., Hartmann,E., Kalies,K.-U. and Rapoport,T.A. (1992b) A mammalian homolog of Sec61p and SecYp is associated with ribosomes and nascent polypeptides during translocation. *Cell*, **71**, 489–503.

Gundelfinger,E.D., Krause,E., Melli,M. and Dobberstein,B. (1983) The organization of the 7SL RNA in the signal recognition particle. *Nucleic Acids Res.*, **11**, 7363–7374.

Haeuptle,M.T., Frank,R. and Dobberstein,B. (1986) Translation arrest by oligodeoxynucleotides complementary to mRNA coding sequences yields polypeptides of predetermined length. *Nucleic Acids Res.*, **14**, 1427–1448.

High,S., Flint,N. and Dobberstein,B. (1991a) Requirements for the membrane insertion of signal-anchor type proteins. *J. Cell Biol.*, **113**, 25–34.

High,S., Görlich,D., Wiedmann,M., Rapoport,T.A. and Dobberstein,B. (1991b) The identification of proteins in the proximity of signal-

anchor sequences during their targeting to and insertion into the membrane of the ER. *J. Cell Biol.*, **113**, 35–44.

High,S., Martoglio,B., Görlich,D., Andersen,S.S.L., Ashford,A.J., Giner,A., Hartmann,E., Prehn,S., Rapoport,T.A., Dobberstein,B. and Brunner,J. (1993) Site-specific photocross-linking reveals that Sec61p and TRAM contact different regions of a membrane inserted signal sequence. *J. Biol. Chem.*, **268**, 26745–26751.

Krieg,U.C., Walter,P. and Johnson,A.E. (1986) Photocrosslinking of the signal sequence of nascent preprolactin to the 54-kilodalton polypeptide of the signal recognition particle. *Proc. Natl Acad. Sci. USA*, **83**, 8604–8608.

Kurzchalia,T.V., Wiedmann,M., Girshovich,A.S., Bochkareva,E.S., Bielka,H. and Rapoport,T.A. (1986) The signal sequence of nascent preprolactin interacts with the 54 K polypeptide of signal recognition particle. *Nature*, **320**, 634–636.

Lauring,B., Sakai,H., Kreibich,G. and Wiedmann,M. (1995) Nascent polypeptide-associated complex protein prevents mistargeting of nascent chains to the endoplasmic reticulum. *Proc. Natl Acad. Sci. USA*, **92**, 5411–5415.

Lentzen,G., Dobberstein,B. and Wintermeyer,W. (1994) Formation of SRP-like particle induces a conformational change in *E. coli* 4.5S RNA. *FEBS Lett.*, **348**, 233–238.

Lingelbach,K., Zwieb,C., Webb,J.R., Marshallsay,C., Hoben,P.J., Walter,P. and Dobberstein,B. (1988) Isolation and characterization of a cDNA clone encoding the 19 kDa protein of signal recognition particle (SRP): expression and binding to 7SL RNA. *Nucleic Acids Res.*, **16**, 9431–9442.

Luirink,J., High,S., Wood,H., Giner,A., Tollervey,D. and Dobberstein,B. (1992) Signal sequence recognition by an *E. coli* ribonucleoprotein particle. *Nature*, **359**, 741–743.

Luirink,J., ten Hagen-Jongman,C.M., van der Weijden,C.C., Oudega,B., High,S., Dobberstein,B. and Kusters,R. (1994) An alternative protein targeting pathway in *Escherichia coli*: studies on the role of FtsY. *EMBO J.*, **13**, 2289–2296.

Lütcke,H. (1995) Signal recognition particle (SRP), a ubiquitous initiator of protein translocation. *Eur. J. Biochem.*, **228**, 531–550.

Lütcke,H., High,S., Römisch,K., Ashford,A.J. and Dobberstein,B. (1992) The methionine-rich domain of the 54 kDa subunit of signal recognition particle is sufficient for the interaction with signal sequences. *EMBO J.*, **11**, 1543–1551.

Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) In *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Miller,J.D., Wilhelm,H., Gierasch,L., Gilmore,R. and Walter,P. (1993) GTP binding and hydrolysis by the signal recognition particle during initiation of protein translocation. *Nature*, **366**, 351–354.

Miller,J.D., Bernstein,H.D. and Walter,P. (1994) Interaction of *E. coli* Ffh/4.5S ribonucleoprotein and FtsY mimics that of mammalian signal recognition particle and its receptor. *Nature*, **367**, 657–659.

Nakamura,K., Imai,Y., Nakamura,A. and Yamane,K. (1992) Small cytoplasmic RNA of *Bacillus subtilis*: functional relationship with human signal recognition particle 7S RNA and *Escherichia coli* 4.5S RNA. *J. Bacteriol.*, **174**, 2185–2192.

Phillips,G.J. and Silhavy,T.J. (1992) The *E. coli ffh* gene is necessary for viability and efficient protein export. *Nature*, **359**, 744–746.

Poritz,M.A., Bernstein,H.D., Strub,K., Zopf,D., Wilhelm,H. and Walter,P. (1990) An *E. coli* ribonucleoprotein containing 4.5S RNA resembles mammalian signal recognition particle. *Science*, **250**, 1111–1117.

Prehn,S., Wiedmann,M., Rapoport,T.A. and Zwieb,C. (1987) Protein translocation across wheat germ microsomal membranes requires an SRP-like component. *EMBO J.*, **6**, 2093–2097.

Rapiejko,P.J. and Gilmore,R. (1992) Protein translocation across the ER requires a functional GTP binding site in the α subunit of the signal recognition particle receptor. *J. Cell Biol.*, **117**, 493–503.

Rapoport,T.A., Heinrich,R., Walter,P. and Schulmeister,T. (1987) Mathematical modeling of the effects of the signal recognition particle on translation and translocation of proteins across the endoplasmic reticulum membrane. *J. Mol. Biol.*, **195**, 621–636.

Ribes,V., Römisch,K., Giner,A., Dobberstein,B. and Tollervey,D. (1990) *E. coli* 4.5S RNA is part of a ribonucleoprotein particle that has properties related to signal recognition particle. *Cell*, **63**, 591–600.

Römisch,K., Webb,J., Herz,J., Prehn,S., Frank,R., Vingron,M. and Dobberstein,B. (1989) Homology of 54K protein of signal-recognition particle, docking protein and two *E. coli* proteins with putative GTP-binding domains. *Nature*, **340**, 478–482.

Römisch,K., Webb,J., Lingelbach,K., Gausepohl,H. and Dobberstein,B. (1990) The 54-kD protein of signal recognition particle contains a methionine-rich RNA binding domain. *J. Cell Biol.*, **111**, 1793–1802.

Siegel,V. and Walter,P. (1986) Removal of the Alu structural domain from signal recognition particle leaves its protein translocation activity intact. *Nature*, **320**, 81–84.

Siegel,V. and Walter,P. (1988) Each of the activities of signal recognition particle (SRP) is contained within a distinct domain: analysis of biochemical mutants of SRP. *Cell*, **52**, 39–49.

Strub,K., Moss,J. and Walter,P. (1991) Binding sites of the 9 and 14 kDa heterodimeric protein subunit of the signal recognition particle (SRP) are contained exclusively in the Alu domain of SRP RNA and contain a sequence motif that is conserved in evolution. *Mol. Cell. Biol.*, **11**, 3949–3959.

Studier,F.W., Rosenberg,A.H. and Dunn,J.J. (1990) Use of T7 RNA polymerase to direct the expression of cloned genes. *Methods Enzymol.*, **185**, 60–89.

Stueber,D., Ibrahimi,I., Cutler,D., Dobberstein,B. and Bujard,H. (1984) A novel *in vitro* transcription-translation system: accurate and efficient synthesis of single proteins from cloned DNA sequences. *EMBO J.*, **3**, 3143–3148.

Summers,M.D. and Smith,G.E. (1987) *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*. Texas Agricultural Experiment Station, Bulletin No. 1555.

Walter,P. and Blobel,G. (1980) Purification of a membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. *Proc. Natl Acad. Sci. USA*, **77**, 7112–7116.

Walter,P. and Blobel,G. (1982) 7SL small cytoplasmic RNA is an integral component of the signal recognition particle. *Nature*, **299**, 691–698.

Walter,P. and Blobel,G. (1983a) Disassembly and reconstitution of signal recognition particle. *Cell*, **34**, 525–533.

Walter,P. and Blobel,G. (1983b) Preparation of microsomal membranes for cotranslational protein translocation. *Methods Enzymol.*, **96**, 84–93.

Walter,P. and Blobel,G. (1983c) Signal recognition particle: A ribonucleoprotein required for cotranslational translocation of proteins, isolation and properties. *Methods Enzymol.*, **96**, 682–691.

Walter,P. and Blobel,G. (1983d) Subcellular distribution of signal recognition particle and 7SL-RNA determined with polypeptide-specific antibodies and complementary DNA probe. *J. Cell Biol.*, **97**, 1693–1699.

Walter,P. and Johnson,A.E. (1994) Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. *Annu. Rev. Cell Biol.*, **10**, 87–119.

Walter,P., Ibrahimi,I. and Blobel,G. (1981) Translocation of proteins across the endoplasmic reticulum. I. Signal recognition protein (SRP) binds to *in vitro* assembled polysomes synthesizing secretory protein. *J. Cell Biol.*, **91**, 545–550.

Wiedmann,B., Sakai,H., Davis,T.A. and Wiedmann,M. (1994) A protein complex required for signal-sequence-specific sorting and translocation. *Nature*, **370**, 434–440.

Wiedmann,M., Kurzchalia,T.V., Bielka,H. and Rapoport,T.A. (1987) Direct probing of the interaction between the signal sequence of nascent preprolactin and the signal recognition particle by specific cross-linking. *J. Cell Biol.*, **104**, 201–208.

Wolin,S.L. and Walter,P. (1989) Signal recognition particle mediates a transient elongation arrest of preprolactin in reticulocyte lysate. *J. Cell Biol.*, **109**, 2617–2622.

Wood,H., Luirink,J. and Tollervey,D. (1992) Evolutionary conserved nucleotides within the *E. coli* 4.5S RNA are required for association with P48 *in vitro* and for optimal function *in vivo*. *Nucleic Acids Res.*, **20**, 5919–5925.

Zopf,D., Bernstein,H.D., Johnson,A.E. and Walter,P. (1990) The methionine-rich domain of the 54 kDa protein subunit of the signal recognition particle contains an RNA binding site and can be crosslinked to a signal sequence. *EMBO J.*, **9**, 4511–4517.

Zopf,D., Bernstein,H.D. and Walter,P. (1993) GTPase domain of the 54-kD subunit of the mammalian signal recognition particle is required for protein translocation but not for signal sequence binding. *J. Cell Biol.*, **120**, 1113–1121.

Received on July 17, 1995; revised on August 14, 1995