

Signal Sequence Processing in Rough Microsomes*

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Secretory proteins are synthesized with a signal sequence that is usually cleaved from the nascent protein during the translocation of the polypeptide chain into the lumen of the endoplasmic reticulum. To determine the fate of a cleaved signal sequence, we used a synchronized *in vitro* translocation system. We found that the cleaved signal peptide of preprolactin is further processed close to its COOH terminus. The resulting fragment accumulated in the microsomal fraction and with time was released into the cytosol. Signal sequence cleavage and processing could be reproduced with reconstituted vesicles containing Sec61, signal recognition particle receptor, and signal peptidase complex.

Signal sequences mediate the entry of proteins into the secretory pathway (Blobel and Dobberstein, 1975a). As soon as they emerge from the ribosome, signal sequences are recognized by the cytosolic signal recognition particle (SRP)¹ (Walter *et al.*, 1981), which targets the nascent proteins to the membrane of the endoplasmic reticulum (ER). Specific binding of the ribosome-nascent chain-SRP complex to the ER membrane occurs through binding to the membrane-bound SRP receptor (also called docking protein) (Gilmore *et al.*, 1982; Meyer *et al.*, 1982). After SRP displacement and insertion of the nascent chain into the translocation complex, the signal sequence is cotranslationally cleaved (Blobel and Dobberstein, 1975b), and the mature part of the protein is translocated into the lumen of the ER.

Although signal sequences display almost no sequence similarities, they share some common features. They can be found at the amino terminus of the respective proteins and consist in most cases of 20–30 amino acid residues. Furthermore, they usually show a characteristic tripartite structure; a positively charged amino-terminal region precedes a central hydrophobic core, which is followed by a COOH-terminal polar region that contains the cleavage site for the signal peptidase (von Heijne, 1985).

The microsomal signal peptidase has been purified as a complex of five subunits (signal peptidase complex (SPC)) with apparent molecular masses of 12, 18, 21, 22/23, and 25 kDa (Evans *et al.*, 1986). The 18-kDa and the 21-kDa subunits, SPC18 and SPC21, are mammalian homologues of the *Esche-*

richia coli leader peptidase (van Dijl *et al.*, 1992), which performs the signal sequence cleavage as a single protein (Zwizinski and Wickner, 1980). Both subunits are also homologous to the yeast SEC11 protein (Greenburg *et al.*, 1989; Shelness and Blobel, 1990), which is an essential component of the signal peptidase complex in *Saccharomyces cerevisiae* (Böhni *et al.*, 1988). All leader peptidase homologues contain a highly conserved serine residue that is supposed to catalyze the actual signal peptidase reaction (Sung and Dalbey, 1992).

In *E. coli*, cleaved signal peptides are digested by signal peptide peptidases (Hussain *et al.*, 1982). This degradation is thought to be initiated by membrane-bound protease IV, which cleaves the peptide within the hydrophobic core (Novak and Dev, 1988). The resulting fragments can be released into the cytosol and further hydrolyzed by oligopeptidase A (Novak and Dev, 1988). Both signal peptide peptidases are endoproteases and account for the majority of signal peptide degrading activity *in vitro* (Novak *et al.*, 1986).

Most likely, signal peptides are also degraded in eukaryotic cells (Habener *et al.*, 1979), but the process itself, the enzymes involved, and the sites of cleavage have not yet been elucidated. We show here that the preprolactin (PPL) signal peptide is further processed in rough microsomes. The resulting COOH-terminal signal peptide fragment could be detected in the cytosolic fraction of translocation assays. Further results suggest that signal peptide processing is required although not sufficient for the release of the signal peptide from the membrane.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes and cycloheximide were from Boehringer Mannheim GmbH (Mannheim, Germany). m7G(5')ppp(5')G (cap analogue) was supplied by New England Biolabs GmbH (Schwalbach, Germany), and [³⁵S]methionine and [³⁵S]cysteine were from Amersham Buchler GmbH (Braunschweig, Germany). Puromycin was supplied by Sigma-Aldrich Vertriebs GmbH (Deisenhofen, Germany), and proteinase K was supplied by E. Merck (Darmstadt, Germany).

Plasmids and Transcription—The plasmid encoding PPL has been previously described (High *et al.*, 1993). Codons 12 and 13 of the coding region were replaced by two methionine (ATG) codons using overlap extension polymerase chain reaction (Ho *et al.*, 1989) to yield the PPLMM mutant. mRNA coding for full-length PPL and PPL86 was produced by linearizing the plasmid with *EcoRI* and *PvuII*, respectively, and subsequent transcription with T7 RNA polymerase. Transcription was performed at 42 °C in the presence of 500 nM m7G(5')ppp(5')G.

Translation and Translocation—Translation was performed in wheat germ extract for 15 min at 25 °C in the presence of [³⁵S]methionine or [³⁵S]cysteine, SRP, and dog pancreatic rough microsomes or reconstituted vesicles. SRP, rough microsomes, and reconstituted vesicles were prepared as previously described (Walter and Blobel, 1983a, 1983b; Görlich and Rapoport, 1993).

After translation of PPL86, samples were put on ice, and the salt concentration was raised to 500 mM KOAc. The samples were incubated for 5 min on ice and layered on top of a 50- μ l cushion containing 500 mM sucrose, 500 mM KOAc, 50 mM Hepes-KOH, pH 7.9, 5 mM Mg(OAc)₂, and 1 mM dithiothreitol. Membranes were pelleted by a 3-min centrifugation at 48,000 rpm and 4 °C in a Beckman TLA 100 rotor. Reconstituted vesicles were sedimented twice through a cushion containing

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¹ The abbreviations used are: SRP, signal recognition particle; ER, endoplasmic reticulum; SPC, signal peptidase complex; PPL, preprolactin; PL, prolactin; PSP, processed signal peptide; SP, signal peptide; MHC, major histocompatibility complex; TAP, transporter associated with antigen presentation.

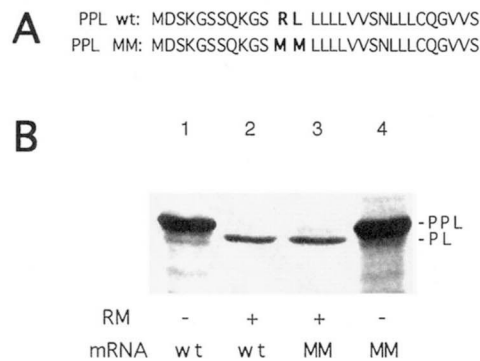


FIG. 1. Characterization of PPLMM. *A*, primary structure of the signal sequences of PPL and PPLMM. Altered amino acids are shown in *boldface*. *B*, signal sequence cleavage of PPL and PPLMM. PPL wild type mRNA (*lanes 1 and 2*) or PPLMM mRNA (*lanes 3 and 4*) was translated in the absence (*lanes 1 and 4*) or presence (*lanes 2 and 3*) of rough microsomes (*RM*). [³⁵S]Methionine-labeled translation products were separated by SDS-polyacrylamide gel electrophoresis and visualized by phosphoimaging.

only 100 mM sucrose under otherwise identical conditions. The supernatants were removed, and the pellets were resuspended in a buffer containing 20 mM Hepes-KOH, pH 7.9, 70 mM KOAc, 10 mM KCl, 3 mM Mg(OAc)₂, 200 μM GTP, and 1 mM dithiothreitol. Nascent chains were released from ribosomes by the addition of puromycin, pH 7.9, to the final concentration of 1 mM and by a 12-min incubation at 28 °C. Membranes were pelleted by centrifuging translocation assays for 3 min at 48,000 rpm and 4 °C in a Beckman TLA 100 rotor.

Proteinase K treatment was performed by incubating translocation assays for 10 min at 25 °C in the presence of 300 μg/ml proteinase K. For controls, proteinase K was omitted or added in the presence of 1% Triton X-100.

Analysis of Translation and Translocation Products—Full-length PPL was analyzed in 12.5% acrylamide gels according to Laemmli (1970). PPL86 and its processed products were separated in 16.5% T, 3% C gels according to Schägger and von Jagow (1987). Labeled proteins were visualized by autoradiography or a Fuji phosphoimager BAS1000. Radioactivity in respective bands was quantified using the phosphoimager and Fuji MacBAS V1.0 software. The amount of radioactivity was determined in the area of relevant peptides, and the corresponding amount in lanes containing unprocessed PPL86 was subtracted as background.

PPL signal peptide-specific molecular weight markers were produced by *in vitro* transcription/translation of PPL TAG18, PPL TAG25 (High *et al.*, 1993), and PPL30 (synthesized by polymerase chain reaction amplification according to Nilsson *et al.* (1994)).

RESULTS

Characterization of the PPLMM Mutant—Signal sequence cleavage and processing was studied in an *in vitro* system that relies on translation in wheat germ extract in the presence of radioactive amino acids (*e.g.* [³⁵S]methionine) and dog pancreatic rough microsomes. For better detection of the cleaved signal peptide, a preprolactin mutant with two additional methionines within the signal sequence was constructed (PPLMM, Fig. 1A). To test whether the signal sequence of this mutant is cleaved by signal peptidase, mRNA encoding PPLMM was translated in the presence of rough microsomes. As can be seen in Fig. 1B, signal peptide cleavage for this mutant is as efficient as for the wild type (compare *lane 3* and *lane 2*). Thus, the mutant can be used for the intended investigation with the advantage of a more easily detectable signal peptide.

Identification of the Processed Preprolactin Signal Peptide—For the analysis of the signal peptide, the plasmids encoding PPL and PPLMM, respectively, were linearized within their coding sequence by *Pvu*II and transcribed *in vitro*. The resulting truncated mRNAs code for the amino-terminal 86 amino acids of PPL (PPL86). Since these mRNAs lack a stop codon, normal termination of translation cannot occur, and the nas-

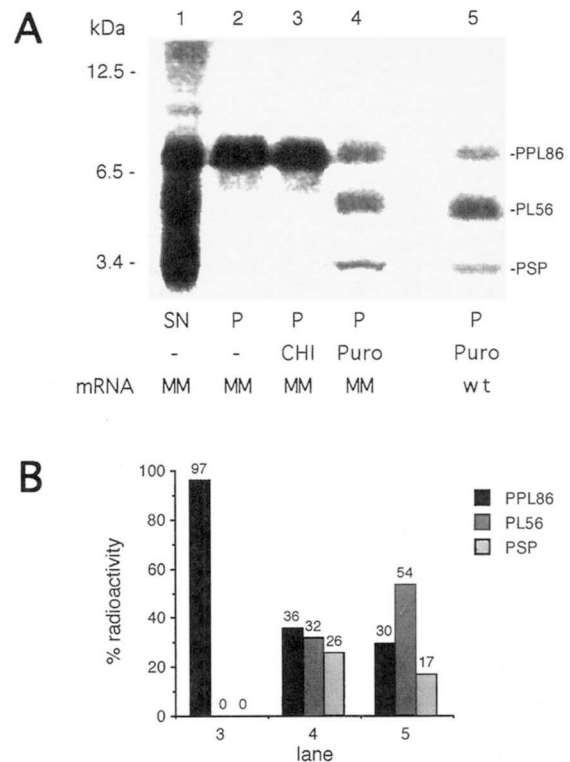


FIG. 2. Puromycin release of ribosome-bound PPL86. *A*, PPLMM86 mRNA (*lanes 1–4*) or PPL86 mRNA (*lane 5*) was translated in the presence of SRP and RM. After translation, membranes were sedimented through a high salt sucrose cushion. The supernatant (*SN*, *lane 1*) was removed, and the pellet (*P*, *lane 2*) was resuspended and incubated with 4 mM cycloheximide (*CHI*, *lane 3*) or 1 mM puromycin (*Puro*, *lane 4*). *B*, quantification of the results shown in *panel A*, *lanes 3–5*. The amount of radioactivity in the area of PPL86, PL56, and PSP was determined by using the phosphoimager as described under “Experimental Procedures.” The amount of radioactivity in unprocessed PPL86 (*A*, *lane 2*) was taken as 100%.

cent chains remain bound to the ribosomes as peptidyl-tRNA (Gilmore *et al.*, 1991). When translation takes place in the presence of SRP and rough microsomes, nascent chains become functionally inserted into translocation complexes. Since ribosome-bound, membrane-inserted PPL86 is too short to be cleaved by signal peptidase, it accumulates in its unprocessed form. Unrelated polypeptides that are also present in our translocation assay and interfere with the analysis of the signal peptide were removed by sedimenting the microsomes through a high salt sucrose cushion (Fig. 2A, *lanes 1 and 2*).

When nascent chains are released from the ribosomes by puromycin, they become translocated across the microsomal membrane (Redman and Sabatini, 1966), and their signal sequence is cleaved. Accordingly, we detected two peptides with apparent molecular masses of about 5 and 3 kDa (Fig. 2A, *lanes 4 and 5*) representing the mature part of PPL86 (PL56) generated by signal sequence cleavage between amino acid residues 30 and 31 (Sasavage *et al.*, 1982) and the processed signal peptide (PSP), respectively. Processing was only observed when puromycin was added and did not occur in the presence of cycloheximide (Fig. 2A, *lane 3*). The ratios of label in the 5- and 3-kDa peptides were roughly 1:1 for the MM mutant (Fig. 2B, *lane 4*) and 3:1 for the wild type (Fig. 2B, *lane 5*). These ratios are consistent with the methionine content of PL56 (three methionines) and of the processed signal peptide (three methionines in MM mutant, one in wild type).

Localization of the Processed Signal Peptide—To determine whether the processed signal peptide is exposed to the cytoplasmic surface of microsomes, we treated translocation assays

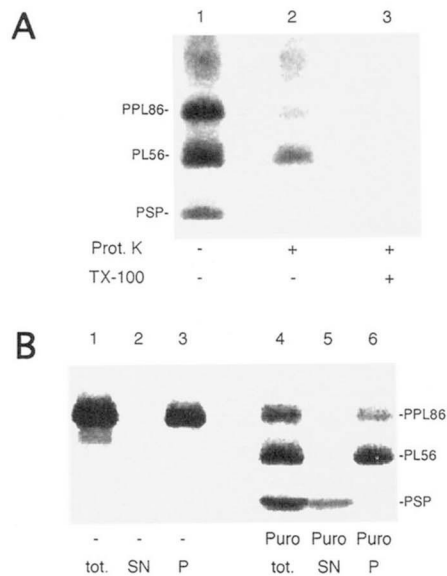


FIG. 3. Localization of the processed signal peptide. *A*, proteinase K treatment of microsomal membranes. Microsomes containing puromycin-released PPLMM86 (lane 1) were incubated with proteinase K in the absence (lane 2) or presence (lane 3) of Triton X-100. *B*, pelleting of microsomes. Assays containing rough microsomes and membrane-inserted PPLMM86 were centrifuged directly (lanes 1–3) or after treatment with puromycin (lanes 4–6) and separated into supernatant (lanes 2 and 5) and pellet (lanes 3 and 6).

with proteinase K. As can be seen in Fig. 3*A*, lane 2, PL56 but not the PSP was protected against proteolysis. This indicates that PL56 was translocated into the lumen of the microsomes, whereas the PSP was in the cytosol or on the cytoplasmic surface of the membranes.

When membranes were pelleted after puromycin release, the PSP was found in the supernatant (Fig. 3*B*, lane 5), whereas PL56 was found in the microsomal pellet (Fig. 3*B*, lane 6). We therefore conclude that the PSP is released into the cytosol.

Characterization of the Processed Signal Peptide—To determine the apparent size of the processed signal peptide, we synthesized marker peptides representing the intact PPL signal peptide (30 amino acid residues) or amino-terminal fragments of 24 and 17 amino acid residues, respectively. Parallel analysis of these peptides with puromycin-released wild type PPL86 (Fig. 4*A*) revealed that the PSP is not the intact signal peptide but only a fragment of about 20 amino acid residues. Since the only methionine in the wild type PPL signal sequence is the initiating methionine, the fragment must be COOH-terminally truncated.

This conclusion was confirmed by labeling PPL86 with [³⁵S]cysteine. The signal sequence of PPL contains one cysteine at position 25, and PL56 contains two cysteines. If the signal peptide were truncated amino-terminally of cysteine 25, the PSP should not be detectable on gels. Indeed, after puromycin treatment of cysteine-labeled PPL86, PL56 accumulated, whereas the PSP remained invisible (Fig. 4*B*, lane 2).

Time Dependence of Signal Sequence Processing and Release—To investigate the time dependence of signal sequence processing, we performed a pulse-chase experiment. Nascent chains were released from the ribosomes by the addition of puromycin followed by an incubation at 25 °C. At the time points indicated, aliquots were taken and frozen in liquid nitrogen. Samples were then thawed on ice, separated into supernatant and pellet, and characterized by SDS-polyacrylamide gel electrophoresis (Fig. 5). 1 min after the addition of puromycin, PL56 and two small peptides of about 3 kDa were detected in the microsomal pellet (Fig. 5, lane 4). Due to its

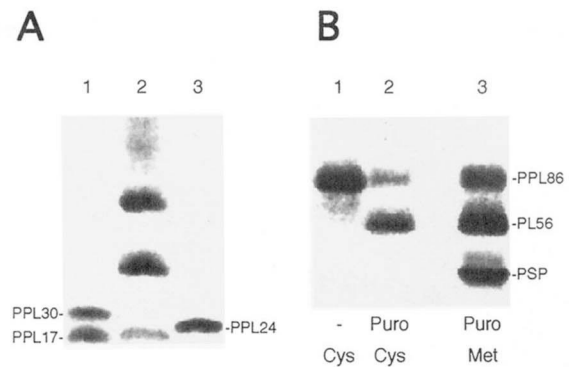


FIG. 4. Characterization of the processed signal peptide. *A*, parallel electrophoretic analysis of puromycin-released wild type PPL86 (lane 2) and marker peptides (lanes 1 and 3). *B*, cysteine labeling of PPL86 and its processed products. PPLMM86 mRNA was translated in the presence of [³⁵S]cysteine (lanes 1 and 2) or [³⁵S]methionine (lane 3). The assay was performed as described for Fig. 2*A*.

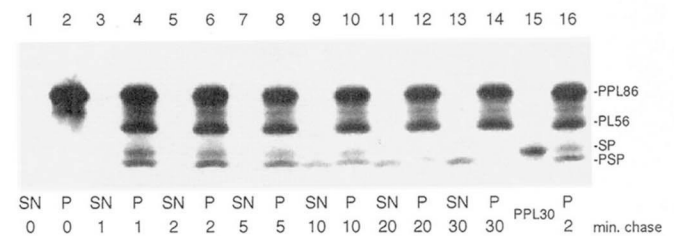


FIG. 5. Pulse-chase experiment. Nascent PPL86 was released from the ribosomes by puromycin and incubated at 25 °C. Aliquots were taken at the time points indicated and separated into supernatant (SN) and pellet (P). Lane 15 shows *in vitro* synthesized intact signal peptide (PPL30).

apparent comigration with *in vitro* synthesized intact signal peptide (Fig. 5, lane 15), we designated the slower migrating peptide signal peptide (SP). Over time, the amount of the SP decreased, whereas the PSP became the predominant small processing product, which was gradually released into the supernatant (Fig. 5, lane 13). This suggests that the cleaved signal peptide is processed to the PSP during the chase. Furthermore, the PSP is retained in the microsomes for several minutes prior to its release into the cytosol.

Signal Sequence Processing in Reconstituted Vesicles—Neither the incubation of translocation assays with several standard protease inhibitors (phenylmethylsulfonyl fluoride, EDTA, chymostatin, leupeptin, aprotinin, pepstatin) nor the use of content-depleted microsomes affected the generation and size of the PSP (data not shown). The latter finding suggests that the signal peptide processing activity resides in the membrane fraction of microsomes. To characterize this activity, we used reconstituted vesicles containing the minimal components for PPL translocation, Sec61 and SRP receptor (Görlich and Rapoport, 1993), and the microsomal SPC. Proteoliposomes were reconstituted from purified Sec61 complex, purified SRP receptor, purified SPC, and a mixture of phospholipids. The SPC preparation used for reconstitution shows the characteristic composition with proteins of 12, 18, 21, 22/23, and 25 kDa (Evans *et al.*, 1986) (Fig. 6*A*). Reconstituted vesicles were tested by *in vitro* translation of PPL in the presence of SRP (Fig. 6*B*). Posttranslational treatment of the assay mixture with proteinase K (Fig. 6*B*, lanes 4–6) revealed protection of processed PPL against proteolytic digestion (Fig. 6*B*, lane 6). This proves the reconstituted vesicles to be competent for membrane translocation and signal sequence cleavage.

To assay for signal peptide processing by the reconstituted vesicles, we used membrane-inserted and puromycin-released

PPL86. When reconstituted vesicles containing only Sec61 and SRP receptor were used, no processing of PPLMM86 was observed (Fig. 7A, lane 1). When reconstituted vesicles containing Sec61, SRP receptor, and SPC were used, a peptide of about 5 kDa and, similar to early steps in microsomes (Fig. 5, lane 4), two peptides of about 3 kDa were generated (Fig. 7A, lane 2). The 5-kDa peptide and the faster migrating 3-kDa peptide comigrated with PL56 and the PSP, respectively (Fig. 7B, lanes 4 and 5). However, the SP* is not the intact signal peptide, as it exhibits a slightly higher electrophoretic mobility when compared to *in vitro* synthesized intact signal peptide (PPL30, Fig. 7C). Thus, reconstituted vesicles can process the signal sequence; however, the processing sites differ from those in microsomes. Furthermore, the considerable amount of SP* suggests that signal peptide processing is inefficient in reconstituted vesicles.

When reconstituted vesicles were pelleted after puromycin treatment, the PSP was found in the supernatant (Fig. 8, lane 5), whereas PL56 and the SP* were detected in the pellet (Fig. 8, lane 6). This suggests that the PSP is released into the cytosol by reconstituted vesicles, whereas the SP* is associated with the membranes.

DISCUSSION

The characterization of the fate of signal sequences after their cleavage from nascent polypeptides is difficult for several reasons: 1) no antibodies could yet be raised against any signal peptide, and thus their identification presents a major problem;

2) signal peptides are small and have to be distinguished from small peptides accumulating in translation systems as a result of premature chain termination; 3) signal peptides can usually not be labeled efficiently as they contain only few methionine or cysteine residues for labeling with ³⁵S; and 4) signal peptides are probably very rapidly processed, making it necessary to identify also fragments derived from processing reactions.

Signal Sequence Processing in Microsomes—The *in vitro* translocation/processing system we describe here makes use of a mutant secretory protein with two additional methionine residues within its signal sequence. This allowed a comparatively easy detection of the processed signal peptide and provided circumstantial evidence for its identity by comparison to the wild type peptide. To overcome the problem with unrelated small peptides in the reaction mixture, we used a two stage *in vitro* system. In the first stage, nascent PPL86 was inserted into membranes with signal peptidase being unable to cleave the signal sequence. Membrane-inserted chains were purified by centrifugation, thus leaving unrelated small peptides in the supernatant. In a second reaction, translocation and signal peptide cleavage were induced by liberation of the nascent chains from the ribosomes by puromycin. This allowed also the synchronization of the translocation and processing reactions.

The PPL signal peptide fragment generated by microsomes and released into the cytosol comprises the amino-terminal, roughly 20 amino acid residues of the signal sequence. The approximate size was estimated from a comparison with defined amino-terminal PPL signal peptide fragments as standards. According to our size estimation, cleavage occurs between the two leucine clusters in the middle of the hydrophobic core of the PPL signal peptide (see Fig. 1A). This indicates that the mammalian signal peptide peptidase like its *E. coli* counterpart cleaves the signal peptide in the hydrophobic core.

After short incubation with puromycin, the apparently intact SP accumulated and was processed to the PSP over time. Initially, the SP as well as the PSP were associated with the microsomes. Only after prolonged incubation, the PSP was released into the supernatant, whereas the SP stayed in the pellet. This suggests that cleavage within the hydrophobic core of the signal peptide is required although not sufficient for its release from the membrane and indicates that the PSP undergoes a time-dependent release into the cytosol. The release of the PSP might be important for subsequent polypeptide translocation across the membrane, a notion being supported by the finding that inhibition of *E. coli* signal peptide peptidase results in inhibition of translocation (Chen and Tai, 1989). Degradation of signal peptides might thus contribute to the maintenance of fast and efficient protein translocation across the ER membrane.

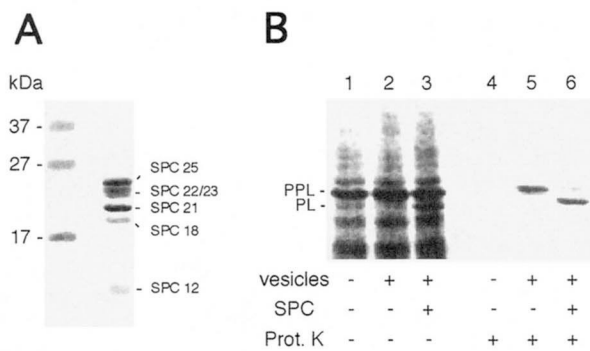
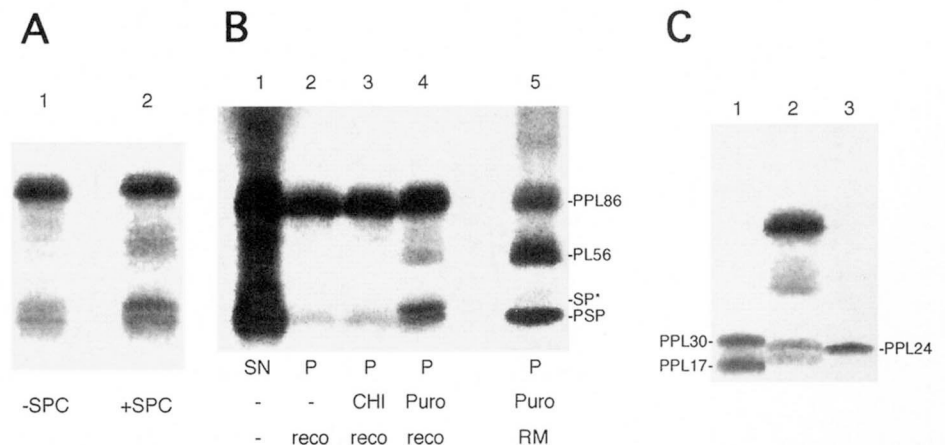


FIG. 6. Membrane translocation and signal sequence cleavage by reconstituted vesicles. A, protein composition of the SPC preparation used for reconstitution. The proteins were separated on a 16% acrylamide gel and stained with Coomassie Brilliant Blue. B, translocation into reconstituted vesicles. PPL mRNA was translated in the presence of SRP and membrane buffer (lanes 1 and 4) or reconstituted vesicles containing Sec61 and SRP receptor (lanes 2 and 5) or reconstituted vesicles containing Sec61, SRP receptor, and SPC (lanes 3 and 6). After translation, half of the samples were treated with proteinase K (lanes 4–6).

FIG. 7. Processing of PPL86 by reconstituted vesicles. A, puromycin release of PPLMM86 bound to reconstituted vesicles with (lane 2) or without (lane 1) SPC. B, PPLMM86 mRNA was translated in the presence of SRP and reconstituted vesicles containing Sec61, SRP receptor, and SPC (lanes 1–4) or rough microsomes (lane 5). After translation, membranes were sedimented through a high salt sucrose cushion. The supernatant (lane 1) was removed, and the pellet (lane 2) was resuspended and incubated with 4 mM cycloheximide (lane 3) or 1 mM puromycin (lane 4). C, comparison of puromycin-released PPLMM86 from reconstituted vesicles (lane 2) with marker peptides (lanes 1 and 3).



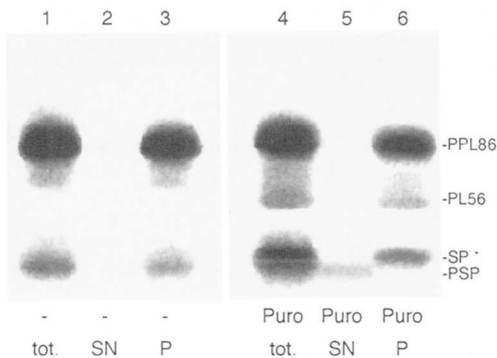


FIG. 8. **Localization of processed signal peptides in reconstituted vesicles.** Assays containing reconstituted vesicles and membrane-inserted PPLMM86 were centrifuged directly (lanes 1–3) or after treatment with puromycin (lane 4–6) and separated into supernatant (lanes 2 and 5) and pellet (lanes 3 and 6).

It has been shown before that the addition of prepromelittin to vesicles reconstituted from rat liver microsomes resulted in the generation of the intact signal peptide (Mollay *et al.*, 1982). This finding does not contradict our results, as prepromelittin signal sequence cleavage occurred from polypeptides not inserted into the membrane, and signal peptides accumulating in the cytosol could have escaped further processing by signal peptide peptidase. Furthermore, an extraction procedure was used that specifically selects for hydrophobic peptides (Mollay *et al.*, 1982), thus excluding processed signal peptide fragments from detection.

Signal Sequence Processing in Reconstituted Vesicles—Signal sequence cleavage has been previously investigated with detergent-solubilized SPC. In contrast to these studies, we used here a reconstituted system where ribosome-attached nascent chains are functionally inserted into membranes prior to cleavage by the SPC. This system maintains the spatial arrangement of the nascent polypeptide at the translocation site, and it can therefore be expected to reproduce the sequentially occurring events of membrane translocation and signal sequence processing. Using reconstituted vesicles we observed signal sequence cleavage as previously described (Görlich and Rapoport, 1993). However, in contrast to microsomes, we did not obtain the predicted intact signal peptide of 30 amino acid residues (Sasavage *et al.*, 1982) but a slightly shorter fragment (SP*). Most likely, this is due to signal peptidase cleavage at a second site between amino acid residues 25 and 26, which is also predicted by the (–1, –3) rule (von Heijne, 1983).

Reconstituted vesicles containing SPC were able to process the signal peptide to the PSP, which was released into the supernatant. This suggests that the reconstituted vesicles containing SPC contained also signal peptide peptidase activity. Signal peptide processing was, however, found to be less efficient with reconstituted vesicles than with microsomes. Whether the signal peptide peptidase is one of the known subunits of the SPC or partially copurified with one of the components used for reconstitution remains to be determined. Signal peptide peptidase from *E. coli* has been characterized before (Ichihara *et al.*, 1986). It shows no homologies to any one of the SPC subunits (Shelness *et al.*, 1988; Greenburg *et al.*, 1989; Shelness and Blobel, 1990; Greenburg and Blobel, 1994). This includes SPC12, of which the cDNA has been recently cloned and sequenced.²

The Fate of Signal Peptide Fragments—Our data suggest that the amino-terminal PPL signal peptide fragment is re-



FIG. 9. **Compilation of so far characterized signal peptide fragments.** The localization of the fragments is indicated below the description of the source signal peptide. A, signal peptide fragments found associated with MHC class I molecules. Data are from Wei and Cresswell (1992) and Henderson *et al.* (1992). B, signal peptide fragment characterized in this study (dots indicate that the COOH-terminal end of the fragment is estimated from comparison with marker peptides) (see Fig. 4A).

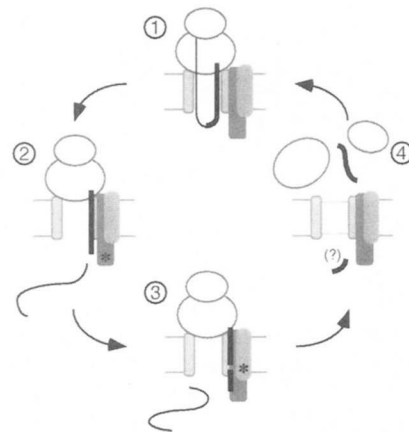


FIG. 10. **Schematic illustration of signal sequence cleavage and processing in rough microsomes.** After membrane insertion of the nascent chain (1), the signal sequence is cleaved by signal peptidase (2), and the signal peptide is further processed by signal peptide peptidase (3). The signal peptide fragments are then released from the translocation complex either to the cytosol or into the ER lumen (4) to allow a new round of translocation. Proteins at the translocation site are shaded, and enzyme activity is indicated by asterisks.

leased into the cytosol, where it may be further processed or degraded by the proteasome, a large multicatalytic protease (Goldberg and Rock, 1992). Since the COOH-terminal PPL signal peptide fragment is too small to be resolved by our gel system, we could not investigate its fate. For the following reason, however, we can assume that COOH-terminal signal peptide fragments are released into the ER lumen. Nascent presecretory proteins are thought to span the membrane in a loop-like conformation with the amino terminus of the signal sequence facing the cytoplasm and the cleavage site for signal peptidase facing the ER lumen (discussed in High and Dobberstein, 1992). Upon cleavage of the signal peptide within its hydrophobic core, the amino- and COOH-terminal fragments may be released to the sites they are exposed to, the cytosol and the lumen, respectively. Furthermore, fragments of the signal peptides from the interferon-inducible protein with a molecular mass of 30 kDa (IP-30) and from calreticulin have been found associated with MHC class I molecules in cells lacking TAP transporters (Wei and Cresswell, 1992; Henderson *et al.*, 1992). TAP transporters are thought to transport peptides generated in the cytosol into the ER lumen. In the presence of the TAP transporters, peptides of cytosolic origin are found associated with MHC class I molecules. Thus, if fragments derived from signal peptides are associated with MHC class I molecules in TAP-deficient cell lines, they are probably released into the ER lumen after their cleavage in the membrane. Like the PPL

² E. Hartmann, S. Prehn, K.-U. Kalies, and T. A. Rapoport, unpublished results.

signal peptide fragment, they result from cleavage in the middle of the hydrophobic core of the signal sequence (Fig. 9). The amino-terminal signal peptide fragment also found among the peptides bound to MHC class I molecules is unusual as it lacks charged amino acid residues. Probably, the hydrophobic nature of this fragment caused its release into the ER lumen.

Steps in Signal Sequence Cleavage and Processing—We can distinguish at least three distinct steps in the signal sequence processing reaction (Fig. 10). 1) after membrane insertion of the nascent chain, the signal sequence is cleaved by signal peptidase; 2) the cleaved signal peptide is further processed by signal peptide peptidase; and 3) the signal peptide fragments are released from the membrane, either into the cytosol or into the ER lumen, where they could be further processed or completely hydrolyzed. The release of the signal peptide allows the clearance of the translocation complex and the subsequent insertion of a new polypeptide chain. This outline shows principal similarities to the corresponding processes in *E. coli*. Here, leader peptidase cleaves the signal sequence from the precursor protein. Processing of signal peptides is initiated by a membrane-bound signal peptide peptidase (protease IV), and the signal peptide fragments are released into the cytosol where they are degraded by cytosolic proteases.

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