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Wild Type and Mutant Signal Peptides of *Escherichia coli* Outer Membrane Lipoprotein Interact with Equal Efficiency with Mammalian Signal Recognition Particle*

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The signal peptide of the outer membrane lipoprotein (OMLP) of *Escherichia coli* was shown to be capable of promoting protein translocation across mammalian microsomal membranes *in vitro*. We assayed translocation of a fusion protein containing the OMLP signal peptide and nine amino acids of OMLP fused in frame to β-lactamase. The efficiency with which the mammalian translocation machinery recognizes and accepts the OMLP signal peptide as substrate is indistinguishable from that of mammalian secretory proteins. Upon translocation mammalian signal peptidase processes the pre-OMLP-β-lactamase protein at different sites than are utilized *in vivo* by *E. coli* OMLP signal peptidase (signal peptidase II) but that can be predicted as mammalian signal peptidase cleavage sites.

Mutants in the OMLP signal peptide were tested for their ability to promote translocation of the fusion protein in this assay system. It has been shown previously that mutants in the positively charged amino acids at the amino terminus of the signal peptide severely delay the translocation of OMLP *in vivo* in *E. coli*. However, these mutants had no detectable effect either on signal recognition by mammalian signal recognition particle or on the efficiency of translocation itself.

Secretory, lysosomal, and most integral membrane proteins contain peptide sequences that act as signals for their specific translocation across the membrane of the endoplasmic reticulum (ER[1] (1)). Usually, the signal peptides are cleaved by signal peptidase located in the luminal side of the ER membrane during or immediately after translocation (2). Biochemical dissection of canine ER fractions capable of *in vitro* protein translocation has led to the characterization of the signal recognition particle (SRP (3)) and the SRP receptor (4–8). These two components function to target nascent secretory proteins to the ER membrane (1) and to initiate the translocation process. SRP has high affinity for ribosomes engaged in synthesis of secretory proteins (9) and upon binding causes pausing of protein synthesis (elongation arrest (10)). Upon interaction of the SRP-ribosome-nascent chain complex with the SRP receptor in the ER membrane, the SRP-dependent arrest is released and the ribosomes engage in a functional ribosome-membrane junction (7, 8, 11). Subsequent translocation of the protein across the membrane proceeds (most likely coupled to translation) by an essentially unknown mechanism.

Several observations indicate that the prokaryotic protein translocation machinery may function by a mechanism similar to that described for the translocation of secretory proteins across the mammalian endoplasmic reticulum membrane. (*a*) Prokaryotic secretory and some integral membrane proteins contain signal peptides that have similar features to that of eukaryotic proteins (12, 13). (*b*) The signal peptide of β-lactamase (a periplasmic enzyme) requires SRP and SRP receptor to be translocated across canine ER membranes *in vitro*, and the translocated protein is correctly processed by the mammalian signal peptidase (14). (*c*) Expression of β-lactamase in *Xenopus* oocytes results in secretion of the correctly processed enzyme (15), indicating that the signal peptide of this bacterial protein is recognized *in vivo* by the eukaryotic translocation machinery. (*d*) The expression in *Escherichia coli* of eukaryotic secretory proteins containing their natural signal peptides results in many cases in localization of the mature protein into the periplasmic space (16–18). (*e*) *In vitro* translocation systems have been developed recently using inverted plasma membrane vesicles from *E. coli* (19, 20). Such a system has been used to show that a soluble factor that can be separated from the membranes is required for their translocation activity (21). Such a factor may act similarly to SRP in that it may somehow prevent precursor proteins from assuming a tertiary structure that would then be incompatible with translocation (47).

In spite of these similarities, prokaryotic and eukaryotic protein secretion appears to differ in the degree of coupling between translation and translocation. Protein translocation in prokaryotic cells can occur post-translationally for many proteins. In contrast, secretory proteins in mammalian cells are translocated while they are synthesized on membrane-bound ribosomes. Recently, however, it was shown in a variety of systems that eukaryotic ER is in principle capable of accepting post-translationally certain proteins as translocation substrates (22–25). Thus, although eukaryotic protein translocation is generally a co-translational event that is tightly coupled to translation, the coupling of the two processes is not a strict prerequisite for translocation.

The evolutionary functional conservation of signal peptides raises the question about the molecular nature of the infor-

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[1] The abbreviations used are: ER, endoplasmic reticulum; SRP, signal recognition particle; OMLP, outer membrane lipoprotein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

mation contained within them, since no primary sequence homology is apparent (12). Compilations of known signal sequences reveal features that appear to be conserved (12, 13, 44, 46). (i) One to three basic amino acids are usually found at the amino terminus of the signal peptide; (ii) 10–15 hydrophobic amino acids follow these basic amino acids; and (iii) amino acids of small side chain (alanine or glycine) are preferentially found at positions −1 and −3 of the signal peptidase cleavage site. Recently, Kaiser *et al.* (48) showed that a remarkable number of random amino acid sequences can function as signal peptides *in vivo*, albeit most at reduced efficiency. Nevertheless, it appears that the specificity with which these peptides are recognized must be surprisingly low and seems to correlate primarily to the overall hydrophobicity (48). Thus, it would seem that other conserved features of signal peptides may indeed be dispensable and may at most add fidelity to the process.

To test specifically which of the conserved features of the signal peptides are relevant to their function, mutations in the signal peptide of the *E. coli* outer membrane lipoprotein (OMLP) have been constructed, and their *in vivo* effects have been determined (26–32). Replacement of the positively charged amino acids of the signal peptide with acidic amino acids, although not completely abolishing translocation, results in accumulation of the precursor in the cytoplasm (26, 27). This cytoplasmic pre-OMLP can be post-translationally translocated across the plasma membrane with a considerable delay relative to the wild type protein (26, 27). Most of the eukaryotic signal peptides contain positively charged amino acids in their amino terminus. However, in contrast to the prokaryotic signal peptides, some of them contain acidic amino acids resulting in a signal peptide with a negatively charged amino terminus (44). This fact raises questions about the functional importance of these charges for protein translocation across eukaryotic ER membranes. To address this question, we have characterized and quantitated the efficiency by which the eukaryotic translocation machinery recognizes OMLP signal peptide mutants.

MATERIALS AND METHODS

Construction of the Plasmids—The construction of a plasmid containing the coding sequences for the signal peptide and the first nine amino acids of the OMLP fused in frame to the coding sequences for mature β-lactamase has been described elsewhere (33). An *Xba*I-*Bam*HI fragment of plasmid pJG300 (33) that contains the coding sequences for the wild type or alternatively mutations in the signal peptide of this fusion protein was inserted between the *Xba*I and *Bam*HI site of the pSP64 vector (34). The resulting plasmids contain the fusion gene in the correct orientation for transcription from the SP6 phage promoter.

Transcription by the SP6 Phage RNA Polymerase—The plasmids were linearized with *Bam*HI and transcribed in 20-μl reactions containing 40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine, 0.5 mM each ATP, CTP, and UTP, 0.1 mM GTP, 0.5 mM G(5′)ppp(5′)G, 10 mM dithiothreitol, 1000 units/ml human placental ribonuclease inhibitor, 0.1 mg/ml linearized plasmid, and 500 units/ml SP6 RNA polymerase. The reactions were incubated at 40 °C for 60 min and were terminated by phenol-chloroform extraction. Nucleic acids were ethanol precipitated, and the resulting pellet was dissolved in 40 μl of water.

In Vitro Translation and Translocation Assays—Wheat germ translation extracts were prepared as described by Erickson and Blobel (35). Pancreatic microsomal vesicles (K-RM) were depleted of SRP and ribosomes by EDTA and salt extraction as described (36). SRP was prepared from canine pancreas as previously described (37). Translation was performed at 26 °C for 1 h as reported (35), except that the magnesium concentration was found optimal at 3.5 mM and that 0.002% Nikkol detergent (octaethylene glycol *n*-dodecyl ether) was included to stabilize SRP activity (3). RNA transcripts of 50 ng of plasmid (contained in 1 μl) were translated in a 25-μl reaction containing 25 μCi of [^{35}S]methionine. Translation products were visualized after overnight exposure after SDS-polyacrylamide gel electrophoresis.

Microsome Sedimentation Assay—Translocation of the processed form of OMLP-β-lactamase was assayed by cosedimentation of this protein with the microsomal membranes. After translation the reactions were transferred to an ice-water bath, and KOAc was added to a concentration of 500 mM. Ten equivalents of K-RM (36) were added as carrier membranes. The reactions were layered on a 100-μl cushion containing 0.5 M sucrose, 0.5 M KOAc, and 2 mM $Mg(OAc)_2$. After centrifugation at 30 p.s.i. in a Beckman Airfuge for 5 min, the proteins from the supernatant and the pellet were trichloroacetic acid-precipitated and subjected to SDS-PAGE and autoradiography. This sedimentation assay was preferable over proteolytic protection assays (2), due to the intrinsic protease resistance of mature β-lactamase (14).

SRP Arresting and Translocation Efficiency Assays—For quantitative interpretation of the SRP-dependent elongation arrest and translocation efficiency, globin mRNA was included as an internal standard of a protein whose translation is not affected by SRP (10). Bands corresponding to the precursor and processed forms of the OMLP-β-lactamase protein and globin were quantified by densitometric scanning of the preflashed autoradiograms. To measure elongation arrest activity, the percentage of synthesis at a SRP concentration equal to (A) was determined by

$$\% \text{ synthesis} = \frac{\text{pre-OMLP-}\beta\text{-lactamase } (A) \times \text{globin } (O)}{\text{pre-OMLP-}\beta\text{-lactamase } (O) \times \text{globin } (A)} \times 100$$

where pre-OMLP-β-lactamase (A) and globin (A) correspond to the intensities of the bands of these proteins made at a SRP concentration equal to A, and pre-OMLP-β-lactamase (O) and globin (O) correspond to the intensities of the bands of these proteins made in the absence of SRP. To quantitate translocation efficiency, the percentage of signal peptide processing at a given SRP concentration (A) was determined relative to the total pre-OMLP-β-lactamase synthesis when no SRP was added. The factor 10/9 was included because one of the 10 methionine residues of pre-OMLP-β-lactamase is removed upon processing. For this calculation the following equation was used.

$$\% \text{ translocation} = \frac{10/9 \text{ OMLP-}\beta\text{-lactamase } (A) \times \text{globin } (O) \times 100}{[\text{pre-OMLP-}\beta\text{-lactamase } (O) + 10/9 \text{ OMLP-}\beta\text{-lactamase } (O)] \times \text{globin } (A)}$$

Note that this term is different from the one previously used to measure the efficiency of translocation (38). Because we determine the percentage of translocated protein relative to the total amount of protein synthesized in the absence of SRP (rather than the total protein made in each reaction, *i.e.* in the presence of various SRP concentrations), this new term reflects more accurately the translocation activity of a given amount of membranes at different SRP concentrations. The distortion of the measurements produced by the translational arrest of the precursor protein observed at high SRP concentration is thereby eliminated.

RESULTS

The signal peptide of the *E. coli* OMLP has all the features commonly found in both eukaryotic and prokaryotic signal sequences (12, 13). Using a standard *in vitro* protein translocation assay, we tested if this prokaryotic signal peptide can be properly recognized by the eukaryotic translocation machinery. In order to avoid complications due to the small size of authentic pre-OMLP (58 amino acids plus the 20-amino acid signal peptide, *i.e.* the signal peptide would just be barely exposed outside the eukaryotic ribosome by the time protein synthesis is terminated), we chose to study the function of the OMLP signal peptide as part of a larger fusion protein. An *in vitro* synthesized mRNA (34) encoding a fusion protein of the signal peptide and the first 9 amino acids of OMLP fused to β-lactamase (pre-OMLP-β-lactamase (33)) was translated in a wheat-germ cell-free extract (Fig. 1, *lane 2*). Addition of salt-extracted microsomal membranes (K-RM) in the presence (Fig. 1, *lane 5*), but not in the absence (Fig. 1, *lane 3*), of SRP led to the formation of an additional band of

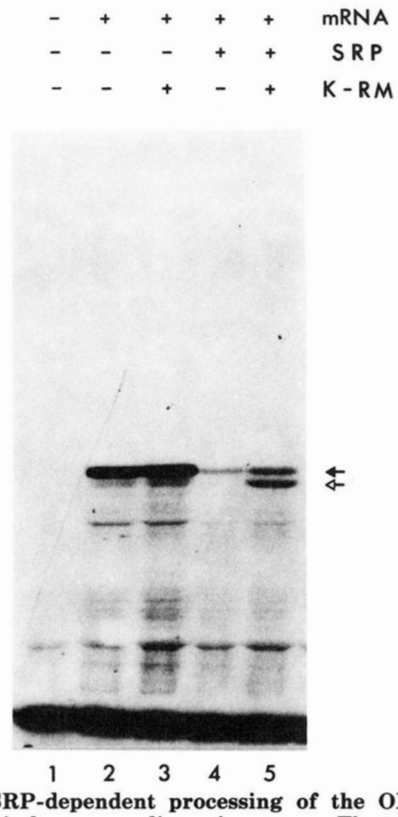

FIG. 1. **SRP-dependent processing of the OMLP-β-lactamase protein by mammalian microsomes.** The translation reactions were carried out in a 25-μl volume. Transcripts from 50 ng of the plasmid were added per reaction. SRP (about 7 nM) and K-RM (1 eq) were added in the indicated reactions. *Lane 1,* no exogenous RNA was included; *lane 2,* only RNA was added; *lane 3,* RNA and K-RM were added; *lane 4,* RNA and SRP were added; *lane 5,* RNA, SRP, and K-RM were added. The *full* and *open arrows* indicate the bands corresponding to the precursor (32 kDa) and the processed form (30 kDa) of the OMLP-β-lactamase protein, respectively.

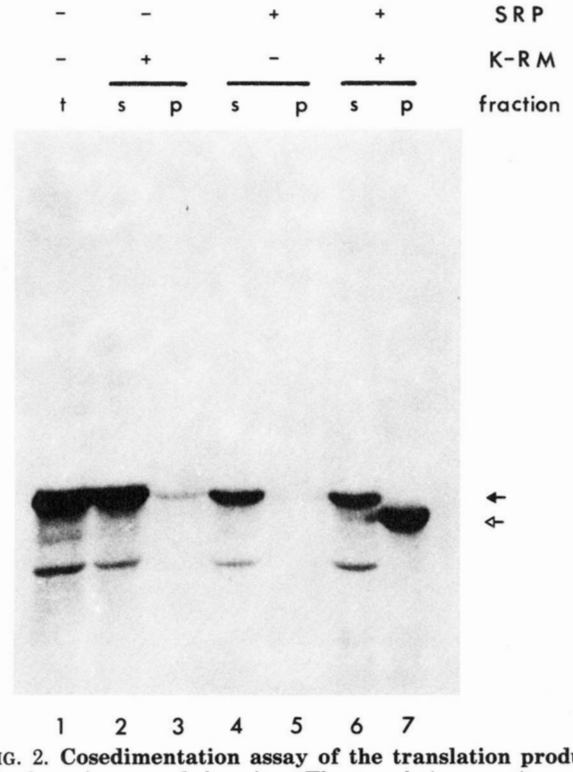

FIG. 2. **Cosedimentation assay of the translation products with the microsomal fraction.** The translation reactions were performed as indicated in the legend to Fig. 1. After translation, the microsomal and soluble fractions were obtained as indicated under "Materials and Methods." *t* indicates the total translation products, *s* indicates the supernatant (soluble fraction), and *p* indicates the pellet (microsomal fraction). The presence of small quantities of pre-OMLP-β-lactamase in the pellet fractions (*lanes 3* and *5*) is not reproducible and probably corresponds to unspecific sticking to the walls of the centrifuge tubes.

lower molecular weight. Both the primary translation product and the lower molecular weight band can be immunoprecipitated by anti-β-lactamase antibodies (data not shown), indicating that the latter corresponds to a processed form of pre-OMLP-β-lactamase protein. Addition of SRP in the absence of microsomal membranes (Fig. 1, *lane 4*) led to a substantial decrease in pre-OMLP-β-lactamase synthesis due to elongation arrest by SRP.

To verify that the processed form of OMLP-β-lactamase is indeed translocated across the membrane of the microsomal vesicles, the translocation reactions were fractionated by sedimentation prior to SDS-PAGE analysis. Translation reactions performed in the absence or presence of SRP and/or K-RM were centrifuged as described under "Materials and Methods." Fig. 2 shows an analysis of the pellets and supernatants by SDS-PAGE. When both SRP and K-RM were included in the translation, the processed form of the fusion protein pelleted quantitatively with the microsomal membranes (Fig. 2, *lane 7*), while, as expected, the precursor protein remained in the supernatant (Fig. 2, *lane 6*). Pre-OMLP-β-lactamase also remained in the supernatant fractions if K-RM (Fig. 2, *lanes 2* and *3*) or SRP (Fig. 2, *lanes 4* and *5*) was added independently. Taken together the results of Figs. 1 and 2 demonstrate that (i) the signal peptide of pre-OMLP is recognized by mammalian SRP and (ii) the OMLP-β-lactamase fusion protein is properly targeted to and translocated into the lumen of the microsomal vesicles where it appears proteolytically processed by signal peptidase.

Most *E. coli* periplasmic and membrane proteins are processed by a signal peptidase (signal peptidase I) that is very similar in its specificity to the mammalian counterpart (39). In contrast, processing of pre-OMLP is coupled to the addition of a fatty acid moiety to OMLP (40), and the proteolytic cleavage step is performed by a specialized signal peptidase (signal peptidase II (39)). This peptidase is sensitive to the peptide antibiotic globomycin (41). To test whether a mammalian counterpart of signal peptidase II exists we added globomycin to the *in vitro* translocation assay. Even at high concentrations (30 μg/ml) of globomycin, no inhibition of the processing of pre-OMLP-β-lactamase was observed (data not shown; 1 μg/ml globomycin completely inactivate *E. coli* signal peptidase II (41)).

To determine the position of the cleavage site in OMLP-β-lactamase by the mammalian signal peptidase, we sequenced a sample of the processed protein. Translation products labeled with [^{3}H]proline in the presence of SRP and K-RM were resolved by SDS-PAGE. The processed form of OMLP-β-lactamase was electroeluted from the gel (42) and subjected to sequential Edman degradation in a gas phase sequenator. Fig. 3*b* shows that peaks of released radioactivity were obtained at cycles 7, 9, 10, and 12. Peaks 7 and 10 (labeled with *closed arrows* in Fig. 3) and peaks 9 and 12 (labeled with *open arrows*) can be aligned with prolines +12 and +15 in OMLP-β-lactamase (labeled with *stars* in Fig. 3*a*). These results indicate that mammalian signal peptidase cleaves at two positions in the OMLP-β-lactamase protein: between serine +3 and asparagine +4, and between alanine +5 and lysine +6 (see Fig. 3*a*). The first cleavage position is somewhat preferred (note that the peak in cycle 9 is larger than that in cycle 7). The processed form of OMLP-β-lactamase is, therefore, a

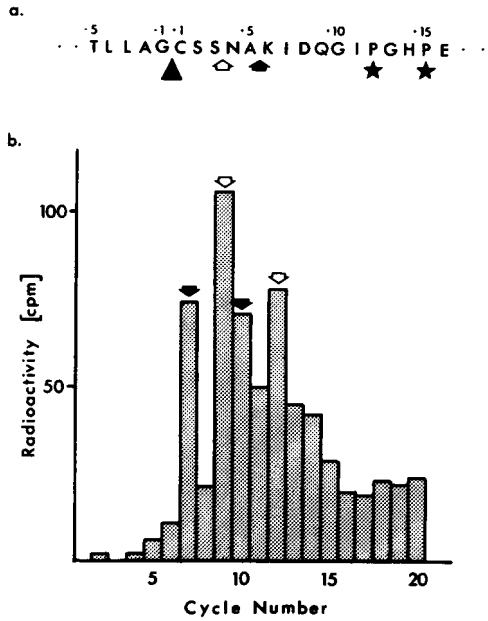

FIG. 3. **Localization of the signal peptidase cleavage site.** *a*, sequence of the OMLP-β-lactamase protein around the signal peptidase cleavage site. The first amino acid of the mature OMLP is indicated as +1. The *full triangle* shows the positions of the cleavage by the OMLP signal peptidase (signal peptidase II). The *full* and *empty arrows* indicate the two cleavage sites by the mammalian signal peptidase. The *stars* show the positions of the two radiolabeled prolines detected in the Edman degradation cycles (*b*). *b*, Edman degradation analysis of the processed form of the OMLP-β-lactamase protein. The [^{3}H]proline-radiolabeled processed form of the OMLP-β-lactamase protein was applied to a gas phase sequenator (see "Results" for details). The products of each reaction cycle were diluted in 10 ml of Aquasol (New England Nuclear) and counted for 5 min two times. The radioactivity levels indicated in the figure represent the average cpm. Background levels (that represent 20% of the total counts of the major peaks) are subtracted from the numbers indicated. the background levels of radioactivity for these experiments were determined by averaging the radioactive contents in the products of two degradation cycles containing no radiolabeled proteins. Similar results were obtained in two other independent Edman degradation experiments (data not shown). We estimate that about 10% of the radioactivity incorporated in each proline residue was recovered in cycle 7.

TABLE I

Amino-terminal signal peptide mutants of the OMLP

Mutant	Amino-terminal sequence	Net charge in the amino terminus
	+ +	
Wild type	+ Met Lys Ala Thr Lys Leu Val. . .	+3
	− − +	
I-4	+ Met Glu Asp Thr Lys Leu Val. . .	0
I-6	+ Met . . . Ala Thr Asn Leu Val. . .	+1
	− −	
I-7	+ Met Glu Asp Thr Asn Leu Val. . .	−1

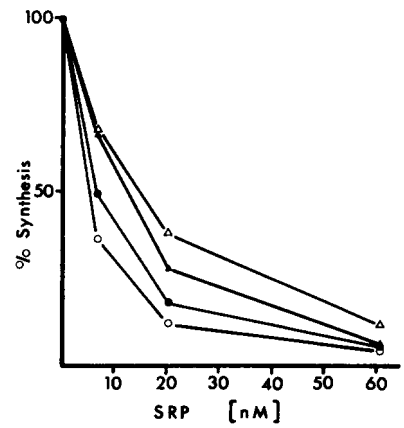

FIG. 4. **SRP-dependent translation arrest of the OMLP-β-lactamase protein containing the wild type and mutant signal peptides.** Translation reactions were performed as indicated under "Materials and Methods," in the presence of varying concentrations of SRP. Rabbit reticulocyte total RNA (coding primarily for the cytoplasmic protein globin) was included in the reactions simultaneously with the mRNA for the wild type or the signal peptide mutants of the OMLP-β-lactamase protein. The products of each translation reaction were resolved in a 10–15% gradient SDS-PAGE. After electrophoresis, the gels were dried and exposed on preflashed Kodak XAR-5 film. The intensities of the bands corresponding to each protein were determined in a LKB scanning densitometer. The percentage of synthesis of the OMLP-β-lactamase in each reaction was determined as indicated under "Materials and Methods." The *symbols* are: *closed circles*, wild type protein; *open triangles*, I-4; *open circles*, I-6; and *closed triangles*, I-7 mutant proteins, respectively.

mixture of two proteins differing by two amino acids at their amino terminus. Both cleavage positions differ from that used by *E. coli* signal peptidase II which cleaves between position −1 and +1 (Fig. 3*a*, *triangle*).

One of the "consensus features" of signal sequences is the presence of basic amino acids at their amino-terminal end (12, 13). It has been argued that these positive charges play a role in the translocation process across the membrane (13, 43, 44). Mutations that alter these amino acids in the OMLP signal peptide have been constructed by site-directed mutagenesis (26, 27) and have been shown to affect OMLP translocation when assayed *in vivo* in *E. coli*. In particular, mutations that change the positive charge to a negative net charge cause a delay in translocation of OMLP (27). To test if such mutations also have an effect on the translocation of the OMLP-β-lactamase across mammalian microsome membranes, we assayed the properties of three of these mutant signal peptides both for signal recognition by SRP (observed as SRP-dependent elongation arrest) and for translocation itself. In the signal peptide of two of these mutants (I-4 and I-7; see Table I) negative charges have been introduced, whereas the third mutant (I-6) contains only neutral amino acid residues in its signal peptide (see Table I).

The relative efficiency of SRP to recognize wild type and mutant pre-OMLP signal peptides was measured by determining elongation arrest at different SRP concentrations. The data in Fig. 4 show that the synthesis of pre-OMLP-β-lactamase containing wild type or mutant signal sequences is inhibited by SRP to very similar degrees, which are about the same as for an authentic eukaryotic secretory protein, bovine preprolactin (data not shown). This indicates that a net positive charge is not essential for an efficient signal peptide-SRP interaction *in vitro*. There are some slight, yet reproducible, variabilities in the relative inhibition of synthesis at low SRP concentrations for the different mutations. This effect does not correlate with the net charge at the amino terminus of the signal peptide and, therefore, may reflect other structural differences of unknown nature that affect signal recognition. In a similar series of experiments we addressed the question of whether the efficiency of membrane translocation is dependent on the terminal charge of the signal peptide. Fig. 5 demonstrates that mutant and wild type proteins are translocated to an indistinguishable extent at all the SRP concentrations assayed. Since post-translational translocation of this protein cannot be detected under the experimental conditions of Fig. 5 (data not shown), the observed processing must correspond to co-translational translocation. Thus, the pre-

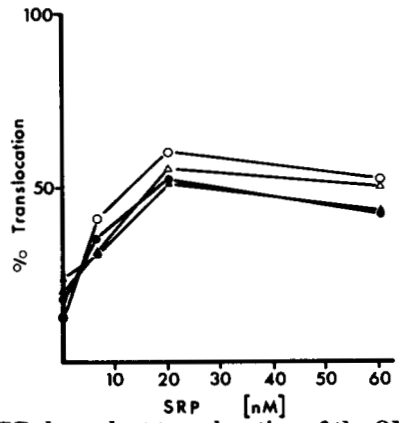

FIG. 5. **SRP-dependent translocation of the OMLP-β-lactamase protein containing the wild type and mutant signal peptides.** Translation reactions were performed as indicated in Fig. 4, with the exception that K-RM (2 eq in a 25-μl reaction) was included in all reactions. The translation products were analyzed as for Fig. 4. The percentage of translocation was determined as indicated under "Materials and Methods." The symbols are as for Fig. 4.

OMLP-β-lactamase protein has a defined period of time to be translocated while it is being synthesized. Therefore, the extent of processing observed in Fig. 5 reflects the rate at which these proteins are translocated. We conclude that neither signal recognition by SRP nor the subsequent targeting to and translocation across the microsomal membrane is measurably affected by a variety of drastic changes in the amino-terminal charge of this signal peptide *in vitro*.

DISCUSSION

We have shown that the signal peptide of the OMLP can direct the translocation of OMLP-β-lactamase across the canine microsomal membrane *in vitro*. Due to the small size of the OMLP (58 amino acids, *i.e.* it would barely span the large subunit of a eukaryotic ribosome) we chose to study the functions of this signal peptide as part of a fusion protein with β-lactamase. The efficiency by which this prokaryotic signal peptide promotes translocation across mammalian microsomes is indistinguishable from that of mammalian signal peptides, both at the level of signal recognition by SRP and at the level of subsequent translocation across the lipid bilayer. It follows that the OMLP signal peptide contains all the information necessary for efficient protein translocation across the mammalian ER membrane. This confirms the notion that signal sequences directing proteins to the prokaryotic plasma membrane and the mammalian ER are functionally conserved and evolutionarily related.

Similar results were previously obtained for β-lactamase, a periplasmic protein of *E. coli*. Its signal sequence is also efficiently recognized by the eukaryotic translocation machinery (14). Translation of the mRNA for this protein in the presence of canine pancreatic microsomes results in translocation and correct processing of its signal peptide. Thus, the mammalian signal peptidase will cleave pre-β-lactamase at the same position as cleaved in *E. coli*. This is in contrast with the processing observed for the OMLP signal peptide, which is cleaved at positions by the mammalian microsomes that are cryptic in *E. coli* (Fig. 3). This difference can be reconciled considering that the localization and processing pathway for the OMLPs in *E. coli* is different from that of other membrane and periplasmic proteins. In particular, the signal peptide of the OMLP is removed by a specialized signal peptidase (signal peptidase II) that is specific for OMLPs, whereas signal peptidase I removes the signal peptides of the other membrane and periplasmic proteins (like β-lactamase). Signal peptidase II requires a covalent modification (the addition of a glyceride moiety) on the cysteine residue at the cleavage site of the OMLP (position +1 in Fig. 3) as a prerequisite for processing (28, 29). If this modification is prevented (either by site-directed mutagenesis of cysteine (+1) into a glycine residue (28) or by inhibition of the signal peptidase II with globomycin (45)), then signal peptide cleavage will not take place. If the same mutation is introduced in the pre-OMLP-β-lactamase fusion protein, the *E. coli* signal peptidase I will cleave at a previously cryptic position (between residues +5 and +6 in Fig. 3*a* (45)). This cleavage is no longer sensitive to the signal peptidase II inhibitor globomycin (45) and, therefore, is performed by signal peptidase I. In the mammalian system, cleavage is observed at the position where signal peptidase I cleaves the glycine (+1) substitution in *E. coli* (see Fig. 3*b*), as well as an alternative site that is less frequently used (between residues +3 and +4 in Fig. 3*a*). As expected, in the mammalian *in vitro* system the glycine (+1) substitution behaves indistinguishably from the wild type (data not shown). These results indicate that the mammalian microsomes lack an activity equivalent to signal peptidase II and that, as for the cleavage of the β-lactamase signal peptide, the *E. coli* signal peptidase I and the mammalian signal peptidase have the same specificity for this particular site. The cryptic cleavage site(s) that get utilized by both prokaryotic signal peptidase I and eukaryotic signal peptidase agree well with the rules described by vonHeijne (44) for signal peptide cleavage.

Mutations that change the positively charged amino acids in the amino terminus of the OMLP signal peptide have no effect on signal recognition by SRP or translocation across mammalian microsomal membranes. These results indicate that a net positive charge in the amino terminus of the signal peptide is not required nor does it increase the efficiency of the process *in vitro*. Functional interactions of the conserved positive charges with the membrane have been suggested (43, 44) to be essential for the translocation of the protein. However, some eukaryotic signal sequences deviate from this "consensus" and have an acidic amino terminus (12, 44), demonstrating that a requirement for an amino-terminal positive net charge is not absolute. Some of the mutants assayed in this study show kinetically delayed translocation in *E. coli in vivo*, both in OMLP (26, 27) or in the fusion protein used here (54). It may be possible that the mammalian translocation machinery overcomes the requirement for a net positive charge by coupling translation to translocation more tightly (by targeting the *nascent* chain via the SRP/SRP receptor) than it is observed in *E. coli*, where at least the mutant proteins of pre-OMLP were clearly shown to be translocated post-translationally (26, 27). Alternatively, the α-amino group of the initiating methionine in eukaryotic cells carries an additional positive charge that is not present when the proteins are synthesized in prokaryotic systems (due to the formylated amino terminus) and which, at least in principle, could compensate for effects that would otherwise be induced by the mutations. Since we cannot construct mutations that completely lack basic groups, our interpretation has to be limited to the effects of the *net* charges on the amino end of signal sequences.

The question remains, what constitutes a "minimal signal sequence" and how can signal sequences, diverse as they are, be efficiently recognized in a protein/protein interaction by mammalian SRP and/or additional signal receptors within the membrane. For the signal sequence/SRP interaction it was clearly shown by cross-linking experiments that the rec-

ognition involves a direct binding of the signal sequence to the 54-kDa polypeptide of SRP (49, 50). Similarly, the subsequent interactions of signal sequences with membrane components also seem to involve protein/protein interactions, although this has only been demonstrated indirectly (51, 52). Molecular models that account for such interactions must take into account the considerable diversity of signal sequences. Thus, it seems reasonable to consider a model in which some features of the signal peptides, such as a certain amount of hydrophobicity together with the ability to assume a particular secondary structure, may constitute a structural moiety that is recognized in the context of its interactions with other components. Interestingly, an analogous model has been proposed to explain the related problem of how a class II major histocompatibility antigen can bind to different peptide antigens (53). Class II major histocompatibility antigens appear to contain a single binding site for different peptides which, once bound, may adopt a similar general structure that is reinforced by the binding site (53). For signal recognition one could envision a hydrophobic patch or grove on the surface of the receptors (SRP or the yet putative membrane receptor) that binds to signal sequences forcing them to assume an α-helical or β-sheet configuration. Thus, signal recognition and antigen presentation may have evolved similar mechanisms to solve a related problem, and it will be interesting to compare the two systems once more detailed structural information about the receptor/ligand interactions becomes available.

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