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A Bacterial Secretory Protein Requires Signal Recognition Particle for Translocation across Mammalian Endoplasmic Reticulum*

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Matthias Müller‡, Ibrahim Ibrahimi§, Chung Nan Chang¶, Peter Walter, and Günter Blobel

From the Laboratory of Cell Biology, The Rockefeller University, New York, New York 10021

***In vitro* transcription of DNA from plasmid pBR322 was coupled to cell-free translation in a wheat germ system. The major translation product was pre-β-lactamase. Upon addition of dog pancreas microsomes, the precursor was processed to authentic β-lactamase as shown by partial NH_2-terminal sequence analysis. Processing was linked to translocation into the microsomal vesicles. Salt-extracted microsomes did not process pre-β-lactamase but could be reactivated by purified signal recognition particle, which is the functional component of the salt wash (Walter, P., and Blobel, G. (1980) *Proc. Natl. Acad. Sci. U. S. A.* 77, 7112-7116). Signal recognition particle alone caused a drastic translation arrest that could be released by salt-depleted membranes. These data are consistent with those obtained for eukaryotic proteins and suggest that co-translational translocation of both bacterial and eukaryotic secretory proteins across the endoplasmic reticulum require identical components.**

Substantial experimental data has recently been provided on the co-translational translocation of proteins across, and integration into, the endoplasmic reticulum membrane. Thus, a peripheral membrane protein complex, originally termed signal recognition protein but now signal recognition particle (SRP)[1] because it also contains a distinct RNA,[2] was purified from dog pancreas rough microsomes (1). SRP selectively recognizes polysomes which are engaged in the synthesis of secretory proteins by interacting with the signal sequence after its emergence from the ribosome (2, 3). SRP is also required for binding of these polysomes to the microsomal membrane (2, 4). In addition, an integral membrane protein was described (5) which is necessary for translocation across rough microsomes, and which turned out to be the recently purified membrane receptor for SRP[3] (6). Proteins that have so far been shown to be translocated in an SRP-dependent manner are prolactin (2-4), apolipoprotein AI (7), acetylcholine receptor δ subunit (8), and cathepsin D.[4]

In prokaryotic cells, far less details on the mechanism of transport of secretory proteins across the cytoplasmic membrane are available. Several findings, however, suggest similarities between the prokaryotic and eukaryotic translocation apparatus. (i) Most secretory proteins from bacteria, like their eukaryotic counterparts, are synthesized as precursor molecules with an NH_2-terminal signal sequence (9). (ii) Precursor proteins bearing a mutation within their signal sequence generally are not secreted and accumulate in the cytoplasm (9-11). (iii) The signal sequence of a secretory protein when attached by gene fusion to a COOH-terminal part of an *a priori* cytoplasmic protein directs secretion of the cytoplasmic protein across the inner membrane of Gram-negative bacteria (9-11). (iv) Bacterial cells, into which the gene for an eukaryotic secretory protein has been introduced, correctly mature and secrete the eukaryotic protein (12-14).

Plasmid-encoded β-lactamase is secreted into the periplasmic space, the space between inner and outer membrane of Gram-negative bacteria. Accordingly, it is synthesized as precursor with a 23 amino acid residue long signal sequence (15). This signal sequence has been shown to guide the secretion of eukaryotic proinsulin across the cytoplasmic membrane of *Escherichia coli* (16). The recent report on the expression of active plasmid encoded β-lactamase in yeast provided for the first time evidence that bacterial preproteins can be processed by eukaryotic cells (17). Here we demonstrate that β-lactamase synthesized *in vitro* is co-translationally translocated into dog pancreas microsomal vesicles by the same translocation mechanism described for eukaryotic secretory proteins.

EXPERIMENTAL PROCEDURES

DNA Preparation—DNA from plasmid pBR322 was prepared by CsCl ethidium bromide centrifugation (18). Plasmid pHI-1 was a generous gift of Drs. Jon Beckwith and Hiroshi Inouye, Harvard Medical School. DNA from this plasmid was obtained by chromatography on hydroxyapatite (19).

In Vitro Transcription—Transcription of 10 μg of plasmid DNA by 4.4 units of *E. coli* K12 RNA polymerase (Miles) in a final volume of 100 μl was carried out as described (20) except for the omission of spermine and the use of potassium acetate instead of KCl. Aliquots were stored at −70 °C.

In Vitro Translation—A nuclease digested S-23 supernatant from wheat germ was prepared according to Ref. 21. Translations were performed for 90 min at 25 °C using 5 μl of wheat germ S-23, 5 μl of transcription mix heated to 55 °C followed by rapid chilling on ice, and 10 μCi of L-[^{35}S]methionine (1000 Ci/mmol; New England Nuclear) in a final volume of 25 μl. The translation mix was composed essentially as previously described (20) with the addition of 5 μM *S*-adenosylmethionine (22) and 0.004 A_{280}/ml of human placental RNAse inhibitor (2). Preparation of dog pancreas rough microsomes and of SRP were described elsewhere (2).

Post-translational Treatments—Translations were stopped by the addition of cycloheximide at a final concentration of 16 μg/ml. When

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‡ Recipient of a fellowship from the Deutsche Forschungsgemeinschaft. To whom correspondence should be addressed at, Laboratory of Cell Biology, Box 292, The Rockefeller University, 1230 York Avenue, New York, New York 10021.

§ Present address, Department of Biology, University of Jordan, Amman, Jordan.

¶ Present address, Genentech, Inc., 460 Point San Bruno Boulevard, S. San Francisco, CA 94080.

[1] The abbreviations used are: SRP, signal recognition particle; SDS, sodium dodecyl sulfate.

[2] P. Walter, and G. Blobel, (1982) *Nature (Lond.)*, in press.

[3] R. Gilmore, P. Walter, and G. Blobel, (1982) *J. Cell Biol.*, in press.

[4] A. Erickson, P. Walter, and G. Blobel, manuscript in preparation.

no further treatment was performed, translation products were precipitated with 5% trichloroacetic acid or processed for immunoprecipitation essentially according to Ref. 23. Anti-β-lactamase antibodies were raised in rabbits by injection of the SDS-denatured and alkylated enzyme which was a gift of Dr. Peter Model, The Rockefeller University. Post-translational proteolysis (for details see legends to figures) was stopped by addition of aprotinin (Boehringer-Mannheim) and L-1-Tosylamido-2-phenylethyl chloromethyl ketone at final concentrations of 20 μg/ml and 0.1 mM, respectively. Microsomal membranes were recovered from the translation mix by centrifugation for 10 min at 135,000 × g_{av} through 25 μl 0.5 M sucrose in a Beckman Airfuge. The resulting pellet was dissolved in electrophoresis sample buffer at 70 °C. Solubilization of trichloroacetic acid precipitates, alkylation of proteins, SDS-polyacrylamide gel electrophoresis, and fluorography were done according to published procedures (2, 23).

Partial Sequence Determination—Translation products were labeled with 2 mCi/ml of L-[3,4,5-^{3}H]leucine (110 Ci/mmol; New England Nuclear), immunoprecipitated, and further treated as described (24).

RESULTS AND DISCUSSION

DNA of the plasmid pBR322 containing the β-lactamase gene (*bla* gene) (15) was transcribed *in vitro* utilizing RNA polymerase from *E. coli*. Transcripts were translated in a nuclease digested cell-free system from wheat germ which had been supplemented with *S*-adenosylmethionine for capping of the bacterial RNA (22). SDS-gel electrophoresis yielded a major translation product of an apparent M_r = 32,000 (Fig. 1, *lane 1*) that cross-reacted with antibodies raised against β-lactamase (Fig. 1, *lane 4*). Thus, our coupled transcription-translation system synthesized primarily the precursor of β-lactamase. An additional polypeptide with an approximate M_r = 26,000 (Fig. 1, *lane 1* and *lanes 10–14*), also immunoprecipitable with anti-β-lactamase antibodies (Fig. 1, *lane 4*), most likely represents a degradation product of this precursor.

In proportion to the amount of dog pancreas microsomal membranes co-translationally added, the precursor was converted into a 29,500-dalton product (Fig. 1, *lanes 2* and *3*). This polypeptide was identified as mature β-lactamase by its immunoreactivity with anti-β-lactamase antibodies (Fig. 1, *lane 4*) and by NH_2-terminal sequence analysis. Consecutive automated Edman degradation of the L-[3,4,5-^{3}H]leucine-labeled protein yielded Leu in positions 5, 15, 24, and 26 (Figs. 2 and 3) as was expected from the known amino acid sequence of authentic β-lactamase (15, 25). No processing occurred when microsomal membranes were added post-translationally (Fig. 1, *lane 5*), which is in contrast to previous *in vivo* findings (26, 27).

To investigate whether processing of the β-lactamase precursor to the mature enzyme involved segregation into the microsomal vesicles, membranes were spun down after translation was completed. Fig. 1 shows that most of the precursor stayed in the supernatant (*lane 6*), whereas most of the β-lactamase sedimented with the membranes (*lane 7*). Translocation across the membrane into the microsomal vesicles should render β-lactamase inaccessible to externally added proteases. In fact, after post-translational treatment of both precursor and processed protein with trypsin and chymotrypsin, only mature β-lactamase appeared to be resistant against the proteases (Fig. 1, compare *lane 2* to *8*). However, proteolytic treatment solely of the precursor did not lead to a complete digestion but surprisingly to a distinct cleavage product which, on SDS gels, was indistinguishable in size from the mature protein (Fig. 1, *lane 9*) although the Ala-His peptide bond linking the signal sequence to the mature part of the protein (15) is not susceptible to either protease.

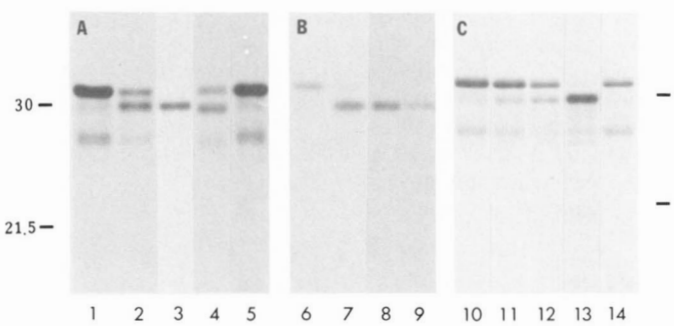

FIG. 1. **SRP-dependent processing of pre-β-lactamase by dog pancreas microsomes.** DNA from plasmid pBR322 was transcribed *in vitro* and the transcripts were translated in a wheat germ system. Shown are fluorographed SDS gels of labeled translation products. *Numbers to the left of A* indicate molecular weight of ^{14}C-methylated (23) carbonic anhydrase (30 kilodaltons) and soybean trypsin inhibitor (21.5 kilodaltons). *A*, processing by rough microsomes. *Lane 1*, total products from 25 μl of translation mix. *Lane 2*, translation products obtained in the presence of 0.4 eq of rough microsomes. One equivalent is the amount of membranes derived from 1 μl of a rough microsome suspension at a concentration of 50 A_{280} units/ml (2). *Lane 3*, same as *lane 2* but 2.4 eq. *Lane 4*, proteins of *lane 2* which react with anti-β-lactamase antiserum. *Lane 5*, post-translational incubation with 2.4 eq of rough membranes. *B*, translocation of processed β-lactamase. *Lanes 6* and *7*, supernatant and pellet, respectively, after post-translational centrifugation of the translation mix containing 0.8 eq of rough microsomes (10 min at 135,000 × g_{av}). *Lane 8*, post-translational incubation with trypsin and chymotrypsin (50 μg/ml of each) for 90 min on ice of proteins translated in the presence of 0.4 eq of rough microsomes. *Lane 9*, post-translational proteolysis of proteins translated in the absence of microsomes. *C*, SRP-dependence of processing. *Lane 10*, same as *lane 1*. *Lane 11*, translation products obtained in the presence of 1 eq of salt-washed membranes. *Lane 12*, as *lane 11* but 2.4 eq. *Lane 13*, as *lane 11* except for addition of 15 units of SRP. For definition of units see Ref. 2. *Lane 14*, as *lane 13* without membranes.

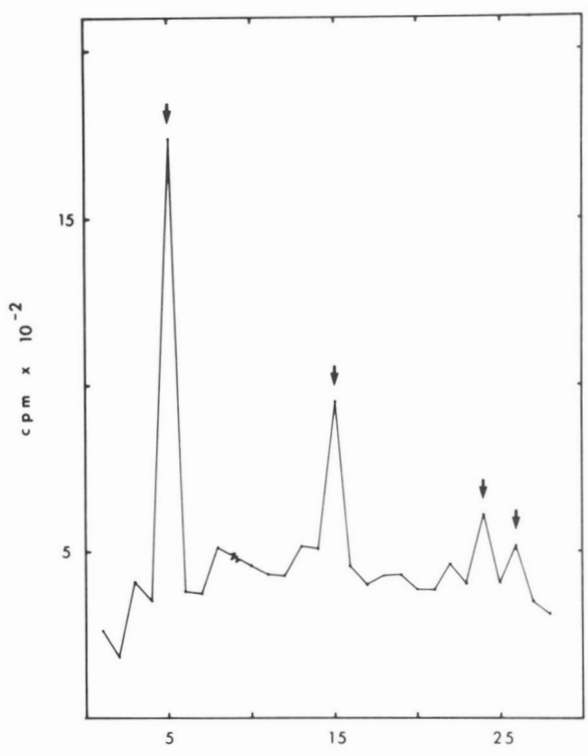

FIG. 2. **NH_2-terminal sequence analysis of β-lactamase processed by dog pancreas microsomes.** Translation was performed in a total volume of 250 μl in the presence of L-[3,4,5-^{3}H]leucine. Processed β-lactamase was recovered from sedimented microsomes by immunoprecipitation. Preparation of the immunoreactive material for automated Edman degradation was as described in Ref. 24. *Arrows* indicate occurrence of Leu within the amino acid sequence.

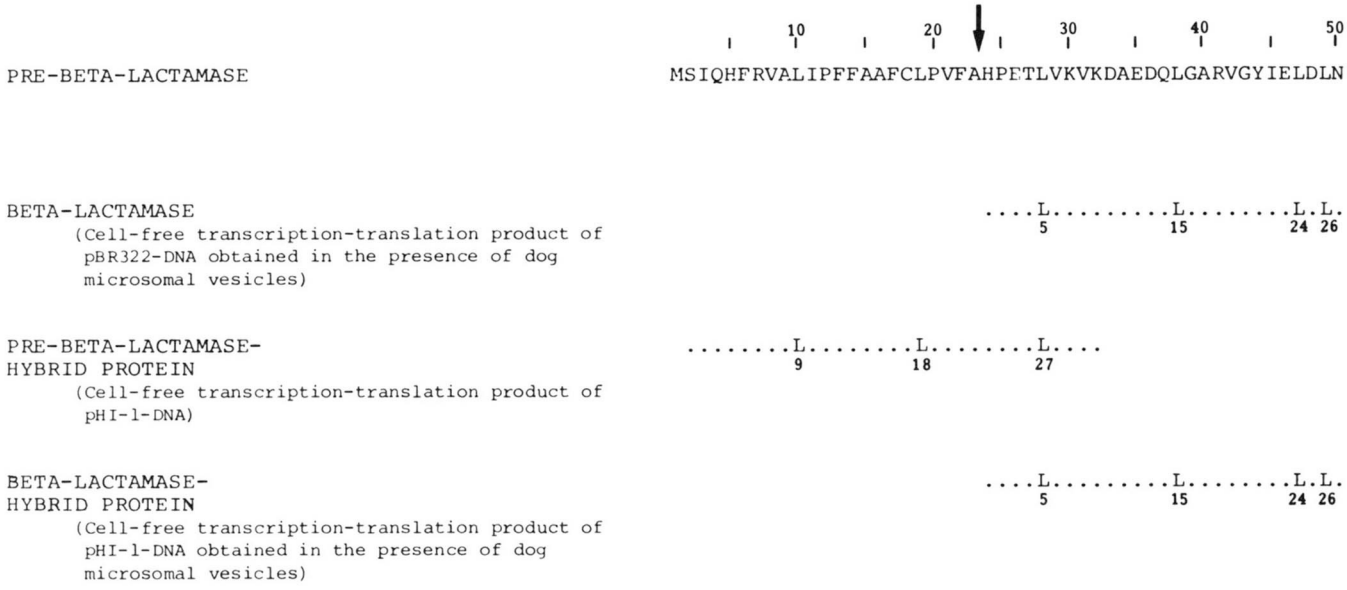

FIG. 3. **Alignment of partial NH_2-terminal sequences of precursor and mature form of β-lactamase and of β-lactamase hybrid protein.** The complete amino acid sequence of the first 50 residues is taken from Ref. 15. The *arrow* indicates the cleavage site of the signal peptidase. For experimental details, see legend to Fig. 2.

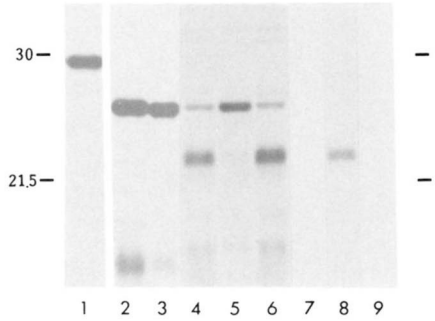

FIG. 4. **Processing and translocation by dog microsomes of a β-lactamase hybrid protein encoded by plasmid pHI-1.** Experimental details including marker proteins are as described in Fig. 1. *Lane 1,* authentic β-lactamase processed by rough microsomes. *Lane 2,* total translation products of pHI-1 transcripts. *Lane 3,* anti-β-lactamase immunoreactive products of *lane 2. Lane 4,* proteins translated in the presence of 0.8 eq of rough microsomes. *Lane 5,* proteins translated in the presence of 0.8 eq of salt-washed microsomes. *Lane 6,* as *lane 5* except for addition of 15 units of SRP. *Lane 7,* post-translational proteolysis with trypsin and chymotrypsin of total translation products obtained in the absence of membranes. *Lane 8,* post-translational proteolysis of proteins translated in the presence of 0.8 eq of rough microsomes. *Lane 9,* as *lane 8* except for the presence of 1% sodium deoxycholate during proteolysis.

TABLE I

SRP arrests translation of β-lactamase

The amount of β-lactamase synthesized either as precursor or mature form was estimated by densitometric scanning of the corresponding bands of the fluorogram shown in Fig. 1*C* (for details see Fig. 1).

	Control	SRP	SRP + K-RM[a]	K-RM
Sum of relative areas under peaks representing precursor and mature protein	100%	52%	107% (1 eq K-RM) 98% (2.4 eq K-RM)	92% (1 eq K-RM) 68% (2.4 eq K-RM)
Corresponding lanes of Fig. 1	10	14	13	11 12

[a] K-RM, salt-washed microsomes.

Under these conditions, it was impossible to prove unambiguously that co-sedimentation of co-translationally processed β-lactamase with membranes was due to translocation across the microsomal membrane. This, however, could be clearly demonstrated for a related secretory protein encoded by the plasmid pHI-1. This plasmid had been derived from plasmid pBR322 by cloning the structural gene for *E. coli* alkaline phosphatase (*phoA*) into the *pst* I site of pBR322 (28). The predominant translation product of pHI-1-DNA in our *in vitro* system (Fig. 4, *lane 2*) was smaller by an approximate M_r = 4,000 than mature β-lactamase (Fig. 4, *lane 1*) and was recognized by anti-β-lactamase antibodies (Fig. 4, *lane 3*). No alkaline phosphatase translation product could be detected under the conditions of the experiment depicted in Fig. 4. Since the *pst* I site of pBR322 into which the *phoA* gene had been inserted lies in the region encoding amino acids 181–182 of β-lactamase (15), the new plasmid pHI-1 may conceivably code for a hybrid protein which comprises the NH_2-terminal part of β-lactamase including the signal sequence. Partial sequence analysis of our cell-free translation product after labeling with L-[3,4,5-^{3}H]leucine confirmed this assumption. Leucyl residues at positions 9, 18, and 27 correspond to the NH_2-terminal sequence of β-lactamase precursor lacking the NH_2-terminal Met (Fig. 3). As expected, the cell-free translation product of the hybrid protein could be co-translationally processed by rough microsomes (Fig. 4, *lane 4*). The difference in the apparent M_r between precursor and processed form of the hybrid protein was considerably greater (4,000 daltons) than in the case of β-lactamase (2,000 daltons). Partial sequence analysis of the processed hybrid protein, however, revealed that cleavage of the signal sequence had occurred at the correct site (Fig. 3). It is therefore not clear whether the apparent loss of 4,000 daltons reflects an additional cleavage at the COOH-terminus or merely a nonlinear electrophoretic mobility of precursor and mature form of the hybrid protein. A similar situation was previously reported for prolactin and growth hormone (29) in which case the processed forms of both hormones differ considerably more in their electrophoretic mobility on SDS gels than the precursors even though the signal sequences are of nearly identical length.

In contrast to what we found for β-lactamase, the precursor of the hybrid protein was completely digested by trypsin and

chymotrypsin (Fig. 4, compare *lane 2* to *lane 7*), whereas the processed material was resistant (Fig. 4, compare *lane 4* to *lane 8*) unless microsomes were disrupted by sodium deoxycholate (Fig. 4, *lane 9*). The extent of protection by microsomal vesicles against proteolytic degradation was about 50–60% (Fig. 4, compare *lane 4* to *lane 8*) and turned out to be independent of the protease concentration used (data not shown). Partial leakiness of the vesicles may account for this incomplete protection (30).

Incidentally, our results with the pHI-1 hybrid protein are in line with those obtained with chain termination mutants of Gram-negative bacteria, where fragments of maltose-binding protein (31) and β-lactamase (9) missing their normal COOH-termini are processed and translocated across the cytoplasmic membrane.

Having established that nascent chains of prokaryotic secretory proteins are correctly translocated across and processed by eukaryotic endoplasmic reticulum, the intriguing question arose whether this translocation was dependent upon the same components of the translocation machinery as that of eukaryotic nascent presecretory proteins. Salt extraction of dog pancreas rough microsomes resulted in an abolishment of their translocation capacity due to the removal of SRP (1). Salt extracted microsomes had also lost most of their ability to process β-lactamase precursor (Fig. 1, compare *lane 2* to *lane 11*, and *lane 3* to *lane 12*) and the precursor of β-lactamase hybrid protein (Fig. 4, compare *lane 4* to *lane 5*). Restoration of processing was achieved upon addition of purified SRP to the depleted vesicles (Fig. 1, *lane 13* and Fig. 4, *lane 6*). This effect was not exerted by SRP alone (Fig. 1, *lane 14*) but required both SRP and salt-washed membranes.

A semiquantitative analysis of the synthesis rate of precursor and authentic β-lactamase revealed that SRP caused a drastic inhibition of precursor synthesis (Table I, compare column 1 to 2). This inhibition was released by the addition of salt-depleted membranes (Table I, column 3). Since high concentrations of salt-washed membranes inhibited translation *per se* (Table I, column 4), the question may arise whether SRP simply overcomes this membrane-induced inhibition or whether the membranes release an SRP-mediated translation arrest. Restoration of translation even with noninhibiting amounts of membranes (Table I, column 3, upper row), however, clearly support the latter interpretation. Thus, SRP was not only required for the translocation of a bacterial secretory protein but also arrested its translation in a membrane-free translation system. Translation-arrest by SRP was first described for the synthesis of preprolactin and shown to be caused by a signal sequence-induced and site-specific arrest in the elongation of nascent secretory chains (2–4). The arrest is maintained until the ribosome-SRP complex binds to the translocation competent sites of membranes followed by a discharge of the nascent chains into the endoplasmic reticulum, thereby coupling elongation to translocation. We conclude, therefore, that the initial events of the co-translational segregation into eukaryotic microsomes are similar for β-lactamase and mammalian secretory proteins. This implies, on the one hand, that the bacterial precursor meets all the structural requirements for being correctly processed by the eukaryotic translocation apparatus. On the other hand, it might suggest an analogous translocation machinery in the cytoplasmic membrane of bacteria.

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