

# Transport and Cleavage of Bacterial Pre- $\beta$ -lactamase by Mammalian Microsomes

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**ABSTRACT** Eucaryotic endoplasmic reticulum and bacterial inner cell membranes use strikingly similar mechanisms to transport proteins from one cellular compartment to another. Using a mammalian linked transcription-translation system, we show here that canine pancreatic microsomes accurately cleave off the signal sequence of  $\beta$ -lactamase, a secreted bacterial protein. We suggest that the apparent differences between bacterial and eucaryotic protein transport may involve only minor modulations of a profoundly conserved mechanism.

Eucaryotic endoplasmic reticulum and bacterial inner cell membranes use strikingly similar mechanisms to transport proteins from one cellular compartment to another (1-4). Precursors of secreted proteins in both procaryotic and eucaryotic cells contain hydrophobic NH<sub>2</sub>-terminal sequences of similar length. The functional importance of these so-called signal or leader sequences is illustrated by (a) mutants defective in secretion in bacteria (5); (b) the correlation of secretion with the presence of a signal sequence in the secreted form of yeast invertase, but not in the cytoplasmic form (6, 7); and (c) the ability of a synthetic signal sequence in a mammalian system to inhibit sequestration of proteins inside canine pancreatic microsomes in vitro (8). Usually, in both procaryotes and eucaryotes, the signal sequence is cleaved from the secreted protein in association with its transport across the membrane. The most striking evidence of evolutionary conservation of the protein transport mechanism is the ability of avian ovalbumin (9) and rat preproinsulin (10) made in *Escherichia coli* to cross the inner cell membrane into the periplasmic space. Further, in *E. coli* preproinsulin is appropriately cleaved to proinsulin (11). Both procaryotes and eucaryotes use a signal sequence to insert proteins into their membranes as well as through the membranes (12-14).

To further probe the relationship between eucaryotic and procaryotic protein transport, we have examined the function of the precursor to bacterial  $\beta$ -lactamase in an in vitro mammalian processing system.  $\beta$ -Lactamase is synthesized with a 24-amino acid signal sequence (15). Koshland and Botstein (16, 17) found completed chains of pre- $\beta$ -lactamase free in bacterial cytoplasm, and "pulse-chase" studies showed that pre- $\beta$ -lactamase is transported to the periplasmic space in association with cleavage of the signal. We have synthesized pre- $\beta$ -lactamase in a rabbit reticulocyte lysate, using RNA transcribed in vitro from the plasmid pBR322, which contains the *bla* gene encoding  $\beta$ -lactamase. We find that canine pan-

creatic microsomes added during protein synthesis cleave off the signal sequence accurately.

## MATERIALS AND METHODS

**Linked Transcription-Translation Reactions:** Transcription and translation of DNA from plasmid pBR322 were performed exactly as described by Coen et al. (18). pBR322 DNA was purified using the cleared lysate technique modified from Clewell and Helinski (19), followed by centrifugation through CsCl-ethidium bromide once. 1-2  $\mu$ g of supercoiled DNA was transcribed in 10- $\mu$ l reactions using Coen's conditions (18) and using *E. coli* RNA polymerase, a gift from D. Coen (Harvard Medical School), purified according to the procedure of Burgess and Jendrisak (20). Translation in nuclease-digested rabbit reticulocyte lysate was modified from Coen only in that translation was performed for merely 30 min, since little incorporation of radioactive amino acids into protein occurred subsequently. Canine pancreatic microsomes, prepared and digested with micrococcal nuclease according to the methods of Shields and Blobel (21), were added during or after protein synthesis. Protein synthesis was performed using [<sup>35</sup>S]methionine or [<sup>3</sup>H]leucine, and the proteins were separated on SDS polyacrylamide gels (22) followed by autoradiography. Messenger RNA from rabbit reticulocytes was obtained from Bethesda Research Laboratories, Rockville, MD.

**Protein Sequence Determination:** Linked transcription-translation of 6  $\mu$ g of pBR322 DNA was performed using pancreatic microsomes. 200  $\mu$ Ci of [<sup>3</sup>H]leucine, 100 Ci/mmol (New England Nuclear, Boston, MA) were used in the translation, whose final volume was 100  $\mu$ l. A parallel reaction used 0.25  $\mu$ g of pBR322 DNA, 100  $\mu$ Ci of [<sup>35</sup>S]methionine, 900 Ci/mmol (Amersham Corp., Arlington Heights, IL) and a final translation volume of 40  $\mu$ l. After translation, the reactions were combined, and the proteins were separated on a 12.5% polyacrylamide SDS slab gel. The gel was dried, autoradiography was performed, and the region of the gel containing  $\beta$ -lactamase was removed with a razor blade. The protein was eluted from the acrylamide by overnight incubation at 37°C in 1 ml of 1% triethylamine, 0.1% SDS, and 200  $\mu$ g/ml bovine serum albumin. The eluate was lyophilized and subjected to automated Edman degradation using a Beckman model 890C protein sequenator (Beckman Instruments, Inc., Fullerton, CA) and a 0.1 M quadrol program. The methionine-labeled protein was added to facilitate detection of the protein by autoradiography.

## RESULTS

DNA from plasmid pBR322 was transcribed into RNA using *E. coli* RNA polymerase, and the RNA was translated into

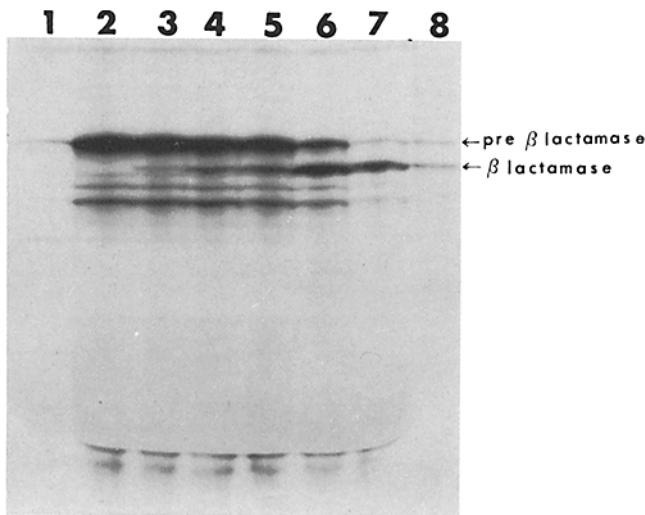


FIGURE 1 Cleavage of pre- $\beta$ -lactamase by canine pancreatic microsomes—titration of microsomes. 1.05  $\mu$ g of pBR322 DNA was transcribed in a reaction volume of 35  $\mu$ l. In a control reaction no DNA was added. 5  $\mu$ l of the transcripts were then added to translations with a final volume of 25  $\mu$ l, including varying amounts of canine pancreatic microsomes as indicated: lane 1, no DNA, no microsomes; lanes 2–7 contain DNA and in lane 2, no microsomes; lane 3, 0.1  $\mu$ l microsomes; lane 4, 0.25  $\mu$ l microsomes; lane 5, 0.5  $\mu$ l microsomes; lane 6, 1  $\mu$ l microsomes; lane 7, 2  $\mu$ l microsomes; lane 8, pre- $\beta$ -lactamase and  $\beta$ -lactamase from pulse-labeled *E. coli* containing pBR322 (a gift from D. Koshland, Massachusetts Institute of Technology). The proteins were separated on a 12.5% polyacrylamide SDS gel.

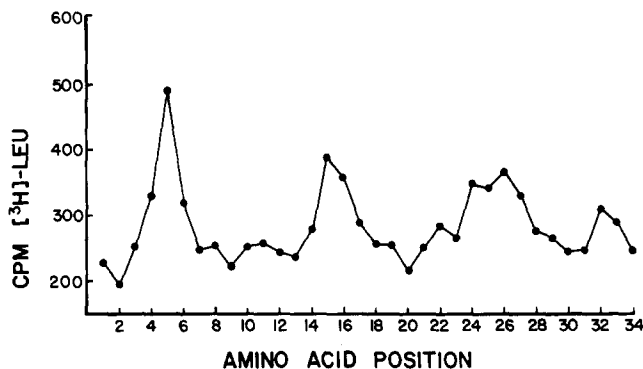


FIGURE 2 NH<sub>2</sub>-terminal sequence analysis of  $\beta$ -lactamase made during linked transcription-translation. See Materials and Methods for methods. Repetitive yield deduced from analysis of carrier myoglobin was 94%.

protein using a rabbit reticulocyte lysate. After electrophoresis through an SDS polyacrylamide gel, the major protein synthesized co-migrated with bacterial pre- $\beta$ -lactamase (Fig. 1, well 2). When the translation was performed in the presence of increasing amounts of microsomes from canine pancreas, progressively less pre- $\beta$ -lactamase was synthesized, and, instead, more of a band co-migrating with bacterial  $\beta$ -lactamase appeared (wells 3–7). When the microsomes were added after protein synthesis was completed, only pre- $\beta$ -lactamase was synthesized (data not shown). To verify that the protein synthesized in the presence of microsomes was  $\beta$ -lactamase, protein was synthesized in the presence of [<sup>3</sup>H]leucine and [<sup>35</sup>S]-methionine (to allow detection by autoradiography), the  $\beta$ -lactamase band was eluted from a polyacrylamide gel, and then automated Edman degradation was performed on the

isolated protein. Fig. 2 shows that the isolated protein has leucine at positions 5, 15, 24, 26 and, 32, just as authentic  $\beta$ -lactamase does. Thus, the microsomes cleave pre- $\beta$ -lactamase at the same residue recognized by the *E. coli* inner cell membrane.

## DISCUSSION

Microsomes from canine pancreas process pre- $\beta$ -lactamase as if it were a precursor of a secreted eucaryotic protein. Amino acid sequence analysis of  $\beta$ -lactamase produced *in vitro* shows that the signal sequence is cleaved off accurately. Because  $\beta$ -lactamase is relatively resistant to proteolytic digestion (reference 17 and our unpublished results), we could not use an assay involving protection of  $\beta$ -lactamase from proteolysis to demonstrate the microsomal sequestration of  $\beta$ -lactamase. However, since the signal peptidase is located on the inner surface of the microsomal membrane (23), appropriate cleavage of the signal peptide implies that at least part of the  $\beta$ -lactamase is inside the microsomal vesicle.

The linked transcription-translation system makes possible the translation of virtually any protein encoded by a cloned DNA sequence. Either an authentic bacterial promoter can be used, as in these experiments, or the ends of linear DNA can be used to transcribe promoter-free DNA (18). Here we have shown that mRNA synthesized *in vitro* can direct the synthesis of proteins that are processed appropriately by canine pancreatic microsomes. This system should be particularly useful for exploring the secretory properties of mutant proteins encoded by genes modified by site-specific mutagenesis. Because such mutant genes are likely to be constructed using plasmids such as pBR322, it may be necessary to separate the mutant genes of interest from the parent plasmids to avoid competition for microsomal sites with pre- $\beta$ -lactamase.

These studies demonstrate the striking evolutionary conservation of the apparatus for protein transport. Further studies of the behavior of bacterial genes and derived mutant genes in the linked system should allow further characterization of the similarities and differences between bacterial and mammalian secretion.

The authors thank Donald Coen for advice and *E. coli* RNA polymerase, Douglas Koshland for helpful discussion, Joseph Majzoub for canine pancreatic microsomes, Gary Lampman for performing the Edman degradations, and John T. Potts, Jr., and Alexander Rich for support, advice, and encouragement.

This work was supported by National Institutes of Health grant AM27758.

Received for publication 4 August 1982, and in revised form 9 December 1982.

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