Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 4599–4603, August 1983
Biochemistry

Importance of secondary structure in the signal sequence for protein secretion

(LamB protein/export-defective mutants/pseudorevertants/DNA sequence analysis)

SCOTT D. EMR* AND THOMAS J. SILHAVY†

Cancer Biology Program, NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, Maryland 21701

Communicated by David Botstein, December 20, 1982

ABSTRACT **Mutant *Escherichia coli* strains in which export of the LamB protein (coded for by the *lamB* gene) to the outer membrane of the cell is prevented have been described previously. One of these mutant strains contains a small (12-base pair) deletion mutation within the region of the *lamB* gene that codes for the NH_2-terminal signal sequence. In this mutant strain, export but not synthesis of the LamB protein is blocked. We have isolated pseudorevertants that restore export of functional LamB protein to the outer membrane. DNA sequence analysis showed that two of the revertants contain a point mutation in addition to the original deletion. These point mutations lead to amino acid substitutions within the signal sequence. Our results indicate that these secondary mutations efficiently suppress the export defect caused by the deletion mutation. Analysis of the secondary structure of the wild-type, mutant, and pseudorevertant LamB signal sequences suggests that the secondary mutations restore export by allowing the formation of a stable α-helical conformation in the central, hydrophobic region of the signal sequence.**

The mechanism of protein secretion in both prokaryotic and eukaryotic cells appears to require the presence of an extra sequence of predominantly hydrophobic amino acids at the NH_2 terminus of the protein. This extra amino acid sequence has been termed the signal sequence. The signal sequence is proposed to participate in binding the translation complex to a specific membrane receptor, thereby allowing cotranslational transfer of the polypeptide across the membrane (1). At present, the nature of the components of the signal sequence that permit this recognition and subsequent initiation of the secretion process are not known.

We have used a genetic approach to study the role of the signal sequence in protein localization. Specifically, we have analyzed export of the *Escherichia coli* outer membrane protein, LamB. This protein facilitates the passage of maltose and maltodextrins through the outer membrane and serves as the receptor for bacteriophage λ (2, 3). Like other exported proteins, LamB is initially synthesized on membrane-bound ribosomes in precursor form with a signal sequence (25 amino acids) at the NH_2 terminus (4–6).

We have demonstrated that the signal sequence of the LamB protein plays an essential role in initiating the localization of this protein to the outer membrane. Export-defective *lamB* mutants have been isolated (7, 8). The mutations present in these strains alter the LamB signal sequence and cause precursor accumulation in the cell cytoplasm (9, 10). Here we report the isolation and characterization of two revertants of one of the export-defective mutants. Our results indicate that the secondary structure of the signal sequence plays a critical role in the initiation of LamB export.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Strains and Phages. *E. coli* strains MC4100 [F⁻ *araD*139 Δ(*lac*)U169 *rpsL relA flbB ptsF thi*], SE2078 (MC4100 *lamBS78*), SE3001 [MC4100 Δ(*malB*)1], and SE3015 [MC4100 Δ(*malB*)15] have been described (7). Phage λap Δ(*malB*)1 is a λ-φ80 hybrid transducing phage carrying all of the *malB* region except for the promoter-distal portion of the *lamB* gene. The deletion Δ(*malB*)1 extends from within the *malK* gene (promoter proximal to *lamB*) into the *lamB* gene (7, 11).

Media, Chemicals, and Enzymes. Media have been described (7, 12). *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was from Sigma. DNA restriction and modifying enzymes were purchased from Bethesda Research Laboratories and radiolabeled compounds were from New England Nuclear.

Genetic Techniques. Standard bacterial and phage techniques and NG mutagenesis were done as described by Miller (12).

DNA Sequence Analysis. Phage DNA strand separation and DNA sequence analysis were done as described (6, 9, 13).

Radiolabeling and Immune Precipitation. [^{35}S]Methionine labeling and immune precipitation of LamB protein were done as described (14).

Cell Fractionation. Bacterial inner and outer membranes were separated by using the selective solubilization technique as described (15).

Polyacrylamide Gel Electrophoresis. LamB immune precipitates and cell fractions were run on 30-cm 9% NaDodSO$_4$/polyacrylamide gels as described (7, 16).

RESULTS

Selection of Mutants that Restore Export of Internalized LamB Protein. We have isolated several mutations (point mutations and deletions) that lead to amino acid alterations in the LamB signal sequence (7–10). These mutations prevent export of the protein. However, they do not block synthesis, and the precursor form of the LamB protein can be detected accumulating in the cytoplasm of the cell with its mutant signal sequence still attached. The only apparent defect in these cells appears to be at the level of signal sequence recognition. By isolating general suppressors of signal sequence mutations, we have, in fact, demonstrated that functional LamB protein can be efficiently exported and processed in the suppressed mutant cells (14, 17). Furthermore, we have shown that several of the

Abbreviations: Genetic nomenclature is from Bachmann *et al.* (29). Phenotype abbreviations are Mal, maltose; Dex, maltodextrin; ^{s}, sensitive; ^{r}, resistant. Other abbreviations are NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; ⟨Pα⟩ and ⟨Pβ⟩, the average α-helix and β-sheet conformational parameters, respectively, for any calculated sequence (19, 20).

* Present address: Division of Biology, California Inst. of Technology, Pasadena, CA 91125.
† To whom reprint requests should be addressed.

signal sequence point mutations and one of the deletion mutations (SE2078) do not cause an absolute export-negative phenotype (7). Rather, it is possible to detect a very small amount of LamB protein in the outer membrane of these cells. Thus, in the case of these mutants, signal sequence recognition can still occur albeit with poor efficiency. This efficiency can be increased by the presence of suppressor mutations that are unlinked to the *lamB* gene. Most likely these suppressor mutations affect other cellular components involved in signal sequence recognition. We reasoned that it might also be possible to restore export by isolating secondary mutations within the *lamB* gene. Analysis of these double mutants could provide information regarding the structural features of the LamB signal sequence that are important for its recognition.

Functional LamB protein in the outer membrane is required for growth on maltodextrins. This fact provides us with a simple method for selecting revertants. We employed the deletion mutant SE2078 in our selection. Because SE2078 contains the *lamBS78* deletion (12 base pairs deleted within the coding region for the signal sequence), back mutation to wild type is not possible. Any reversion mutations isolated in this strain must be the result of second-site mutations and should therefore be enriched for the desired class of pseudorevertants.

Spontaneous and NG-induced maltodextrin-positive (Dex^+) revertants of SE2078 were selected on minimal Dex plates. Eight independent revertants were picked (four NG-induced and four spontaneous) for further analysis. The revertants were tested for sensitivity to bacteriophage λ, a very specific and sensitive indicator for the presence of LamB protein in the outer membrane of the cell (18). All eight exhibited a λ-sensitive (λ^s) phenotype. The simultaneous appearance of a Dex^+ and λ^s phenotype strongly indicates that LamB protein is present in the outer membrane of these cells.

Mapping of the Suppressor Mutations. To determine whether the Dex^+ λ^s revertants of strain SE2078 are due to secondary mutations linked to the *lamB* gene, P1 lysates made on the revertants were used to transduce strain SE3001 to Mal^+. Strain SE3001 contains a deletion, Δ(*malB*)1, that removes a portion of the *lamB* gene (corresponding to the signal sequence) and, in addition, a portion of the promoter-proximal gene *malK*. Growth on maltose minimal medium does not require LamB protein; however, functional MalK protein is essential. Consequently, Mal^+ transductants selected in this way must have acquired both a functional *malK* gene and, because of the nature of the Δ(*malB*)1 deletion, the promoter-proximal portion of the *lamB* gene. Because the signal sequence deletion mutation, *lamBS78*, present in strain SE2078 maps under Δ(*malB*)1 (7), this mutation must also be present in the transductants. Several (6) Mal^+ transductants from each of the eight Dex^+ λ^s revertants were purified and tested for sensitivity to phage λ. If the secondary mutations present in the Dex^+ λ^s revertants are linked to *lamB*, then at least some of the Mal^+ transductants should show a λ^s phenotype. However, if these mutations are unlinked to *lamB*, then the Mal^+ transductants should only acquire the *lamB*S78 deletion and therefore a λ-resistant (λ^r) phenotype.

When the Mal^+ transductants from each of the eight Dex^+ λ^s revertants were tested in this way, five were found to give rise only to λ^r transductants and three gave rise only to λ^s transductants. This suggested that five of the eight mutants contain suppressors unlinked to *lamB* and the three remaining contain secondary mutations linked to *lamB*. To determine how tightly linked the three secondary mutations are to the deletion present in strain SE3001, 50 additional Mal^+ transductants from each were screened in the same manner. Again, 100% of the Mal^+ transductants exhibited a λ^s phenotype, indicating that the secondary mutations present in these three strains are very tightly linked to the promoter proximal portion of the *lamB* gene (the region coding for the signal sequence). Two pseudorevertants were derived from NG mutagenesis. The remaining mutant is a spontaneous pseudorevertant. We used two of these Dex^+ λ^s pseudorevertants for further studies. One, SE2078r1, was derived from NG mutagenesis, and the other, SE2078r2, was the single spontaneous mutant.

The remaining five Dex^+ λ^s revertants of SE2078 appear to contain suppressor mutations unlinked to the *lamB* gene. We previously identified three chromosomal loci (*prlA*, *prlB*, and *prlC*) unlinked to *lamB* that can cause suppression of *lamB* signal sequence mutations (14). To see if the five revertants might represent previously unrecognized isolates of mutations at any of these three *prl* loci, two of the five were further mapped by methods previously described (14). Both were found to map at a position on the *E. coli* chromosome corresponding to the location of the *prlA* locus. Therefore, these unlinked suppressors appear to represent previously unrecognized isolates of one of the previously identified *prl* loci. For this reason, no further analysis of these unlinked mutations will be discussed.

Effect of the Secondary Mutations on LamB Protein Synthesis. LamB export is prevented in the *lamB* mutant SE2078. The precursor form of LamB protein accumulates in the cytoplasm of such cells (7). This precursor can be detected by specific immune precipitation of LamB protein from [^{35}S]methionine-labeled whole cell extracts of SE2078 (17). When [^{35}S]-methionine-labeled extracts of the Dex^+ λ^s revertants of SE2078 (SE2078r1 and SE2078r2) were treated in a similar fashion, two bands were detected on $NaDodSO_4$/polyacrylamide gels (Fig. 1). One band corresponds to the precursor LamB protein that had accumulated in SE2078 and the other comigrates with wild-type mature LamB protein. Efficient processing of precursor LamB protein is detected in strains containing the secondary mutations. Although this processing appears to occur at the normal site, verification must await NH_2-terminal amino acid analysis of the processed form of the protein.

We also analyzed the level of synthesis of LamB protein in these double mutants. An excess of anti-LamB protein sera was used to precipitate LamB protein from an equal portion of la-

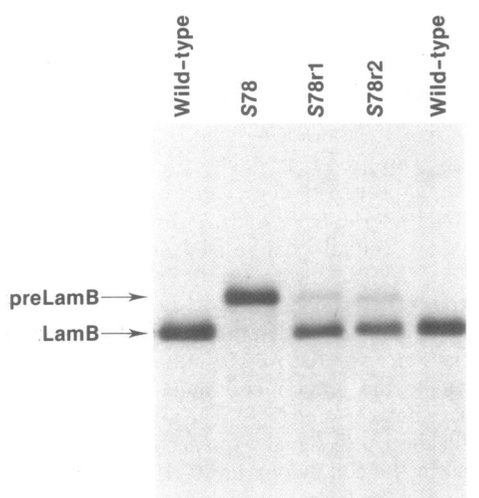

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis of LamB and preLamB protein immune precipitated from [^{35}S]methionine-labeled wild-type (MC4100), signal sequence mutant (SE2078), and double mutant cells (SE2078r1 and SE2078r2). Only the relevant portion of the gel is shown. The *lamB* alleles of the various strains used are given above each lane. The positions of precursor LamB (preLamB) and LamB on the gel are indicated by arrows.

beled wild-type and double mutant cells. When the immune precipitates were run on a $NaDodSO_4$/polyacrylamide gel and the LamB protein gel bands were excised and the radioactivity counted, the total amount of LamB protein detected in the different strains was similar, indicating that LamB protein synthesis is unaffected by the secondary mutations. In addition, the growth rate of the Dex^+ λ^s revertants of SE2078 appears normal. Cells containing the secondary mutations exhibit doubling times similar to that of wild-type cells in both rich, Luria broth medium, and minimal maltose medium.

Localization of LamB Protein in the Double Mutants. The fact that the secondary mutations lead to a Dex^+ λ^s phenotype indicates that LamB protein is present in the outer membrane of these cells. In addition, our results indicate that precursor LamB protein is efficiently processed in the double mutants. We have previously shown that precursor processing serves as an indicator of export for the LamB protein (17). To determine the level of LamB protein export in SE2078r1 and SE2078r2, cells were labeled with [^{35}S]methionine and then fractionated into the soluble, inner membrane and outer membrane cellular fractions. When we assayed the fractions using $NaDodSO_4$/polyacrylamide gel electrophoresis, processed LamB protein was detected only in the outer membrane fraction of the cell (Fig. 2). No precursor LamB protein was seen in the outer membrane. Approximately 75–80% of the wild-type level of LamB protein was detected in the outer membrane of both double mutants SE2078r1 and SE2078r2. Other proteins from the outer membrane, inner membrane, and soluble cell fractions appeared to be unaffected by the secondary mutations.

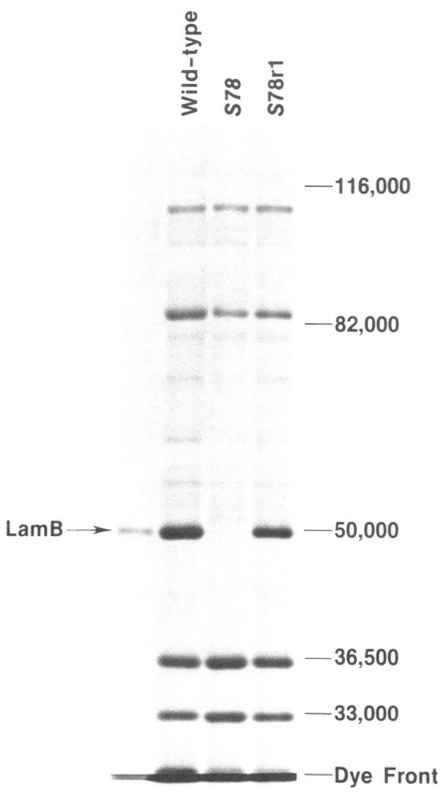

FIG. 2. $NaDodSO_4$/polyacrylamide gel electrophoresis of outer membranes isolated from wild-type (MC4100), mutant (SE2078), and double mutant (SE2078r1) cells. Wild-type mature LamB protein was run in the far left lane. Its position on the gel is indicated by an arrow. The relevant *lamB* genotype of each strain is listed above each gel lane. The positions of molecular weight markers are indicated along the right side of the gel. An outer membrane pattern essentially identical to that shown here for SE2078r1 was obtained with strain SE2078r2.

DNA and Amino Acid Sequence Analysis of the Secondary Mutations. The very tight linkage of the secondary mutations to the Δ(*malB*)1 deletion suggested that these mutations mapped within or close to the portion of the *lamB* gene that codes for the signal sequence. We previously subjected to sequence analysis a variety of signal sequence deletions and point mutations that map under the Δ(*malB*)1 deletion (9). The sequence of the secondary mutations has been determined by using a similar approach. The mutations were first recombined onto the λap Δ(*malB*)1 phage by P1 transduction, as already described (7). We confirmed the presence of the secondary mutations on the λap phage by demonstrating that the phages could rescue strain SE2078 to Dex^+ λ^s by recombination. For the DNA sequence analysis, we used DNA primers that permitted us to sequence the mutations from both of the separated strands of the phage DNA. This ensured accuracy of the sequence analysis results.

The results show that the 12-base-pair signal sequence deletion present in the parent strain SE2078 is, as expected, still present in both of the Dex^+ λ^s revertants analyzed. In addition, each strain also contains a point mutation within the region of *lamB* coding for the signal sequence. The point mutations lead to amino acid substitutions in the signal sequence. In strain SE2078r1, a glycine (GGC) residue at position 13 of the mutant signal sequence is replaced by a cysteine (TGC). In strain SE2078r2, the proline (CCC) residue at position 9 is replaced by a leucine (CTC).

The nucleotide changes in the DNA of the revertants does not appear to lead to the formation or removal of any significant nucleic acid secondary structures. However, analysis of polypeptide secondary structure does suggest certain alterations. By using the method of Chou and Fasman (19, 20), the predicted amino acid secondary structure of the wild-type LamB signal sequence and that of SE2078 and its Dex^+ λ^s revertants were calculated (21, 22). The analysis showed that the most probable conformation of the wild-type LamB signal sequence is an α helix (Fig. 3). In particular, the central segment of the signal sequence (residues 10 through 16) exhibits a significantly higher potential for α-helix formation ($\langle P\alpha \rangle = 1.29$) than for β-sheet formation ($\langle P\beta \rangle = 1.15$). This sequence is flanked by amino acid residues known to break α-helix and β-sheet conformations (proline at position 9 and glycine at position 17). The central α helix is predicted to terminate in the region of these two residues.

When a similar analysis was made of the LamB signal sequence in strain SE2078, the most striking feature was the predicted absence of a central α-helical segment. The deletion present in this strain removes four amino acid residues present in this α-helical segment. The loss of these amino acids should prevent the nucleation of an α helix in this region. No stable structure is predicted and this sequence probably exists as a random coil (Fig. 3). With Dex^+ λ^s revertants of SE2078, the amino acid substitutions, either glycine for cysteine or proline for leucine, should restore an α-helical conformation to the central segment of the signal sequence. In the first case the α helix would extend into this region from the COOH-terminal portion of the signal sequence; in the second case it would extend in from the NH_2-terminal portion of the signal sequence.

The analysis also predicts a β turn just beyond the processing site for the LamB signal sequence. This feature has also been predicted in many other signal sequences that have been analyzed in a similar manner (21–25).

DISCUSSION

We have described a genetic approach that has allowed us to isolate a class of mutants that provides evidence for the role of

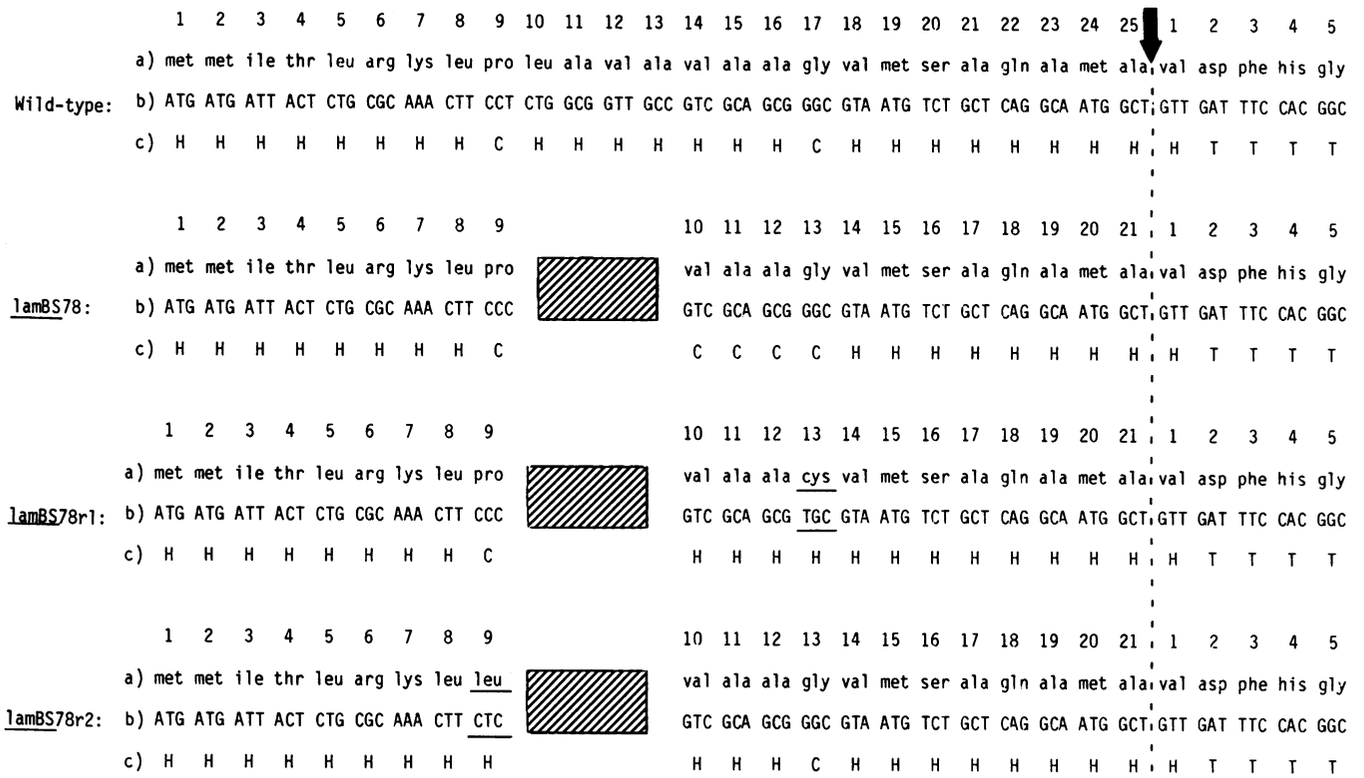

FIG. 3. DNA and amino acid sequence analysis of the LamB signal sequence from wild-type (MC4100), mutant (SE2078), and double mutant (SE2078r1 and SE2078r2) cells. The amino acid sequence, DNA sequence, and predicted secondary structure of the wild-type and mutant signals are listed. The site of LamB signal sequence processing is indicated by a vertical arrow and dashed line. Peptide secondary structure predictions were made as described by Chou and Fasman (19, 20). The letters H, C, and T stand for α-helix, random coil, and β-turn structures, respectively. The various *lamB* alleles analyzed are listed next to each sequence. Numbers correspond to the amino acid residue positions in either the precursor or mature LamB protein sequence. The *lamBS78* deletion is indicated by a shaded bar. Amino acid substitutions are underlined.

signal sequence secondary structure in the initiation of protein export. To select such mutants, we have used a previously described *lamB* mutation that alters the LamB protein signal sequence and thereby prevents export of this protein to the outer membrane (7, 9). Several revertants of this mutation have been isolated. Two have been found to contain mutations that map in the portion of the *lamB* gene that encodes the LamB signal sequence. Efficient export and apparently normal processing of the LamB protein is restored in the revertants. We suggest that the signal sequence alterations caused by these mutations suppress the export defect present in the original mutant by affecting the secondary structure of the signal sequence.

Strains containing the secondary mutations were selected as $Dex^+ \lambda^s$ pseudorevertants of the $Dex^- \lambda^r$ *lamB* signal sequence deletion mutant SE2078. Of eight such revertants isolated, three were found to be genetically linked to the *lamB* gene and five were found to be unlinked. Of the five, two were tested for linkage to loci (*prlA*, *prlB*, and *prlC*) that have been shown previously to allow general suppression of both *lamB* and *malE* (codes for the periplasmic maltose-binding protein) signal sequence mutations (14, 17). Both were found to be linked to the *prlA* locus.

Two of the three linked mutations were chosen for further characterization (one NG-induced and one spontaneous mutant). Immune precipitation of LamB protein from SE2078 and both of these double mutants indicated that the secondary mutations allow efficient processing of precursor LamB protein. Based on the observed reduction in molecular weight, this processing appears to occur at the normal site (Fig. 1). Cell fractionation of the mutants revealed further that the processed LamB protein detected in these cells is localized exclusively to the outer membrane (Fig. 2). Approximately 75–80% of the wild-type levels of LamB protein are present in the outer membrane of the double mutants. Other outer membrane proteins as well as cellular proteins in general appear unaffected by the secondary mutations. In addition, no growth defects have been detected in these strains. This absence of any detectable cellular defects and the dramatically efficient export and processing of LamB protein in the double mutants suggest that LamB protein export is occurring via its normal route.

DNA sequence analysis shows that the NG-induced secondary mutation leads to an amino acid substitution of a leucine (CTC) for a proline (CCC) at residue 9 of the mutant signal sequence (Fig. 3). This mutation corresponds to a G:C to A:T transition, an event known to be induced by NG mutagenesis (26). On the other hand, the spontaneous secondary mutation is the result of a transversion (G:C to T:A), in which a glycine (GGC) is replaced by a cysteine (TGC) residue at position 13 of the mutant signal sequence.

Analysis of the secondary structure of the wild-type LamB signal sequence, as well as that of SE2078 (21, 22) and the two double mutants, suggests that the export defect present in strain SE2078 is the result of the inability of the central region of the mutant LamB signal sequence to maintain an α-helical conformation. The secondary mutations are predicted to restore an α-helical conformation to this portion of the signal sequence. Each leads to a change in a residue known to inhibit α-helix propagation (proline and glycine) to residues that are commonly found in α helices (19, 20).

It is important to note that the deletion mutation present in strain SE2078 does not remove any of the amino acids that can be altered to yield an export-negative phenotype (7, 9). Mul-

tiple isolates of several of these export-defective point mutants have been found, suggesting that these amino acids represent a subset of key residues that are important for recognition during the initiation of export. The data suggest that the deletion in the LamB signal sequence of strain SE2078 prevents export by disrupting an important secondary structure in this critical segment of the signal sequence. Certain amino acids present in this segment of the signal sequence must be in the correct conformation to participate in specific recognition by the export machinery.

Additional support for this proposal comes from a general analysis of signal sequence peptides. The amino acid sequence of many secreted protein precursor segments have been determined, yet no apparent clear homologies have been detected at the primary sequence level. This has led to the suggestion that certain general conformational features of the signals are functionally important for recognition. These include the presence of a predominantly hydrophobic central segment and the presence of one to three charged basic amino acid residues near the NH_2-terminal end of most signal sequences. The involvement of signal peptide secondary structure in signal sequence recognition has also been suggested by the apparent similarity of the predicted structure for several different signals from both prokaryotic and eukaryotic cells (21–25). In fact, Rosenblatt *et al.* (27) have recently demonstrated through spectroscopic studies that the signal sequence peptide of pre-parathyroid hormone assumes a highly structured α-helical conformation in a nonpolar environment. They further showed that the structure for this sequence as predicted by the Chou and Fasman rules (19, 20) agreed well with these studies. Besides the *lamB* deletion mutant described here, an export-defective mutational alteration in the maltose-binding protein signal sequence has also been suggested to prevent export by altering signal sequence secondary structure. This mutation leads to the exchange of a proline for a leucine at amino acid residue 10 or the signal sequence (21, 22, 28). The continued application of a genetic approach similar to that described here should yield additional insights into the role of other features of the signal sequence that are critical for its efficient recognition in export initiation.

This work was sponsored by the National Cancer Institute, under Contract N01-C0-75380 with Litton Bionetics, Inc.

1. Blobel, G. & Dobberstein, D. (1975) *J. Cell Biol.* **67,** 835–851.
2. Szmelcman, S., Schwartz, M., Silhavy, T. J. & Boos, W. (1976) *Eur. J. Biochem.* **65,** 13–19.
3. Randall-Hazelbauer, L. & Schwartz, M. (1973) *J. Bacteriol.* **116,** 1436–1446.
4. Randall, L. L., Hardy, S. J. S. & Josefsson, L.-G. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1209–1212.
5. Marchal, C., Perrin, D., Hedgpeth, J. & Hofnung, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1491–1495.
6. Hedgpeth, J., Clement, J.-M., Marchal, C., Perrin, D. & Hofnung, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2621–2625.
7. Emr, S. D. & Silhavy, T. J. (1980) *J. Mol. Biol.* **141,** 63–90.
8. Emr, S. D., Hall, M. N. & Silhavy, T. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5802–5806.
9. Emr, S. D., Hedgpeth, J., Clement, J.-M., Silhavy, T. J. & Hofnung, M. (1980) *Nature (London)* **285,** 82–85.
10. Emr, S. D., Hall, M. N. & Silhavy, T. J. (1980) *J. Cell Biol.* **86,** 701–711.
11. Raibaud, O., Clement, J.-M. & Hofnung, M. (1979) *Mol. Gen. Genet.* **174,** 261–267.
12. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
13. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
14. Emr, S. D., Hanley-Way, S. & Silhavy, T. J. (1981) *Cell* **23,** 79–88.
15. Schnaitman, C. A. (1971) *J. Bacteriol.* **108,** 545–552.
16. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
17. Emr, S. D. & Bassford, P. J., Jr. (1982) *J. Biol. Chem.* **257,** 5852–5860.
18. Schwartz, M. (1972) *J. Mol. Biol.* **103,** 521–536.
19. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47,** 251–276.
20. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47,** 45–148.
21. Bedouelle, H. & Hofnung, M. (1981) in *Intermolecular Forces,* ed. Pullman, B. (Reidel, Hingham, ME), pp. 361–372.
22. Bedouelle, H. & Hofnung, M. (1981) in *Membrane Transport and Neuroreceptors* (Liss, New York), pp. 399–403.
23. Chan, S. J., Patzelt, C., Duguid, J. R., Quinn, P., Labrecque, A., Noves, B., Keim, P., Henrikson, R. L. & Steiner, D. F. (1979) in *From Gene to Protein: Information Transfer in Normal and Abnormal Cells,* eds. Russell, T. F., Brew, K., Gaver, H. & Schultz, J. (Academic, New York), pp. 361–378.
24. Austen, B. M. (1979) *FEBS Lett.* **103,** 308–313.
25. Cohn, D. V., Smardo, F. L., Jr., & Morrissey, J. J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1469–1471.
26. Coulondre, C. & Miller, J. H. (1977) *J. Mol. Biol.* **117,** 577–606.
27. Rosenblatt, M., Beaudette, N. V. & Fasman, G. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3983–3987.
28. Bedouelle, H., Bassford, P. J., Jr., Fowler, A. V., Zabin, I., Beckwith, J. & Hofnung, M. (1980) *Nature (London)* **285,** 78–81.
29. Bachmann, B. J., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40,** 116–167.