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Genetics

# Intragenic regions required for LamB export

**(outer membrane/signal sequence/sorting signal/β-galactosidase/hybrid protein)**

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ABSTRACT **_Escherichia coli_ strains containing a series of _lamB–lacZ_ fusions have been isolated and characterized. Each of these fusions specifies a hybrid protein with LamB sequences at the $NH_2$ terminus and a large functional COOH-terminal fragment of β-galactosidase. The amount of LamB present in the various hybrid proteins ranges from as few as 4 amino acids to a complete signal sequence (25 amino acids) plus 49 amino acids of the mature protein. With respect to hybrid protein export these fusions fall into three classes. Hybrid proteins with an incomplete LamB signal sequence or those that have a complete signal sequence plus 27 or fewer amino acids of the mature LamB protein are not exported and remain in the cytoplasm. In contrast, fusion strains attempt to export hybrid proteins that contain a complete signal sequence plus 39 or 43 amino acids of mature LamB. However, these proteins are not localized to the outer membrane. Finally, a hybrid protein that is slightly larger, containing 49 amino acids of mature LamB, is found in the outer membrane in appreciable amounts. These fusions, together with previously described _lamB–lacZ_ fusions, have enabled us to define more precisely the minimal amount of _lamB_ required to initiate the process of protein export. Moreover, they genetically locate a signal that appears to guide LamB to the outer membrane. This signal is within a region of amino acid homology shared by other major outer membrane proteins [Nikaido, H. & Wu, H. C. P. (1984) _Proc. Natl. Acad. Sci. USA_ 81, 1048–1052].**

The cellular envelope of the bacterium *Escherichia coli* is composed of three major layers: an inner membrane that surrounds and contains the cytosol, a cell wall that provides structural integrity, and an outer membrane that forms the first selective barrier to the environment. Between the two membranes lies the periplasmic space, a specialized cellular compartment. The machinery required for the synthesis of the protein components indigenous to each of these cellular locations lies within the cytosol. The signals required and the mechanisms of protein localization are subjects of considerable research, and several theories as to how these processes might occur have been proposed (for review see ref. 1). However, a majority of the relevant experimental data address only the early steps of the export process and are centered on the function of the signal sequence and the genetic identification of cellular machinery involved in its recognition (2–7). Very little, if any, is known about how the cell recognizes proteins destined for a particular location and routes them correctly.

We have been studying the localization of the inducible outer membrane protein LamB. This protein functions both as a pore for maltose and maltodextrins (8) and as the receptor for several bacteriophages, including λ (9). Using techniques of gene fusions, we have identified several intragenic regions required for export (1). We report here the isolation and characterization of several additional *lamB–lacZ* fusion strains. Our results allow us to define more precisely the signals required for export to the outer membrane.

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## MATERIALS AND METHODS

**Media and Chemicals.** Media and chemicals are described elsewhere (10–12).

**Bacteria.** The bacterial strains used are derivatives of strain MC4100: F⁻ Δ*lac*U169 *araD139 rpsL150 relA1 flbB5301 deoC7 rbsR ptsF25* (13). Pop3105 (*lamB–lacZ* 61-4), Pop3168 (*lamB–lacZ* 52-4), Pop3191 (*lamB–lacZ* 42-1), and Pop3299 (*lamB–lacZ* 42-12) are MC4100 strains that carry the designated fusions (13). The BRE strains isolated in this study are derivatives of Pop3299. The various *lamB–lacZ* fusions have been designated by either the published allele number (13) or, in the case of the new *lamB–lacZ* fusions, by allele numbers that are the same as the strain number—i.e., the *lamB–lacZ* fusion present in strain BRE100 is referred to as fusion 100.

**Selection of Mutants.** A culture of Pop3299 that carries the *lamB–lacZ* fusion 42-12 was grown overnight in L broth and 0.2-ml aliquots (≈$10^9$ cells) were plated on lactose/tetrazolium agar and incubated at either 30°C or 42°C until lactose-utilizing (Lac⁺) colonies appeared in the lawn of lactose-nonutilizing (Lac⁻) cells. Lac⁺ colonies, identified as red papilli, appeared after 3–5 days.

**Maltose Sensitivity.** Sensitivity to maltose was quantitated on the basis of maltose-induced inhibition of growth. A lawn of cells was plated on minimal (M63) glycerol agar in 2.5 ml of F top agar. Seven-millimeter filter discs were then placed on the lawn, and 15-μl aliquots of 5%, 3%, 1%, and 0.5% maltose were placed on individual disks. Plates were incubated overnight at 30°C and the zones of inhibition were then measured.

**Cloning and Sequencing of the *lamB–lacZ* Fusion Joints.** The procedure used to generate a small plasmid vector carrying the *lamB–lacZ* fusion joint has been previously described (12). First the *lamB–lacZ* fusion was cloned from a λ transducing phage onto a small high-copy-number plasmid derived from pBR322 (14). This plasmid was then used as a source of DNA for sequence analysis using the bacteriophage M13 vector (15) and the dideoxy method of Sanger *et al.* (16). A fragment of DNA carrying the fusion joint between the *Sal* I site in *malK* and the *Pvu* II site corresponding to amino acid 30 of *lacZ* was inserted into the general sequencing vector M13 MP11 (from M. Berman) at the *Sal* I and *Sma* I sites present in the phage. Insertion of this piece of DNA destroys the ability of the phage to exhibit *lacZ* α complementation, resulting in white phage plaques on media containing the chromogenic substrate 5-bromo-4-chloro-3-indolyl galactoside (XG). The sequence of the inserted fragment carrying the *lamB–lacZ* fusion joint was then determined. An M13 cloning and sequencing kit (P-L Biochemi-

Abbreviations: Lac⁺ and Lac⁻, lactose-utilizing and -nonutilizing; Mal$^s$ and Mal$^r$, maltose-sensitive and -resistant.

cals) was used as described by the manufacturer.

**Membrane Separation.** S-200 and P-200 fractions were prepared from cells grown in the presence of the inducer maltose. Cells were plasmolyzed with 25% (wt/vol) sucrose in Hepes (0.05 M, pH 7.5) and quickly broken by two passages through a French pressure cell. Unbroken cells were removed by low-speed centrifugation (1000 × *g*) for 10 min. The resulting supernatant was then centrifuged for 1 hr at 200,000 × *g*. The supernatant (S-200) contains soluble proteins; membrane proteins are contained in the pellet (P-200). Inner and outer membrane fractions were prepared as described by Crowlesmith *et al.* (17), with the modifications described by Hall *et al.* (13).

**Polyacrylamide Gels.** The gels used were 10% (wt/vol) acrylamide and 0.13% (wt/vol) bisacrylamide and were prepared as described by Benson *et al.* (18).

**Quantitation of Protein in Various Fractions.** The amount of hybrid protein was determined by densitometer scanning of a $NaDodSO_4$/polyacrylamide gel stained with Coomassie blue. The area under the protein band was quantitated by an integrator attachment on the densitometer. All protein quantitations were adjusted according to the amount of protein loaded and by a comparison to an internal standard protein band.

## RESULTS

**Rationale for Mutant Isolation.** The largest *lamB–lacZ* fusion (42-12) specifies a hybrid protein containing 241 amino acids of LamB fused to a large COOH-terminal fragment of β-galactosidase (LacZ) (19). This hybrid protein is localized with more than 85% efficiency to the outer membrane (13). Strains containing this fusion exhibit two unusual and characteristic phenotypes. First, these strains are sensitive to the inducer maltose (maltose sensitivity). Maltose sensitivity has been proposed to result from the attempted localization of large amounts of the hybrid protein (20). This appears to inhibit growth by jamming the export machinery and thereby partially or totally preventing other essential exported proteins from reaching their proper location. This explanation is supported by the observed accumulation of the precursor form of exported proteins in maltose-induced cultures (21). This sensitivity is relieved by signal sequence mutations that block export of the hybrid protein (21).

Second, these strains are unable to metabolize the sugar lactose. Strains that contain this fusion and carry a deletion of the chromosomal *lac* genes are phenotypically $Lac^-$, apparently because the hybrid LamB–LacZ protein is efficiently localized to the outer membrane. In such a location the hybrid protein presumably cannot assume an enzymatically active conformation. Mutations that block export of the hybrid protein (19) restore β-galactosidase activity and confer the ability to grow on lactose (a $Lac^+$ phenotype). Thus, the basal level of hybrid proteins in noninducing media is sufficient to allow growth on lactose if the hybrid protein is not efficiently localized from the cytoplasm.

In general, strains containing *lacZ* gene fusions specifying an exported hybrid protein exhibit phenotypes similar to those described above (1). In several cases, these phenotypes have been exploited to obtain cells containing mutations that cause defects in export. Such mutations alter the signal sequence of the product of the gene to which *lacZ* is fused, or they alter a component of the cellular export machinery. However, in all cases, the export process is blocked at an early step, as shown by the fact that these mutations cause the accumulation of precursors to one (in the case of signal sequence mutants) or more exported proteins in the cytoplasm of mutant cells.

We wish to obtain mutants in which a later step in the export process is altered. We have previously shown that certain *lamB* deletions when present on the *lamB–lacZ* fusion lower the degree of localization of the hybrid protein to the outer membrane. These fusions confer a $Lac^+$ phenotype and simultaneously increase maltose sensitivity (12). In contrast, mutations that alter the signal sequence and block localization at an early step simultaneously lose maltose sensitivity (19). By selecting for growth on lactose and screening for retention of maltose sensitivity, cells with mutations that block or compromise localization can therefore be obtained. Accordingly, we have searched for $Lac^+$ mutant derivatives of strain Pop3299 (*lamB–lacZ* 42-12) that remain sensitive to maltose.

**Isolation of $Lac^+$ Derivatives of the *lamB–lacZ* 42-12 Fusion.** Selection for $Lac^+$ mutants on lactose/tetrazolium agar was done as described in *Materials and Methods*. These mutants appear as papilli in a lawn of cells and occur at a frequency of $<10^{-10}$. Two hundred and seventy-five independent $Lac^+$ colonies were isolated and purified by restreaking. All 275 mutants were screened initially for growth on various lactose and maltose indicator plates at both 30°C and 42°C. As expected, two classes of mutants were obtained: those that remain sensitive to maltose ($Mal^s$) and those that no longer exhibit this phenotype ($Mal^r$). Most (222) of the $Lac^+$ mutants are $Mal^r$. The remaining 53 $Lac^+$ mutants exhibit various degrees of maltose sensitivity. Although the mutants were scored for either a temperature-sensitive or a cold-sensitive phenotype, neither was found. A representative subset of 12 mutants was selected for further study.

**Quantitation of the $Mal^s$ Phenotype.** The degree of maltose sensitivity exhibited by each of the 12 selected $Lac^+$ mutants was quantitated and the results are shown in Table 1. As can be seen, considerable variation in maltose sensitivity was observed: from total resistance to greater sensitivity than that exhibited by the parent fusion strain. Previously characterized *lamB–lacZ* fusions 61-4 (Pop3105), 52-4 (Pop3194), and 42-1 (Pop3186) and the parent 42-12 fusion (Pop3299) are included for comparison.

**Physical Characterization of the $Lac^+$ Mutants.** Initial biochemical analysis of the 12 representative mutants indicated that, in all cases, the $Lac^+$ phenotype was the consequence of a genetic alteration that reduced the size of the hybrid protein (data not shown). This suggested that the mutations were deletions contained within the hybrid gene.

To determine more precisely the amount of *lamB* DNA that had been removed by the various deletions, we inserted a DNA fragment from the *Eco*RI site in *malK* to the *Eco*RI site in *lacZ* from each of the fusions into a small plasmid as described in *Materials and Methods*. The size of the various deletions was then estimated by physical analysis of the cloned fragment, using a variety of restriction enzymes. These results are summarized in Table 1.

The results shown in Table 1 reveal a striking correlation. Strains containing deletions that remove >700 base pairs are $Mal^r$, whereas strains containing smaller deletions are $Mal^s$. Presumably, these larger deletions that confer a $Mal^r$ phenotype extend into regions of *lamB* required for early steps of protein localization. Six of the mutants, BRE100, -103, -104, -120, -330, and -367, were selected for DNA sequence analysis to determine the exact location of the deletion within the hybrid gene.

**DNA Sequence Analysis of the Deletion Mutations.** Using techniques summarized in *Materials and Methods*, we determined the DNA sequence of the deletion mutations. Results show that in all cases the deletions remove both *lamB* and *lacZ* DNA (Table 2). Accordingly, the deletions remove the parent fusion joint and result in the formation of a new *lamB–lacZ* fusion. Each of the new fusions specifies a hybrid protein that contains portions of LamB at the $NH_2$ terminus and a large, enzymatically active COOH-terminal

Table 1. Maltose sensitivity and restriction analysis of various fusion strains

| Strain | *lamB–lacZ* fusion | Growth sensitivity to maltose | Diameter of maltose inhibition zone, mm | | | | Size of deletion, base pairs |
|---|---|---|---|---|---|---|---|
| | | | 5% | 3% | 1% | 0.5% | |
| MC4100 | — | R | 0 | 0 | 0 | 0 | NA |
| Pop3105 | 61-4 | R | 0 | 0 | 0 | 0 | NA |
| Pop3194 | 52-4 | R | 0 | 0 | 0 | 0 | NA |
| Pop3186 | 42-1 | $S^s$ | 26 | 24 | 19 | 16 | NA |
| Pop3299 | 41-12 | $R^s$ | 17 | 16 | 0 | 0 | NA |
| BRE100 | 100 | R | 0 | 0 | 0 | 0 | 835 |
| BRE103 | 103 | S | 19 | 14 | 9 | 0 | 674 |
| BRE104 | 104 | S | 19 | 14 | 11 | 0 | 650 |
| BRE120 | 120 | $S^s$ | 22 | 21 | 16 | 11 | 636 |
| BRE144 | 144 | S | 19 | 16 | 9 | 0 | 620 |
| BRE181 | 181 | R | 0 | 0 | 0 | 0 | 819 |
| BRE187 | 182 | S | 21 | 19 | 16 | 10 | 620 |
| BRE200 | 200 | R | 0 | 0 | 0 | 0 | 720 |
| BRE294 | 294 | R | 0 | 0 | 0 | 0 | 842 |
| BRE330 | 330 | R | 0 | 0 | 0 | 0 | 710 |
| BRE351 | 351 | R | 0 | 0 | 0 | 0 | 715 |
| BRE367 | 367 | R | 0 | 0 | 0 | 0 | 704 |

The sizes of the various deletions were determined by restriction digest analysis of *lamB–lacZ* fusion plasmids as described by Benson and Silhavy (12). R, resistant; $R^s$, resistant–sensitive; S, sensitive; $S^s$, extreme sensitivity; NA, not applicable.

fragment of β-galactosidase.

The DNA sequence analysis (Table 2) and results presented in Table 1 allow us to determine the minimal amount of *lamB* required for a *lamB–lacZ* fusion to confer a $Mal^s$ phenotype. Fusions that specify a hybrid protein containing the signal sequence plus 39 or more amino acids of mature LamB confer maltose sensitivity. In contrast, fusions that specify a hybrid protein containing as many as 27 amino acids of mature LamB grow normally in the presence of maltose.

**Cellular Localization of the Hybrid Proteins Specified by the $Lac^+$ Mutants.** As a first step to determine the cellular location of the various hybrid proteins, mutant fusion strains were fractionated by high-speed centrifugation into soluble (cytoplasm and periplasm, S-200) and particulate (inner and outer membranes, P-200) fractions. The amount of hybrid protein present in each was quantitated, and the results of these experiments (Table 3) are consistent with data obtained previously with other *lamB–lacZ* fusion strains.

Hybrid proteins that do not contain a complete LamB signal sequence, such as those specified by the *lamB–lacZ* fusions 100 or 61-4 (13), are located almost exclusively in the cytoplasm. The small amount of hybrid protein found in the particulate fraction probably represents cross-contamination.

The *lamB–lacZ* 52-4 fusion specifies a hybrid protein that contains the complete LamB signal sequence plus 15 amino acids of mature LamB (13). Strains carrying this fusion are $Mal^r$, and the hybrid protein is located in the cytoplasm (13). Two of the fusions reported here, 330 and 367, behave identically to 52-4. Fractionation data obtained with these fusion strains reveal a reproducible increase in the amount of hybrid protein associated with the particulate fraction when compared to fusions such as 61-4 or 100. This was observed previously with the 52-4 fusion and in other fusion strains and may reflect nonspecific binding of the hydrophobic signal sequence to membrane vesicles.

BRE103 and BRE104 define an additional class of *lamB–lacZ* fusion strains. Although these strains are $Mal^s$, the hybrid protein produced appears to be located predominantly in the cytoplasm. Since the $Mal^s$ phenotype is associated with hybrid protein export, this result seems anomalous.

Table 2. Sequence analysis of various *lamB–lacZ* fusion joints

| Strain | *lamB–lacZ* fusion | Amount of LamB present in fusion | Amino acid position of fusion joints in LacZ |
|---|---|---|---|
| Pop3105 | 61-4 | −21 of SS | 26* |
| Pop3194 | 52-4 | SS + 15 | 20* |
| Pop3186 | 42-1 | SS + 173 | ND |
| Pop3299 | 42-12 | SS + 241 | 20 |
| BRE100 | 100 | −16 of SS | 25 |
| BRE103 | 103 | SS + 39 | 23 |
| BRE104 | 104 | SS + 43 | 24 |
| BRE120 | 120 | SS + 49 | 26 |
| BRE330 | 330 | SS + 20 | 25 |
| BRE367 | 367 | SS + 27 | 20 |

The amounts in *lamB* are numbers starting at amino acid 1 of the mature protein; therefore, the initiation methionine is amino acid −25. For Pop3186, the exact position of the *lamB–lacZ* fusion joint has not been determined by sequence analysis. The amount of *lamB* present has been estimated by detailed restriction digest analysis. SS, signal sequence; ND, not determined.

*The amount of LacZ at the fusion joint was determined by Moreno *et al.* (22).

Table 3. Cellular location of various LamB–LacZ fusion proteins

| Strain | *lamB–lacZ* fusion | Amount of LamB present in fusion | % of total fusion protein | | |
|---|---|---|---|---|---|
| | | | S-200 | P-200 | Outer membrane |
| Pop3105 | 61-4 | −21 of SS | >97 | >3 | <0.5 |
| Pop3194 | 52-4 | SS + 15 | 90 | 10 | <0.5 |
| Pop3186 | 42-1 | SS + 173 | 33 | 67 | 33 |
| Pop3299 | 42-12 | SS + 241 | >15 | >85 | >65 |
| BRE100 | 100 | −16 of SS | >97 | >3 | <0.5 |
| BRE103 | 103 | SS + 39 | 90 | 10 | 1.4 |
| BRE104 | 104 | SS + 43 | 90 | 10 | 1.5 |
| BRE120 | 120 | SS + 49 | 40 | 60 | 20 |
| BRE330 | 330 | SS + 20 | 92 | 8 | <0.5 |
| BRE367 | 367 | SS + 27 | 88 | 12 | <0.5 |

The various cellular fractions were prepared and the percent of hybrid protein present in each fraction was quantitated by densitometer scanning. The level of detectability is approximately 0.5%. SS, signal sequence.

However, it may reflect the inherent problems often encountered when fractionating fusion strains. Ito *et al.* (23) have studied the cellular localization of hybrid proteins produced by *malE–lacZ* fusion strains (*malE* specifies the periplasmic maltose-binding protein). They found that when fractionation was done under conditions of low ionic strength, the hybrid protein was soluble. However, under conditions of higher ionic strength, the hybrid protein was found in the particulate fraction. Ito *et al.* (23) interpreted these results as indicating a weak hydrophobic interaction between these hybrid proteins and the membrane. Although we have not done fractionation studies under conditions of higher ionic strength, we suspect that the hybrid proteins specified by the *lamB–lacZ* fusions 103 and 104 would behave in a manner analogous to the MalE–LacZ hybrid proteins because strains containing these fusions exhibit nearly identical phenotypes.

The fusion strain BRE120 produces a hybrid protein that is found predominantly in the particulate fraction. To more accurately determine the location of this hybrid protein, we separated the inner and outer membranes. Although results of cellular fractionation experiments with Mal$^s$ fusion strains can be difficult to interpret (13), data presented in Fig. 1 show that a significant amount of the hybrid protein produced by this strain copurifies with the major outer membrane proteins OmpF, OmpC, and OmpA. In this regard this strain is similar to the previously described fusion strain Pop3186 (*lamB–lacZ* 42-1). Both strains produce a hybrid protein that is exported inefficiently to the outer membrane, and both strains exhibit extreme maltose sensitivity (Table 1). In contrast, the amount of hybrid protein in the outer membrane of strain BRE103 is only about 1.5% of the total (Fig. 1). Thus, despite the fact that BRE120 and BRE103 exhibit similar degrees of maltose sensitivity, only the former produces a hybrid protein that contains sufficient information to direct export to the outer membrane.

## DISCUSSION

We have developed a selection procedure that has yielded strains carrying a series of *lamB–lacZ* fusions. Each of these fusions specifies a hybrid protein containing LamB sequences at the $NH_2$ terminus and a large functional COOH-terminal fragment of $\beta$-galactosidase. DNA sequence analysis shows that each of the fusions differs in the amount of *lamB* contained within the hybrid gene, whereas the amount of *lacZ* is essentially constant. The phenotypes conferred by these fusions together with fractionation data regarding the cellular location of the various hybrid proteins provide a means to identify regions within *lamB* that are required for export from the cytoplasm and localization to the outer membrane.

The Mal$^s$ phenotype exhibited by certain *lamB–lacZ* fusion strains is correlated with hybrid protein export (1, 12, 21). If export of the hybrid protein to the outer membrane is efficient, then the degree of maltose sensitivity is relatively low [(*lamB–lacZ* 42-12) Table 2 (13)]. Mutations that decrease export efficiency to the outer membrane exacerbate maltose sensitivity (12). On the other hand, mutations that prevent export altogether confer resistance to maltose (1, 11, 19–21). Thus, the Mal$^s$ phenotype appears to be a sensitive

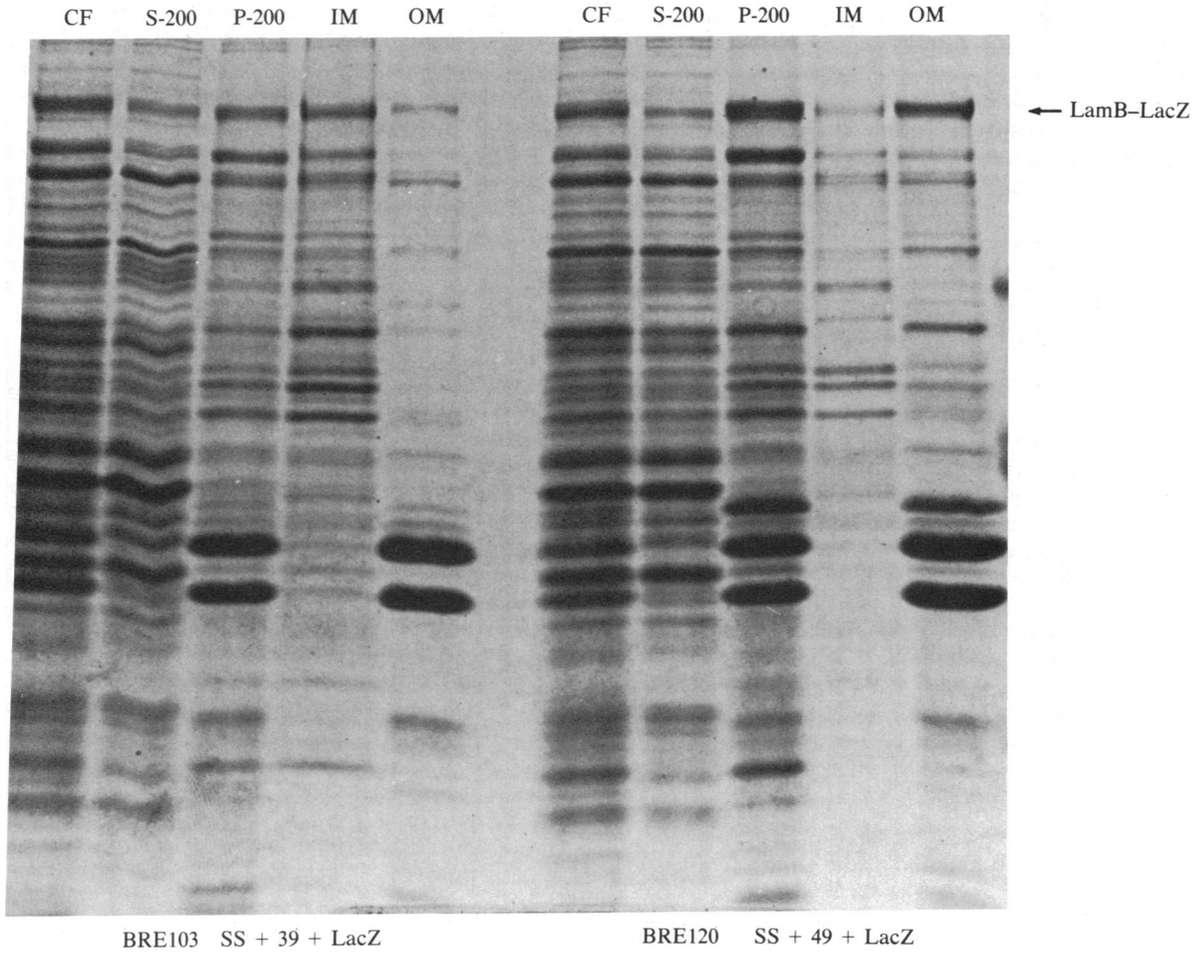


FIG. 1. Cellular location of LamB–LacZ hybrid protein in BRE120 and BRE103. Cellular extracts were prepared and the gel electrophoresis was performed as described in *Materials and Methods*. CF, total cell fraction; IM, inner membrane; OM, outer membrane; SS, signal sequence.

indicator of the cells' attempt to localize the hybrid protein.

The *lamB–lacZ* fusions reported here allow us to more precisely define the minimal amount of *lamB* required to confer a Mal$^s$ phenotype. Fusions that specify a hybrid protein containing the complete signal sequence plus as many as 27 amino acids of LamB do not confer sensitivity to maltose. In contrast, fusions that specify a hybrid protein containing the signal sequence plus as few as 39 amino acids of LamB confer a high degree of maltose sensitivity. Since the Mal$^s$ phenotype is correlated with export, we suggest that at least a portion of an essential export signal is contained in a region of the *lamB* gene corresponding to amino acids 27 to 39 of the mature protein. If this region is not present, export does not occur and the hybrid protein remains in the cytoplasm.

Unlike other *lamB–lacZ* fusions, the fusion that specifies a hybrid protein containing the signal sequence plus 39 amino acids of LamB behaves in a manner analogous to large *malE–lacZ* fusions. These fusions, which contain as much as 90% of the *malE* structural gene (24), confer a high degree of maltose sensitivity, and they specify a hybrid protein that appears to be weakly associated with the inner membrane. This association seems to be a consequence of the aborted attempt by the cell to export the hybrid protein to the periplasm. Using the same reasoning, we suggest that the information in *lamB* necessary to direct export from the cytoplasm is contained at the 5′ end of the structural gene prior to the codon specifying amino acid 40 of the mature protein. The signal sequence is clearly a necessary part of this information. However, for this signal to function, additional information is required as well. At least a portion of this additional information appears to lie in a region of *lamB* between the codons for amino acids 27 and 39.

In order to direct β-galactosidase to the outer membrane, a *lamB–lacZ* fusion must contain more information than that which corresponds to the signal sequence and 39 amino acids of mature protein. The smallest fusion we have isolated that appears to direct appreciable amounts of product to the outer membrane specifies a hybrid protein containing the signal sequence plus 49 amino acids of LamB. This result suggests that at least a portion of the information that specifies an outer membrane location must be present between the codons for amino acids 39 and 49 of the mature protein. If this region is absent, the cell attempts to export the hybrid protein but very little reaches the outer membrane.

It seems reasonable to propose that many outer membrane proteins will contain a common signal that directs sorting to the correct cellular membrane. If this sorting signal is confined to a small region, then it should be possible to identify similar signals within other outer membrane proteins by amino acid sequence comparison. Recently, Nikaido and Wu (25) have developed a computer program to search for such homologous sequences. With this program they have identified a common region in the major outer membrane proteins OmpF, OmpA, PhoE, and LamB. In LamB, this common region overlaps the amino acids 39 to 49. This striking convergence lends credence to both genetic and computer approaches and supports the proposal that outer membrane proteins contain a common sorting signal.

Neither the results described here nor sequence analysis of signal-containing regions provides any clues as to the nature or role of the intragenic information required for export from the cytoplasm or sorting to the outer membrane. Although the signal sequence and the sorting signal (amino acids 39 to 49) are likely to function at the amino acid level, other information, such as the region corresponding to amino acids 27 to 39, may be read from the mRNA. Moreover, the regions identified may function in an indirect manner by allowing the formation of specific, recognizable conformational domains (26).

It can always be argued that by fusing β-galactosidase to various segments of LamB we have perturbed the system to the point at which results are not physiological. Given past successes using gene fusions as a tool, we believe that the results presented here identify regions of *lamB* that are important for export of the protein to the outer membrane. However, conclusive proof must await specific modification of these regions in an otherwise wild-type *lamB* gene.

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