Localization and Processing of Outer Membrane and Periplasmic Proteins in *Escherichia coli* Strains Harboring Export-specific Suppressor Mutations*

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Mutations at three genetic loci (termed prlA,B,C) were previously shown to specifically suppress signal sequence mutations in the lamB gene encoding the outer membrane phage λ receptor protein of *Escherichia coli* (Emr, S. D., Hanley-Way, S., and Silhavy, T. J. (1981) *Cell* 23, 79-88). The majority of these suppressor mutations map at the prlA locus and are thought to result in an altered ribosomal protein. In this study, we demonstrate that prlA mutations also phenotypically suppress signal sequence mutations in the malE gene encoding the periplasmic maltose-binding protein. For both lamB and malE mutations, suppression is achieved by transporting the export-defective protein to its correct extracytoplasmic location, in some instances with near 100% efficiency. With a single exception, the mutant-exported protein is apparently processed to its normal mature form. These results indicate that prlA-mediated protein export occurs via the usual route, and additional data suggest that the prlA product directly interacts with the mutant signal sequence to restore export. The single prlC allele also suppresses malE signal sequence mutations, whereas the single prlB allele only phenotypically suppresses lamB signal sequence mutations. However, with these latter two suppressors, there is some indication that export of the phage λ receptor to the outer membrane is not accomplished by the usual route.

The study of protein localization in the prokaryotic gram-negative bacterium *Escherichia coli* is believed to represent a valid model system for the study of analogous processes in eukaryotic cells, due to the many striking similarities between the two systems (for reviews, see refs. 1-5). In *E. coli*, most outer membrane and periplasmic proteins are initially synthesized with an extra amino-terminal sequence of 15 to 30 predominantly hydrophobic amino acid residues termed the "signal sequence". It is hypothesized that this signal sequence binds the ribosome to the cytoplasmic membrane and initiates the co-translational transfer of the nascent polypeptide chain through that membrane. At some point either prior to the completion of translation or very shortly thereafter, the signal sequence is enzymatically removed by a processing enzyme located in the cell envelope (6). In approximately the same time interval, the "mature" protein reaches its final cellular location (7). The exact sequence of events in the maturation and localization of exported proteins has not been firmly established. The precise role of the ribosome in the export process, and the nature of the export machinery in the cytoplasmic membrane, if any, are unknown.

Confirmation of the essential role of the signal sequence in initiating protein export was provided by the isolation and characterization of *E. coli* mutants that are defective in either the export of the phage λ receptor protein to the outer membrane (8-10), or the maltose binding protein to the periplasm (11, 12). As a direct consequence of mutational alterations in the signal sequence, these proteins accumulate in the cytoplasm in their higher molecular weight precursor (i.e. unprocessed) forms. As one approach to identifying other cellular components involved in the export process, a number of extragenic suppressor mutations were recently isolated that restore export of mutant LamB protein having a defective signal sequence (13). The three genetic loci in which such suppressor mutations were obtained are designated prlA, B and C (for protein localization). The prlA locus includes the great majority of suppressor mutations, and fine structure genetic mapping assigned this locus to an operon known to code for only ribosomal proteins (the Pspc operon at 72 min on the *E. coli* linkage map). In this study, we have further investigated the effect of various prl suppressor mutations on the localization and processing of wild-type and export-defective LamB protein and MBP.  

**MATERIALS AND METHODS**

Media, Chemicals and Genetic Techniques—Cells were grown and, when required, radiolabeled in minimal medium M63 (14) containing 0.2% maltose as the carbon source. Strains were tested for their Dex+ phenotype on Eosin Methylene Blue minimal Dextrin agar plates (9) containing 0.7% Dextrin (biological grade, Type 1, Sigma). Tryptone yeast extract agar was prepared as described (14). 35S-methionine was obtained from New England Nuclear; 14C-unlabeled methionine was obtained from Sigma.

*The abbreviations used are: LamB protein, phage λ receptor protein; preLamB protein, precursor phage λ receptor protein; MBP, maltose-binding protein; preMBP, precursor maltose-binding protein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.*

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whether labeled amino acids were obtained from ICN, formalinized Staphylococcus aureus (Mr. Geobert) was purchased from the Enzyme Center, Inc., Boston, MA. Generalized transduction with bacteriophage Pirvir was performed as described (14).

**Dipteral Strains:** Construction of lamB prl and malE prl Double Mutants—Because of the large number of different strains used in this study, we have chosen to refer throughout the text and figures to the lamB prl mutant rather than specific strain designations. All of the strains are derivatives of the E. coli K12 strain SE2600 (F araD139 lacIq lacY189 rpsL relA thi lamBSGO; see Ref. 13). To introduce a particular lamB signal sequence mutation into a strain harboring a particular allele, a small malE-lamb deletion (malE/lamB deletion, Ref. 9) was moved into the appropriate periplasm strain by phage P1-mediated co-transduction with the transposon Tn5 positioned on the E. coli chromosome adjacent to the malB region (selecting resistance to the antibiotic kanamycin). The resultant periplasm recombinant strain is unable to utilize maltose as a carbon source due to deletion of a portion of the malB gene. This same deletion removes the promoter-proximal portion of the lamB gene encoding the signal sequence of the LamB protein. Transduction of this strain to growth on maltose (malK') by phage P1 grown on the appropriate lamB donor strain (described in Ref. 10) requires that the malBlB deletion in the recipient strain be replaced by homologous DNA from the donor that includes the lamB mutation. Similarly, the wild-type lamB* allele was introduced into prl strains. To introduce a particular malE signal sequence mutation into a given periplasm, a malE-malK deletion (malB/lamB deletion) was introduced into the appropriate periplasm strain by co-transduction with Tn5. This deletion includes the promoter-proximal portion of the malE gene encoding the signal sequence of the MBP. Transduction of the prl malB/lamB strain to growth on maltose by phage P1 grown on the appropriate malE donor strain (described in Ref. 12) required incorporation of the malE mutation into the chromosome. In the case of both lamB and malE mutant strains, only those recombinant strains that had lost the Tn5 transposition in the second transduction step were saved for further study.

**Radiolabeling and Immune Precipitation—** Anti-MBP serum was prepared by subcutaneously injecting a 6-lb, female New Zealand White rabbit with 1.0 mg of purified MBP (prepared as described in Ref. 15) in complete Freund's adjuvant, boosting subcutaneously at 2 weeks with 0.75 mg of purified MBP, also in complete adjuvant, and bleeding beginning 10 days later. To precipitate MBP and preMBP, 0.5 ml cultures (A600 of 0.5) growing at 30°C with vigorous aeration were labeled with 2.5 pCi of 14C-uniformly labeled amino acids for 10 min and then placed on ice. Cells were transferred to a 1.5-ml Eppendorf tube, washed once with 0.5 ml of 10 mM Tris (pH 8.0), and then resuspended in 0.1 ml of 10 mM Tris (pH 8.0), 0.15 mM EDTA, and then also redissolved in 0.1 ml of 10 mM Tris (pH 8.0). The immune precipitate was precipitated with 50 ml of SDS sample buffer, heating in a boiling water bath for 2.5 min, and centrifuged at 10,000 g for 10 min at room temperature (supernatant contained immune precipitate). An aliquot (50 ml) of each precipitate was used for SDS-PAGE. This procedure is nearly identical to that previously described (16). Recovery of antigen is near 100% (16).

A similar protocol was used to precipitate LamB protein and preLamB protein (described in Ref. 13). However, in this case 1 ml cultures were labeled with 10 ml of 10 ml of 3H-methionine for 4 min prior to preparation of antigen extract. Anti-LamB protein serum was kindly provided by J. Gabay of the Institut Pasteur.

**Cell Fractionation—** Bacterial inner and outer membranes were separated by a selective solubilization procedure (17), beginning with 500 ml cultures growing exponentially in maltose minimal medium. A periplasmic fraction was prepared for immune precipitation as follows: cultures (2.0 ml) were grown, labeled with 14C-amino acids and placed on ice as described above. A 0.5-ml portion was used to prepare the whole cell antigen extract. The remaining cells were osmotically shocked with a suspension of the bacterial strain in 8 M urea and lyophilized, and then resuspended in 0.15 ml of 10 mM Tris (pH 8.0) containing 1% SDS, 1 mg EDTA. The pellet (shocked cells) was washed once with 10 ml of cold Tris (pH 8.0) and then also redissolved in 0.15 ml of the same SDS buffer. Antigen extracts were prepared and immune precipitation performed exactly as described above.

**Polyacrylamide Gel Electrophoresis—** The polyacrylamide slab gel system used has been described previously (9). Outer membrane fractions and immune precipitates of LamB protein and preLamB protein were run on 30-cm 9% polyacrylamide gels. Immune precipitates of MBP and preMBP were run on 21-cm 10% gels. Gels were dried down for autoradiography. In some instances, gels were rehydrated, relevant lanes cut out, frozen on a block of dry ice, and cut into 1-mm slices. These were solubilized in 0.1 ml of 30% H2O2 at 50°C and then counted following addition of 10 ml of appropriate scintillation mixture.

**RESULTS**

**Phenotypes of Suppressor Strains—** Mutations that prevent the export but not the synthesis of the LamB protein and the MBP have been previously described (8–12; summarized in Fig. 1). Those strains that fail to export the LamB protein to the outer membrane are phenotypically resistant to bacteriophage λ (λ') and are unable to utilize maltodextrins as a carbon source (Dex+). The various prl mutations were originally selected for their ability to suppress a lamB signal sequence deletion mutation (lamBSGO, see Fig. 1)(13). Subsequently, these prl mutations were tested for their ability to suppress the other lamB signal sequence mutations shown in Fig. 1. If the prl mutation did suppress a given lamB mutation, the lamB prl double mutant strain would score as Dex+. Suppression to λ' for two lamB mutations, S71 and S60, is illustrated in Fig. 2. Several points are readily apparent. One is that among the many independent prlA mutations isolated, some exhibit much stronger suppression (prlA4) than others (prlA3). In addition, the two prlA mutations shown appear to exhibit somewhat stronger suppression than prlB, which in turn seems stronger than prlC. Full induction of the malB-lamb operon by growth on maltose is required to observe significant suppression by either of the latter two mutations. It is also apparent in Fig. 2 that the point mutation S71 is more readily suppressed by prlA alleles than the S60 deletion that eliminates 12 amino acids from the LamB protein signal sequence. In fact, by this and other criteria (see below), each of the four lamB point mutations, as well as the smaller S78 deletion, are all suppressed to a significantly greater extent by prlA mutations than the S60 deletion. In contrast, we consistently observe maximum phenotypic suppression by prlB in combination with the S60 deletion (Fig. 2). Results obtained by scoring lamB prl double mutants for their Dex+ phenotype were fully consistent with those obtained by scoring sensitivity to bacteriophage λ (data not shown).

Those strains that fail to export the MBP to the periplasm are deficient in their ability to utilize maltose or maltodextrins as a carbon source (Mal'). The ability of various prlA, B and C alleles to phenotypically suppress signal sequence mutations in the malE gene encoding the MBP was also investigated by determining doubling times for malE prl double mutants utilizing 0.2% maltose as the sole carbon source (Table I). As previously discussed (11, 12), none of the five classes of malE signal sequence mutants characterized to date are totally defective in the export of mature MBP to the periplasm. Thus, the malE prl strains do grow in maltose minimal
maltose. The amino-terminal 32 residues for both the LamB protein prl alleles we worked with have any adverse effect on growth respectively, are two of five genes whose products are components of the maltose transport system of E. coli. These five genes are organized into two operons that diverge from a common promoter region. Expression of both operons is induced by growth in the presence of and the preMBP that prevent protein export.

The prlB mutation can phenotypically suppress malE mutations (10-1), and the prlC mutation can phenotypically suppress malE mutation. In contrast, there is no indication that suppression by two different prlA alleles results in increased processing of preLamB protein. Note that suppression by two different prlA alleles results in the appearance of a protein band apparently identical to mature LamB protein, with a corresponding decrease in the intensity of the preLamB protein band. (In the case of the lamBS71 prlC and lamBS78 prlC strains, a protein band that appears to migrate just slower than mature LamB protein is observed, indicating that export of the LamB protein resulting from deletion mutations is indicated by shaded bars. The corresponding allelic designations are also given. See text for additional details and references.)

Medium at rates that correlate with the amount of mature MBP exported. Most of the malE prlA double mutants constructed show a significant decrease in doubling time compared to its isogenic malE prlB strain. In general, the prlA4 allele seems to be the strongest suppressor, but good suppression is also observed with the A2 and A3 alleles. Somewhat weaker suppression is observed in malE prlC double mutants, not inconsistent with the weak suppression described above for lamB prlC strains. In contrast, there is no indication that the prlB mutation can phenotypically suppress malE mutants; doubling times are essentially unchanged in the presence or absence of this mutation. Interestingly, none of the prl alleles we worked with have any adverse effect on growth of malE strains in maltose minimal medium. Also, it is important to note here that, as was previously and rigorously demonstrated in the case of lamB mutations (13), we do not observe suppression by any prl allele of a number of malE nonsense or missense mutations mapping promoter distal to the signal sequence region (data not shown).

Immune Precipitation of LamB Protein from Wild-type and Mutant Strains—Precipitation of 35S-labeled lamB+ cells with anti-LamB protein serum yields a single, major protein of approximately 50,000 molecular weight on SDS-PAGE, the mature LamB protein (Fig. 3). Precipitation of cells harboring the lamBS78 or the lamBS71 mutations yields a single, major protein of some 2,000-3,000 greater molecular weight corresponding to the mutant LamB precursor protein (preLamB protein) that accumulates in these signal sequence mutant strains (8, 9). A small amount of mature LamB protein also is precipitated from the lamBS78 strain but not the lamBS71 strain, indicating that export of the LamB protein is not totally prevented by the S78 mutation. This is in agreement with previous results (9) that indicated that strains harboring the lamBS78 mutation are slightly more sensitive to passage through the preLamB processing site. Precipitation of the preLamB protein is not inconsistent with the weak suppression described above for lamB prlC strains, although phenotypic suppression to AS is observed (Fig. 2). In the case of the lamBS71 prlC and lamBS78 prlC strains, a protein band that appears to migrate just slightly slower than mature LamB protein is observed, indicative perhaps of incorrect processing of preLamB protein. Even though a fairly significant amount of processed LamB protein is detected in these prlC strains (particularly the lamBS78 prlC strain), the prlC allele exhibits only very weak
phenotypic suppression of the lamB signal sequence mutants (see Fig. 2). This could be further indication that the presence of the prlC mutation results in improper processing and/or localization of the mutant LamB protein. Finally, Fig. 3 indicates that the efficiency of suppression of the lamBS78 mutation generally is greater than that of lamBS71. This may be because, as mentioned above, lamBS71 is a stronger signal sequence mutation than lamBS78. Results comparable to those shown in Fig. 3 were also obtained for other lamB signal sequence mutations (except for the lamBS60 mutation, see below).

For each of the lamB mutations shown in Fig. 1, the most efficient suppression was exhibited by the prlA4 suppressor allele. Precipitation of LamB and preLamB protein from the different signal sequence mutant strains harboring either prlA4 or prlA4 alleles is shown in Fig. 4. Note the following. (i) The prlA4 allele has no effect on maturation of the wild-type LamB protein. (ii) Certain lamB mutations are suppressed more efficiently than others (particularly compare the S78 and S69 mutations), suggesting there is some allele specificity to the suppression pattern. (iii) No processing of the lamBS60 product can be detected although, again, suppression to a λ6Dex phenotype is observed in this strain (although the suppression is relatively weak, processing is not observed even with significantly longer film exposure). The 36 base pair S69 deletion does not eliminate the processing site between residues 25 and 26 of the preLamB protein. However, processing of preLamB protein coded for by the lamBS60 allele has not been observed in any suppressor strain isolated to date. (iv) The preLamB protein that accumulates in the lamBS69 point mutant strain migrates with an apparent molecular weight slightly smaller than that seen in the other point mutant strains. The single amino acid substitution resulting from the lamBS69 mutation (Met to Arg at position 19) may change the protein conformation, or the SDS-binding capacity of the protein, sufficiently to lead to an altered migration pattern on SDS-PAGE. This was previously observed for a single amino acid substitution (Arg to Cys) in the histidine binding protein of E. coli (19). Alternatively, the lamBS69 mutation may result in the nonspecific enzymatic removal of a few amino-terminal residues of the internalized preLamB protein.

To further illustrate suppression by the prlA4 allele, several lanes from the gel shown in Fig. 3 were sliced and counted, as presented in Fig. 5. One can see that suppression of the 12 base pair S78 deletion is remarkably efficient. Suppression of the S71 point mutation, in contrast, is much less efficient. Note the relative positions of preLamB protein. Elimination of residues 10–13 in the preLamB protein by the S78 deletion results in a detectably smaller precursor. The processed mature protein, however, runs identical to the wild-type lamB+ product in both cases.

**Immune Precipitation of MBP from Wild-type and Mutant Strains.** Precipitation of 14C-labeled wild-type cells with anti-MBP serum yields a single, major protein of approximately
Protein Export in prl Suppressor Strains

FIG. 3. Slab gel SDS-PAGE of preLamB and LamB protein immune precipitated from various wild-type and mutant strains. See "Materials and Methods" for experimental details. Top gel, precipitates obtained from lamB' strains and strains harboring the lamBS78 mutation. Lower gel, precipitates obtained from strains harboring the lamBS99 mutation. The relevant prl genotypes are given above each lane. Arrows indicate the positions of preLamB and LamB protein.

FIG. 4. Slab gel SDS-PAGE of preLamB and LamB protein immune precipitated from various prl' and prlA4 strains. See "Materials and Methods" for experimental details. Only the relevant portion of autoradiograph is shown. Relevant lamB and prl genotypes are given above each lane. Arrows indicate the positions of preLamB and LamB protein. It should be noted that in the case of the lamBS99 mutant, and the lamBS99 prlA4 double mutant, immune precipitation was performed with five times more labeled cells than was used for other strains. This was necessary to obtain similar numbers of radioactive counts in the immune precipitates of these two strains.

39,000 molecular weight on SDS-PAGE, the mature MBP (Fig. 6). Precipitation of cells having malE signal sequence mutations yields a protein of molecular weight approximately 3,000 greater which corresponds to the mutant MBP precursor (preMBP). As previously stated, none of the MBP signal sequence mutants are absolutely defective in MBP export; a small amount of mature MBP can be detected in each mutant strain (Fig. 6). The mutations malE18-1 and malE19-1 are strongest in terms of preventing MBP export; malE16-1 is the weakest. As can also be seen in Fig. 6, the presence of the prlA4 allele in combination with each malE mutation significantly increases the amount of mature MBP detected in each strain with, as expected, a corresponding decrease in the intensity of the preMBP protein band. Note that the prlA4 allele has no effect on maturation of the wild-type MBP. It should also be noted here that these results are certainly consistent with the ability of the respective malE prl' and malE prlA4 strains to utilize maltose as a carbon source.

FIG. 5. SDS-PAGE profiles of preLamB and LamB protein immune precipitated from representative prl' and prlA4 strains. See "Materials and Methods" for experimental details. The relevant lamB and prl genotype is given for each profile.
Protein Export in prl Suppressor Strains

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FIG. 6. Slab gel SDS-PAGE of preMBP and MBP immune precipitated from various prl+ and prlA4 strains. See "Materials and Methods" for experimental details. Only the relevant portion of autoradiograph is shown. Relevant malE and prl genotypes are given above each lane. Arrows indicate the position of preMBP and MBP.

(Table 1). Those strains that exhibit only a very small amount of mature MBP in Fig. 6 are definitely deficient in their ability to utilize maltose; where the prlA4 mutation results in a marked increase in the relative proportion of mature MBP, there is a corresponding increase in the efficiency of the strain for maltose utilization.

As we previously observed for the various lamB signal sequence mutations, certain malE mutations are more efficiently suppressed by the prlA4 allele than others, again suggesting a degree of allele specificity to the suppression pattern. To illustrate this, several lanes from the gel pictured in Fig. 6 were sliced and counted (Fig. 7). Note the marked difference in suppression of the 16-1 and 19-1 point mutations. However, one could argue that what we are observing here is not really allele specificity, but rather relative increases in suppression efficiency due to the strength of the individual mutations. Since the 19-1 mutation is considerably stronger than the 16-1 mutation, one might reasonably expect less efficient suppression of the former. The argument for allele specificity is strengthened by the suppression of malE signal sequence mutations by two additional prlA alleles, A2 and A3 (Fig. 8). For example, both these prlA alleles suppress the malE19-1 mutation significantly more efficiently than the 18-1 mutation, even though these two malE mutations are virtually identical in terms of amino acid alteration and effect on MBP export. Even more striking, note that prlA3 quite clearly suppresses the 19-1 mutation more efficiently than the weaker 14-1 mutation.

When MBP and preMBP are precipitated from malE prlB double mutants, we detect no appreciable difference in the ratio of the two MBP species, compared to that seen in isogenic prl+ strains (data not shown). We do observe slight increases in the proportion of mature MBP in each of the malE prlC double mutants (i.e. weak suppression, as predicted by the results in Table 1). However, there is no indication of aberrant processing of preMBP in these strains (data not shown).

Localization of LamB Protein and MBP in prlA Suppressor Strains—We expected that the phenotypic suppression of lamB signal sequence mutations would require export of the mutant LamB protein to the outer membrane, with or without subsequent processing. Outer membrane fractions were prepared from several strains and run on SDS-PAGE (Fig. 9). The mature LamB protein is easily discerned as a major outer membrane protein in wild-type maltose-grown cells. There is no readily apparent LamB protein (mature or precursor) in the outer membrane of the lamBS78 prl+ strain (although, as discussed above, we expect that there is a very small amount of mature LamB protein present, owing to the nature of this particular mutation). The preLamB protein that this strain accumulates has previously been localized to the cytoplasm (8, 9). Mature LamB protein is, however, readily detected in the outer membrane of the lamBS78 prlA4 double mutant. No preLamB protein is detected in the outer membrane, indicating that virtually all of the preLamB protein exported via prlA-mediated suppression is processed. The preLamB
**Protein Export in prl Suppressor Strains**

**Fig. 8.** Slab gel SDS-PAGE of preMBP and MBP immune precipitated from malE prlA3 and malE prlA4 double mutants. See "Materials and Methods" for experimental details. Top gel, precipitates obtained from strains harboring the prlA2 mutation. Lower gel, precipitates obtained from strains harboring the prlA3 mutation. The relevant malE mutation is specified above each lane. Only the relevant portion of each autoradiograph is shown. Arrows indicate the positions of preMBP and MBP.

| A          | WT     | 18-1 prlA2 | 16-1 prlA2 | 10-1 prlA2 | 19-1 prlA2 | 14-1 prlA2 |
|------------|--------|------------|------------|------------|------------|------------|
| pre MBP    |        |            |            |            |            |            |
| MBP        |        |            |            |            |            |            |

**Fig. 9.** Slab gel SDS-PAGE of whole outer membrane proteins of representative wild-type and mutant strains. See "Materials and Methods" for additional experimental details. Pre-LamB protein was run in far left lane; LamB protein was run in far right lane. The positions of these two proteins are indicated by arrows. The positions where known molecular weight protein standards migrate are indicated along right side of gel. The relevant protein that this strain fails to export remains in the cytoplasm; however, no mature LamB protein is detected in this cell fraction (data not shown). Similar results were found for each of the additional strains we fractionated having various combinations of lamB and prlA mutations, with the exception of strains harboring the lamBS60 deletion mutation. As previously mentioned, there is no indication of processing of this mutant precursor protein in any suppressor strain. With prolonged exposure of autoradiographs, we can discern unprocessed lamBS60 product in outer membranes prepared from the lamBS60 prlA4 strain (data not shown).

In the case of suppression of malE signal sequence mutations by prlA suppressor alleles, we likewise expected that this would require export of the mutant MBP to the periplasm. Periplasmic proteins are usually defined operationally as those soluble proteins released from cells by a cold osmotic shock procedure (18). We precipitated MBP and preMBP from whole cells, shocked cells, and shockates prepared from a number of strains. Typical results are shown in Fig. 10. Nearly all of the mature MBP is released from wild-type cells by the shock procedure. In contrast, only a miniscule (probably insignificant) amount of preMBP is released from prl' strains harboring malE signal sequence mutations. The preMBP in these strains has previously been localized to the cytoplasm (11). The small amount of mature MBP detected in whole cells of these strains is efficiently released. Suppression of malE mutations by prlA4 results in a significant increase in the amount of mature MBP, almost all of which appears in the periplasmic fraction. No significant amount of preMBP is released, suggesting that all of the exported MBP is processed (or that nonprocessed, exported MBP is not released). We also examined whole shockates prepared from these strains on SDS-PAGE (not shown). Just as we observe in the case of outer membrane proteins (see Fig 9), the prlA genotype of strains from which the outer membranes were prepared is indicated above each lane.
We have presented evidence that demonstrates that prlA-mediated suppression is accomplished by facilitating the transport of export-defective LamB protein or MBP to its correct extracytoplasmic location.

First, the $\lambda^{10}$Dex$^+$ and Mal$^+$ phenotypes characteristic of lamB and malE signal sequence mutants, respectively, are suppressed to $\lambda^{10}$Dex$^-$ and Mal$^-$. The simplest way to interpret these results, as well as the failure to suppress other kinds of lamB and malE mutations, is to assume that export of these proteins is restored, despite the presence of a defective signal sequence. In particular, the simultaneous suppression of both the $\lambda^{10}$ and Dex$^+$ phenotypes to $\lambda^{10}$Dex$^-$ in lamB mutants strongly suggests that bonafide LamB protein is being exported to the cell surface.

Second, there is an oftentimes dramatic increase in the amount of mature LamB protein or mature MBP that can be immune precipitated from prlA strains as compared to iso- genic prl$^+$ strains. Previous results have indicated that internalized LamB protein and MBP is not processed in prl$^+$ strains (8, 9, 11). With the exception of strains harboring the lamBS60 deletion mutation, there is fairly good correlation between the degree of phenotypic suppression of lamB or malE mutations, and the amount of mature LamB protein or mature MBP detected in prlA strains. Within the limit of resolution of the SDS-PAGE system we employ, the process-

**FIG. 10.** Slab gel SDS-PAGE of preMBP and MBP immune precipitated from (W) whole cells, (S) shocked cells, and (P) periplasm of wild-type and mutant strains. Relevant genotypes of strains from which fractions were prepared are given at top. Arrows indicate the relative positions of preMBP and MBP. For each indi-

suppressors do not appear to have any effect on export or processing of normal periplasmic proteins synthesized by these strains. In fact, when proteins of each of the different cellular fractions (cytoplasm, cytoplasmic membrane, periplasm, outer membrane) from these strains are given at SDS-PAGE, no obvious differences can be detected between corresponding cellular fractions (not shown).

**DISCUSSION**

The great majority of signal sequence suppressor mutations isolated to date, and those about which we have the most information, map at what is probably a single genetic locus termed prlA. These mutations suppress signal sequence muta-
tions in both the lamB and malE genes, in some cases with very high efficiency. The prlA4 allele appears to be a particular-

ularly strong suppressor, capable of achieving near 100% sup-

pression of certain signal sequence mutations. The prlA locus has been genetically mapped on the E. coli chromosome to a site within the Pwse ribosomal protein operon, indicating that the prlA product is a ribosomal protein (13). As shown previ-
ously, there is no evidence that prlA mutations result in translational misreading, or that they exhibit any other kind of generalized suppressor activity. Rather, these mutations appear to specifically suppress only those lamB and malE mutations that alter the signal sequence in such a way as to produce an export-defective protein.

We have presented evidence that demonstrates that prlA-mediated suppression is accomplished by facilitating the transport of export-defective LamB protein or MBP to its correct extracytoplasmic location.

The expression of homologous signal sequences in E. coli and S. aureus has been used to argue for a direct interaction between the two proteins and the signal sequence.

**Protein Export in prl Suppressor Strains**

**FIG. 10.** Slab gel SDS-PAGE of preMBP and MBP immune precipitated from (W) whole cells, (S) shocked cells, and (P) periplasm of wild-type and mutant strains. Relevant genotypes of strains from which fractions were prepared are given at top. Arrows indicate the relative positions of preMBP and MBP. For each indi-

vidual strain, the amount of antigen extract added to the three precipitation mixtures was derived from an equal number of cells. See "Materials and Methods" for additional details. Only the relevant portion of the autoradiograph is shown.

Third, we can detect mature LamB protein in the outer membrane of lamB prlA double mutants, and mature MBP in the periplasm of malE prlA double mutants. In fact, all of the mature form of these two proteins that we find in these strains would appear to be correctly localized. That which is not exported remains in its unprocessed, precursor form in the cytoplasm.

Strains harboring prlA mutations manifest no obvious growth defects and exhibit no apparent defect in the export of wild-type LamB protein, MBP or other envelope proteins. This might suggest that prlA mutations have not greatly altered the normal export pathway, or possibly that prlA-mediated export is not accomplished via a route that is em-
ployed in these strains to any significant degree. On the other hand, the correct localization and processing of both defective LamB protein and MBP strongly suggest that these proteins are being exported by the usual route, of which the prlA product is a component. There is one additional observation that suggests that the prlA product is involved in the normal secretory pathway of the cell. Recently, a new genetic locus (secA) was described in which conditional lethal (temperature-sensitive) mutations were obtained that were pleiotropically defective in secretion of a number of envelope proteins (20). It was found that prlA secA double mutants, in contrast to the prl$^+$ secA strain, are mucoid and grow poorly at the permissive temperature. Although these double mutants have not been analyzed further, this result may indicate that the cell has difficulty accommodating these two alterations in its export apparatus.

In considering the role of the prlA product in protein export, we do observe a fair degree of allele specificity in the suppres-
sion of signal sequence mutations by various prlA mutations, i.e. certain prlA alleles suppress certain signal sequence muta-
tions more efficiently than others. Such allele specificity has in the past been used to argue for a direct interaction between two mutational altered proteins (e.g. see Refs. 21 and 22). Likewise, the prlA product may interact directly with the defective signal sequences of the LamB protein and the MBP to initiate the export of these proteins through the cytoplasmic membrane. Since it appears that the prlA product is indeed a ribosomal protein, our results suggest that the ribosome can play an active role in determining the cellular location of proteins.

The remaining two classes of suppressors, those designated prlB and prlC, may represent mutational alterations in other components of the export apparatus of the cell. The single prlB allele isolated to date is unusual in several respects. (i) It phenotypically suppresses lamB but not malE signal sequence mutations. (ii) It appears to suppress the lamBS60 36

2. D. Oliver and J. Beckwith, personal communication.
base pair deletion mutation better than other lamB signal sequence mutations, including point mutations. (iii) Export of LamB protein is apparently achieved without any indication of processing. The single prIC allele thus far obtained exhibits fairly weak suppression of both lamB and malE signal sequence mutations, but there is an indication of aberrant processing of the preLamB protein in these strains. The failure to observe authentic processing of the preLamB protein in either prIB or prIC strains suggests that this protein is not being exported to the cell surface via its normal route. However, our information concerning these suppressors is still quite limited, and the localization and processing of both the LamB protein and the MBP in these strains is being studied further. Also, attempts are currently underway to isolate additional prIB and prIC mutations.

The demonstration that signal sequence alterations in both an outer membrane protein and a periplasmic protein can be compensated for by mutations at prlA and prlC indicates that there may be common components involved in the export machinery for proteins destined for different regions of the cell envelope. Similar ideas were recently proposed using a totally different experimental approach (16). Although each has a unique primary amino acid sequence, the signal sequence of the LamB protein, MBP, and a number of other prokaryotic exported proteins share certain general features (see Refs. 1-5). Similarly, lamB and malE point mutations that prevent export of the LamB protein and the MBP from the cytoplasm (see Fig. 1) are quite similar in the nature of the amino acid substitutions. This kind of evidence suggests that the various signal sequences are functionally equivalent; that their role is to simply initiate protein export from the cytoplasm. As has been suggested previously (2, 5, 23), there are probably other signals in the mature protein that are the ultimate determinants of cellular location.

Finally, we would like to make two points concerning the processing of export-defective LamB protein and MBP. First, although each of the signal sequence alterations shown in Fig. 1 is sufficient to largely inhibit export, only one of these mutations, the lamB5680 deletion, prevents protein maturation if the block in export is circumvented by a prlA suppressor mutation. Obviously, whatever signal sequence secondary structure is required to initiate export, requirements are not the same for recognition by the processing enzyme (termed "signal peptidase" in the recent literature (24, 25)). The processing enzyme perhaps must recognize something more than just the cleavage site, since this is intact in the lamB5680 product. That the requirements for export and processing do differ is also supported by the identification of mutational alterations in the murein lipoprotein signal sequence (Gly to Asp at residue 14 (26)) and the mature phase M13 coat protein (Glu to Leu at residue 2 (25, 27)) that prevent processing but not localization of these membrane proteins. Secondly, we would like to emphasize that, in our system, export appears to be an absolute prerequisite for protein maturation. It was recently reported that large, nonsense fragments of the periplasmic enzyme β-lactamase that were not exported from the cytoplasm were, nonetheless, correctly processed at their NH2 termini (28). Although we have described instances where a mutant LamB protein can be exported without apparent processing, we see no evidence that the reverse can also occur. We have never observed maturation of internalized preLamB protein or preMBP in the cytoplasm. We therefore believe that, for at least the LamB protein and the MBP, processing serves as a strong indication of export.

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S D Emr and P J Bassford, Jr

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