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In vivo selection and characterization of internal deletions in the *lamB*::*lacZ* gene fusion

(Recombinant DNA; protein export; phage Mu; lactose utilization; *E. coli*; nucleotide sequences; spontaneous mutants)

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SUMMARY

Strain Pop3299 contains the *lamB*::*lacZ*42-12 gene fusion that encodes a hybrid protein that is efficiently exported to the cellular envelope of *Escherichia coli*. As a result of this efficient export, this strain is killed by the inducer maltose and unable to grow on minimal media supplemented with lactose. In an attempt to isolate mutants in which export of the hybrid protein is altered, we selected Lac$^+$ mutants of strain Pop3299 on lactose tetrazolium media. Unlike mutants previously isolated on lactose minimal media, all the mutants we obtained carried large deletions within the *lamB*::*lacZ* gene fusion. Thus, it appears that the type of selection employed affects the type of mutations obtained. We have analyzed the nucleotide sequences of representative mutants, and demonstrate a correlation between the deletion size and the export-related maltose and lactose phenotypes. In addition, we demonstrate that the deletions do not appear to arise from regions of micro-homology.

INTRODUCTION

Gene fusions using the *lac* genes have been used to study a variety of biological processes in both bacteria and higher organisms (for review, see Silhavy and Beckwith, 1985; Weinstock et al., 1983). One important advantage of the *lac* system is the large variety of compounds and media available for genetic selections and analysis. In addition, gene fusions to *lacZ* (which specifies the large soluble protein, β-galactosidase) can specify chimeric proteins with unusual properties that can be exploited to obtain mutants which are not otherwise readily selectable (Silhavy et al., 1983; Bankaitis et al., 1985).

We have used such a system to study the export of the outer membrane protein LamB in *E. coli*. Strains carrying *lamB*::*lacZ* fusions which specify hybrid proteins that are exported have several unique and useful phenotypes (Benson, 1985). Such strains are killed by the inducer maltose (Mal^S) and are

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Abbreviations: aa, amino acid(s); bp, base pair(s); cfu, colony-forming units; Δ, deletion; Mal, maltose; PAGE, polyacrylamide gel electrophoresis; ^{R}, resistant; ^{S}, sensitive; SDS, sodium dodecyl sulfate; XGal, 5-bromo-4-chloro-indoyl-β-D-galactoside; ::, novel joint.

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unable to utilize lactose for growth (Lac^-). The first property is believed to result from lethal jamming of the export machinery by high-level synthesis and attempted export of the hybrid protein (Emr and Silhavy, 1980). The second property is believed to result from the inability of the β-galactosidase portion of the hybrid protein to yield an enzymatically active conformation within the confines of a membrane structure (Silhavy et al., 1983).

The strain Pop3299 carries the *lamB*::*lacZ*42-12 fusion that encodes a hybrid protein which is efficiently exported from the cytoplasm (Hall et al., 1982). This strain is Mal^S and Lac^-. By selecting for Lac^+ cells on complex media containing the indicator tetrazolium and the sugar lactose, we have obtained over 290 independent Lac^+ mutants in which a significant amount of the hybrid protein remains in the cytoplasm and thus confers a Lac^+ phenotype (Benson et al., 1984). We present here a detailed genetic and physical analysis of these mutants and demonstrate that they carry large deletions which change the *lamB*::*lacZ* fusion joint. Our results suggest that, in contrast to previous reports (Farabaugh et al., 1978; Albertini et al., 1982), small areas of homology are not instrumental in the formation of these deletions. Moreover, we found that the selection conditions we used strongly influenced the type of mutant we recovered.

MATERIALS AND METHODS

(a) Media and chemicals

Bacteriological media and chemicals are as described previously (Benson et al., 1984; Silhavy et al., 1984; Miller, 1972).

(b) Bacterial strains

The bacterial strains used are derivatives of strain MC4100: F^- Δ*lac*U169 *araD*139 *rpsL*150 *relA*1 *flbB*5301 *deoC*7 *rbsR*· *ptsF*25 (Casadaban, 1976). Pop3299 (*lamB*::*lacZ*42-12) is derived from MC4100 (Hall et al., 1982). Strain ECB141 is a derivative of Pop3299 which carries a C to A substitution at bp 44 of the *lamB* signal sequence (S.A.B., unpublished data). Strain SE3015 is a derivative of MC4100 which carries the deletion, Δ(*malB*)15 (Emr and Silhavy, 1980). The BRE strains isolated in this study are derivatives of Pop3299. The various *lamB*::*lacZ* fusions and deletions have been designated 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 and the deletion as Δ100.

(c) Selection and characterization of Lac^+ mutants

Cultures of Pop3299 were grown overnight in L broth at 37°C and 0.2-ml aliquots (approx. 10^9 cells) were plated on lactose tetrazolium agar and incubated at either 30°C or 42°C for 3 to 5 days. Lac^+ colonies appear as red papilli in a white lawn of Lac^- cells. One colony from each plate was selected for further characterization. All mutants were purified by streaking twice on lactose-tetrazolium agar and then on L agar. Individual colonies were then tested for their Lac and Mal phenotypes by streaking on the following media at both 42°C and 30°C: lactose-tetrazolium, lactose-MacConkey, lactose-minimal, maltose-tetrazolium, maltose-MacConkey, maltose-minimal spread with the chromogenic indicator dye XGal, and L plates spread with XGal. Maltose sensitivity was determined by colony size and growth response on maltose minimal medium or by a disc-sensitivity test as previously described (Benson et al., 1984). Hybrid proteins were visualized on SDS–polyacrylamide gels by analyzing total cellular extracts as described (Silhavy et al., 1984).

(d) Cloning and sequencing of the *lamB*::*lacZ* fusions joints

The λ prophages adjacent to each *lamB*::*lacZ* fusion was induced by UV irradiation (Silhavy et al., 1984) and specialized transducing phages carrying the entire *lamB*::*lacZ* fusion were isolated. These transducing phages were shown to carry the Mal and Lac phenotypes by lysogenizing them into the strain SE3015 and scoring for growth response on maltose and lactose media. DNA from these λ phages was used to clone the various *lamB*::*lacZ* fusions into the plasmid vector pMLB524 as described previously (Benson and Silhavy, 1983). Restriction analysis and nucleotide sequencing of the fusion joints was carried out as described (Benson et al., 1984).

Base pairs were numbered from the first bp of the AUG initiation codon of the protein's coding sequence.

(e) **Reconstruction of the selection system**

Strains Pop3299 and ECB141 were grown overnight in L broth. The ECB141 culture was diluted 10^{-5} and 10^{-6}, and 0.1 ml of each dilution was added to a sample of the Pop3299 strain. Mixed and non-mixed cell cultures (0.2 ml) were then plated on lactose-minimal, lactose-MacConkey, and lactose-tetrazolium plates and incubated at 37°C for 48, 96, or 120 h. The number of cfu per ml was determined on each of the starting cultures before the cultures were mixed.

RESULTS

(a) **Isolation and characterization of Lac^+ derivatives of the *lamB*::*lacZ*42-12 fusion**

Lac^+ mutants were obtained as described in MATERIALS AND METHODS, section **c**. The mutants appeared as red papilli in a lawn of Lac^- cells after 3 to 5 days incubation. We estimate that the frequency of the mutants was $<10^{-10}$. 295 independent colonies were purified and tested at both 42°C and 30°C on a series on lactose and maltose indicator plates as described in MATERIALS AND METHODS, section **b**. All the mutants failed to show either a cold-sensitive or a temperature-sensitive phenotype and could be grouped into the four classes on the basis of their Lac and Mal phenotypes as shown in Table I. We determined the size of the hybrid proteins in 25 mutants by SDS–PAGE. In all cases, the hybrid protein was significantly smaller than the parent fusion protein (not shown). This observation suggests that the Lac^+ phenotype resulted from a deletion event within the *lamB*::*lacZ*42-12 gene fusion. A representative subset of the mutants was selected for further analysis.

(b) **Physical characterization of the Lac^+ mutants**

To determine the exact nature of the genetic lesion, we cloned the *lamB*::*lacZ* fusion from 15 representative strains. Restriction analysis of the resulting plasmids showed all of the fusions carried deletions of greater than 600 bp which remove the *Hin*fI site at bp 783 of *lamB* but leave the *Pvu*II site at bp 102 of *lacZ* (Fig. 1). Furthermore, this analysis showed a correlation between the size of the deletion and the Mal phenotype. Strain BRE246, a class-4 mutant, carries a deletion of more than 900 bp that removes part of the *malK* gene (Fig. 1). Since the coding region of *malK* is impaired in this strain, the Mal^- phenotype is explained. The remaining three classes of mutants have smaller deletions and are Mal^+ but vary in their sensitivity to killing by the inducer maltose. The class-1 mutants have deletions that range in size from 678–842 bp and are Mal^R. In contrast, class-2 mutants are Mal^S and carry deletions ranging in size from 620–649 bp. Strain BRE263, a class-3 mutant, is an exception to this pattern: it carries a deletion of 637 bp, has a Mal^R phenotype, grows slowly on lactose-minimal media, and synthesizes less hybrid protein than the mutants of the other three classes (not shown).

Previous studies have indicated that sensitivity to maltose is related to the cell's attempt to export the hybrid protein from the cytoplasm (Hall et al., 1982;

TABLE I

Phenotypic characterization of the Lac^+ mutants

Class	Number of isolates	Lactose phenotype[a]	Maltose phenotype[a]
1	225	Lac^+	Mal^+, Mal^R
2	58	Lac^+	Mal^+, Mal^S
3	5	$Lac^{+/-}$	Mal^+, Mal^R
4	7	Lac^+	Mal^-

[a] +, growth; –, no growth; +/–, slow growth on minimal media supplemented with lactose or maltose.
R and S, resistant and sensitive, respectively, to the inducer maltose.

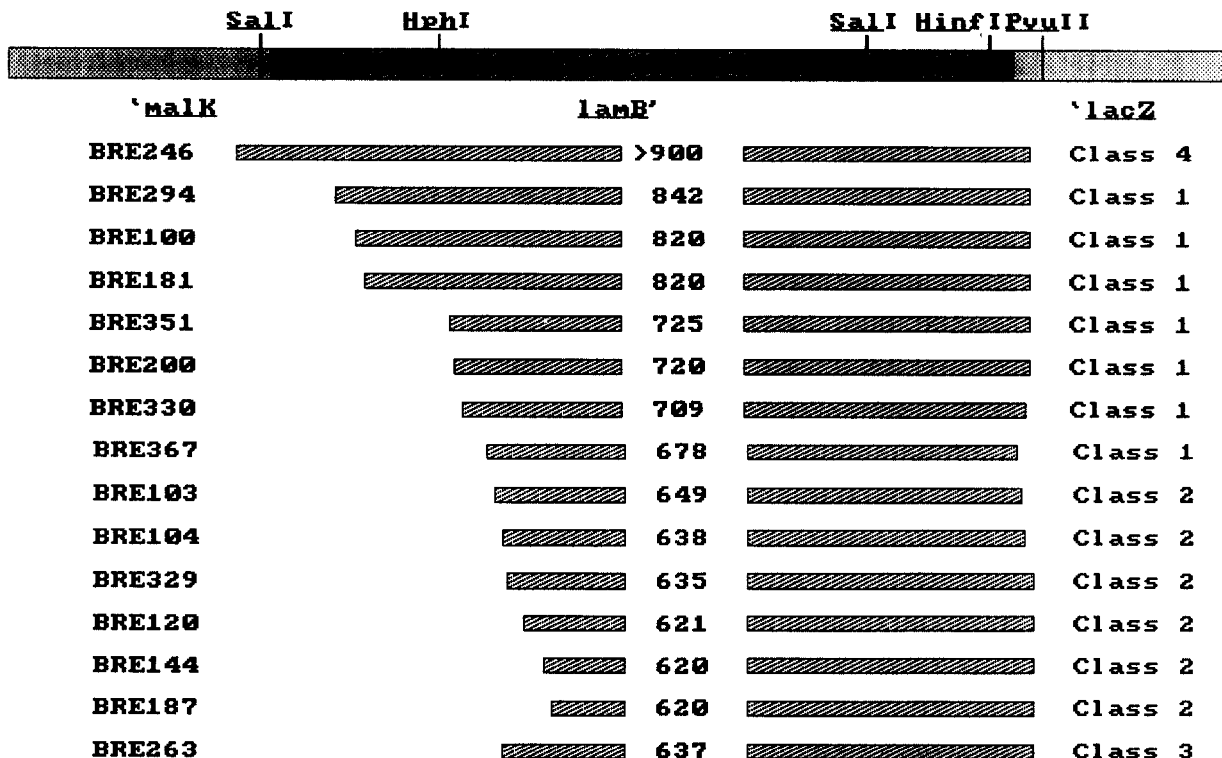

Fig. 1. Deletions present in the various mutants. (Top) A segment of the DNA sequence from the center of the *malK* gene through the beginning of the *lacZ* gene is shown. The solid bars depict the nucleotide sequences between the coding regions. The positions of the *Sal*I, *Hin*fI, *Hph*I, and *Pvu*II restriction sites are shown. (Bottom) The extent of the deletion present in various mutant strains is depicted by hatched bars. The size of the deletions in bp is given in the center of the hatched bar. The DNA and deletions are represented to scale.

Benson et al., 1984) and that the amount of LamB present on the hybrid protein is an important factor in conferring this phenotype. Our analysis of the deletions present in class 1 and class 2 more precisely defines the amount of LamB sequence necessary to confer a Mal^S phenotype. The strain BRE367 with the smallest class-1 deletion retains 156 bp of *lamB*, while strain (BRE103) with the largest class two deletion retains 192 bp of *lamB*. Thus the Mal^S phenotype appear to require at least 192 bp of the *lamB* coding sequence.

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Wild type lamB

238 239 240 241 242 243 244 245 246 247 248 249 250 251 253 254 255 256 257
TCG ATG ACC TCG CAG AAA GGG CTG TCG CAG GGT TCT GGC GTT GCA TTT GAT AAC GAA
===         ===         ===     ===                             ===

lamB::lacZ42-12 fusion joint

238 239 240 241 242                                             19  20  21  22  23
TCG ATG ACC TCG CTG AAG CGG CGC ACG AAA AAC GCG AAA GCG TTT GGC GTT ACC CAA
===         ===         ===     ===                             ===

  lamB sequence                  Mu sequence                   lacZ sequence
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Fig. 2. Nucleotide sequence of the *lamB*::*lacZ*42-12 fusion joint. Sequence was determined as described in MATERIALS AND METHODS, section **d**, and from Clement and Hofnung (1981). The numbers above the codons refer to the amino acid number of mature LamB or LacZ protein. Phage Mu sequence is homologous with sequences present in the *S* end of Mu (Kahmann and Kamp, 1979). Codons which are infrequently found in highly expressed gene (Grosjean and Fiers, 1982) are double-underlined.

(c) Sequence analysis of the deletion end points

To determine precisely the deletion end points, we sequenced the *lamB*::*lacZ* fusion joints in seven of the mutants as well as that of the original *lamB*::*lacZ*42-12 fusion. We found that in the *lamB*::*lacZ*42-12 fusion, codon 242 of *lamB* was joined to codon 19 of *lacZ* by 32 bp of DNA from the *S* end of phage Mu (Fig. 2) (Kahmann and Kamp, 1979). The open reading frame through this segment of Mu DNA contains several codons which are infrequent in highly expressed genes of *E. coli* (Grosjean and Fiers, 1982). Interestingly, the corresponding region of *lamB* carries an equal number of such codons at nearly identical positions (Fig. 2). It appears that the deletions obtained do not begin within or extend from the Mu DNA present at the original fusion joint. In six of the mutants analyzed, the deletions remove the complete Mu sequence and extend to different positions in *lacZ* (Fig. 3). In the remaining deletion (Δ367), only the final T residue of the 3′ end of the Mu segment is retained (Fig. 3).

Because base-pair homology has been reported to be a factor in the formation of deletions (Farabaugh et al., 1978; Albertini et al., 1982), we looked for homology in the regions of *lamB* and *lacZ* adjacent to the fusion joints. The results are shown in Table II. In six of the seven cases tested, the homology surrounding the deletion end points ranged from 20–35%. Always, many other alignments which exhibited greater homology were possible (Table II). If we consider only homology alignments that do not

```
lamB    C T G C G C A A A C T T C C T C T G G C G G T T G C C G T C
lacZ    G T T A C C C A A C T T A A T C G C C T T G C A G C A C A T
BRE100  C T G C G C A A A C T T C C T/C G C C T T G C A G C A C A T
          *       *   * * * * *     * *           *     * *

lamB    G G C A A C G A A T G T G A A A C T T A T G C T G A A T T A
lacZ    G C G T T T G G C G T T A C C C A A C T T A A T C G C C T T
BRE103  G G C A A C G A A T G T G A A/C A A C T T A A T C G C C T T
        *           *         *                 *     *         *

lamB    A A A C T T A T G C T G A A T T A A A A T T G G G T C A G G
lacZ    T T G G C G T T A C C C A A C T T A A T C G C C T T G C A G
BRE104  A A A C T T A T G C T G A A T/T T A A T C G C C T T G C A G
                      *   *     * *   *   * *                     *

lamB    A T T G G G T C A G G A A G T G T G G A A A G A G G G C G A
lacZ    C G T T A C C C A A C T T A A T C G C C T T G C A G C A C A
BRE120  A T T G G G T C A G G A A G T/T C G C C T T G C A G C A C A
            *         * *                 *         *     * *   * *

lamB    A A C T T A T G C T G A A T T A A A A T T G G G T C A G G A
lacZ    T T G G C G T T A C C C A A C T T A A T C G C C T T G C A G
BRE263  A A C T T A T G C T G A A T T/T T A A T C G C C T T G C A G
                    *           *         * * *   *     *

lamB    T A G C G G C G G T G A A C A A C A G T G T T T C C A G A C
lacZ    T G G C G T T A C C C A A C T T A A T C G C C T T G C A G C
BRE330  T A G C G G C G G T G A A C A/T A A T C G C C T T G C A G C
            * * *             * * *             *     *           *

lamB    G T G T T T C C A G A C T A C C G G T G C T C A A A G T A A
lacZ    A A A C G C G A A A G C G T T T G G C G T T A C C C A A C T
BRE367  G T G T T T C C A G A C T A C/T G G C G T T A C C C A A C T
                        *     *         * *   *   *
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Fig. 3. The DNA sequences of various *lamB*::*lacZ* fusion joints. The position of each newly formed fusion joint is indicated by a slash. The corresponding regions of wild-type *lamB* and *lacZ* are also shown above each fusion and homologous base pairs are designated by an asterisk.

TABLE II

Percent of homology between *lamB* and *lacZ* at the deletion end points

Deletion No.	Parent strain	% homology [a]	Number of matches with greater homology	
			All	In frame [b]
Δ100	BRE100	50	0	0
Δ103	BRE103	20	127	50
Δ104	BRE104	35	22	7
Δ120	BRE120	20	115	41
Δ263	BRE263	35	22	– [c]
Δ330	BRE330	30	44	19
Δ367	BRE367	30	52	21

[a] For each mutant a 20-bp region spanning the deletion end point of the *lacZ* sequence was used to search for homology within the first 255 bp of the *lamB* sequence. The computer program utilized was from IBI Inc.

[b] In-frame homologies are alignments in which the reading frames of *lamB* and *lacZ* overlap in register.

[c] Strain BRE263 contains a deletion that shifts the reading frame between *lamB* and *lacZ* sequences.

change the reading frames between the *lamB* and *lacZ* sequences, then the number of possible alignments is reduced (Table II). However, even with this restriction, a significant number of alignments with homology greater than that of the deletion isolated were found. In the seventh mutant, BRE100, the regions surrounding the deletion end points exhibit a 50% homology and no other alignments share a higher degree of homology. Thus, homology does not appear to be important in the formation of the *lamB*::*lacZ* deletions described here.

(d) Internal translational start signal with *lamB*

As already mentioned, strain BRE263 (a class 3 mutant) shows several unusual phenotypes. To gain insight into the nature of this deletion, we sequenced the deletion end points. The deletion extends from bp 205 of *lamB* to bp 79 of *lacZ*, resulting in misalignment of the reading frames of these genes (Fig. 4). Strains carrying this fusion are Lac$^+$ (Table I). Thus, the *lacZ* portion of the hybrid gene is expressed. Inspection of the *lamB* sequence reveals an ATG 10 bp upstream from the *lacZ* sequence that is in-frame with *lacZ* but out of frame with *lamB*. The sequence that is 5′ to this ATG contains a region which can base pair with the 3′ end of the 16S rRNA molecule (Fig. 4). The combined regions fit the requirements for a translational initiation site (Steitz, 1979), and most likely serves as an internal translational start signal for expression of the fusion. The resulting hybrid protein would contain 4 aa at the N terminus of the β-galactosidase moiety (Fig. 4). As expected, such an internal translational start signal is inefficient, shown by the fact that strain BRE263 produces much less hybrid protein and grows slower on lactose-minimal media than the other mutants we analyzed.

(e) Analysis of the selection system

All 25 of the mutants analyzed carried deletions that reduced the size of the hybrid protein. This finding was surprising since previous selections for a Lac$^+$ phenotype on lactose minimal plates using strain Pop3299 had yielded both deletions and point

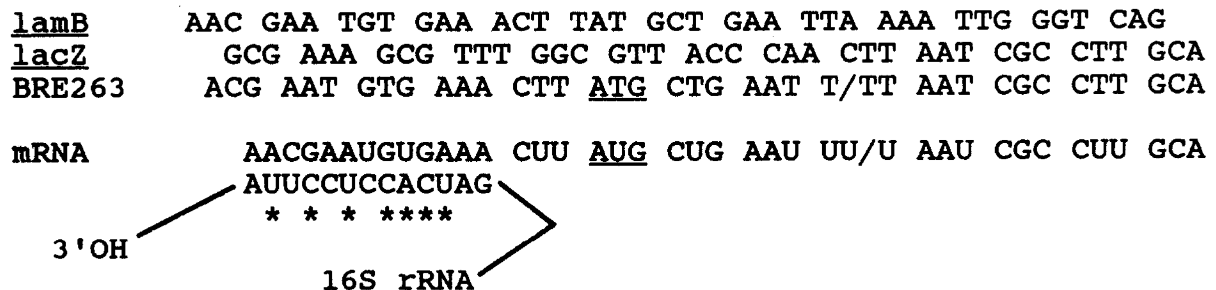

Fig. 4. The sequence surrounding the BRE263 fusion joint. The reading frames of *lamB* and *lacZ* are shown together with the sequence of the fusion joint of the class 3 mutant, BRE263. A potential initiation codon and ribosome-binding site are shown. The sequence of the rRNA is from Steitz et al. (1979). Nucleotide sequence of *lamB* is from bp 180–229, and the *lacZ* sequence is from bp 48–87; sequences were obtained as described in MATERIALS AND METHODS, section **d**, and from Clement and Hofnung (1981) and Kalnins et al. (1983). The symbols are as designated in Fig. 3.

mutations that altered the LamB signal sequence (S.A.B., data not shown). To determine why mutants of this type were not obtained on lactose-tetrazolium media, we performed a reconstruction experiment. Strain ECB141 is a derivative of strain Pop3299 containing a single-base-pair alteration in the *lamB* signal sequence that changes aa 15 from an Ala to a Glu residue. This alteration is identical to a previously isolated signal sequence mutation (S70) obtained in a selection using the smaller, Mal^S fusion *lamB*::*lacZ*42-1 (Emr and Silhavy, 1980). This mutation has been shown to prevent export of both LamB and LamB-LacZ hybrid proteins from the cytoplasm. The Lac^+ Mal^R phenotypes of strain ECB141 is identical to that of the class 1 mutants described in this study.

To determine if this strain would produce Lac^+ papilli in a lawn of Lac^- cells, dilutions of ECB141 were added into an overnight culture of Pop3299. The cell mixture was plated on lactose-minimal, lactose-MacConkey, and lactose-tetrazolium media. On lactose-minimal medium, the number of Lac^+ colonies detected correlated with the number of ECB141 cells added to the sample plates. However, on lactose-MacConkey and lactose-tetrazolium media, no Lac^+ papilli were observed in the first 48 h. In a second similar experiment, it took 96 h for papilli to appear on the lactose-MacConkey plates seeded with ECB141 and several additional days before papilli appeared on the un-seeded plates. Approximate equal numbers of Lac^+ papilli (3 to 5) were observed on lactose-tetrazolium media irrespective of whether or not the inoculum had been seeded with ECB141 cells. Thus, the type of lactose indicator media used to select Lac^+ derivatives of the *lamB*::*lacZ*42-12 fusion influences the type of mutant recovered.

DISCUSSION

We have been studying the export of the LamB protein to the outer membrane of *E. coli* using *lamB*::*lacZ* gene fusions. Previous attempts to isolate mutants in which export was altered have utilized selections for a Lac^+ phenotype on lactose minimal media. These selections yielded signal sequence mutations, many of which were identical (Emr and Silhavy, 1980; S.A.B., unpublished data). In an attempt to isolate different types of mutations, we selected Lac^+ derivatives of strain Pop3299. We obtained exclusively deletion mutations which generated new *lamB*::*lacZ* fusions. A reconstruction experiment with a known signal sequence mutation indicates that different lactose media favor the selection of different types of mutants. This is consistent with the work of Shapiro (1984) on *lacZ* fusion formation which suggests that under non-lethal conditions medium composition, genetic background, and the environment surrounding the potential clone all influence the selection process. We do not know why the selection procedure we employed yielded only deletion mutants. None the less, our results suggest that the use of different lactose media in selecting Lac^+ mutants can result in the isolation of a wider variety of mutations.

Since the Lac^+ selection we used favored the recovery of deletion mutants, we tested to see if the deletion formation was sensitive to the DNA structure at the original *lamB*::*lacZ*42-12 fusion joint. It has been reported that small regions of homology may be involved in the formation of deletions (Albertini et al., 1982). In one of the mutants we analyzed (Δ100), it is possible that homology near the deletion end points determined the position of the deletion. However, in six other mutants this was clearly not the case. In light of this, it appears that these deletions were generated by a mechanism that does not require homology at the end points.

Sequence analysis of the fusion joint present in Pop3299 revealed the presence of 32 bp from the S end of phage Mu. It is well known that Mu can cause deletions of chromosomal material in the vicinity of its insertion point (Toussaint and Resibois, 1983). However, the Mu sequence present at the *lamB*::*lacZ*42-12 fusion joint does not appear to be involved in the formation of the deletions.

We were able to group the mutants we obtained into four distinct classes based on their maltose and lactose phenotypes (Table I). By restriction and nucleotide sequence analysis we have determined a relationship between the size of the deletion and these phenotypes.

The class 1 and 2 mutants were the most interesting because the export related Mal^S and Mal^R phenotypes could be correlated with size of the deletions. The class 1 mutants (Mal^R), were the

predominant class and appeared to contain no more than 27 aa of mature LamB in the hybrid protein. We have previously shown a relationship between the Mal^R phenotype and the cytoplasmic location of the hybrid protein (Benson et al., 1984). Approx. 20% of the mutants were in class 2 and exhibited a Mal^S phenotype. These mutants appear to contain at least 39 aa of mature LamB in the hybrid protein. The Mal^S phenotype is observed only when the export process for the hybrid protein has been at least initiated. This has been demonstrated not only for *lamB*::*lacZ* fusions (Benson et al., 1984) but for *malE*::*lacZ* gene fusion as well (Bankaitis et al., 1985).

Mutants in class 3 and 4 form only a very small fraction of the mutants we isolated. Since the class 4 mutants show a Mal^- phenotype, it seems likely that these mutants contain deletions that extend into *malK*. Indeed, analysis of one of these mutants, BRE246, showed that it contains a large deletion that removes part of the *malK* coding region. The Mal^R $Lac^{+/-}$ phenotype of the class 3 mutants was at first puzzling. However, when one of these mutants, BRE263, was analyzed in detail we found that the deletion disrupted the *lamB*::*lacZ* reading frame. We believe that the weak Lac^+ phenotype of this strain results from an internal translational start signal within the *lamB* gene near bp 204 of *lamB* (Fig. 3). We assume that the other class 3 mutants had similar disruptions. The existence of this class of mutants points out one of the caveats of the selection employed.

We have previously shown that *lamB*::*lacZ* fusions which specify hybrid proteins containing more than 70 aa of mature LamB are exported from the cytoplasm with increased efficiency and consequently have low β-galactosidase activity (Benson and Silhavy, 1983). All of the Lac^+ mutants obtained in this study apparently contain less than 50 aa residues of mature LamB present in the hybrid protein. We assume that these results reflect a requirement for increased β-galactosidase activity in the selection we imposed, since the amount of β-galactosidase material present in each of the hybrid proteins is nearly constant. The first *lacZ* codon found in each of the fusions ranges from codon 20 to codon 26. This is consistent with previous data suggesting that aa 26 of β-galactosidase is required for the formation of enzymatically active tetramer of this protein (Fowler and Zabin, 1983).

All of the fusions characterized at the DNA level remove the Mu DNA at the original fusion joint. Previously constructed in vitro deletions that remove a region of *lamB* from approximately codon 70 to codon 220 of the mature sequence do not alter localization of the hybrid protein or LamB itself (Benson and Silhavy, 1983). Deletions that extended past codon 220 and possibly into the Mu sequences reduced the efficiency of localization (Benson and Silhavy, 1983). Thus, this region of *lamB* and possibly the Mu sequences may increase the efficiency of the translocation to the outer membrane (Benson and Silhavy, 1983; Benson, 1985). At least one other fusion, *lamB*::*lacZ*4218, which is nearly identical to the *lamB*::*lacZ*42-12 fusion has similar Mu sequences at it fusion joint (D. Jackson, personal communication). Taken together, these results suggest that the Mu sequences may affect localization, possibly because the Mu DNA specifies a number of codons that are infrequently used in highly transcribed genes of *E. coli*. Interestingly, a similar number of rare codons are present in the corresponding region of the wild-type *lamB* sequence. Such condons may be involved in modulating the rate of translation within a specific region of the message. Slowing or pausing may facilitate the export process by allowing needed factors to assemble or alternatively allowing the nascent polypeptide chain to form a required conformation.

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