Isolation of Mutations in the α Operon of *Escherichia coli* That Suppress the Transcriptional Defect Conferred by a Mutation in the Porin Regulatory Gene *envZ*

S. GARRETT^{1,2} AND T. J. SILHAVY^{2*}

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218,¹ and Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544²

Received 29 September 1986/Accepted 16 December 1986

One class of mutations in the *envZ* gene of *Escherichia coli* K-12 confers a pleiotropic defect on the expression of several genes, including *ompF*, *lamB*, and *phoA*, that are otherwise not commonly regulated. Four second-site mutations that suppress this transcriptional defect have been isolated by using a procedure that circumvented the problem of intragenic suppressors, including true revertants. All four mutations have been mapped to the genes of the α operon and have been assigned tentatively to the gene *rpoA*, which specifies the α subunit of RNA polymerase. The mutations, referred to as *sez* (for suppressor of *envZ*), did not appear to confer a phenotype on an otherwise wild-type strain and did not suppress the transcriptional defects conferred by several other phenotypic classes of *envZ* mutations, including amber mutations. Our results led us to postulate that the α subunit or some other component of the α operon plays a role in determining the specificity of gene expression.

Synthesis of the major outer membrane proteins OmpF and OmpC of *Escherichia coli* is regulated by the products of the genes *ompR* and *envZ* (8, 11, 21). These genes form an operon, termed the *ompB* locus, that maps to 74 min on the *E. coli* chromosome (8, 19, 30). Studies with strains carrying defined chain-terminating and deletion mutations of the *ompR* and *envZ* genes suggest that both regulatory proteins are required to activate expression of *ompF* and *ompC* but that neither is essential for the expression of genes specifying other envelope proteins (8, 9). These results were somewhat surprising since certain *envZ* mutants are pleiotropically depressed for the expression of a variety of genes that specify noncytoplasmic proteins, including *lamB* and *phoA* (27–29). For example, one mutant of this class carries the allele *envZ473* and exhibits an OmpF[–] OmpC⁺ LamB[–] PhoA[–] phenotype. Since *envZ* amber mutations are recessive in diploid analysis, whereas the pleiotropic *envZ* alleles exhibit codominance with the wild-type allele, we have previously suggested that the pleiotropic *envZ* alleles specify an altered EnvZ protein (8, 26). As the EnvZ protein does not function as a positive activator for *lamB* and *phoA*, the altered EnvZ protein of the pleiotropic mutants must somehow interfere with their transcription. If we understood the molecular nature of this defect, we might have some insight into the regulation of the porin genes as mediated by the EnvZ protein.

We reasoned that by isolating extragenic mutations that simultaneously suppressed the transcriptional defect for all of the genes affected in the *envZ473* strain, we might be able to define the genes for one or more of the components common to their expression. Here we describe a method that has allowed us to isolate four such mutations without having to screen these from a background of intragenic suppressors, including true revertants. Genetic analysis of the four independent mutants suggests that each contains a mutation that is linked to, or within, the gene *rpoA*, which specifies the α subunit of RNA polymerase.

MATERIALS AND METHODS

Media and chemicals. Most growth media were prepared as described previously (18, 24). The inducing medium (P_i-poor rich medium) for alkaline phosphatase assays was made from noninducing medium (LB medium [24]) by a method described previously (13). Noninducing medium and inducing medium for *malE* expression were, respectively, LB medium and LB medium containing 0.2% maltose. Nutrient broth was purchased from Difco Laboratories and served as the low-osmotic-strength medium, whereas high-osmotic-strength medium was prepared by adding sucrose to a final concentration of 15% (wt/vol). Carbon sources were added at 0.2% (wt/vol), except to the MacConkey and tetrazolium indicator agars, which contained sugars at 1%. L-Biotin was from Sigma Chemical Co. and was added to a final concentration of 25 μ g/ml where indicated. The antibiotics were used at the following concentrations unless otherwise noted: tetracycline, 20 μ g/ml; kanamycin, 40 μ g/ml; streptomycin, 100 μ g/ml; spectinomycin, 800 μ g/ml; and ampicillin, 125 μ g/ml. The final tetracycline concentration in indicator agars was always 8 μ g/ml. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine was purchased from Sigma and was used as a stock solution of 2.5 mg/ml in 95% ethanol. Assays of alkaline phosphatase and β -galactosidase activities were carried out as previously described (4, 18) after growth of the cultures in inducing or noninducing medium as previously described (3, 8, 13).

Manipulations of bacteria and bacteriophage. The bacterial strains, bacteriophages, and plasmids used are shown in Table 1. Standard microbiological techniques were used for determining bacteriophage titers, generalized transduction, lysogen formation and induction, and bacterial growth (18, 24).

Localized nonmutagenesis. Ten 2-ml cultures of strain SG901 [Φ (*ompF-lac*) Δ *ompB76*] were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by standard procedures (24) and incubated overnight for phenotypic expression. Samples of 0.2 ml from each mutagenized culture were

* Corresponding author.

TABLE 1. Bacterial strains

Strain or phage	Genotype	Source or reference
Bacterial strains		
MC4100	F ⁻ <i>araD139</i> Δ (<i>argF-lac</i>) <i>U169</i> <i>rpsL150</i> <i>relA</i> <i>flb5301</i> <i>ptsF25</i> <i>deoC1</i>	5
MH1471	MC4100 <i>envZ473</i>	11
MH1461	MC4100 <i>envZ11</i>	28
SG499	MH1471 <i>malPQ::Tn5</i>	This study
MH1160	MC4100 <i>ompR101</i>	11
RT3	MC4100 <i>envZ3 malPQ::Tn5</i>	26
SG477	MC4100 <i>envZ22</i> (Am)	8
SG463	MC4100 <i>ompR331</i> (Am)	8
SG480 Δ 76	MC4100 Δ (<i>envZ-malT</i>)76	9
MH225	MC4100 Φ (<i>ompC-lacZ</i> ⁺)10-25	11
MH513	MC4100 Φ (<i>ompF-lacZ</i> ⁺)16-13	11
SG901	MH513 Δ (<i>envZ-malT</i>)76	This study
SG950	MH513 <i>envZ473</i>	This study
SG903	SG950 <i>sez-18</i>	This study
SG904	SG950 <i>sez-85</i>	This study
SG912	SG950 <i>sez-44</i>	This study
SG913	SG950 <i>sez-611</i>	This study
SG340	MC4100 [λ Φ (<i>malE-lacZ</i>) 4-81(Hyb)]	3
SG341	MH4100 [λ Φ (<i>malE-lacZ</i>) 4-81(Hyb)] <i>envZ473 sez85</i>	This study
SG351	MH4100 [λ Φ (<i>malE-lacZ</i>) 4-81(Hyb)] <i>envZ473</i>	This study
SG265	F ⁻ Δ (<i>gpt-lac</i>) <i>XIII ara</i> <i>argE</i> (Am) <i>gyrA</i> <i>rpoB</i> <i>thi</i> <i>supP</i> (P1 <i>cry</i>)	24
SG404	F ['] 141/MC4100 <i>asd</i> (P1CM <i>clr-100</i>)	24
SE4003	MC4100 <i>aroE24 rpsE lamB60</i>	23
MS367	Hfr KL1699 <i>thi-1</i> <i>drm-3</i> <i>relA1</i> <i>recA1</i> <i>srl::Tn10</i>	11
KLF41	F ['] 141/ <i>leuB6</i> <i>hisG1</i> <i>recA1</i> <i>argG6</i> <i>metB1</i> <i>lacY1</i> <i>gal-6</i> <i>xyl-7</i> <i>mtl-12</i> <i>rpsL104</i> <i>fhuA</i> <i>tsx-1</i> <i>supE44</i>	17
NO1294	NO1230 (λ <i>trkA</i> , λ <i>cI857</i> S7 <i>b515</i> <i>b519</i> <i>xis6</i>)	16
NO1267	NO1230 (λ <i>spc1</i> , λ <i>cI857</i> S7)	16
NO1340	NO1230 <i>recA</i> (λ <i>spc2</i> , λ <i>cI857</i> S7)	16
SE4003-2	SE4003 <i>recA</i> (λ <i>spc2-I2</i> , λ <i>cI857</i> S7)	Scott Emr
SE4003-16	SE4003 <i>recA</i> (λ <i>spc2-II6</i> , λ <i>cI857</i> S7)	Scott Emr
NO1428	NO1230 (λ <i>spc2-Δ9</i> , λ <i>cI857</i> S7)	15
Phages		
λ <i>vir</i>		Laboratory stock
K20		12
hy2		2
P1 <i>vir</i>		Laboratory stock
λ NK561	<i>b221</i> <i>cI::Tn10</i> <i>Oam29</i> <i>Pam80</i>	7

mixed with 0.1 ml of a P1 *vir* lysate grown on strain MH1471, adsorbed for 20 minutes, and then spread on glucose minimal agar containing sodium citrate. After 2 days of incubation at 37°C, the agar plates were replicated onto glucose minimal agar and lactose MacConkey agar. The next day colonies were scored for their ability to grow on glucose minimal agar (Bio⁺) and their metabolism of the sugar lactose.

Isolation of strains carrying Tn10 close to the *envZ473* suppressor alleles. The method of choice is a two-step

procedure described in detail elsewhere (24). A culture of a strain lysogenic for the generalized transducing phage P1CM *clr-100* was mixed with a high-titer lysate of λ NK561 propagated on strain SG265. Strain SG265 contains a cryptic P1 phage (24); consequently, the λ phage is resistant to P1-mediated restriction on infection into the strain containing P1CM *clr-100*. The mixture was incubated at 30°C for phenotypic expression of the Tet^r determinant, spread on L agar containing sodium citrate and tetracycline, and incubated overnight at 30°C. The Tet^r colonies were removed from the agar, pooled, washed several times in L-citrate broth, and finally diluted 1/1000 into 10 ml of L broth containing tetracycline at 25 μ g/ml. This culture was incubated at 30°C until it reached an optical density at 600 nm of 0.2, and then it was shifted to a higher temperature for 3 h, or until lysis.

Samples of the P1CM *clr-100* lysate from the Tn10-transposition pool were used to transduce each of the suppressor-containing strains to Tet^r on lactose MacConkey agar containing 8 μ g of tetracycline per ml. The next day Tet^r colonies were scored for their ability to utilize lactose and sensitivity to λ *vir*.

Complementation tests with transducing phages carrying the *spc* and α ribosomal operons. The defective λ transducing phages λ *trkA*, λ *spc1*, and λ *spc2* carry various amounts of chromosomal DNA from the ribosomal gene cluster at 72 min (16). Since these phages carry a temperature-sensitive repressor, the lysates were prepared by heat induction of lysogens containing the respective phage along with a temperature-inducible helper phage (λ *cI857* S7). The mixed lysates were added to the recipient cells so that the multiplicity of infection was ≥ 5 . Serial dilutions of each mixture were then spread on minimal medium agar and incubated at 30°C for 2 days.

RESULTS

Rationale and isolation of suppressors of *envZ473*. We have suggested that the *envZ473* allele specifies an altered gene product that blocks transcription of *ompF*, *lamB*, and *phoA* by interacting in a detrimental way with one or more components common to their expression. We reasoned that by isolating extragenic mutations that simultaneously suppressed the transcriptional defect for *ompF* and *lamB*, we could define the genes for those transcriptional components. In turn, this could provide insights into the nature of EnvZ function and the mechanism of porin regulation. Implicit in this reasoning was the assumption that the interactions between the product of *envZ473* and the transcriptional component, although altered, reflected a normal association between the wild-type products of their genes. Even if this was incorrect, delineation of the suppressor gene(s) would shed light on the pleiotropic phenotype conferred by the *envZ473* allele.

The isolation of second-site suppressor mutations relies on the ease with which revertant colonies can be distinguished from the parental strain. The availability of *ompF-lac* fusions simplified this task since the parental *envZ473* strain is Lac⁻ and the revertant strains will be Lac⁺ as the result of the increased expression of *ompF*. An additional requirement is the ability to differentiate the desired extragenic mutations from a background of intragenic mutations, including true revertants. To eliminate this problem, we used a procedure which allows all of the chromosome to be mutagenized, except for that region carrying the initial mutation. Strain SG901 (Fig. 1) contains an *ompF-lac* operon fusion, and a

large region that includes the *ompR*, *envZ*, and *bioH* genes has been deleted (9). Among other phenotypic characteristics conferred by this deletion, SG901 requires the addition of exogenous biotin for growth on minimal media. Ten independent cultures of this strain were mutagenized with nitrosoguanidine and then transduced to biotin prototrophy with a P1 *vir* lysate propagated on a strain carrying the *envZ473* allele. Accordingly, all of the Bio⁺ transductants must have acquired the *ompB* region from the nonmutagenized donor, including the *envZ473* mutation. By mutagenizing all of the chromosome except for the *ompB* region, we hoped to eliminate the potential problem of true revertants and second-site intragenic mutations. After sufficient growth, the Bio⁺ colonies were replicated to lactose MacConkey agar and glucose minimal agar and then scored the next day for their ability to ferment lactose.

The majority of the colonies displayed a pink color on the lactose indicator agar that is characteristic (26) of the parental *envZ473* Φ (*ompF-lacZ*) fusion strain. The pink reflects the low level of lactose metabolism that is found in the *envZ473* Φ (*ompF-lacZ*) fusion strain, in contrast with the red (high-level lactose metabolism) of the *envZ*⁺ Φ (*ompF-lacZ*) fusion strain. Approximately 1% of the Bio⁺ transductants appeared as white colonies, whereas another 1% were more red (Lac⁺) than the *envZ473* strain was. The Lac⁺ colonies were purified and then streaked against the phage λ *vir* to score for the expression of the *lamB* gene. Approximately 10% (8 of 78) of the colonies that exhibited increased expression of *ompF* also appeared to contain elevated amounts of LamB protein, as judged by their sensitivity to λ *vir*.

In addition to the gene *lamB*, all of the structural genes of the maltose regulon showed decreased transcription in the pleiotropic *envZ* mutant strains (28). To ensure that the increased amounts of LamB were due to enhanced expression of the *lamB* gene, each of the eight mutants was streaked on maltose MacConkey and maltose tetrazolium indicator agars to monitor expression of the complete maltose regulon. Single colonies of all eight strains appeared to express the Mal⁺ phenotype more than the control SG950 did. Thus, the LamB⁺ phenotype is most easily accounted for by an increase in transcription of all of the genes of the maltose regulon.

To eliminate the possibility that any of the revertants contained an intragenic suppressor mutation, each of the revertants was transduced to resistance to kanamycin with a P1 lysate grown on strain SG499 (*envZ473 malPQ::Tn5*). All kanamycin-resistant transductants from each of the mutants retained the characteristic Lac⁺, λ *vir*^s phenotype. In addition, P1 *vir* lysates generated on each of the mutants were used to transduce strain SG901 to prototrophy for biotin. For each of the mutants, 100% of the Bio⁺ transductants appeared as pink colonies on lactose MacConkey agar. These results are consistent with the suppressor mutations mapping outside the *ompB* region.

Mapping the *envZ473* suppressor mutations. Since most of the revertants displayed phenotypes intermediate to those of the wild-type and *envZ473* strains, we chose to map a drug resistance marker that was linked to each suppressor mutation. Accordingly, the transposable element Tn10 was placed next to the suppressor mutations by transducing each of the revertants to Tet^r with a P1 lysate propagated on a Tn10 transposition pool generated by the method described in Materials and Methods. The Tet^r transductants that appear pink (Lac^{-/+}) on lactose MacConkey agar would contain a Tn10 linked to the wild-type allele of the suppres-

FIG. 1. Chromosomal map of an Δ (*envZ-malT*) Φ (*ompF-lac*) strain used for localized nonmutagenesis. The circle represents the chromosomal map of *E. coli* K-12 and the indicated map positions, 0/100 min, *ompF* (21 min), and *ompR envZ* (74 min) are from Bachmann (1), except that *ompR* and *envZ* are reversed (8). The enlarged region at 21 min shows the structure of the *ompF-lac* operon fusion with the adjacent prophage. The enlarged region at 74 min shows the chromosomal region around the *ompB* locus and the genetic material missing as a result of the $\Delta 76$ allele.

sor gene (24). Among the Tet^r transductants of each of the eight revertants, about 1% of the colonies were white, and 1% exhibited the dark-red phenotype of an *envZ*⁺ strain. Subsequent tests revealed that the former colonies had lost the *ompF-lac* fusion and that the latter colonies had replaced the mutant *envZ* allele with the wild-type gene.

An additional 1% of the colonies appeared to have regained the characteristic pink color of the *envZ473* fusion strain. These pink colonies were tested for expression of the *lamB* gene by the methods described above. Four of the eight revertants had obtained Tn10 insertions linked to a single suppressor mutation, as judged by the simultaneous return to the *envZ473* (λ *vir*^r Lac⁻ Mal⁻) phenotype. The revertant strains that contained a single suppressor by this criterion were designated SG903, SG904, SG912, and SG913. Two Tn10 insertions linked to the suppressor of strain SG904 yielded 55 and 90% Lac⁻ λ *vir*^r Tet^r colonies, respectively, upon transduction into the strains SG903, SG904, SG912, and SG913. All four mutations were subsequently moved into an unmutagenized strain, SG950 [Φ (*ompF-lac*) *envZ473*], by using the Tet^r selection of the Tn10 insertion that exhibits 55% linkage. Neither of these Tn10 insertions generated Lac⁻ or λ *vir*^r colonies on transduction into the four remaining strains. Repeated transductions of these four revertants failed to generate any Tet^r Lac⁻ colonies that simultaneously became λ *vir*^r. These strains were presumed to contain multiple suppressor mutations and were dropped from further analysis.

In the course of mapping the Tn10 insertions by Hfr-mediated conjugation, it was determined that the insertions were all tightly linked to the *rpsL150* allele of strain SG904, which confers resistance to streptomycin (Str^r). Subsequent mapping of one of the Tn10 insertions by P1 transduction

FIG. 2. Linkage map of the *sez* (suppressor of *envZ473*) region. The numbers represent P1 cotransduction frequencies between the markers indicated. Arrows point from the unselected marker to the selected marker. For each selected marker, at least 400 transductants were purified and screened for the presence or absence of the remaining three unselected markers.

showed that it was approximately 90% linked to the *rpsL150* allele. The suppressor mutations will hereafter be referred to as *sez* (suppressor of *envZ473*), and the allele numbers 18, 85, 44, and 611 have been assigned to the mutations in strains SG903, SG904, SG912, and SG913, respectively.

One of the *sez* mutations (*sez*-85) was mapped more precisely by determining its linkage to two genetic markers whose map position relative to the *rpsL* gene is known. These two genes are *rpsE* (*Spc*^r, resistance to spectinomycin) and *aroE* (*Aro*⁻, requirement for aromatic amino acids). By preparing P1 *vir* lysates on several different donor strains and by using these lysates to transduce appropriately marked recipients, we have determined the linkage of *sez*-85 to each of these known markers. Results of these experiments are summarized in Fig. 2. Although only one of the suppressor mutations was mapped by this method, our previous results had suggested that all four mutations were closely linked. The linkage between *sez*-85 and *rpsE* was so great that it is difficult to say with certainty on which side of *rpsE* the mutation actually maps; however, the data are consistent with the order shown in Fig. 2.

Complementation analysis of the *sez* suppressor mutations. To determine whether the suppressor mutations were recessive or dominant, a strain carrying the *sez*-85 allele was made merodiploid by mating it with an appropriate male strain containing an episome covering the region between *argG* (68 min) and *asd* (75 min). Since the episome of choice, F'141, includes the *ompB* locus, the *envZ473* mutation was first recombined onto F'141 (11). The recipient strain contained, in addition to the *envZ473* and *sez*-85 alleles, the *aroE* mutation to allow selection of the episome and the *recA* (recombination deficiency) mutation. Exconjugants were streaked on lactose indicator agar and against λ *vir* to determine the expression of the *ompF* and *lamB* genes, respectively. All *sez*-85/*sez*⁺ merodiploids became simultaneously Lac⁻ and λ *vir*^r. Thus, the *sez*-85 allele is recessive.

The availability of a series of defective λ transducing phages which carry various amounts of chromosomal DNA in the region of the *sez* mutations has enabled us to map more precisely the *sez*-85 mutation (15, 16). These phages contain, in addition to the DNA coding for the ribosomal proteins of the *spc* and α operons, the wild-type *aroE* gene. Thus, by transducing *aroE* derivatives of the *sez*-85 strain to *Aro*⁺ with this series of phages, strains can be isolated that are merodiploid for various amounts of the ribosomal gene cluster. The results of this experiment are summarized in Fig. 3. It is apparent from these results that the chromosomal DNA carried by λ *spc1* is sufficient to complement the *sez*-85 mutation, whereas the transducing phage λ *trkA* does not appear to contain, or express, the *sez* gene since lysogens of that phage retain the Lac⁺ λ *vir*^s phenotype of the *sez*-85

parent strain. Mutations that were isolated as being polar on expression of the *rpsE* gene of the *spc* operon (6) still allow expression of the *sez* gene, as evidenced by the Lac⁻ λ *vir*^r phenotype of λ *spc2-116* and λ *spc2-Δ2* lysogens. However, the phage λ *spc2-Δ9* does not complement the *sez*-85 allele. This phage is not proficient for synthesis of the genes in either the *spc* or α operons, as determined by complementation analysis (14). Collectively, these results demonstrate that the *sez* gene is part of the α operon.

Since many of the genes of the major ribosomal gene cluster have been cloned, we attempted to delineate further the position of the *sez* mutations by carrying out the complementation analysis with plasmids carrying various portions of this region. The chromosomal DNA carried by these plasmids, along with the results of the complementation tests, are shown in Fig. 3. Previous results (23) suggest that at least one of the genes, *prlA*, of the *spc* operon is expressed from the chromosomal material carried by plasmid pRLA4, and it is presumed that the other genes (*rp10*, *rpmD*, and *X*) contained on this plasmid are as well. Plasmids pLL31 and pRAG1003 contain, respectively, the 5' and 3' regions of the α operon (J. Zengel and H. Kestler, personal communication). The DNA fragment of pLL31 includes the genes encoding the S13, S11, and S4 ribosomal proteins, as well as the 5' portion of *rpoA* and the promoter for the α operon. Plasmid pRAG1003 contains an 8-kilobase *EcoRI*-*Bam*HI fragment that includes the 3' portion of the *rpoA* gene and extends past the downstream gene for the L17 ribosomal protein. None of these plasmids were able to complement the *sez* mutation.

Characterization of the *sez* mutations. A trivial explanation for the phenotypes conferred by the *sez* suppressor mutations is that they cause the increased expression of *ompF* (*ompC*?) and *lamB*, independent of the particular *envZ* allele. That is, they bypass the need for EnvZ. To test this, we transduced the *sez*-85 allele into several *envZ* and *ompR* mutant strains by using one of the previously isolated Tn10 insertions (*zhc3::Tn10*) as the selectable marker. Whereas 55% of the Tet^r transductants of an *envZ473* Φ (*ompF-lac*)

FIG. 3. Complementation of the *sez*-85 allele with DNA fragments from the *spc* and α ribosomal operons. The first line shows the chromosomal region of the suppressor mutation as determined by genetic mapping. The lines below this indicate the genetic material carried by the respective phages and plasmids as outlined in the text. The column to the right of the diagram indicates the ability (+) or inability (-) of each phage and plasmid to complement the *sez*-85 allele. Complementation was assumed to have occurred if the merodiploid exhibited the *OmpF*⁻ *LamB*⁻ phenotype of the *envZ473* *sez*⁺ strain.

fusion strain became Lac^+ and $\lambda \text{ vir}^s$, all of the strains containing *envZ* and *ompR* alleles of different phenotypic classes, including *ompR*⁺ $\Phi(\text{ompF-lac})$, *ompR331*(Am) $\Phi(\text{ompF-lac})$, *envZ22*(Am) $\Phi(\text{ompF-lac})$, *envZ3* $\Phi(\text{ompC-lac})$, and *ompR101* $\Phi(\text{ompF-lac})$, always retained their characteristic phenotypes. To confirm that the *sez-85* allele had been transduced into the various strain backgrounds, P1 lysates generated on eight Tet^r colonies from each transduction were used to transduce strain SG950 to Tet^r. Lysates capable of transducing SG950 to Tet^r Lac^+ were assumed to have been generated on strains carrying the *sez-85* allele. Approximately 50% of all *ompR* and *envZ* mutant strains had acquired the *sez-85* allele by this criterion. Thus, the *sez* mutations are not bypass suppressors. In contrast, a strain containing the allele *envZ11* (formerly *tpo-11*), which confers the same phenotype as *envZ473* (28), exhibits a Lac^+ $\lambda \text{ vir}^s$ phenotype on transduction to *sez-85*.

To quantitate the suppression of *envZ473* by *sez-85*, we determined the β -galactosidase activities of *ompF-lac* and *malE-lac* fusion strains containing the *envZ*⁺, *envZ473*, and *envZ473 sez-85* alleles. We also determined the effect of *sez* on one of the other genes known to be affected by *envZ473* by measuring the alkaline phosphatase activity of the *ompF-lac* fusion strain. Whereas expression of *malE* and *phoA* is reduced 10- and 15-fold, respectively, by *envZ473*, an *envZ473 sez-85* double mutant exhibits near wild-type expression of both genes. Moreover, while the absolute levels of *malE* and *phoA* expression were higher in inducing medium (see Materials and Methods) than in noninducing medium, the relative effects of both *envZ473* and *sez-85* were the same in both media. That is, whereas the *sez* mutation restores the expression of *malE* and *phoA* in an *envZ473* strain, it does so by preventing the *envZ473* defect, rather than by causing their constitutive expression. The *sez-85* mutation also appeared to affect *ompF* expression in a similar fashion, although the absolute effects of both *envZ473* (11) and *sez-85* on *ompF* expression were less. For example, the *envZ473 sez-85* $\Phi(\text{ompF-lac})$ fusion strain exhibited β -galactosidase activity that was 2- to 2.5-fold lower than that exhibited by the *envZ*⁺ fusion strain and 2- to 2.5-fold higher than that of the *envZ473* fusion strain. Again, although the β -galactosidase activities of the *ompF-lac* fusion strains were higher in medium of low osmotic strength (inducing) than in medium of high osmotic strength (noninducing), the activity exhibited by the *envZ473 sez-85* strain was 2- to 2.5-fold lower and higher, respectively, than the activity exhibited by the *envZ*⁺ and *envZ473* strains, regardless of the medium in which they were grown. Thus, the *sez-85* mutation restores, to various degrees, the expression of at least three of the genes known to be affected by the pleiotropic *envZ* allele. In addition, it appears to do so by specifically blocking the *envZ473* defect rather than by causing the constitutive expression of the three genes.

The *rpoA109* allele was isolated as a mutation that prevented the growth of the phage P2 due to a block in expression of the late genes required for phage morphogenesis (14, 25). To determine if the *sez-85* mutation also prevented phage P2 growth, we examined the efficiency of plating of P2 on several isogenic *sez*⁺ and *sez-85* strains. No difference was detected for either plaque number or morphology. Therefore, if these mutations are within the same gene, the phenotypes they confer are different.

DISCUSSION

We have used a procedure, which we call localized nonmutagenesis, to allow us to isolate four second-site

suppressor mutations that specifically alleviate the pleiotropic transcriptional defect of an *envZ473* strain. The advantage of this method is that it appears to eliminate the high background of intragenic suppressor mutations, including true revertants, that are often isolated in this type of selection. For example, none of the OmpF^+ LamB^+ revertants isolated by this method arose as a result of a secondary mutation within the *envZ* or *ompR* gene.

The effect of the *sez* (suppressor of *envZ473*) suppressors on a variety of different *ompR* and *envZ* mutations has been examined. Expression of an *ompF-lacZ* operon fusion was restored to approximately 50% of the wild-type levels in an *envZ473* background. Moreover, the LamB^+ phenotype of the *envZ473 sez-85* pseudorevertant is apparently due to increased expression of the *lamB* gene since all of the products of the maltose operon were induced, as determined by the elevated expression of a *malE-lac* fusion and an increase in maltose metabolism. Finally, the *PhoA* activity of an *envZ473* strain was increased almost 10-fold by *sez-85*, to levels that approach those exhibited by wild-type strains. Thus, the *sez* mutations suppress many, if not all, of the phenotypes conferred by the pleiotropic class of *envZ* mutations. Moreover, our results suggest that the suppressor mutations exert their effect by blocking specifically the transcriptional defect imposed by the *envZ473* gene product, rather than by causing a general increase in the expression of the affected genes. First, the relative effect of *sez* on the expression of *malE*, *phoA*, and *ompF* remained the same, regardless of whether the strains were grown in inducing or noninducing media. Thus, *sez* did not appear to overcome the *envZ473* defect by causing the constitutive expression of the three genes. Second, none of the other phenotypic classes of *ompR* or *envZ* mutations were affected by *sez*, including the *envZ3* allele which is also thought to specify an altered *EnvZ* protein (26). Finally, we have been unable to detect any effect of the suppressor mutations in an otherwise wild-type background.

All of the *sez* suppressors have been mapped to the ribosomal gene cluster at about 72 min (1) and appear, within the limits of cotransduction analysis, to be within the same gene. Although each of the *sez* alleles suppresses the transcriptional defect to a different degree, the general pattern of suppression for each is identical. One of the suppressor alleles, *sez-85*, has been mapped precisely. Using transducing phages that carry various portions of the ribosomal gene cluster (6, 15), we have shown clearly that the mutations map within the α operon. This is evidenced by the fact that insertion and deletion mutations in the *spc* operon that are known to disrupt synthesis of genes distal to the promoter have no effect on the ability of the respective transducing phages to complement the *sez-85* allele. By contrast, disruptions of the α operon prevent complementation.

The simplest explanation for the suppression of the transcriptional defect caused by *envZ473* is that a component of the transcriptional machinery has been altered in the revertant strains. The α operon harbors the gene for the α subunit of RNA polymerase, as well as the genes for the ribosomal proteins S13, S11, S4, and L17 (16). Of these, only the α protein is known to function directly in transcription. Unfortunately, attempts to further define the *sez* gene by using high-copy-number plasmids containing defined portions of the α operon have been unsuccessful.

If the *sez* mutations map in *rpoA*, then the transcriptional defect of an *envZ473* strain may be due to a detrimental interaction between the altered *EnvZ* protein and the α subunit of RNA polymerase. Accordingly, the *sez* mutations

overcome the detrimental interaction and restore normal expression to the set of genes affected. It should be noted that the interaction between EnvZ and RpoA may not involve direct physical contact. Indeed, it may be mediated by another protein or even a small molecule. Allele-specific suppression would provide evidence favoring direct interaction; however, our data are inconclusive in this regard since the only other available allele of this phenotypic class (*envZ11*) was also suppressed by the *sez* mutations.

There are at least two simple explanations to account for the defective interaction between the mutant EnvZ protein and α . The first and more interesting possibility is that the wild-type EnvZ protein interacts, directly or indirectly, with the α subunit of RNA polymerase. According to this model, the *envZ473* allele specifies a protein that alters this normal interaction in a detrimental fashion. The second model postulates that EnvZ normally does not interact with the α subunit, but has been altered by the *envZ473* mutation so as to interfere with α function. Although we have no direct proof, we favor the former possibility because the first model suggests an alteration of a normal interaction, whereas the second model requires the creation of a new interaction. That is, the first alternative is more simple. In addition, EnvZ has been shown to be required for the expression of *ompF* and *ompC* (8) and so has been implicated in at least one facet of the transcriptional process.

Regardless of which, if either, of these models is correct, the specificity of the transcriptional defect exhibited by the *envZ473* strain is an enigma since many of the genes affected do not appear to require the EnvZ protein for expression (8, 9). We have suggested previously that the *envZ473* allele specifies an altered protein that interferes with the expression of a specific set of genes (8), presumably by impinging on the function of one or more components common to the expression of these genes. Since a single extragenic mutation (*sez*) has been isolated which suppresses the transcriptional defect of at least two of the unrelated genes, it is possible that the product of the suppressor gene is the component common to the transcription of *ompF*, *lamB*, and *phoA*. That is, the product of the *sez* (*rpoA*?) gene might determine the specificity of the transcriptional defect of the *envZ473* mutant. Although we have no direct evidence for this hypothesis, support for the idea that the α subunit plays a role in selective gene expression comes from the analysis of two other mutations that have been reported to map in the α gene. One mutation, *rpoA109*, blocks late transcription of the bacteriophage P2 (22, 25). The other mutation, *phs*, confers a deficiency for growth on melibiose, glutamate, and arabinose, as well as a requirement for either methionine or cysteine supplementation (10, 20). At least for arabinose, the growth defect is due to a 20-fold decrease in expression of the *araBAD* operon (20). Our mutants do not exhibit the phenotype characteristic of the *rpoA109* strain and appear not to require a supplement of methionine or cysteine for growth on minimal media since they were selected for biotin prototrophy on minimal agar. It is unlikely, however, that any of the three represent the phenotype conferred by a null mutation.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant GM35791 (to T.J.S.) from the National Institutes of Health. S.G. was supported during the initial stages of this work by a postgraduate scholarship from the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

- Bachmann, B. J. 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. 47:180-230.
- Bassford, P. L., Jr., D. L. Diedrich, C. L. [sic] Schnaitman, and P. Reeves. 1977. Outer membrane proteins of *Escherichia coli*. VI. Protein alteration in bacteriophage-resistant mutants. J. Bacteriol. 131:608-622.
- Bassford, P. L., Jr., T. J. Silhavy, and J. R. Beckwith. 1979. Use of gene fusion to study secretion of maltose-binding protein into *Escherichia coli* periplasm. J. Bacteriol. 139:19-31.
- Brickman, E., and J. Beckwith. 1975. Analysis of the regulation of *Escherichia coli* alkaline phosphatase synthesis using deletions and 080 transducing phages. J. Mol. Biol. 96:307-316.
- Casadaban, M. J. 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. 104:541-555.
- Emr, S. D., S. Hanley-Way, and T. J. Silhavy. 1981. Suppressor mutations that restore export of a protein with a defective signal sequence. Cell 23:79-88.
- Foster, T. J., V. Lundblad, S. Hanley-Way, S. M. Halling, and N. Kleckner. 1981. Three *Tn10*-associated excision events: relationship to transposition and role of direct and inverted repeats. Cell 23:215-227.
- Garrett, S., R. K. Taylor, and T. J. Silhavy. 1983. Isolation and characterization of chain-terminating nonsense mutations in a porin regulator gene, *envZ*. J. Bacteriol. 156:62-69.
- Garrett, S., R. K. Taylor, T. J. Silhavy, and M. L. Berman. 1985. Isolation and characterization of $\Delta ompB$ strains of *Escherichia coli* by a general method based on gene fusions. J. Bacteriol. 162:840-844.
- Giffard, P. M., G. C. Rowland, R. G. Kroll, L. M. D. Stewart, E. P. Bakker, and I. R. Booth. 1985. Phenotypic properties of a unique *rpoA* mutation (*phs*) of *Escherichia coli*. J. Bacteriol. 164:904-910.
- Hall, M. N., and T. J. Silhavy. 1981. Genetic analysis of the *ompB* locus in *Escherichia coli* K-12. J. Mol. Biol. 151:1-15.
- Hancock, R. E. W., and P. Reeves. 1975. Bacteriophage resistance in *Escherichia coli* K-12: general pattern of resistance. J. Bacteriol. 121:983-993.
- Inouye, H., S. Michaelis, A. Wright, and J. Beckwith. 1981. Cloning and restriction mapping of the alkaline phosphatase structural gene (*phoA*) of *Escherichia coli* and generation of deletion mutants in vitro. J. Bacteriol. 146:668-675.
- Jaskunas, S. R., R. R. Burgess, L. Lindahl, and M. Nomura. 1976. Two clusters of genes for RNA polymerase and ribosome components in *Escherichia coli*, p. 539-552. In R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Jaskunas, S. R., A. M. Fallon, and M. Nomura. 1977. Identification and organization of ribosomal protein genes of *Escherichia coli* carried by $\lambda fus2$ transducing phage. J. Biol. Chem. 252:7323-7336.
- Jaskunas, S. R., L. Lindahl, and M. Nomura. 1975. Specialized transducing phages for ribosomal genes of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 72:6-10.
- Low, K. B. 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. 36:587-607.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Mizuno, T., E. T. Wurtzel, and M. Inouye. 1982. Osmoregulation of gene expression. II. DNA sequence of the *envZ* gene of the *ompB* operon of *Escherichia coli* and characterization of its gene product. J. Biol. Chem. 257:13692-13698.
- Rowland, G. C., P. M. Giffard, and I. R. Booth. 1985. *phs* locus of *Escherichia coli*, a mutation causing pleiotropic lesions in metabolism, is an *rpoA* allele. J. Bacteriol. 164:972-975.
- Sarma, V., and P. Reeves. 1977. Genetic locus (*ompB*) affecting a major outer-membrane protein in *Escherichia coli* K-12. J. Bacteriol. 132:23-27.
- Scaife, J. 1976. Bacterial RNA polymerases: the genetics and control of their synthesis, p. 207-226. In R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

23. Shultz, J., T. J. Silhavy, M. L. Berman, N. Fiil, and S. D. Emr. 1982. A previously unidentified gene in the *spc* operon of *Escherichia coli* K-12 specifies a component of the protein export machinery. *Cell* **31**:227-235.
24. Silhavy, T. J., M. L. Berman, and L. W. Enquist. 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. Sunshine, M. G., and B. Sauer. 1975. A bacterial mutation blocking P2 phage late gene expression. *Proc. Natl. Acad. Sci. USA* **72**:2770-2774.
26. Taylor, R. K., M. N. Hall, and T. J. Silhavy. 1983. Isolation and characterization of mutations altering expression of the major outer membrane porin proteins using the local anaesthetic procaine. *J. Mol. Biol.* **166**:273-282.
27. Verhoef, C., B. Lugtenberg, R. van Boxtel, P. deGraaff, and H. Verheij. 1979. Genetics and biochemistry of the peptidoglycan-associated proteins b and c of *Escherichia coli* K-12. *Mol. Gen. Genet.* **169**:137-146.
28. Wandersman, C., F. Moreno, and M. Schwartz. 1980. Pleiotropic mutations rendering *Escherichia coli* K-12 resistant to bacteriophage TP1. *J. Bacteriol.* **143**:1374-1383.
29. Wanner, B. L., A. Sarthy, and J. Beckwith. 1979. *Escherichia coli* pleiotropic mutant that reduces amounts of several periplasmic and outer membrane proteins. *J. Bacteriol.* **140**:229-239.
30. Wurtzel, E. T., M.-Y. Chou, and M. Inouye. 1982. Osmoregulation of gene expression. I. DNA sequence of the *ompR* gene of the *ompB* operon of *Escherichia coli* and characterization of its gene product. *J. Biol. Chem.* **257**:13692-13698.