JOURNAL OF BACTERIOLOGY, June 1985, p. 1054–1060
0021-9193/85/061054-07$02.00/0

Vol. 162, No. 3

Mutations that Define the Promoter of *ompF*, a Gene Specifying a Major Outer Membrane Porin Protein

RONALD K. TAYLOR,[1]† STEPHEN GARRETT,[2] ERICA SODERGREN,[3]‡ AND THOMAS J. SILHAVY[3]§*

Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228[1]; Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218[2]; and Laboratory of Genetics and Recombinant DNA, LBI-Basic Research Program, Frederick Cancer Research Facility, National Cancer Institute, Frederick, Maryland 21701

Received 21 December 1984/Accepted 20 March 1985

Expression of the major porin structural genes, *ompF* and *ompC*, is influenced by medium osmotic strength and requires the products of two regulatory genes, *ompR* and *envZ*. To help define the sites required for the expression of both porin genes, we have used a novel selection to identify mutations that decrease *ompF* transcription. From our assignment of the mRNA start site by the primer extension method, these mutations appear to delineate poorly conserved −10 and −35 consensus promoter regions. In addition, one mutation provides the first genetic evidence that an A residue at position −45 may be important for RNA polymerase recognition.

The major porin proteins of *Escherichia coli* K-12, OmpF and OmpC, facilitate the passage of small molecules across the outer membrane permeability barrier. Although the structure and function of these proteins are similar, the OmpF pore has a slightly larger effective diameter and is consequently more efficient (27, 28). The total amount of porin present in the cell is nearly constant; however, synthesis is regulated to permit a fluctuation in the relative amounts of OmpF and OmpC. The major environmental property responsible for this fluctuation is medium osmotic strength. In media of low osmolarity, OmpF is synthesized preferentially, whereas in media of high osmolarity, OmpC is synthesized preferentially (2, 16, 17, 37). The observed fluctuation in the levels of synthesis for both porins is approximately sixfold (37). We wish to understand the mechanisms by which *E. coli* cells sense osmotic strength and direct synthesis of the appropriate porin protein.

Previous studies have shown that the osmolarity-dependent fluctuation in porin protein synthesis is accomplished by controlling the transcription of the structural genes *ompF* and *ompC* (9, 10). Two regulatory genes, *ompR* and *envZ*, have been characterized (11). These genes form an operon, with *ompR* being promoter proximal (8, 25). The complete DNA sequence of this operon has been determined (25, 42), and the protein products of *ompR* and *envZ* (29 and 44 kilodaltons, respectively) have been identified (25, 35, 42).

Available data on the mutants suggest that OmpR functions to stimulate transcription at both *ompF* and *ompC* (11). Mutations in the second gene of the operon, *envZ*, have been isolated by various selections. Such mutant strains show a wide range of effects that alter normal porin fluctuation, and one class of *envZ* mutations causes a pleiotropic decrease in the expression of other exported proteins (36, 39, 40). The recent isolation of defined null mutations in *envZ* has demonstrated that this gene product is required, in addition to *ompR*, for normal transcriptional activation of the porin genes but not for expression of genes that encode other exported proteins (8, 8a). EnvZ may be required to sense medium composition and to direct OmpR to the appropriate porin gene promoter (12). However, the details of this regulatory system remain obscure. Moreover, other genetic loci may also participate in porin regulation (18, 24, 26, 32).

Although careful examination of the DNA sequence upstream of the coding regions of *ompF* and *ompC* reveals several regions of shared homology (6, 15, 23), these regions do not suggest an obvious model for regulation. Mizuno et al. (23) identified a 9-base-pair (bp) sequence in *ompF* and *ompC* that was identical except for a mismatch at the center. However, it is found at position −75 of *ompC* (+1 is the transcriptional start site) and at position +30 of *ompF* (6, 14), and it is difficult to envision a regulatory protein that would function at such different locations. They also observed a region of good homology just upstream from the Shine and Dalgarno sequences of both genes, which might suggest that OmpR or some other factor acts as a transcription antiterminator. It should be noted, however, that although the promoter of *ompC* shares homology with consensus −35 and −10 sequences, in scripto analysis has failed to identify consensus sequences for *ompF*. It therefore seems likely that *ompF* transcription requires a factor to facilitate polymerase recognition.

To provide further evidence to support the role of OmpR as a positive transcriptional activator and to analyze the mechanism of *ompF* expression, we have identified and characterized a series of *cis*-dominant mutations that decrease the transcription of *ompF*. These mutations appear to define at least part of the *ompF* promoter.

MATERIALS AND METHODS

Media, chemicals, and enzymes. All liquid and solid media have been described previously (22, 33). The melibiose concentration in melibiose MacConkey plates was 0.4%. Avian myeloblastosis virus reverse transcriptase was purchased from Life Sciences, Inc. Other enzymes were purchased from New England BioLabs or Bethesda Research Labora-

* Corresponding author.
† Present address: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.
‡ Present address: Department of Biochemistry, University of Texas Medical School at Houston, Houston, TX 77025.
§ Present address: Department of Molecular Biology, Princeton University, Princeton, NJ 08544.

TABLE 1. Bacterial strains, phages, and plasmids

Strain, phage, or plasmid	Genotype or relevant genes carried	Origin or reference
Bacteria		
JM103	Δ(*lac-pro*) *thi rpsL supE endA sbcB15 hsdR4* F′ *traD36 proAB lacI*^{q} *lacZM15*	20
LE30	*mutD5 rpsL azi galU95*	33
MC4100	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 ptsF25 deoC1*	7
MH760	MC4100 *ompR472*	10
MH1160	MC4100 *ompR101*	10
SG523	MC4100 (*ompF′-lacZ*::*neo1081.1*)*1* [λ*p*1(209) c*I857*]	This study
SG477	MC4100 *envZ22*(Am)	8
SV101	MC4100 *malPQ*::Tn*10*	Laboratory collection
Phages		
K20		Laboratory stock
λp16-21	(*ompF′-lacZ*)hyb*16-21 lacY*$^+$ *lacA′*	12
M13mp8		21
P1 *vir*		Laboratory stock
Plasmids		
pORF1		41
pRT100		This study
pRT240		This study

tories and used in accordance with the specifications of the manufacturer. β-Galactosidase activity was assayed as described by Miller (22). The chromogenic indicator 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG) was purchased from Bachem, Inc., and used at a concentration of 20 μg/ml.

Bacteria, bacteriophages, and plasmids. Bacteria, bacteriophages, and plasmids used in this study are listed in Table 1.

Bacteria and bacteriophage manipulations. Standard microbiological techniques were used for titrating bacteriophages, generalized transduction, lysogen formation and induction, and bacterial growth (22, 33). When applicable, mutagenesis of the phage λp16-21 was performed by growth through the *mutD* strain, LE30, as described previously (33).

DNA sequence analysis. To determine the alterations caused by the *ompF* mutations, DNA was first isolated from each of the mutant λp16-21 transducing phages by a rapid purification technique (4). This DNA was digested with *Eco*RI and *Bgl*II and ligated with *Eco*RI-*Bam*HI-digested M13mp8 replicative-form DNA (3, 21, 38). Single-stranded DNA was isolated from phage that formed white plaques on JM103 and that contained the appropriate 1.8-kilobase insert. The DNA sequence was determined by the dideoxynucleotide method (30) with the universal *lacZ* primer as described previously (20).

Determination of the *ompF* mRNA start site. The *ompF* mRNA transcription start site was determined by a primer extension procedure. Total cellular RNA was prepared from strains MC4100 (*ompR*$^+$), MH1160 (*ompR101*), and MH760 (*ompR472*) essentially as described previously (1). A DNA probe for the *ompF* mRNA was generated by end labeling *Bam*HI-digested pORF1 DNA (see Fig. 5) with ^{32}P by using polynucleotide kinase (19) and subsequently digesting with *Mnl*I. This yielded a radioactively labeled DNA fragment encoding the NH_2 terminus of OmpF but not extending as far as the region believed to include the message start. The RNA preparations were hybridized overnight with the probe, precipitated, and then incubated with avian myeloblastosis virus reverse transcriptase and nucleoside triphosphates to allow primer extension (5). The resultant DNA fragment was run on a urea-polyacrylamide DNA sequencing gel alongside a chemical cleavage (G+A) sequencing reaction of a DNA fragment labeled at the same *Bam*HI site (19).

RESULTS

Experimental design and rationale. Using techniques developed by Casadaban (7), we have isolated and characterized several different *ompF-lacZ* gene fusions (9, 10). The genetic structure of one of these fusions, Φ(*ompF′-lacZ*) hyb*16-21*, including adjacent chromosomal regions, is shown in Fig. 1 of the companion paper by Sodergren et al. (34). A λ transducing phage carrying this fusion has been isolated and used as a source of DNA to clone and analyze this fusion at the DNA sequence level (6). This analysis shows that the *ompF-lacZ* fusion specifies a hybrid protein containing the NH_2-terminal OmpF signal sequence plus 12 amino acids of the mature protein fused to a large, functional COOH-terminal fragment of β-galactosidase. The OmpF sequences present direct export of this hybrid protein to the outer membrane at low efficiency (15 to 20%) (12).

λ transducing phage carrying this *ompF-lacZ* gene fusion grow normally on wild-type *E. coli* strains. However, on strains that carry the *ompR472* mutation, the efficiency of plating is reduced by five orders of magnitude. The *ompR472* mutation causes constitutive, high-level expression of the *ompF-lacZ* fusion. This, coupled with the increase in hybrid gene dosage caused by phage replication, leads to a detrimental overproduction of the hybrid protein and an inhibition of productive phage infection. Mutations that prevent hybrid protein synthesis or alter the OmpF signal sequence and prevent hybrid protein export overcome this inhibition and allow normal phage growth (34).

We reasoned that mutations that alter the *ompF* promoter and decrease hybrid gene expression should restore growth of the λ *ompF-lacZ* transducing phage on strains that carry *ompR472*. The challenge was to identify these against the high background of mutations that abolish hybrid protein synthesis by other mechanisms. We solved this problem by using the logic of Scaife and Beckwith (31). They identified promoter mutations in *lac* by searching for mutants in which both *lacZ* and *lacY* expression were reduced but not abolished. Thus, by screening for mutant λ *ompF-lacZ* transducing phages that grow on an *ompR472* strain and exhibit decreased β-galactosidase and lactose permease activities we should be able to greatly enrich for the desired *cis*-acting mutations.

Expression of *lacZ* was monitored in mutant phage plaques by using the chromogenic substrate XG. To monitor *lacY* expression we took advantage of the promiscuous nature of the lactose transport system. In *E. coli* K-12 strains, expression of the melibiose transport system is temperature sensitive. At high growth temperature, >38°C, the lactose transport system is the only available route for efficient melibiose entry. Accordingly, *lacY* expression can be monitored by streaking lysogens of the mutant phage on melibiose indicator agar at 42°C.

Isolation of mutant phages exhibiting decreased expression of an OmpF-LacZ hybrid protein. Lysates of λp16-21 were

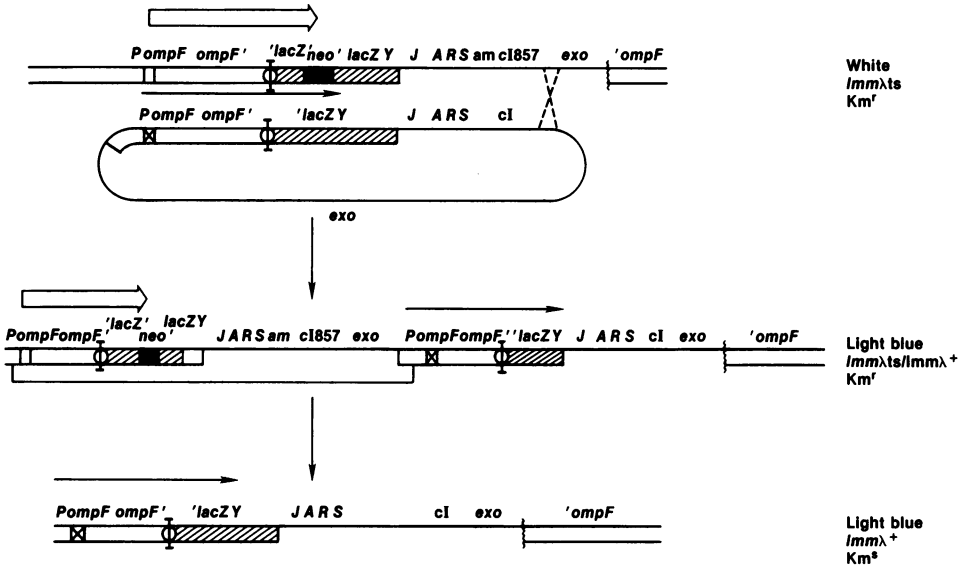

FIG. 1. Replacement of the wild-type *ompF* promoter with a mutant allele by homologous recombination. Integration of a mutant *ompF-lacZ*::*neo* fusion strain is shown by a dashed line. Symbols: ———, λ sequences; ▭, *ompF* DNA; and ▨, *lac* DNA; ▬, *neo* insert. The open arrow above the *ompF-lac* region represents strong transcription from the wild-type *ompF* promoter, whereas the thin solid arrow indicates weak expression from the mutant *ompF* promoter. The relevant phenotypes of the starting strain, the intermediate strain, and the segregant strain are shown to the right. Excision of the prophage is shown in the second line by a line connecting homologous *ompF* sequences upstream from the promoter mutation.

propagated on the *ompR101* strain, MH1160, to prevent expression of the OmpF-LacZ hybrid protein. These lysates, which contained 10^9 to 10^{10} PFU/ml when titrated on MH1160 and on the isogenic parent MC4100 (*ompR*$^+$), showed an efficiency of plating of 10^{-5} when titrated on strain MH760. This greatly decreased efficiency of plating is specific for the fusion phage (34).

To identify phages that plaque on strain MH760 as a result of decreased expression of the hybrid protein, we included the chromogenic indicator XG in the top agar to monitor LacZ activity. Phages producing even small amounts of LacZ activity (as little as 10 U) yield pale-blue plaques, whereas the parental phage, λp16-21, forms dark-blue plaques on strain MC4100. Phages that exhibited decreased but not abolished LacZ activity were chosen as candidates for decreased promoter activity.

Characterization of mutant phages to identify those carrying *ompF* promoter mutations. Promoter mutations should pleiotropically alter the expression of the entire operon. In the case of the λp16-21 fusion phage, such a mutation would cause a decrease in the expression of the downstream gene, *lacY*, that encodes lactose permease. To test *lacY* expression, mutant phage were lysogenized into *ompR*$^+$ and *ompR472* backgrounds and the resulting lysogens were streaked onto melibiose MacConkey indicator agar at 42°C. For the lysogens to ferment melibiose at 42°C and form red colonies on the indicator agar, they must express *lacY*. Those that formed white colonies or colonies with a less intense color and therefore harbored a prophage that expressed *lacY* at a reduced level were tested further.

The mutations carried by these phages were shown to be *cis* dominant, since Lac$^-$ lysogens of these mutant phage still express the chromosomal *ompF* gene. This was demonstrated by their sensitivity, in cross-streaks, to the phage K20 which utilizes the OmpF porin as its receptor. The mutations were then mapped to the *ompF* region of each phage by scoring for K20^r, Lac$^-$ recombinants among the lysogens, demonstrating that the mutations could recombine at the chromosomal *ompF* locus. Phages that carried a mutation that met all of these criteria were assumed to be strong candidates for harboring *ompF* promoter-down mutations. The 2-11 mutation causes only a slight decrease in LacZ and LacY activity and does not appear to confer an OmpF$^-$ phenotype when crossed onto the wild-type *ompF* gene. Consequently, we were unable to localize the mutation to the *ompF* region genetically.

Activities of the mutant *ompF* promoters. To assay the effect of each putative promoter mutation, we measured β-galactosidase activities from an *ompF-lac* fusion strain containing the various mutations. Strain SG523 is an *ompF-lac* operon fusion strain in which the *lacZ* gene has been disrupted with a kanamycin resistance determinant (*neo*) and the repressor gene of the adjacent λ prophage has been converted to the temperature-sensitive allele. The procedure for this conversion is outlined elsewhere (8a). As such, this strain is Lac$^-$, Km^r, and temperature sensitive. On lysogenization of strain SG523 with λp16-21 or any of the mutant derivatives (Fig. 1), the strain becomes temperature resistant and very pale blue to dark blue on XG, depending on the strength of the promoter mutation. These dilysogens were scored for Km^s segregants that left the *ompF* promoter mutation *cis* to the intact *lacZ* gene as a result of the excision of one of the prophages. Such segregants arose at a frequency of about 0.05%.

To test the effect of OmpR on the expression of *ompF* in each of these mutants, the null mutation *ompR101* was introduced by transduction by using the linkage between *ompR* and a Tn*10* in the *malPQ* operon. These strains, together with their *ompR*$^+$ parents, were grown in NB and TSB media (9) to elicit the two extremes of porin fluctuation. The β-galactosidase activity of these strains is presented in Table 2. The assay values in Table 2 show that all but one (2-11) of the mutations cause a large decrease in *ompF-lacZ* expression. All of the mutations that allow measurable

TABLE 2. β-Galactosidase activities of *ompR*$^+$ *ompF-lac* and *ompR101 ompF-lac* fusion strains with the *ompFp* mutations[a]

ompF allele[b]	Activity (in β-galactosidase activity units) of strain on medium:			
	ompR101		*ompR*$^+$	
	TSB	NB	TSB	NB
wt[c]	6	10	594	2,946
1-3	1	1	1	1
1-4	ND[d]	ND	11	144
1-5	1	3	17	184
2-11	3	6	250	1,968
2-12	1	2	5	33
20-1	1	1	1	1
20-4	1	1	1	1
40-1	1	1	1	15

[a] β-Galactosidase was assayed as described by Miller (22) after growth to mid-logarithmic phase in TSB or NB media.
[b] Each strain contained the *ompF-lac* fusion with one of the *ompFp* alleles and either the *ompR*$^+$ or the *ompR101* mutation as indicated.
[c] wt, Wild type.
[d] ND, Not determined.

expression of *ompF* (> 1 U of β-galactosidase activity) still appear responsive to OmpR as judged by the lower β-galactosidase levels in the *ompR101* background. Moreover, these strains continue to exhibit the medium-dependent fluctuation characteristic of the wild-type strain.

Sequence alterations determined by the promoter mutations. To shed light on the mechanism by which the mutations decrease *ompF* expression, the DNA sequence was determined and compared with the wild-type *ompF* sequence. The appropriate DNA fragment from each of the mutant phages was cloned and the DNA sequence was determined as described above. The results of this analysis are presented in Fig. 2. Of 12 independent spontaneous mutants and one isolated after passage of the phage through the *mutD* strain LE30, 10 different lesions were found. Eight of the mutations cluster in a region approximately 120 to 155 bp upstream of the translation start site. These mutations probably define the *ompF* promoter. Two mutations, not shown, are described in more detail elsewhere (34) and are alluded to below.

Determination of the *ompF* mRNA start site. To provide further evidence that the mutations described above define the *ompF* promoter, we determined the 5′ end of the *ompF* message. This was done by using a primer extension technique previously described (5). We reasoned that the message should start just downstream of the mutation cluster and therefore selected a DNA probe that would hybridize to a portion of the message distal to this region. To isolate this probe, we digested pORF1 DNA (41) with *Bam*HI, labeled the 5′ ends with ^{32}P by polynucleotide kinase, and then cleaved the DNA with *Mnl*I, which recognizes a site upstream of the *Bam*HI site located within the message. This probe was hybridized with total mRNA isolated from strains MC4100 (*ompR*$^+$), MH760 (*ompR472*), or MH1160 (*ompR101*) and subsequently incubated with reverse transcriptase in the presence of unlabeled nucleoside triphosphates. The resultant extended primer was run alongside a chemical cleavage reaction on a denaturing acrylamide DNA sequencing gel (Fig. 3). This gel shows that the *ompF* message starts at an A within a *Pst*I site just downstream of the promoter mutations. No message was detected in the strain carrying the *ompR101* allele. It is important to note that the message begins at the same base position in both *ompR*$^+$ and *ompR472* strains.

Elements necessary for transcriptional regulation of *ompF* by OmpR and EnvZ are located upstream of the promoter. To further demonstrate that the *ompF* promoter defined by these mutations shows correct transcriptional regulation by the products of the *ompB* locus, we used in vitro methods to construct an *ompF-lacZ* operon fusion that creates a fusion joint at base +2 of the *ompF* mRNA. This construction is outlined in Fig. 4. The β-galactosidase activities of *ompR*$^+$, *ompR101*, and *envZ22* strains harboring a plasmid (pRT240), as well as the parental vector pRT100, were assayed after growth in L broth. The pRT240 plasmid yields 3,255 U in strain MC4100 (*ompR*$^+$), 675 U in strain MH1160 (*ompR101*), and 1,229 U in strain SG477 (*envZ22*), showing clearly that OmpR activates this fusion. Because of the high plasmid copy number, these values are abnormally high. Indeed, the parental plasmid (pRT100), which has no promoter at all, yields 533 U in strain MC4100 under the same conditions. Repeated attempts to measure osmotic fluctuation were inconclusive. This may be due simply to the high gene dosage of the fusion plasmid. Alternatively, the failure to observe osmotic fluctuation may be due to the fact that this construct lacks important regulatory sequences located near the 5′ end of the mRNA (24, 32). In any event, these results indicate that the *cis*-acting elements required for transcriptional activation of *ompF* by OmpR are not located in the 5′ untranslated region of the *ompF* gene.

DISCUSSION

We devised a method to enrich for promoter mutations from among the plethora of mutations that could prevent *ompF* expression or functionally alter the OmpF pore. This selection takes advantage of the overproduction lethality commonly observed with fusions between genes encoding exported proteins and the *lacZ* gene encoding β-galactosidase. Sodergren et al. (34) showed that λ phage carrying one such fusion, (*ompF′-lacZ*)hyb*16-21*, exhibit a decreased (10^{-5}) efficiency of plating on strains in which *ompF* expression is increased as a result of the *ompR472* regulatory mutation. Potential promoter mutations were selected by picking phages that plaque on this strain and that exhibit decreased β-galactosidase activity. By screening these phages for decreased *lacY* expression and mapping the lesion to *ompF*, we were able to identify at high frequency a number of promoter mutations. The *ompF-lacZ* fusion also allowed us to measure conveniently the effect of each mutation on *ompF* expression by assaying β-galactosidase activity.

Mutations that decrease *ompF* expression may prevent or weaken recognition by either the positive regulatory factor(s) or RNA polymerase. We suspect that the mutations described here hinder functional recognition by RNA polymerase. Two lines of evidence support this claim. First, in all cases in which meaningful results can be obtained, the mutationally altered promoters are still activated by OmpR. Second, seven of the eight mutations can be aligned to show correlation between decreased expression and deviation from the common promoter consensus sequence (13). The mutations closest to the message start site cluster into a possible −10 region. Since the −10 consensus region is TATAAT (13, 29), the *ompF* −10 region is most probably AAAGAT. Even though only three of these bases match the consensus, four of the mutations, 40-1, 1-3, 20-4, and 20-1, alter the two most conserved consensus bases. The mutation 1-4, which reduces *ompF* expression to a lesser degree,

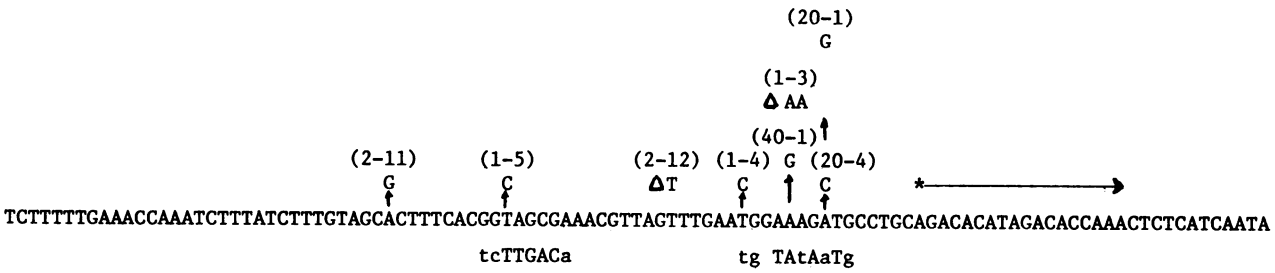

FIG. 2. DNA sequence of the *ompF* promoter region and the NH_2-terminal portion of the structural gene. Each mutation that causes decreased promoter activity is indicated in parentheses along with the resultant base change. The most probable locations of promoter determinants deduced from these mutations are indicated by the consensus −35 and −10 sequences written in below the promoter sequence. The position of the *ompF* mRNA start site as determined by primer extension is indicated by an asterisk.

alters a base that appears to be weakly conserved in promoters previously studied (13).

As with most positively regulated promoters, the −35 region of the *ompF* promoter does not show extensive homology with the −35 consensus region. Considering the mutations 2-12 and 1-5, we have designated the sequence TAGCGA as the −35 region, compared with the TTGACA consensus sequence (13, 29). Again, the logic that the promoter mutations should cause deviations from consensus was used. Here the T of TAGCGA could conform to either the first or second T of the consensus sequence. If it was the first T of the consensus sequence, the spacing between the −10 and −35 regions would be 17 bp. The 2-12 mutation would thus change the spacing from 17 to 16 bp. Assuming 17 bp to be optimal (13), this assignment is probably correct, since the 2-12 mutation should otherwise have caused a promoter-up effect by making the spacing more optimal. Consequently, we assume that the 1-5 mutation alters the first T of the consensus sequence. Recently, Inokuchi et al. (14) have used deletion mutagenesis to define sequences required for *ompF* expression. Their assignment of the −10 and −35 regions is in agreement with ours.

The final mutation, 2-11, does not alter any of the base pairs so far identified genetically and ascribed to a promoter consensus sequence. In this case, the A normally found at position −45 is substituted for by a G. Although the effect of this mutation is not large, we believe it is significant since the same lesion occurred in two independent mutant isolates. Indeed, Hawley and McClure (13) have noticed that an A is found at this position at a frequency commensurate with several other weakly conserved promoter sequences. Therefore, the mutation 2-11 may provide the first genetic evidence that an A at position −45 is an important part of the promoter.

Two mutations do not lie in the putative promoter region and represent other classes of mutations that are characterized in the accompanying paper (34). The 1-9 mutation is an insertion of an A·T base pair that creates a frameshift in the signal sequence coding portion of *ompF*. A second mutation, 20-2, changes the codon for the second amino acid of the mature OmpF protein to an ochre nonsense codon. The mutant phage that carries this allele is unable to form plaques on ochre suppressing strains that contain the *ompR472* allele. Thus, even though these two mutations do not alter the *ompF* promoter, they confer a phenotype that is similar to a strong promoter-down mutation.

Our assignment of the *ompF* promoter, as well as its transcriptional start site, shows that *ompF* contains a large 5′ untranslated region of mRNA. This result raises the possibility that OmpR activates *ompF* expression by functioning as an antiterminator. Using an *ompF-lacZ* fusion constructed in vitro (Fig. 4), we have shown that sequences specific for OmpR- and EnvZ-activated transcription must be located within a region starting at +2 of the mRNA transcript and extending approximately 200 bp upstream of the −35 region. These results are consistent with those obtained with many other positively regulated systems and

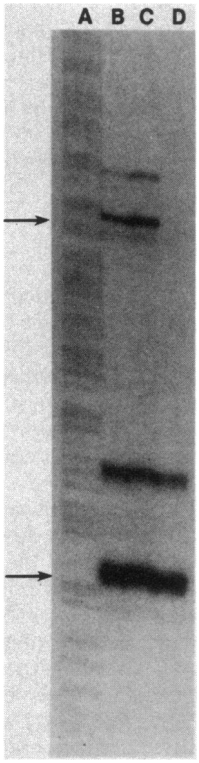

FIG. 3. Location of the *ompF* mRNA start site as determined by primer extension. Lanes: B, MC4100 (*ompR*$^+$); C, MH760 (*ompR472*); D, MH1160 (*ompR101*). The lowest band (indicated with an arrow) is the primer fragment from pORF1 labeled at the *Bam*HI site. The second band from the top (also indicated with an arrow) is the extended primer after hybridization of the primer with total cellular mRNA and incubation with avian myeloblastosis virus reverse transcriptase. The second band from the bottom represents the same primer with an added linker containing the *Bam*HI site. The top band is this larger primer extended to the same mRNA start point. The DNA sequence shown in lane A is a Maxam-Gilbert (G+A) reaction which gives C+T of the coding strand shown in the figure.

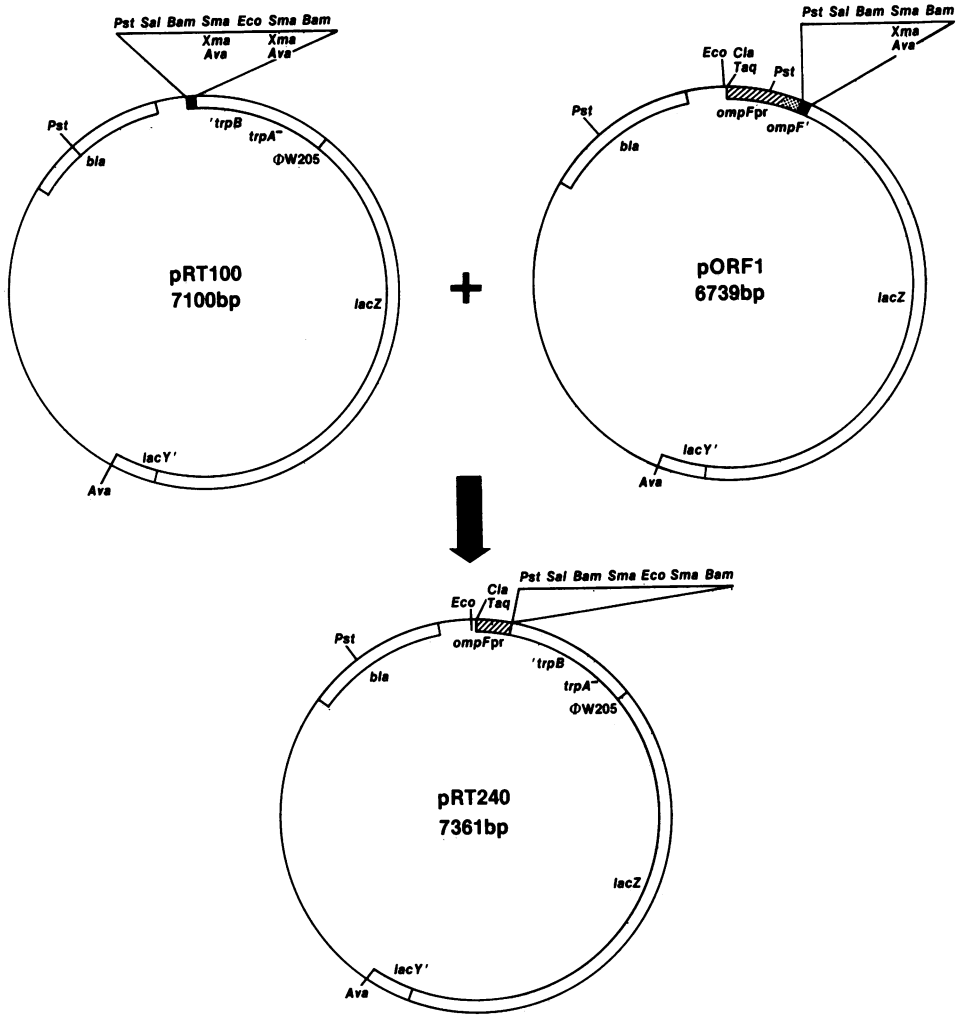

FIG. 4. Construction of the *ompF-lacZ* operon fusion plasmid pRT240. A 240-bp DNA fragment encompassing the *ompF* promoter region was previously subcloned during the construction of the plasmid pORF1 (41). This plasmid was used to isolate a DNA fragment that spans the region from the *PstI* site within the β-lactamase gene of pORF1, through 240 bp of *ompF* DNA, to the *PstI* site at the beginning of the *ompF* message. The operon fusion vector pRT100 is a derivative of plasmid pMLB1010 (6) with a different multiple cloning site. pRT100 was digested with *PstI*, and the large fragment was ligated with the small, *PstI*-generated fragment from pORFI, reconstituting the β-lactamase gene. Strain MH1160 was transformed with the ligation mix, and Ap^r colonies were selected in the presence of XG. Blue colonies were shown to contain the plasmid pRT240. This plasmid contains 240 bases of the *ompF* promoter region DNA, beginning at base +2 of the *ompF* message and including the −10 and −35 sequences plus 203 bp upstream.

they rule out the possibility that OmpR and EnvZ function as antitermination factors. They are also consistent with the results of Inokuchi et al. (14), who showed that the region necessary for OmpR activation falls between base positions +17 and −90 of the *ompF* gene.

ACKNOWLEDGMENTS

We thank G. Weinstock and M. Berman for helpful discussions and C. Benyajati for help with the primer extension method. We also thank Vickie Koogle, Sylvia Lucas, Karen Toms, and Julie Ratliff for their help in the preparation of this manuscript.

This research was sponsored by the National Cancer Institute under contract no. N01-CO-23909 with Litton Bionetics. S.G. was supported by a postgraduate scholarship from the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

1. **Aiba, H., S. Adhya, and B. de Crombrugghe.** 1981. Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. J. Biol. Chem. **256**:11905–11910.
2. **Bassford, P. J., Jr., D. L. Diedrich, C. A. Schnaitman, and P. Reeves.** 1977. Outer membrane proteins of *Escherichia coli*. VI. Protein alteration in bacteriophage-resistant mutants. J. Bacteriol. **131**:608–622.
3. **Benson, S. A.** 1984. A rapid procedure of isolation of DNA fragments from agarose gels. Biotechniques **2**:66–67.
4. **Benson, S. A., and R. K. Taylor.** 1984. A rapid small-scale procedure for isolation of phage λ DNA. Biotechniques **2**:126–127.
5. **Benyajati, C., and J. F. Dray.** 1984. Cloned *Drosophilia* alcohol dehydrogenase genes are correctly expressed after transfection into *Drosophilia* cells in culture. Proc. Natl. Acad. Sci. U.S.A. **81**:1701–1705.
6. **Berman, M. L., D. Jackson, A. Fowler, I. Zabin, L. Christensen, N. Fiil, and M. Hall.** 1984. Gene fusion techniques: cloning vectors for manipulating *lacZ* gene fusions. Gene Anal. Tech. **1**:43–51.
7. **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.

8. **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.
8a. **Garrett, S., R. K. Taylor, T. J. Silhavy, and M. L. Berman.** 1985. Isolation and characterization of Δ*ompB* strains of *Escherichia coli* by a general method based on gene fusions. J. Bacteriol. **162**:840–844.
9. **Hall, M. N., and T. J. Silhavy.** 1979. Transcriptional regulation of *Escherichia coli* K-12 major outer membrane protein 1b. J. Bacteriol. **140**:342–350.
10. **Hall, M. N., and T. J. Silhavy.** 1981. The *ompB* locus and the regulation of the major outer membrane proteins of *Escherichia coli* K-12. J. Mol. Biol. **146**:23–43.
11. **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.
12. **Hall, M. N., and T. J. Silhavy.** 1981. Genetic analysis of the major outer membrane proteins of *Escherichia coli*. Annu. Rev. Genet. **15**:91–142.
13. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11**:2237–2255.
14. **Inokuchi, K., J. Furukawa, K. Nakamura, and S. Mizushima.** 1984. Characterization by deletion mutagenesis *in vitro* of the promoter region of *ompF*, a positively regulated gene of *Escherichia coli*. J. Mol. Biol. **178**:653–668.
15. **Inokuchi, K., N. Mutoh, S. Matsuyama, and S. Mizushima.** 1982. Primary structure of the *ompF* gene that codes for a major outer membrane protein of *Escherichia coli* K12. Nucleic Acids Res. **10**:6957–6968.
16. **Kawaji, H., T. Mizuno, and S. Mizushima.** 1979. Influence of molecular size and osmolarity of sugars and dextrans on the synthesis of outer membrane proteins O-8 and O-9 of *Escherichia coli* K-12. J. Bacteriol. **140**:843–847.
17. **Lugtenberg, B., R. Peters, H. Bernheimer, and W. Berendsen.** 1976. Influence of cultural conditions and mutations on the composition of the outer membrane proteins of *Escherichia coli*. Mol. Gen. Genet. **147**:251–262.
18. **Lundrigan, M. D., and C. F. Earhart.** 1984. Gene *envY* of *Escherichia coli* K-12 affects thermoregulation of major porin expression. J. Bacteriol. **157**:262–268.
19. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
20. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–321.
21. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digit restriction fragments. Gene **19**:269–276.
22. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Mizuno, T., M.-Y. Chou, and M. Inouye.** 1983. A comparative study of the genes for three porins of the *Escherichia coli* outer membrane: DNA sequence of the osmoregulated *ompC* gene. J. Biol. Chem. **258**:6932–6940.
24. **Mizuno, T., M.-Y. Chou, and M. Inouye.** 1984. A unique mechanism regulating gene expression: translational inhibition by a complementary RNA transcript (mic RNA). Proc. Natl. Acad. Sci. U.S.A. **81**:1966–1970.
25. **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.
26. **Morona, R., and P. Reeves.** 1982. The *tolC* locus of *Escherichia coli* affects the expression of three major outer membrane proteins. J. Bacteriol. **150**:1016–1023.
27. **Nikaido, H., and E. Y. Rosenberg.** 1983. Porin channels in *Escherichia coli*: studies with liposomes reconstituted from purified proteins. J. Bacteriol. **153**:241–252.
28. **Nikaido, H., E. Y. Rosenberg, and J. Foulds.** 1983. Porin channels in *Escherichia coli*: studies with β-lactams in intact cells. J. Bacteriol. **153**:232–240.
29. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–353.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467.
31. **Scaife, J., and J. R. Beckwith.** 1966. Mutational alteration of the maximal level of *lac* operon expression. Cold Spring Harbor Symp. Quant. Biol. **31**:403–408.
32. **Schnaitman, C. A., and G. A. McDonald.** 1984. Regulation of outer membrane protein synthesis in *Escherichia coli* K-12: deletion of *ompC* affects expression of the OmpF protein. J. Bacteriol. **159**:555–563.
33. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Sodergren, E. J., J. Davidson, R. K. Taylor, and T. J. Silhavy.** 1985. A selection for mutants altered in the expression and export of the outer membrane porin OmpF. J. Bacteriol. **162**:1047–1053.
35. **Taylor, R. K., M. N. Hall, L. Enquist, and T. J. Silhavy.** 1981. Identification of OmpR: a positive regulatory protein controlling expression of the major outer membrane matrix porin proteins of *Escherichia coli* K-12. J. Bacteriol. **147**:255–258.
36. **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.
37. **van Alphen, W., and B. Lugtenberg.** 1977. Influence of osmolarity of the growth medium on the outer membrane protein pattern of *Escherichia coli*. J. Bacteriol. **131**:623–630.
38. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
39. **Wandersman, C., F. Moreno, and M. Schwartz.** 1980. Pleiotropic mutations rendering *Escherichia coli* K-12 resistant to bacteriophage TP1. J. Bacteriol. **143**:1374–1383.
40. **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.
41. **Weinstock, G. M., C. ap Rhys, M. L. Berman, B. Hampar, D. Jackson, T. J. Silhavy, J. Weisemann, and M. Zweig.** 1983. Open reading frame expression vectors: a general method for antigen production in *Escherichia coli* using protein fusions to β-galactosidase. Proc. Natl. Acad. Sci. U.S.A. **80**:4432–4436.
42. **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**:13685–13691.