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Transcriptional Regulation of *Escherichia coli* K-12 Major Outer Membrane Protein 1b

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Eleven independent insertion mutations were isolated that prevented expression of major outer membrane protein 1b. Seven of the mutations were Mucts insertions located at *ompB*. These *ompB*::Mucts strains fell into two phenotypic classes with regard to expression of proteins 1a and 1b. The remaining four mutants were comprised of one Tn*5* and three Mucts insertions mapping at *par*. The Mucts insertions at *par* were used to construct fusions of the *lac* operon to the *par* promoter. Expression of β-galactosidase in these fusion strains reflected known regulatory properties of protein 1b. When an *ompB* allele was introduced into the *par-lac* fusion strains, β-galactosidase activity was reduced 14- to 31-fold. Transcriptional regulation of the *par* gene and the existence of two functions at *ompB* are discussed. The results suggest that *par* is the structural gene for protein 1b and that an *ompB* gene product is a diffusible, positive regulatory element controlling expression of *par*.

Escherichia coli protein 1 is a major outer membrane protein (4, 12, 13, 16, 18, 27). In K-12 strains, protein 1 can be electrophoretically separated into two components, 1a and 1b (14, 26). Functionally, these proteins aggregate to form aqueous channels or pores in the outer membrane, allowing diffusion of certain metabolites (2, 20, 22, 31). A second role is that of receptor for various phages (1, 6, 11). In addition, mutational loss of protein 1a results in tolerance to certain colicins (7, 10). Mutations in at least three widely spaced chromosomal loci are known to affect the production of proteins 1a and 1b. The loci *tolF*, mapping at 21 min, and *par*, mapping at 48 min, are defined by mutations that result in the loss of 1a and 1b, respectively (1, 10, 32). A mutation at another locus, *ompB*, mapping at 74 min, results in the loss of expression of 1a or 1b, or both (25, 33). Conflicting models have been proposed to account for the existence of three genetic loci but only two known roles. Bassford et al. (1) have reported that 1a and 1b have similar cyanogen bromide fragments and thereby suggested that *ompB* is the structural gene for a protein precursor that is then modified to produce two species. Other investigators (15, 33) have presented biochemical evidence indicating that the two proteins are different. They suggested that *tolF* and *par* are structural genes for the respective proteins. Genetic evidence also supports the contention that *tolF* is the structural gene for protein 1a (25a) and that *par* is the structural gene for protein 1b (30).

The synthesis of proteins 1a and 1b is regulated. The relative and absolute amounts of 1a and 1b fluctuate depending on the growth medium (1, 19, 29). This may be so that the outer membrane can constantly be adjusted to adapt to the external environment and the available nutrients. The two protein species are also principles in lysogenic conversion by phage PA-2. In a PA-2 lysogen, proteins 1a and 1b are supplanted by a new major outer membrane protein, designated protein 2 (8, 28).

The purpose of this work was to investigate the discrepancy in the number of known genes and roles and to elucidate the regulatory phenomena listed above. We isolated strains in which DNA sequences were inserted in the genes involved in the expression of protein 1b. This allowed us to study mutants in which the functions of these genes had been abolished. The DNA elements inserted were the Tn*5* translocon, determining kanamycin resistance, and the genome of bacteriophage Mu. We used certain of the Mu insertions to construct fusions of the lactose operon to the *par* gene. These fusions provided a tool for studying transcriptional regulation at *par*.

MATERIALS AND METHODS

Media and chemicals. Nutrient broth (NB) and

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tryptic soy broth (TSB) were purchased from Difco Laboratories. TY broth was 1% tryptone and 0.5% yeast extract at pH 7.2. All other media were prepared as described previously (21). 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (XG) was purchased from Bachem Inc. Nalidixic acid and kanamycin were used in concentrations of 20 and 40 μg/ml, respectively. Ultrapure urea was purchased from Schwarz/Mann.

Bacteria and bacteriophages. Bacterial and phage strains are listed in Table 1.

Assay of β-galactosidase activity. β-Galactosidase was assayed as described by Miller (21).

Purification of colicin L. Colicin L was prepared as described by Foulds (9).

Polyacrylamide gel electrophoresis. Outer membrane samples were prepared by Triton X-100 solubilization of the envelope fraction as previously described (8). Electrophoresis was performed, as described previously (17), with 8 M urea in the separation gel (23). The acrylamide concentration was 12%.

Isolation and mapping of mutants resistant to bacteriophage hy-2. Drops of a Mucts lysate were deposited on a lawn of MH70 and incubated overnight at 30°C. Lysogens were streaked from the plaques onto tryptone-yeast extract agar that had been previously spread with 5×10^8 hy-2 phage. hy-2 is a virulent phage that uses protein 1b as its receptor. Survivors were purified, and resistance to hy-2 was verified by cross-streaking against phage hy-2. Ten independent hy-2^r Mucts lysogens were isolated. The Mucts insertions that resulted in hy-2 resistance were mapped to either one of two loci, *par* or *ompB*. The *par* Mucts insertions were mapped by transduction of the candidates to resistance to nalidixic acid (Nal^r) with phage P1 grown on a *nalA par*$^+$ donor. Transductants were checked for simultaneous loss of the Mucts prophage

TABLE 1. *Bacteria and phages used*

Bacterium/phage	Genotype/bacterial genes carried	Origin/reference
Bacterium		
MC4100	F$^-$ Δ*lacU169 araD139 rpsL relA thiA*	5
MH70	MC4100 *malQ7*	This study
MH72	MH70 *nalA*	This study
MH110	MH70 *par*::Mucts10	This study
MH111	MH70 *par*::Mucts11	This study
MH118	MH70 *par*::Mucts18	This study
MH1106	MH70 *ompB*::Mucts6	This study
MH702	MH70 *ompB*::Mucts2	This study
MH704	MH70 *ompB*::Mucts4	This study
MH705	MH70 *ompB*::Mucts5	This study
MH710	MH70 *ompB*::Mucts10	This study
MH719	MH70 *ompB*::Mucts19	This study
MH720	MH70 *ompB*::Mucts20	This study
MH150	MC4100 *par*::Tn*5* 12	This study
MH221	MH110 λp1(209) ΔMu Φ(*par-lacZ*$^+$)10-21	This study
MH225	MH110 λp1(209) ΔMu Φ(*par-lacZ*$^+$)10-25	This study
MH212	MH111 λp1(209) ΔMu Φ(*par-lacZ*$^+$)11-12	This study
MH215	MH111 λp1(209) ΔMu Φ(*par-lacZ*$^+$)11-15	This study
MH221.1	MH221 *malQ7*	This study
MH225.1	MH225 *malQ7*	This study
MH212.1	MH212 *malQ7*	This study
MH215.1	MH215 *malQ7*	This study
MH762	MC4100 *ompB108*	This study[a]
MH764	MC4100 *ompB4*	This study
MH1160	MC4100 *ompB101*	This study[b]
MH1169	MC4100 *ompB9*	This study
PB112778		Philip Bassford[c]
Phage		
Mucts61		Laboratory stock
hy-2		1
TuIa		6
λp1(209)	*trp'BA'*-ΔW209-*lac'OZYA'*	5
λ*b*221*c*I857*O*am29 *P*am80rex::Tn*5*		Michael Lichten
λp10-21	Φ(*par-lacZ*$^+$)10-21 *lacY lacA'*	This study
λp10-25	Φ(*par-lacZ*$^+$)10-25 *lacY lacA'*	This study
λp11-12	Φ(*par-lacZ*$^+$)11-12 *lacY lacA'*	This study
λp11-15	Φ(*par-lacZ*$^+$)11-15 *lacY lacA'*	This study
λ*c*I*h*80Δ(int)9		Laboratory stock

[a] The *ompB* allele in this strain is derived from strain PB108 (1).
[b] The *ompB* allele in this strain is derived from strain P530 (25).
[c] This is a colicin L-producing *Serratia marcescens* strain.

and restoration of an intact *par* gene. An approximately 40% cotransduction frequency was observed for *nalA* and *par*. Restoration of *par* was screened by cross-streaking the Nal^r transductants against hy-2. The Nal^r transductants were screened for loss of the Mucts prophage by stabbing into a lawn of a strain sensitive to Mucts and checking for release of phage at the nonpermissive temperature. The insertion mutants whose Nal^r transductants exhibited a simultaneous loss of the Mucts prophage and restoration of hy-2 sensitivity were kept as the desired *par*::Mucts strains. The *ompB* Mucts insertions were mapped by a similar method, using *malQ* as the cotransducible, selected trait.

A Tn*5* insertion at *par* was isolated. The translocation of Tn*5* from λ*b*221c1857*O*am29*P*am80*rex*::Tn*5* onto the bacterial chromosome was performed as described previously (3). After the translocation, hy-2 was preadsorbed to the pooled Kan^r bacteria at a multiplicity of 3. The adsorbed mixture was spread on kanamycin-containing tryptone-yeast extract agar that had been previously spread with 5×10^8 hy-2 phage. Several Kan^r hy-2^r candidates were purified, of which approximately 25% were *par* Tn*5* insertions. The site of the translocon insertion was mapped as described above for mapping Mucts insertions. In this case, Nal^r transductants were screened for simultaneous loss of kanamycin resistance and restoration of hy-2 sensitivity.

Spontaneous *ompB* mutant MH764 was isolated from strain MH70. The hy-2 resistance was mapped to *ompB* by cotransduction with *malQ*. Spontaneous *ompB* mutant MH1169 was selected by resistance to both hy-2 and colicin L. In this case, a mixture of hy-2 phage and colicin L was deposited on a lawn of MH70. The simultaneous colicin L tolerance and hy-2 resistance was mapped to *ompB* as described above.

Construction of *par-lac* operon fusions. Operon fusions were constructed as described previously (5), using the *par* Mucts insertions discussed above. The techniques involved in this construction are summarized in Fig. 1. The deletion event that generates the *par-lac* fusions does not result in the formation of a hybrid gene. Rather, the deletion event simply places the *lac* genes under the regulatory control of the *par* promoter. Such fusions are referred to as operon fusions.

Isolation of *par-lac* fusion transducing phages. Transducing phages carrying the *par-lac* fusions were isolated by induction of the lambda phages that reside adjacent to the fusions as a result of the fusion technique (5) (Fig. 1).

Genetic mapping of fusion joints in *par*. Presumptive *par-lac* fusions were shown to contain a fusion joint within *par* by a genetic cross with a *par* Tn*5* insertion (see Fig. 2). Similar techniques have been described previously for the genetic characterization of other fusion strains (3a; M. Débarbouillé and M. Schwartz, J. Mol. Biol., in press). Strain MH150 carrying a *par* Tn*5* insertion was infected at a multiplicity of infection of 0.1 with the fusion transducing phages. A low multiplicity of infection was used to avoid the complications of double lysogens. The phages were adsorbed by incubation at room temperature for 20 min. The infection mixtures were then

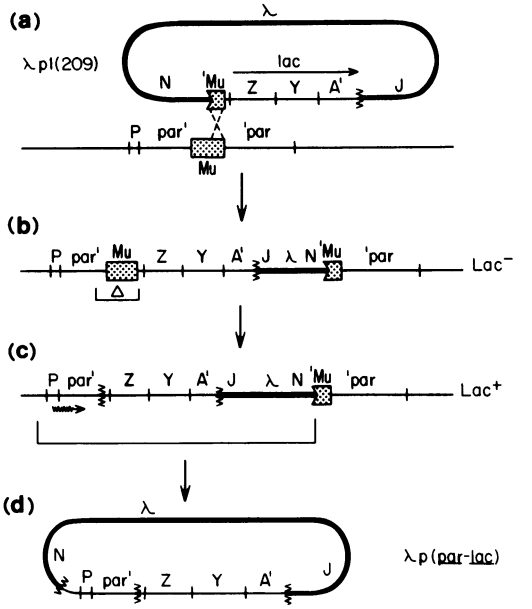

FIG. 1. *Scheme for construction of par-lac fusions and isolation of fusion transducing phages. (a) A Mucts phage is inserted in the par gene. The phage can be inserted in either orientation; however, only those which have been inserted with the immunity end promoter distal will yield fusions with the λp1(209) phage. The par::Mucts strain is lysogenized with λp1(209). The λp1(209) phage can integrate only by homologous recombination of the shared Mu sequences since λp1(209) is deleted for its attachment site and the recipient is deleted for the lac genes. (b) The λp1(209) lysogen is Lac⁻ as a result of the polar block imposed by the Mucts prophage. (c) Since the Mucts prophage is temperature inducible, selection for Lac⁺ survivors at 42°C often yields fusions of the lac operon to the par promoter. (d) A fusion transducing phage can be easily isolated by UV induction of the λ phage residing adjacent to the fusion. Transducing phages carrying the fusion are detected by screening for blue plaques on 5-bromo-4-chloro-3-indolyl-β-D-galactoside indicator medium.*

diluted 10-fold with LB medium and incubated at 37°C for 20 min with aeration. Lysogens were selected by being spread on tryptone-yeast extract agar that had been previously spread with 2×10^9 λc1*h*80Δ(*int*) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside. The galactoside facilitated screening of Lac$^+$ and Lac$^-$ lysogens. The Lac phenotype is determined by the region where the integrative recombinational event of the transducing phage occurs (see Fig. 2). Integration of a λ*par-lac* phage promoter distal to the Tn*5* insertion results in a lysogen with a *Lac*$^-$ phenotype due to the polar effects of the Tn*5* insertion. This lysogen also exhibits a Par$^+$ phenotype, since a wild-type *par* gene is regenerated by the integrative recombinational event. Such a Lac$^-$ Par$^+$ lysogen can be obtained only if the fusion carried by the transducing phage is a *par-lac* fusion. This provides genetic evi-

dence confirming that the fusion joint is in the *par* gene.

RESULTS

Analysis of mutants resistant to bacteriophage hy-2. Bacteriophage hy-2 uses protein 1b as a receptor. We obtained 11 independent insertion mutants missing protein 1b by selecting for resistance to phage hy-2 (see above). The insertion sites were determined to be linked either to the *ompB* locus, by cotransductional linkage to *malQ*, or to the *par* locus by linkage to *nalA*. The 11 mutants included 10 Mucts insertions and one Tn*5* insertion.

Purified outer membranes of the hy-2^r insertion mutants were run on a urea-sodium dodecyl sulfate-polyacrylamide gel (see Fig. 3). Two types of patterns emerged. Strain MH1106, an *ompB*::Mucts strain, was lacking both 1a and 1b, consistent with a previously reported OmpB phenotype. As expected, this strain was also resistant to phage TuIa whose receptor is protein 1a. The remaining 10 insertion mutants, consisting of six *ompB*::Mucts strains, three *par*::Mucts strains, and one *par*::Tn*5* strain, had protein 1a but were missing protein 1b.

The 1a$^+$ 1b$^-$ *ompB*::Mucts strains were further studied to rule out the possibility that the apparent 1a protein was a new outer membrane protein (23). These mutants were cross-streaked against phage TuIa. All were TuIa sensitive, providing evidence that the protein possessed the same receptor specificity as protein 1a and was therefore, most likely, not a new outer membrane protein. The 1a$^+$ 1b$^-$ *ompB* strains were then transduced to *malQ*$^+$ with phage P1 grown on a 1a$^-$ 1b$^-$ *ompB* mutant. Mal$^+$ transductants were screened for resistance to TuIa. TuIa-resistant transductants were obtained at the frequency expected from the approximately 80% cotransduction between *malQ* and *ompB* (25). Such transductants consistently lost the Mucts prophage and no longer exhibited protein 1a when analyzed by polyacrylamide gel electrophoresis, again indicating that this band did actually correspond to protein 1a.

Based on these results, the hy-2^r insertion mutants were categorized into three classes. Class I, the least frequently occurring, with only one isolate, includes the *ompB*::Mucts strain lacking both 1a and 1b. Class II consists of the 1a$^+$ 1b$^-$ *ompB* Mucts insertions and is represented by six independent isolates. Class III includes the three *par*::Mucts isolates and one *par*::Tn*5* isolate.

Differential phage resistance. The *par* and *ompB* mutants exhibited two easily differentiated types of resistance to phage hy-2. Upon cross-streaking against hy-2, both classes of *ompB* Mucts insertions gave a slight clearing zone. When hy-2 was spotted on the *ompB*:: Mucts strains, the virulent phage produced a faint plaque. Conversely, cross-streaking hy-2 against the *par* insertions gave no clearing, and spotting hy-2 on the class III insertions resulted in no visible plaque. In short, an insertion mutation at *par* confers complete resistance to hy-2, whereas a similar insertion at *ompB* results in incomplete resistance. This suggests that *par*, and not *ompB*, is the structural gene for protein 1b.

Construction of *par-lac* fusions. To study regulation of the *par* gene, we isolated a series of strains in which the *lac* genes are fused to the *par* promoter. The techniques employed for this construction are summarized in Fig. 1. Since these techniques are described in detail elsewhere (5), we will mention only the problems encountered specifically in the construction of *par-lac* fusions.

The first step is the isolation of a Mucts insertion in the *par* gene (see above). The phage can insert into the gene in either one of the two possible orientations; however, only the *par* Mucts insertions that place the immunity end of the prophage distal to the *par* promoter will yield fusions with the λp1(209) phage. Since the position of the *par* promoter relative to the *par* gene is unknown, it was impossible to identify *par* Mucts insertions that were in the correct orientation. To overcome this problem, three independent *par*::Mucts insertion strains were employed. We assumed that at least one of these strains would contain the Mu prophage in the correct orientation.

The three *par*::Mucts strains were lysogenized with λp1(209), and Lac$^+$ thermoresistant clones were selected. All three strains yielded Lac$^+$ clones at approximately equal frequencies. The majority of the Lac$^+$ thermoresistant clones derived from a strain containing a Mu prophage in the proper orientation should be *par-lac* fusions. Conversely, the Lac$^+$ thermoresistant clones derived from a strain containing a prophage in the wrong orientation are, in all probability, fusions to a promoter other than the *par* promoter. The simplest way to identify the *par-lac* fusions was to demonstrate that the *lac* genes are regulated in the same manner as the wild-type *par* gene product. Results described above suggested that protein 1b is the product of the *par* gene. Therefore, we reasoned that the β-galactosidase activity of the *par-lac* fusions might be regulated in a manner analogous to that observed for protein 1b.

The amount of protein 1b that is found in a

bacterium, as seen by gel electrophoresis, is known to fluctuate depending on the medium (1, 19, 29). Two media that elicit two extremes in the production of protein 1b are TSB and NB (1). Strains grown in TSB produce an excess of protein 1b relative to the amount of protein 1b in the same strain cultured in NB (see Fig. 3). Fusion candidates derived from each of the three Mucts insertions were cultured in TSB and NB and assayed for β-galactosidase activity. The majority of the fusions derived from insertion strains MH110 and MH111 grown in TSB exhibited a three- to fourfold increase in β-galactosidase activity over the same fusion grown in NB (see Table 2). Fusions derived from Mucts insertion strain MH118 did not give this induction effect. These results suggested that the Lac$^+$ thermoresistant clones derived from strains MH110 and MH111 were *par-lac* fusions. The Mu prophage in strain MH118 is presumably in the incorrect orientation.

Genetic proof for fusion of *lac* to *par*. The technique used to verify that the *lac* genes are fused to the *par* promoter is depicted in Fig. 2. If a mutation that blocks transcription of the wild-type *par* gene also blocks transcription of the *lac* operon, it can be concluded that the *lac* genes are fused to the *par* promoter.

Transducing phages carrying the presumed *par-lac* fusions were isolated (Fig. 1). These phages were then lysogenized into a strain carrying a Tn*5* insertion (a polar mutation) in the *par* gene. Since the recipient strain is deleted for the *lac* genes and the transducing phages are deleted for the attachment site, lysogenization occurs primarily via a recombinational event at the homologous *par* DNA (Fig. 2). If this recombination occurs at a site promoter distal to the Tn*5* insertion, expression of the *lac* genes is prevented owing to the polar nature of the Tn*5* insertion. In addition, a wild-type *par* gene is regenerated. This experiment was performed with two presumptive *par-lac* fusions derived from strain MH110 and two from strain MH111. The transducing phages carrying fusions 11-12 and 11-15 yielded Lac$^-$ lysogens at a frequency of 5%. Transducing phages carrying fusions 10-25 and 10-21 yielded Lac$^-$ lysogens at frequencies of 10 and 30%, respectively. In all cases, approximately 200 lysogens were screened for the Lac phenotype. Several of the Lac$^+$ and Lac$^-$ lysogens were subsequently screened for *par*$^+$, i.e., hy-2 sensitivity. Of the Lac$^-$ and Lac$^+$ lysogens, 90% were hy-2 sensitive and resistant, respectively. The remaining 10% that did not exhibit the predicted Par phenotype can be explained by a homogenotization event. This is supported by the observation that the *lac*$^+$ *par*$^+$ lysogens were no longer Kan^r, demonstrating that the Tn*5* insertion had been lost. These results indicate that all four fusions examined have a fusion joint within *par* mapping promoter distal to the Tn*5* insertion of strain MH150. Accordingly, expression of the *lac* genes in these

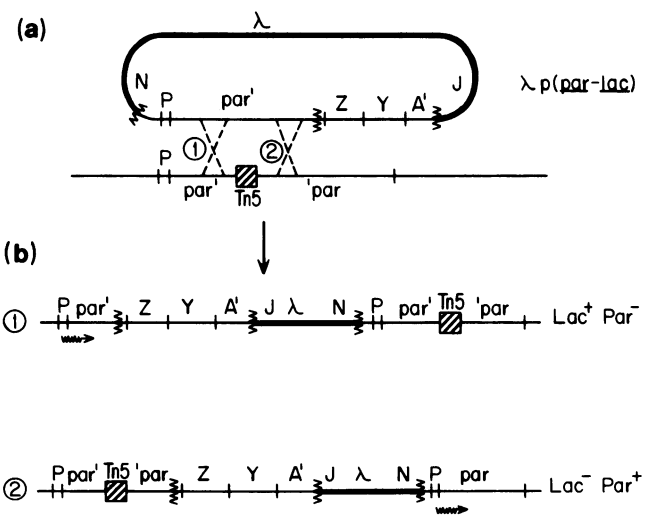

FIG. 2. *Verification of par-lac fusions. (a) A par::Tn5 strain is lysogenized with a par-lac transducing phage. Should the Tn5 insertion reside promoter proximal to the fusion joint, two classes of lysogens will result depending on where (1 or 2) the integrative recombination event occurs. (b) Integration by a homologous recombination promoter proximal to the Tn5 insertion, 1, will generate a Lac$^+$ Par$^-$ lysogen. Integration promoter distal to the Tn5 insertion, 2, will generate a Lac$^-$ Par$^+$ lysogen. This latter class of lysogens is possible only if the transducing phage carries a par-lac fusion.*

fusion strains must be under the transcriptional control of the *par* promoter.

Transcriptional regulation of the *par* gene. Fusions of the *lac* operon to the *par* gene were constructed such that the transcriptional regulatory properties of this gene are reflected in the levels of β-galactosidase activity. As mentioned previously, the β-galactosidase activity of the *par-lac* fusion strains varies in a manner analogous to protein 1b after growth in different media (Fig. 3, Table 2). Accordingly, the medium-dependent fluctuation of the *par* gene product is regulated at the transcriptional level.

If the absence of protein 1b in an *ompB* mutant is the result of a defect in the transcriptional regulation of *par*, an *ompB* mutation in a *par-lac* fusion should show a reduction in β-galactosidase activity. The four fusion strains were P1 transduced to *ompB* $1a^-$ $1b^-$ by cotransduction with *malQ*. The *ompB* derivatives were identified by screening for resistance to TuIa, i.e., protein $1a^-$. The *ompB* transductants of the fusion strains were assayed for β-galactosidase activity. The *ompB* strains became Lac^-, exhibiting a 14- to 31-fold reduction in β-galactosidase activity relative to the fully expressed *ompB*$^+$ derivatives. They also failed to show the induction effect obtained from growing in different culture media (Table 2). These same results were obtained for alleles from two independent *ompB* mutants, MH1160 (25) and MH1169.

The *ompB* alleles exhibiting the $1a^+$ $1b^-$ phenotype, corresponding with the class II insertions, from strains MH762 and MH764 were also transduced into the fusion strains, by cotransduction with *malQ*. The Mal^+ transductants that had acquired the *ompB* allele were detected by a subsequent P1 transduction experiment in which the Mal^+ transductants were used as donors. The isolates that could confer hy-2 resistance on a *par*$^+$ recipient by a *malQ*-linked trait were kept as the desired strains. The *ompB* $1a^+$ $1b^-$ *par-lac* genotype also correlated with a dark-red phenotype on lactose-tetrazolium agar, as opposed to the white *ompB*$^+$ *par-lac* recipients. The red colonies are Lac^- and the white colonies are Lac^+. Fusion strains containing the *ompB* $1a^+$ $1b^-$ allele exhibited only a 5- to 17-fold reduction in β-galactosidase activity. Unlike the *ompB* $1a^-$ $1b^-$ *par-lac* fusion strains, these strains still exhibited the induction effect observed in the *ompB*$^+$ fusions (Table 2).

The class I and class II *ompB*::Mucts mutations were shown to have the same effect on the *par-lac* fusions as the *ompB* alleles described above. This was determined by lysogenizing the *ompB*::Mucts strains with λp transducing phages carrying the *par-lac* fusions. β-Galactosidase activities of these lysogens (which are merodiploids, *par*$^+$, *par-lac*), after growth in different media, were similar (data not shown) to the results shown in Table 2. It should be noted that the β-galactosidase activities of an *ompB*$^+$ lysogen also correlated with the β-galactosidase activities of the corresponding haploid fusion strain. Accordingly, the *par* gene product is not involved in the transcriptional regulation of the *par* gene.

PA-2 lysogens of *E. coli* K-12 no longer ex-

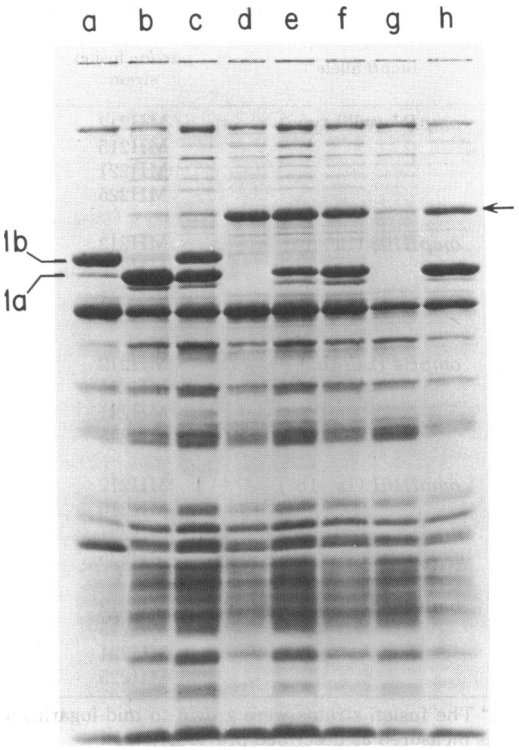

FIG. 3. *Major outer membrane protein expression in parental and representative ompB and par mutant strains. The strains were grown to mid-logarithmic phase in the media indicated. Outer membrane fractions were prepared (8) and urea-sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed (23). (a) Parental strain MC4100 grown in TSB; (b) MC4100 grown in NB; (c) MC4100 grown in TY; (d) class I ompB::Mucts strain MH1106 grown in TY; (e) class II ompB::Mucts strain MH704 grown in TY; (f) par::Mucts strain MH110 grown in TY; (g) spontaneous class I ompB strain MH1169 grown in TY; (h) spontaneous class I ompB strain MH764 grown in TY. Major outer membrane proteins 1a and 1b are labeled. The protein (designated by an arrow) overproduced by certain mutants was not identified. The nature of the protein that appears directly below protein 1a when protein 1a is expressed is also unknown.*

TABLE 2. *β-Galactosidase activities of par-lac fusion strains*[a]

ompB allele	*par-lac* fusion strain	Activity of strain grown on:		TSB/NB induction ratio	Ratio avg
		TSB	NB		
ompB$^+$ (wild type)	MH212	582	184	3.2	
	MH215	577	180	3.2	3.55
	MH221	537	144	3.7	
	MH225	679	164	4.1	
ompB108 (1a$^+$ 1b$^-$)	MH212	83	14	5.9	
	MH215	130	29	4.5	
	MH221	71	20	3.6	
	MH225	89	26	3.4	
					3.56
ompB4 (1a$^+$ 1b$^-$)	MH212	59	16	3.7	
	MH215	78	24	3.3	
	MH221	34	17	2.0	
	MH225	39	19	2.1	
ompB101 (1a$^-$ 1b$^-$)	MH212	28	21	1.3	
	MH215	39	29	1.3	
	MH221	19	23	0.8	
	MH225	22	22	1.0	
					1.04
ompB9 (1a$^-$ 1b$^-$)	MH212	27	29	0.9	
	MH215	40	30	1.3	
	MH221	18	27	0.7	
	MH225	22	22	1.0	

[a] The fusion strains were grown to mid-logarithmic phase in the media indicated. β-Galactosidase activity was measured as described previously (21).

press protein 1b (8, 28). If this were the result of a block in transcription promoter proximal to the fusion joint of our *par-lac* fusions, a reduction in β-galactosidase activity would be observed in these fusion strains. When PA-2 was lysogenized into the *par-lac* fusion strains, β-galactosidase activity remained essentially unaltered (data not shown). Thus, transcription of the *par* gene promoter proximal to the fusion joints of the fusion strains is unaffected by PA-2.

DISCUSSION

We constructed a series of strains in which the *lac* operon is fused to the *par* gene. These fusions were constructed in a manner such that the transcriptional regulatory properties of the *par* gene are reflected in the levels of β-galactosidase activity. Analysis of these *par-lac* fusions established a significant role for transcriptional regulation in the expression of the *par* gene product.

The levels of β-galactosidase expression in the *par-lac* fusion strains coincided with observed regulatory phenomena of major outer membrane protein 1b. First, depending on the growth medium, protein 1b can give either an intense band or a very faint band as seen on a polyacrylamide gel (1, 19, 29) (Fig. 3). By growing the fusion strains in two different media that elicit these two extremes in protein 1b production, we observed a similar disparity in β-galactosidase activities. The activity of the fusions cultured in TSB was three to four times what it was when they were grown in NB (Table 2). Second, production of protein 1b can be prevented by a mutation at *ompB* (Fig. 3). When an *ompB* mutation was introduced into the fusion strains, β-galactosidase activity was reduced (Table 2).

The results indicate that an *ompB* gene product is a diffusible positive regulatory element, acting at the level of transcription. Transcriptional activity at the *par* promoter, as monitored by β-galactosidase activity in the fusion strains, was virtually turned off by a mutation in *ompB*, mapping 26 min away on the chromosome. A role for *ompB* as a positive regulatory element would also account for why a mutation at either one of the loci, *par* or *ompB*, results in the absence of protein 1b. If an *ompB* gene product were exclusively a modifying or processing enzyme acting at a posttranscriptional level, β-galactosidase activity produced by the fusion strains would be unaffected.

A regulatory role for *ompB* and the pronounced similarities between the regulation of β-galactosidase in the *par-lac* fusion strains and protein 1b in a wild-type strain suggest that *par*

is the structural gene for protein 1b. Further evidence for this is provided by the different types of hy-2 resistance exhibited by the *ompB* and *par* insertion mutants. The *par* mutants were completely resistant to the phage, a predictable phenotype for a strain lacking a receptor. The *ompB* mutants were still slightly sensitive to the phage, indicating perhaps that the strain is not lacking a receptor but rather only has reduced amounts. This contention is consistent with the model that *par* is the structural gene for the receptor and *ompB* is a regulatory element, in the absence of which only basal levels of the receptor are expressed.

Analysis of the hy-2^r insertion mutants also raises some interesting questions. Two classes of mutants can be obtained by insertions at *ompB*. Class I, although the least represented in this study with only one insertion mutant, is the OmpB phenotype commonly described and discussed in the literature. The class I mutant lacks both proteins 1a and 1b. Class II mutants are 1a$^+$ 1b$^-$. Since the molecular lesion yielding these mutants is an insertion, it is unlikely that the presence of protein 1a can be attributed to an incomplete knockout of the gene. The simplest explanation for these results is that the *ompB* locus actually codes for two functions.

A comparison of β-galactosidase activities from fusion strains containing the class I and class II *ompB* mutations possibly provides some insight into the nature of *ompB* and the inference of two functions (Table 2). A class II *ompB* allele reduces expression of the *par-lac* fusions, yet the induction ratio obtained from culturing the fusions in different media remains unchanged. The absolute values are reduced, but the relative amounts are unaltered. A class I *ompB* allele reduces both the absolute and relative amounts of β-galactosidase activity such that the fusions do not exhibit any regulatory properties. This evidence may suggest that *par* is subject to two separable modes of regulation. One is simply required to turn on the gene. The second mode of regulation mediates the inducibility of *par* and the flexible composition of the outer membrane. Both regulatory modes are transcriptional and appear to be controlled by *ompB*.

E. coli K-12 is able to regulate the transcriptional activity of the gene of at least one of its major outer membrane proteins such that the composition of the outer membrane varies. This complex regulation is sensitive to certain conditions imposed by the growth medium and requires an *ompB* gene product. The *par-lac* fusions that we isolated should provide a useful tool for further defining this regulation and the role of the *ompB* locus.

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