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Regulatory Mutations Conferring Constitutive Synthesis of Major Outer Membrane Proteins (OmpC and OmpF) in *Escherichia coli*

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An *ompB* strain of *Escherichia coli* K-12 lacking major outer membrane proteins OmpC and OmpF was used to isolate a pair of mutants that have restored the ability to synthesize either OmpC or OmpF protein. These mutants were found to produce the respective proteins constitutively under the several conditions where the synthesis in the wild-type strain was markedly repressed; namely, in the absence of the *ompB* gene function, under restrictive medium conditions, or upon lysogenization with phage PA-2. The mutations *ompCp1* and *ompFp9* responsible for such synthesis were shown to be located in the close vicinity of the corresponding structural genes, *ompC* and *ompF*. Moreover, the mutations affect the expression of these genes in a *cis*-dominant fashion. Taken together with other evidence, it was suggested that *ompCp1* and *ompFp9* represent regulatory site mutations occurring at the promoter regions of *ompC* and *ompF*, respectively. Relevance of these findings to the genetic control of outer membrane protein synthesis is discussed.

Proteins OmpC and OmpF represent a class of major proteins of the outer membrane of *Escherichia coli* K-12. These and analogous proteins of *Salmonella typhimurium* are supposed to play an important role in uptake (diffusion) of low-molecular-weight substances (14-16). Three major classes of mutations are known to cause apparent loss or decrease of OmpC or OmpF protein: *ompC*, *ompF*, and *ompB*, which are located at about 47 min, 21 min, and 74 min, respectively, on the genetic map (see references 1 and 21 for revised symbols for major outer membrane protein genes). Current evidence indicates that *ompC* and *ompF* represent the structural genes for OmpC and OmpF proteins, respectively (26, 28). The *ompB* gene, whose mutations often lead to the defective synthesis of both proteins (22), is supposed to regulate transcription of the *ompC* gene (6) and presumably of the *ompF* gene as well. Although the mutations *divE* (25) and *perA* (32) were reported to reduce the amount of OmpF protein, the role of these genes has not been well defined. Beside these mutations, the levels of OmpC and OmpF proteins have been shown to be influenced markedly by osmolarity of medium used for growing the cells (9, 29). The effect of culture conditions on OmpC protein synthesis also seems to be exerted at the transcriptional level (6).

On the other hand, prolonged incubation or storage of cultures of strains lacking both OmpC and OmpF proteins leads to accumulation of mutants capable of synthesizing new outer membrane proteins with functions presumably similar to those of OmpC and OmpF (5, 7, 12, 20, 30). Mutations of at least three genes have been found to cause synthesis of such new proteins (20). We have also frequently encountered mutants that can synthesize either OmpC or OmpF protein in the stock culture of an *ompB* strain lacking both proteins. It will be shown in this paper that these mutants arise as a result of *cis*-dominant mutations presumably at the promoter regions of the structural genes *ompC* or *ompF*, and can produce the respective protein constitutively under the several conditions where the synthesis is repressed in the wild-type strain. The mode of regulation of these proteins will be discussed in the light of these findings.

MATERIALS AND METHODS

Bacterial and phage strains. Bacterial strains were all derivatives of *E. coli* K-12 and are listed in Table 1. Bacteriophages TuIa and TuIb (4) were kind gifts of U. Henning. Lambdoid phage PA-2 (27) was obtained by UV light induction of a lysogen donated by S. Mizushima. Phage λ $vir h^{PA-2}$, a hybrid between phages λ *vir* and PA-2, was isolated as a plaque former on *E. coli* indicator bacteria that are resistant to λ and lysogenic for PA-2.

Media and growth conditions. Polypeptone glucose (PG) broth contained 10 g of polypeptone (Wako Pure Chemical Co.), 5 g of NaCl, and 1 g of glucose per liter (pH 7.4). L broth contained 10 g of tryptone

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TABLE 1. *Bacterial strains*

Strain	Known genetic markers[a]	Derivation or reference
KY2201	*ompCp1;* other markers as in KY2562	Spontaneous mutant of KY2562
KY2202	*malA*$^{+}$[b] *ompB*$^{+}$; other markers as in KY2201	KY2201, transduced with P1 *vir* grown on KY2517
KY2205	*glpT6 nalA;* other markers as in KY2201	Derivative of KY2201
KY2206	*glpT6 nalA;* other markers as in KY2562	Derivative of KY2562
KY2209	*ompFp9;* other markers as in KY2562	Spontaneous mutant of KY2562
KY2210	*malA*$^{+}$ *ompB*$^{+}$; other markers as in KY2209	KY2209, transduced with P1 *vir* grown on KY2517
KY2215	*aroB tsx ompCp1 glpT6 nalA recA thi*	Derivative of KY2201
KY2216	*thr metE pyrD34 rpoB endA1100 tsx ompFp9 recA*	Derivative of KY2340 (26)
KY2218	*ompC;* other markers as in KY2215	Spontaneous TuIb-resistant mutant of KY2215
KY2219	*ompF219;* other markers as in KY2216	Spontaneous TuIa-resistant mutant of KY2216
KY2224	*thr leu thi rpsL tonA supE lac glpT6 nalA recA*/F'*glpT*$^{+}$ *nalA*$^{+}$ *ompCp*$^{+}$ *ompC*$^{+}$ (KYF2224)	Derivative of E101 (33)
KY2225	*thr metE thyA rpoB endA1100 tsx pyrD34 serC13 ompB101*	Derivative of KY2340 (26)
KY2229	*pyrD*$^{+}$ *ompFp9;* other markers as in KY2225	KY2225, transduced with P1 *vir* grown on KY2209
KY2274	*thr metE trypE9829*(Am) *endA1100 tsx rpoB asnS211*(Ts)	(26)
KY2304	*thi-1 tyrA pyrD34 his-38 thyA33 recA1 mtl-2 xyl-7 malA1 galK35*/F'*recA*$^{+}$ *tyrA*$^{+}$	Trp^{+} revertant of KL259/KLF43 (B. J. Bachmann)
KY2323	*thi-1 pyrD34 his-68 trp-54 recA1 mtl-2 xyl-7 malA1 galK35 rpsL118*/F'*ompFp*$^{+}$ *ompF*$^{+}$ *pyrD*$^{+}$ *trp*$^{+}$ (KYF2323)	(25)
KY2517	*thi tsx aroB*	AB2847 (18), transduced with P1 *vir* grown on P530 (22)
KY2562	*thi tsx malA ompB101*	AB2847 (18), transduced with P1 *vir* grown on P530 (22)
KY2563	*thi tsx malA*	AB2847 (18), transduced with P1 *vir* grown on P530 (22)

[a] Gene symbols are mostly as described by Bachmann and Low (1). (Am) denotes an amber mutation, and (Ts) denotes a temperature-sensitive conditionally lethal mutation.

[b] *malA* refers to a mutation within the *malQPI* operon.

(Difco), 5 g of yeast extract (Difco), 5 g of NaCl, and 1 g of glucose per liter (pH 7.0). Minimal medium E (31) was supplemented with 2 μg of thiamine per ml and 0.5% glucose. Nutrient broth (Difco) and a high-salt-minimal-glucose-Casamino Acids medium containing 3% NaCl (HS medium; reference 17) were used for growth of cells under conditions of limited synthesis of OmpC or OmpF protein, respectively (17). Minimal medium containing 25 μg each of shikimic acid, L-phenylalanine, L-tryptophan, and L-tyrosine per ml was used for growing *aroB* strains. Growth supplements for strains with other requirements were as described previously (26).

Transduction and conjugation. Transduction was carried out with phage P1 *vir* according to Ikeda and Tomizawa (8). Procedures for bacterial conjugation were described previously (23).

Isoelectric focusing. Cells were harvested from overnight cultures, washed in saline, and treated with 5% trichloroacetic acid. After standing at 4°C for several hours, the precipitates (whole-cell protein) collected by low-speed centrifugation were washed in acetone and dispersed in lysis buffer for isoelectric focusing (24). Isoelectric focusing was carried out with a column gel (2.5 by 130 mm) containing 4% acrylamide, 8 M urea, 2% Triton X-100, 1.6% ampholines (pH 4 to 6) (LKB), and 0.4% ampholines (pH 3.5 to 10) (LKB), as described previously (26).

Immunoprecipitation of OmpC and OmpF proteins. Cells were washed in 10 mM Tris-hydrochloride (pH 8.1) and suspended in 10 mM Tris-hydrochloride (pH 8.1) containing 1% sodium dodecyl sulfate (ca. 10^{10} cells per ml). After sonication and heating in boiling water for 5 min, the extracts were centrifuged at 17,000 × *g* for 15 min. The resulting supernatant fluids were diluted more than 10-fold with 30 mM Tris-hydrochloride (pH 8.1), containing 3 mM EDTA, 2% Triton X-100, and 150 mM NaCl, and treated with antisera against OmpC and OmpF proteins (donated by Y. Anraku) at 4°C for 2 days. The precipitates were collected by centrifugation at 17,000 × *g* for 15 min, washed three times with the Tris-EDTA-Triton-NaCl buffer described above, and finally dissolved in sample buffer for gel electrophoresis (10). After heating in boiling water for 2 min, 2 volumes of lysis buffer for isoelectric focusing were added, and samples were subjected to isoelectric focusing.

Peptide mapping. The procedures of Cleveland et al. (3) were followed. Gel pieces corresponding to OmpC or OmpF protein, cut out from the stained gel,

were rinsed with 125 mM Tris-hydrochloride (pH 8.1) containing 0.1% sodium dodecyl sulfate, 1 mM EDTA, and 20% glycerol (vol/vol) at 37°C for 30 min and inserted into the slot of the gel (85 by 180 mm; 1 mm thick). The gel pieces were overlaid with the same buffer and then with 10 μl of bovine pancreas α-chymotrypsin (Sigma) solution (1 mg/ml) in the same buffer but containing 10% glycerol. The discontinuous buffer system of Laemmli (10) using 15% acrylamide gel with 1 mM EDTA was used, and electrophoresis was carried out at 25 mA. When the dye front reached the middle of the stacking gel, electrophoresis was interrupted for 1 h to promote digestion of proteins, and then continued until the dye front reached the bottom of the gel.

Staining of gels and fluorography. Gels were stained with Coomassie brilliant blue as described previously (24). Fluorography was performed by exposure of dried gels to Fuji X-ray films according to the method of Laskey and Miles (11).

RESULTS

Isolation of mutants. The parental strain KY2562, carrying the *ompB101* mutation, lacks both OmpC and OmpF proteins and is resistant to phages TuIa and TuIb (or λ *virh*$^{PA-2}$), which use OmpF and OmpC protein, respectively, as a receptor. When cultures of this strain were stored at room temperature for a few weeks, they accumulated mutants that had regained sensitivity to phage TuIa, TuIb, or λ *virh*$^{PA-2}$. In one culture of the *ompB101* strain (KY2562), the majority of cells (more than 90%) were found to be sensitive to TuIb and λ *virh*$^{PA-2}$ though resistant to TuIa, and most of the others were sensitive to TuIa but resistant to TuIb and λ *virh*$^{PA-2}$. The representative mutants of these two classes, KY2201 and KY2209, were used for further study. In accordance with their phage sensitivities, the pair of mutants was found to synthesize new proteins which migrate, upon isoelectric focusing, exactly like OmpC and OmpF proteins, respectively, of the wild-type (*ompB*$^+$) strain. No appreciable differences in apparent molecular weight on sodium dodecyl sulfate-gel electrophoresis were found either between the protein produced by KY2201 and OmpC protein or between the protein produced by KY2209 and OmpF protein (data not shown).

The new proteins found in these mutants can be precipitated by antiserum against a mixture of OmpC and OmpF proteins, indicating that they are immunologically related to OmpC or OmpF (data not shown). Furthermore, chymotryptic peptides of the new proteins produced by KY2201 and KY2209 showed electrophoretic patterns that were very similar to those of OmpC and OmpF proteins, respectively. These results strongly suggest that KY2201 synthesizes OmpC protein and KY2209 synthesizes OmpF protein. This conclusion was further supported by the following genetic experiments.

Ten independent TuIb-resistant derivatives spontaneously isolated from KY2201 were found to lack the OmpC-like protein. These mutations were all mapped by phage P1-mediated transduction within the narrow region in which the structural gene for OmpC protein (*ompC*) is located (data not shown). Similarly, five independent TuIa-resistant derivatives isolated from KY2209 lacked the OmpF-like protein, and all the mutations were mapped near the *ompF* gene (data not shown). Based on this immunological, biochemical, and genetic evidence, we have concluded that the new proteins produced by KY2201 and KY2209 represent OmpC and OmpF proteins, respectively. As described below, these mutants are suggested to bear a promoter-like mutation which is responsible for the *ompB*-independent synthesis of OmpC or OmpF protein. We tentatively designate the mutations in KY2201 and KY2209 *ompCp1* and *ompFp9*, respectively.

Transductional analysis of the *ompB* region. To examine whether the mutations that lead to the synthesis of OmpC or OmpF protein have occurred within the *ompB* locus, the *ompB* region of the chromosome in the mutant strains KY2201 and KY2209 was transduced by phage P1 into strain KY2517 (*aroB ompB*$^+$) by selecting for Aro$^+$ transductants. About half (40 to 48%) of the transductants became resistant to both phages TuIa and TuIb (data not shown). This, together with the observed distribution of *malA* among the transductants, indicates that the *aroB*$^+$-*ompB101-malA* region has been transduced from the donor strain. Thus the *ompB101* allele is retained in the mutants KY2201 and KY2209, and a mutation outside of the *ompB* region must be responsible for the observed synthesis of OmpC or OmpF protein. It appears that the synthesis of OmpC or OmpF protein does not depend on the functional *ompB* gene product in these mutant strains.

In the reciprocal crosses, the *ompB*$^+$ allele of KY2517 was transduced into KY2201 or KY2209 by selecting for Mal$^+$ transductants. About half of the transductants derived from either cross were sensitive to both TuIa and TuIb, unlike the recipient strains, which are sensitive to only one of the phages, suggesting that they represent *ompB*$^+$ transductants (data not shown).

Effects of culture media on the synthesis of OmpC and OmpF proteins. To further characterize the nature of the mutations involved, we examined the synthesis of OmpC and OmpF proteins in the *ompCp1* (KY2201) and

ompFp9 (KY2209) mutants grown on various media: minimal medium E, nutrient broth, and HS medium. The synthesis of OmpC protein in the wild type was markedly reduced upon growth in nutrient broth (Fig. 1, lane 7), whereas it was repressed only partially in the *ompCp1* mutant (lane 8). Similarly, the synthesis of OmpF protein in the wild type was strikingly reduced when grown in HS medium (lane 13), whereas it was virtually unaffected in the *ompFp9* mutant (lane 14). Similar effects of growth media on OmpC-OmpF protein synthesis were observed in the *ompB*$^+$ derivatives of these mutants (Fig. 1). OmpC protein synthesis in the *ompCp1 ompB*$^+$ strain (KY2202) was observed even in nutrient broth, whereas OmpF protein synthesis was repressed normally in HS medium. Likewise, OmpF protein synthesis in the *ompFp9 ompB*$^+$ strain (KY2210) was observed even in HS medium, whereas OmpC protein synthesis was repressed in nutrient broth. It is evident that the effects of culture media are exerted independently on the syntheses of OmpC protein and OmpF protein. These results on the synthesis of OmpC and OmpF proteins (in both the *ompB*$^+$ and *ompB101* backgrounds) have been completely correlated with the sensitivities of these mutants grown on each of the different media to phages TuIa, TuIb, and λ *virh*$^{PA-2}$ (Table 2). We have concluded from these combined data that OmpC protein synthesis in the *ompCp1* mutants and OmpF protein synthesis in the *ompFp9* mutants are constitutive, because they are more refractory to repression normally observable when the wild-type strain is grown in certain media.

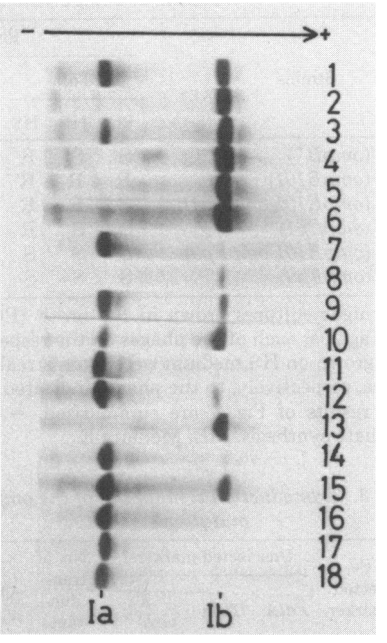

FIG. 1. *Effect of growth media on the synthesis of OmpC (Ia) and OmpF (Ib) proteins. Cells were harvested from overnight cultures grown at 37°C in medium E (lanes 1 to 3, 10 to 12), HS medium (lanes 4 to 6, 13 to 15), or nutrient broth (lanes 7 to 9, 16 to 18). Washed-cell suspensions were treated with trichloroacetic acid, and whole-cell proteins were subjected to isoelectric focusing, as described in the text. Only the acidic region of the stained gels is presented. The arrow indicates the polarity of electrofocusing. The protein bands for OmpC and OmpF have been identified as described previously (24, 26). Lanes 1, 4, 7, 10, 13, and 16, Strain KY2563 (ompB*$^+$*); lanes 2, 5, and 8, KY2201 (ompB101 ompCp1); lanes 3, 6, and 9, KY2202 (ompB*$^+$* ompCp1); lanes 11, 14, and 17, KY2209 (ompB101 ompFp9); lanes 12, 15, and 18, KY2210 (ompB*$^+$* ompFp9).*

Mapping of the *ompCp1* and *ompFp9* mutations. The results presented above suggested that the *ompCp1* and *ompFp9* mutations may have occurred in the promoter region of the respective structural gene operons. To test this possibility, P1 transduction experiments were carried out using KY2562 (*ompB101*) as donor and KY2205 (*ompB101 glpT nalA ompCp1*) as recipient. Examination of Glp$^+$ transductants obtained has revealed that *ompCp1* and *nalA* cotransduced with *glpT* at 9.5 and 54.5%, respectively, the gene order being *glpT-nalA-ompCp1* (experiment I, Table 3). This suggests that *ompCp1* is located near *ompC*, the structural gene for OmpC protein that has been mapped previously (2). Similar results were obtained in the cross in which KY2201 (*ompB101 ompCp1*) was used as donor and KY2206 (*ompB101 glpT nalA*) as recipient (Experiment II, Table 3). Furthermore, each of the transductants tested (50 for each experiment) exhibited the same sensitivity to phage λ *virh*$^{PA-2}$ whether PG broth or nutrient broth was used. This suggests that a single mutation, *ompCp1*, is responsible for the OmpC protein synthesis found in the absence of *ompB* function and for its constitutive synthesis observed in nutrient broth.

Similar analysis of linkage between *ompFp9* and *ompF* has been made by a series of transduction experiments (Table 4). The results clearly indicate that *ompFp9* is located between *serC* and *pyrD*, the cotransduction frequency between *ompFp9* and *serC* being 24.9%. These results, taken together with our previous data on *ompF* (26), indicate a close linkage between *ompFp9* and *ompF*, the structural gene for OmpF protein. Again, the phage sensitivity of the transductants tested (50 for each experiment) was not affected by whether HS medium

TABLE 2. *Phage sensitivity of ompCp1 and ompFp9 mutants*[a]

Strains	Phage sensitivity to:								Synthesis[b]					
	TuIa			TuIb		λ *virh*$^{PA-2}$			OmpF			OmpC		
	PG	NB	HS	PG	HS	PG	NB	HS	ME	NB	HS	ME	NB	HS
KY2563 (*ompB*$^+$)	S	S	R	S	S	S	R	S	+	+	−	+	−	+
KY2562 (*ompB101*)	R	R	R	R	R	R	R	R						
KY2201 (*ompB101 ompCp1*)	R	R	R	S	S	S	S	S	−	−	−	+	±	+
KY2202 (*ompCp1*)	S	S	R	S	S	S	S	S	+	+	−	+	±	+
KY2209 (*ompB101 ompFp9*)	S	S	S	R	R	R	R	R	+	+	+	−	−	−
KY2210 (*ompFp9*)	S	S	S	S	S	S	R	S	+	+	+	±	−	+

[a] Overnight cultures grown in PG broth (PG), nutrient broth (NB), or HS medium at 37°C were cross-streaked against each of the phages on the respective agar medium, and the plates were incubated at 37°C. The cultures grown on HS medium were cross-streaked on PG agar containing 1.5% NaCl. S and R, Sensitivity and resistance, respectively, to the phages indicated.

[b] The results of Fig. 1 are summarized. + and −, Synthesis and little or no synthesis, respectively; ±, intermediate synthesis. ME, Medium E.

TABLE 3. *Transductional mapping of the ompCp1 mutation*[a]

Expt	Selected marker	Unselected markers[b]			No. of transductants	Frequency (%)
		nalA	TuIa	λ *virh*$^{PA-2}$		
I	*glpT*$^+$	−	R	S	90	45.0
		+	R	S	91	45.5
		+	R	R	18	9.0
		−	R	R	1	0.5
II	*glpT*$^+$	−	R	R	109	54.8
		+	R	R	54	27.1
		+	R	S	34	17.1
		−	R	S	2	1.0

[a] Donors: Experiment I, KY2562 (*ompB101*); experiment II, KY2201 (*ompB101 ompCp1*). Recipients: Experiment I, KY2205 (*ompB101 glpT6 nalA ompCp1*); experiment II, KY2206 (*ompB101 glpT6 nalA*). Transductants were selected at 37°C and were tested for their properties after purification.

[b] + and −, Wild-type and mutant alleles, respectively. S and R, Sensitivity and resistance, respectively, to the phages indicated as determined by cross-streak tests on PG agar.

TABLE 4. *Transductional mapping of the ompFp9 mutation*[a]

Expt	Selected marker	Unselected markers[b]			No. of transductants	Frequency (%)
		TuIa	TuIb	*pyrD*		
I	*serC*$^+$	S	R	+	158	77.8
		R	R	+	35	17.8
		R	R	−	9	4.4
		S	R	−	1	0.5
II	*serC*$^+$	R	R	−	116	71.6
		S	R	−	35	21.6
		S	R	+	10	6.2
		R	R	+	1	0.6

[a] Donors: Experiment I, KY2304 (*pyrD34*); experiment II, KY2209 (*ompB101 ompFp9*). Recipients: Experiment I, KY2229 (*ompB101 serC13 ompFp9*); experiment II, KY2225 (*ompB101 serC13 PyrD34*). Transductants were selected at 37°C and were tested for their properties after purification.

[b] The symbols are as in Table 3.

or PG broth was used. Thus a single mutation, *ompFp9*, appears to be responsible for the synthesis of OmpF protein in the absence of *ompB* function and for the constitutive synthesis observed in HS medium.

The linkage between *ompFp9* and *ompF* was examined further by transduction between the double mutant KY2219 (*ompFp9 ompF219*) and KY2274 (*asnS*), selecting for Asn$^+$ (ability to grow at 42°C) transductants. Strain KY2219 is a TuIa-resistant derivative of the *ompFp9* mutant and produced inactive OmpF protein isoelectrically similar to the wild-type OmpF protein. This mutation (*ompF219*) cotransduced with *asnS* at about 90% and mapped to the opposite side of *asnS* from *pyrD* (data not shown). That KY2219 retains the *ompFp9* mutation was suggested by the insensitivity of OmpF protein synthesis to 3% NaCl in growth medium. Fifty Asn$^+$ transductants were examined for production of OmpF protein upon growth in PG broth containing 3% NaCl and for sensitivity to phage TuIa on PG agar. The results gave no indication of occurrence of crossing over between *ompFp9* and *ompF219*, showing that these two mutations are very closely linked to each other. Figure 2 summarizes the results of all the transduction experiments presented above.

Dominance tests of the *ompCp1* and *ompFp9* mutations. It was important to examine whether the *ompCp1* and *ompFp9* mutations were dominant or recessive to the respective wild-type allele in considering the possible mechanisms of constitutive expression of the

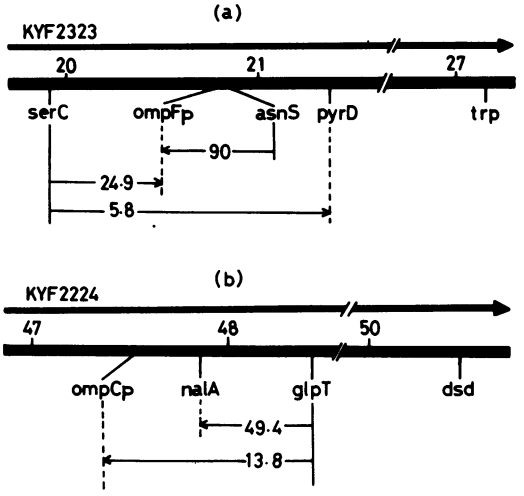

FIG. 2. *Diagrams showing the location of the ompCp1 and ompFp9 mutations and F′ plasmids used. The map positions of the ompFp9 (a) and ompCp1 (b) mutations were based on the transductional data described in Tables 3 and 4. The numbers indicate the frequencies of cotransduction. The arrowheads represent the origin and direction of transfer of the F′ plasmids (KYF2224 and KYF2323).*

ompC and *ompF* operons in the mutants. We first isolated, by the method of Low (13), an F′*ompC*$^+$ plasmid (KYF2224) carrying the *ompC-nalA-glpT* region of the *E. coli* chromosome; the F′ plasmid was obtained from one of the Glp$^+$ transconjugants arising from a cross between an Hfr (KY2301; reference 26) and an F$^-$ *glpT recA* strain. An *ompCp1* mutant (KY2215; F$^-$ *aroB glpT nalA ompCp1 recA*) was then mated with an F′ strain carrying KYF2224, and Glp$^+$ transconjugants were selected. Five clones were established that are merodiploid for the *ompC* region (*glpT nalA ompCp1*/F′*glpT*$^+$ *nalA*$^+$ *ompCp*$^+$). They were sensitive to UV light (Rec$^-$) and to nalidixic acid (Nal$^+$), required aromatic amino acids for growth (Aro$^-$), and transferred *glpT*$^+$ into an F$^-$ *glpT recA* strain at high frequency. The presence of *ompCp*$^+$ on the plasmid was confirmed by observing P1-mediated cotransduction of *ompCp*$^+$ and *glpT*$^+$ from these strains into KY2205 (*glpT ompCp1 ompB101*) at appropriate frequencies. These merodiploid strains were all found to be sensitive to phage λ *virh*$^{PA-2}$ both in nutrient broth and in PG broth, suggesting that *ompCp1* is dominant to the wild-type allele (*ompCp*$^+$).

The F′*ompC*$^+$ plasmid was then introduced into the double mutant carrying *ompCp1* and *ompC* (KY2218). Five clones of the resulting merodiploid strains (*ompCp1 ompC*/F′*ompCp*$^+$ *ompC*$^+$) were all sensitive to phage λ *virh*$^{PA-2}$ in PG broth, unlike the parental F$^-$ strain, which was resistant. Nalidixic acid-resistant derivatives isolated from each merodiploid clone were shown to be simultaneously Glp$^-$ and resistant to λ *virh*$^{PA-2}$ (F$^-$ segregants). Interestingly, these merodiploid strains were resistant to λ *virh*$^{PA-2}$ when grown in nutrient broth, in contrast to the merodiploids of genotype *ompCp1 ompC*$^+$/F′*ompCp*$^+$ *ompC*$^+$ (see above). These results indicate that the *ompCp1* mutation affects expression of the *ompC* gene on the chromosome but does not affect expression of the *ompC*$^+$ gene located on the F′ plasmid; *ompCp1* represents a *cis*-dominant mutation.

Similar tests of dominance for the *ompFp9* mutation were carried out using an F′*ompF*$^+$ plasmid (KYF2323; references 25 and 26). An *ompFp9 recA* strain carrying KYF2323 was constructed, and the genetic constitution (*ompFp9*/F′*ompFp*$^+$) was verified by the strain's capacity to transfer *pyrD*$^+$ by mating and to donate *pyrD*$^+$ and *ompF*$^+$ jointly by P1 transduction into appropriate recipients, as in the case of merodiploids for the *ompC* region. The merodiploid strain thus established was found to be sensitive to phage TuIa when grown in HS medium as well as in minimal medium E, suggesting the dominance of *ompFp9* over its wild-type allele (*ompFp*$^+$).

The *ompFp9 ompF* double mutant carrying the F′ plasmid KYF2323 (*ompFp9 ompF*/F′*ompFp*$^+$ *ompF*$^+$) was then constructed and found to be sensitive to phage TuIa in PG broth, unlike the F$^-$ parent used (KY2219). However, it was resistant to phage TuIa when tested on HS medium, in contrast to the *ompFp9*/*ompFp*$^+$ strain. Thus the *ompFp9* mutation did not affect expression of the *ompF* gene located in *trans*, but did affect *ompF* located in *cis*. All the results presented in this section clearly indicate that the *ompCp1* and *ompFp9* mutations occurring near the respective structural genes are responsible for the constitutive synthesis of OmpC and OmpF proteins, respectively, and that they exhibit *cis*-dominant effects. We conclude that *ompCp1* and *ompFp9* represent regulatory site mutations directly affecting the expression of *ompC* and *ompF*, respectively.

OmpC protein synthesis in the PA-2 lysogens. Lysogenization of wild-type *E. coli* cells with phage PA-2 causes decrease of proteins OmpC and OmpF and appearance of a new phage-directed protein called protein 2 in the outer membrane (27). When the wild-type strain (KY2563) and its PA-2 lysogen were grown in L broth without glucose, very little synthesis of OmpC protein was observed in the lysogen; the effect was less striking for the synthesis of OmpF protein (Fig. 3). In contrast, OmpC protein syn-

thesis in the *ompCp1 ompB*$^+$ mutant was hardly affected by lysogenization with PA-2. It thus appears that the presumptive promoter mutation (*ompCp1*) rendered the expression of the *ompC* operon insensitive to "repression by lysogenization." Such repression in the PA-2 lysogens may therefore occur at the level of transcription. Since similar amounts of protein 2 are synthesized by the lysogens of both *ompCp1* and *ompCp*$^+$ strains, the observed decrease in OmpC protein does not seem to be related to the production of protein 2. When tryptic soy broth was used instead of L broth without glucose, OmpC protein was synthesized normally even by the PA-2 lysogen of the wild-type strain (KY2563) (Fig. 3). Thus the reduced synthesis of OmpC protein observed in the PA-2 lysogen is a growth medium-dependent phenomenon.

DISCUSSION

E. coli strains lacking both OmpC and OmpF proteins (*ompB* mutants or *ompC ompF* double mutants) have great disadvantages in growth, presumably due to the transport defects (19). Revertants which have restored the capacity to produce OmpC-or OmpF-like protein or new outer membrane protein(s) easily accumulate in cultures of these strains (5, 7, 30). Revertants that produce OmpC- or OmpF-like protein, such as those studied here, become sensitive to phage TuIa, TuIb or PA-2, whereas those producing new proteins remain resistant to these phages. Analysis of the latter revertants has identified several new membrane proteins with functions similar to OmpC or OmpF (12, 20). We have studied "revertants" obtained from the *ompB101* strain, *ompCp1* and *ompFp9*, which have recovered the ability to synthesize OmpC or OmpF protein, respectively.

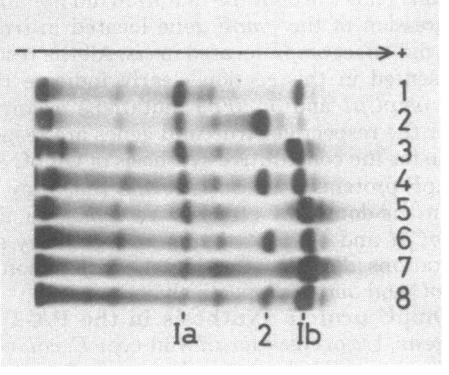

FIG. 3. *Isoelectric focusing patterns of proteins synthesized by the PA-2 lysogens. Cells were harvested from overnight cultures grown in L broth without glucose (lanes 1 to 4) or in tryptic soy broth (Difco) (lanes 5 to 8), and the whole-cell proteins were subjected to isoelectric focusing. Lanes 1 and 5, KY2563 (ompCp*$^+$*); 2 and 6, a PA-2 lysogen of KY2563; 3 and 7, KY2202 (ompCp1); 4 and 8, a PA-2 lysogen of KY2202.*

OmpC or OmpF protein synthesis in these mutants (revertants) was found to be constitutive, namely, virtually independent of *ompB* gene function, medium conditions, and lysogenization with PA-2. Both of these mutations are located very closely linked to the respective structural genes and clearly exhibit *cis*-dominant effects. We have concluded that *ompCp1* and *ompFp9* represent regulatory site mutations directly affecting OmpC-OmpF protein synthesis in *E. coli*. Such regulatory sites may include promoter, operator, attenuator, and terminator (for an upstream operon) that are involved in transcriptional control. Besides, the mutations might have created a new promoter that permits transcription of the *ompC* or *ompF* gene. Though direct evidence is lacking, a promoter mutation or a new promoter seems to explain the available results best (see below).

Hall and Silhavy (6) have recently shown that β-galactosidase synthesis directed by the *ompC* promoter in the *ompC-lacZ* fusion strain is regulated by the *ompB* gene, whose (diffusible) product may work as a positive regulator for transcription of the *ompC* gene. The effect of growth media on *ompC* expression was also observed in this system, suggesting that it is exerted at the transcriptional level (6). The results reported in this paper strongly support these conclusions and extend the transcriptional model of regulation to include both the *ompC* and *ompF* operons. In this connection, our data seem to exclude the possibility that the *ompB* gene product or the medium conditions primarily affect the synthesis of one of the proteins (OmpC or OmpF), and that an increase or a decrease of this protein secondarily affects synthesis of the other. The effects therefore seem to be directed to the promoter regions of both the *ompC* and *ompF* operons.

Kawaji et al. (9) reported that the levels of OmpC and OmpF proteins are influenced by addition to the medium of substances that are not permeable to the outer membrane. It is not known how transcription of *ompC* and *ompF* genes is affected by alteration of medium osmolarity. According to Hall and Silhavy (6), the residual level of *ompC* expression in OmpC$^-$ OmpF$^+$ type *ompB* mutants is affected by medium conditions, but that in OmpC$^-$ OmpF$^-$ type *ompB* mutants is not. This suggests that the *ompB* gene product may be involved in modulation of OmpC-OmpF protein synthesis by medium osmolarity. The facts that synthesis of OmpC or OmpF protein in the *ompCp1* or

ompFp9 mutant is independent of regulation by the *ompB* gene product and by medium conditions can be explained on the same basis. In view of these considerations, the *ompCp1* and *ompFp9* mutations most likely affect a region of the respective promoter which is specifically involved in the interaction with a hypothetical positive regulator molecule (*ompB* gene product).

Regulation of the major outer membrane protein synthesis is also affected by lysogenization of cells with phage PA-2; the synthesis of OmpC protein in the wild-type strain was markedly repressed by prophage PA-2 in the present experiments. Interestingly, OmpC protein synthesis in the *ompCp1* mutant is insensitive to this prophage-mediated repression. In addition, analyses of these PA-2 lysogens suggested that the reduced synthesis of OmpC protein is brought about at the transcriptional level and is independent of the newly acquired capacity of the lysogen to synthesize the phage-directed outer membrane protein 2. On the other hand, β-galactosidase synthesis in the above *ompC-lacZ* fusion strain was reported to be not significantly affected by lysogenization with PA-2 (6). Since the decrease of OmpC protein in the PA-2 lysogens is a medium-dependent phenomenon (Fig. 3), the failure to detect the prophage-mediated repression in the fusion strain might be due to the particular growth medium used.

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