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Pleiotropic Mutations Rendering *Escherichia coli* K-12 Resistant to Bacteriophage TP1

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tpo mutations, located at 74 min on the genetic map, rendered *Escherichia coli* K-12 resistant to TP1, a phage which can use either the OmpF protein or the LamB protein as its receptor. *tpo* mutants synthesized decreased amounts of OmpF and LamB proteins but increased amounts of the OmpC product, another outer membrane protein. The effect of the *tpo* mutations on *lamB* gene expression was transcriptional. It is one facet of the following effect on the maltose regulon: strong decreases in the syntheses of the LamB protein and the periplasmic MalE protein occurred when the regulon was uninduced; a lesser decrease occurred in the syntheses of the LamB protein the MalE protein, and the cytoplasmic MalQ protein (amylomaltase) when the regulon was induced. The *tpo* mutants were found to be phenotypically identical to the *perA* mutant recently described by Wanner et al. (J. Bacteriol. **140**:229-239, 1979) and to some of the *ompB* mutants described by Verhoef et al. (Mol. Gen. Genet. **169**:137-146, 1979). Mapping and complementation analysis suggested that these three types of mutations belong to the same cistron. Our results bring to at least four the number of clearly distinct phenotypes which can result from mutations at, or close to, *ompB*, a locus which appears increasingly complex.

We recently described a coliphage which can use either of two outer membrane proteins as its receptor (29). This bacteriophage, called TP1, is a host range mutant of phage TuIa (4). As its receptor it can use either the OmpF protein, which is the only protein recognized by parental phage TuIa, or the LamB protein, which is otherwise known as the receptor for phage λ (16). (The outer membrane proteins will be referred to by the name of their structural genes. The nomenclature for these genes will be that which has been proposed by Reeves [17] and which will be used in the next edition of the *E. coli* genetic map. Hence, *ompF*, which was previously called *tolF*, codes for a protein which has been called Ia, 1a, b, or O9 by different authors [7, 24]. Gene *ompC* was previously called *par* or *meoA* and codes for a protein called Ib, 1b, c, or O8 [7, 24].) The OmpF protein is one of the porins allowing the diffusion of several low-molecular-weight compounds across the outer membrane (3, 14), whereas the LamB protein, whose structural gene is part of the maltose regulon (6, 15), is involved in the transport of maltose and maltodextrins (30). We previously reported that TP1-resistant mutants could be isolated in a single step from an *ompF*$^+$ *lamB*$^+$ strain (29). The analysis of such mutants was thought likely to provide information regarding the biosyntheses of the OmpF and LamB proteins and, more generally, on the biogenesis of the outer membrane. In this paper, we describe some of these mutants and demonstrate that they are impaired in the expression of the *ompF* gene and of the whole maltose regulon and that they carry a mutation in a region (74 min on the genetic map) in which other mutations occur (20, 28, 31) that affect the synthesis of the envelope proteins.

MATERIALS AND METHODS

Strains and media. The bacterial strains used are listed in Table 1. Phage strains λ V*h*°, λ V*h*434, λ cI *h*80, and 434 were from our laboratory collection. Phages λ p Φ(*lamB-lacZ*$^+$), λ p Φ(*lamB-lacZ*)$_{hyb}$61-4, λ Y*h*°434(lB8), TuIa, and TP1 were described previously (4, 12a, 19, 26, 29). Complete medium (ML), minimal medium (M63), and eosin-methylene blue medium were also previously described (10).

Isolation of TP1-resistant mutants. Spontaneous mutants resistant to TP1 were isolated as follows. Exponential cultures of strain pop1010 were infected with phage TP1 at a multiplicity of infection of about 5. The cultures were incubated for 2 h at 37°C, with aeration, until lysis occurred. Portions of these cultures were plated on ML agar coated with 10^9 plaque-forming units of phage TP1. The clones that had grown within 24 h were isolated and verified to be TP1 resistant by cross-streaking and spot testing. All of the mutants obtained by this procedure were still sensitive to host range mutants present at a frequency of about 10^{-5} in the TP1 phage stocks. Therefore, the

TABLE 1. *Bacterial strains used*

Strain	Genotype[a]	Origin
pop3350	F⁻ *araD139 malT ΔlacU169 rpsL relA thi*	Débarbouillé and Schwartz (5)
pop1010	Hfr *his metA aroB rpoB*	Laboratory collection
pop1263	Hfr *his metA aroB tpo-11 rpoB*	Spontaneous TP1-resistant mutant of pop1010
pop1267	Hfr *his metA aroB tpo-22 rpoB*	Spontaneous TP1-resistant mutant of pop1010
pop1371	Hfr *his metA aroB ompB101 rpoB*	This work
pop1372	Hfr *his metA aroB malT rpoB*	Spontaneous λ-resistant Mal⁻ mutant of pop1010
pop1378	Hfr *his metA tpo-11 rpoB*	This work
pop1379	Hfr *his metA tpo-22 rpoB*	This work
pop1384	Hfr *his metA malT1*[c] *tpo22 rpoB*	This work
pop1385	Hfr *his metA malT1*[c] *rpoB*	This work
pop1386[b]	Hfr *his metA aroB lac rpoB*	Spontaneous Lac⁻ mutant of pop1010
pop1387	Hfr *his metA lac tpo-11 rpoB*	This work
pop1388	Hfr *his metA tpo-22 lac rpoB*	This work
pop1389	Hfr *his metA ompB101 lac rpoB*	This work
pop1390	Hfr *his metA aroB lac rpoB* (λ p Φ(*lamB-lacZ*)$_{hyb}$61-4)	This work
pop1391	Hfr *his metA lac tpo-11 rpoB* (λ p Φ(*lamB-lacZ*)$_{hyb}$61-4)	This work
pop1392	Hfr *his metA lac tpo-22 rpoB* (λ p Φ(*lamB-lacZ*)$_{hyb}$61-4)	This work
pop1393	Hfr *his metA lac ompB101 rpoB* (λ p Φ(*lamB-lacZ*)$_{hyb}$61-4)	This work
pop1406	Hfr *his metA lac rpoB* (λ p Δ(*lamB-lacZ*⁺))	This work
pop1407	Hfr *his metA lac tpo-11 rpoB* (λ p Φ(*lamB-lacZ*⁺))	This work
pop1408	Hfr *his metA rpoB lac tpo-22* (λ p Φ(*lamB-lacZ*⁺))	This work
popx409	Hfr *his metA lac rpoB ompB101* (λ p Φ(*lamB-lacZ*⁺))	This work
pop1409	Hfr *his metA lac rif ompB101* (λ p Φ(*lamB-lacZ*⁺))	This work
pop1411[c]	Hfr *his metA rpoB malT* (λ Y*h*°434(lB8))	This work
pop1412[c]	Hfr *his metA rpoB malT tpo-11* (λ Y*h*°434(lB8))	This work
pop1413[c]	Hfr *his metA rpoB malT tpo-22* (λ Y*h*°434(lB8)	This work
JC1553 (KLF41)	F′ *argG*⁺ *rpsL*⁺ *malT*⁺/*leu metB his recA argG rpsL*	Hofnung et al. (11)
pop667	F⁻ *thr leu metA argH tpo-11*	This work
pop668	F⁻ *thr leu metA argH aroB tpo-11*	This work
pop670	F⁻ *thi thr leu argH metA tonA rpsL glpD*	This work
pop674	F⁻ *thr leu metA argH ompB101*	This work
pop675	F⁻ *thr leu metA argH aroB ompB101*	This work
pop678	F⁻ *thi thr leu argH metA tonA aroB glpD tpo-11 recA srl*::Tn*10*	This work
pop680	F⁻ *thi thr leu argH metA tonA rpsL aroB glpD ompB101 recA srl*::Tn*10*	This work
pop1639	F⁻ *thi thr leu argH metA tonA rpsL glpD perA recA srl*::Tn*10*	This work
pop1643	F⁻ *thi thr leu argH tonA aroB glpD ompB101 recA srl*::Tn*10*	This work
pop1665	KLF41/pop680	This work
pop1667	KLF41/pop678	This work
pop1669	KLF41/pop1639	This work
pop1675	KLF41 *rpsL ompB101 aroB*/pop680	This work
pop1677	KLF41 *rpsL ompB101 aroB*/pop678	This work
pop1679	KLF41 *rpsL ompB101 aroB*/pop1639	This work
pop1685	KLF41 *tpo-11 aroB*/pop1643	This work
pop1689	KLF41 *tpo-11 aroB*/pop1639	This work
CE1121, CE1122 and CE1123	*thr leu thi pyrF codA thyA argG ilvA his lacY tonA tsx rpsL deoC supE uvrB ompB*	Verhoef et al. (28)

[a] [d]

TABLE 1—*Continued*

[a] Genetic nomenclature is from Bachmann et al. (2) except for: *tpo*, which stands for "TPone" resistance and is defined in this work; *perA*, defined in reference 31; and the notations $\Phi(lamB\text{-}lacZ)_{hyb}61\text{-}4$ and $\Phi(lamB\text{-}lacZ^+)$, which designate a gene and an operon fusion, respectively (see Table 6).

[b] This strain was selected from pop1010 by plating on minimal arabinose agar supplemented with 5×10^{-3} M isopropyl-β-D-thiogalactoside. For unknown reasons, this medium allows for the selection of mutants devoid of lactose permease (J. Monod, personal communication). Among these, some also lack β-galactosidase, presumably because they carry a polar mutation in *lacZ* or a *lacZ-lacY* deletion. Strain pop1386 is one such mutant. It is totally devoid of β-galactosidase activity.

[c] The prophage present in this strain, λ *Yh*°434(1B8), carries a *lacZ-lamB*$^+$ fusion constructed in vitro (12a).

[d] These mutants were designated as *ompB* by Verhoef et al. (28). This nomenclature will probably have to be reconsidered (see text).

apparent frequency of the TP1-resistant mutants (about 10^{-8}) is probably an underestimation of the true mutation rate. Indeed, many of the TP1-resistant mutants probably never appeared on the selection plates because they were killed by the host range phage mutants.

Complementation tests. The dominance analyses between the mutant (*tpo*, *ompB*, or *perA*) alleles and the wild-type alleles were performed by crossing JC1553 (KLF41) with *aroB glpD recA srl*::Tn*10* F$^-$ strains carrying, in addition, one of the mutant alleles. Aro$^+$ Glp$^+$ Tet^r sexductants were selected and analyzed.

The dominance analyses between different mutant alleles required that they be transferred onto KLF41. The wild-type episome was first introduced into *recA*$^+$ F$^-$ strains carrying one of the mutant alleles. These merodiploid strains had a wild-type phenotype with respect to TuIa, TP1, and λ V*h*434 sensitivity (see Results). Homogenotes carrying the mutant alleles on the episome as well as on the chromosome could be selected by their resistance to the appropriate phage. Two such homogenotes were constructed. From an *rpsL aroB ompB101 malT* strain carrying a wild-type KLF41 episome selection for λ V*h*434 resistance yielded a homogenote carrying a KLF41 *rpsL aroB ompB101 malT*$^+$ episome. On the other hand, from an *rpsL aroB tpo-11 malT* strain carrying a wild type KLF41 episome selection for TuIa resistance yielded a homogenote carrying a KLF41 *rpsL aroB tpo-11 malT*$^+$ episome. The episomes were then transferred into *glpD recA srl*::Tn*10* F$^-$ strains carrying a mutant (*tpo*, *perA*, or *ompB101*) allele. Glp$^+$ Tet^r sexductants were selected and analyzed. The genotypes of all merodiploid strains were verified by transferring the episome into another genetic background and by analyzing segregants which had lost the episome.

RESULTS

Selection of TP1-resistant mutants; location of the mutations. Spontaneous TP1-resistant mutants are obtained at a frequency of about 10^{-6} when one starts with a strain which fails to express either *lamB* or *ompF* (29). TP1-resistant mutants could also be obtained from wild-type strains but at a lower frequency (i.e., about 10^{-8}). This frequency of TP1-resistant mutants is higher than would be expected from the simultaneous occurrence of two mutations affecting the expression of *lamB* and *ompF*, respectively (see above).

Twelve independant TP1-resistant mutants were isolated from strain pop1010. All were found unable to adsorb TP1. Their mutation was called *tpo* (for "TPone" resistance). They all grew more slowly than the parental strain in various media. The *tpo-11* strain, for instance, had a doubling time of 45 min instead of 30 min in complete medium (ML) at 37°C and of 120 min instead of 90 min in minimal (M63) glycerol. The growth defect was more pronounced in minimal maltose medium and maltodextrin medium. Thus, the *tpo-11* strain had a doubling time of 180 min in minimal maltose medium compared to 90 min for the parental strain and did not grow on minimal maltodextrin agar. On maltose indicator agar, these mutants appeared to be "leaky" Mal$^-$. Strain pop1010, from which the 12 TP1-resistant mutants were isolated, was *aroB*. When the mutants were transduced to Aro$^+$, using a P1 phage grown on an *aroB*$^+$ *tpo*$^+$ strain, about 60 to 80% of the transductants were found to be TP1 sensitive. The three-point tests described in Table 2 demonstrated that the *tpo* mutations were located between *aroB* and *malT*, i.e., at about 74 min on the genetic map. Another locus in the same area was *ompB*. Mutations such as *ompB101* strongly decreased the synthesis of the OmpF protein as well as of another porin (the OmpC protein) (17, 20). However, unlike *tpo* strains, the *ompB101* strains (20) were not resistant to TP1. The three-point tests described in Table 3 demonstrated that *tpo-11* was extremely close to *ompB101* (cotransduction frequency, 95% or more). The order seemed to be *aroB-ompB101-tpo-11*, although this conclusion was based only on a twofold difference in the number of *ompB*$^+$ *tpo*$^+$ recombinants in the two reciprocal crosses.

Other mutations located close to *ompB101*, and also affecting the synthesis of the OmpF protein, have recently been described (28, 31). A comparison of their properties with those of the *tpo* mutations will be provided at the end of the Results section.

TABLE 2. *Ordering of aroB, tpo, and malT by P1 transduction*

Donor strain	Acceptor strain	No. of AroB$^+$ transductants analyzed	Unselected characters[a]			
			MalT$^-$ Tpo$^+$	MalT$^-$ Tpo$^-$	MalT$^+$ Tpo$^+$	MalT$^+$ Tpo$^-$
pop3550 (*malT tpo$^+$ aroB$^+$*)	pop1263 (*malT$^+$ tpo-11 aroB*)	100	41	0	38	21
pop3550 (*malT tpo$^+$ aroB$^+$*)	pop1267 (*malT$^+$ tpo-22 aroB*)	100	35	0	33	32
pop1378 (*malT$^+$ tpo-11 aroB$^+$*)	pop1372 (*malT tpo$^+$ aroB*)	100	36	17	0	47
pop1379 (*malT$^+$ tpo-22 aroB$^+$*)	pop1372 (*malT tpo$^+$ aroB*)	100	34	22	2	42

[a] P1 transductions were carried out under standard conditions. The cultures were plated on minimal glucose medium supplemented with histidine and methionine. In each transduction, recombinants were reisolated and scored for unselected characters: Mal$^+$ on eosin-methylene blue-maltose agar (the *tpo* mutants are leaky Mal$^-$ and can be easily distinguished from *malT* mutants on this medium) and Tpo by resistance to TP1.

TABLE 3. *Ordering of aroB, tpo-11, and ompB101 by P1 transduction*

Donor strain	Acceptor strain	No. of AroB$^+$ transductants analyzed	Unselected characters[a]			
			OmpB$^+$ Tpo$^-$	OmpB$^-$ Tpo$^+$	OmpB$^+$ Tpo$^+$	OmpB$^-$ Tpo$^-$
pop667 (*aroB$^+$ ompB$^+$ tpo-11*)	pop675 (*aroB ompB101 tpo$^+$*)	548	388	139	21	—[b]
pop674 (*aroB$^+$ ompB101 tpo$^+$*)	pop668 (*aroB ompB$^+$ tpo-11*)	650	198	442	10	—[b]

[a] The cultures were plated on glucose minimal agar supplemented with threonine, leucine, arginine, and methionine. After purification on the same medium, the colonies were tested for unselected characters: OmpB (resistance to λ V*h*434) and Tpo (resistance to TP1).

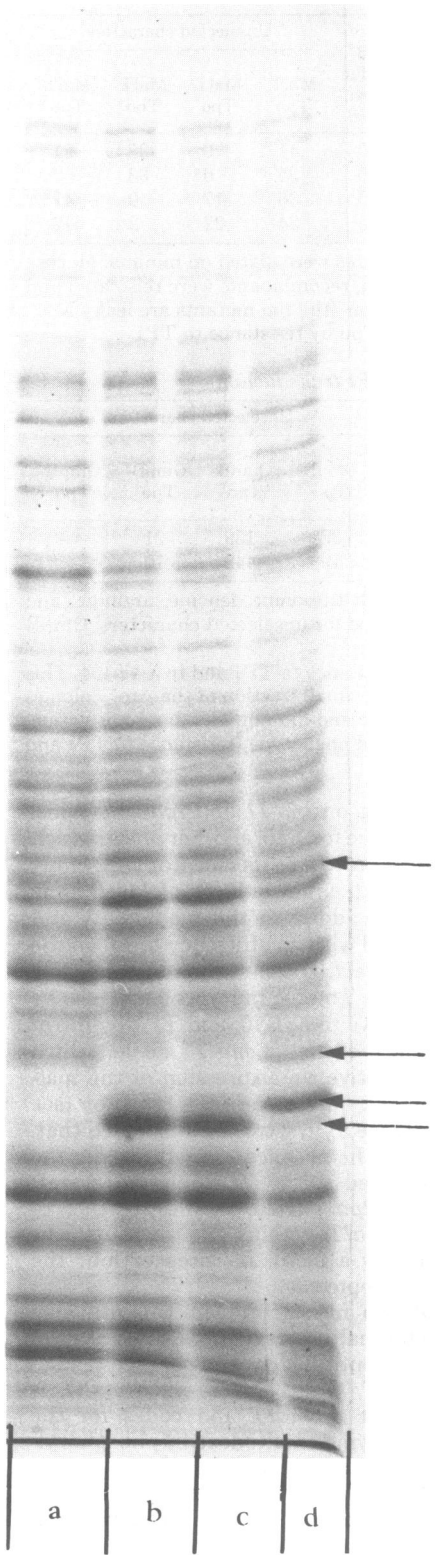
[b] No stable recombinant was obtained displaying this phenotype, i.e., resistance to TP1 and to λ V*h*434. This may have been due to an epistasis of *ompB* on *tpo* or vice versa. However, a small fraction of the Aro$^+$ colonies did seem to be resistant to the two phages, but this character was unstable and did not withstand reisolation. Therefore, we believe that *ompB101 tpo-11* recombinants have the expected phenotype (resistance to TP1 and λ V*h*434) but that they are strongly counterselected.

tpo mutations affect production of the OmpF and LamB proteins. Several lines of evidence suggested that the TP1 resistance of the *tpo* mutants resulted from a defect in the production of the two proteins which TP1 can use as its receptor.

Firstly, the *tpo* strains produced reduced amounts of OmpF protein (Fig. 1). However, unlike the *ompB101* strain, they still produced the OmpC protein. In fact, they even produced more OmpC protein than did the wild-type strain. The pattern of phage sensitivity (Table 4) was in agreement with these observations, since the *ompB101* strain was resistant both to TuIa (receptor, OmpF protein [4]) and to 434 (receptor, OmpC protein [9]), whereas the *tpo* strains were resistant to TuIa but sensitive to 434.

Secondly, the *tpo* strains were affected in the production of LamB protein. However, this effect of the *tpo* mutations could be partially suppressed by induction of the maltose regulon, to which gene *lamB* belongs. This was demonstrated by the pattern of phage sensitivity (Table 4), the assay of λ receptor activity in vitro (Table 5), and the analysis of crude extracts on polyacrylamide gels (Fig. 1). Under the usual plating conditions, phage λ had a reduced efficiency of plating on *tpo-11* and *tpo-22* strains. The same was true for K10, another phage which uses the LamB protein as its receptor (18). However, if maltose (i.e., an inducer of *lamB* gene expression) was added to the medium, λ and K10 again plated with an efficiency of 1.0 on the *tpo* strains. In fact, these strains also became sensitive to TP1. The *tpo* mutants could also be rendered sensitive to λ, K10, and TP1 by introducing into the strains a *malT^c* allele, which renders constitutive the expression of the maltose regulon (6). The assay of λ receptor (see footnote of Table 5) in vitro demonstrated that, when grown in the absence of maltose, the *tpo* mutants produced 100 to 500 times less LamB protein than a *tpo$^+$* strain. In the presence of maltose, or in *malT^c* strains, the *tpo* mutations reduced only by a factor of three or four the amount of λ receptor.

Effect of *tpo* mutations on the maltose regulon. The maltose regulon is comprised of three operons controlled by the positive regulatory gene *malT*. One operon, *malP-malQ*, is located adjacent to *malT* and codes for the cytoplasmic enzymes maltodextrin phosphorylase and amylomaltase. The other two operons, *malE-malF-malG* and *malK-lamB*, are adjacent to one another at 90 min on the map and



code for proteins involved in the transport of maltose and maltodextrins. The effect of the *tpo* mutations on the production of LamB protein was reported in the preceding section. A very similar effect was obtained on the MalE product, which is a maltose-binding protein located in the periplasm (Table 5; Fig. 1). The *tpo* mutations also affected amylomaltase production (Table 5); however, in this case the effect (a fourfold decrease) only concerned the induced level.

In conclusion, the *tpo* mutations affected strongly the production of the LamB and MalE proteins in the uninduced state, and also, but to a lesser extent, in the induced state. The production of MalQ protein was affected like that of LamB and MalE proteins in the induced state but not at all in the uninduced state.

Although they are pleiotropic, the *tpo* mutations only affected the production of a limited number of proteins. This can be seen in Fig. 1, where the above-mentioned changes in the production of OmpF, OmpC, LamB, and MalE proteins are among the most prominent. In addition, we found that the uninduced and fully induced levels of β-galactosidase were unaffected by the *tpo* mutations (data not shown).

The effect of the *tpo* mutations on *lamB* gene expression is transcriptional. To determine at which level the *tpo* mutations affected the expression of gene *lamB*, we made use of previously described strains carrying gene (or operon) fusions (12a, 19, 26). On the one hand, we used strains in which *lacZ* is under the control of the *malK-lamB* promoter. The production of β-galactosidase was then found to be affected by *tpo* mutations in the same way as is the production of the LamB protein (Table 6). On the other hand, we used a strain in which *lamB* is controlled by the promoter of the *lac* operon. In this case, the production of LamB protein was not significantly affected by the *tpo* mutations, whether the *lac* operon was uninduced or induced with various concentrations of

FIG. 1. *Analysis of cell extracts from wild-type, tpo, and ompB strains. The different strains were grown in 5 ml of M63 glycerol medium plus the appropriate amino acid supplements. When they reached an optical density at 600 nm of 1.0, the cells were harvested and suspended in 0.25 ml of distilled water. Portions (25 μl) of the suspensions were boiled for 5 min in sample buffer and subjected to electrophoresis on a 30-cm-long polyacrylamide slab gel (9% acrylamide) in the presence of sodium dodecyl sulfate by the technique of Laemmli (12) as modified by Anderson et al. (1). The positions of the OmpF, OmpC, MalE, and LamB proteins are shown by arrows. (a) strain pop1389 (ompB101); (b) strain pop1388 (tpo-22); (c) strain pop1387 (tpo-11); (d) strain pop1386 (wild type).*

TABLE 4. *Average plating efficiencies of phages on bacterial strains carrying tpo or ompB mutations*[a]

Bacterial strains	Relevant genotype	Plating efficiency of:				
		TuIa	434	TP1	λ V*ho*	K10
pop1010[b]	*tpo*$^+$ *ompB*$^+$	1	1	1	1	1
		1	1	1	1	1
pop1371	*tpo*$^+$ *ompB101*	10^{-5}	10^{-5}	1	1	1
		10^{-5}	10^{-5}	1	1	1
pop1263	*tpo-11 ompB*$^+$	5×10^{-4}	1	5×10^{-5}	0, 1[c]	5×10^{-4}[c]
		5×10^{-4}	1	0, 1 to 0, 5	1[d]	1[d]
pop1267	*tpo-22 ompB*$^+$	5×10^{-4}	1	5×10^{-5}	0, 1[c]	5×10^{-4}[c]
		5×10^{-4}	1	0, 1 to 0, 5	1[d]	1[d]
pop1385	*tpo*$^+$ *malT1*^{c}	1	1	1	1	1
		NT[e]	NT	NT	NT	NT
pop1384	*tpo-22 malT1*^{c}	5×10^{-5}	1	0, 1 to 0, 5	1[d]	1[d]
		NT	NT	NT	NT	NT

[a] Average plating efficiencies, as determined by spot tests. For each strain, the numbers on the first line give the efficiencies when the cells were grown and plated in the absence of maltose, whereas those on the second line correspond to cells grown and plated in the presence of this sugar.

[b] The plating efficiency on this strain was taken as a reference for all phage strains.

[c] The plaques were turbid.

[d] The plaques were clear.

[e] NT, Not tested.

TABLE 5. *Effect of the tpo mutations on the maltose regulon*[a]

Bacterial strain	Relevant genotype	LamB protein		MalE protein		MalQ protein	
		Uninduced	Induced	Uninduced	Induced	Uninduced	Induced
pop1010	Wild type	900	3,600	182	793	11	239
pop1371	*ompB101*	1,200	3,600	NT[b]	NT	9	166
pop1263	*tpo-11*	12	900	≤30	422	7	69
pop1267	*tpo-22*	2	1,200	≤30	153	12	65
pop1385	*malT1*^{c}	5,400	NT	NT	NT	NT	NT
pop1384	*malT1*^{c} *tpo-22*	1,200	NT	NT	NT	NT	NT

[a] The cells were grown in M63 glycerol (uninduced) and M63 glycerol-maltose (induced) supplemented with appropriate amino acids. The amount of LamB protein (number of λ receptor sites per bacterium) was assayed in cholate-EDTA extracts (16) and calculated as described previously (23). The amount of MalE protein (maltose-binding protein) was determined in the shock fluid and expressed as picomoles of maltose bound per milligram of periplasmic protein (25). The amount of MalQ protein (amylomaltase) was determined in sonic extracts and expressed in units per milligram of protein (21).

[b] NT, Not tested.

isopropyl-β-D-thio-galactoside (Table 7). These results demonstrated that the effect of the *tpo* mutations on *lamB* gene expression depended upon the promoter by which this gene is controlled. Therefore, the *tpo* mutations seem to affect the initiation of transcription of the *malK-lamB* operons rather than the translation of the *lamB* gene or some posttranslational event involved in the export of the LamB protein to the outer membrane.

***tpo* mutants can still accumulate maltose.** The possibility was considered that the effect of the *tpo* mutations on the maltose regulon results from leakiness of the membrane. The effect of these mutations on the basal expression of the *lamB* and *malE* genes would then result from a leakage of a hypothetical internal inducer (22), whereas the effect on the induced expression would result from an inability to concentrate externally added maltose. This interpretation could in fact also account for the effect of *tpo* mutations on the synthesis of OmpF protein, because this synthesis could also be controlled by a low-molecular-weight inducer. However, the experiment shown in Fig. 2 argues against this interpretation. The level of maltose transport in *tpo* cells was lower than in *tpo*$^+$ cells, but this can easily be explained by the presence of

TABLE 6. *Effect of tpo mutations on lamB-lacZ fusions*

Strain	Relevant genotype	Prophage carried[a]	β-Galactosidase activity	
			Uninduced	Induced
pop1406	Wild type	λ p Φ(*lamB-lacZ*$^+$)	21	157
pop1407	*tpo-11*	λ p Φ(*lamB-lacZ*$^+$)	1	55
pop1408	*tpo-22*	λ p Φ(*lamB-lacZ*$^+$)	0	45
pop1409	*ompB101*	λ p Φ(*lamB-lacZ*$^+$)	25	130
pop1390	Wild type	λ p Φ(*lamB-lacZ*)$_{hyb}$61-4	156	1,128
pop1391	*tpo-11*	λ p Φ(*lamB-lacZ*)$_{hyb}$61-4	3	306
pop1392	*tpo-22*	λ p Φ(*lamB-lacZ*)$_{hyb}$61-4	3	259
pop1393	*ompB101*	λ p Φ(*lamB-lacZ*)$_{hyb}$61-4	218	1,400

[a] The notation Φ(*lamB-lacZ*$^+$) corresponds to an operon fusion in which an intact *lacZ* gene is inserted in *lamB*, whereas Φ(*lamB-lacZ*)$_{hyb}$61-4 corresponds to a gene fusion which leads to the synthesis of a *lamB-lacZ* hybrid protein. The strains were grown in M63 glycerol (uninduced) and M63 glycerol-maltose (induced) supplemented with appropriate amino acids. β-Galactosidase activity was assayed by the method of Miller (13). Portions of the cultures were plated and tested to determine the frequency of the prophage loss (less than 1%) and the frequency of the reversion of the *tpo* mutations (less than 1%).

TABLE 7. *Synthesis of LamB protein in lysogens carrying a lacZ-lamB operon fusion*

Strain	Relevant genotype[a]	LamB protein			
		No IPTG	10^{-6} M IPTG	10^{-5} M IPTG	10^{-4} M IPTG
pop1411	*malT tpo*$^+$	4	10	280	2,400
pop1412	*malT tpo-11*	3	6	180	1,800
pop1413	*malT tpo-22*	3	10	180	1,800

[a] The three strains are lysogenic for prophage λ Y*h*°434(lB8) which carries a *lacZ-lamB* operon fusion (12b). The number of phage lambda receptor sites per bacterium (LamB protein) was determined in extracts of cells grown in M63 glycerol containing the indicated concentrations of isopropyl-β-D-thiogalactoside (IPTG).

smaller amounts of the transport proteins in the mutants. The transport of thiomethyl-β-D-galactoside by the *lac* permease was also measured and found to be normal in the *tpo* strains (data not shown). Finally, the expression of the maltose regulon was found to be the same in the presence of 10^{-1} M maltose as it was in the presence of a 10^{-3} or 10^{-2} M concentration of the same sugar (data not shown).

Phenotypic comparison of *tpo*, *perA*, and *ompB* mutants. In all of the experiments described above, the effect of the *tpo* mutations was compared with that of *ompB101*. The only character in common was the reduction in OmpF protein. The *tpo* mutants were also compared with other strains bearing a mutation in the same region. These comparisons were performed after transduction of these other mutations in the same genetic background as that of the *tpo* mutants (pop1010).

The mutants which have been called *ompB* (28) can be subdivided into three clearly distinct classes according to their phenotypes. Those of the first class, of which *ompB101* is a representative, synthesize very little OmpF and OmpC protein. Those of the second class synthesize less OmpC protein but normal or increased amounts of OmpF protein. Those of the third class, iso-

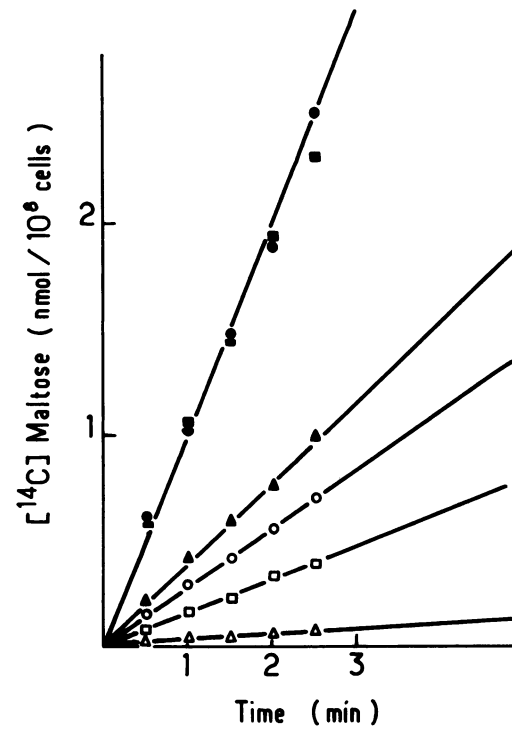


FIG. 2. *Maltose transport in tpo-11, ompB101, and wild-type strains. The assays (27) were performed in the presence of 10^{-5} M [^{14}C]maltose, at 25°C. The cells were grown in glycerol minimal medium (uninduced) or in glycerol-maltose minimal medium (induced). Symbols: pop1386 (wild type), uninduced* (○) *and induced* (●); *pop1387 (tpo-11), uninduced* (△) *and induced* (▲); *pop1389 (ompB101), uninduced* (□) *and induced* (■).

lated as TuIa resistant, synthesize decreased amounts of OmpF protein but not of OmpC protein. Since the mutants in this last class resembled the *tpo* mutants, three of them were analyzed. Two (CE1121 and CE1123) indeed behaved like *tpo* mutants. In the absence of maltose, they were resistant to TP1 and K10 and partially resistant to λ, whereas they were sensitive to these phages in the presence of maltose. These two mutants produced very low levels of LamB protein in the absence of maltose and about four times less LamB protein than the wild-type strain in its presence. By contrast, the other mutant (CE1122) was not affected in the production of LamB protein and was sensitive to TP1, λ, and K10 even in the absence of maltose. Therefore, according to our results, the third class of *ompB* mutants must be further subdivided in two subclasses, one affected in *lamB* expression and the other unaffected.

Another mutant very similar to *tpo* is the *perA* mutant described by Wanner et al. (31). This mutant was isolated as deficient in the production of alkaline phosphatase, but it turned out to have pleiotropic defects, including a decrease in the synthesis of OmpF protein and an increase in that of OmpC protein. The *perA* mutation maps very close to *ompB*. We found that the *perA* mutant behaves like the *tpo* mutants with respect to phage sensitivity and synthesis of LamB protein. In addition, like *perA*, *tpo-11* was found to affect the production of several periplasmic proteins (data not shown), including alkaline phosphatase (B. Wanner, personal communication). Therefore, *tpo* and *perA* apparently lead to identical phenotypes.

Preliminary complementation studies of the *ompB* region. In view of the complexity of the phenotypes which can result from mutations in the *ompB* region, it becomes essential to define the number of cistrons located in this region and the dominance relationship between the different types of mutations. As a first attempt to do so, we performed complementation analyses with *ompB101*, *perA*, and *tpo-11*. The three alleles were transferred onto the KLF41 episome, and the necessary merodiploid strains were all constructed in a *recA* background (for details, see Materials and Methods). The complementation was tested by analyzing the pattern of phage sensitivity of the merodiploid strains and by evaluating the amount of OmpF protein and OmpC protein on polyacrylamide gels (Fig. 3). The results can be summarized as follows. (i) If we consider only the production of OmpF protein, the three mutations are recessive to the wild type. In addition, the *perA* and *tpo-11* mutations both complement *ompB101* but fail to complement one another. The complementing diploids are fully sensitive to TuIa, TP1, and λ and produce wild-type amounts of OmpF protein. (ii) If we consider the production of OmpC protein, the *ompB101* mutation still appears recessive to the wild type since, like the wild-type strains under the same growth conditions, the *ompB*$^+$/*ompB101* merodiploid strains produce much more OmpF protein than OmpC protein. The *perA* mutation, on the other hand, appears dominant to the wild type, since the *perA*$^+$/*perA* merodiploid strains produce more OmpC protein than OmpF protein. The case of *tpo-11* is less clear. In the experiment shown in Fig. 3, *tpo-11* appeared recessive to the wild type. However, in other experiments (not shown here), it behaved like *perA*. Because of this variability, we feel that the results regarding the synthesis of OmpC protein in the various merodiploid strains are inconclusive at present.

DISCUSSION

Mutants resistant to phage TP1 can be isolated in a single step from a wild-type strain.

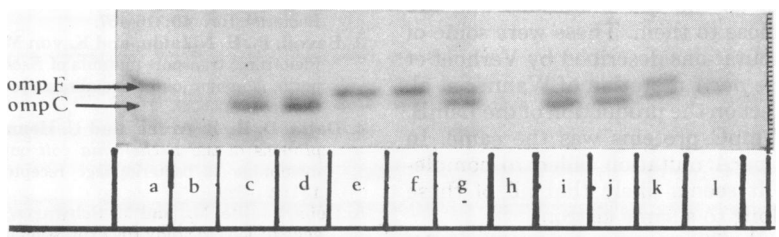


FIG. 3. *Synthesis of OmpF and OmpC proteins by merodiploid strains. The different strains were grown in 5 ml of ML medium. Cell extracts were prepared and subjected to electrophoresis as described in the legend to Fig. 1. Portions of the cultures were plated onto indicator plates to determine the frequency of episome loss, which was less than 1%. The positions of OmpF and OmpC proteins are shown by arrows. (a) pop670 (wild type); (b) pop674 (ompB101); (c) pop678 (tpo-11); (d) pop1639 (perA); (e) pop1665 (F'ompB*$^+$*/ompB101); (f) pop1667 (F'tpo*$^+$*/tpo-11); (g) pop1669 (F'perA*$^+$*/perA); (h) pop1675 (F'ompB101/ompB101); (i) pop1677 (F' ompB101 tpo*$^+$*/ompB*$^+$* tpo-11); (j) pop1679 (F'ompB101 perA*$^+$*/ompB*$^+$* perA); (k) pop1685 (F'ompB*$^+$* tpo-11/ ompB101 tpo*$^+$*); (l) pop1689 (F' tpo-11 perA*$^+$*/tpo*$^+$* perA).*

They produce very small amounts of OmpF and LamB proteins. The mutations, called *tpo*, are located very close to other mutations (*ompB*, *perA*) which affect the production of several envelope proteins including the OmpF protein. We shall first discuss the effect of the *tpo* mutations on the production of LamB protein and then compare the *tpo* mutants with *ompB* and *perA* strains.

The *tpo* mutations led to a marked decrease (about 100 times) in the basal level of *lamB* gene expression. The experiments performed with strains carrying operon or gene fusions strongly suggested that this effect of the *tpo* mutations is at the level of transcription initiation. A similar effect was observed for the basal expression of *malE*, another gene of the maltose regulon, which codes for a periplasmic protein. In contrast, the basal expression of *malQ*, which codes for the cytoplasmic enzyme amylomaltase, was unaffected by the *tpo* mutations. The effect of the *tpo* mutations on *lamB* and *malE* expression was in part suppressed when the maltose regulon was induced. Even under these conditions, however, the *tpo* mutants synthesized three to four times less LamB protein and MalE protein than did the wild type. In this case, the synthesis of MalQ protein was also affected to similar extent.

The effect of the *tpo* mutations on the expression of the maltose regulon did not seem to result from a defect in inducer uptake or retention (Fig. 2). Therefore, the possibility must be considered that the *tpo* gene codes for a protein involved in the transcription of the *mal* operons. The main function of this protein could be to maintain a certain expression of two of the operons, *malEFG* and *malK-lamB*, in the absence of maltose. It should be noted in this respect that the basal expression of these two operons has generally been found to be higher than that of the *malP-malQ* operon (22).

Two classes of mutations seemed to affect the same function as the *tpo* mutations and are located very close to them. These were some of the "*ompB*" mutations described by Verhoef et al. (28) and the *perA* mutation of Wanner et al. (31). Their effect on the production of the LamB, OmpF, and OmpC proteins was the same. In addition, the *perA* mutation failed to complement *tpo-11*. It seems likely that all of these mutations belong to a same cistron.

Verhoef et al. (28) suggested that all of the mutations located in the *ompB* region, and affecting the production of the OmpF protein or the OmpC protein or both, are located in a same regulatory gene. On the other hand, Hall and Silhavy (8; personal communication) provided evidence that the *ompB* region contains at least two different cistrons, both involved in the transcriptional regulation of the *ompC* and *ompF* genes. Our results do not provide further insight regarding the number of cistrons in this region. They demonstrate, however, that one class of the previously known "*ompB*" mutations, i.e., those leading to an $OmpF^-$ $OmpC^+$ phenotype, must now be subdivided in two classes, depending on their effect on *lamB* gene expression.

We concluded above that the *tpo* and *perA* mutations are probably located in a same cistron. On the other hand, we concluded that in the *tpo* mutants it is the transcription of the *lamB* gene that is affected, whereas Wanner et al. concluded that in the *perA* mutant the synthesis of phosphatase is affected at a posttranscriptional level. It seems improbable that the same gene product can act at these two levels. Therefore, it appears likely that one of these two effects or perhaps both are indirect consequences of the *tpo* (*perA*) mutations. In any case, the *ompB* locus now appears to be increasingly complex. Even though *ompB* is mainly involved in the synthesis of envelope proteins, some mutations in this region do affect to some extent the production of amylomaltase, a cytoplasmic protein. The number of polypeptides encoded in this region is unknown. Their function is still a matter of speculation.

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