

# New Locus (*ttr*) in *Escherichia coli* K-12 Affecting Sensitivity to Bacteriophage T2 and Growth on Oleate as the Sole Carbon Source

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The nature of resistance to phage T2 in *Escherichia coli* K-12 was investigated by analyzing a known phage T2-resistant mutant and by isolating new T2-resistant mutants. It was found that mutational alterations at two loci, *ompF* (encoding the outer membrane protein *OmpF*) and *ttr* (T-two resistance), are needed to give full resistance to phage T2. A *ttr::Tn10* mutation was isolated and was mapped between *aroC* and *dsdA*, where the *fadL* gene (required for long-chain fatty acid transport) is located. The receptor affected by *ttr* was the major receptor used by phage T2 and was located in the outer membrane. Phage T2 was thus able to use two outer membrane proteins as receptors. All strains having a *ttr::Tn10* allele and most of the independently isolated phage T2-resistant mutants were unable to grow on oleate as the sole carbon and energy source, i.e., they had the phenotype of *fadL* mutants. The gene *fadL* is known to encode an inner membrane protein. The most likely explanation is that *fadL* and *ttr* are in an operon and that *ttr* encodes an outer membrane protein which functions in translocating long-chain fatty acids across the outer membrane and also as a receptor for phage T2.

Many proteins of the outer membrane of *Escherichia coli* K-12 can serve as receptors, or components thereof, for bacteriophages (34). Most of these proteins are normally involved in the transport of various substrates across the outer membrane or have a structural role (16). Usually, one type of phage recognizes only one type of protein. Exceptions are known in which a phage can use two or three different outer membrane proteins as receptors. This has been demonstrated by the use of mutant strains deficient in one or more well-characterized outer membrane proteins and by the isolation of resistant mutants in strains already lacking one of the receptor proteins (3, 21, 23, 37).

In this communication we describe an analysis of strains resistant to phage T2. This phage has been reported to use the outer membrane protein *OmpF* as its receptor (10), since phage T2-resistant mutants have reduced amounts of or lack this protein, and also purified *OmpF* protein and lipopolysaccharide are able to neutralize the phage. In contrast, however, it is well known that *E. coli* mutants resistant to phage T2 are difficult to isolate and occur at low frequencies (10, 12). This suggested that resistance may involve multiple loci. We have found that mutation at a second locus apart from *ompF* is needed for full resistance to phage T2. This locus, designated *ttr*, also affects growth of cells on oleate as the sole carbon and energy source. The *ttr* locus was mapped very close to *fadL* (1), a gene encoding an inner membrane protein which functions in transporting long-chain fatty acids into *E. coli* cells (28).

## MATERIALS AND METHODS

**Bacterial strains, phages, and growth media.** The bacterial strains used are described in Table 1. The bacteriophages used have been described elsewhere (23), with the exception of T2L, which was obtained from B. Keller (Biozentrum, University of Basel, Switzerland). The growth media used

were L-broth (LB) and the minimal medium was M9 (20). These media were supplemented with 0.2% (vol/vol) Triton X-100 and 0.1% (wt/vol) oleic acid (10% [wt/vol] stock solution in 10% [vol/vol] Triton X-100) as required. Tetracycline was used at 10 µg/ml, but this was increased to 20 µg/ml when *ompF* mutants were contraselected. Liquid cultures were grown at 37°C without aeration unless specified.

**Genetic manipulations.** P1 transduction with phage P1 *vir* and Hfr transfer matings were performed as described previously (26). Transduction with T4GT7 (38) was performed as described for transduction with P1 *vir*. *recA* derivatives of strains P456 and 72-6 were obtained by transduction with phage P1 *vir* grown on strain JC10240 and selection for tetracycline resistance; *recA* transductants were identified by their sensitivity to UV irradiation. Plasmid DNA was extracted by the method of Ish-Horowitz and Burke (11), and bacterial cells were made competent and transformed as recently described (27).

**Transposon insertion mutagenesis: isolation of strain RMT218.** Strain RMT210 was grown to an OD<sub>650</sub> of 0.6 in 30 ml of LB containing 0.2% (wt/vol) maltose. The culture was centrifuged and suspended in 1 ml of LB containing 10 mM MgCl<sub>2</sub> (prewarmed to 42°C). To these cells, 5 × 10<sup>10</sup> PFU of λNK561 phage (7) was added. After 20 min of incubation at 42°C, 20 ml of prewarmed LB was added. After a further 20 min of incubation at 42°C, the cells were collected by centrifugation, and the pellet was suspended in 1 ml of LB. The cells were plated on LB plates containing 10 µg of tetracycline per ml and 10 mM tetrasodium pyrophosphate, and the plates were incubated at 42°C. After 48 h, the resulting tetracycline-resistant colonies (~20,000) were pooled, washed, suspended in a 2:3 mixture of 80% (vol/vol) glycerol and 1% (wt/vol) peptone, and stored frozen. A portion of these cells were diluted into LB, grown for 30 min, and then plated on LB agar plates with phage T2 to isolate resistant mutants. Resistant mutants occurred at a frequency of 5 × 10<sup>-4</sup>. One of these was called RMT218.

**Phage sensitivity testing and phage neutralization.** Sensitivity was quantitated by placing the test culture in an overlay

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TABLE 1. Bacterial strains

Strain	Characteristics <sup>a</sup>	Source <sup>b</sup> or reference
P400	F <sup>-</sup> <i>thi argE proA thr leu mtl xyl galK lacY rpsL supE non</i>	P. Reeves (35)
P460	P400 <i>ompA</i>	P. Reeves (35)
P400-MI	P400 <i>ompA2008</i>	23
P400-6	P400 <i>ompC</i>	32
P456	P400 <i>ompF680</i>	P. Reeves (9)
PS160	F <sup>-</sup> <i>proC leu-6 trpE38 entA thi fep ompF::Tn5</i>	C. F. Earhart (16)
PS170	F <sup>-</sup> <i>proC leu-6 trpE38 entA thi fep ompC::Tn5</i>	C. F. Earhart (16)
72-6	Hfr PO2A <i>fhuA22 phoA8 fadL701 relA1 (malA-glpR)14 pit-10 spoT1 glpT ompF627 (T2')</i>	K. Hantke (5, 34)
P2811	P400 <i>pyrD34 zcb::Tn10-43 rfa</i>	J. Hackett (29)
P2125	F <sup>-</sup> <i>fhuA pyrD</i>	P. Reeves (24)
JC10240	Hfr PO45 <i>thr-300 recA56 srl-300::Tn10 relA1 ilv-318 spoT1 thi-1 rpsE2300</i>	
P1700	P400 <i>trp supE<sup>+</sup></i>	P. Reeves (26)
W620	<i>thi pyrD gltA galK rpsL</i>	H. U. Schairer (30)
AB2557	F <i>purF1 dsdA1 aroC4 ilvD188 thi-1 lacY1, or lacZ4, malA1 xyl-7 mtl-2 rpsL8, rpsL9 or rpsL14, fhuA2 or fhuA22, tsx-23 or tsx-25, supE44</i>	B. Bachmann
CC673	<i>araD139 ΔlacU169 relA rpsL thi ompA927</i>	C. Manoil (20)
RMT196	P2125 <i>pyrD<sup>+</sup></i>	CC673, T4GT7
RMT209	P2125 <i>pyrD<sup>+</sup> ompF680</i>	P456, T4GT7
RMT210	P2125 <i>pyrD<sup>+</sup> ompF627</i>	72-6, P1 <i>vir</i>
RMT218 <sup>c</sup>	RMT210 <i>ttr::Tn10</i>	
RMT237	RMT196 <i>ttr::Tn10</i>	RMT218, P1 <i>vir</i>
RMT238	RMT209 <i>ttr::Tn10</i>	RMT218, P1 <i>vir</i>
RMT239	RMT210 <i>ttr::Tn10</i>	RMT218, P1 <i>vir</i>
RMT240	P1700 <i>ttr::Tn10</i>	RMT218, P1 <i>vir</i>
RMT241	P1700 <i>ttr::Tn10</i>	RMT218, P1 <i>vir</i>
RMT242	W620 <i>ttr::Tn10</i>	RMT218, P1 <i>vir</i>
RMT247	P456 <i>ttr::Tn10</i>	RMT218, P1 <i>vir</i>
RMT253, 254, 256, 261, 262, 267 <sup>d</sup>	P456	
KL983	Hfr <i>xyl lac mglP</i>	B. Low (13)
KL16	Hfr <i>thi rel</i>	B. Low (13)

<sup>a</sup> Nomenclature is according to Bachmann (1).

<sup>b</sup> Strains were obtained by transduction with the strains listed as the donor and the indicated phage. Selection was for tetracycline resistance.

<sup>c</sup> Construction described in Materials and Methods.

<sup>d</sup> Selected as phage T2 resistant.

on LB agar plates, and then serially 10-fold-diluted portions of phage were applied. Phage neutralization was performed as described recently (23). Bacterial culture ( $5 \times 10^8$  to  $10^9$  cells, treated with chloroform), or outer membrane material was incubated with  $5 \times 10^5$  PFU of phage T2 in a volume of 2 ml. After incubation at 37°C for 60 min, 0.1-ml samples were plated with strain P400 to determine the fraction of surviving phage.

**Isolation of phage-resistant mutants.** About  $10^8$  cells from independently grown cultures were plated with  $10^9$  PFU of phage T2 on LB plates. After incubation at 37°C for 20 h, several resistant colonies from each plate were streak-purified several times before retesting.

**Preparation of outer membranes.** Outer membranes were prepared from 100 ml of cells grown to an OD<sub>650</sub> of 1.0 in LB essentially by the method of Manning et al. (18). The insoluble material obtained after extraction of whole-cell envelopes with Triton X-100 and MgCl<sub>2</sub> was called outer membrane material. It was suspended in distilled water and stored at -20°C. The amount of protein present was determined by the method of Lowry et al. (14) with bovine serum albumin as the standard. This material was used for the phage neutralization experiments described in Table 5. For small-scale preparation of outer membranes, bacterial cells (10 ml) were grown in the appropriate medium for 16 h with aeration. Cell envelopes were then prepared as described previously (26) by a procedure which involves treatment with lysozyme and EDTA. The cell envelopes were then

extracted with Triton X-100 and MgCl<sub>2</sub> as described previously (18). The resulting insoluble material was washed once with distilled water and finally suspended in sample buffer. Samples were boiled before being loaded onto polyacrylamide gels.

**Proteinase K treatment.** Cells were treated with proteinase K (75 μg/ml) for 30 min as described previously (27). The enzyme was then inhibited with 2 mM phenylmethylsulfonyl fluoride which was also included in all solutions used for isolation of outer membranes from treated and control cells. All samples were resuspended in sample buffer containing 2 mM phenylmethylsulfonyl fluoride.

**SDS PAGE.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as recently described (27) with 11% linear acrylamide gels. Gels were stained in 0.1% (wt/vol) Coomassie blue R250-25% (vol/vol) isopropanol-10% (vol/vol) acetic acid, then briefly destained in the same solution without dye, and finally destained in 7% (vol/vol) acetic acid. For estimating molecular weights, the following standard proteins (Combithek, Boehringer Mannheim) were used: soy bean trypsin inhibitor (molecular weight 20,100), lactate dehydrogenase (36,500), glutamate dehydrogenase (55,400), and phosphorylase B (97,400).

## RESULTS

**Is the OmpF protein the only receptor for phage T2?** While studying the effect of proteinase K treatment on the ability of

TABLE 2. Phage plating ability of *E. coli* K-12 mutants

Strain (relevant marker)	Sensitivity or resistance to phage <sup>a</sup> :		
	T2 <sup>b</sup>	Tula	Tulb
P400	S	S	S
P400.6 ( <i>ompC</i> )	S	S	R
P456 ( <i>ompF</i> )	S	R	S
PS170 ( <i>ompC::Tn5</i> )	S	S	R
PS160 ( <i>ompF::Tn5</i> )	S	R	S
72.6 ( <i>ompF</i> )	R	R	S

<sup>a</sup> S, Fully sensitive; R, fully resistant.<sup>b</sup> The same results were obtained with phage T2 from K. Hantke (Tübingen) and T2L phage from B. Keller (Basel).

cells to neutralize phages (27), we also tested phage T2. Cells of strains P460 or P400-MI were treated with proteinase K and subsequently assayed for their ability to neutralize T2. The results (data not shown) showed that proteinase K digestion greatly decreased the ability of the cells to neutralize the phage. We have shown by SDS-PAGE that this treatment has no detectable effect on the OmpF protein in intact cells (27), and the same was observed in this instance (data not shown). We then determined the efficiency of plating of phage T2 in comparison to phages Tula (OmpF specific) and Tulb (OmpC specific) (6) on various mutant strains. Whereas phage Tula was unable to grow on the *ompF* mutants (strains P456, PS170, and 72-6), phage T2 grew on all strains except 72-6 (Table 2).

Strain 72-6 is derived from an old *E. coli* K-12 cell lineage (B. Bachmann, personal communication) and the T2<sup>r</sup> marker arose very early in its history. Since the data suggested that the OmpF protein was not the phage T2 receptor but biochemical evidence has demonstrated that it can be a phage T2 receptor (10), a genetic analysis of phage T2 resistance was undertaken.

**Genetic analysis of resistance to phage T2.** The *ompF* alleles from strains P456 and 72-6 were transferred into strain P2125 by cotransduction with *pyrD*. The transductants tested were all sensitive to phage T2, but resistance to phage Tula was cotransduced (Table 3, data for P456 not shown). Strain 72-6 was transduced to tetracycline resistance with T4GT7 grown on P2811, having *Tn10* near *ompF*<sup>+</sup>. In this case, sensitivity to both phages Tula and T2 was cotransduced into this strain (Table 3). Finally, a recombinant plasmid carrying the *ompF* gene, pJP33 (36), was transformed into *recA* derivatives of strains P456 and 72-6. The resulting transformants were found to be sensitive to phages T2 and Tula in the 72-6 derivative and sensitive to phage Tula in the P456 derivative. In summary, these data suggested that the OmpF protein can act as a phage T2 receptor, but that it is not the only receptor for the phage.

**Isolation of *ttr::Tn10*.** To facilitate genetic mapping of a

TABLE 4. Phage sensitivity of strains with the *ttr::Tn10* insertion

Strain (relevant markers)	Sensitivity <sup>a</sup> to phage:	
	T2	Tula
RMT196 ( <i>ompF</i> <sup>+</sup> <i>ttr</i> <sup>+</sup> )	S	S
RMT237 ( <i>ttr::Tn10</i> )	0.1Z	S
RMT209 ( <i>ompF</i> )	S	R
RMT238 ( <i>ompF ttr::Tn10</i> )	R	R
RMT210 ( <i>ompF</i> )	S	R
RMT239 ( <i>ompF ttr::Tn10</i> )	R	R
RMT218 ( <i>ompF ttr::Tn10</i> )	R	R
P456 ( <i>ompF</i> )	S	R
RMT247 ( <i>ompF ttr::Tn10</i> )	R	R
P1700 ( <i>ompF</i> <sup>+</sup> <i>ttr</i> <sup>+</sup> )	S	S
RMT240 ( <i>ttr::Tn10</i> )	0.3Z	S
W620 ( <i>ompF</i> <sup>+</sup> <i>ttr</i> <sup>+</sup> )	S	S
RMT242 ( <i>ttr::Tn10</i> )	0.3Z	S

<sup>a</sup> S, Sensitive; R, resistant. Numbers indicate the efficiency of plating relative to the parental strain (RMT196 for RMT237, P456 for RMT247, P1700 for RMT240, and W620 for RMT242); Z indicates smaller plaque size.

second locus presumably involved in phage T2 receptor activity, a *Tn10* insertion mutation in such a locus was isolated. An *ompF* mutant strain, RMT210, was mutagenized with transposon *Tn10* by the use of  $\lambda$  NK561. In the resulting pool of cells, T2-resistant mutants were detected at a frequency of  $5 \times 10^{-4}$ . P1 *vir* transducing phage were grown on one of these isolates (strain RMT218), and the lysate was used to transduce the *ompF* mutants RMT209, RMT210, and P456 as well as the *ompF*<sup>+</sup> strain RMT196 to tetracycline resistance. Analysis of the transductants showed that resistance to phage T2 was transduced into RMT209, RMT210, and P456, but RMT196-derived transductants remained phage T2 sensitive; other *ompF*<sup>+</sup> strains with the *ttr::Tn10* allele gave the same result (Table 4).

These data confirmed our prediction that a second locus apart from *ompF* was needed for phage T2 sensitivity. This new locus was called *ttr*.

The *ttr::Tn10* insertion in strain RMT218 was located on the chromosome (2) by using strain RMT241 in matings with several Hfr donors. Of nine different donors tested (strains KL99, Hfr6, HfrH, RA-2, KL25, KL208, KL96, KL983, and KL16 [13]), only Hfr strains KL983 and KL16 could transfer tetracycline sensitivity to strain RMT241. Hence, the *ttr::Tn10* allele is located between min 45 and 51.5 on the *E. coli* genetic map (2).

The *ttr::Tn10* allele was located more precisely by transduction. Transducing phages P1 *vir* and T4GT7 grown on strains RMT218 and RMT247, respectively, were used to transduce strains with suitable markers located in the above-mentioned region of the chromosome. No linkage between tetracycline resistance and *ompC*, *gyrA*, or *his* was detected.

TABLE 3. Transduction mapping of T2<sup>r</sup> in strain 72-6

Donor	Recipient	Selected marker <sup>a</sup>	Transductant classes <sup>b</sup> (no. with phenotype/no. tested)			
			T2 <sup>r</sup> Tula <sup>r</sup>	T2 <sup>r</sup> Tula <sup>s</sup>	T2 <sup>s</sup> Tula <sup>r</sup>	T2 <sup>s</sup> Tula <sup>s</sup>
72-6 (T2 <sup>r</sup> Tula <sup>r</sup> )	P2125	<i>pyrD</i> <sup>+</sup>	0/36	13/36	23/36	0/36
P456 (T2 <sup>s</sup> Tula <sup>r</sup> )	RMT199	<i>pyrD</i> <sup>+</sup>	0/20	9/20	11/20	0/20
P2811	72-6 (T2 <sup>r</sup> Tula <sup>r</sup> )	Tet <sup>r</sup>	20/40	20/40	0/40	0/40
P2811	72-6 (T2 <sup>r</sup> Tula <sup>r</sup> )	<i>fadL</i> <sup>+</sup>	0/20	0/20	20/20	0/20

<sup>a</sup> Transducing phage were grown on the indicated donor strain, and the lysate was used to transduce the indicated recipient strain: *pyrD*<sup>+</sup>, selected on minimal glucose medium; Tet<sup>r</sup>, selected on LB agar with tetracycline; *fadL*<sup>+</sup>, selected on minimal Triton X-100-oleate medium.

<sup>b</sup> Transductants were scored by cross-streaking against phage Tula to score *ompF* and against phage T2.

TABLE 5. Neutralization of phage T2 by cells or outer membranes<sup>a</sup>

Strain	% Surviving phage			
	Cells	Outer membranes <sup>b</sup>		
		200 µg	20 µg	2 µg
RMT196	0.3	0.3	0.3	3
RMT210 ( <i>ompF</i> )	0.9	0.3	0.3	12
RMT237 ( <i>ttr</i> )	49	52	92	100
RMT239 ( <i>ttr ompF</i> )	100	100	100	100
RMT218 ( <i>ttr ompF</i> )	100	100	100	100
72-6	100	100	100	100

<sup>a</sup> Phage neutralizations were performed as described in Materials and Methods, except that cells (LB grown) were at 10<sup>9</sup>/ml and outer membranes derived from the strains indicated (0.1 ml, in water) were made up to 1 ml with LB and were used after treatment with chloroform. Results are expressed relative to controls containing LB only.

<sup>b</sup> Amount of protein in a 2-ml incubation mixture.

Transduction into strain AB2557 showed that the *ttr::Tn10* allele was linked to *aroC*, *dsdA*, and *purF*, with linkages of 47, 55, and 25%, respectively (a total of 100 transductants were screened for each marker). The order of the markers is thus probably *dsdA-ttr::Tn10-aroC-purF*.

The only other marker known to be in this region is *fadL* (1). Since strain 72-6 is known to harbor an *fadL* mutation (B. Bachmann, personal communication), we investigated the possibility that *ttr* and *fadL* are related.

**Resistance to phage T2 and inability to grow on oleate.** Strains with and without the *ttr::Tn10* mutation were tested for their ability to grow with oleate as the sole carbon and energy source. All strains carrying *ttr::Tn10* were unable to grow on oleate (OA<sup>-</sup> phenotype). Strain 72-6 was confirmed as being *fadL* since it also did not grow on oleate minimal medium. Strain 72-6 was then transduced with T4GT7 grown on strain P2811, and *fadL*<sup>+</sup> transductants were selected. All transductants tested were phage T2 sensitive but had remained phage T2 resistant (Table 3).

From these results we conclude that either the *ttr* mutation is the same as *fadL* or that a mutational alteration in one locus is polar on the other locus. To determine whether mutational alterations like *ttr::Tn10* causing an OA<sup>-</sup> phenotype are common when selection is made for resistance to phage T2, the *ompF* strain P456 (10 independent cultures) was plated with phage T2. Four mutants from each selection were tested for their ability to grow on oleate minimal medium. Each selection resulted in mutants that were resistant to phage T2 and unable to grow on oleate as the sole carbon and energy source. However, one mutant strain was isolated that was phage T2 resistant but could grow on oleate minimal medium. This experiment confirmed that the OA<sup>-</sup> phenotype of strains with *ttr::Tn10* is very common among phage T2-resistant mutants isolated in an *ompF* background. The *ttr/fadL* locus is thus needed for phage T2 sensitivity in *ompF* mutants. Since *fadL* is known to encode an inner membrane protein involved in long-chain fatty acid transport into *E. coli* cells (4, 8), we hypothesized that *ttr* may affect an outer membrane protein in this system, by analogy with several other transport systems (15).

**Relative roles of *ompF* and *ttr* in phage T2 receptor activity.** Tables 2 and 4 show that an *ompF* mutation had no effect on the efficiency of plating of phage T2. However, a *ttr* mutation alone had a significant effect on the efficiency of plating of the phage (Table 4). This was confirmed by examining phage neutralization. Cells or isolated outer membranes from strain RMT210 (*ompF*) could inactivate phage T2 much more

efficiently than cells or outer membranes from strain RMT237 (*ttr::Tn10*) (Table 5). Cells or outer membranes from strains RMT239 (*ompF ttr::Tn10*), RMT218, and 72-6 were unable to inactivate phage T2.

The results suggested that the major receptor for phage T2 is the *ttr* component rather than the *OmpF* protein. This explains why proteinase K treatment of intact cells had a drastic effect on phage T2 receptor activity without cleaving the *OmpF* protein.

**Outer membrane proteins induced by growth on oleate.** The *fadL* gene has been relatively well characterized (4, 8, 28) and encodes a 35-kilodalton (kDa) inner membrane protein which is needed to transport C<sub>12</sub> or greater chain length fatty acids into the cell. The transport system is inducible by long-chain fatty acids (17). Since *ttr* appears to affect an outer membrane component, we grew cells in LB medium with and without oleate and examined their outer membrane protein composition electrophoretically. A protein of about 52 kDa and several proteins of high molecular weight were induced in parental but not *ttr::Tn10* transductant strains (Fig. 1). The former protein was also seen in the outer membranes from uninduced cells of certain wild-type strains (RMT196 and RMT210). In other outer membrane preparations the latter proteins were not easily detected, but the induced 52-kDa protein was always seen.

This observation was confirmed by examining outer membranes isolated from oleate-grown cells of strain P456 and its phage T2-resistant derivatives. A 52-kDa protein was present in strain P456 (Fig. 2). Strains RMT247, RMT253, RMT254, RMT256, RMT261, and RMT267 did not produce the 52-kDa protein; however, strain RMT262 (Fig. 2, lane 8) produced reduced amounts of the 52-kDa protein. As mentioned above, this mutant strain was phage T2 resistant but grew on oleate minimal medium.

In summary, an outer membrane protein of 52 kDa can be induced by growth with oleate in parental but not in phage T2-resistant mutant strains having the OA<sup>-</sup> phenotype (i.e., typical *ttr* mutants). Other membrane protein alterations

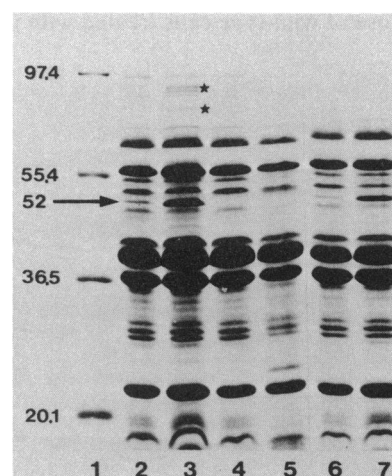


FIG. 1. SDS-PAGE of outer membranes of wild-type, *ttr::Tn10*, and *ompF* mutant strains. The membranes were derived from cells grown in LB plus Triton X-100 with and without oleate. Lanes: 1, standard proteins; 2, RMT196; 3, RMT196 plus oleate; 4, RMT237 (*ttr::Tn10*); 5, RMT237 plus oleate; 6, RMT210 (*ompF*); 7, RMT210 plus oleate. The 52-kDa protein is indicated by the arrow, and high-molecular-weight proteins induced by oleate are shown by stars. Molecular sizes (in kilodaltons) are shown to the left.

have also been detected. One of these proteins may be a candidate for the second outer membrane protein receptor for phage T2.

**Effect of proteinase K on oleate-induced outer membrane proteins.** Since the phage T2 receptor is apparently inactivated by proteinase K (see above), we examined the effect of proteinase K on the outer membrane proteins of cells grown with oleate. Oleate-grown cells of strains RMT196 and RMT237 were treated with proteinase K. Outer membranes were then isolated and analyzed by SDS-PAGE. Several proteins were cleaved, including OmpC protein (27), but not the OmpF protein (Fig. 3). The oleate-induced 52-kDa protein and higher-molecular-weight proteins were cleaved by proteinase K. The results clearly indicate that these proteins are cell surface exposed. The OmpA protein remained undigested, an indicator that the enzyme acted only at the cell surface (27).

The effect of proteinase K on the ability of the cells to inactivate phage T2 was also examined. Proteinase K treatment reduced the ability of RMT196 cells to inactivate phage T2 almost 100-fold. The residual phage T2 neutralization activity of RMT237 cells (Table 5) was not affected by proteinase K treatment and was comparable to that found for proteinase K-treated RMT196 cells. Hence, proteinase K treatment closely simulated a *ttr* mutation in its effect on phage T2 receptor activity.

## DISCUSSION

The results presented in this communication demonstrate that phage T2 is able to use two different cell surface components as receptors. One of these receptors is the OmpF protein, as has been reported (10). We have found that the major receptor is a second outer membrane protein whose presence is affected by mutation at the *ttr* locus. Thus, complete resistance to phage T2 only occurs when both *ompF* and *ttr* loci are mutated. This also explains the observation that mutation to resistance to phage T2 is very rare. Outer membranes from strains with the *ttr*::Tn10 mutation were able to inactivate phage T2 only poorly and resembled those of wild-type cells treated with proteinase K

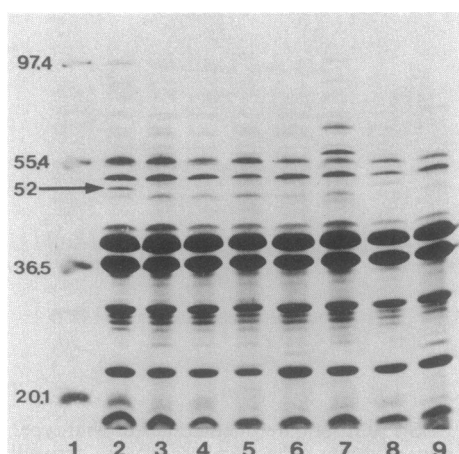


FIG. 2. SDS-PAGE of outer membranes of strain P456 (*ompF*) and phage T2-resistant mutants obtained from it. The membranes were isolated from cells grown as indicated in the legend to Fig. 1. Lanes: 1, standard proteins (see Fig. 1); 2, P456; 3 through 9, RMT247, 253, 254, 256, 261, 262, and 267, respectively. Arrow, 52-kDa protein.

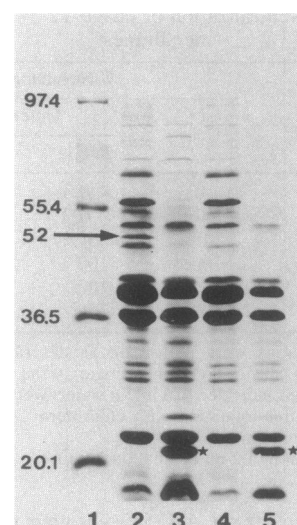


FIG. 3. SDS-PAGE of outer membranes from cells grown with oleate and treated with proteinase K. Lanes: 1, standard proteins (see Fig. 1); 2, RMT196; 3, RMT196 treated with proteinase K; 4, RMT237 (*ttr*::Tn10); 5, RMT237 treated with proteinase K. The OmpC and OmpF proteins were not separated in this gel system; however, efficient cleavage of the OmpC protein can be seen from the presence of one of the resulting fragments (stars in lanes 3 and 5) (27). Arrow, 52-kDa protein.

in this respect. This demonstrates that the *ttr* mutation results in a defective outer membrane receptor for phage T2.

The relationship between *ttr* and *fadL* is interesting but not yet clear. The *ttr*::Tn10 mutation results in resistance to phage T2 (in an *ompF* background), absence of a 52-kDa outer membrane protein, and inability to grow on a long-chain fatty acid as the sole carbon and energy source. The *fadL* mutation in strain 72-6 may be responsible (together with *ompF*627) for the T2 resistance phenotype of this strain, because all *fadL*<sup>+</sup> transductants were sensitive to phage T2. Alternatively, strain 72-6 has a *ttr* mutation which is closely linked to *fadL*. The *ttr*::Tn10 allele was located on the genetic map between *aroC* and *dsdA*, which is identical to the map position of *fadL* (28); hence, *ttr* may be present in an operon with the latter gene.

The *ttr* locus affects the expression of a 52-kDa outer membrane protein which itself is induced by growing wild-type (*ttr*<sup>+</sup>) strains with oleate. Ginsburg et al. (8) observed that wild-type cells produced a 33-kDa inner membrane protein and a 57.5-kDa outer membrane protein when induced by growth with oleate. The former protein was absent in all *fadL* mutants, whereas the latter 57.5-kDa protein was absent in only 2 of 16 independent *fadL* mutants. The 57.5-kDa outer membrane protein observed by these authors most likely corresponds to the 52-kDa outer membrane protein reported here. The discrepancy in the molecular size of the protein is probably due to the membrane sample preparation prior to electrophoresis: our samples were solubilized at 100°C, whereas Ginsburg et al. (8) solubilized their membrane samples at 25°C. We have also observed other changes in the outer membrane protein profile from wild-type and *ttr*::Tn10 mutant strains grown with oleate, although the effect on the 52-kDa protein was the most prominent. Finally, the 52-kDa protein was cell surface exposed, since it was readily cleaved by proteinase K acting on intact cells.

By analogy with other transport systems which have an

outer membrane component, we propose that the transport system for long-chain fatty acids has an outer membrane component(s). This component is a protein which also acts as a receptor for phage T2 and is affected by the *ttr* locus. Although the best candidate for the major T2 receptor is the 52-kDa protein, we did not demonstrate this directly.

One observation is seemingly at variance with the proposal that the major receptor for phage T2 is a component of the system used for the uptake of long-chain fatty acids, namely, that selection for T2 resistance very often but not always yielded the OA<sup>-</sup> phenotype. However, if the *ttr* product is required for growth on oleate, the protein could easily undergo mutational alterations affecting T2 resistance but not affecting uptake of the fatty acid.

T2 may now be added to the list of phages which are able to recognize more than one outer membrane protein (see Introduction). As discussed previously, one possibility for this ability would be that such proteins expose areas at the cell surface which are homologous (22). However, evidence has been presented that phage T4 can recognize the outer membrane protein OmpC or lipopolysaccharide (39), completely unrelated structures. Hence the interesting alternative exists that a phage may possess more than one receptor recognition site. This possibility is consistent with the recent demonstration that the host range of a T-even-type phage can be determined by more than one gene (31).

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