

## *fii*, a Bacterial Locus Required for Filamentous Phage Infection and Its Relation to Colicin-tolerant *tolA* and *tolB*

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We describe mutations in a new bacterial locus, designated *fii*, which do not allow the filamentous bacteriophage f1 to infect bacteria harboring the F plasmid. Mutations at this locus do not affect the ability of F plasmid-containing bacteria to undergo conjugation or be infected by the F plasmid-specific RNA phage f2. The filamentous phage can still adsorb to the F sex pilus, but the DNA is unable to enter the bacteria. All *fii* mutants become tolerant to colicins E1, E2, and E3. Strains with amber mutations in *fii* also are unable to plaque P1, even though they can be infected with this phage. Mutations in *fii* also prevent infection of bacteria harboring the N plasmid by the filamentous bacteriophage IKe. The *fii* locus maps adjacent to *tolA*, mutants of which demonstrate tolerance to high levels of the E and K colicins. The three genes *tolA*, *tolB*, and *fii* are shown to reside on a 4.3-kilobase fragment of the *Escherichia coli* chromosome. Each gene has been cloned into a chimeric plasmid and shown to complement, in *trans*, mutations at the corresponding chromosomal locus. Studies in maxicells show that the product of *fii* appears to be a 24-kilodalton protein which copurifies with the cell envelope. The product of *tolA* has been identified tentatively as a 51-kilodalton protein. Data from cloning, Tn5 mutagenesis, and P1 transduction studies are consistent with the gene order *sucA-fii-tolA-tolB-aroG* near 17 min on the *E. coli* map.

Most of the single-stranded DNA filamentous phage that have been described infect bacteria which harbor conjugative plasmids. The specificity of a particular phage for its host appears to reside in the type of pilus encoded by the plasmid (11, 46). Thus, the filamentous phage f1, fd, and M13 infect cells which produce the F pili encoded by the F plasmid, whereas phage IKe infects bacteria containing the N plasmid and consequently producing N pili.

Infection of *Escherichia coli* by f1 apparently is initiated by the binding of one end of the phage to the tip of the F pilus (7, 42). This binding involves the amino-terminal portion of the phage gene III protein (1, 15, 16, 29, 33), which has been shown to reside at one end of the bacteriophage (14, 48). It is postulated that the filamentous phage is brought to the cell surface by progressive depolymerization of the pilus (5, 17, 24), perhaps by using the same mechanism of pilus retraction hypothesized to occur in the formation of mating pairs during conjugation (5, 10, 24, 47). Once at the cell surface, the phage presumably interacts with some sort of receptor or infection site which allows the DNA to enter the cytoplasm. During this process, the virion proteins depolymerize and become incorporated into the bacterial membrane (38, 41, 46).

The nature of the infection site at the bacterial surface is unknown. It might be part of the protein membrane complex used during the normal conjugation mechanism and consequently be encoded in the *tra* operon on the F plasmid. Alternatively, the proteins involved in such an infection site may be encoded on the chromosome; this would be consistent with the early observation that partial disruption of bacteria lacking pili makes them efficient hosts for M13 phage (23). One possible component of such an infection site is the product of the *tolA* chromosomal locus. This protein is required for the E and K colicins to be able to reach their target of action after these bacteriocins have adsorbed to the bacteria (18, 28). Mutants at the *tolA* locus not only become

tolerant to the E and K colicins but also are unable to be infected by f1 (39, 50). These facts, together with the observation that bacteria infected with f1 phage or bacteria containing a plasmid expressing the phage gene III protein are tolerant to reasonably high concentrations of colicins E1, E3, and K, suggest that gene III protein may interact with the *tolA* product during infection (4, 50).

To help clarify the nature of the infection process, we have attempted to isolate mutants specifically defective for infection by the filamentous phage. In this paper we described the characterization of a chromosomal locus, *fii*, mutations in which render the cells resistant to infection by phage f1 and IKe but allow infection by the male-specific icosahedral RNA phage f2 or other single-stranded DNA phage such as St-1 and φX174. This gene is located adjacent to *tolA*, and a 4.3-kilobase (kb) fragment of chromosomal DNA has been isolated which contains the *fii*, *tolA*, and *tolB* genes. The product of the *fii* gene is a 24-kilodalton (kDa) protein which appears to interact with the bacterial envelope.

### MATERIALS AND METHODS

**Bacteria, bacteriophage, and plasmids.** The bacterial strains used in this study are listed in Table 1. They were obtained from B. Bachmann (Yale University), M. Russel (The Rockefeller University), R. Konings (University of Nijmegen, The Netherlands), and D. Steege and J. Walsh (Duke University). The filamentous bacteriophage f1, a gene VII amber mutant, R100, and the RNA bacteriophage f2 were obtained from N. Zinder (The Rockefeller University). Bacteriophage St-1, φX174, P1 (Cm<sup>r</sup> *clr-100*) and λ467 (cI857 *b221 Oam29 Pam80 rex::Tn5*) were gifts from M. Russel. The filamentous phage IKe was a gift from R. Konings.

Plasmids pUC9 and pUC18 were obtained from J. Messing (30, 43). J. I. Horabin (this laboratory) supplied pJIH12. This plasmid is essentially a derivative of pSCC31 (8), which contains the gene for the *EcoRI* restriction endonuclease under control of the left promoter of phage λ. It also contains

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TABLE 1. *E. coli* strains

Strain	Relevant characteristics	Construction and immediate source
P90C	<i>ara</i> $\Delta$ ( <i>lac pro</i> ) <i>thi</i>	D. Steege
GM1	P90C/F' <i>lac pro</i>	D. Steege
JM1	F <sup>-</sup> <i>rpsL pro lac thr leu</i>	P. Modrich
TPS13	GM1 <i>fii-1</i>	UV mutagenesis
TPS30, -56, -58, -66, -94	GM1 <i>fii-2</i> through -6	Ethyl methanesulfonate mutagenesis
JE2571/N <sub>3</sub>	<i>leu thr</i> /N <sub>3</sub>	R. N. H. Konings
W3110::Tn10	Wild-type <i>E. coli</i> K-12::Tn10	J. Walsh
C131	TPS13 <i>fii<sup>+</sup> zbg-1::Tn10</i>	Transduction by P1 (W3110::Tn10 pool)
T131, T30, T56, T58, T66, T946	TPS13, -30, -56, -58, -66, -94, <i>zbg-1::Tn10</i>	Transduction by P1 (C131)
X697	F <sup>-</sup> <i>leuB purE trpE his argG meta</i> (or -B) <i>lacY galK rpsL</i>	B. J. Bachmann
Hfr Broda 8, KL99	Hfr	B. J. Bachmann
JRG94	F <sup>-</sup> <i>bioA gal chlD</i>	B. J. Bachmann
AB3257	F <sup>-</sup> <i>aroG galK</i>	B. J. Bachmann
WGA <sub>suc26</sub>	<i>sucA8 gal-25</i>	B. J. Bachmann
K37, K38	HfrC <i>supD</i> ; HfrC <i>sup<sup>+</sup></i>	This laboratory
A592	F <sup>-</sup> <i>tolA</i>	B. J. Bachmann
A593	F <sup>-</sup> <i>tolB</i>	B. J. Bachmann
A5921, A5931	A592, A593/F <sup>+</sup> ::Tn10	F <sup>+</sup> ::Tn10 was transferred from K866 (M. Russel)
A5922, A5932	A592, A593 <i>recA56 srl-300::Tn10</i>	P1 transduction from strain JC10240 (M. Russel)
TPS1301, TPS9401	TPS13, TPS94 <i>recA56 srl-300::Tn10</i>	P1 transduction from strain JC10240 (M. Russel)
KMBL3752	<i>fii<sup>+</sup> tolA<sup>+</sup> tolB<sup>+</sup></i>	P. Modrich
CSR603	<i>recA uvrA</i>	D. Steege

the *cI857* repressor gene. There is one *Bgl*II site in the plasmid, and this is contained in the *Eco*RI gene.

**Media and chemicals.** Bacteria were routinely grown in tryptone broth (50) or LB medium (26). The Pro<sup>-</sup> MTPA salts medium was that described by Vinuela et al. (44), but supplemented with 0.2% glucose and 1 mM of each amino acid except proline. Antibiotics were used in the following concentrations: ampicillin (60 µg/ml), tetracycline (20 µg/ml), kanamycin (50 µg/ml), and streptomycin (200 µg/ml). Deoxycholate sensitivity was scored on LB plates containing 0.5% deoxycholate. Transductions mediated by P1 (Cm<sup>r</sup> *clr-100*) were performed by the method of Miller (26), except that the transduction mixtures were plated onto DO salts-based plates (45) supplemented with 0.2% Casamino Acids, 0.2% glucose, and antibiotics as required. The TG salts medium used in maxicell experiments was as described by Garen and Garen (13), except that peptone was omitted. L-[<sup>35</sup>S]methionine (1,000 Ci/mmol) was purchased from New England Nuclear Corp., and [<sup>32</sup>P]ATP (9,000 Ci/mmol) was the gift of P. Modrich (Duke University). Restriction endonucleases and other enzymes used in the cloning and physical mapping studies were obtained from Bethesda Research Laboratories and P-L Biochemicals Inc. Purified colicins E1, E2, and E3 were gifts of K. Jakes (The Rockefeller University).

**Isolation of f1-resistant mutants.** UV mutagenesis and ethyl methanesulfonate mutagenesis were performed on strain GM1 by the methods of Miller (26) to give survivals of 1 and 50%, respectively. After overnight growth at 42°C in Pro<sup>-</sup> MTPA medium to permit segregation, the mutagenized culture was diluted 20-fold in tryptone broth, and growth was allowed to continue at 42°C. When the bacteria reached a concentration of  $2 \times 10^8$  cells per ml, the culture was infected with R100 (multiplicity of infection of 100) and incubated for 60 min to achieve 95% killing. Infection of nonpermissive hosts with different filamentous phage amber mutants has been shown to result in termination of cell growth (32). The culture was then washed, diluted, plated on

Pro<sup>-</sup> MTPA plates, and incubated at 42°C overnight. Streaks of individual bacterial colonies were tested for sensitivity to f2 phage at 34°C. Colonies that grew f2 were streaked to test their sensitivity to both f1 and f2 phages at 42°C. Those which grew f2 but not f1 were retested by spot testing on lawns of the mutant bacteria. To determine whether the mutation resided on the chromosome or the F' plasmid, each mutant was conjugated with JM1, and the transconjugants tested for f1 and f2 plaque formation at various temperatures. Chromosomal mutations which did not allow f1 infection were named *fii*.

**Mapping the *fii* mutations.** The mapping procedure was performed as described by Kleckner et al. (20) and Russel and Model (35), with slight modification. Each mutant was transduced with a P1 lysate grown on a pool of Tet<sup>r</sup> clones derived from W3110::Tn10 (a gift from Jim Walsh, Duke University), and several Fii<sup>+</sup> Tet<sup>r</sup> transductants were isolated. P1 lysates were prepared from each Fii<sup>+</sup> Tet<sup>r</sup> transductant and were transduced back into original mutants to measure the frequency of Fii<sup>+</sup> Tet<sup>r</sup> cotransduction. The cotransduction frequencies varied between 25 and 90%.

One of the Tn10 insertions in strain C131 which cotransduced approximately 85% of the time with *fii* was mapped by introducing it into several Hfr strains (by P1 transduction) and scoring it as an unselected marker in crosses with a suitable marked F<sup>-</sup> strain X697 as described by B. Low (21). The Tn10 insertion was located as a proximal marker in crosses with strain Hfr Broda 8 and as a distal marker in crosses with strain KL99 at around 17 min on the *E. coli* map. Strains JRG94, AB3257, and WGA<sub>suc26</sub> carrying markers around 17 min were used for further mapping the Tn10 site by P1 transduction with P1 lysates from T946.

**Cloning of *fii*, *tolA*, and *tolB* (wild-type) genes from wild-type chromosomal DNA.** Standard cloning techniques of Maniatis et al. (22) were used. Chromosomal DNA from KMBL3752 partially digested with *Sau*3AI was ligated to pBR322 completely digested with *Bam*HI. The ligation mix-

ture was used to transform TPS13 *recA* cells and select for deoxycholate-resistant, Amp<sup>r</sup> transformants. A chimeric pBR322 plasmid pTPS1 containing a 2.5-kb insert was isolated which could complement *fii*. To isolate a larger region of the chromosome, chromosomal DNA that was partially digested with *Bgl*II was ligated with pJIH12 that had been completely digested with *Bgl*II, the resulting mixture was transformed into TPS13 *recA*, and colonies selected for deoxycholate-resistant, Amp<sup>r</sup> transformants. A pJIH12 chimeric plasmid pTPS202 containing an 8.7-kb insert was isolated and found to complement *tolA*, *tolB*, and *fii*. The plasmid DNAs of all deoxycholate-resistant, Amp<sup>r</sup> clones were prepared and retransformed into TPS13 *recA* strain to rule out the possibility of revertants.

To subclone *fii*, *tolA*, and *tolB*, various restriction endonuclease fragments from the 8.7-kb insert in pTPS202 were ligated into pUC9 or pUC18. pTPS301, pTPS304, and pTPS306 carry *Bgl*II-*Ava*I, *Alu*I-*Hinc*II, and *Sph*I-*Ava*I fragments, respectively, blunt end ligated in the filled-in *Bam*HI site of pUC9 (see Fig. 3). pTPS308 contains the *Bgl*II-*Eco*RI fragment inserted into the *Bam*HI-*Eco*RI sites of pUC9. pTPS310 has the *Bgl*II-*Eco*RV fragment inserted between the *Bam*HI-*Hinc*II sites of pUC18. pTPS302 contains the *Eco*RI-*Ava*I fragment whose *Ava*I end was first converted to a *Bam*HI site by adding the *Bam*HI linker, in *Eco*RI-*Bam*HI sites of pUC9.

**Restriction mapping.** Mapping was performed by the method of Smith and Birnstiel (40), with slight modification as described by Maniatis et al. (22). pTPS1 was digested with *Hind*III, 5' end labeled with [ $\gamma$ -<sup>32</sup>P]ATP, and further digested with *Sal*I. The *Hind*III-*Sal*I fragment containing the 2.5-kb insert was purified by agarose gel electrophoresis and electroelution. The resulting fragment labeled at one 5' end was partially digested with restriction enzymes (one for each reaction), and the size distribution of the fragments was analyzed by gel electrophoresis on 1.5% agarose in 0.08 M Tris phosphate (pH 7.5), followed by autoradiography. pTPS301 was digested with *Eco*RI, at a site on the vector six nucleotides from the *Ava*I junction with the chromosomal insert. After the 5' end was labeled with <sup>32</sup>P, the chromosomal fragment was isolated by digestion with *Sal*I, which cleaved six nucleotides upstream of the *Bgl*II junction with the chromosomal DNA. The *Eco*RI-*Sal*I fragment carrying the insert was purified and mapped by the same procedure described above.

**Tn5 mutagenesis and isolation of plasmids containing Tn5 insertions in the *tolA*, *tolB*, and *fii* genes.** Tn5 mutagenesis was performed as described by Russel and Model (36). Strain GM1 carrying either pTPS301 or pTPS308 was infected by  $\lambda$ 467. The resultant kanamycin- and ampicillin-resistant colonies ( $>10^5$ ) were pooled and subcultured in LB medium containing kanamycin and ampicillin. pTPS301 plasmid DNA prepared from this culture was used to transform strains A592 (*tolA*) and A593 (*tolB*). pTPS308 plasmid DNA was used to transform strains TPS13, A592, and A593. Kan<sup>r</sup> Amp<sup>r</sup> transformants were selected and were scored for inactivation of *tolA*, *tolB*, and *fii* genes by testing the f1 phage sensitivity or colicin E1 and E2 sensitivities at 37°C. The location of each Tn5 element was determined by restriction mapping with *Hae*II and *Hpa*I endonucleases. Tn5 has *Hae*II and *Hpa*I sites located 150 and 185 base pairs (bp) from each end of the element, whereas both chimeric plasmids have only one *Hpa*I site in the chromosomal insert.

**Maxicell preparation and labeling of proteins.** Maxicells were prepared by the method of Sancar et al. (37), with slight modification. CSR603 (*recA uvrA*) and its derivative contain-

ing pUC9, pTPS301, pTPS302, pTPS304, pTPS306, and pTPS310 were grown in 10 ml of M9 medium plus 1% Casamino Acids (Difco Laboratories) to  $2 \times 10^8$  cells per ml, irradiated with UV light, and incubated at 37°C for 1 h with shaking. Cycloserine (100  $\mu$ g/ml) was added; after incubation for 6 h with shaking at 37°C, the cells were centrifuged, washed, and suspended in 1 ml of TG medium for 30 min at 37°C. [<sup>35</sup>S]methionine (50  $\mu$ Ci) was added; 30 min later the cells were collected and washed with TG salts, and the resulting pellets were suspended in 200  $\mu$ l of sodium dodecyl sulfate sample buffer (8% sodium dodecyl sulfate, 1%  $\beta$ -mercaptoethanol, 5% glycerol, 0.004% bromophenol blue, and 0.125 M Tris hydrochloride [pH 6.8]). The proteins were separated on 12% sodium dodecyl sulfate-polyacrylamide gels, and the labeled proteins were identified by autoradiography. Cell envelope and cytoplasmic fractions were obtained from the labeled maxicells by method of Russel and Model (34), in which the spheroplasts were formed with lysozyme and lysed by osmotic shock and sonication, and the cytoplasmic and envelope fraction was isolated by centrifugation.

**Tests for tolerance and adsorption of colicin.** Colicin tolerance was measured by spotting dilutions of purified colicins onto a lawn of bacteria to be tested. The method of Nagel De Zwaig and Luria (28) was used to measure the adsorption of colicin E1. Samples (0.2 ml) of late-log-phase cultures in LB broth of the streptomycin-sensitive (Str<sup>s</sup>) strains to be tested were mixed with 0.1 ml colicin dilutions and incubated at 37°C for 25 min. The mixtures were then centrifuged, and supernatants were transferred to new tubes containing 0.2-ml samples with 800 to 1,000 cells of an Str<sup>r</sup> colicin E1-sensitive indicator strain (JM1). After 10 min of incubation at 37°C, 2.5 ml of melted soft agar containing streptomycin was added, and the mixture was poured on streptomycin agar plates. The colonies of the Str<sup>r</sup> strain were counted after overnight incubation at 37°C.

## RESULTS

**Isolation and characterization of *fii* mutants.** Strain GM1 bacteria were treated with UV light or ethyl methanesulfonate, and mutants were selected which allowed growth of the F pilus-specific RNA bacteriophage f2, but not of the filamentous phage f1, at 41°C as described in Materials and Methods. To determine whether the mutations reside on the chromosome or on the F' plasmid, each mutant was conjugated with JM1, and the transconjugants were tested for f1 and f2 plaque formation at various temperatures. Using this procedure, six mutants, TPS13, -30, -56, -58, -66, and -94, were determined to be chromosomal and were further characterized (Table 2). All six mutants fail to plaque f1 but still produce F pili and grow f2 phage at all temperatures. The defects in the mutants do not affect F pilus function, since conjugation with various recipient strains is normal and f1 phage can be observed to bind to the tip of the pilus by electron microscopy (data not shown). However, these mutants are impaired in their ability to allow the DNA of the infecting phage to enter the bacteria, since transformation of these defective bacteria with f1 single-stranded DNA leads to normal phage production. The function required to get the DNA in must be specific for filamentous phage infection, since all six mutants can support the growth of the isometric single-stranded DNA phages St-1 and  $\phi$ X174. Consistent with this hypothesis is the observation that the related filamentous phage IKE is not able to grow on these mutants when they harbor the N plasmid (Table 2). Based on these

TABLE 2. Characterization of *fii* mutants

Strain <sup>a</sup>	Plaque formation		F pilus formation	Phage production after transformation with f1 ss DNA <sup>b</sup>	Conjugation	P1 transduction	Plaque formation			
	f1, M13, fd	f2					P1	φX174 <sup>c</sup>	St-1	IKe <sup>d</sup>
GM1	+	+	+	+	+	+	+	+	+	+
TPS13	—	+	+	+	+	+	—	+	+	—
TPS58	—	+	+	+	+	+	+	+	+	—
TPS94	—	+	+	+	+	+	+	+	+	—

<sup>a</sup> TPS30, -56, -58, and -66 had the same results as TPS13.

<sup>b</sup> Cured wild-type and mutant cells were transformed with single-stranded (ss) DNA from fd-tet, an fd phage carrying a determinant for tetracycline resistance (29). Tet<sup>r</sup> transformants were selected and tested for fd phage production.

<sup>c</sup> P1 lysates from T131, -58, and -946 were used to transduce wild-type *E. coli* strain C. Tet<sup>r</sup>, colicin E1-resistant transductants were selected and tested for φX174 plaque formation.

<sup>d</sup> P90C and TPS13, -58, and -94 cured of F' were conjugated with JE2571/N<sub>3</sub>, and the transconjugants were tested for IKe plaque formation.

data, we named the mutation(s) *fii* for filamentous phage infection.

The six *fii* isolates can be grouped into three classes (Table 2). The first class, which consists of TPS13 (*fii-1*), TPS30 (*fii-2*), and TPS56 (*fii-3*), is distinguished by the observation that they cannot plaque P1 phage, even though they can be recipients for P1 transduction. The second class, TPS58 (*fii-4*) and TPS66 (*fii-5*), can plaque P1 phage. The third class, TPS94 (*fii-6*), was shown to be an allele of *tolA* after cloning of *fii* (see below).

**Mapping of the *fii* mutations.** Several Hfr strains were isolated, as described in Materials and Methods, which contained a Tn10 element at the same position close to *fii*. These Tet<sup>r</sup> Hfr strains were crossed with strain X697, and Tet<sup>r</sup> was scored as an unselected marker in the resulting transconjugants. The Tn10 element transferred proximal to the point of origin for strain Broda 8 (8.5 min, counterclockwise) and distal to the point of origin for strain KL99 (22 min, counterclockwise). The results from these and other similar crosses place the Tn10 element and consequently *fii* near 17 min on the *E. coli* map. This Tn10 insertion is designated *zbg-1::Tn10*.

Transduction of *zbg-1::Tn10* into strains JRG94, AB3257, and WGA<sub>suc26</sub> carrying markers around 17 min was done by using a P1 lysate from strain C131. The cotransduction frequencies of these markers with *zbg-1::Tn10* are shown in Fig. 1. To determine the location of *fii-6* relative to *zbg-1::Tn10* and other markers, a P1 lysate was prepared from the *fii-6*, *zbg-1::Tn10* strain T946 and used to transduce the *aroG* strain AB3257. The 80% cotransduction frequency of *fii-6 aroG*<sup>+</sup> *zbg-1::Tn10* suggests that *zbg-1::Tn10* is inserted between *fii-6* and *aroG* around 16.9 min on the *E. coli* map. This is the position where *tolA* and *tolB* mutants had been mapped previously (2, 3), suggesting that some or all of the *fii* mutants may be alleles of *tolA*, mutants of which had previously been shown to be resistant to f1 infection (39). Comparison of the properties of *fii*, *tolA*, and *tolB* mutants show that all of the *fii* mutants, like *tolA* mutants, are insensitive to colicin E1 and E2 and sensitive to deoxycholate (Table 3). However, the *tolA* mutant can grow P1, whereas the TPS13 class of *fii* mutants cannot plaque P1 phage.

The insensitivity of *fii* mutants to colicins resembles the tolerant phenotype exhibited by *tolA* mutants since the colicin receptor coded for by the *btuB* locus is intact as judged by the ability of the *fii* mutants (Table 3) to plaque phage BF23. This phage requires a functional *btuB* receptor for infection (6). When the binding of colicin E1 to the

various mutants was assayed by the method of Nagel De Zwaig and Luria (28), it was found that GM1, A592, TPS58, and TPS94 all bound the same amount of colicin E1 per cell. The TPS13 class of *fii* mutants appeared to consistently bind 30 to 50% of the amount of colicin E1 bound by the other strains, but the significance of this is unclear.

**Cloning of the *fii*, *tolA*, and *tolB* genes.** To determine whether the TPS13 and TPS58 classes of *fii* mutations are in a closely linked but distinct gene from *tolA*, a number of chimeric plasmids containing the wild-type *fii* gene was isolated as described in Materials and Methods. The chimeric plasmid pTPS1, which contains a 2.5-kb insert of chromosomal DNA, can complement the TPS13 and TPS58 classes of *fii* mutations. It cannot complement TPS94 or the *tolAB* mutations and thus defines *fii* as a gene separate from *tolA*. Plasmid pTPS202 contains an 8.7-kb *Bgl*III insert and can complement not only the TPS13, TPS58, and TPS94 classes of *fii* mutation but also the *tolA* and *tolB* mutations. A 3.3-kb fragment of this 8.7-kb insert was subcloned into pUC9, and the resulting chimeric plasmid, pTPS301, was found to complement both *tolA* and *tolB* mutants. Analysis of this insert and the 2.5-kb insert in pTPS1 with restriction endonucleases showed that they overlapped, thus confirming that the *tolAB* and *fii* loci are close if not adjacent to each other (Fig. 2).

Fragments of the 5-kb *Bgl*III-*Sau*3AI insert shown in Fig. 2

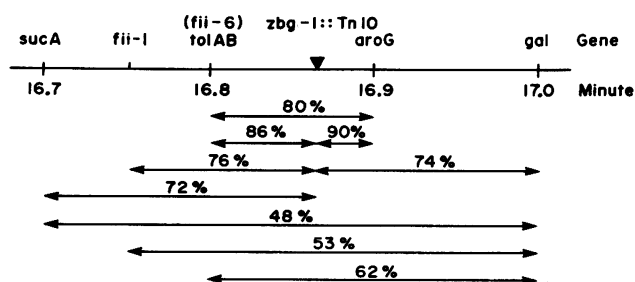


FIG. 1. Location of *zbg-1::Tn10* and *fii* on the *E. coli* chromosome. P1 lysates grown on C131, T946, or T131 containing pTPS1 were used to transduce strains JRG94, AB3257, WGA<sub>suc26</sub>, A592, and A593. The resulting transconjugants were selected for Tet<sup>r</sup> and scored for the desired markers. The numbers above each line represent the percentage of cotransduction of the two markers present at each end of the arrows. The transduction frequencies are accurate to  $\pm 3\%$ . *fii-1* represents the *fii* mutation in TPS13, and *fii-6* is an allele of the *tolA* locus (see Fig. 3).

TABLE 3. Characterization of strains

Strain	Plaque formation			Sensitivity (S) or resistance (R) to:			Plaque formation	
	f1	f2	E1	Colicin E1	Colicin E2	Deoxycholate	BF23	IKe <sup>a</sup>
GM1	+	+	+	S	S	R	+	+
TPS13	—	+	—	R	R	S	+	—
TPS58	—	+	+	R	R	S	+	—
TPS94	—	+	+	R	R	S	+	—
A592 ( <i>tolA</i> )	—	+	+	R	R	S	+	—
A593 ( <i>tolB</i> )	+	+	+	S	R	S	+	+

<sup>a</sup> A592 and A593 were conjugated with P90C/N<sub>3</sub>, and transconjugants were tested for IKe plaque formation.

were subcloned from pTPS202 into pUC9 or pUC18, and a number of chimeric plasmids were obtained with the properties shown in Fig. 3. All three genes, *tolA*, *tolB*, and *fii*, are present in the 4.3-kb *Bgl*II-*Eco*RI fragment in chimeric plasmid pTPS308. Plasmid TPS304 contained a 1.3-kb *Alu*I-*Hinc*II fragment inserted in pUC9 and can complement the TPS13 and TPS58 classes of *fii* mutations (*fii*-1 through -5). The presence of this plasmid in the TPS13 class of mutants allowed the growth of P1 as well as f1 phage. The 2.0-kb *Sph*I-*Ava*I fragment found in pTPS306 can complement the *tolA* mutation and the TPS94 class of *fii* mutations (*fii*-6). Thus, the *fii*-6 mutation in TPS94 is an allele of the *tolA* locus and will be referred to as a *tolA* mutation. Similar analysis showed that the *tolB* locus is contained in the 1.4-kb *Bgl*II-*Eco*RV fragment found in the pUC18 chimeric plasmid pTPS310. None of these chimeric plasmids appeared to require transcription from the *lac* promoter to get complementation (data not shown). Comparison of the physical maps of the inserts from the different plasmids shown in Fig.

3 defined the order of the genes to be *tolB*-*tolA*-*fii*. To confirm the position of the genes, pTPS301 or pTPS308 was subjected to Tn5 mutagenesis as described by Russel and Model (36), and the sites of insertion of Tn5 were determined as described in Materials and Methods. The results (Fig. 3) are entirely consistent with the location of the genes determined by the subcloning experiments.

Both cloning and Tn5 mutagenesis experiments indicated that the TPS13 (*fii*-1 through -3) and TPS58 (*fii*-4 and -5) classes of mutations were located in the same gene, yet they could be distinguished by their ability to plaque P1 (Table 2). One possible difference might be the nature of the mutation; one class might be a nonsense mutation and the other may be a missense mutation. To test this, the mutants in each class were transferred together with *zbg*-1::Tn10 by P1 transduction to K37 (*supD*) and K38 (*sup*<sup>+</sup>). This was possible for the TPS13 class of *fii* mutations, since the TPS13 can grow P1 when pTPS1 containing the cloned *fii* gene is present. The f1-resistant, colicin E1-resistant phenotype of T58 (*fii*-4) and

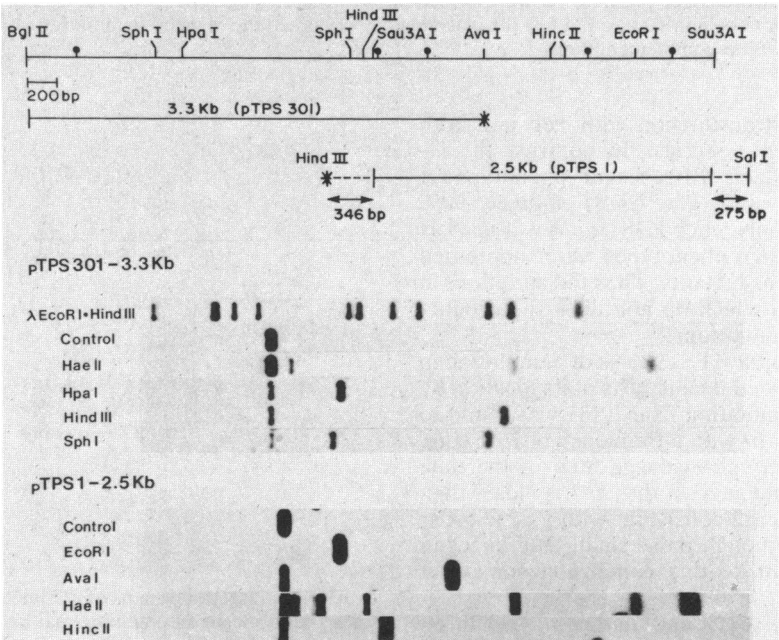


FIG. 2. Physical map of the *tolA*, *tolB*, and *fii* region as defined by various restriction endonucleases. pTPS1 was digested with *Hind*III, the resulting 5' ends were labeled with <sup>32</sup>P, and the chromosomal fragments were isolated from pTPS1 after digesting with *Sal*I. The 3.3-kb chromosomal fragment was isolated from pTPS301 in a similar manner as described in Materials and Methods. The resulting labeled fragments are shown at the top of the figure. Symbols: \*, end labeled with <sup>32</sup>P; -----, pBR322 DNA; —, chromosomal DNA; and —, *Hae*II sites. The <sup>32</sup>P-labeled fragments were partially digested with various restriction enzymes, and the size of the resulting fragments was determined by electrophoresis on 1.5% agarose. The bottom of the figure shows an example of the results obtained with a subclass of the restriction enzymes used. The size markers used were λ DNA digested with *Eco*RI and *Hind*III. Control lanes refer to undigested <sup>32</sup>P-labeled chromosomal fragments isolated from pTPS301 and pTPS1.

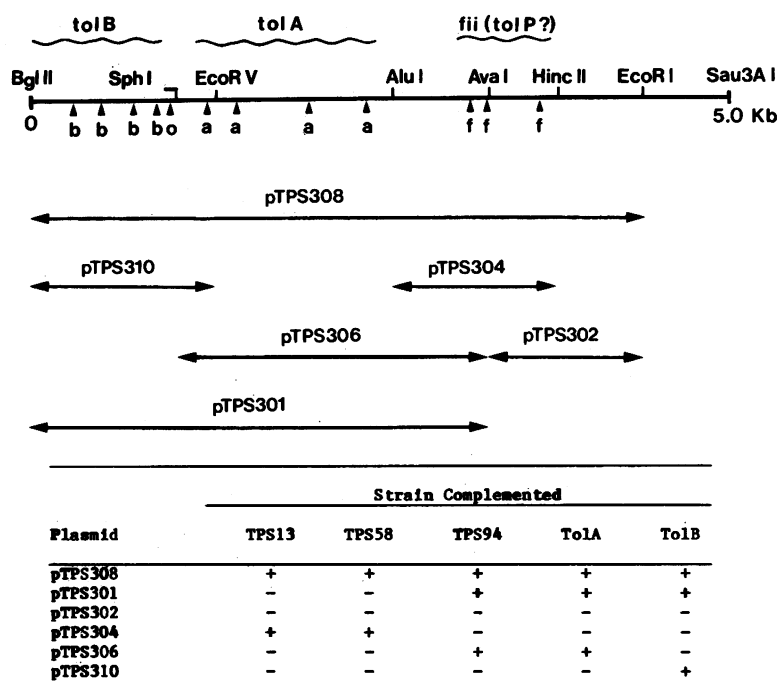


FIG. 3. Locations of *tolA*, *tolB*, and *fii* genes. The top of the figure shows the position of some restriction enzyme sites determined as described in the legend to Fig. 2. The arrows a, b, and f represent sites of Tn5 insertions which inactivated the *tolA* gene, *tolB* gene, and *fii* gene, respectively. A Tn5 insertion at position O did not inactivate any of these three genes. The wavy lines represent the sizes of the genes as estimated from restriction mapping, Tn5 mutagenesis, and the size of the proteins produced in maxicells (see Fig. 4). The lines with arrows at each end in the middle of the figure represent the size of the chromosomal insert individually subcloned. The number above each line represents the name of the chimeric plasmid containing that chromosomal fragment. pTPS301, pTPS302, pTPS304, pTPS306, and pTPS308 are chimeric plasmids of pUC9. pTPS310 is a chimeric plasmid of pUC18. The bottom of the figure shows the properties of the chimeric plasmids described in the middle of the figure. Each plasmid was transformed into *recA* strains of TPS13, 58, 94, A592 (*tolA*), and A593 (*tolB*), and the cells were tested for sensitivity to colicin E1 and E2. (+) the plasmid complemented the respective mutation, and the strain became sensitive to colicin E1 or E2 or both; (-) no complementation.

T66 (*fii-5*) showed 90% cotransduction with Tet<sup>r</sup> into both K37 (*supD*) and K38 (*sup<sup>+</sup>*) strains. In contrast the fl-resistant, colicin E1-resistant, P1-resistant phenotype of T131 (*fii-1*), T30 (*fii-2*), and T56 (*fii-3*) showed 90% cotransduction with Tet<sup>r</sup> only when K38 (*sup<sup>+</sup>*) was used as the recipient. All three mutant phenotypes were suppressed by *supD* in the Tet<sup>r</sup> K37 transductants. Thus the mutations in TPS13, TPS30, and TPS56 which do not allow the production of P1 must be amber mutations.

The ability to grow P1 on the TPS13 class of *fii* mutation in the presence of pTPS1 allowed the analysis of its location by P1 transduction. The *fii-1* mutation from T131 was found to cotransduce with *zbg-1::Tn10* with a frequency of 76% (Fig. 1). Since the *fii-6* mutation cotransduced 86% of the time with *zbg-1::Tn10*, this would suggest that *fii-1* is closer than *fii-6* to *sucA*. These results are consistent with a gene order *sucA-fii-tolA-tolB-aroG*, although these small differences in transduction frequencies make this conclusion somewhat tentative.

**Identification of the *fii*, *tolA*, and *tolB* gene products.** CSR603 (*uvrA recA*) strains carrying pUC9, pTPS301, pTPS302, pTPS304, pTPS306, and pTPS310 (Fig. 3) were used to prepare maxicells. Proteins coded by these plasmids in the maxicells were labeled with [<sup>35</sup>S]methionine and electrophoresed on sodium dodecyl sulfate-polyacrylamide gels as described in Materials and Methods. Cells containing pUC9 produced two major protein bands with approximate molecular masses of 31 and 29 kDa, representing β-

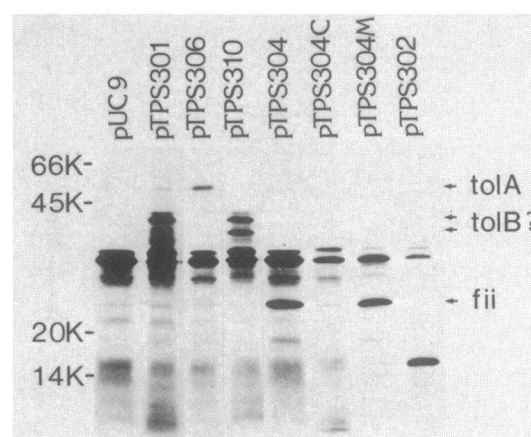


FIG. 4. Autoradiograms of [<sup>35</sup>S]methionine-labeled proteins synthesized in plasmid-containing maxicells. Maxicells were prepared from CSR603 and its derivatives containing pUC9, pTPS301, pTPS302, pTPS304, pTPS306, or pTPS310 and labeled with [<sup>35</sup>S]methionine, and the proteins were analyzed by sodium dodecyl sulfate-gel electrophoresis as described in Materials and Methods. The genes contained in each plasmid are described in the legend to Fig. 3. pTPS304C and pTPS304M indicate the analysis of the cytoplasmic and envelope fractions from pTPS304 containing maxicells. The size markers are bovine serum albumin, ovalbumin, soybean trypsin inhibitor, and lysozyme.



lactamase and its precursor coded by the *amp* gene (Fig. 4). Maxicells containing pTPS301 which complement both *tolA* and *tolB* mutations synthesized one extra band migrating at a position expected for a protein of 51 kDa and several extra bands corresponding to proteins of between 30 and 40 kDa. The 51-kDa protein is synthesized in maxicells harboring pTPS306, which contains only the *tolA* gene, suggesting that this is the *tolA* gene product. Two extra major proteins of 40 and 37 kDa were synthesized in cells carrying pTPS310, which only contains the *tolB* gene together with three other minor bands of 30 to 40 kDa. These results are consistently found for plasmids carrying the *tolB* gene, suggesting that the 37- and 40-kDa proteins may be related to the *tolB* product.

Cells harboring pTPS304, which can only complement *fii* mutations but not *tolA* or *tolB*, produced one major protein of 24 kDa. This 24-kDa protein did not appear from cells carrying pTPS302 which only contain part of the *fii* gene and cannot complement *fii* mutations. We concluded that the 24-kDa protein is the *fii* protein. This is also supported by the genetic data which show that the *fii* protein is coded by a region less than 1.3 kb long. Cell fractionation experiments showed that the *fii* protein is in the cell envelope fraction (Fig. 4).

### DISCUSSION

Six chromosomal mutants of *E. coli* were isolated which are defective for infection by the filamentous single-stranded DNA phage *f1* or IKe but still sensitive to the isometric single-stranded DNA phage  $\phi$ X174 or St-1. Other phenotypes exhibited by these mutants are an increased sensitivity to deoxycholate and an increased tolerance to the E class of colicins. The receptor for colicin binding, coded for by the *btuB* locus, is normal as judged by the ability of these mutant bacteria to plaque phage BF23 and bind colicin. These phenotypes are similar to the ones found for strains carrying a mutation in the *tolA* locus at 16.9 min on the *E. coli* map. A chimeric plasmid pTPS306 was isolated which can only complement isolate TPS94 and the mutant phenotype exhibited by *tolA* mutations, suggesting that the mutation present in TPS94 is an allele of *tolA*. The mutations present in the other five isolates could be complemented by a different chimeric plasmid (pTPS304), which contains a fragment of *E. coli* DNA normally contiguous to the fragment in pTPS306 containing the *tolA* gene. Thus these five mutations define at least one locus which is distinct from, but adjacent to, the *tolA* gene. This region is named *fii*, for filamentous phage infection.

The evidence is consistent with all five mutations occurring in the same gene. Maxicells containing pTPS304 synthesize a unique 24-kDa protein. Approximately 660 nucleotides would be necessary to encode such a protein, assuming an average residue molecular mass of 110 Da. None of the *fii* mutations is complemented by pTPS302, which contains a 550-bp overlap with the 1.3-kb insert found in pTPS304. However, a Tn5 insert located 200 bp leftward from the *HincII* site of the *E. coli* insert in pTPS304 is located in this overlapping region and inactivates all of the complementing activity for *fii* mutations (Fig. 3). If the Tn5 insert is at the end of the 660 bp necessary for synthesis of the 24-kDa protein, then the region containing all five *fii* mutations cannot extend much more than 860 bp to the left of the *HincII* site of the 1.3-kb insert in pTPS304. Another Tn5 insert located 685 bp leftward from the *HincII* site also inactivates the complementing activity for all five *fii* mutations. This would suggest that all five *fii* mutations are alleles

of the same gene, assuming no overlapping genes or polar effects of one gene on another. The latter reservation must be considered, since the three *fii* mutations which cannot grow P1 are amber mutations as judged by their suppression in a strain carrying the *supD* suppressor gene. Knowledge of the sequence of the 1.3-kb insert in pTPS304 should resolve this question.

The combination of cloning, Tn5 mutagenesis and P1 transduction studies described in this paper gives a gene order of *sucA-fii-tolA-tolB-aroG* for this region of the *E. coli* genome. Bernstein et al. (3) previously reported that the E colicin-tolerant mutants mapping near 17 min fall into three complementation groups with the order *tolP-tolA-tolB*. Thus *tolP* mutants map in the same position and possibly may be allelic to *fii*. However, *tolP* mutations are only partially tolerant to colicins (3), whereas *fii* mutations resemble *tolA* mutations in the concentration of colicins they can tolerate.

The role that the products of the *fii-tolA-tolB* group of genes have in the normal cell metabolism, in allowing the colicins to be active, or in allowing the filamentous phage to enter the cytoplasm is unknown. The E colicins, phage BF23, and vitamin B12 share the same outer membrane protein as a receptor, the product of the *btuB* gene located at 90 min in the *E. coli* map (2, 6, 12, 19). This implies that the products of the *fii-tolA-tolB* group of genes must act at some step which occurs between the adsorption and the final target of the colicins. Since mutation in these genes result in many phenotypic changes such as increased sensitivity to various detergents and antibiotics, it is suggested that these proteins might be associated with the bacterial membrane and help facilitate the translocation of the colicins across the cytoplasmic membrane. Similarly, the filamentous phages have a receptor system, the tip of the sex pili coded by specific conjugative plasmids, whose function is independent of the *tolA fii* group of genes. Thus, the products of these latter genes probably are involved in transmitting the phage DNA through the membrane, presumably after the pilus has withdrawn, bringing the attached phage to the surface (17).

Present studies do not define the relation of *fii* to *tolA* or *tolB*. The product of *fii* is a protein, as judged from the results with maxicells containing the cloned *fii* genes and the fact that three mutants in this gene are suppressible amber mutations. Its probable association with the bacterial envelope suggests that it may be directly involved in translocating colicins or the phage DNA into the cytoplasm, perhaps by forming some complex with the product of *tolA* or *tolB* or both. An alternate possibility is that the *fii* gene product is a regulatory protein, needed for the expression of the *tolA* and *tolB* products. In addition, the *fii* protein may regulate other genes responsible for the assembly of P1. This might explain why amber mutations in *fii* do not allow the growth of P1. It is equally possible that the *fii* protein plays a direct role in P1 assembly. Present investigations on the transcription products from the *fii-tolA-tolB* region may resolve some of these questions.

The attachment of the filamentous phage to the tip of the pilus is via the phage gene III protein, which is located at one end of the phage particle (46). In the case of the filamentous phage specific for the F pilus (*f1*, *fd*, and M13), the gene III protein also may interact with the products of *tolA* or *fii* or both, since cells infected with *f1* phage or cells harboring a chimeric plasmid expressing gene III have been shown to have a much higher tolerance to the effects of colicins E1 and E3 and exhibit a higher sensitivity to deoxycholate (4, 50). Boeke et al. (4) suggest that the first glycine-rich region in the gene III protein may be responsible for these pheno-

types. Both colicins E1 and E2 have similar glycine-rich regions (9, 49), suggesting that the gene III protein may be competing for the same sites on the *tolA* or *fli* gene products or both, leading to the observed phenotype in f1-infected bacteria. However, any interaction of gene III protein and colicins with the *tolA* or *fli* gene products may be more complicated than such a competition, since cells containing gene III are also more tolerant to colicin E3, a molecule which has no such glycine-rich region (25, 27). In addition, we found that cells infected with IKE do not demonstrate an increased sensitivity to deoxycholate or increased tolerance to colicin E1 (data not shown), even though functioning *tolA* and *fli* genes are necessary for infection with this phage. Peeters et al. (31) also mentioned that Ike gene III protein does not have the same effects on the host cell membrane as does f1 gene III protein. Resolution of the interaction of gene III with the *tolA* and *fli* proteins should lead to a better understanding of how complex molecules can traverse the bacterial membrane.

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