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A Bacterial Gene, *fip*, Required for Filamentous Bacteriophage f1 Assembly

MARJORIE RUSSEL* AND PETER MODEL

The Rockefeller University, New York, New York 10021

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An *Escherichia coli* mutant which does not support the growth of filamentous bacteriophage f1 allows phage f1 DNA synthesis and gene expression in mutant cells, but progeny particles are not assembled. The mutant cells have no other obvious phenotype. On the basis of experiments with phage containing nonlethal gene I mutations and with mutant f1 selected for the ability to grow on mutant bacteria, we propose an interaction between the morphogenetic function encoded by gene I of the phage and the bacterial function altered in this mutant. The bacterial mutation defines a new gene, *fip* (for filamentous phage production), located near 84.2 min on the *E. coli* chromosome.

The requirement for bacterial gene products in the growth and assembly of phages has been defined by the isolation of bacterial mutants on which wild-type phage cannot grow. Many of the mutations consist of nonlethal changes in essential genes that specify proteins such as RNA polymerase (5, 9, 12, 29) and proteins required for DNA replication (11, 13). Other mutations, such as those that block morphogenesis of phages λ, T4, and T5 (14), are in genes that specify abundant proteins of unknown function (30).

Unlike the lysis-released phages affected by the bacterial mutants described above, the filamentous single-stranded DNA phages (f1, M13, and fd) are secreted into the medium as the host continues to grow and divide. The assembly process of filamentous phages is intimately associated with the bacterial cytoplasmic membrane. The major coat protein (pVIII) and one minor (pIII) coat protein are synthesized with signal sequences (31, 37, 41) and are transmembrane proteins before they appear in phage (3, 39). Several of the remaining filamentous phage proteins have been shown to be membrane associated (48).

We initially set out to isolate bacterial mutants that were unable to support filamentous phage growth in the hope that signal peptidase mutants or other mutants with mutations affecting protein transport into membranes would be included in such a collection. Several mutants with mutations in at least three different genes (including *rep*) were isolated. However, none of these mutations appears to directly affect signal peptidase function or protein insertion into membranes. Instead, we found a mutation which appears to hinder phage assembly. In this paper we describe some of the properties of this mutant. The mutation in uninfected cells has no obvious phenotype, but mutant cells infected with wild-type phage f1 at a high temperature fail to produce progeny phage. Phage mutants that can grow at a high temperature on this mutant strain were isolated and characterized. A modification of the technique of Kleckner et al. (21) that makes it possible to isolate transposons near genes for which there is no selectable phenotype allowed us to locate the mutation in a new gene, *fip* (filamentous phage production), at 84.2 min on the *Escherichia coli* chromosome.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used in this study are shown in Table 1. The filamentous phage strains used were from our laboratory collection. λ467 (*c*I857 *b*221 *O*am29 *P*am80 *rex*::Tn*5*) was obtained from C. Lichten via N. Kleckner.

Bacteria were routinely grown in Ty medium (24). The growth conditions used for labeling cells with [^{35}S]methionine have been described previously (34). P1 Cm^r *clr*100 transducing lysates were prepared and transductions were performed by the methods of Miller (27), except that the transduction mixtures were plated onto DO salts-based (47) minimal plates supplemented with 0.2% glucose and with amino acids (50 μg/ml each or 0.2% Casamino Acids) and antibiotics as required. The high concentration of citrate in DO salts reduced infection of transductants by P1 on the selective plates. All unselected markers except *cya* were scored by standard replicating techniques; *cya* was scored by colony size. Cells containing the Δ*cya-854* allele formed very small colonies on DO salts-glucose plates. Kanamycin (Bristol Laboratories) was used at a concentration of 50 μg/ml, and tetracycline was used at a concentration of 20 μg/ml. Nitrosoguanidine mutagenesis and bacterial matings were performed by the methods of Miller (27).

TABLE 1. *E. coli* strains

Strain(s)	Genotype	Comments and/or construction
K38	HfrC *supD* (λ)	
K91	K38 (λ⁻)	
K569	K38 *fip-1*	Nitrosoguanidine mutagenesis
A18	K569 *malA*	Selected with λvir
A19-A27	A18 *fip*⁺ *zie/zid-1–9*::Tn*5*	Transduction by P1(Tn*5* hop pool)
A28	K569 *zie-1*::Tn*5*	Transduction by P1(A19);*fip*
A37	K569 *zid-6*::Tn*5*	Transduction by P1(A*24*);*fip*
A95	K38 *ilv fip-1* *zie-1*::Tn*5*	*ilv* from strain AB3059[a]
A96	K38 *ilv zie-1*::Tn*5*	*ilv* from strain AB3059[a]
K748	K38 *recA*	
K815	K38 Δ*cya-854* *metE163*::Tn*10*	
K37	HfrC *supD* (λ)	
A130	K37 *ilv fip-1* *zie-1*::Tn*5*	Transduction by P1 (A95)
K832	K38 *supE*	
A131	K832 *ilv fip-1* *zie-1*::Tn*5*	Transduction by P1 (A95)
K833	K38 *supF*	
A132	K833 *ilv fip-1* *zie-1*::Tn*5*	Transduction by P1 (A95)

[a] Strain AB3059 was transduced by P1(A28) to Kan^r, and P1 lysate prepared on an *ilv zie-1*::Tn*5* transductant was used to transduce strain K38 to Kan^r; transductants were scored for the *ilv fip* markers to isolate strain A95. Strain A96 was similarly isolated, but from an initial transduction by P1(A19).

Bacterial colonies were streaked across a line of f1$^+$ phage (a preparation containing 10^8 phage per ml, which had dried into the plate before streaking) and incubated at 42°C to assay for Fip. We found that many strains that contained useful markers were poor f1 indicators at 42°C. Thus, markers were first introduced into strain K38, and *fip* was mapped with respect to these markers in a uniform genetic background in cells that propagated f1 well at 42°C.

Sample preparation and electrophoresis. Sample preparation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled cell proteins were performed as described previously (2, 22). Synthesis of f1 single-stranded DNA and double-stranded replicative-form (RF) DNA was measured in whole-cell lysates by the modification of the procedure of Twigg and Sheratt (44) described by Lerner and Model (23). Gels were stained in 0.5 μg of ethidium bromide per ml after electrophoresis and destained in water.

DNA manipulations. Phage RF and plasmid DNAs were isolated by a modification of the method described by Davis et al. (6) for plasmid isolation from 10-ml cultures. Treatment with diethyl oxydiformate was omitted. The supernatant collected after heating to 70°C was brought to 10% polyethylene glycol and 0.5 M NaCl, incubated at 4°C for 2 to 4 h, and centrifuged. The resulting pellet was suspended in 0.3 ml of 10 mM Tris-hydrochloride (pH 8)–10 mM NaCl–0.2 mM EDTA and incubated with 30 μg of pancreatic RNase per ml at 37°C for 60 min. The DNA was extracted with 0.3 ml of phenol that had been freshly saturated with 0.02 M sodium borate (pH 8), and the DNA was precipitated twice with ethanol. DNA digestions by restriction enzymes and ligations were performed as suggested by the manufacturers. Single-stranded DNA was prepared by extracting phage twice with phenol that had been freshly saturated with 0.02 M sodium borate (pH 8) and then precipitating the DNA twice with ethanol. DNA sequence analysis was performed by the dideoxy method of Sanger et al. (36).

Marker rescue. Single-stranded DNA was prepared from amber mutant phage stocks in which the frequency of amber$^+$ revertants was less than 10^{-4}. Single-stranded DNA (3 μg) was mixed with *gfip* RF DNA (1 μg) that had been digested to completion with an appropriate restriction enzyme in 80 μl of DNA buffer (10 mM Tris-hydrochloride [pH 8], 10 mM NaCl, 0.2 mM EDTA). This represented a six-fold excess of single-stranded DNA with respect to complementary strands from the *gfip* RF DNA. After 5 μl of 0.5 M EDTA (tetrasodium salt) and 12.5 μl of 1 N NaOH (freshly diluted from a 10 N solution) were added, the mixture was incubated for 5 min at room temperature. Samples were brought to pH 8 by adding 12.5 μl of 1 M Tris-hydrochloride (pH 8.0) and 23 μl of 1 N acetic acid, and they were incubated at 65°C for 30 min. The DNA mixture was precipitated in ethanol, dried, and suspended in 20 μl of DNA buffer. Strain K748 cells were transfected by the $CaCl_2$ technique (26) with 6 to 12 μl of the hybridized mixture to select amber$^+$ phage. Amber$^+$ plaques were picked (usually 23 from each transfection), replicated on strains K38 and K569, and incubated at 42°C to score for the *gfip* allele. Under these conditions (i.e., excess single-stranded DNA containing a low level of amber$^+$ revertants), the absolute efficiency of marker rescue to amber$^+$ was low (about 1%), but the stimulation compared with the revertant background was usually more than 100-fold.

RESULTS

Mutant isolation. Strain K38 cells were treated with nitrosoguanidine for 15 min as described by Miller (27). After overnight growth to permit segregation, the mutagenized culture was plated at 37°C. Mutagenesis was monitored by scoring the accumulation of *lac* mutants; 4% of the colonies were red on lactose-tetrazolium plates. A total of 2,005 individual colonies were cross-streaked in duplicate against phages f1 and f2 and incubated at 34 and 42°C. Although both of these phages are male specific and adsorb to F-pili, f1 and f2 are otherwise unrelated. Clones that failed to plaque both phages were discarded as potential F-pilus mutants. We isolated 12 clones that plaqued f2 at both temperatures and f1 at neither temperature (7 clones) or only at 34°C (5 clones).

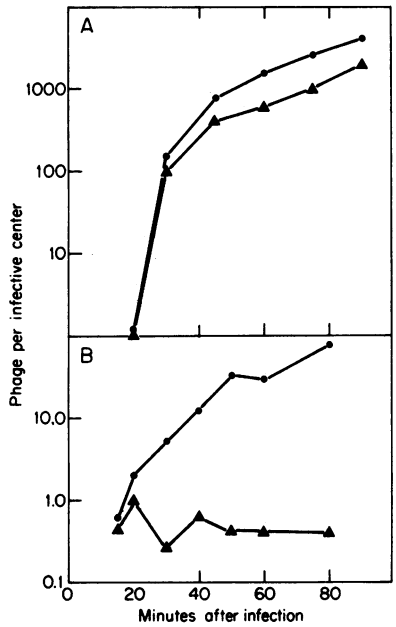


FIG. 1. Phage production at 34°C (A) and 41.5°C (B). Symbols: ●, strain A96 (*fip*$^+$); ▲, strain A96 (*fip*). Bacteria grown in tryptone broth to a density of about 2×10^8 cells per ml were infected with wild-type f1 at a multiplicity of infection of about 50. After a 5-min adsorption period, f1 antiserum was added (final k of 5 such that the rate of phage inactivation was 100-fold per min) to remove unadsorbed phage. The cultures were diluted 10^4-fold 5 min later. Samples of each culture were withdrawn into chloroform at different times and then diluted and assayed on strain K38.

Although cells infected by wild-type f1 continue to grow and divide, cells infected by most f1 mutant phage are killed (17), presumably due to the intracellular accumulation of phage products. Certain *rep* mutants which do not support filamentous phage DNA replication have been described (7). f1 gene expression is severely reduced in *rep* cells, and unlike wild-type cells, *rep* cells are not killed by infection with mutant phage (unpublished data). Thus, our 12 clones were screened for the ability to be killed by R12, a gene IV amber mutant phage which kills nonsuppressing (*sup*$^+$) cells very effectively. All seven of the clones that failed to grow f1 at both temperatures and one of the clones that was able to grow f1 only at 34°C were resistant to R12 killing. Gene expression, which was measured in five of the former clones and the latter clone, was severely reduced, and the lesion responsible for the defect in f1 growth was shown to cotransduce with *ilv*, as classic *rep* mutants do. These isolates were presumed to be *rep* mutants. Two of the clones which were killed by R12 infection had severe growth defects, and the mutations of these clones were not mapped. The remaining two clones that failed to grow f1 at 42°C contained mutations that mapped in different regions of the *E. coli* chromosome. The mutation in one mapped between *metC* and *nupG* and will be described elsewhere. The mutation described here was designated *fip* (for filamentous phage production). Strains containing the *fip* mutation have no obvious phenotype when they are not phage infected. The growth rate of the mutant was indistinguishable from that of wild-type cells at 34 and 41.5°C in Ty medium and in minimal medium. The mutant was also as resistant as wild-type cells to outer membrane perturbants, as it formed colonies at normal efficiency at 42°C on Ty medium plates containing 0.3% deoxycholate or 1% sodium dodecyl sulfate. The *fip* mutant plated a variety of other phages (T4, λ, P1, ST-1 (15), and f2) at normal efficiencies (0.75 to 1.2) at the higher temperature.

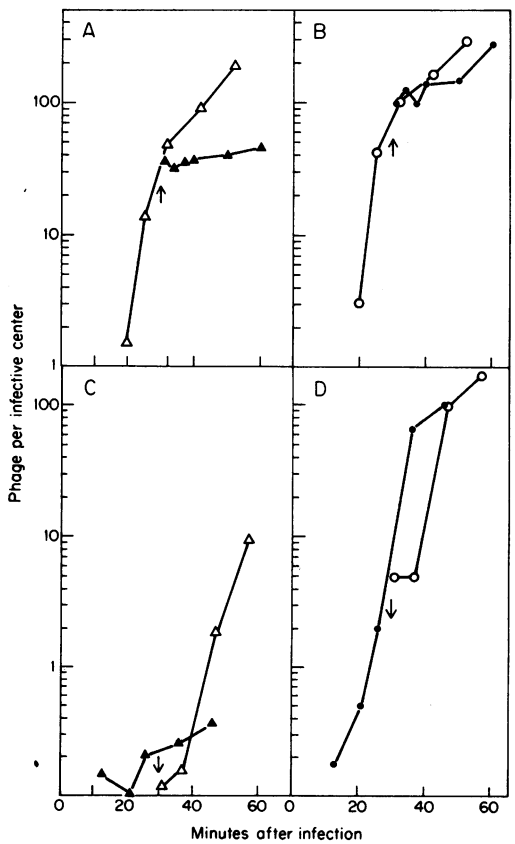


FIG. 2. Phage production after a shift from 34 to 41.5°C (A and B) or from 41.5 to 34°C (C and D). (A and C) Strain A95 (*fip*). Symbols: △, 34°C; ▲, 41.5°C. (B and D) Strain A96 (*fip*$^+$). Symbols: ○, 34°C; ●, 41.5°C. Infections were performed as described in the legend to Fig. 1, except that the final dilution from antiserum to prewarmed medium at the appropriate temperature was at 30 min.

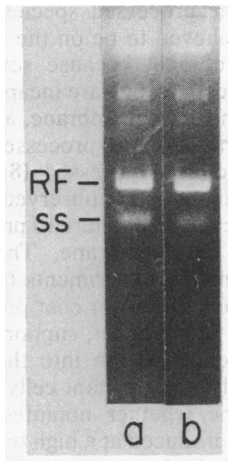


FIG. 3. DNA synthesis in *fip* bacteria. Lane a, strain A96 (*fip*$^+$); lane b, strain A95 (*fip*). Bacteria grown at 42°C in Ty broth were harvested 30 min after infection with f1 amber mutant R12 (multiplicity of infection, about 100). The cells were concentrated and electrophoresed on a 1% agarose–Tris-acetate gel along with f1 RF and single-stranded (ss) DNA standards, as described by Lerner and Model (23).

Characterization of *fip* mutant cells. The efficiency of f1 plaque formation on the *fip* mutant at 34 and 37°C was identical to the efficiency of f1 plaque formation on the wild-type *E. coli* strain from which the mutant was derived; at temperatures above 42°C, the efficiency was less than 10^{-6}. Phage production was measured in f1-infected strain A95 (*fip*) and A96 (*fip*$^+$) cells grown at 34 or 41.5°C (Fig. 1). At 34°C, the rate of phage production was essentially identical for the two infections. Filamentous phage production is intrinsically somewhat temperature sensitive (e.g., *fip*$^+$ at 34 and 41.5°C); however, at the high temperature the *fip* culture produced no detectable progeny phage.

Figure 2 shows that the defect in f1 phage production was readily reversible; strains A95 and A96 were grown and infected at 34°C and then shifted to 41.5°C (Fig. 2A and B) or grown and infected at 41.5°C and then shifted to 34°C (Fig. 2C and D). Phage production in the mutant cells stopped within about 1 min of the temperature shift to 41.5°C, and it resumed rapidly after the shift to 34°C. We infer from this result that the block in f1 phage production observed in *fip* mutant cells at the high temperature was not simply due to an indirect effect of irreversible membrane deterioration or of cell death.

At what level does f1 phage production fail in *fip* cells grown at the nonpermissive temperature? *fip*$^+$ and *fip* cultures were infected with R12, an f1 mutant blocked in assembly, so that no phage production occurred in the control culture, and DNA was isolated as described previously (23). Figure 3 shows the phage RF DNA and single-stranded DNA synthesized in infected *fip* and control cells grown at 42°C. The intracellular pool of single-stranded phage DNA was barely detectable in either infection at this temperature, as has been shown previously by Timmis and Marvin (43). The levels of phage-specific DNA were comparable in the two infections; the block in phage production (more than 100-fold [Fig. 1]) cannot be accounted for by an effect on synthesis of phage RF DNA or single-stranded DNA.

Figure 4 shows that synthesis of those phage-encoded proteins that can be detected in vivo was normal in *fip* bacteria grown and infected by f1$^+$ phage at 41.5°C. These proteins included pVIII (the major coat protein), pV (the single-stranded DNA-binding protein), and pIV′ (a truncated product of gene IV) (28; unpublished data). The f1 protein required for phage DNA replication, pII, and a minor coat protein, pIII,

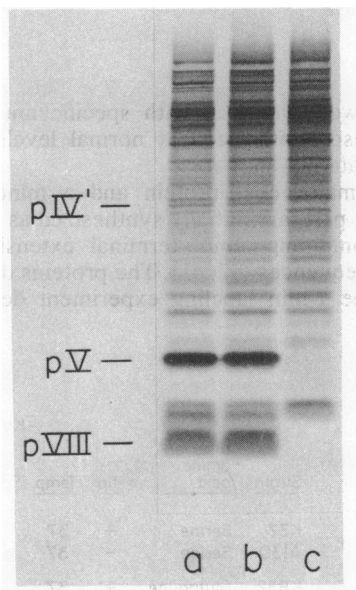


FIG. 4. Synthesis of f1-encoded proteins in *fip* bacteria. Lane a, f1-infected strain K569 (*fip*); lane b, f1-infected strain K38 (*fip*$^+$); lane c, uninfected cells. Bacteria grown at 41.5°C in minimal medium lacking methionine (4) to a density of about 4×10^8 cells per ml were infected with wild-type phage (multiplicity of infection, about 100). [^{35}S]methionine (10 μCi; 1 mCi/mmol) was added to 200-μl culture samples at 20 min after infection, and 1 ml of 5% trichloroacetic acid was added 1 min later. After centrifugation, the trichloroacetic acid precipitates were rinsed with acetone and suspended in 25 μl of 4% sodium dodecyl sulfate. Portions were diluted into sample buffer and electrophoresed directly on a 23% acrylamide–sodium dodecyl sulfate–urea gel (34).

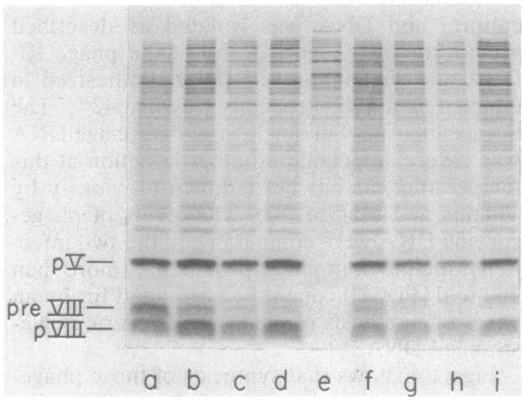
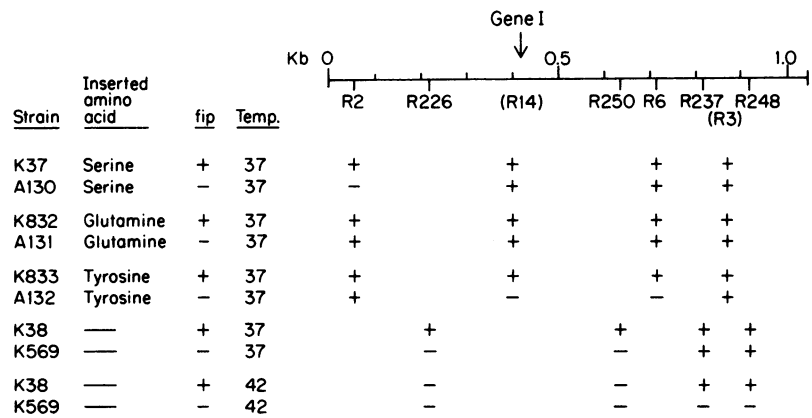


FIG. 5. Rate of precursor processing in *fip* bacteria. Lanes a through d, Strain A95 (*fip*); lanes f through i, strain A96 (*fip*$^+$); lanes a and f, 5-s chase; lanes b and g, 20-s chase; lanes c and h, 40-s chase; lanes d and i, 90-s chase; lane e, uninfected cells. Bacteria were grown, infected, and labeled as described in the legend to Fig. 4, except that nonradioactive methionine was added to a final concentration of 1 mg/ml 15 s after [^{35}S]methionine was added. Samples were placed into 5% trichloroacetic acid after 5, 20, 40, and 90 s of chasing.

which were detected with specific antiserum, were also synthesized at normal levels in *fip* cells (data not shown).

The major coat protein and a minor coat protein, pIII, are initially synthesized as precursors containing amino-terminal extensions or signal sequences (37, 41). The proteins detected after the 1-min labeling experiment described above were the processed species. The signal peptidase is believed to be on the outer face of the inner membrane, because several mutant precursor proteins, which are incapable of being inserted into the inner membrane, accumulate in vivo, although they are processed when the insertion defect is suppressed (8). Thus, the block in phage production observed in *fip* bacteria must not be due to a failure to properly insert proteins into the membrane. The results of NaOH fractionation experiments (35) (data not shown), in which the major coat protein (pVIII) was recovered in the pellet, support the conclusion that protein insertion into the membrane occurs normally in *fip* mutant cells.

To determine whether noninfectious phage particles were produced at a high temperature in *fip* bacteria, phage lysates were prepared from mutant and control cells that had been infected by f1$^+$ phage and grown at 41.5°C. The lysates were concentrated and electrophoresed on agarose gels to assay for the presence of phage particles. No particles were detected in the mutant lysate under conditions that permitted detection of particles at 5% of the *fip*$^+$ control level (data not shown). Thus, we conclude that the *fip* gene function is required for virion assembly. In the experiment shown in Fig. 5, f1-infected wild-type and *fip* bacteria were labeled with [^{35}S]methionine for 15 s and chased with nonradioactive methionine for different times. The rate of processing of precoat protein was reduced in *fip* cells; the fraction of coat protein that remained unprocessed after a 20-s chase was comparable to the fraction detected in the control after a 5-s chase. Except for the instance



Strain	Inserted amino acid	fip	Temp.	R2	R226	(R14)	R250	R6	R237	R248 (R3)
K37	Serine	+	37	+		+		+	+	
A130	Serine	−	37	−		+		+	+	
K832	Glutamine	+	37	+		+		+	+	
A131	Glutamine	−	37	+		+		+	+	
K833	Tyrosine	+	37	+		+		+	+	
A132	Tyrosine	−	37	+		−		−	+	
K38	—	+	37		+		+		+	+
K569	—	−	37		−		−		+	+
K38	—	+	42		−		−		+	+
K569	—	−	42		−		−		−	−

FIG. 6. Growth of gene I mutants on *fip* at a semipermissive temperature. R237 consists of a two-amino acid insertion (Asn-Ser), and R226, R250, and R248 consist of a Tyr insertion; the locations of these insertion mutants are defined by the loss of particular restriction sites (2). The locations of the amber mutations indicated by a line (R2 and R6) are derived from DNA sequence information, and the approximate locations of R14 and R3 (given in parentheses) are based on marker rescue experiments with purified restriction fragments (33). The mutant phages were diluted and spotted onto plates seeded with the bacterial strains indicated. Kb, Kilobase.

of a mutation in the coat gene (34), a slowing of precoat protein processing has been invariably associated with failure to extrude particles (19). Presumably the accumulation of large amounts of the major coat protein in the membrane alters the capacity of the cell to process the preprotein at maximal rates. Infection with mutant phage, which leads to the formation and extrusion of noninfectious particles, does not show the processing delay (19).

We tested the ability of mutant phage to grow on *fip* strains under semipermissive conditions. Mutant phages (containing an amber mutation in one of the nine phage genes) were plated at 37°C on strain K37 (*supD*) and on strain A130, a *fip* derivative of strain K37. Amber mutants in genes II through IX plated with equal efficiency on strain K37 and its *fip* derivative. However, R2, a gene I amber mutant, did not; the efficiency of plating of this mutant (about 10^{-6}) was similar to the frequency of amber$^+$ revertants in the stock, and, indeed, the plaques obtained on strain A130 were revertants. A series of gene I amber mutants and one or two codon insertion mutants in gene I (4) were tested for the ability to plaque at 37°C on *fip*$^+$ and *fip* strains containing various amber suppressors (Fig. 6). Failure to plaque on the *fip* strain at 37°C was allele specific and suppressor specific. For example, R2 failed to plaque on the serine-inserting *fip* strain (strain A130) at 37°C, but did plaque on the glutamine-inserting (strain A131) and tyrosine-inserting (strain A132) derivatives; R14 and R6 plaqued on strains A130 and A131, the serine- and glutamine-inserting *fip* strains, but not on strain A132, the tyrosine-inserting derivative. The most C-terminal gene I amber mutant, R3, plated on all three suppressor-containing *fip* strains. The gene I amber mutants plaqued at near-normal efficiencies on these strains at 34°C, although the plaques were smaller and more turbid than the plaques on the *fip*$^+$ suppressor strains. The gene I insertion mutants varied in their ability to plaque on *fip* cells at 37°C; those that are intrinsically temperature sensitive (on strain K38) (mutants R226 and R250) did not plaque on *fip* cells at 37°C, whereas the more C-terminal mutants (mutants R237 and R248), which are capable of growth on strain K38 at 42°C, also grew on *fip* cells at 37°C. These observations indicate that phage carrying mutations in the proximal two-thirds of gene I are particularly sensitive to the *fip* defect. Phage carrying mutations in the distal one-third of gene I do not exhibit this sensitivity.

Isolation and characterization of *gfip* mutants of f1. Spontaneous f1 mutants were selected by plating the entire contents of single f1$^+$ plaques on *fip* strain K569 at 42°C. Mutants arose at a frequency of about 10^{-7}; these phage, designated *gfip*, plated on strain K569 at 42°C with an efficiency of 1. Figure 7 shows the rates of phage production of *gfip*- and f1$^+$-infected *fip*$^+$ (Fig. 7A) and *fip* (Fig. 7B) bacteria at 41.5°C. The rates of *gfip* phage production were identical in *fip*$^+$ and *fip* cells. In fact, *gfip* phage were produced at a rate slightly higher than the rate of production of f1$^+$ in the *fip*$^+$ control infection.

A complementation test was performed to determine whether the *gfip* mutation was dominant to the f1$^+$ allele and whether the mutation was *cis* or *trans* active. A *gfip*-gene VII amber double mutant was constructed (see below). Strains K38 (*fip*$^+$) and K569 (*fip*) were each infected with this double mutant and a gene IV amber mutant or with a gene VII amber single mutant and the gene IV amber mutant. Amber mutant phage were used to infect these nonsuppressing hosts so that dominance of the *gfip* allele could be determined from cells that had been infected by both input phages. The rate of phage production at 41.5°C was measured by plating on permissive cells (*supD fip*$^+$). Figure 8A shows that in *fip*$^+$ cells the gene IV and VII

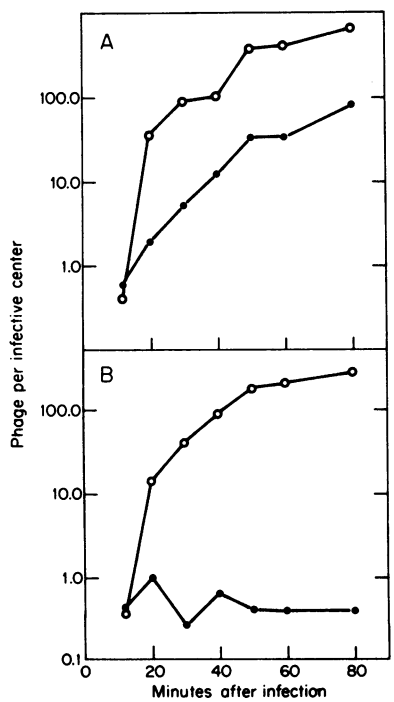


FIG. 7. Rate of *gfip* phage production in *fip*$^+$ and *fip* bacteria. (A) Strain K38 (*fip*$^+$). (B) Strain K569 (*fip*). Symbols: ○, *gfip*; ●, f1. Bacteria grown in Ty broth at 41.5°C were infected by f1$^+$ or *gfip* (R317) phage at multiplicities of infection of about 20. The cultures were treated with anti-f1 serum, diluted, sampled, and assayed on strain K38 at 37°C as described in the legend to Fig. 1.

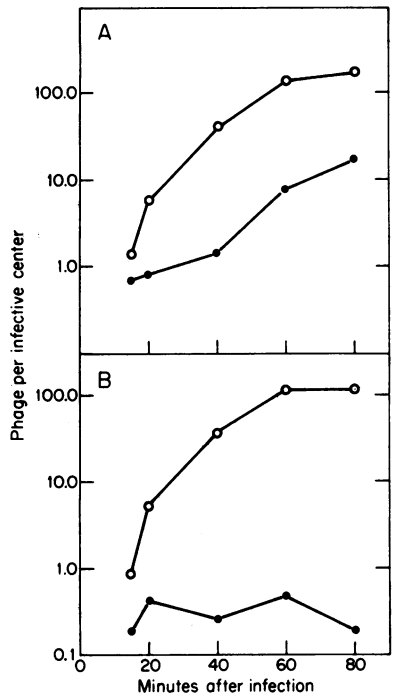


FIG. 8. Dominance of *gfip* to the wild-type allele. (A) Strain K38 (*fip*$^+$). (B) Strain K569 (*fip*). Symbols: ○, *gfip*-VII$_{amber}$ + IV$_{amber}$; ●, VII$_{amber}$ + IV$_{amber}$. Nonsuppressing bacteria grown at 41.5°C as described in the legend to Fig. 1 were infected with a mixture of *gfip*-VII$_{amber}$ (R318) and IV$_{amber}$ (R12) phages or with a mixture of VII$_{amber}$ (R148) and IV$_{amber}$ (R12) phages. Each phage was added at a multiplicity of infection of 20. The cultures were treated as described in the legend to Fig. 1, except that infective centers and progeny phage were assayed on strain K37 (*supD*), a permissive host for the amber mutant phages.

amber mutants complemented, regardless of the *gfip* mutation. Figure 8B shows that in *fip* cells, complementation occurred only in the infection in which one of the input phages contained the *gfip* mutation. Thus, *gfip* is dominant to the wild-type allele.

The genotypes of progeny from the *gfip*-gene VII amber × gene IV amber mixed infections shown in Fig. 8 were determined by replicating plaques on strains K37 (*supD*) and A130 (*supD fip*) at 42°C. The fraction of *gfip* phage among the progeny from the mixed infection of *fip*$^+$ (0.17) or from *fip* (0.25) was similar to the input frequency (0.22). Thus, the *gfip* mutation acts in *trans* to allow phage production in *fip* cells at the nonpermissive temperature.

Mapping the *gfip* mutation. The frequency of filamentous phage recombination is low, and it is difficult to distinguish recombinants from double-length phage that contain two phage genomes (25). Therefore, as an initial attempt to map the *gfip* mutation, we constructed recombinants in vitro. Double-stranded RF DNA was prepared from a gene VII-gene IV double amber mutant phage and from *gfip* phage. Each RF DNA was digested with restriction enzymes *Asu*I and *Bam*HI. These enzymes cleave f1 RF DNA once, and the cleavage sites are located at opposite positions on the circular genome, generating two fragments of approximately equal length. As shown in Fig. 9, the gene VII amber mutation is in one of these fragments, whereas the gene IV lesion is in the other. The fragments from both RF DNAs were mixed and ligated, and the ligated DNA was used to transfect permissive (*supD fip*$^+$) bacteria. Individual transfection plaques were tested for each marker by using standard complementation techniques. Two types of nonparental phages were recovered (gene VII amber-*gfip* double mutants and gene IV amber single mutants). Neither gene IV-*gfip* double mutants nor gene VII single mutants were obtained. Thus, *gfip* must be located in the same segment of the genome as gene IV.

The *gfip* mutation was further localized in a series of marker rescue experiments. Restriction fragments from *gfip* RF DNA and single-stranded DNAs from various amber mutant phages, were hybridized and used to transfect strain K748 (*sup*$^+$) cells, thereby selecting amber$^+$ phage. Individual plaques were scored for the *gfip* allele by using replica-plating techniques. The rationale for these experiments was that

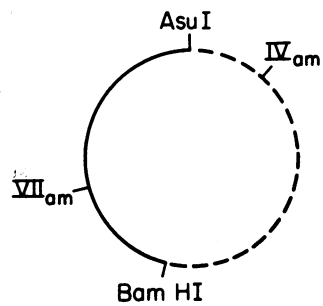


FIG. 9. Simplified map of f1. The single *Bam*HI and *Asu*I sites relative to the locations of the gene IV and gene VII amber mutations are indicated. RF DNAs from R36 (IV$_{amber}$-VII$_{amber}$) and R317 (am$^+$-*gfip*) were digested to completion with *Asu*I and *Bam*HI. The fragments were phenol extracted, mixed, and coprecipitated in ethanol; the DNA mixture was resuspended at a concentration of 0.1 mg/ml and incubated for 24 h at 4°C with T4 DNA ligase. Strain K37 (*supD*) cells were transfected with the ligated DNA. Single plaques were picked and replicated onto strain K569 (*fip*) at 42°C to assay for the *gfip* allele and onto strain K38 (*sup*$^+$) seeded with IV$_{amber}$ or VII$_{amber}$ phage to assay for the amber alleles.

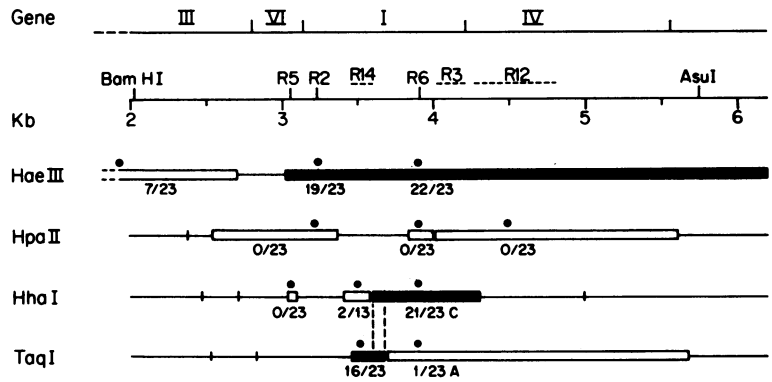


FIG. 10. Rescue of amber mutations by restriction fragments to map the *gfip* mutation. Restriction fragments of RF DNA of *gfip* phage were used to rescue various amber mutations as described in the text. The amber mutation rescued for each restriction enzyme digest is indicated by a solid circle. The location of each amber mutation indicated is based on DNA sequence information or on the results of marker rescue experiments with purified restriction fragments. The fragments that simultaneously rescued amber mutations and introduced the *gfip* mutation are indicated by solid bars; those that rescued amber mutations but did not introduce the *gfip* mutation are indicated by open bars. In each case, the number of amber$^+$ phage tested and the number that exhibited the *gfip* phenotype (*gfip*/total) are indicated below the bars. Kb, Kilobases.

under conditions of single-strand DNA excess, the fraction to which more than one restriction fragment hybridized would be low; thus, simultaneous rescue of the amber$^+$ allele and *gfip* would occur if they both were located on the same restriction fragment. This was the case, as Fig. 10 shows; the frequency with which *gfip* phage were obtained from fragments outside a small region within gene I was low, usually undetectable. By contrast, fragments that included the central portion of gene I brought in the *gfip* allele at high frequencies (70 to 97%). These experiments with unfractionated restriction fragments defined a 96-base region (between the *Hha*I fragment L-*Hha*I fragment C border and the *Taq*I fragment J-*Taq*I fragment A border) within which *gfip* must be located.

This conclusion was confirmed by marker rescue experiments with purified restriction fragments. *Hha*I fragment C and *Taq*I fragment A were purified from *gfip* DNA and were used to rescue R3 single strands to amber$^+$. Of 23 plaques obtained from purified *Hha*I fragment C, 10 were *gfip*; none of the 15 plaques obtained from purified *Taq*I fragment A were *gfip*. These fragments were also used in marker rescue experiments with single strands prepared from wild-type f1 phage, in which the *gfip* phenotype was selected directly. Plaques were selected by transfecting *fip* cells at 42°C and were obtained from *Hha*I fragment C, but not from *Taq*I fragment A.

The sequence of a region of the *gfip* genome that included the 96-base region implicated by marker rescue experiments was determined by the dideoxy method of Sanger et al. (36). Four independent *gfip* isolates contained the same adenine-to-thymine transversion at nucleotide 3,619. This mutation (AAT to TAT) changed amino acid 142 in gene I from asparagine to tyrosine. This was the only change detected in the more than 200 bases sequenced. Interestingly, this is the site of the only amino acid difference in gene I between f1 and its close relative, M13; the M13 codon, CAT, specifies histidine (1, 16). Another related filamentous phage, fd, also codes for histidine (CAC) at this position, although two additional differences within gene I distinguish this phage from f1 and M13 (1, 16). We wanted to determine whether wild-type M13 and fd grew on *fip* bacteria at the nonpermissive temperature. Both phages formed small, turbid plaques on strain K569 at 42°C, and the phage yields in experiments similar to the experiment shown in Fig. 7 were about 10% of the *gfip* yield. More than 80% of the amber$^+$ plaques obtained in a marker rescue experiment in which R6 was rescued to amber$^+$ by M13 DNA digested with *Hha*I were able to grow on *fip* cells at 42°C, indicating that this phenotype must be due to the histidine at position 142 of the M13 gene I protein.

Mapping the *fip-1* mutation. We devised a modification of the procedure described by Kleckner et al. (21) for identifying cells in which a selectable transposable element has been inserted near a gene of interest to map the *fip-1* mutation carried by strain K569. Strain K91 (*sup*$^+$ λ^-) cells were infected by a Tn*5*-containing lambda phage, λ467, and kanamycin-resis-

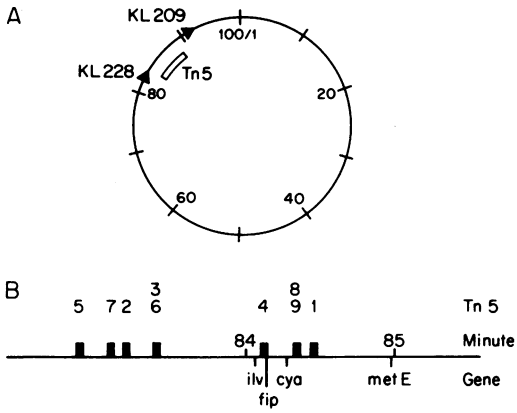


FIG. 11. Map locations of Tn*5* insertions near *fip*. (A) Diagram of *E. coli* chromosome and Hfr strains used to determine the approximate locations of Tn*5* insertions near *fip*. (B) More detailed map locations of independent Tn*5* insertions near *fip* based on the results of the P1 transduction experiments presented in Tables 2 and 3. The bars indicate the approximate locations of Tn*5* insertions *zie/zid-1* through *-9*.

tant colonies were selected. λ467 (*c*I857 *b*221 *O*am29 *P*am80 *rex*::Tn*5*) is not able to replicate in or lysogenize this host effectively, and most Kan^r clones result from transposition of Tn*5* into the bacterial chromosome. A P1 lysate was prepared on a pool of about 1,000 Kan^r clones (representing about 1,000 independent events). This lysate was used to transduce strain A18 (*fip* λ^r) to Kan^r. Individual Kan^r clones were scored for the *fip*$^+$ allele by cross-streaking. Although λ467 fails to replicate effectively in cells that do not contain an amber suppressor, enough phage are produced to severely contaminate a P1 lysate. These phage confer a Kan^r phenotype on sensitive cells, and the frequency of true Kan^r P1 transductants is too low to detect. We have found that if a λ^r recipient is used for this first transduction, the enormous background of cells rendered Kan^r by contaminating λ467 phage was avoided. Several independent Kan^r *fip*$^+$ transductants (strains A19 through A27) were isolated, and P1 lysates prepared on each of these strains were used to transduce *fip* strain K569 to Kan^r; the frequency of Kan^r *fip*$^+$ cotransduction was determined for each strain by cross-streaking. The cotransduction frequencies varied between about 10 and 100%, suggesting that Tn*5* insertion occurred at several different sites near *fip*.

P1 lysates were prepared on several Kan^r *fip* transductants of strain K569. These lysates were used to transduce strain K38 to Kan^r, and the Fip phenotype was scored by cross-streaking; the cotransduction frequencies (to Kan^r Fip$^-$) varied between about 5 and 85%. This transduction of the mutant phenotype into a clean genetic background indicates that the mutant phenotype does not involve multiple mutations in widely dispersed regions of the chromosome.

One of the Tn*5* insertions was mapped by introducing it into several Hfr strains (by P1 transduction) and scoring it as an unselected marker in crosses with a suitably marked F$^-$ strain. The Tn*5* element behaved as a proximal marker in crosses with strain KL209 and as a distal marker in crosses with strain KL228, implicating the interval between 84 and 91 min on the *E. coli* chromosome, as shown in Fig. 11A. A subsequent transduction analysis, in which strain K815 (Δ*cya metE*::Tn*10*) was the recipient, showed that this Tn*5* (*zie-1*, from strain A19) cotransduced with both *cya*$^+$ (84%) and *metE*$^+$ (47%); the frequency of *cya*$^+$ *metE*$^+$ Kan^r transductants (30%) suggests that the Tn*5* insertion lies between *cya* and *metE* at about 84.5 min.

P1 lysates derived from each of the indepen-

TABLE 2. Cotransduction of Tn*5* near *fip* with *cya* and *metE*[a]

Donor strain	No. of Kan^r colonies	No. of Kan^r *cya*$^+$ colonies	% of Kan^r *cya*$^+$ cotransductants	% of *metE*$^+$ colonies among Kan^r *cya*$^+$	% of *metE*$^+$ colonies among Kan^r *cya*
A19 *zie-1*::Tn*5*	768	717	93	40	90
A20 *zid-2*::Tn*5*	2,170	109	5	0	0
A21 *zid-3*::Tn*5*	2,510	270	11	0	0
A22 *zie-4*::Tn*5*	1,234	1,060	86	25	0
A23 *zid-5*::Tn*5*	2,728	8	0.3	0	0
A24 *zid-6*::Tn*5*	2,950	250	8	0	0
A25 *zid-7*::Tn*5*	2,790	90	3	0	0
A26 *zie-8*::Tn*5*	1,320	1,160	88	18	12.5
A27 *zie-9*::Tn*5*	1,700	1,600	94	32	50

[a] Strain K815 (Δ*cya-854 metE*::Tn*10*) was transduced by P1 prepared on the donor strains. Kan^r colonies were selected and scored for the *cya* allele on the basis of colony size. The numbers of Kan^r transductants and *cya*$^+$ Kan^r cotransductants are indicated (see text). A total of 50 *cya*$^+$ and 50 *cya* colonies from each transduction were scored for the *metE* allele.

TABLE 3. Mapping *fip* by three-factor P1 transductions

Expt	Recipient strain	P1 donor strain	Selected marker	Scored marker(s)[a]	No. scored/ total no.	%
1	K38 (*ilv*$^+$ *fip*$^+$)	A95 (*ilv fip zie-1*::Tn*5*)	Kan^r	*ilv*$^+$ *fip*$^+$	11/33	33
				ilv$^+$ *fip*	2/33	6
				ilv fip$^+$	0/33	0
				ilv fip	20/33	61
2	K815 (Δ*cya*)	A37 (*fip zid-6*::Tn*5*)	Kan^r	*cya*$^+$	160/1,200	12
				fip cya$^+$	10/10	100
				fip cya	0/7	0
3	K818 (*ilv fip*$^+$)	A37 (*ilv*$^+$ *fip zid-6*::Tn*5*)	*ilv*$^+$	*fip* Kan^r	6/32	19
				fip Kan^s	12/32	38
				fip$^+$ Kan^r	4/32	13
				fip$^+$ Kan^s	10/32	31

[a] Transductants in experiments 1 and 3 were tested for cotransduction of the unselected markers by replication onto appropriate selective plates and by cross-streaking. Transductants in experiment 2 were scored for the *cya* allele; 10 *cya*$^+$ Kan^r clones and 7 *cya* Kan^r clones were subsequently scored for *fip*.

dently isolated Tn*5* insertions near *fip* (i.e., from strains A20 through A27) were similarly used to transduce strain K815 to Kan^r (Table 2). The Tn*5* insertions from five of these strains (those containing *zid-2*, *-3*, *-5*, *-6*, and *-7*) showed reduced linkage to *cya*, and they did not bring in *metE*$^+$ at all. Thus, these insertions are located on the *cya* side of the *cya-metE* interval. One insertion (*zie-4*) was more tightly linked to *cya*; Kan^r *metE*$^+$ cotransductants were obtained, and they were all *cya*$^+$. Thus, *zie-4*::Tn*5* must be closer to *cya* than the insertions which do not bring in *metE*, but must still be outside the interval. The pattern was different for insertions *zie-1*, *-8*, and *-9*. These insertions were closely linked to *cya*, but Kan^r transductants that were *cya* acquired the *metE*$^+$ allele at appreciable frequencies. Thus, these Tn*5* insertions occurred within the *cya-metE* interval. An approximate map of these insertions, based on data presented in Tables 2 and 3, is shown in Fig. 11B.

zie-4 is very tightly linked to *fip*$^+$; segregation of these markers has not been observed, although several hundred Kan^r transductants of strain K569 were scored for retention of the *fip* allele. Since *zie-4* was shown to map very near *cya*, just outside the *cya-metE* interval, the gene order in this region of the chromosome must be *fip-cya-metE*. The results of additional transduction experiments (Table 3) indicate that the order is *ilv-fip-cya*. Insertion *zie-1* was shown to map between *cya* and *metE*, and the first transduction showed that all of the Kan^r recipients which were cotransduced for the *ilv* allele also received the *fip* allele; 20 of the 22 Fip$^-$ transductants became Ilv$^-$, indicating that *fip* is closely linked to *ilv* and probably lies between *ilv* and *cya*. This conclusion is supported by the results of transduction experiments with a P1 donor containing *zid-6*::Tn*5*; this Tn*5* insertion lies outside the *cya-metE* interval, on the *ilv* side of *cya*. All of the Kan^r recipients which were cotransduced for the *cya*$^+$ allele also received the *fip* allele, suggesting that *fip* lies between *zid-6*::Tn*5* and *cya*. The results of the third transduction (in which a *fip zid-6* donor was used) show that Kan^r and Fip$^-$ segregate independently after selection for Ilv$^+$, indicating that they lie on opposite sides of *ilv*. The *fip* gene can be located within the *zid-6–cya* interval and outside the *zid-6–ilv* interval only if the gene order is *zid-6–ilv–fip–cya*.

The map position of *fip* was defined more precisely with the λd *ilv* transducing phage isolated by Jorgensen et al. (20) and used by Tessman et al. (42) to map the *rep* gene; λ37 and λ22, but not λ58, provide Rep$^+$ function (42). An *ilv fip* strain (strain A95) was transduced to *ilv*$^+$ with the lambda transducing phage shown in Fig. 12. λ22, but not λ37 or λ58, provided *fip* function. Thus, *fip* is not *rep*, and *fip* is located between *rep* and *cya* in interval 2 or at the junction of intervals 1 and 2. The *rho* gene has been assigned to interval 2 (42, 45). We have cloned the *fip* gene (manuscript in preparation); the resulting clones provide *fip* function, but they do not allow growth of a temperature-sensitive *rho* mutant at 42°C, and they do not provide *rep* function (data not shown). Thus, *fip* is not *rho*.

Dominance. The *fip*$^+$ gene was dominant to the mutant allele. An F′ factor that covers the *ilv* region, F′197 from strain JG85 (49), was introduced into strain A89 (F$^-$ *ilv zie-1*::Tn*5* *fip recA*), and *ilv*$^+$ Kan^r derivatives were purified and tested for the ability to plaque f1 at 42°C. F′197-containing *fip* derivatives were able to support f1 growth at 42°C, whereas strain A89 containing an F$^+$ factor was not.

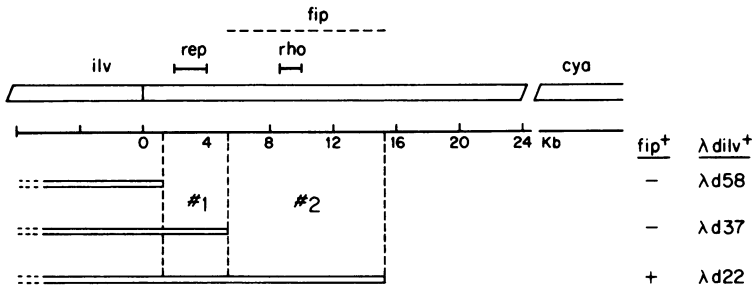


FIG. 12. Fine structure mapping of *fip* with λd *ilv* transducing phage. Log-phase strain A95 cells (*fip ilv*) were infected with a mixed lysate of λ helper and λd *ilv* phages at a multiplicity of infection of about 0.1, and *ilv*$^+$ transductants were selected on minimal plates lacking isoleucine and valine. The transductants were purified on selective plates, and individual *ilv*$^+$ colonies were cross-streaked against f1 and incubated at 42°C to assay for the Fip phenotype. The diagram of the *ilv-cya* region is adapted from the diagrams of Tessman et al. (42) and Uzan et al. (45). Kb, Kilobases.

DISCUSSION

We isolated and characterized a mutation in a previously undescribed bacterial gene (*fip*) which is required for filamentous phage f1 assembly. We have not detected an effect of this mutation on bacterial growth or on the growth of several other phages. Filamentous phage injection, DNA replication, and gene expression did not appear to be altered in the mutant host. Membrane insertion and signal peptide cleavage of phage-encoded preproteins also occurred normally. However, at the nonpermissive temperature the block in phage production was absolute; fewer than one phage per infected cell was recovered at 41.5°C. This block was reversible; phage production resumed within a few minutes of a shift from 41.5°C to a permissive temperature. Similarly, phage production ceased rapidly after a shift from 34°C to the nonpermissive temperature. The rapidity with which the mutant phenotype can be induced or reversed suggests that the *fip* mutation affects filamentous phage assembly directly.

This defect appears to affect the capacity of a phage gene product to participate in assembly of the virions. This conclusion is based on two observations. First, several f1 gene I mutants (unlike mutants in the other eight phage genes) were unusually sensitive to the *fip* mutation, failing to grow at an otherwise permissive intermediate temperature, 37°C. The more stringent requirement for gene I function in *fip* cells is for quality, not quantity, of gene I protein. The gene I amber mutation in R2 is identical to 1H7 and arose from a glutamine codon (CAG-UAG) (unpublished data; 46). R2 grows on the *fip* glutamine-inserting suppressor, despite the fact that *supE* has been shown to be inefficient in many contexts (10, 38, 40). Tyrosine, but not serine, suffices for R2 to grow on *fip*, although both *supD* and *supF* are efficient suppressors in many contexts (10, 38, 40). Second, spontaneous f1 mutants that are capable of normal growth on *fip* cells at the nonpermissive temperature have been isolated. These phage contain a gene I mutation, designated *gfip*, that consists of an adenine-to-thymine transversion at nucleotide 3,619. Wild-type f1 encodes asparagine at this position (amino acid 142 in gene I), and *gfip* specifies tyrosine. M13, a closely related filamentous phage that differs from f1 at only four (16) or eight (1) sites that cause amino acid differences over the entire genome, contains histidine at this position. This is the only amino acid difference between these phages in gene I. M13 grows, albeit poorly, on *fip* strains at the restrictive temperature. Thus, this strain-specific difference can have phenotypic consequences and most likely reflects slightly different selective pressures. The *gfip* mutation again underscores the close relationship between the function of the phage gene I product and the bacterial *fip* product. These allele-specific effects suggest that the gene I and *fip* products interact to promote filamentous phage assembly.

We know nothing of the role of the gene I protein in phage assembly, except that phage DNA synthesis and gene expression occur but no phage particles are produced (32). This protein has not been detected in vivo, and only very small amounts are synthesized in vitro (18, 28). Further characterization of the *fip* gene product, of gene I protein, and of the gene I mutants that overcome the *fip* assembly defect should provide a means to begin to understand how filamentous phage assemble in the cytoplasmic membrane and how they are extruded from living cells.

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