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Replacement of the *fip* Gene of *Escherichia coli* by an Inactive Gene Cloned on a Plasmid

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To determine whether the *fip* gene of *Escherichia coli*, which is required for filamentous phage assembly, is required for cell viability, we replaced the chromosomal copy of the gene with an inactive copy introduced on a plasmid. We found that the *fip* gene is dispensable. The method we devised, which should be generally useful, was also tested with an inactivated *rho* gene. As expected, the *rho* gene is essential.

The *fip* gene of *Escherichia coli* is defined by a single mutation, *fip-1*, that renders the cell temperature sensitive for filamentous phage assembly (18). Genetic evidence suggests that the *fip* protein normally interacts with a phage-encoded morphogenetic protein, pI, to promote assembly and that this interaction is blocked at 42°C, but not at 34°C, in the mutant. The *fip* gene has been mapped (18) and cloned (19); it is located between *rep* and *rho*, at 84.7 min on the *E. coli* chromosome (1) and is transcribed in the same direction as *rho*. The *fip* protein is small (12.5 kilodaltons) and cytoplasmic (19).

Bacterial mutations that restrict bacteriophage development are often subtle, sublethal changes in essential genes (2, 4–7). We were interested in determining whether the *fip* gene product is essential and what role it plays in the life of the cell. To accomplish the first goal, we replaced the chromosomal gene with a nonfunctional copy of the gene cloned in a *polA*-dependent plasmid. The method involves integrating the cloned inactive gene (with plasmid) into the chromosome (8, 9, 23) and then selecting for cells in which the plasmid has excised. Within this population are cells in which the nonfunctional gene copy is in the chromosome and the functional gene is in the plasmid. Such chromosomal mutant copies can be transduced by phage P1 into the chromosome of a *polA*$^+$ recipient strain. Successful transduction into strains that contain two or more functional copies of the gene, but not into strains that contain a single wild-type gene copy, indicates that the gene is essential. This procedure ensures the presence of a functional gene copy at all times, so that cells with an inactive copy of an essential gene in the chromosome can be recovered. With this technique, we show that cells lacking an intact *fip* gene are viable, whereas those lacking an intact *rho* gene are not.

Gutterson and Koshland (9) have also used plasmid integration as a method for gene inactivation; however, their method is applicable only to nonessential genes.

MATERIALS AND METHODS

Bacterial strains, phage, and plasmids. The bacterial strains, phage, and plasmids used are listed in Table 1. Bacteria were routinely grown in Ty medium or on Ty plates (13), except for transduction mixtures, which were plated on minimal medium plates as described previously (18). Plasmid pEG25, obtained from S. Adhya, contains a *Hin*dIII-*Pvu*II fragment from the *E. coli* chromosome that includes a complete *rho* gene; this fragment replaces the *Hin*dIII-*Pvu*II *tet* gene fragment from pBR322. Plasmid pSKS101 was kindly provided by M. Casadaban (20); it contains the *kan* gene derived from Tn*5* flanked by *Eco*RI-*Bam*HI-*Sal*I-*Pst*I polylinkers in a pBR322-derived Amp^r vector.

DNA manipulations. Plasmid and f1 phage replicative-form DNA were prepared as described previously (19). Transformation was by the method of Mandel and Higa (14). Standard procedures (15) were used for restriction enzyme digestions, nuclease S1 digestion, ligations, ethidium bromide-agarose gel electrophoresis, and Southern transfer and hybridization.

Immunoblot procedure. Cell lysates were electrophoresed on a 6 M urea–sodium dodecyl sulfate–acrylamide gel (20% acrylamide/0.075% bisacrylamide) containing 0.09 M NaCl. The gel slab was briefly swollen in water, carefully placed on two nitrocellulose sheets (0.45-μm pore size; Schleicher & Schuell), and sandwiched between several pieces of filter paper (Whatman 3MM) cut to the size of the gel. Electrophoretic transfer and immunological transfer of protein were essentially as described by Haid and Suissa (10), with the following modifications: (i) no methyl green was used; (ii) the transfer buffer was 20 mM sodium phosphate (pH 8.0); (iii) transfer was for 2 to 3 h at 300 to 400 mA; (iv) all incubations of the nitrocellulose sheet were in phosphate-buffered saline plus 10 mM Tris-hydrochloride (pH 7.5)–0.1% Triton X-100–0.02% sodium dodecyl sulfate–2.5 mg of gelatin per ml (PBS-M); (v) the nitrocellulose sheet was preadsorbed with PBS-M containing 2.5 mg of bovine serum albumin per ml, fraction V (Pentex), before antibody addition; and (vi) the second antibody was affinity-purified, horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Jackson Immuno Research Laboratories, Inc.).

RESULTS

Construction of an interrupted cloned *fip* gene. The *fip* gene has been cloned in pBR325 and subcloned in phage f1 (19). The single *Ava*II restriction endonuclease cleavage site in one such subclone, R316, is located within the 300- to 400-base-pair *fip* coding region (19). Thus, to inactivate the cloned gene, R316 replicative-form DNA was digested with *Ava*II, treated with S1 nuclease to remove the 5′-extending single-stranded nucleotides, ligated to *Bam*HI linkers, and redigested with *Ava*II. Cells were transfected by this DNA, and phage containing the new *Bam*HI site in *fip* (R316-A) (Fig. 1) were isolated. The *fip* gene was inactive in these phage.

To provide a selectable marker for the interrupted *fip* gene, an antibiotic resistance gene was cloned into the new *Bam*HI site in *fip*. Plasmid pSKS101 contains a kanamycin resistance gene flanked by multiple restriction enzyme

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TABLE 1. Bacterial strains, phage, and plasmids

Strain	Genotype	Construction/source or reference
Bacteria		
K38	HfrC (λ)	
K549	F⁺ *thyA rha lac rpsL polA12*(Ts)	F⁺ derivative of MM383 (I. R. Lehman)
K817	K38 *ilvC7 Δcya-854*	This study
A179	K817 *ilv*⁺ *cya*⁺ *fip*::*kan*	This study
K819	K38 *ilv-683 rho-702*(Ts) *metE163*::Tn*10*	This study
K844	F⁻ *thi thr leu ilvC*::Mu-1 Tl^r λ *c*I857 *S*am7, λ *c*I857 *S*am7 *dilv-DAC62*	L62 of P. Jorgensen (21, 22)
K866	F⁺-1::Tn*10 his-871 relA rpsL181 gal-3 rep-71*	F⁺ derivative of IT1101 (I. Tessman [21])
Phage		
R316	*fip*⁺ f1⁺	*fip* gene cloned in f1 phage (19)
R316-A	*fip* f1⁺	*Ava*II site in *fip* converted to *Bam*HI site; this study
R316-AK	*fip*::*kan* f1⁺	*kan* gene cloned in the R316-A *Bam*HI site in *fip*; this study
Plasmids		
pPMR5	*fip*⁺ *cam tet*	*fip* gene cloned in pBR325 (19)
pPMR5-AK	*fip*::*kan cam tet*	*kan* gene cloned in *Bam*HI site in *fip*; this study
pPMR7	*fip*⁺ *amp*	*fip* gene cloned in pIN-A1 (19)
pEG25	*rho*⁺ *amp*	*rho* gene cloned in pBR322 (19; Guletta and Adhya, personal communication)
pEG25-H	*rho amp*	*Hpa*I site in *rho* converted to *Bam*HI site; this study
pEG25-HK	*rho*::*kan amp*	*kan* gene cloned in the pEG25-H site in *rho*; this study
pSKS101	*kan amp*	20

cleavage sites (20); pSKS101 and R316-A DNAs were digested with *Bam*HI and ligated, and phage that rendered host bacteria Kan^r were isolated. The structures of several isolates were analyzed to confirm that the 2-kilobase (kb) *kan* gene fragment had been inserted into the *Bam*HI site in *fip* (Fig. 1). The resulting phage is called R316-AK.

Next, we used in vivo recombination to transfer the *fip*::*kan* region from R316-AK to pPMR5, a pBR325 derivative containing the 3.4-kb *Pst*I fragment from the *E. coli* chromosome that includes the *fip* gene cloned in the *amp* gene (19). To facilitate recovery of *fip*::*kan* on pPMR5 and to counterselect the phage, a *rep* strain (K866) containing pPMR5 was infected by R316-AK. Filamentous phage replication is blocked in this strain (data not shown). After 60 min of growth at 37°C, Kan^r Cm^r colonies were selected, and plasmid DNA was prepared from several isolates. In addition to material that comigrated with pPMR5, substantial amounts of a new plasmid, about the size predicted for the pPMR5-*fip*::*kan* recombinant (Fig. 1), were detected in two of the DNA preparations. These DNA mixtures gave rise to Kan^r Cm^r transformants at high frequency (21 to 50%) relative to Cm^r transformants. Restriction endonuclease analysis of plasmid DNA prepared from one Kan^r Cm^r transformant confirmed that the *kan* gene had been incorporated into the *fip* gene of pPMR5, creating pPMR5-*fip*::*kan*.

Replacement of the chromosomal *fip* gene by *fip*::*kan*. ColE1-type plasmids cannot stably transform *polA* mutant strains because their replication depends on DNA polymerase I, the product of the *polA* gene (12). Stable transformants can be obtained if the ColE1-derived plasmid contains DNA homologous to the host chromosome (8, 9, 23); the plasmid is maintained by its integration into the chromosome at the locus of the homologous DNA (8). Yamaguchi and Tomizawa (23) also showed that complete restoration of *polA* function is lethal to cells with such an integrated plasmid.

We took advantage of these observations to select transformants of a *polA* mutant strain in which pPMR5-*fip*::*kan* had recombined into the chromosome and then restored *polA* function to select for cells in which the plasmid had excised. We anticipated that this excision could occur in two ways: it could restore *fip*::*kan* to the plasmid and leave *fip*⁺ on the chromosome, or it could leave *fip*::*kan* on the chromosome and *fip*⁺ on the excised plasmid (Fig. 2).

A *polA*(Ts) strain (K549) was transformed by pPMR5-*fip*::*kan*, and transformants were selected at 30°C. Individual colonies were suspended and plated on antibiotic-containing plates at 30 and at 42°C. Colonies were obtained at a frequency of 10^{-2} to 10^{-3} at 42°C. Two high-temperature survivors were suspended and assayed at 30 and 42°C on antibiotic-containing plates. Their colony-forming ability was the same at both temperatures. One was subcultured at 30°C in the expectation that some excision would take place.

To obtain cells in which *fip*::*kan* (rather than the wild-type allele) remained in the chromosome, a P1 lysate (no. 1) prepared on this 30°C-grown culture was used to transduce strain K817 or K817(pPMR7) (*amp fip*⁺ [19]), with selection for markers that flank the *fip* gene (*ilv*⁺ and *cya*⁺). Flanking chromosomal markers were selected to avoid simply transducing free plasmid. Control experiments showed that free, nonintegrated pPMR5-*fip*::*kan* (11-kb) plasmid could be transduced by P1 at a frequency similar to that of the chromosomal markers *ilv*⁺ and *cya*⁺, but cotransduction of free plasmid and chromosomal markers was not observed (0 of 90 scored).

Cells transduced to *ilv*⁺ *cya*⁺ by the experimental P1 lysate (no. 1) were scored for cotransduction of Kan^r and for loss of Cm^r Tc^r (antibiotic resistance markers carried by the vector portion of pPMR5-*fip*::*kan*) (Table 2). None of the transductants (0 of 10) of K817 that had lost the vector markers were Kan^r, whereas two of six of those from K817(pPMR7) were; this bias implies that cells lacking an intact *fip* gene cannot grow or cannot grow well (see below). Most of the *ilv*⁺ *cya*⁺ transductants were also Kan^r Cm^r Tc^r [86% for K817, 98% for K817(pPMR7)], indicating that selection for cells in which the plasmid had excised from the chromosome before preparation of P1 lysate no. 1 had been inefficient.

However, two Kan^r Cm^s Tc^s transductants were obtained (from the recipient that provided *fip* function in *trans*). A second P1 lysate (no. 2) prepared on one of these transductants was used to transduce K817 or K817(pPMR7) to *ilv*⁺ Kan^r *cya*⁺. Similar numbers of transductants were obtained with either recipient. Kan^r transductants of K817 formed small colonies (data not shown); this may explain our failure to detect this class from the first transduction. These results

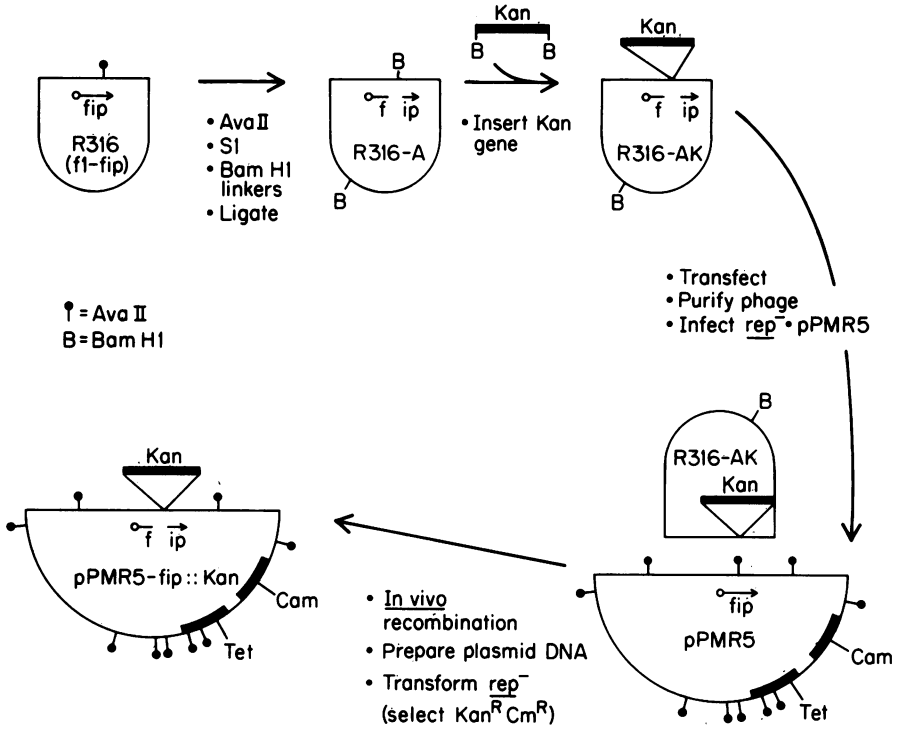

FIG. 1. Construction of a selectable null allele of the *fip* gene cloned on a *polA*-dependent plasmid. The single *Ava*II site (f) in R316, located within the *fip* coding region (19), was converted to a *Bam*HI site with *Bam*HI linkers; the resulting phage, R316-A, which contains two *Bam*HI sites (B), was partially digested by *Bam*HI and ligated to a *Bam*HI digest of pSKS101 (20), the *kan* gene donor. Kan^r phage were isolated, and one (R316-AK) in which the 2-kb *kan* gene had been inserted in the new *Bam*HI site in *fip* was used to infect strain K866(pPMR5). Plasmid DNA prepared from several infected colonies was used to transform strain K866 to Kan^r Cm^r to clone the recombinant plasmid, pPMR5-*fip*::*kan*.

suggest that although the absence of *fip* impairs cell growth, *fip* function is not essential for cell viability under the conditions employed.

Confirmation of gene replacement. Four criteria were used to confirm gene replacement in the construct, strain A179: (i) linkage of the *kan* gene to the markers that flank *fip*; (ii) failure to support filamentous phage growth; (iii) absence of the *fip* protein; (iv) alteration of the chromosomal DNA fragment containing the *fip* gene.

A P1 lysate prepared on strain A179 was used to transduce strain K817 to *ilv*$^+$ *cya*$^+$; 99% of the transductants were Kan^r. The Kan^s transductants are presumably the product of rare multiple recombination events. When this P1 lysate was used to transduce a *thr leu* strain to prototrophy, none of the transductants were Kan^r. Thus, the *kan* gene originally introduced into the cloned *fip* gene (R316-AK) was localized in the appropriate region of the A179 chromosome.

The phenotype of the original mutant that defined the *fip* gene is its failure to support f1 phage growth at high temperature (efficiency of plating, ca. 10^{-6}) (18). Strain A179 failed to support f1 phage growth at any temperature (efficiency of plating, $<10^{-7}$); R316, the f1 phage derivative that contains a functional cloned *fip* gene, does grow on strain A179 (efficiency of plating, ca. 1), suggesting the absence of an endogenous functional *fip* gene in A179.

Figure 3 shows the result of an immunoblot of whole-cell proteins from strain A179 along with a *fip*$^+$ control strain. No *fip* protein could be detected in the A179 lysate (although a high-molecular-weight contaminating antigen recognized by this antiserum was present at identical levels in the two lysates). The small size of full-length *fip* protein (12.5 kilodaltons) and the location of the *kan* gene insertion early in the gene in A179 (Russel and Model, unpublished data) suggest that a truncated *fip* polypeptide would have been too small to detect in this gel system.

To confirm that the *fip* gene had been replaced by *fip*::*kan* and that the vector had been excised in strain A179, we performed a Southern gel analysis. The *fip* gene was originally isolated on a 3.4-kb *Pst*I fragment from an *E. coli Pst*I fragment library; the *kan* gene from pSKS101, cloned by its *Bam*HI ends into *fip*, retains a *Pst*I site a few base pairs in from each *Bam*HI site (20). Thus, the *kan* gene insertion into *fip* can be visualized as a new *Pst*I site that splits the original 3.4-kb insert into 2.2- and 1.2-kb *Pst*I fragments.

Total DNA was prepared from our strains: A179 (chromosomal *fip*::*kan*), K38 (chromosomal *fip*$^+$), K38(pPMR5-*fip*::*kan*) (chromosomal *fip*$^+$, plasmid *fip*::*kan*), and K38(pPMR5) (chromosomal *fip*$^+$, plasmid *fip*$^+$). Each DNA was digested with *Pst*I, electrophoresed, transferred to nitrocellulose, and hybridized to ^{32}P-labeled, nick-translated pPMR5 DNA (Fig. 4). The 3.4-kb *fip*-containing fragment

TABLE 2. Isolation of chromosomal *fip*::*kan* by P1 transduction[a]

Recipient strain	Selected markers	Scored markers		
		Cm^r Tc^r Kan^r	Cm^s Tc^s Kan^r	Cm^s Tc^s Kan^s
K817(pPMR7)	*ilv*$^+$ *cya*$^+$	292	2	4
	ilv$^+$ Kan^r *cya*$^+$	139	0	
K817	*ilv*$^+$ *cya*$^+$	61	0	10
	ilv$^+$ Kan^r *cya*$^+$	150	0	

[a] The recipients were transduced by a P1 lysate prepared on K549(pPMR5-*fip*::*kan*) cells grown at 30°C derived from a colony selected and purified at 42°C.

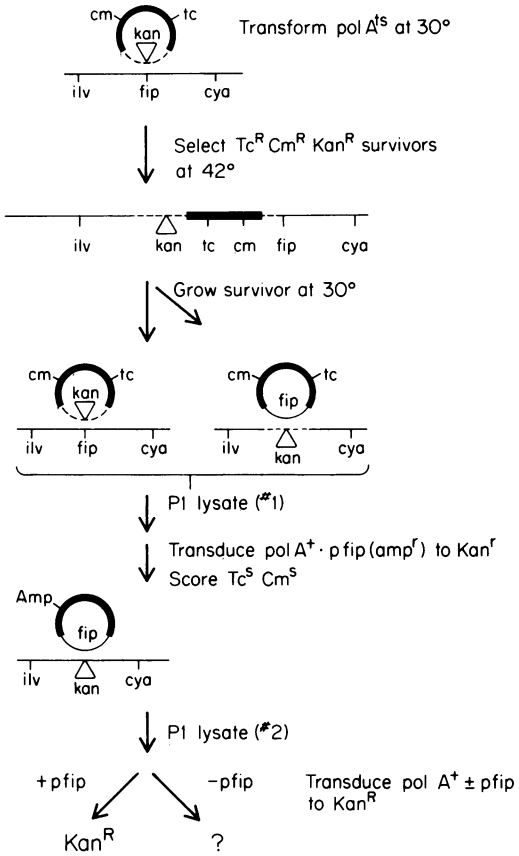

FIG. 2. Gene replacement scheme. The *polA*(Ts) strain K549 was transformed by pPMR5-*fip*::*kan* at the permissive temperature (30°C). Clones in which the plasmid had integrated into the chromosome were selected and purified on tetracycline-chloramphenicol-kanamycin plates at 42°C, and cells in which the plasmid had excised were selected by culturing a 42°C survivor at 30°C. A phage P1 lysate (no. 1) prepared on this 30°C culture was used to transduce K817(pPMR7) (*ilv cya* chromosome; *amp fip*$^+$ plasmid) to *ilv*$^+$ *cya*$^+$ or *ilv*$^+$ *cya*$^+$ *kan*. Transductants were scored for Tc^s and Cm^s, and a Kan^r Tc^s Cm^s transductant was purified. The P1 lysate (no. 2) prepared on this isolate was used to transduce K817(pPMR7) and K817 to Kan^r.

cloned in pPMR5 (lane c) was detected at lower abundance in wild-type *E. coli* DNA (lane e). The *Pst*I sites introduced along with the *kan* gene cloned in *fip* in pPMR5-*fip*::*kan* split the 3.4-kb fragment as predicted (lane b). Note that the 3.4-kb fragment derived from the intact chromosome of K38(pPMR5-*fip*::*kan*) was still present. The *fip*::*kan* strain (A179) contained only the 2.2- and 1.2-kb *Pst*I fragments characteristic of the interrupted *fip* gene (lane d). No other bands indicative of residual segments of vector DNA or duplicated portions of the cloned *fip* region were found. Thus, the chromosomal *fip* gene was replaced by the inactive cloned copy.

Gene replacement procedure to define essential genes. Because the procedure we developed was intended to determine whether a particular cloned gene is essential in *E. coli* and the *fip* gene was dispensable, we wanted to confirm the method with a gene known to be essential. We chose the *rho* gene for this purpose (3, 11). pEG25 is a pBR322-derived plasmid that contains an intact, functional *rho* gene (S. Adhya and E. Gulletta, personal communication). It contains only two *Hpa*I sites, which are located a few base pairs apart in the first third of the coding region of *rho* (17). The *kan* gene of pSKS101 was cloned into *rho* at the *Hpa*I sites by using *Bam*HI linkers, the *rho*::*kan* plasmid was forced into the chromosome, and a P1 lysate was prepared, all as described above for *fip*. Strain K844, which contains two copies of *rho* by virtue of a second copy carried on the lysogenized tranducing phage λ d*ilv62* (22), was transduced to Kan^r, and a transductant that had lost the antibiotic resistance gene contained on the vector portion of pEG25 (Amp) was identified. A second P1 lysate, prepared on this Kan^r Amp^s derivative of K844, was used to transduce K819 or K819(pEG25), selecting for Kan^r or a flanking marker (*metE*$^+$) or both. Kan^r transductants were obtained with the latter strain, but none were obtained from the former strain, which contains a single gene copy of *rho*. All (27 of 27) *met*$^+$ transductants of K819(pEG25), but none (0 of 51) of the *met*$^+$ transductants of K819, were Kan^r. Thus, this technique serves to define essential genes of *E. coli*.

DISCUSSION

This method for replacing a wild-type copy of a chromosomal gene with a mutated copy of the gene cloned on a plasmid was predicated on three observations: (i) cells (*polA*) can be selected in which a plasmid that contains DNA homologous to the host chromosome has integrated at the homologous site (8, 9), thereby forming a duplication of the homologous region (interrupted by the vector portion of the plasmid); (ii) restoration of *polA* function is lethal to cells containing an integrated plasmid (23); and (iii) P1 phage packages DNA >80 kb in length and has been observed to

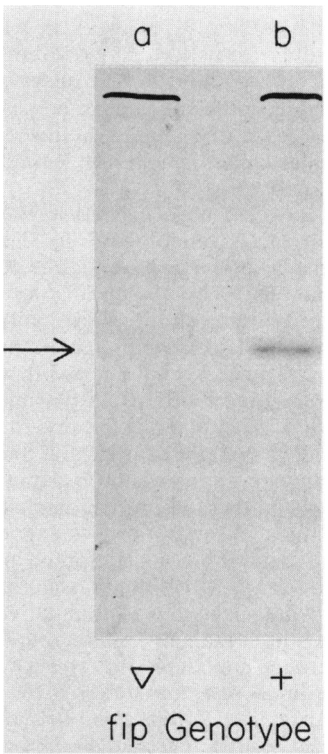

FIG. 3. Immunoblot of *fip* protein. Whole-cell lysates from A179 (lane a, *fip*::*kan*) and K38 (lane b, *fip*$^+$) were electrophoresed on a sodium dodecyl sulfate–urea–20% polyacrylamide gel. Proteins were transferred from the gel to a nitrocellulose sheet, incubated with rabbit anti-*fip* serum and then with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G, and visualized with 4-chloro-1-napthol (see the text).

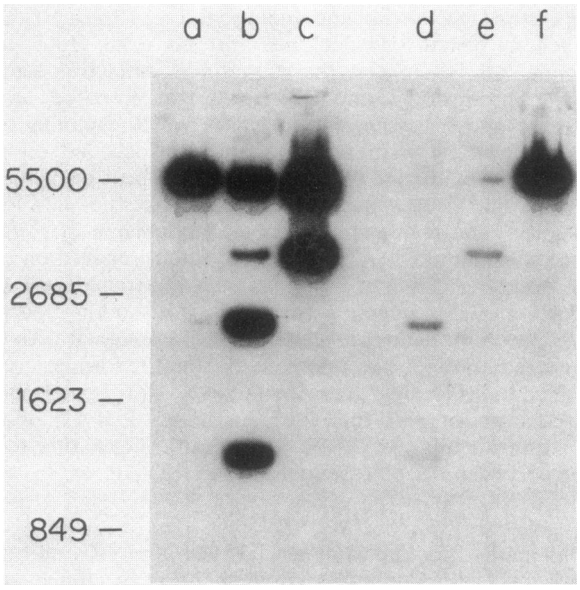

FIG. 4. Southern gel analysis of *fip*::*kan* DNA. Total cellular DNA prepared from K38(pPMR5) (*fip*$^+$ chromosome, *fip*$^+$ plasmid), K38(pPMR5-*fip*::*kan*) (*fip*$^+$ chromosome, *fip*::*kan* plasmid), K38 (*fip*$^+$ chromosome), and A179 (*fip*::*kan* chromosome), along with marker DNA, was digested with *Pst*I, electrophoresed on a 1% agarose gel, transferred to nitrocellulose, and hybridized to ^{32}P-labeled, nick-translated pPMR5 DNA (15). (a) pBR325 marker. (b) K38(pPMR5-*fip*::*kan*). (c) K38(pPMR5). (d) A179; (e) K38. (f) pBR325 marker.

transduce free plasmids only in cases in which very large inserts and multimer formation have combined to fulfill this size requirement (16). Although these observations were not uniformly adaptable to the scheme we envisaged, we developed a straightforward and simple method for determining whether particular cloned genes are essential by a gene replacement technique.

The starting *polA*(Ts) cells carried a plasmid that contained 3.4 kb of DNA homologous to the chromosome, interrupted by the 2-kb *kan* gene, which is not homologous. Antibiotic-resistant survivors (integrants) arose at a frequency of ca. 10^{-2} to 10^{-3} upon shift to 42°C. None ($<10^{-9}$) were detected if the plasmid lacked homology to the chromosome.

Yamaguchi and Tomizawa have noted that *polA*(Ts) cells containing an integrated ColE1-type plasmid could not be lysogenized with λ *polA*$^+$ phage or reverted to full PolA function (23). They concluded that full PolA function is lethal to cells carrying an integrated plasmid (although they noted that free plasmid could protect against this lethality). On the basis of these observations, we expected that return of *polA*(Ts) cells containing an integrated plasmid to 30°C would select for cells in which the plasmid had excised. We found, however, that survival was unimpaired. For the most part, these cells still contained an integrated plasmid, since the plasmid markers encoding Cm^r and Tc^r were cotransduced with chromosomal markers by P1. Although this proved a nuisance, the frequency of excision was high enough (2 to 14%) so that segregants could easily be identified.

O'Connor and Zusman (16) reported that the efficiency of plasmid transduction by P1 is very low for plasmids of less than 18 kb, but becomes more efficient as the plasmid size increases. We found that free pPMR5-*fip*::*kan* (11 kb) was transduced about as efficiently as a chromosomal marker, whereas smaller plasmids were transduced at the lower limits of detection (data not shown). Cotransduction of chromosomal and plasmid markers was not observed when the donor cells contained only free (nonintegrated) plasmids. Thus, the use of flanking chromosomal markers to select for transduction of the *fip*::*kan* gene replacement simplifies the analysis but is not required. Direct selection for Kan^r followed by screening for the loss of vector (antibiotic) markers should be sufficient to identify cells which have undergone gene replacement.

We chose to replace the chromosomal *fip* gene by a null allele with a selectable phenotype. It should be possible, however, to introduce mutant genes with less drastic alterations. Two of the six transductants that had lost the vector markers had undergone chromosomal gene replacement. Thus, screening alone should be sufficient to detect the desired recombinant among the segregants. If outside markers with which to cotransduce the mutant gene were not available, a selectable marker (such as the *kan* gene), inserted in a nonessential region of the cloned homologous DNA segment near the mutated gene of interest, would be essential. This method should also be useful for transferring a chromosomal mutation onto a plasmid containing the cloned wild-type gene. About half the plasmids resulting from the resolution of an integration event should contain the mutant allele.

With the use of this gene replacement technique, the *fip* gene has been shown to be nonessential for cell viability, although *fip*-null mutants grow poorly. The *fip*-null mutants are being examined in an attempt to determine the basis of this physiological effect. Initially we had thought that the *fip* gene product might be involved in the assembly of pili; however, A179 supports the growth of the male-specific phage f2 with high efficiency and acts as an effective host for an f1 phage which carries its own copy of the *fip* gene and transfers its genetic markers as effectively as the parent (data not shown). Thus, pilus assembly does not seem to require *fip* function.

After this manuscript was submitted, we found that the *fip* protein is bacterial thioredoxin. Evidence for this assertion will be published separately.

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