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Characterization of the Cloned *fip* Gene and Its Product

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A DNA fragment encoding the *fip* (filamentous phage production) gene from *Escherichia coli*, when cloned in a filamentous phage vector, restored to the phage the ability to assemble progeny in *fip* mutant hosts. The *fip* gene was located just upstream of and transcribed in the same direction as the *rho* gene. Minicells containing *fip*$^+$ phage or plasmids synthesized a 12,500-dalton protein that was missing or truncated when the Fip$^+$ phenotype was inactivated by insertion of Tn*5*. The *fip* protein was cytoplasmic and was partially purified.

We recently described an *Escherichia coli* mutant that is unable to support assembly of the filamentous single-stranded DNA phage f1 (22). The mutation responsible for this phenotype defines a new gene, designated *fip* (filamentous phage production). We also presented genetic evidence for an interaction between the *fip* gene product and a phage-encoded morphogenetic protein, the product of gene I. These proteins, along with another phage product, pIV, are presumed to act as facilitators of phage assembly, as they are absolutely required for particle formation (13) but are not present in the final structure (24).

Although the self-assembly of complex macromolecules, such as ribosomes (17) and at least parts of the capsids of icosahedral bacteriophage (4), has been studied in great detail, remarkably little is understood of facilitated assembly, i.e., of filamentous phage or of other structures, such as pili. We are interested in characterizing the interaction between the phage gene I and the host *fip* products and in determining how these proteins (and pIV) act to assemble f1 phage. Because of the unusual nature of filamentous phage release from infected cells—they are secreted or extruded without affecting cell viability (7)—and because of their intimate association with the bacterial membrane—the major coat protein exists as a transmembrane protein before it appears in mature phage (18)—an understanding of their assembly will surely provide useful insights into the mechanism of delivery of macromolecular structures to the extra cytoplasmic milieu as well as into assembly mechanisms in general.

We report here the successful cloning of *fip*, the identification of the *fip* gene product, and the partial purification of the protein.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used and their genotypes are listed in Table 1. The filamentous phage cloning vector CGF3 (provided by G. Vovis) is a derivative of f1 that contains a unique *Pst*I site at the *Hpa*II-A–*Hpa*II-H border (28). The construction of various f1 or plasmid derivatives that contain the *fip* gene is described in the text. The plasmid pIN-II-A1 (13), a derivative of pBR322, was kindly provided by M. Inouye. λ467 (*c*I857 *b*221 *O*am29 *P*am80 *rex*::Tn*5*) was obtained from M. Lichten via N. Kleckner.

DNA manipulations. Plasmid and phage replicative form (RF) DNA was prepared by a modification of the method of Davis et al. (5) as described previously (22). DNA from *fip* phage or plasmids was always isolated from *recA* mutant cells. Restriction enzyme digestions, treatment with alkaline phosphatase, and ligations with T4 DNA ligase were performed by the methods of Maniatis et al. (15).

Isolation of plasmids containing Tn*5* insertions in *fip*. A lambda-sensitive, Sup$^+$ strain (K91) containing pPMR5 (a pBR325 derivative containing the *fip* gene segment inserted at the *Pst*I site) was infected by λ467, and kanamycin-resistant colonies were selected as described by Kleckner et al. (11). The colonies (>10^5), each representing an independent transposition event, were pooled and subcultured in medium containing kanamycin (50 μg/ml), chloramphenicol (50 μg/ml), and tetracycline (20 μg/ml). Plasmid DNA prepared from this mixed culture was used to transform strain A140 to Kan^r Cm^r. Transformants were initially screened for inactivation of *fip* by cross-streaking them against f1 phage at 42°C; the phenotypes were confirmed by plating for single plaques.

Minicell isolation, labeling, and electrophoresis. Plasmid-containing strains K311 or K867 and K849 were grown in DO salts (27) supplemented with glucose (0.4%), thiamine (5 μg/ml), and 19 amino acids (1 mM each; no methionine) to about 10^9 cells per ml. For experiments with strain K849, phage were added at a multiplicity of at least 20 when the cells had grown to about 2×10^8/ml. The cultures (100 to 200 ml) were centrifuged in a Sorvall GSA rotor at 3,500 rpm for 8 min to remove whole cells, and the whole-cell pellet was resuspended in DO salts and recentrifuged (GSA rotor; 3,500 rpm, 8 min). The minicell-containing supernatants from both centrifugations were pooled and then centrifuged in the GSA rotor at 8,000 rpm for 15 min to pellet minicells.

The crude minicell pellet was resuspended in about 0.2 ml of DO salts, layered on a 12.5-ml, 5 to 20% sucrose gradient (in DO salts), and centrifuged in an SW40 rotor (4,000 rpm, 15 min, 4°C). A broad band, visible in the upper portion of the tube, was collected by puncturing the cellulose nitrate tube with a 5-ml syringe. The minicells (usually in a volume of about 2 to 4 ml) were diluted twofold with DO salts, centrifuged to pellet the minicells (8,000 rpm for 15 min in a Sorvall SS-34 rotor), and resuspended in growth medium (as described above, lacking methionine). From a 200-ml culture (optical density at 660 nm, about 1.0), 1 ml of minicells (at an optical density at 660 nm of 0.6 to 2.0) were recovered. Fewer than one whole cell per 10^3 minicells was visible under a phase-contrast microscope, and fewer than 10^5 viable cells contaminated the minicell preparation. The

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TABLE 1. *E. coli* strains

Strain	Genotype	Comments and construction
K38	HfrC *Sup*$^+$ *phoA*(Am) (λ)	Standard laboratory strain, originally S26 from A. Garen
K91	HfrC *Sup*$^+$ *phoA*(Am)	K38 cured of λ
A95	K38 *ilv fip-1 zie-1*::Tn*5*	Reference 22
A116	A95 *recA56 srl-300*::Tn*10*	P1 transduction from strain JC10240 (A. J. Clark)
A156	A116 *lacI*^{Q}	*LacI*^{Q} derived from LS286 (L. Soll)
A140	K38 *fip-1 recA56 srl-300*::Tn*10*	P1 transduction of K569 (22) by strain JC10240 (A. J. Clark)
K311	F$^-$ *minA minB gal thr leu thi lacY rpsL*	P678-54 λ^s of Adler et al. (1)
K849	F$^+$ K311	
K867	K311 *recA56 srl-300*::Tn*10*	P1 transduction from strain JC10240 (A. J. Clark)
K841	F$^-$ *rho-15 his relA rpsL gal ilvY*::Tn*10*	IT1021 from I. Tessman (26)
K866	F$^+$-1::Tn*10*/*rep-71 his relA rpsL gal*	Male derivative of IT1011 from I. Tessman (26)

purified minicells were incubated at 37°C for 10 min before the addition of [^{35}S]methionine (10 μCi; 10^3 Ci/mmol), and incubation was continued for 2 min after the addition of the radioactive label. Trichloroacetic acid (0.05 ml, 100%) was added, and after 10 min on ice, the samples were centrifuged in an Eppendorf centrifuge for 10 min. The precipitates were washed with acetone, air-dried, and resuspended in 100 μl of sample buffer. Samples of 20 μl were electrophoresed on 19% acrylamide–6 M urea–sodium dodecyl sulfate gels as described previously (8).

RESULTS

Cloning the *fip* gene. The temperature-sensitive *fip-1* mutation prevents f1 plaque formation at 42°C but not at 37°C (22). The block at 42°C is tight enough for a uniform bacterial lawn to form even when mutant cells are plated with 10^6 f1 phage. Merodiploid studies showed that *fip*$^+$ is dominant to *fip* (22). We selected phage containing a functional *fip* gene from an *E. coli* library that we had constructed in a filamentous phage cloning vector. DNA prepared from *fip*$^+$ *E. coli* (K91) was partially digested with *Pst*I and ligated to CGF3 replicative-form (RF) DNA that had been digested to completion with *Pst*I and treated with alkaline phosphatase to prevent self-ligation. The ligation mixture was used to transfect *fip recA* cells (A116) by the $CaCl_2$ procedure of Mandel and Higa (14). Transfected cells were plated at 37°C to determine the efficiency of ligation and at 42°C to select *fip*$^+$-containing phage. Plaques at 42°C were obtained at a frequency of 3.5×10^{-4}.

Phage isolated from plaques that formed at 42°C plated with equal efficiency on *fip* and *fip*$^+$ cells at 37 and 42°C; however, on mutant cells incubated at the high temperature, the plaques were small. This result was in contrast to that obtained with mutants of f1 phage, designated *gfip*, which have overcome the host block as a result of a single-base-pair (bp) mutation in the phage gene I (22); *gfip* phage form normal-sized plaques on both hosts at both temperatures (22).

Characterization of *fip*$^+$-containing phage. RF DNA was prepared from three of the new isolates, and the DNA was digested with *Pst*I. Two of the isolates, R313 and R314, contained a single new *Pst*I fragment of about 3.4 kilobases (kb); a third, R315, contained 3.4- and 0.5-kb *Pst*I fragments. Subsequent analysis with a variety of restriction enzymes indicated that these isolates contained a common segment of DNA and that R314 differed from R313 and R315 in the orientation of the cloned segment.

The 3.4-kb insert provided *fip*$^+$ function. To show this, we digested R313 RF with *Pst*I and religated at a high dilution (to favor intramolecular ligation). After transfection of *fip*$^+$ cells, 10 plaques were tested on *fip* bacteria at 42°C; none of the transfectants retained the ability to plaque on the mutant strain at high temperature.

The *fip* gene has been mapped at 84.2 min on the *E. coli* chromosome, between *rep* and *cya* and within about 5 kb of the *rho* gene (22). We therefore wished to determine whether the DNA that provided *fip* function complemented *rep* or *rho* mutations. The 3.4-kb fragment was cloned into the *Pst*I site of pBR325, generating two plasmids that contained the insert in opposite orientations, pPMR5 and pPMR6. These plasmids restored to *fip* cells the ability to produce f1 phage at high temperature. A *rep* strain (K866) and a *rho*(Ts) strain (K841) were transformed with these plasmids. The K866 transformants were tested for the ability to plate the *rep*-dependent phages f1 and ST-1, and K841 transformants were tested for temperature resistance. Neither the *rep* nor the *rho* strain was complemented by pPMR5 or pPMR6. Thus, the 3.4-kb insert does not contain a complete, functional copy of either *rep* or *rho*. However, pEG25, a plasmid derived from a λ d*ilv* phage that contains a 2.9-kb *Pvu*II-*Hin*dIII fragment including a functional *rho* gene cloned in pBR322 (E. Gulletta and S. Adhya, personal communication), did complement the *fip* mutation. Thus, *fip* must be located within 2.9 kb of *rho*.

A restriction map of the *fip*-containing insert cloned in phage R315 is shown in Fig. 1. Brown et al. (3) have analyzed a *rho*-containing lambda phage, establishing its orientation with respect to the genetic map. We have aligned the restriction map of R315 with that of the *rho* plasmid, p39, that they derived from the phage (3) and that of the apparently identical *rho* plasmid, pEG25 (E. Gulletta and S. Adhya, personal communication). This alignment established that the DNA fragments containing the *fip* and *rho* genes overlap and oriented R315 with respect to the genetic map (Fig. 1).

Location of the *fip* gene. R314 RF DNA was digested to completion with *Hae*II, and the mixture was ligated to f1 that had been partially digested with *Hae*II. f1 contains three *Hae*II sites, one of which (at the A-B border) occurs in a noncoding region of the genome and is a viable cloning site (10). Again, *fip* f1 phage were selected directly by plating transfected *fip recA* cells (A116) at 42°C. One *fip* phage thus isolated, R316, was characterized. As diagrammed in Fig. 1, it contained a 1,500-bp insert derived from the 1,900-bp *Hae*II fragment present in R314. About 400 bp that include one of the *Hae*II ends (which derived from the vector portion of R314) and *rho* coding sequences up to and including the *Ava*II site in *rho* were lost during cloning.

In addition, an *Hae*III fragment from R313 that consisted of 1,400 bp of insert and 120 bp of vector DNA was ligated to *Hin*dIII linkers and then cloned into the unique *Hin*dIII site of the plasmid pIN-II-A1 (16), creating pPMR7 and pPMR8 (Fig. 1). These subclones (inserted in opposite orientations) retained Fip function. Thus, the *fip* gene must be located within the 1-kb region upstream of the *rho* gene common to R316 and pPMR7-pPMR8.

To locate *fip* more precisely, we obtained Tn*5* insertions that inactivated the *fip* gene cloned in pPMR5 and mapped the sites of insertion. The Tn*5* element was introduced into

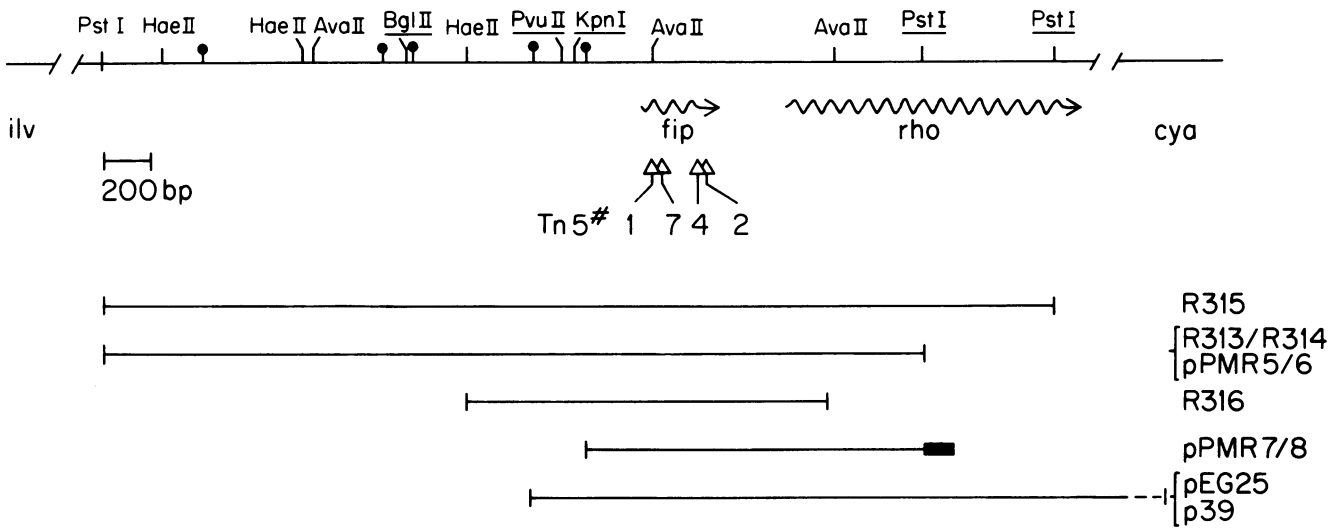

FIG. 1. (A) Restriction nuclease map of *fip* phage and plasmids. The position of *fip* with respect to the *E. coli* chromosome was deduced by aligning the underlined restriction sites mapped in R315 with those of the *rho*-containing plasmids, p39, mapped by Brown et al. (3) and Pinkham and Platt (20) or pEG25, mapped by Gulletta and Adhya (personal communication). ↑, *Hae*III sites; ■, f1 sequences subcloned with *fip*. The direction of transcription of *rho* is from Brown et al. (3); that of *fip* is based on the positions of Tn*5* insertions in *fip* that truncate the *fip* protein (see text). (B) Structure of *fip*$^+$-containing fragments cloned into phage and plasmids.

pPMR5-containing cells via λ467 by the method of Kleckner et al. (11), and plasmid DNA prepared from a pool of $>10^5$ kanamycin-resistant colonies was used to transform *fip recA* cells (A140) to Kan^r Cm^r. Individual transformants were tested for Fip function by cross-streaking them against f1 phage at 42°C; 5 of 111 transformants tested had lost Fip function.

The Tn*5* element contains *Hae*II sites about 100 bp from its extremities (9); thus, its insertion into a defined region of DNA can be treated as if it generates a new *Hae*II site. Plasmid DNA was prepared from strains in which the plasmid copy of *fip* had been inactivated (pPMR5 *fip*::Tn*5*-1, -2, -4, and -7) or in which it remained functional (pPMR5::Tn*5*-3, -6, -8, -12, and -13), and the DNA was digested with *Hae*II. A 2,800-bp *Hae*II fragment (Fig. 2, arrow) present in pPMR5 remained intact in plasmids that retained Fip function (Fig. 2, lanes d, g, h, and i); it was replaced by two shorter fragments, whose sum was about 2,800 bp, in plasmids that had lost Fip function (Fig. 2, lanes a, b, c, and e). Double digests of these DNAs by *Hae*II and *Pst*I (not shown) demonstrated that in each case the longer of the fragments generated by the "new" *Hae*II site contained a *Pst*I site. This observation fixed the position of Tn*5* insertions in *fip* on the restriction map (Fig. 3).

The digest shown in Fig. 2, lane f, consists of fragments from a mixture of two DNAs, one of which had a Tn*5* insertion within the 2,800-bp fragment, generating ca. 1,550- and 1,300-bp fragments. Cells (*fip*) transformed by this DNA mixture which retained only the plasmid that contained this new *Hae*II site were still *fip*$^+$; thus, this Tn*5* insertion occurred in the cloned DNA but not in *fip* (Fig. 3). The locations of the remaining Tn*5* insertions outside of *fip* were not determined.

The localization of *fip* on the cloned segment by Tn*5* insertion predicted that the single *Ava*II site present in R316 (f1 has no *Ava*II sites) was within the *fip* gene (see Fig. 1). This prediction was confirmed by mutagenesis at the *Ava*II site. R316 RF DNA was digested by *Ava*II, treated with nuclease S1, ligated, and redigested with *Ava*II. Permissive cells (*fip*$^+$) were transfected by this DNA, and single plaques were picked and tested for the ability to grow on *fip* strains at the nonpermissive temperature. Of the 40 phage tested, 35 were unable to plaque on *fip* cells at 42°C, indicating that the cloned copy of *fip* was no longer functional. Analysis of RF DNA prepared from three of these R316 derivatives that had lost Fip function confirmed that the *Ava*II site had been eliminated; two clones that remained *fip*$^+$ retained the *Ava*II site.

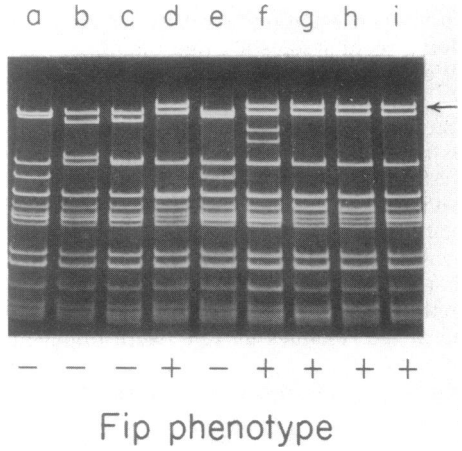

FIG. 2. Mapping Tn*5* insertions in the cloned *fip* gene. DNA from pPMR5 derivatives containing independent Tn*5* insertions (5μg) were digested with *Hae*II and electrophoresed on a 2.5 to 7.5% acrylamide gradient gel (40 mM Tris-acetate, pH 7.2) which was subsequently stained with ethidium bromide. The Fip phenotype of each plasmid is indicated. Tn*5*-3 (lane f) is a mixture of two Tn*5*-containing pPMR5 derivatives, both of which retain the Fip$^+$ phenotype. The arrow indicates the 2.8-kb fragment that is disrupted by Tn*5* insertions in *fip*. Lane a, Tn*5*-1; lane b, Tn*5*-2; lane c, Tn*5*-4; lane d, Tn*5*-6; lane e, Tn*5*-7; lane f, Tn*5*-3; lane g, Tn*5*-8; lane h, Tn*5*-12; lane i, Tn*5*-13.

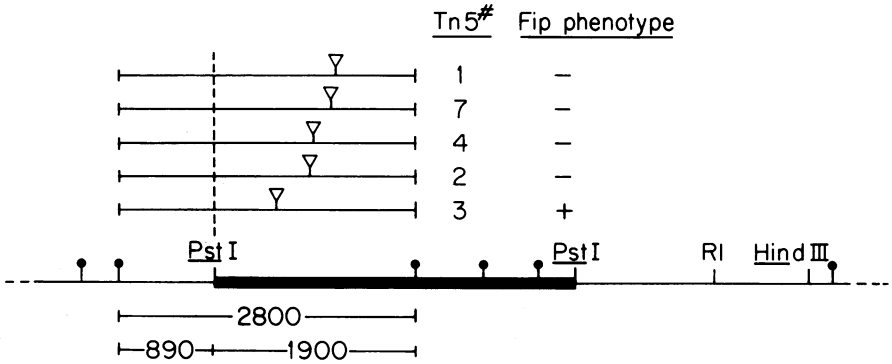

FIG. 3. Schematic diagram indicating the basis for mapping Tn*5* insertions in *fip*. The pBR325 portion of the map, derived from Sutcliff (25) and Bolivar (2), is indicated by a line; the insert is indicated as a fill bar. ♦, *Hae*II sites; ▽, Tn*5* elements ("new" *Hae*II sites). Tn*5* insertions in *fip* introduce new *Hae*II sites that split the ca.-2,800-bp pBR325-*fip* *Hae*II fragment into two fragments (one fragment must be larger than the 890-bp pBR325 portion and the other must be smaller than the 1,900-bp insert portion of the original 2,800-bp fragment). Double digestion by *Hae*II and *Pst*I orients these two fragments by cleaving the pBR325-*fip* joint fragment.

Identification of the *fip* protein. Several insert-specific proteins were detected in lysates from appropriately prepared minicells (Fig. 4). Minicells were purified from K849 (Fig. 4, lane a), from K849 that had been infected by f1, R316, R313, or R314 (lanes b through e), or from K311 transformed by pBR325, pPMR5, or pPMR6 (lanes f through h). The minicells were labeled with [^{35}S]methionine and denatured. The samples were electrophoresed on a sodium dodecyl sulfate-urea-acrylamide gel capable of resolving proteins as small as the f1 major coat protein (6,300 kilodaltons [kd]). Several proteins were synthesized by the purified minicells, including the following: the major outer membrane proteins of *E. coli* (ompC-ompC/F, ompA, and lipoprotein) which are synthesized from unusually stable mRNAs (6) and were present in all lysates; phage- and plasmid-specified products such as phage single-stranded DNA binding protein and coat protein and plasmid-specified chloramphenicol transacetylase. The orientation-specific bands that migrated faster than chloramphenicol transacetylase in lane g and slower than chloramphenicol transacetylase in lane h may be β-lactamase related, as the *bla* gene was disrupted by cloning into the *Pst*I site of pBR325 to create pPMR5-pPMR6. Three additional protein bands were detected in lysates that contained the entire 3.4-kb *Pst*I fragment on which *fip* was located: a protein with an apparent molecular weight of 38,000 that migrated more slowly than ompC and ompF and was best seen in the plasmid lysates (Fig. 4, lanes g and h); a protein of ca. 23 kd visible in the R313 and R314

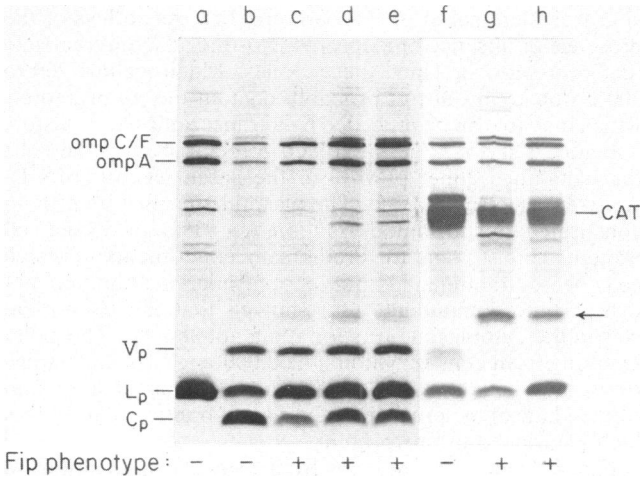

FIG. 4. Proteins specified by *fip* phage and plasmids in minicells. Minicells purified from phage-infected K849 or plasmid-containing K311 were labeled with [^{35}S]methionine, dissolved in sample buffer, and electrophoresed (see text). The Fip phenotype of each phage or plasmid is indicated. The arrow indicates the presumptive *fip* protein. CAT, Chloramphenicol acetyltransferase; ompC/F and ompA, major outer membrane proteins ompC-ompF and ompA, respectively; L_p, lipoprotein; V_p, f1 gene V (single-strand DNA binding) protein; C_p, f1 major coat protein. Lane a, uninfected cells; lane b, f1$^+$-infected cells; lane c, R316-infected cells; lane d, R313-infected cells; lane e, R314-infected cells; lane f, pBR325; lane g, pPMR5; lane h, pPMR6.

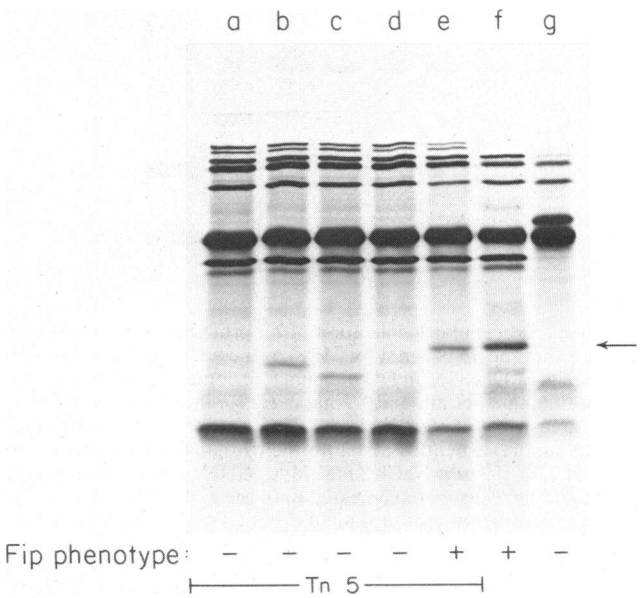

FIG. 5. Identification of *fip* protein; inactivation by Tn*5*. Minicells purified from K867 cells containing pPMR5 derivatives into which Tn*5* had been inserted were labeled with [^{35}S]methionine, dissolved in sample buffer, and electrophoresed as described in the text. The Fip phenotype of each plasmid is indicated. The arrow indicates full-length *fip* protein. Lane a, Tn*5*-1; lane b, Tn*5*-2; lane c, Tn*5*-4; lane d, Tn*5*-7; lane e, Tn*5*-8; lane f, pPMR5; lane g, pBR325.

lysates that is presumably obscured by the 24-kd (23) chloramphenicol transacetylase band present in the plasmid lysates; and a 12.5-kd protein that was efficiently synthesized by plasmid-containing minicells but barely detectable in phage-infected minicells. R316, the *fip*$^+$ derivative of f1 containing a 1,500-bp subfragment of the original 3.4-kb insert, specified the 12.5-kd protein but neither of the other proteins (Fig. 4, lane c), suggesting that the 12.5-kd protein may be the *fip* gene product.

This hypothesis was verified by examining the protein products of pPMR5 derivatives in which *fip* had been inactivated by the insertion of Tn*5*. Minicells purified from K867 that contained plasmids pPMR5 *fip*::Tn*5*-1, -2, -4, or -7 (Fig. 5, lanes a through d), from the *fip*$^+$ controls pPMR5::Tn*5*-8 (lane e) and pPMR5 (lane f), and from pBR325 (lane g) were labeled with [^{35}S]methionine. The 12.5-kd band was eliminated by Tn*5* insertions 1 and 7 and truncated by Tn*5* insertions 2 and 4. Assuming that Tn*5* causes chain termination shortly after the point of its insertion, the missing and truncated proteins, together with the restriction mapping data for each insertion (Fig. 3), are consistent with the direction of transcription for *fip* shown in Fig. 1.

Subcellular localization and partial purification of p*fip*. In an effort to amplify the synthesis of the 12.5-kd *fip* protein, we subcloned the *fip* gene downstream from the *lpp* and *lacZ* promoters in pIN-II-A1 (16) as described above and diagrammed in Fig. 1. The resulting plasmids, pPMR7 and pPMR8, contained the *fip* gene in opposite orientations. Expression of several genes cloned in pIN-II plasmids (without their original promoters) has been shown to be under *lac* control (16, 19). However, the level of *fip* expression was identical in pPMR7 and pPMR8, regardless of the presence or absence of isopropyl-β-D-thiogalactopyranoside (data not shown). Thus, these clones, which include 200 to 300 bp upstream of *fip*, probably contain the *fip* promoter, which may in turn be preceded by a transcription terminator.

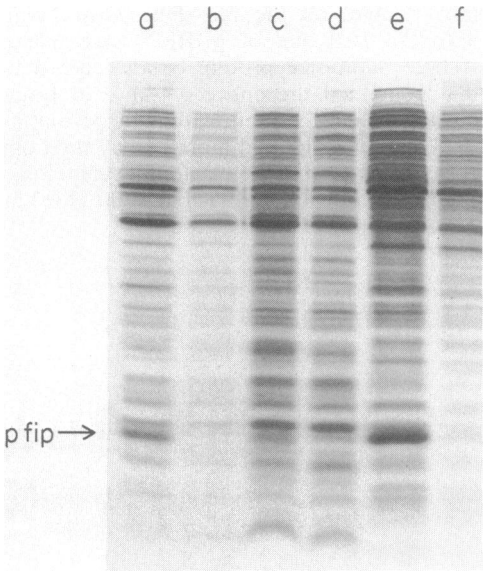

FIG. 6. Subcellular localization of *fip* protein. Cells (A156) containing either the *fip* plasmid (pPMR8) or the vector plasmid (pIN-II-A1) were grown at 37°C in DO medium (26) containing 0.4% glucose, 5 μg of thiamine per ml, and 1.5 mg of Casamino Acids per ml to an optical density at 660 nm of 1 to 2. The cultures were centrifuged, washed, and resuspended at 1/10th their original volume in 50 mM Tris (pH 7.5)–100 mM NaCl–2 mM $MgCl_2$. DNase I was added to 20 μg/ml, and the cells were sonicated until the cell number (measured by phase-contrast microscopy) had dropped >10-fold. After a low-speed spin to remove intact cells, the lysate was centrifuged in a Beckman type 40 rotor for 2 h at 40,000 rpm. The pellet was resuspended in 2% sodium dodecyl sulfate–50 mM Tris (pH 7.5)–100 mM NaCl. Samples from whole cells, the high-speed pellet, and the high-speed supernatant representing approximately equal numbers of cells (4×10^8) were electrophoresed, and the gel was stained with Coomassie blue. Lane a, Fip, cells; lane b, control, cells; lane c, Fip, pellet resulting from centrifugation at 200,000 × *g*; lane d, control, pellet resulting from centrifugation at 200,000 × *g*; lane e, Fip, supernatant; lane f, control, supernatant.

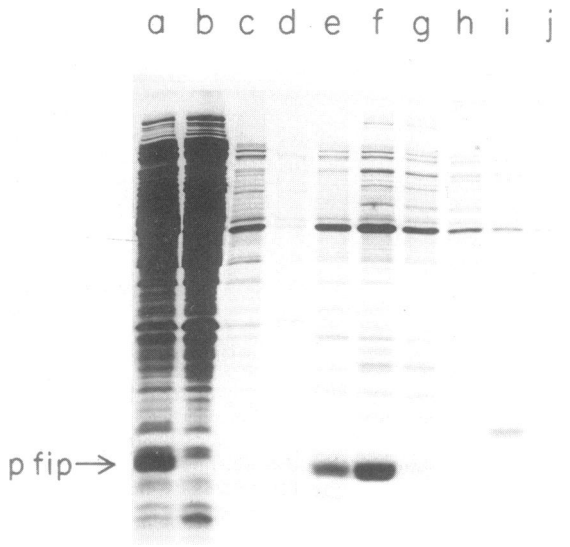

FIG. 7. Partial purification of *fip* protein on DEAE-Sephadex. The high-speed supernatant from A156 cells containing pPMR8 (shown in Fig. 7, lane e) from 10^{12} cells in 50 mM Tris (pH 7.5)–50 mM NaCl–1 mM $MgCl_2$ was applied to a DEAE-Sephadex column (2.5 by 7.5 cm). A total of 55 6-ml fractions were eluted over a 100 to 500 mM NaCl gradient. Samples of fractions pooled successively in batches of five are shown.

Figure 6 shows a gel on which proteins from A156 cells that contained either pPMR8 or the parent vector, pIN-II-A1, were electrophoresed. The plasmid-specified *fip* protein constituted a substantial fraction (ca. 1%) of whole-cell proteins (Fig. 6, lane a). Preliminary experiments in which cells were fractionated into periplasmic, membrane, and cytoplasmic components (21) showed that the *fip* protein was in the cytoplasmic fraction (data not shown). The pellet resulting from centrifugation at 200,000 × *g*, which contains ribosomes and membrane fragments, contained little p*fip* (Fig. 6, lane c), whereas the supernatant fraction (lane e) was highly enriched in this protein.

Figure 7 shows the proteins from a supernatant clarified at high speed and eluted from a DEAE-Sephadex column. The bulk of the protein loaded (Fig. 7, lane a) eluted in the 0.1 M NaCl wash (lane b), whereas p*fip* eluted at about 0.25 M NaCl (lanes e and f). Additional purification was achieved by eluting p*fip* from a 10% nondenaturing acrylamide gel (pH 8.8), on which it migrated as a single band.

DISCUSSION

A bacterial gene, *fip*, that is required for filamentous phage f1 assembly was cloned in a single step; f1 *fip*$^+$

plaques were selected directly from a mixture of *E. coli* DNA fragments ligated to f1 RF by selecting transfectants in a *fip* mutant strain. The structure of these *fip* clones was determined, and the cloned region was oriented with respect to nearby genes and the *E. coli* genetic map by aligning it with the restriction enzyme maps of the *ilvC-rho* region (3, 20). The alignment predicts that the 3.4-kb fragment on which *fip* was isolated should also include a 5′ portion of the *rho* gene that should encode a polypeptide fragment of ca. 22 kd. More detailed analysis of *fip*-containing subclones and of Tn*5*-inactivated *fip* plasmids showed that *fip* is located just upstream of the *rho* gene and is transcribed in the same direction as *rho* (3). We detected two proteins in addition to the *fip* proteins encoded by the full-length *fip* clones (pPMR5 and pPMR6). One, about 23 kd, is presumably the truncated *rho* polypeptide predicted above. The second protein, which migrates more slowly than the 36-kd outer membrane proteins ompC and ompF and has an apparent molecular weight of 38,000, has not been identified as the product of any known gene.

The *fip* protein has an apparent molecular weight of 12,500, suggesting that it consists of about 100 amino acids encoded by about 300 bp. The Tn*5* insertions into *fip* span about 250 bp. Less p*fip* is synthesized in phage-infected than in plasmid-containing minicells. The former may not have achieved a steady-state level of phage DNA, as the minicell-producing cultures were infected only about two generations before their purification; the reduced amount of the 38-kd protein detected in phage-infected minicells relative to that detected in plasmid-containing minicells is consistent with this explanation.

In fractionation experiments with cells that overproduce p*fip*, the protein was recovered in the soluble, cytoplasmic fraction. We assume that this localization holds for cells containing normal levels of p*fip*, but this assumption remains to be established. Filamentous phage assembly is believed to take place at the cytoplasmic membrane of infected cells. Thus, the finding that *fip* protein is located in the cytoplasm and that in preliminary purification experiments it behaves as a soluble protein with no particularly hydrophobic character was somewhat surprising. Of the two phage-encoded morphogenetic proteins, pIV has been detected in membrane fractions (12; unpublished results); pI has not been detected at all in vivo, but its predicted sequence suggests that it should be quite hydrophobic.

Further purification of *fip* protein and isolation of anti-p*fip* serum are under way. These reagents will allow us to determine whether p*fip* has an enzymatic or specific binding activity and, hence, to attack the hitherto refractory problem of filamentous phage assembly from a different perspective.

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