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A *traC* Mutant That Retains Sensitivity to f1 Bacteriophage But Lacks F Pili

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An F *lac pro* mutant which was temperature sensitive for infection by the filamentous bacteriophage f1 but resistant to the F-specific icosahedral RNA phage f2 was isolated. Cells carrying the F′ mutation failed to elaborate F pili at all temperatures. Mutant cells were able to pair with recipient cells during bacterial conjugation, but transfer of conjugal DNA occurred at a greatly reduced frequency. Complementation analyses showed the F′ mutation to be in the *traC* gene. When a plasmid carrying *traC* was introduced into hosts harboring the F′ mutation, phage sensitivity, the ability to elaborate F pili, and conjugation efficiency were restored. The mutation was named *traC1044*. The F *lac pro traC1044* mutant appears to be unique among *traC* mutants in retaining host sensitivity to the filamentous phage f1 in the absence of expression of extended F pili. Phage f1 attachment sites appeared to be present at the cell surface in *traC1044* mutants. The reduced accessibility of these sites may account for the reduced efficiency of phage f1 infection of *traC1044* hosts, although the possibility that a defect was present in the receptor site itself was not eliminated. Membranes of hosts carrying the F′ mutation contained a full complement of mature F-pilin subunits, so the product of *traC* is presumably required for pilus assembly but not for pilin processing. This, together with the deficiency in conjugal DNA transfer, suggests that *traC* may be part of a membrane-spanning *tra* protein complex responsible for pilus assembly and disassembly and conjugal DNA transmission.

F pili are filamentous appendages found on the surfaces of bacteria harboring the F plasmid. They function as receptors for specific bacteriophages and are also involved in bacterial conjugation. Attachment sites for the F-specific filamentous phages f1, fd, and M13 are found at the tip of the F pilus (7), while F-specific icosahedral RNA phages such as f2, R17, and Qβ initiate their infection process by attaching to the sides of the structure (8). After phage binding, the pilus retracts, bringing the phage particle to the cell membrane, where infection can proceed (20, 34). F pili are also involved in the initial stages of mating pair formation in bacterial conjugation (41). The tip of the pilus recognizes the recipient cell, while the sides of the pilus may be involved in other nonspecific interactions with the recipient (1, 33). After these contacts are made, pilus disassembly is thought to bring the donor and recipient into wall-to-wall contact, allowing a stable mating pair to form.

The production of F pili is a complex process involving at least 13 F transfer (*tra*) genes, in addition to *traJ*, the positive regulatory element for the *tra* operon (13, 30, 31, 41). Purified pili, however, seem to be simple structures composed of only one protein, F pilin (4, 6, 9, 14). F pilin is synthesized as a 13,200-dalton (Da) precursor molecule encoded by the *traA* gene (22, 28). The precursor is efficiently processed to a form of approximately 7,000 Da in the presence of the product of *traQ* (18, 31). Further modification occurs in the presence of at least one additional *tra* gene product to yield the antigenically mature molecule with a molecular weight of 7,200 (23). Mature F pilin has an acetylated amino terminus and may have hexose and phosphate residues associated with it (4, 6, 12). The specific *tra* genes required for these modifications or the *tra* genes needed to actually assemble and disassemble the pilin subunits during pilus elaboration and retraction have not been identified. Pilin subunits appear to be assembled from an inner membrane pool (29), and Bayer (5) has shown that pili emerge from adhesion zones or Bayer junctions, where the inner and outer membranes of the cell are contiguous. No basal F-pilus structure has been identified, although other *tra* gene products are thought to form a membrane-spanning complex which may mediate pilin assembly and disassembly and function in conjugal DNA transmission.

This paper reports the isolation of a mutant that is temperature sensitive for infection by the filamentous phage f1 but is completely resistant to the F-specific RNA phage f2. The mutant lacks F pili at all temperatures. Conjugation occurs at a severely reduced efficiency when mutant cells are used as donors, but mating pair formation is not greatly impaired. The defect has been mapped to *traC* and appears to affect pilus assembly and conjugal DNA transmission.

MATERIALS AND METHODS

Bacteria, phages, and plasmids. The *Escherichia coli* strains used in this study are listed in Table 1. For strain construction, bacterial matings were performed as described by Miller (27). P1 transductions were mediated by P1 *cml clr-100* as described by Miller (27), with the modifications of Lopez and Webster (24). P1 *cml clr-100* was a gift from M. Russel. Phages f1, f2, and R100, a gene VII amber mutant of f1, were laboratory stocks. The EDλ transducing phages (21, 42) were obtained from N. Willetts. Monoclonal antibody JEL 93 (11) was a gift from Laura Frost. Plasmid pKI326 is an F TraC$^+$ pUC18 (32) derivative, the construction and properties of which will be detailed elsewhere (2; S. Maneewannakul, P. Kathir, D. Moore, L.-A. Le, J. Wu, and

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TABLE 1. Bacterial strains

Strain	Relevant genotype[a]	Source
GM1	*thi ara* Δ(*lac pro*)/F′ *lac pro*	D. Steege
TPS44	*thi ara* Δ(*lac pro*)/F′ *lac pro traC1044*	T.-p. Sun
JC6563	Δ*lac trp supD*/F′ *lac traC5*	M. Achtman (3)
VL584	F⁻ *thi ara* Δ(*lac pro*) Δ(*uxu fimD*) *rpsL*	B. Eisenstein
VS2	VL584/F′ *lac pro*	This study
VS4	VL584/F′ *lac pro traC1044*	This study
VS5	VL584/F′ *lac traC5*	This study
JC6612	*lac*Δ*X74 gal his trp lys rpsL*/F′ *lac traC12*	M. Achtman (3)
VSP1	VL584(pKAS1)	This study
VSP2	VS2(pKAS1)	This study
VSP4	VS4(pKAS1)	This study
VSP5	VS5(pKAS1)	This study
K17	F⁻ *thi thr leu lac supE* Str^r	N. Zinder
K91	HfrC *thi*	M. Russel
W3110	F⁻	J. Walsh
KAS8[b]	HfrC *thi* Tet^r Δ(*uxu fimD*)	This study
JC3051	F⁻ *lac*Δ*X74 rpsL his trp* λ^r	M. Achtman (3)
XK5456[c]	F⁻ *lac*Δ*X74 rpsE his trp tsx ton*	This study

[a] *rpsL* and *rpsE* confer resistance to streptomycin and spectinomycin, respectively.

[b] A P1 lysate grown on W3110 containing random insertions of Tn*10* was used to transduce VS2, followed by selection for Tet^r *uxu*$^+$ to produce KAS1. A P1 lysate grown on KAS1 was used to transduce VS2 to Tet^r *uxu* to produce KAS6. A P1 lysate grown on KAS6 was used to transduce K91 to Tet^r *uxu*, followed by electron microscopy to select for the Fim⁻ phenotype. The precise location of Tn*10* in KAS8 has not been determined. Transposon Tn*10* confers resistance to tetracycline.

[c] Derived by curing JC5455 (3) of λ.

K. Ippen-Ihler, submitted for publication). The 3.14-kilobase (kb) *Eco*RI-*Bam*HI insert in pKI326 consists of 2.95 kb of F *traC* DNA (*Eco*RI-*Eco*RV [Fig. 1A]) and 0.19 kb of pBR322 DNA (*Eco*RV-*Bam*HI). For generation of plasmid pKAS1, the 3.14-kb *Eco*RI-*Bam*HI fragment of pKI326 was blunt end ligated into the *Hpa*I site of pJIH1 (15) (Fig. 1b). In pKAS1, *traC* is expressed from the left promoter of lambda and is under the control of the temperature-sensitive *c*I857 repressor. The standard cloning techniques of Maniatis et al. (26) were used throughout.

Media and chemicals. Bacteria were grown in tryptone-yeast (TY) medium (25) or minimal MTPA medium (39) supplemented with 0.2% glucose and a 1 mM concentration of each amino acid, except where noted. Streptomycin and spectinomycin were used at a concentration of 100 μg/ml, ampicillin was used at 60 μg/ml, and tetracycline was used at 20 μg/ml as needed. The Lac$^+$ phenotype was selected on minimal lactose plates or scored on MTPA plates supplemented with the appropriate amino acids, 0.2% lactose, and 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) per ml. Streptomycin, tetracycline, X-gal, and keyhole limpet hemocyanin were purchased from Sigma Chemical Co. Sodium ampicillin (Polycillin-N) was obtained from Bristol Laboratories. Restriction endonucleases and other enzymes used in subcloning were from Bethesda Research Laboratories, Inc., and P-L Biochemicals, Inc.

Isolation and localization of the mutation. Mutants with altered phage sensitivities were isolated by T.-p. Sun as previously described (37). Briefly, strain GM1 was mutagenized with ethyl methanesulfonate and grown overnight in Pro⁻ MTPA medium to permit segregation. The culture was infected with the f1 mutant phage R100 at 42°C to select against cells susceptible to infection with f1, as infection of suppressor-negative strains with f1 amber mutants results in cell death (35). Survivors were tested for sensitivity to both f1 and f2 phages at 34 and 42°C.

One mutant, TPS44, was chosen for further study. The mutation in TPS44 could be transferred to a recipient cell by conjugation and thus is carried by the F′ episome. A set of lambda transducing phages carrying different segments of the F *tra* operon was used for the mapping of the mutation. The phages were purified by banding on CsCl gradients, and the set of *tra* genes expected to be expressed by each preparation (21, 42) was confirmed in transfer complementation spot tests (42) with known F *lac tra* mutant donors. Similarly, 0.1 ml each of log-phase cultures of TPS44 and the Lac⁻ Str^r λ^r recipient, JC3051, was spread on a minimal lactose plate and spotted with 0.01 ml of various dilutions of each purified phage preparation. After 3 h of incubation at 37°C, 0.3 ml of a 100-mg/ml streptomycin solution was inserted under the agar layer. Transfer complementation was scored by the subsequent growth or absence of Lac$^+$ Str^r colonies within each phage spot.

Measurement of the efficiency of f1 infection. The filamentous phages do not normally kill their hosts, so a simple assay, such as measuring cell death from infection, could not be used to determine the efficiency with which strains were infected. Therefore, the following assay was developed. Bacteria carrying F *lac pro* were grown in Pro⁻ MTPA medium with the appropriate antibiotic selection to a density of 6×10^8 cells per ml. The culture was then infected with f1 at the desired multiplicity of infection (MOI). Strains carrying plasmid pKAS1 were grown to 4×10^8 cells per ml at 34°C and shifted to 40°C for 1 h before infection with f1. After a 10-min infection period, the cells were collected by

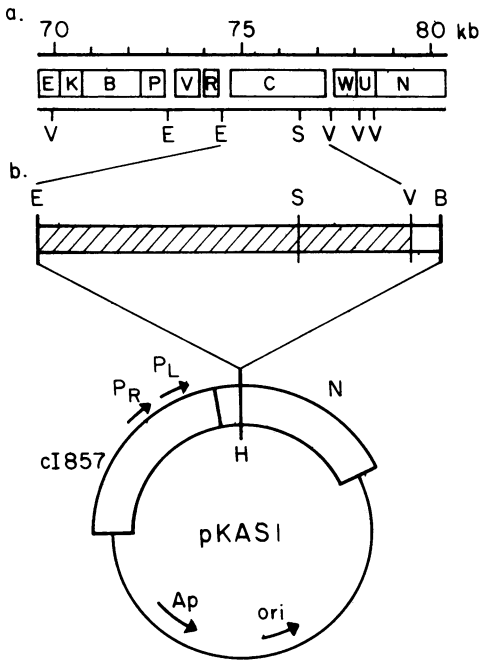

FIG. 1. Plasmid pKAS1. (a) The *traC*-containing region corresponds to F coordinates 74.3 to 77.25 on the 100-kb map of F (17). other *tra* genes are shown in boxes. Sites for restriction endonucleases are *Eco*RI (E), *Sal*I (S), *Bam*HI (B), *Eco*RV (V), and *Hpa*I (H). (b) Plasmid pKAS1 contains a 3.14-kb *Eco*RI-*Bam*HI fragment (derived from pKI326) which was inserted by blunt end ligation into the *Hpa*I site of pJIH1. A hatched area denotes the 2.95-kb *tra* DNA segment that contains *traC*.

centrifugation, washed once with MTPA salts to remove free phages, resuspended in MTPA salts, diluted, and plated on TY plates. Following overnight growth at 36°C, the individual colonies were picked onto TY plates seeded with a lawn of KAS8 and onto plates of Pro^- MTPA supplemented with lactose and X-gal. The plates were incubated overnight at 36°C. A halo appeared in the KAS8 lawn around each inoculum that contained a colony of infected cells extruding progeny phages. A corresponding blue patch on the X-gal plate confirmed the presence of F *lac pro*. Infection was dependent on the presence of the F factor, since VL584 produced no infective centers, even at the highest MOI.

Measurement of conjugation efficiency. For quantitative conjugation experiments, freshly isolated colonies were used to inoculate 5 ml of TY medium, which contained ampicillin for plasmid-bearing strains, and were grown without aeration at 37°C overnight. The cells were then pelleted, resuspended in fresh medium, diluted, and grown with aeration to a cell density of 4×10^8 cells per ml. Samples of 0.1 ml of donor culture and 0.1 ml of XK5456 were then mixed, incubated for a 60-min mating period at the desired temperature, diluted, and plated on selective medium. Transfer frequencies (percent transconjugants) are the number of Lac^+ Spc^r transconjugants obtained per 100 viable donor cells in the mating mixture.

Mating pair formation. Quantitation of the ability to form mating pairs was accomplished by photomicroscopy. Strains were grown in MTPA medium at 36°C to a density of 6×10^8 cells per ml. After the cultures were vortexed for 30 s, equal volumes of the donor strain being tested and the K17 recipient were combined and incubated at 36°C for 30 min. Separate donor and recipient cultures were carried through the experiment as controls. After 30 min, the mating mixtures were diluted 10-fold with MTPA salts, and a 5 μl sample was placed in a Petroff-Hauser chamber and viewed by phase microscopy with a Zeiss photomicroscope III. Photos of five different fields of view were taken for each mating mixture, including the separate donor and recipient culture controls. The elapsed time from dilution through completion of photomicroscopy was only 7 min. The number of lone cells present in each mating mixture and individual culture was tabulated from the photographs; cells in aggregates were not counted. A lone cell was one not in association with any other cell. Cells that appeared to be dividing were counted as two lone cells. The number of lone cells in a mating mixture was subtracted from the average of the numbers of lone cells present in the corresponding donor and recipient cultures. This difference was divided by the average of the numbers of lone cells in the donor and recipient cultures to give the percent decrease in the number of lone cells. This percent decrease in the number of lone cells present in the mating mixture reflected the ability of the donor and recipient cells to form mating pairs or aggregates.

Pilus elaboration. Cells were grown in MTPA medium with the appropriate amino acids and antibiotic selection at the desired temperature to a density of 6×10^8 cells per ml. To simply determine if cells were piliated, we applied samples directly to Formvar grids and stained them as described below or fixed them first with 1% formaldehyde or with 1% formaldehyde–0.6% glutaraldehyde for 1 h before adsorption to grids. To observe complementation of pilus elaboration by pKAS1, we grew cultures at 33°C and then shifted them to 40°C when a density of 4×10^8 cells per ml was reached. Aliquots were removed at various times and mixed with f2 at an MOI of 1,000. RNA phages were added to aid in viewing the pili produced. The samples were immediately placed onto 400 mesh carbon-coated Formvar grids. After allowing 1 min for adsorption, we washed the grids once with 0.1 M ammonium acetate and three times with distilled water and stained them for 1 min with 2% uranyl acetate. The grids were examined in a JEOL-100C electron microscope at 80 kV. The number of pili per cell was determined by counting more than 100 cells at each time point. The cells were counted only if the entire cell and the tips of all its pili were visible.

Preparation of antipilin antibodies. Pili were purified from KAS8 as described by Armstrong et al. (4), but the bacteria were grown in liquid TY medium rather than on agar plates. The purified pili were dissociated and electrophoresed through a 10 to 16% polyacrylamide gradient gel (29), and the pilin subunits were electroeluted from the gel (16). The pilin was cross-linked to an equal weight of hemocyanin with 8% glutaraldehyde. The equivalent of 20 μg of pilin, cross-linked to hemocyanin, was combined with an equal volume of complete Freund adjuvant and injected subcutaneously into a New Zealand White rabbit. The animal was given a booster injection 2 weeks after the initial injection and was bled 2 weeks later. The blood was allowed to clot, and the resultant serum was passed over a Sepharose 4B-protein A affinity column equilibrated with 0.05 M sodium phosphate (pH 7.2)–0.15 M NaCl. The immunoglobulin G fraction was eluted with 0.58% acetic acid–0.15 M NaCl, dialyzed against 0.05 M sodium phosphate (pH 7.2)–0.15 M NaCl, and stored at −20°C.

RESULTS

Isolation and characterization of a mutant with altered sensitivity to f1 and f2. Strain GM1 was mutagenized as described in Materials and Methods. Survivors were tested for sensitivity to both f1 and f2 at 34 and 42°C. One of the isolates, TPS44, was not able to plaque f1 at 42°C, but plaques could be detected on plates incubated at 34 to 36°C. These plaques were smaller, more turbid, and less numerous than those observed with infection of the parent strain, GM1. The mutant could not plaque RNA phage f2 at either temperature. TPS44 acted as a donor in conjugation at a greatly reduced efficiency at both temperatures. The transconjugants exhibited the same phage sensitivities as did TPS44; thus, the mutation was carried by the F′ episome. To eliminate complications from possible secondary chromosomal mutations induced in TPS44 and to aid in electron microscopy, we transferred the mutant F plasmid by conjugation to VL584, a recipient that lacks type 1 fimbriae. The resultant F *lac pro* TPS44-VL584 derivative, strain VS4, was used in further characterizations. VS4 exhibited all the phenotypes of TPS44. When VS4 was examined in the electron microscope, no F pili were visible on the cell surface at either the permissive (Fig. 2b) or the nonpermissive temperature. Hundreds of cells were examined, but none were piliated, and no free pili were observed in the background. The absence of pili was evident even under conditions of rapid fixation with formaldehyde or formaldehyde and glutaraldehyde.

The ability of VS4 to be infected by f1 at the permissive temperature despite its lack of F pili suggested that a functional receptor for the phage was still present on these cells but was located at the cell surface. Such a receptor would be less accessible to the phage than would the normal attachment sites found at the tip of an extended F pilus. To investigate this idea, we determined the efficiency of infection (expressed as percent cells infected) for VS4 and the

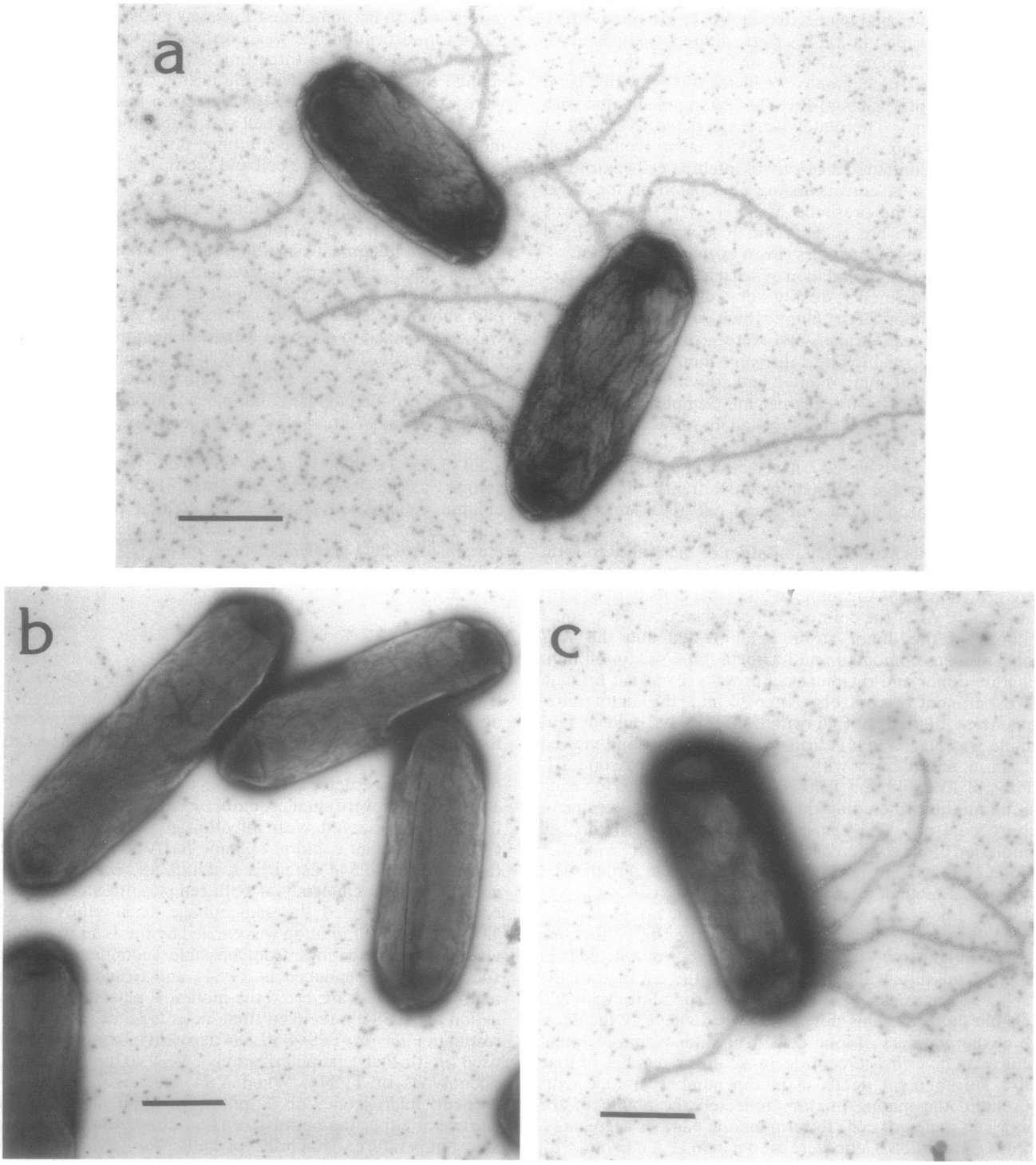

FIG. 2. State of piliation, as determined by electron microscopy. VS2 and VS4 were grown to 6×10^8 cells per ml at 36°C and adsorbed to grids without fixation. VSP4 was grown at 34°C to 4×10^8 cells per ml and then shifted to 40°C to induce the p_L promoter. After 1 h of induction, VSP4 was prepared for electron microscopy as described in the text. The icosahedral RNA phage f2 was added to bind to the sides of the F pili and to aid in visualization. (a) VS2; (b) VS4; (c) VSP4. Bars, 1 μm.

wild-type parent, VS2, during a 10-min period at various MOIs as described in Materials and Methods. At 36°C, mutant VS4 was infected at an efficiency 1,000-fold below that of the same strain carrying the parental F′ plasmid, VS2 (Fig. 3a). However, the infection process could be driven by increasing the ratio of phage to cells (Fig. 3a), suggesting that a functional phage receptor was probably present on every cell. Interestingly, this assay also detected f1 infection of VS4 at 42°C, while spot tests showed that VS4 could not plaque f1 at 42°C. This discrepancy was probably due to the

vastly different conditions (time of exposure of phage to cells and MOI) of the two assays. The decrease in the efficiency of infection seen for VS4 is most easily explained by the inaccessibility of the phage receptor normally found at the tip of the pilus, although the presence of a defect in the phage receptor itself cannot be ruled out.

The tip of the F pilus not only acts as a receptor for F-specific filamentous phages but also is important in establishing mating pairs in conjugation. Therefore, we looked at the ability of VS4 to form mating pairs or aggregates with a wild-type recipient, K17. Because of the difficulty in determining the number of cells present in a mating aggregate, we actually measured the number of lone cells present in the mating mixture and compared this number to the number of lone cells present in separate donor and recipient cultures. A drop in the number of lone cells in the mating mixture should reflect the formation of mating pairs and aggregates. A mating mixture containing the wild-type parental F′ donor, VS2, showed 59% fewer lone cells than did the individual donor and recipient cultures (Table 2). This decrease reflected the appearance of large aggregates of cells in the mating mixture. When the F^- recipient, K17, and the F^- host, VL584, from which the donors had been derived, were mixed, there was no overall decrease in the number of lone cells in the mating mixture, and no mating pairs were observed. When mutant VS4 was used as a donor, a 16% decrease in the number of lone cells was observed, but only pairs and small clusters of cells were seen. This result suggested that VS4 can form mating pairs of some kind but fourfold less efficiently than can wild-type cells. These data

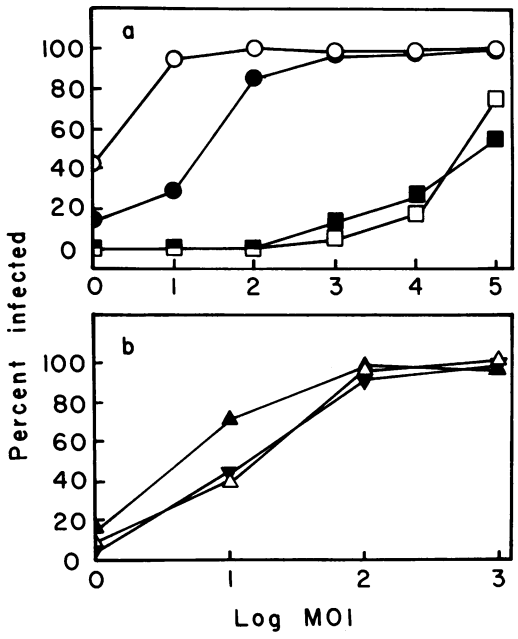

FIG. 3. Temperature dependence and complementation of f1 infection efficiency in VS4. (a) Bacteria were grown to 6×10^8 cells per ml and infected with f1 at various MOIs at the indicated temperatures. After 10 min, the cells were washed of free phage and plated to score for infective centers as described in Materials and Methods. (b) Cells carrying plasmid pKAS1 were grown to 4×10^8 cells per ml at 34°C and shifted to 40°C for 1 h before infection. The infection efficiency (percent infected) was determined as described above. Symbols: ●, VS2 (parental F′ plasmid), 36°C; ○, VS2, 42°C; ■, VS4 (F′ *traC1044*), 36°C; □, VS4, 42°C; ▲, VSP2; ▼, VSP4; △, VSP5 (F′ *traC5*).

TABLE 2. Formation of mating pairs

Mating mixture	No. of lone cells (avg)[a]	Avg % drop in no. of lone cells[b]
K17	138 } (131)	
VS2	124 }	
K17 + VS2	54	59 ± 4 (6)
K17	221 } (218.5)	
VS4	216 }	
K17 + VS4	186	16 ± 4 (9)
K17	152 } (169.5)	
VL584	187 }	
K17 + VL584	180	−5 ± 5 (9)
K17	180 } (172)	
VS5	164 }	
K17 + VS5	174	−2 ± 5 (7)

[a] Data are presented for one experiment only.
[b] Data represent the mean ± standard deviation. The numbers in parentheses indicate the number of times the experiment was repeated.

are also consistent with the idea that a functional receptor that can recognize f1 phage and recipient cells in bacterial conjugation exists at the cell surface in VS4.

The efficiency with which VS4 actually transferred conjugal DNA to a recipient cell was also measured in quantitative 1-h mating experiments. Table 3 shows that VS4 was 10,000-fold less efficient at DNA transfer during conjugation than was its wild-type parent, VS2; at 37°C, VS4 had a transfer frequency (percent transconjugants per donor cell in the mating mixture) of 0.004%, while that of VS2 was 47%. Raising the recipient/donor ratio (up to 50) or performing matings on filters succeeded in raising the VS4 transfer frequency only about 10-fold (data not shown), suggesting that the *traC1044* defect may affect additional conjugative functions besides the kinetics of mating pair formation. In contrast, the 1,000-fold decrease in the efficiency of f1 infection seemed to reflect the reduced accessibility of the phage receptor of the host. The *traC1044* mutation could affect functions essential to conjugal DNA transfer that play no role in f1 infection. This difference in conjugal DNA transmission and phage DNA translocation observed in VS4 presumably reflects the different mechanisms used for DNA transfer in bacterial conjugation and phage infection. It has been shown that the products of the chromosomal loci *tolA*

TABLE 3. Complementation of transfer efficiency in *traC1044* and *traC5* strains

Donor strain	Plasmid(s) in donor	Transfer efficiency[a] at:	
		37°C	42°C
VS2	F *lac pro*	47	208
VS3	F *lac*	35	239
VS4	F *lac pro traC1044*	4×10^{-3}	3×10^{-2}
VS5	F *lac traC5*	$<1 \times 10^{-5}$	7×10^{-4}
VSP2	pKAS1 and F *lac pro*	50	92
VSP3	pKAS1 and F *lac*	42	128
VSP4	pKAS1 and F *lac pro traC1044*	5	55
VSP5	pKAS1 and F *lac traC5*	14	54

[a] Data are expressed as percent transconjugants per donor cell in the initial mating mixture. The higher values obtained at 42°C reflect the higher efficiency of transfer at this temperature (3) and the higher ratio of recipients to donor cells (2:1) in the 42°C mating mixtures. In the 37°C mating mixtures, the ratio was 1:1. All donor strains are derivatives of VL584; the recipient strain was XK5456 in all cases.

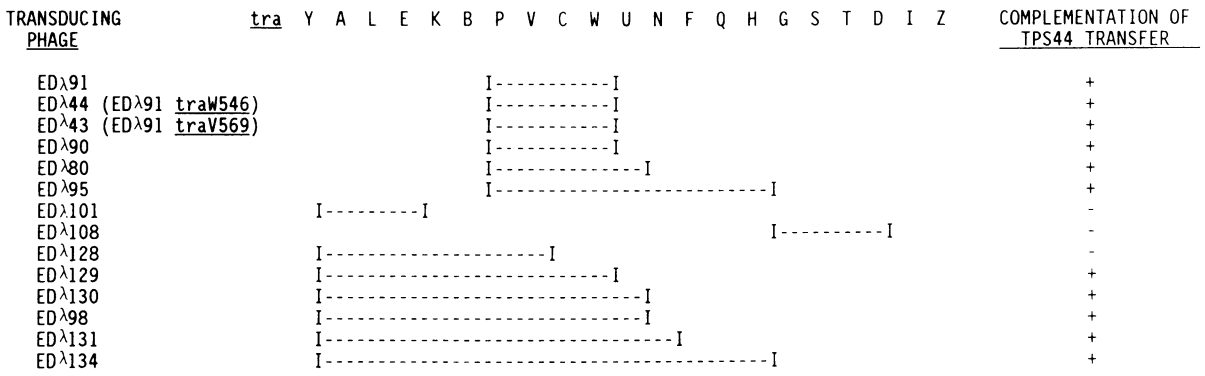

FIG. 4. Mapping of the TPS44 mutation by transductional complementation. The set of F *tra* genes expressed by each EDλ transducing phage is indicated by a dashed line. The transfer capacity of TPS44 donors infected with these phages was assayed as described in Materials and Methods. A positive (+) complementation result indicates that phage infection restored donor mating proficiency, as judged by confluent growth of Lac^+ Str^r transconjugants within the area of the phage spot.

and *fii* are required for f1 DNA to cross the cell membrane, but mutations in these genes do not affect conjugation (36, 37, 43).

Mapping the mutation. Since the mutation in VS4 was associated with the F factor and affected conjugation and the expression of F pili, it was reasonable to presume that an F *tra* region defect was responsible for the VS4 phenotype. To locate the mutation more precisely, we tested the capacity of lambda *tra* transducing phages to complement the transfer defect. These phages carry different, overlapping segments of the *tra* operon (Fig. 4). Infection with phages that carry the *traCW* region markedly enhanced TPS44 transfer proficiency. Since no transducing phage with *tra* DNA endpoint between *traC* and *traW* was available (21), derivatives of the *tra(B)VCW* phage EDλ91 that carry the *traV569* or *traW546* amber mutation were tested. Both derivatives (EDλ143 and EDλ144) were still able to complement the TPS44 transfer defect. Therefore, these results strongly suggested that the TPS44 transfer defect was in *traC*.

Further evidence for this map location was obtained by complementation with a $TraC^+$ plasmid. The *traC* region was subcloned to produce plasmid pKAS1 as described in Materials and Methods and diagrammed in Fig. 1. When the quantitative mating experiments were repeated with VS4(pKAS1), the donor transfer frequency was restored to near wild-type levels (Table 3). Plasmid pKAS1 carries *traC* but not *traW* or any other identifiable *tra* gene (17; Maneewannakul et al., submitted). The ability of the cloned *traC* to complement the VS4 donor transfer defect supported the conclusion that the VS4 mutation was in *traC*, so the mutation was labeled *traC1044*.

We note that *traC* mutants have been the most frequent among all *tra* mutants isolated. A number of missense, nonsense, and frameshift *traC* mutants have been reported (3, 40). All previously described *traC* mutants were shown to be resistant to f1 and f2 by spot testing, and the mutant transfer frequencies of most of these mutants were lower than that of the *traC1044* mutant. Thus, *traC1044* appears to be unique among *traC* mutants in retaining sensitivity to f1 phage. We looked further at two previously isolated *traC* amber mutants, *traC5* and *traC12* (3). In our efficiency-of-infection assay, neither amber mutant could be infected with f1, even at the highest MOI tested (data not shown). The mating pair formation assay showed that VS5, the *traC5*-carrying strain, did not form mating pairs (Table 2), but the assay is only sensitive enough to detect mating pair formation at a level approximately 20-fold below wild-type levels. The transfer frequency of the F *lac traC5* strain was typically 50- to 100-fold lower than the transfer frequency of the F *lac pro traC1044* strain (Table 3). The inability to detect f1 infection and mating pair formation in VS5 suggested that in the absence of the TraC protein, there was no detectable pilus tip structure or phage receptor and recipient cell receptor activity.

***traC*-dependent f1 infection and pilus production.** The cloned *traC* carried by pKAS1 was also tested for its ability to complement the altered phage sensitivities exhibited by VS4 and VS5. Derivatives of both *traC* mutant donors into which pKAS1 had been introduced could plaque f1 and f2. Because of the temperature-sensitive lambda repressor carried by pKAS1 (Fig. 1), this complementation was weak at 36°C but much better at 40°C. The efficiency of infection was also measured in pKAS1 derivatives of the mutants after 1 h of incubation at 40°C to induce expression from the p_L promoter. The bacteria carrying either the *traC1044* or the *traC5* mutation together with the pKAS1 plasmid could be infected at the same efficiency as could the wild-type control, VSP2 (Fig. 3b).

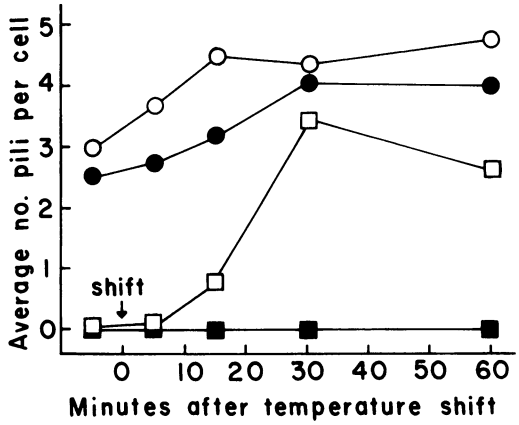

FIG. 5. Complementation of pilus production by pKAS1. Cells were grown at 33°C to 4×10^8 cells per ml and shifted to 40°C at time zero. Aliquots were removed at the times shown, combined with f2, and immediately placed on grids for electron microscopy. The average number of pili per cell at each time point was determined by counting more than 100 cells from two independent experiments. Symbols: ●, VS2; ○, VSP2; ■, VS4; □, VSP4.

We also looked at the ability of cells carrying pKAS1 to elaborate F pili. VSP4 reached a wild-type level of piliation when the lambda p_L promoter was induced (Fig. 2). The time required for cells to become piliated was determined as described in Materials and Methods. At 33°C, when expression of *traC* from p_L is extremely low, VS4(pKAS1) was not significantly piliated (Fig. 5). Upon induction of the lambda p_L promoter and increased expression of *traC*, about 30 min was required for VSP4 to acquire the same average number of pili per cell and range of piliation as in the wild-type control. Donor strains carrying pKAS1 did not become hyperpiliated, suggesting that *traC* alone is not responsible for controlling the number of pili produced per cell or that the expression of *traC* is regulated so that it is not overproduced in donor strains. Preliminary experiments have failed to detect the overproduction of the *traC* product in VSP4 and in the corresponding recipient strain, VSP1 (data not shown).

Previous studies have shown that the product of *traC* is not required for the processing of the 13,200-Da pilin precursor to its mature 7,200-Da form and that normal quantities of F-pilin polypeptide are present in the membranes of F *lac traC* amber mutant strains (23). We used dot blot analysis to confirm that the pilin subunits in VS4 were processed to the antigenically mature 7,200-Da form. The cell envelope of VS4 and its parent, VS2, were disrupted in a French pressure cell and fractionated on modified Osborn gradients (19). Aliquots of the gradient fractions corresponding to the inner membrane, intermediate zone, and outer membrane peaks were transferred to nitrocellulose and probed with antipilin antibodies, followed by ^{125}I-protein A (38). Autoradiography showed that VS4 had the same amount of F-pilin antigen in the same membrane fractions as did VS2, with the concentration of pilin relative to total protein highest in the inner membrane (data not shown). The same result was obtained with monoclonal antibody JEL 93, which has been shown to be specific for the acetylated amino-terminal region of the mature F-pilin molecule (11). These data confirm the results of previous studies (23) that suggested that mutations in *traC* affect the pathway for assembling the mature pilin subunits into pili and not the complex pilin-processing pathway.

DISCUSSION

We reported the isolation and partial characterization of a mutation, *traC1044*, that causes cells to become temperature sensitive for filamentous phage f1 infection and completely resistant to RNA phage f2. Cells carrying *traC1044* do not elaborate F pili at either the permissive or the restrictive temperature. Mating pair formation occurs when *traC1044* strains are used as donors, but conjugal DNA transfer is severely impaired. Although other *traC* mutations have been isolated (3, 40), *traC1044* is unique in that cells carrying this mutation can still plaque f1 at the permissive temperature. The other known *traC* mutants are resistant to f1 and f2, as well as being deficient in conjugation and lacking F pili. A TraC$^+$ plasmid which expresses *traC* from the lambda p_L promoter was constructed. This plasmid complemented the altered phage sensitivities, conjugation deficiency, and inability to elaborate F pili exhibited by *traC1044* strains.

Although there is no reliable f1 phage-binding assay that is independent of a measurement of infection, the ability to increase the infection efficiency of the F *lac pro traC1044* mutant by increasing the ratio of phage to cells suggests that a functional phage receptor or pilus tip structure is present on the cell surface in strains bearing this mutation. This hypothesis is substantiated by the fact that *traC1044* mutant donors can also form some kind of mating pairs. Whether a normal or altered pilus tip structure is present is not clear. However, the fact that filamentous phage infection and mating pair formation can occur in the absence of F-pilus outgrowth in VS4 suggests that one role of the pilus in f1 infection and conjugation is purely a kinetic one. That is, the presence of an extended F pilus increases the frequency with which the phage attachment proteins come in contact with the receptor at the tip of the pilus and increases the efficiency with which a donor cell can associate with a recipient cell in conjugation. This suggestion is consistent with the hypothesis that extended pili can withdraw, presumably by depolymerization into the membrane, bringing the phage to the cell surface, where infection can proceed (20, 34), and the mating cells into wall-to-wall contact during bacterial conjugation (41).

We do not know whether the site on *traC1044* cells to which f1 phage and recipient cells attach is a normal F-pilus tip or whether it is actually an abnormality in the F-pilus tip structure that interferes with phage infection, conjugation, and pilus assembly. We were unable to isolate temperature-sensitive F mutant strains which were resistant to f1 infection (altered tip) but sensitive to f2 infection (normal pilus length). Others have also failed to isolate mutants with this phenotype, despite scoring hundreds of F factor mutants. Thus, it seems likely that mutations which affect the pilus tip structure also interfere with the polymerization of F-pilin subunits. If the pilus tip is F pilin, it is reasonable to assume that the amino acid residues involved in f1 binding could also be important in the proper interaction of internal pilin subunits in the assembly of F pili. Alternatively, although no protein other than F pilin has been identified in purified F pili, it is still possible that a unique protein is required to form the pilus tip and that this structure must be formed before F-pilin polymerization can be initiated. It seems unlikely that the TraC protein itself would constitute the tip structure, since this large, 92,000-Da polypeptide (17) would probably have been detected in purified pilus preparations and would be likely to form an identifiable knob structure at the end of the pilus, a structure which has never been observed. However, TraC could be required to modify or arrange subunits to give the proper pilus tip structure, and a defect in this activity could lead to the phenotype associated with *traC* mutants.

Regardless of whether the product of *traC* affects the pilus tip structure, TraC is certainly involved in the assembly of pilin subunits into a mature pilus. The large number of *tra* gene products concerned with pilus outgrowth are thought to form an inner membrane-outer membrane-spanning complex which mediates pilin assembly and disassembly and may form the channel for conjugative DNA transmission. Pilus assembly appears to be energy dependent and may involve chemical modification or conformational alteration in the subunit polypeptide or both; thus, the TraC protein may interact directly with pilin subunits during assembly, be involved in transfer complex energizing reactions, or simply be essential to the proper structure of the complex itself. Since the pilin found in membranes of *traC1044* mutants exhibited normal reactivity with anti-F pilin serum, it should have an acetylated amino terminus (10, 12, 23). Nevertheless, assembled F pilin may also contain phosphate and hexose residues (4, 12), and differences in such modifications resulting from the *traC1044* mutation might not have been detected. However, our results are consistent with a model

in which a complex of transfer proteins containing the *traC1044* product does form a transmembrane structure with an F-pilus tip exposed at the cell surface, but the resultant complex is incapable of polymerizing pilin subunits into an extended filament. The other known *traC* mutants, such as the *traC5* amber mutant, may be unable to form the transmembrane complex itself or be incapable of assembling the pilus tip structure on the cell surface, leading to resistance to f1 and the inability to form mating pairs.

One other piece of data must be considered. Strains carrying F *lac pro traC1044* can form mating pairs but are unable to efficiently transfer conjugal DNA to a recipient cell. This result implicates the product of *traC* in conjugal DNA transmission. If the *traC* protein is a component of the membrane-spanning complex of *tra* proteins through which both pilus assembly and disassembly and conjugal DNA transmission occur, the *traC* protein could be directly involved in DNA transmission or could secondarily affect transmission by altering the structure of the conjugal pore. An improperly formed complex containing the defective TraC protein may interfere with the function of other *tra* proteins required for conjugation. It would be expected that such a defective *tra* gene protein complex would have no effect on f1 phage DNA translocation, since this process occurs by a different mechanism. Sun and Webster (37) and Smilowitz (36) have implicated the products of the *fii* and *tolA* loci of the chromosome as components of a different membrane protein complex required for f1 infection. Mutations in these genes have no effect on bacterial conjugation.

The ability of cells carrying the *traC1044* mutation to be infected by f1 despite the lack of F pili can be exploited to study the features of F strains required for f1 infection. These strains should also be helpful in establishing the nature and order of events in pilus elaboration.

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