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Location of F Plasmid Transfer Operon Genes *traC* and *traW* and Identification of the *traW* Product

SUMIT MANEEWANNAKUL, PUSHPA KATHIR, DEANNA MOORE, LAN-ANH LE, JUNE H. WU, AND KARIN IPPEN-IHLER*

Department of Medical Microbiology and Immunology, Texas A & M University, College Station, Texas 77843

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As part of an analysis of the conjugative transfer genes associated with the expression of F pili by plasmid F, we have investigated the physical location of the *traC* and *traW* genes. We found that plasmid clones carrying a 2.95-kilobase *Eco*RI-*Eco*RV F transfer operon fragment were able to complement transfer of F *lac traC* mutants and expressed an approximately 92,000-dalton product that comigrates with TraC. We also found that *traW*-complementing activity was expressed from plasmids carrying a 900-base-pair *Sma*I-*Hin*cII fragment. The *traW* product was identified as an approximately 23,000-dalton protein. The two different F DNA fragments that expressed *traC* and *traW* activities do not overlap. Our data indicate that the *traC* gene is located in a more-*tra* operon promoter-proximal position than suggested on earlier maps and that *traW* is distal to *traC*. These results resolve a long-standing question concerning the relationship of *traW* to *traC*. The clones we have constructed are expected to be useful in elucidating the role of proteins TraC and TraW in F-pilus assembly.

Many gene functions that are essential to conjugative transfer of the *Escherichia coli* K-12 fertility factor F have been identified through complementation analyses of transfer-deficient mutants (for reviews, see references 6, 21, and 22). Genes *traC* and *traW* are among the large class of F transfer operon loci that are required for production of F pili. Cells carrying F *lac traC* and F *lac traW* amber derivatives appear resistant to RNA and DNA male-specific bacteriophages such as f2, R17, Qβ, f1, and M13 but are capable of synthesizing pilin subunits, suggesting that F-pilus assembly is defective in these strains (2, 3, 13, 14). Since F *traC* mutant plasmids complemented the transfer of *traW546*, the single F *traW* mutant available, Miki et al. (13) concluded that *traC* and *traW* express functionally separable activities. However, subsequent studies did not achieve physical separation of these two cistrons, and the map position of *traW* became unclear. Although initial analyses of transducing phages had suggested that *traW* was located between *traV* and *traC* (13), cloned F DNA fragments that include this region did not express *traW* activity (8, 16), and the possibility that *traC* and *traW* overlap has been considered (8, 21).

In this paper, we present evidence that *traC* and *traW* can be physically separated and that *traW* lies promoter distal to *traC*. The *traW* gene product was identified as a polypeptide with an apparent molecular weight of 23,000.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial host strains used in this study are described in Table 1. The origin or derivation of these strains has been described previously (16). Plasmids were introduced into strain SE5000 (for maxicell protein analyses) or into strains EM1205, EM12546, and EM9546 (for transfer complementation analyses) by transformation. Plasmid pRS29 and a plasmid pRS29 *traW546* mutant derivative were obtained from R. Skurray and N. Willetts, respectively. The structures of these and other plasmids carrying regions of the F *tra* operon that were used in this analysis are summarized in Table 2. The vectors used in plasmid constructions were pBR322 (19), pACYC177 (4), pUC18 (23), and pQTE (5).

Plasmid construction and analysis. Our procedures for plasmid DNA manipulation, restriction analysis, and cloning have been detailed elsewhere (16, 22a). They are essentially the same as those described by Maniatis et al. (11).

Plasmid products were labeled for 1 h with [^{35}S]methionine in a maxicell host (SE5000) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography, as indicated in previous publications (16, 22a). However, maxicell cultures containing pUC18 or pQTE vector derivatives were transferred to a medium containing glycerol instead of glucose before labeling of plasmid products, and isopropyl-β-D-thiogalactopyranoside was present to induce the vector *lac* promoter throughout the labeling period. The polyacrylamide gels (10.5 or 22.5 cm) contained an exponential gradient of 10 to 16% acrylamide. In addition to unlabeled molecular weight standards, samples of [^{35}S]methionine-labeled proteins expressed by the transducing phage EDλ134 and its parental vector EDλ4 in a UV-irradiated host (XK1800) were also usually analyzed on our gels to provide a set of labeled polypeptides that identify various *tra* gene products. The structure of the *tra*(*Y*)*ALEKBPVRCWUNQH*(*G*) transducing phage EDλ134 and parent phage EDλ4 ha been described by Johnson and Willetts (9); labeling procedures and experiments leading to identification of transducing phage *tra* products have also been reported previously (7, 10, 15, 16).

Complementation of F *lac tra* mutants. Plasmids to be tested were introduced into an F *lac traC5* or F *lac traW546* host by transformation. The ability of the plasmids to provide an active *traC* or *traW* product was assessed by mating these donors with XK3051. The efficiency of transfer was determined as described previously (16) and is expressed as the percent Lac$^+$ transconjugants obtained per donor cell. Sensitivity to male-specific phages was also determined by spotting lysates of f2, MS2, R17, Qβ, f1, and fd on agar layers inoculated with the culture to be tested.

* Corresponding author.

TABLE 1. Bacterial strains and genotypes

Strain	Genotype
EM1200	F *lac*/XK1200
EM1205	F *lac traC5*/XK1200
EM12546	F *lac traW546*/XK1200
EM9000	F *lac*/XK5456
EM9546	F *lac traW546*/XK5456
JC3051	F⁻ *lacΔX74 his trp rpsL tsx mal* (λ)
SE5000	F⁻ *araD139 lacΔU169 rpsL relA thi recA56*
XK1200	F⁻ *lacΔU124* Δ(*nadA gal attλ bio*) *gyrA*
XK1800	F⁻ Δ(*lac-pro*) *gal rpsL ilv pro uvr*
XK5456	F⁻ *lacΔX74 his trp rpsE tsx ton*

RESULTS

Cloning of the *traC* gene. Previous evidence had demonstrated that the *Sal*I site at F *tra* coordinate 9.77 lies within *traC* (8, 16, 20; see Fig. 1). Although previous maps also suggested that the *Sma*I site at coordinate 10.57 was within *traC*, recent data obtained by Moore et al. (16) have indicated that *traC* instead extends over the *Sma*I site at coordinate 8.02 and may even include the *Hpa*I site at coordinate 7.62. Thus, it seemed likely to us that *traC* was situated in a more-promoter-proximal location. On the basis of the restriction map devised by Wu et al. (22a), we predicted that the entire *traC* gene could be contained within an *Eco*RI-*Eco*RV fragment that spans the region between coordinates 7.57 and 10.52. Since the map suggested that other *Eco*RV fragments from the large F *Eco*RI f1 fragment carried by pSH1 might also be useful in *tra* gene analysis, we cloned *Eco*RV and *Eco*RI-*Eco*RV fragments from pSH1. The structures of the *tra* plasmids that we isolated (pKI270, pKI159, pKI272, and pKI273) are described in Table 2 and Fig. 1.

Although plasmid pKI270 carries the 2.95-kilobase (kb) *Eco*RI-*Eco*RV *traC* region fragment, the construction of pKI270 left no appropriate promoter for expression of the *tra* DNA insert. Therefore, two additional plasmids, pKI326 and pKI376, were derived from pKI270 by moving the *tra* DNA segment into appropriate sites on vectors pUC18 and pQTE (Table 2). These vectors contain a *lac* and a λ p_R' promoter, respectively, from which the *tra* fragment can be transcribed after isopropyl-β-D-thiogalactopyranoside induction (in vector pQTE, expression from the λ p_R' promoter is regulated by inducing transcription of the λ *Q* gene from a *lac* promoter [5]).

Analysis of [^{35}S]methionine-labeled proteins synthesized in maxicells carrying pKI326 or pKI376 showed that both plasmids expressed a protein that migrates at approximately 92 kilodaltons (kDa); expression from pKI326 was more readily detected at our usual film exposures, and we used this plasmid for additional analysis. As shown in Fig. 2, the pKI326 92-kDa protein comigrated with the *traC* products of pSH1 and of the λ*tra*-transducing phage EDλ134. Table 3 shows that a transfer-deficient F *lac* plasmid carrying the amber mutation *traC5* was effectively complemented by pKI326. Expression of this plasmid caused the F *lac traC5* strain to become sensitive to pilus-specific phages and to transfer at a normal frequency. Therefore, the 2.95-kDa *Eco*RI-*Eco*RV fragment appeared to contain a fully competent F *traC* gene.

Location of the *traW* gene. To determine the location of the *traW* gene, we tested the capacity of a large number of

TABLE 2. Plasmid sources and vector cloning sites

Plasmid no.	*tra* fragment size (kb) and ends[a]	Vector and vector cloning site(s)	Source of *tra* DNA and reference
pKI153	0.94 *Sma*I-*Hin*cII	pACYC177 *Sma*I	pKI157 (*traW546* mutant) (this paper)
pKI154	0.94 *Sma*I-*Hin*cII	pACYC177 *Sma*I	pKI183 (wild-type F DNA) (this paper)
pKI157	1.74 *Hin*cII	pACYC177 *Hin*cII	pRS29 *traW546* (this paper)
pKI159	2.56 *Eco*RV[b]	pACYC177 *Hin*cII	pSH1 fragment (this paper)
pKI169	2.55 + 6.2 *Ava*I	pACYC177 *Xma*I	pSH1 fragment (22a)
pKI171	1.15 *Ava*I[c]	pACYC177 *Xma*I	EDλ92 fragment (22a)
pKI172	2.55 *Ava*I	pACYC177 *Xma*I	pSH1 fragment (22a)
pKI175	6.2 *Ava*I	pACYC177 *Xma*I	pKI206 fragment (22a)
pKI182	1.77 *Pst*I	pACYC177 *Pst*I	pSH1 fragment (22a)
pKI183	1.74 *Hin*cII	pACYC177 *Hin*cII	pKI206 fragment (22a)
pKI184	4.1 *Hin*cII	pACYC177 *Hin*cII	pKI206 fragment (22a)
pKI202	5.6 (*Bam*HI)-*Sal*I	pBR322 *Bam*HI-*Sal*I	EDλ92 fragment (16)
pKI206	7.15 *Sal*I-*Hpa*I[d]	pBR322 *Sal*I-*Bam*HI	EDλ92 fragment (22a)
pKI270	2.95 *Eco*RI-*Eco*RV	pBR322 *Eco*RI-*Eco*RV	pSH1 fragment (this paper)
pKI272	3.8 *Eco*RV	pBR322 *Eco*RV	pSH1 fragment (this paper)
pKI273	3.29 *Eco*RV	pBR322 *Eco*RV	pSH1 fragment (this paper)
pKI326	2.95 *Eco*RI-*Eco*RV[e]	pUC18 *Eco*RI-*Bam*HI	pKI270 *Eco*RI-*Bam*HI (this paper)
pKI376	2.95 *Eco*RI-*Eco*RV	pQTE *Eco*RI-*Eco*RV	pKI270 *Eco*RI-*Eco*RV (this paper)
pSH1	13.85 *Eco*RI	RSF2124 *Eco*RI	F *Eco*RI f1 fragment (1)
pRS29	15.15 *Eco*RI	pSC101 *Eco*RI	F *Eco*RI f15, f1 fragments (18)
pRS29 *traW546*	15.15 *Eco*RI	pSC101 *Eco*RI	pRS29 × F *lac traW546* recombinant (N. Willetts)

[a] Except as noted, the orientation of *tra* DNA in all pKI plasmids is such that transcription from the promoter of the vector *amp*, *tcy*, or *kan* gene that contains the insert is in the *tra* operon direction (left to right in Fig. 1).

[b] Attempts to clone this fragment into the *Eco*RV site of pBR322 such that *tra* orientation matched *tcy* orientation were unsuccessful. It was cloned into pACYC177 *amp* without difficulty.

[c] An atypical *Ava*I fragment that presumably originated by spontaneous mutation of EDλ92; Southern blot and restriction enzyme analyses demonstrate that it includes a promoter-proximal segment of the F *tra* 6.2-kb *Ava*I fragment as indicated in Fig. 1. Clones carrying the typical 1.15-kb *Ava*I F fragment which stems from the *traV* region have been described previously (16).

[d] Orientation is opposite to the vector *tcy* promoter.

[e] pKI326 carries the *Eco*RI-*Bam*HI fragment from pKI270. This fragment includes the pBR322 sequence between *Eco*RV-*Bam*HI, as well as the *tra* DNA indicated.

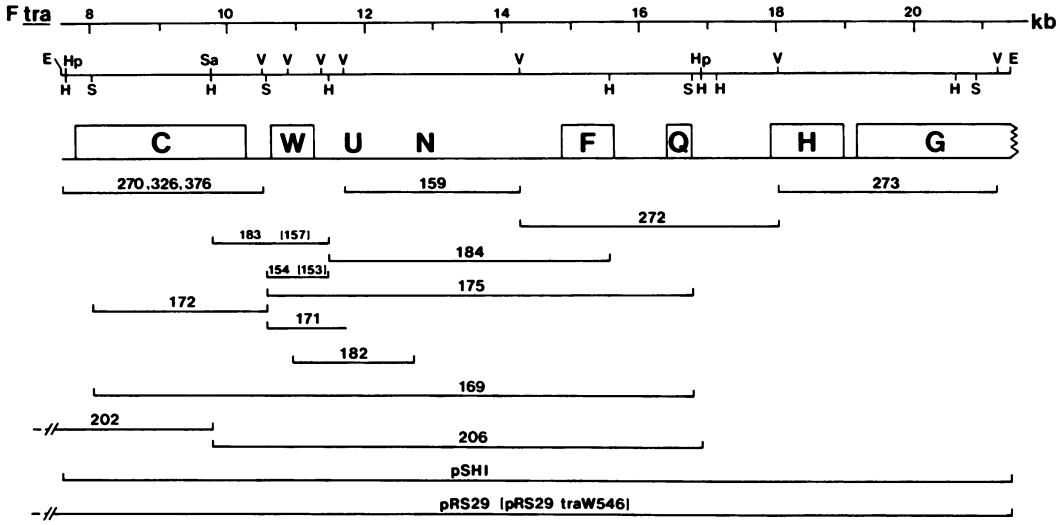

FIG. 1. F *tra* region segments carried by plasmid vectors. A map of the F *tra* region within the *Eco*RI fragment f1 is shown. Positions of restriction sites are as determined by Wu et al. (22a). Numbered line segments indicate the segment of *tra* operon DNA carried by the plasmids (pKI unless otherwise indicated) listed in Table 2. Plasmid numbers noted in parentheses are those that carry the *traW546* amber mutation; plasmids pKI202 and pRS29 include *tra* operon proximal regions not shown. F *tra* region kilobase coordinates at the top of the map are assigned in accordance with the map of Moore et al. (16), where coordinate 0 is the F origin of transfer and the 13.85-kb f1 fragment extends from 7.57 to 21.42 (22a). Large dark letters indicate the relative position of *tra* genes; box lengths denote gene sizes approximated from product analyses (22a; this work). Restriction sites indicated are for *Eco*RI (E), *Eco*RV (V), *Hin*cII (H), *Hpa*I (Hp), *Sal*I (Sa), and *Sma*I (S).

chimeric plasmids that carry *tra* DNA to complement the transfer defect of F *lac traW546* mutant strains. These experiments were complicated by reversion and leaky transfer of the F *lac traW546* mutant plasmid and by poor growth of some plasmid-bearing derivatives. Therefore, we performed the complementation experiments in two different strain backgrounds and averaged the results of several tests of each donor. We also took the precaution of freezing numerous samples of a single competent culture of each F *lac traW546* host so that all plasmids to be tested could be transformed into an identical population of mutant donor cells. The *tra* segments carried by the plasmids that we tested for *traW* activity are diagrammed in Fig. 1; the results of our complementation analysis are given in Table 3. A few representative results, presented previously as part of an analysis of plasmids carrying segments derived from the *traB-traC* region, are included (Table 3) for reference (16).

We initially tested derivatives of the donor EM12546. The

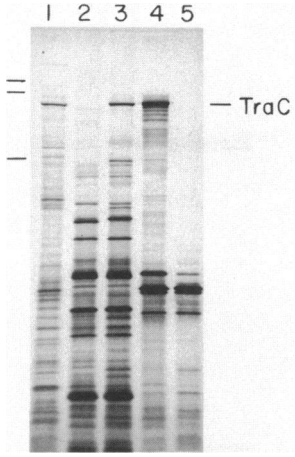

FIG. 2. pKI326 expression of TraC. Shown is an autoradiogram of a polyacrylamide gel of [^{35}S]methionine-labeled polypeptides expressed in maxicells carrying pKI326 (lane 4), pUC18 (lane 5), or pSH1 (lane 1) or in UV-irradiated cells infected with EDλ134 (lane 3) or EDλ4 (lane 2). The position of the approximately 92-kDa TraC protein detectable in lanes 1, 3, and 4 is indicated on the right. The positions of (top to bottom) β-galactosidase, phosphorylase *b*, and catalase molecular weight markers are indicated along the left side.

TABLE 3. Complementation of F *lac tra* mutants

Coresident plasmid	Transfer frequency[a] of mutant F *lac* from:		
	EM1205 (*traC5*)	EM12546 (*traW546*)	EM9546 (*traW546*)
None[b]	$<4 \times 10^{-4}$	0.08	2×10^{-3}
pBR322[b]	$<7 \times 10^{-4}$		
pACYC177[b]			4×10^{-3}
pKI153		0.36	2×10^{-3}
pKI154		20	0.19
pKI157		0.32	8×10^{-3}
pKI169[b]	8×10^{-3}[c]		
pKI172[b]	2×10^{-3}[c]		8×10^{-3}
pKI175	$<4 \times 10^{-4}$	14	0.73
pKI171		18	0.33
pKI182		0.33	3×10^{-3}
pKI183		9	0.23
pKI184			2×10^{-3}
pKI202[b]	$<3 \times 10^{-4}$	0.16	
pKI206	0.02[c]	5.2	
pKI270[d]	0.05[c]		2×10^{-3}
pKI326	30		4×10^{-3}
pRS29[b,e]	0.64	6.2	0.43
pSH1[b]	10	16	0.97

[a] Percent transconjugants per donor cell in mating mixture. The wild-type F *lac* transfer frequency was 55% from strain EM1200 and 14% from EM9000.

[b] These values, provided for reference, were reported previously by Moore et al. (16).

[c] Level is presumed to reflect transfer of TraC$^+$ recombinants.

[d] There is no appropriate vector promoter for expression of *traC* from this plasmid.

[e] Plasmid pRS29 typically gives low complementation levels but does carry both *traC* and *traW*. Our values are similar to those reported by others (1, 13).

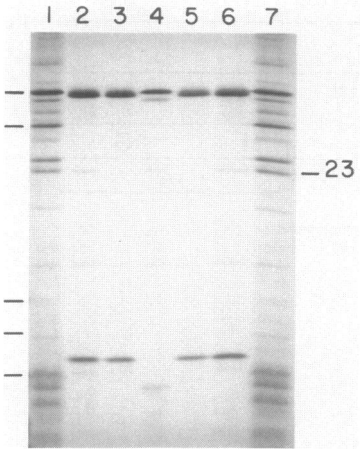

FIG. 3. Analysis of [^{35}S]methionine-labeled polypeptides expressed by pKI plasmids in maxicells. Samples from strains carrying pKI175 (lanes 1 and 7), pKI183 (lanes 2 and 6), pKI157 (lanes 3 and 5), and pKI171 (lane 4) were examined. The position of the 23-kDa product detected in all TraW$^+$ plasmids is indicated on the right. Only a portion of the autoradiogram of the 22.5-cm gel is shown; the positions at which (top to bottom) carbonic anhydrase, α-chymotrypsin, β-lactoglobulin, myoglobin, and lysozyme migrated were determined from the stained gel and are indicated on the left.

background transfer of F *lac traW546* from this strain was typically in the range from 0.05 to 0.5%, whereas derivatives carrying plasmid pRS29 or pSH1 transferred more efficiently at frequencies averaging 10 to 30% of wild-type F *lac* transfer levels. This result indicated that *traW* activity could be expressed from the F *Eco*RI fragment f1. While inconsistent with the conclusion of Miki et al. (13) that pRS8 (which carries F *Eco*RI fragments f1, f17, f19, f2, f12, f16, and f10 [18]) could yield Tra$^+$ recombinants but not complement transfer of F *lac traW546*, our result was consistent with other reports indicating that *traW* is not located at the *Eco*RI site in the *traB-traC* interval (8, 16). The presence of plasmids pKI175, pKI171, and pKI183 also enhanced transfer from the F *lac traW546* host to about 15 to 30% of the wild-type transfer level and caused the strain to become sensitive to F-pilus-specific phages. Thus, these plasmids also appeared to express *traW*.

We also used the donor EM9546 for a series of complementation tests. In this strain, the background transfer frequency of F *lac traW546* was typically $<5 \times 10^{-3}$%. Although the transfer efficiency remained less than 1% in the presence of plasmids pRS29 and pSH1, the transfer frequency observed constituted a 200- to 500-fold increase over background. We therefore interpreted this increase as a positive result. Since the difference between background transfer and complementation could be detected more easily and reliably with EM9546, we continued to use this host in assays of *traW* activity. We do not know the basis for the overall reduction of transfer levels observed; it is possible that in constructing this strain, we inadvertently selected a secondary mutation that also affected overall transfer efficiency.

Plasmids pKI175, pKI171, and pKI183 also substantially increased the level of F *lac traW546* transfer from EM9546. Thus, complementation analysis of both *traW546* donor strains tested strongly suggested that *traW*-complementing activity derived from the small region of *tra* DNA carried in common by these plasmids (Fig. 1). This region is entirely distal to the DNA present in our *traC* clone.

The *traW* product. Analysis of ^{35}S-labeled polypeptides synthesized in maxicells carrying pKI171, pKI175, or pKI183 showed that all three of these plasmids expressed an approximately 23-kDa polypeptide (Fig. 3). As a control for this experiment, we cloned the 1.74-kb *Hin*cII fragment (analogous to that carried by pKI183) from a DNA source containing the *traW546* mutation. This plasmid, pKI157, did not complement F *lac traW546* transfer (Table 3) and did not express the 23-kDa polypeptide (Fig. 3). The protein profile of pKI157 was otherwise identical to that of pKI183.

This result demonstrated that a *tra* operon gene encoding a 23-kDa product lies within the approximately 0.9-kb region of overlap between the segments carried by pKI175 and pKI183 (*Sma*I coordinate 10.57, *Hin*cII coordinate 11.51). It also showed that the sequence alteration in the *traW546* mutation *Hin*cII fragment affects expression of the 23-kDa product. This finding suggested that the 23-kDa protein was TraW. However, since the *Hin*cII fragment carried by pKI157 and pKI183 also contained the carboxy-terminal region of the *traC* gene, the possibility remained that the *traW546* mutation lay within the *traC* sequence and exerted a polar effect on expression of the 23-kDa protein. Therefore, we constructed two plasmids that carry only the small 0.9-kb *Sma*I-*Hin*cII fragment distal to *traC* by using pKI157 and pKI183 as a source of *traW546* mutant and wild-type *tra* DNA, respectively. Plasmid pKI154 was able to complement F *lac traW546* transfer and express the 23-kDa polypeptide (Table 3, Fig. 4). However, the *traW546* derivative, pKI153, was unable to express *traW* function or the 23-kDa product. We concluded from these experiments that the *traW* gene lies within the 900-base-pair *tra* region carried by these plasmids and that its product is the 23-kDa polypeptide.

DISCUSSION

In a prior analysis of activities and products associated with the *traB-traC* region of the F transfer operon, Moore et al. (16) suggested that the *traC* gene should have a more-promoter-proximal location than suggested on previous maps (9, 21), since the size of a *traC* fusion polypeptide appeared to be affected by small deletions generated from the *Hpa*I site at *tra* coordinate 7.62. The results presented

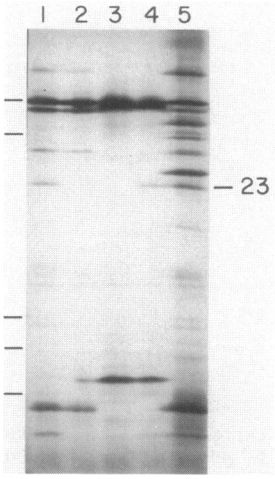

FIG. 4. Analysis of [^{35}S]methionine-labeled polypeptides expressed by pKI plasmids in maxicells. Samples from strains carrying pKI154 (lane 1), pKI153 (lane 2), pKI157 (lane 3), pKI183 (lane 4), and pKI175 (lane 5) were examined and protein products were detected as described in the legend to Fig. 3.

here show that a plasmid carrying the *Eco*RI-*Eco*RV fragment originating from between *tra* DNA coordinates 7.57 and 10.52 expresses an approximately 92-kDa product that appears fully competent to complement *traC* mutations and comigrates with the product expressed by TraC$^+$ vectors that carry longer *tra* DNA segments. The 2.95-kb fragment we cloned is more than large enough to express a 92-kDa protein and might contain sequences in addition to *traC*. However, because molecular weight estimates for proteins of this size lack precision, it is possible that *traC* extends over most of the fragment. It is even possible that *traC* extends beyond the *Eco*RV site at *tra* coordinate 10.52, since a slight foreshortening of the carboxy terminus of the large TraC protein would not have been detected on our gels. Nevertheless, it is clear that no essential portion of the *traC* gene extends distal to coordinate 10.52.

In contrast, we were able to clone the sequence necessary to complement *traW* mutations within a *Sma*I-*Hin*cII fragment located between *tra* coordinates 10.57 and 11.50. Furthermore, comparison of the protein products expressed by a plasmid containing this small fragment cloned from a wild-type F DNA source with those expressed by an otherwise identical plasmid constructed with *traW546* mutant DNA showed that loss of *traW* activity was associated with loss of expression of a 23-kDa polypeptide. Thus, our results show that the F factor *traC* and *traW* genes can be cloned on two different nonoverlapping DNA fragments and that the activities of these genes are associated with two different polypeptide products. These findings resolve a long-standing puzzle in the characterization of the *traW* and *traC* genes and indicate that the two genes do not overlap.

Miki et al. (13) originally suggested placement of *traW* to the left of *traC* on the basis of two findings. First, complementation tests with the large plasmid pRS8 appeared to be negative, and although transfer levels were enhanced, all transconjugants tested appeared to be TraW$^+$ recombinants. Since pRS8 includes the F *Eco*RI f1 fragment (and other distal *tra* fragments [18]), Miki et al. (13) suggested that the f1 fragment could not include an intact *traW* gene. However, our tests indicate that plasmid pSH1 complements the *traW* mutation quite well. Thus, we assume that the pRS8 result was misleading, perhaps because the frequency at which recombinants were generated with this large plasmid approached the transfer complementation frequency which was obtainable. Typically, transfer complementation with plasmid pRS8, like pRS29, is relatively low (13). Second, Miki et al. (13) reported that no transfer complementation of *traC* or *traW* was detectable in tests with the transducing phage EDλ86 but that TraC$^+$ recombinants were obtained. Since EDλ86 stemmed from a lambda insert in *traH* and exhibited complementing activity for *traU*, *traN*, and *traF*, this result appeared to preclude placement of *traW* between *traC* and *traU*. Since the phage result disagrees with our current evidence, we attempted to resolve the contradiction by repeating the EDλ86 complementation tests. Tests of an EDλ86 phage lysate prepared in our laboratory from a lysogen provided by N. Willetts showed a TraC$^-$W$^-$U$^+$N$^+$F$^+$ transfer complementation pattern identical to that reported by Miki et al. (13). However, no TraC$^+$ recombinants were obtained during the tests with our lysate. Thus, our results gave no indication that *traC* region DNA was carried by the phage and were not in conflict with our map position for *traW*. Nevertheless, because it is our experience that individual lysates produced after induction of this type of transducing phage can occasionally carry variant phage types, it is possible that the original lysate used by Miki et al. (13) did contain phages that included *traW* and a portion of the *traC* gene. Expression of *traW* from their phage population may simply have been insufficient to be detected in the complementation test.

The *traC* gene product is one of the few large proteins known to have a role in expression of F-pilus filaments. A relatively large number of the collection of transfer-deficient mutants isolated by Achtman et al. (3) were deficient in *traC*. Recently we also used a derivative of our *traC* clone to demonstrate that the phenotype of an F *lac pro* mutant which seems unable to express extended F-pilus filaments, but retains detectable sensitivity to the filamentous DNA phage f1, is the result of a *traC* mutation (17). Previous studies suggest that the TraC protein can be found associated with inner membrane fractions (10, 12). Analysis of membrane preparations of cells carrying pKI175 indicates that the *traW* product expressed in maxicells is also associated with these fractions (J. H. Wu, unpublished data). We expect the plasmids we have constructed to be useful in future characterizations of the TraC and TraW proteins and in defining the contribution of these two proteins to elaboration of the pilus filament.

ACKNOWLEDGMENT

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