JOURNAL OF BACTERIOLOGY, Aug. 1990, p. 4263–4270
0021-9193/90/084263-08$02.00/0

Vol. 172, No. 8

Characterization of the F-Plasmid Conjugative Transfer Gene *traU*

DEANNA MOORE,[1] KESMANEE MANEEWANNAKUL,[1] SUMIT MANEEWANNAKUL,[1] JUNE H. WU,[1]†
KARIN IPPEN-IHLER,[1]* AND DAVID E. BRADLEY[2]

Department of Medical Microbiology and Immunology, Texas A&M University, College Station, Texas 77840,[1] and Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3V6[2]

Received 11 January 1990/Accepted 3 May 1990

We characterized the *traU* gene of the *Escherichia coli* K-12 conjugative plasmid F. Plasmids carrying segments of the F transfer operon were tested for their capacity to complement F *lac traU526*. The protein products of TraU$^+$ clones were identified, and the nucleotide sequence of *traU* was determined. *traU* mapped between *traW* and *trbC*. It encodes a 330-amino-acid, M_r 36,786 polypeptide that is processed. Ethanol caused accumulation of a precursor polypeptide; removal of ethanol permitted processing of the protein to occur. Because F *lac traU526* strains appear to be resistant to F-pilus-specific phages, *traU* has been considered an F-pilus assembly gene. However, electron microscopic analysis indicated that the *traU526* amber mutation caused only a 50% reduction in F-piliation. Since F *lac traU526* strains also retain considerable transfer proficiency, new *traU* mutations were constructed by replacing a segment of *traU* with a kanamycin resistance gene. Introduction of these mutations into a transfer-proficient plasmid caused a drastic reduction in transfer proficiency, but pilus filaments remained visible at approximately 20% of the wild-type frequency. Like *traU526* strains, such mutants were unable to plaque F-pilus-specific phages but exhibited a slight sensitivity on spot tests. Complementation with a TraU$^+$ plasmid restored the wild-type transfer and phage sensitivity phenotypes. Thus, an intact *traU* product appears to be more essential to conjugal DNA transfer than to assembly of pilus filaments.

Genes essential to conjugal DNA transmission of the *Escherichia coli* K-12 fertility factor, F, are known to be clustered within a 33.3-kilobase (kb) region of this plasmid. Previous work has identified numerous genes in this region (9, 22), many of which encode products involved in the expression of F-pili. These filaments, which extend from the donor cell surface, provide receptors for F-pilus-specific RNA and DNA phages and are thought to initiate contact with recipient cells during conjugation.

Miki et al. (15) discovered the F *traU* gene during an analysis of amber-suppressible transfer-deficient F *lac* mutants and mapped this locus within the F *tra* operon segment between *traC* and *traN*. They found that the F *lac traU526* amber mutant was "leaky" and transferred from an Su$^-$ host at 0.1 to 0.5% of its transfer level in an Su$^+$ host. Since this was the sole *traU* mutant characterized, it has been unclear whether the *traU* function is completely essential to F transfer or has only a contributory role. However, as the *traU526* mutation appeared to confer resistance to both DNA and RNA F-pilus-specific phages (f1, f2, and Qβ), Miki et al. (15) tentatively concluded that *traU* is required for F-pilus formation.

This functional designation placed *traU* together with the large group of other F genes (including *tra* genes *A*, *L*, *E*, *K*, *B*, *V*, *C*, *W*, *F*, *Q*, *H*, and *G*) which are required for elaboration of F-pili. Two of these loci have been assigned roles in synthesis of the F-pilin subunit: *traA*, the structural gene for the F-pilin (3), and *traQ*, which appears to be required for processing the *traA* product (23, 25). The remainder are thought to contribute to pilin subunit assembly, since F-pilin has been detected in the membranes of F derivatives carrying mutations in these genes (17).

* Corresponding author.
† Present address: Department of Microbiology & Immunology, Chang-Gung Medical College, Kwei-San, Tao-Yuan 33332, Taiwan, Republic of China.

As part of an investigation into the nature of the various gene products that contribute to F-pilus expression, we have cloned segments of the *tra* operon that complement *traU526* and identified the protein product and nucleotide sequence of *traU*. We also constructed two new *traU* mutant F derivatives and investigated the contribution of *traU* to F-pilus production and conjugal DNA transfer.

MATERIALS AND METHODS

Bacterial strains and plasmids. The construction of plasmids carrying F *tra* region DNA segments is summarized in Table 1. The *tra* restriction fragments carried by these plasmids are diagrammed in Fig. 1. Plasmids pOX38 (5) and pOX38-Km (2), which include an intact F *tra* region and repFIA replicon but no IS or transposon Tn*1000* sequences, were kindly provided by R. Deonier. Plasmid pUC-4K, the source of the Tn*903 kan* fragment used for mutant construction, was purchased from Pharmacia Inc., Molecular Biology Division.

Strain ED2244, an amber-suppressed F *lac traU526* donor, was originally obtained from the collection of S. McIntire. It was used in standard matings to construct F *lac traU526* derivatives of strains XK1200 [F$^-$ *lac*Δ*U124* Δ(*nadA gal attλ bio*) *gyrA*], XK5456 [F$^-$ *lac*Δ*X74 his trp tsx ton rpsE*], JC3272 [F$^-$ *lac*Δ*X74 his trp lys tsx rpsL*(λ)], and VL584 [F$^-$ *thi ara* Δ(*lac-pro*) Δ(*uxu fimD*) *rpsL*]. Recipients for subsequent crosses were either XK5456 or JC3051 [F$^-$ *lac*Δ*X74 his trp rpsL tsx ton*(λ)]. Strain SE5000 (F$^-$ *araD139 lac*Δ*U169 rpsL relA thi recA56*) was used for maxicell experiments. The origin of these strains is described elsewhere (19, 21).

DNA cloning and sequencing. Cloning and restriction enzyme analysis of DNA fragments were performed by standard procedures (25). DNA sequence analysis of M13mp8 and M13mp9 clones was performed with the Sequenase system (United States Biochemical Corporation) and synthetic oligonucleotide primers. Both dGTP and dITP reac-

TABLE 1. Plasmids[a]

Plasmid	F fragment		Vector	Vector junction site(s)	Construction or reference
	Size (kb)	End(s)			
pKI141	1.4	*Pst*I	pACYC177	*Pst*I	*Eco*RV deletion of pKI182
pKI145	1.6	*Pvu*I-*Pst*I	pACYC177	*Pvu*I-*Pst*I	*Sca*I-*Pst*I fragment ofpKI281 to *Sca*I-*Pst*I sites of pACYC177[b]
pKI175	6.2	*Ava*I	pACYC177	*Xma*I	Wu et al. (25)
pKI182	1.8	*Pst*I	pACYC177	*Pst*I	Wu et al. (25)
pKI183	1.7	*Hin*cII	pACYC177	*Hin*cII	Wu et al. (25)
pKI184	4.1	*Hin*cII	pACYC177	*Hin*cII	Wu et al. (25)
pKI281	1.6	*Pvu*I-*Pst*I	pBR322	*Pvu*I-*Pst*I	*Pvu*I deletion of pKI182
pKI282	1.8	*Pst*I	pBR322	*Pst*I	1.8-kb *Pst*I fragment of pKI182 to pBR322 *Pst*I
pKI346	1.4	*Pst*I	pUC18	*Pst*I	1.4-kb *Pst*I fragment of pKI141 to pUC18
pKI347	1.4	*Pst*I (+ *kan* insert)	pUC18	*Pst*I	Blunt-end ligation of *kan* in *Eco*RV site of pKI346 (*kan* oriented in *traU* direction)
pKI348	1.4	*Pst*I (+ *kan* insert)	pUC18	*Pst*I	As for pKI347, but opposite *kan* orientation
pOX38	45.4	*Hin*dIII			Circularized F fragment (5)
pOX38-Km	45.4	*Hin*dIII			Carries Tn*5* Km^r *Hin*dIII fragment insertion in *Hin*dIII site of pOX38 (2)
pOX38 *traU347*	45.0	*Hin*dIII (+ *kan* insert)			In vivo recombination, pKI347 and pOX38
pOX38 *traU348*	45.0	*Hin*dIII (+ *kan* insert)			In vivo recombination, pKI348 and pOX38

[a] In all pACYC177 and pBR322 derivatives listed, transcription from the vector *amp* promoter (or *kan* promoter for pKI175) proceeds into the *tra* DNA inserts in the *tra* operon direction (left to right in Fig. 1). The Tn*903 kan* gene was inserted as a 1.3-kb *Hin*cII fragment purified from pUC-4K (Pharmacia).
[b] In this construction, the *Sca*I-*Pvu*I segment of the fragment is from the pBR322 *bla* gene and restores the *bla* sequence removed from the pACYC177 vector.

tion mixtures were analyzed, and both DNA strands were sequenced.

Complementation experiments. For bacterial matings, donors and recipients were grown in LB medium (16) to a density corresponding to approximately 2×10^8 cells per ml, and a mixture of 0.1 ml of donor, 0.1 ml of fresh LB, and 0.2 ml of recipient was incubated for 45 min before dilutions were plated on selective plates. Phage sensitivities were determined by spot tests or plaque counts on LC plates (LB with 2.5 mM $CaCl_2$, 0.1% glucose, and 1% agar). Standard phage lysates (e.g., 7×10^{11} PFU of M13 per ml, 3×10^{10} PFU of R17 per ml) were used. For electron microscopic examination of F-piliation properties, derivatives of the Fim$^-$ strain VL584 were used. Fresh overnight cultures were diluted 10-fold, grown at 37°C in a shaking water bath to an OD_{620} of 1.0, and incubated statically for 30 min before being mounted on grids as described by Bradley (1).

RNA phage infection experiments. In all experiments, the number of plaque-forming units per milliliter present in infected cultures at the indicated times was determined by titering dilutions of 0.1-ml culture samples on an XK1200 (F *lac*) strain with H top agar (16) and LC plates; all cultures were grown in LC medium and aerated in a gyratory shaking water bath at 37°C. Initially, approximately 10^8 f2 phage (in 0.1 ml of LC) were added to a 300-ml flask containing 10 ml of an F *lac* or F *lac traU526* culture grown to a density of about 10^8 cells per ml to give a multiplicity of infection of about 0.1. Phages in these cultures were counted immediately (time zero) and after 30, 40, and 70 min. At 30 min, a 1-ml sample of each culture was also added to a tube containing 0.1 ml of anti-f2 serum, incubated for a further 10 min, diluted 10^4-fold in LC, and counted immediately to determine the number of infectious centers present. Controls were performed to ensure that the concentration of antiserum used was appropriate for inactivation of free f2 particles present during the 10-min incubation period but insufficient to inactivate phages released after the 10^4-fold dilution. To determine the effect of RNase, a 1-ml sample of the phage-infected culture was also added to a tube containing 40 μl of RNase solution (1 μg of RNase A per ml; Sigma Chemical Co.) immediately after addition of the phages (within 20 s of time zero). After further incubation, phages in this mixture were also counted at the indicated times.

Detection and localization of plasmid protein products. Plasmids were introduced into strain SE5000 by transformation. Maxicell cultures were prepared and labeled with [^{35}S]methionine as described previously (19). When indicated, ethanol (final concentration, 10%) was added prior to addition of the radioactive label. The procedures for analysis of samples by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography have been described elsewhere (10, 18, 19). Gels were poured with an exponential gradient of 11 to 15% acrylamide and were run in a cooled chamber at 10 W constant power with a model 221 or Protean II gel apparatus (Bio-Rad Laboratories).

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been submitted to GenBank/EMBL (accession no. M34695).

RESULTS

In order to locate the region of the F *tra* operon that encodes *traU*, we first performed complementation tests with the series of plasmids carrying cloned *tra* segments described by Wu et al. (25). Plasmids pKI175, pKI182, pKI183, and pKI184 were individually transformed into an XK1200 (F *lac traU526*) donor, and each derivative was tested for conjugative transfer. As shown in Table 2, the

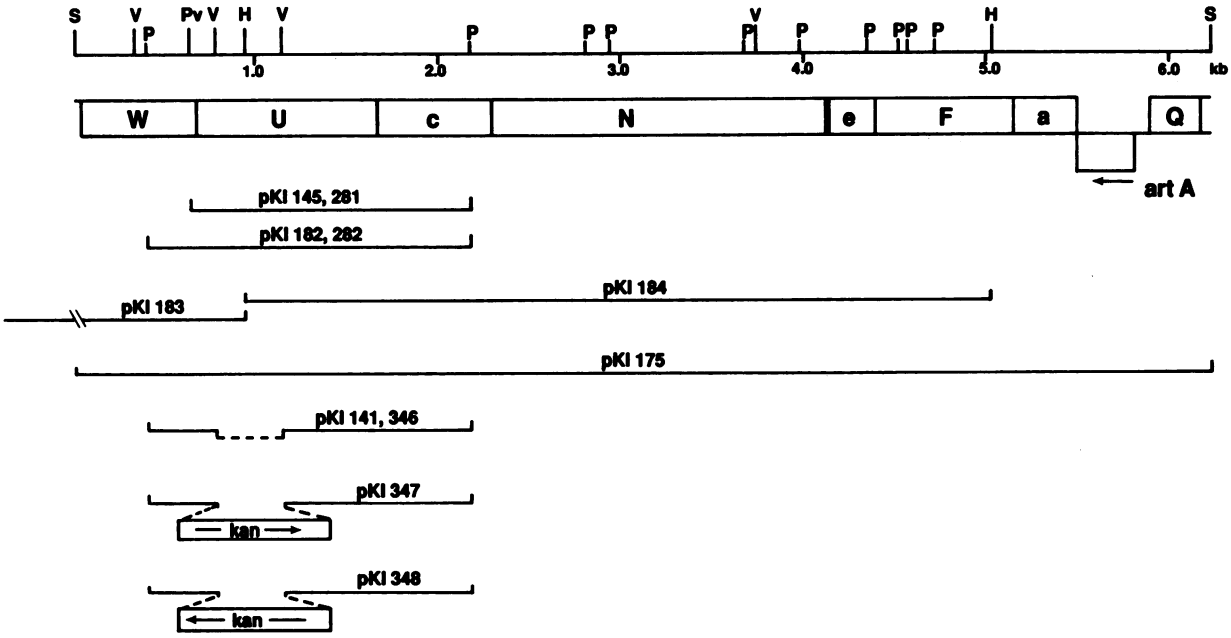

FIG. 1. Map of F transfer genes within the 6.2-kb *Sma*I fragment carried by pKI175. Boxes indicate the position and size of genes within the region. Numbered lines below the map indicate DNA segments carried by various pKI plasmids; a dotted line indicates a deletion, and slanted parallel lines indicate that the segment extends beyond the *tra* region shown. The location and orientation of kanamycin gene insertions are also indicated. At the top of the map, numbers indicate the length in kilobases; letters indicate positions of restriction sites for *Eco*RV (V), *Hin*cII (H), *Pst*I (P), *Pvu*I (Pv), and *Sma*I (S).

level of F *lac traU526* transfer obtained from control strains was approximately 0.2% of the wild-type F *lac* transfer frequency. Neither pKI183 nor pKI184 enhanced F *lac traU526* transfer, but plasmids pKI175 and pKI182 complemented the *traU* mutation. Introduction of these plasmids raised the transfer frequency to normal levels and restored full sensitivity to F-pilus-specific bacteriophages. These results indicated that *traU* spanned the *Hin*cII site distal to *traW* (13) and was contained within the *Pst*I segment carried by pKI182 (Fig. 1).

Identification of the *traU* product. We examined [^{35}S]methionine-labeled proteins in maxicells carrying our plasmid derivatives. Initial studies with 22.5-cm gels suggested that the only product that the *tra* segments of both pKI182 and pKI175 expressed in common was a 20-kilodalton (kDa) band. However, when samples were separated with a 16.5-cm (Protean II) gel system, pKI182 products that comigrated with 33- and 34-kDa pKI175 products were also resolved from background bands expressed by the pACYC177 vector (Fig. 2A). To determine which polypeptide(s) corresponded to the *traU* product, we tested whether either the 20-kDa or 33-kDa polypeptide might derive from a fusion between the amino-terminal end of the vector *bla* gene sequence and the *tra Pst*I fragment in pKI182, by introducing a *Pvu*I deletion into this region of the sequence. For restriction site convenience, pKI282 (in which the same *tra* fragment carried by pKI182 was cloned in vector pBR322) was digested with *Pvu*I to obtain the deletion derivative pKI281. As shown in Fig. 2B, the 20-kDa polypeptide expressed by pKI182 and pKI282 was not synthesized by pKI281. Weak expression of a 33-kDa polypeptide was observed in all three samples, although the *tcy* gene product expressed by pBR322 comigrated with the 34-kDa pKI182 product, precluding detection of an additional 34-kDa band in pKI282 and pKI281 samples. Transfer tests demonstrated that the *Pvu*I deletion did not affect the capacity of pKI282 to provide *traU*-complementing activity (Table 2), and we concluded that the 20-kDa product expressed by pKI182 and pKI282 was not associated with *traU* activity. These results suggested that the 33-kDa band expressed by pKI182 was a *traU* product.

***traU* product processing.** To test whether the 34-kDa polypeptide was a *traU* product precursor to the 33-kDa protein, we examined the effect of ethanol on polypeptide expression. To avoid expression of the *tcy* product in these experiments, we first constructed pKI145, a pACYC177 derivative that carries the same *Pvu*I-*Pst*I *tra* segment remaining in pKI281. Like pKI281, pKI145 expressed *traU*-complementing activity (Table 2) and did not express the 20-kDa fusion polypeptide.

TABLE 2. F *lac traU526* complementation analysis

Donor strain	Additional plasmid	No. of transconjugants/100 donors[a]	Phage sensitivity[b]	
			f1, M13, fd	Qβ, f2, R17
XK1200 (F *lac traU526*)	None	0.5	(R)	(R)
	pACYC177	0.2	(R)	(R)
	pKI145	209.0	S	S
	pKI175	96.0	S	S
	pKI182	190.0	S	S
	pKI183	0.4	(R)	(R)
	pKI184	0.4	(R)	(R)
	pKI281	133.0	S	S
	pKI282	228.0	S	S
XK1200 (F *lac*)	None	240.0	S	S

[a] Lac$^+$ Sm^r transconjugants of the recipient JC3051 were selected.
[b] (R) indicates incomplete resistance; plaques are not formed, but limited lysis is detectable in spot tests. S indicates plating efficiency comparable to that of the F *lac* control.

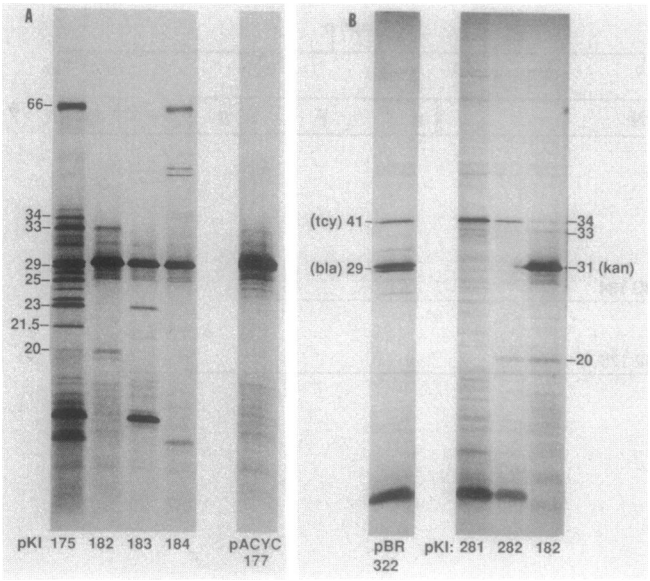

FIG. 2. Analysis of [^{35}S]methionine-labeled polypeptides expressed in maxicells carrying various plasmids. Selected lanes of the autoradiograph of a sodium dodecyl sulfate-polyacrylamide gel are shown. (A) Comparison of samples from cells carrying TraU$^+$ plasmids (pKI175 and pKI182), TraU$^-$ plasmids (pKI183 and pKI184), or vector pACYC177 as indicated below each lane. Numbers at the left indicate the position and size (in kilodaltons) of pKI175 products. (B) As in panel A, except that samples were from cells carrying the TraU$^+$ plasmids pKI281, pKI282, and pKI182 or vector pBR322, as labeled. The positions and sizes of vector *tcy*, *bla*, and *kan* gene products and the positions of the 34-, 33-, and 20-kDa plasmid products are also indicated.

In the presence of ethanol, the 34-kDa polypeptide accumulated in maxicells carrying either pKI145 (Fig. 3, lane 6) or pKI175 (lane 3). Both the 33-kDa and 34-kDa polypeptides could be detected in cells labeled in the absence of ethanol (lanes 4 and 5) or when a sample which had been labeled for 30 min in the presence of ethanol was incubated for a further 30 min in the absence of this inhibitor (lane 2). These results indicated that the 34-kDa polypeptide is a *traU* product precursor and that the 33-kDa polypeptide is generated after signal sequence processing.

The origin of the additional band just above the 34-kDa polypeptide that also appeared to accumulate in ethanol-treated pKI175 samples (Fig. 3, lane 3) was unclear. Since the poor labeling efficiency of *traU* products obtainable with pKI145 and pKI182 may have precluded detection of an analogous band in these samples, it might also stem from *traU*. Possibly, an alternative conformation of the pKI175 34-kDa product is resolved on our gels, or the *traU* product might undergo more than one processing or modification step.

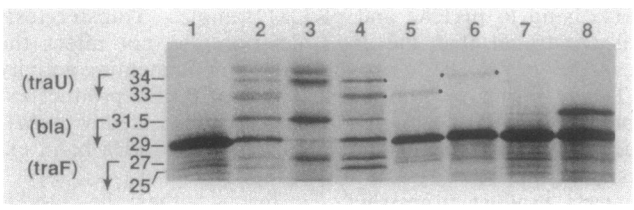

FIG. 3. Effect of ethanol on the [^{35}S]methionine-labeled polypeptides synthesized in maxicells carrying TraU$^+$ plasmids. Cultures carrying pKI175 (lanes 2 to 4), pKI145 (lanes 5 and 6), or the pACYC177 vector (lanes 1, 7, and 8) were labeled without (lanes 1, 4, 5, and 7), or with ethanol (lanes 3, 6, and 8) or with ethanol followed by a 30-min chase without ethanol (lane 2). The size (in kilodaltons) and position of the products of *traU*, *bla*, and *traF* are indicated at the left. Arrows indicate precursor-to-product relationships. Black dots mark *traU* bands in lanes 4, 5, and 6.

Examination of labeled pKI182 products in maxicells sampled at intervals (Fig. 4A) showed that the 33-kDa polypeptide band gradually increased in intensity during a 60-min labeling period. In contrast, the 34-kDa product was most prominent in samples labeled for 5, 10, or 20 min and diminished in intensity after longer labeling periods. A pulse-chase experiment (Fig. 4B) also confirmed that the 33-kDa polypeptide was derived from a higher-molecular-weight precursor. The 34-kDa pKI175 product was visible in samples labeled for only 2 or 5 min (lanes 1 and 2). When samples that had been labeled for 5 min were incubated in an excess of nonradioactive methionine for an additional 10, 30, or 50 min (lanes 3 to 5), the 33-kDa product became increasingly visible and the 34-kDa polypeptide band diminished in intensity.

Nucleotide sequence of *traU*. We also determined the nucleotide sequence of the *traU* region. As shown in Fig. 5, the *traU* segment was found to encode a 330-amino-acid, M_r 36,786 polypeptide. Immediately following three positive charges (Lys-2–Arg-3–Arg-4), there was a typical hydrophobic signal sequence segment in the amino-terminal region of this protein, as expected from our evidence for *traU* product processing. Several additional regions of hydrophobic character also occurred in the portion of the TraU amino acid sequence that was proximal to Glu-244; although charged residues were distributed throughout the molecule, the majority were located in the regions Lys-114 to Pro-140, Glu-155 to Asp-173, and the hydrophilic C-terminal segment that extends from Glu-244 to Arg-325.

Characterization of *traU526*. Miki et al. (15) classified *traU*

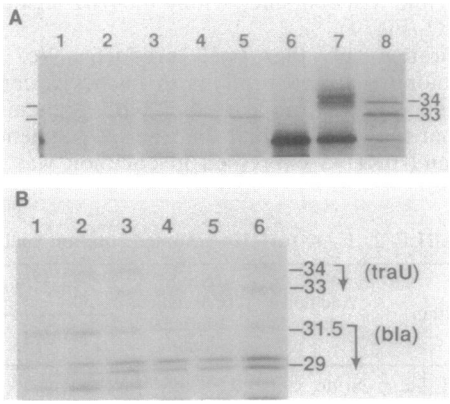

FIG. 4. Expression of *traU* products. (A) Maxicells carrying pKI182 were sampled after labeling for 5 (lane 1), 10 (lane 2), 20 (lane 3), 30 (lane 4), or 60 (lane 5) min. Reference samples carried pKI175, labeled for 60 min with (lane 7) or without (lane 8) ethanol, or pACYC177 labeled for 60 min with ethanol (lane 6). Only a portion of the autoradiograph is shown. The positions of the 34- and 33-kDa products are indicated at the right. (B) Maxicells carrying pKI175 were labeled for 2 min (lane 1), 5 min (lane 2), or 60 min (lane 6). Alternatively, after 5 min to incorporate label, excess unlabeled methionine was added and samples were removed after 10 min (lane 3), 30 min (lane 4), or 50 min (lane 5). The positions of the 34- and 33-kDa *traU* products and the 31.5- and 29-kDa *bla* products are indicated.

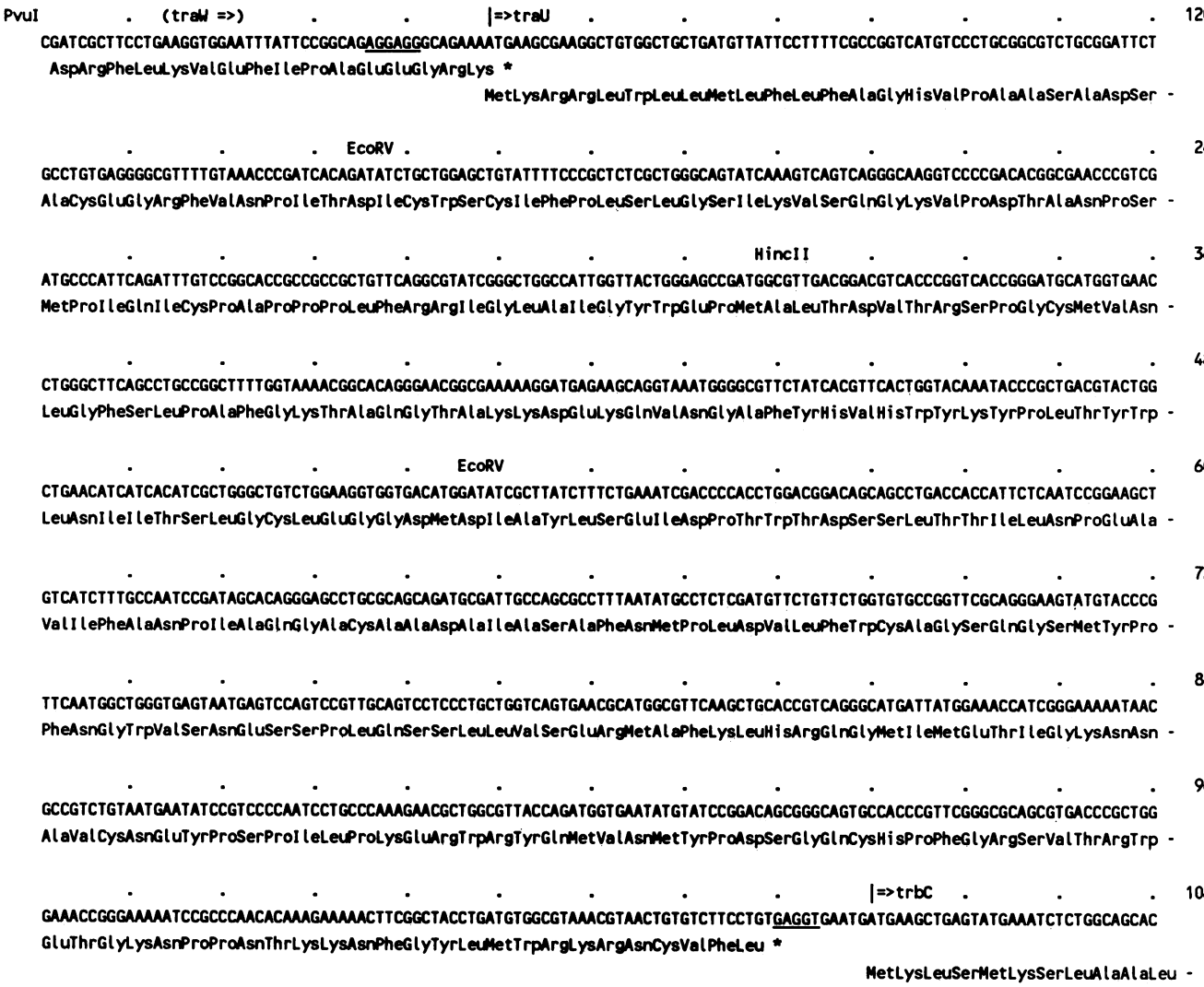

FIG. 5. Nucleotide sequence of *traU*. The translation product of the 330-amino-acid *traU* open reading frame is shown, as are amino acids encoded by the 3′ end of *traW* and the 5′ end of *trbC*. Potential ribosome-binding sequences are underlined, and the positions of *Pvu*I, *Eco*RV, and *Hinc*II restriction sites are indicated. *, Stop codon for *traW* or *traU*.

as a pilus assembly gene on the basis of the resistance to F-pilus-specific phages conferred by *traU526*. However, we observed that our *traU526* derivatives did exhibit some sensitivity in phage spot tests (Table 2). This same degree of sensitivity was exhibited by F *lac traU526* derivatives constructed in several host backgrounds (strains XK5456, JC3272, and VL584 were tested). Clearing was faint, particularly in RNA phage spots, but lysis was usually discernible, especially when log-phage cultures and fresh plates were used. Although none of the phages tested formed plaques on *traU526* strains, our results suggested that such cultures might contain cells which produce F-pili. We therefore examined VL584 (F *lac traU526*) cells by transmission electron microscopy.

It was clear from this analysis that F *lac traU526* strains do express a substantial number of F-pili (Table 3). Both the percentage of piliated cells and the average number of pili per cell approximated 50% of the values obtained for the F *lac* control. Like F *lac* pili, F *lac traU526* pili also disappeared after exposure to 56°C, indicating that disassembly of the filaments could occur under these conditions. Anti-F-pilus serum also appeared to adsorb normally to *traU526* pili.

The effect of *traU526* on the efficiency with which male-specific phages adsorbed and infected was more difficult to interpret; the relatively small reduction in the number of pili produced seemed unlikely to account for the substantial reduction in phage sensitivity exhibited by the mutant strain (Table 2). Electron microscopy with adsorbed RNA phages showed that at least some *traU526* pili bore typical clumps of virions, although incubation with phage Qβ offered little interference with heat-induced pilus retraction (Table 3). This result suggests that most of the virions observed were reversibly adsorbed and unable to prevent retraction. That lysis could be observed on spot tests clearly indicated, however, that at least some irreversible adsorption and successful infection did occur; no RNA phage lysis was observed when RNase was added to the plates.

Table 4 summarizes the results of a series of experiments in which f2 infection of strains carrying either F *lac* or F *lac traU526* was monitored. After addition of approximately 10^7 PFU of f2 to a culture containing approximately 10^8 cells of

TABLE 3. Electron microscopic characterization of F *lac traU526* derivatives[a]

Plasmid(s) present	Heat treatment	% Piliated cells[b]	Avg no. of pili/cell[b]
Before incubation with Qβ			
F *lac*	−	80	1.74
	+	26	0.38
F *lac traU526*	−	42	0.78
	+	0	0
F *lac traU526* + pKI182	−	74	0.96
After incubation with Qβ			
F *lac*	−	46	0.63
	+	30	0.37
F *lac traU526*	−	50[c]	0.9[c]
	+	6.7	0.1

[a] All strains examined were derivatives of the bald strain VL584.
[b] A total of 50 cells were examined, except for experiments with Qβ, in which a total of 30 cells were examined.
[c] Fifteen cells had no visible pili; 15 had 27 pili, all of which appeared to be free of adsorbed phages. However, during examination of additional cells, pili carrying groups of attached phages were found.

TABLE 4. Bacteriophage f2 infection of F *lac* and F *lac traU526* strains

Strain	Time after f2 addition[a] (min)	PFU ratio[b]	
		−RNase	+RNase
XK1200 (F *lac*)	0	1.0	
	30	2.1	0.05
	40	2.4 (2.1)	
	70	1.1×10^3	
XK1200 (F *lac traU526*)	0	1.0	
	30	1.2	0.54
	40	1.4 (0.75)	0.40
	70	2.6×10^2	0.31
XK1200 (F^-)	0	1.0	
	30	1.01	1.07
	40	1.01 (<0.001)	
	70	1.09	1.02

[a] Time period between addition of f2 (0 min) and determination of PFU.
[b] Ratio determined as PFU per milliliter at indicated time after addition of f2 divided by the PFU of f2 per milliliter determined immediately after addition of bacteriophage to the culture (0 min; multiplicity of infection, approximately 0.1). Values are averages of values determined in five separate experiments. +RNase, Culture samples were mixed with RNase A immediately after infection. Numbers in parenthesis are number of infectious centers per added PFU: at 30 min after addition of phages, a sample of the infected culture was mixed with anti-f2 serum; 10 min later, it was diluted, and infectious centers were counted.

the F *lac* control strain per ml, the number of PFU per milliliter had doubled after 30 min of incubation at 37°C, presumably reflecting the doubling of infectious centers during this growth period. Indeed, infected cells accounted for at least 90% of the total PFU obtained from the cultures, since the number of PFU was not markedly reduced in samples incubated with anti-f2 serum during the period from 30 to 40 min postinfection.

In contrast, cell infection proceeded much more slowly in the F *lac traU526* culture. The increase in PFU caused by multiplication of infected cells was less marked, and only about 50% of the total PFU survived the incubation with anti-f2 serum. The results of experiments performed in the presence of RNase were consistent with this finding. In this case, the rate of decrease in PFU reflects the rate at which infectious phages enter the RNase-sensitive RNA ejection stage that occurs prior to RNA penetration into the cell (20). In the F *lac* control culture, only 5% of the initial PFU could be recovered after a 30-min incubation with RNase. In the F *lac traU526* culture, the decrease in PFU was more gradual. After a 30-min incubation with RNase, about 50% of the initial PFU could still be recovered, and even after 70 min of incubation with F *lac traU526*, 30% of the infectious phages present had, apparently, not entered the RNase-sensitive stage. Similar RNase inactivation results were also obtained with R17 (data not shown).

Construction of additional *traU* mutants. As it was possible that both the piliation and leaky transfer levels of F *lac traU526* amber mutant strains reflected a residual activity attributable to a *traU526* polypeptide product, we constructed additional *traU* mutants to test the contribution of *traU* to these phenotypes. We used an approach that has been successful for construction of other *tra* operon mutant derivatives (Kathir et al., unpublished data; Maneewannakul et al., unpublished data). In this case, we first constructed plasmids pKI141 and pKI346 by removing a 372-nucleotide *Eco*RV fragment (nucleotides 159 to 530 in Fig. 5) from the *traU* sequence. We then replaced this with a 1,282-nucleotide pUC-4K *Hinc*II fragment that encodes and expresses the Tn*903* kanamycin resistance gene. We selected one derivative (pKI347) in which the *kan* gene insert was oriented and expressed from its own promoter in the same direction as *traU*, and another (pKI348) in which the *kan* orientation was opposite to that of *traU* (Fig. 1). These insertion mutations were then crossed by in vivo recombination onto pOX38. The pOX38 plasmid was made by self-ligation of the large *Hin*dIII fragment of F (5) and carries the entire F *tra* region as well as the IncFIA replicon. To obtain mutant pOX38 recombinants, a Nal^s donor strain carrying both pOX38 and pKI347 was mated with strain XK1200, and Kan^r Nal^r Tet^s transconjugants were examined. All of these exhibited a transfer-deficient phenotype. Purification and restriction enzyme analysis of plasmid DNA purified from one transconjugant confirmed that it carried a pOX38 plasmid derivative (pOX38 *traU347*) that contained the same *traU* region *kan* insertion we had constructed in pKI347. Plasmid pOX38 *traU348*, which carries *kan* in the opposite orientation (so that transcription of *kan* opposes transcription of *tra*), was obtained in an analogous fashion by recombination with pKI348.

The transfer-related properties of these derivatives are shown in Table 5. Both mutant plasmids were much more transfer deficient than the *traU526* amber mutant. Neither the pOX38 *traU347* nor the pOX38 *traU348* plasmid transferred at a frequency detectable in our standard mating test (Table 5). When larger quantities of culture were plated after mating on filters for 1 h, a few transconjugants were detected, but transfer levels were still only 3.7×10^{-8} transconjugants per pOX38 *traU347* donor per h and 5.3×10^{-9} transconjugants per pOX38 *traU348* donor per h by this method. Like F *traU526*, however, pOX38 *traU347* and *pOX38 traU348* rendered their host slightly sensitive to pilus-specific phages in spot tests. Electron microscopy showed that strains carrying either of the pOX38 *traU* mutant derivatives were still capable of F-pilus expression, although the number of pili produced was definitely reduced in comparison with wild-type numbers (Table 5). The transfer frequency, phage sensitivity, and piliation properties of the pOX38 *traU347* and pOX38 *traU348* mutant strains were all restored to levels approximating that of the wild-type strain by introduction of plasmid pKI281. Since the only intact *tra* gene carried by pKI281 is *traU*, these complementation results indicated that the mutant phenotype of pOX38

TABLE 5. Analysis of pOX38 *traU* mutants

Plasmid(s) present	No. of transconjugants/ 100 donors[a]	Phage sensitivity[b]		% Piliated cells[c]	Avg no. of pili/ cell[c]
		f1, M13, fd	Qβ, f2, R17		
pOX38-Km[d] (wild type)	136	S	S	76	1.20
pOX38 *traU347*	<0.0004	(R)	(R)	14	0.18
pOX38 *traU348*	<0.0004	(R)	(R)	22	0.24
pOX38 *traU347* +:					
pACYC177	<0.0004	(R)	(R)		
pKI175	221	S	S		
pKI281	160	S	S	52	0.84
pOX38 *traU348* +:					
pACYC177	<0.0003	(R)	(R)		
pKI175	125	S	S		
pKI281	133	S	S	50	0.72

[a] Donors were XK1200 derivatives; Km^r Sm^r transconjugants of the recipient JC3051 were selected.
[b] See Table 2, footnote *b*.
[c] Derivatives of strain VL584 were used; a total of 50 cells were examined.
[d] pOX38-Km contains the normal F *tra* operon sequence. In this plasmid, a *kan* gene inserted into the pOX38 *Hin*dIII site provides a selectable marker.

traU347 and pOX38 *traU348* is entirely a consequence of *traU* deficiency. Thus, neither insertion mutation appeared to exert any significant effect on expression of other *tra* operon genes.

DISCUSSION

Our results demonstrate that the F *tra* operon gene *traU* encodes a 330-amino-acid, M_r 36,786 polypeptide which undergoes signal sequence processing. The *traU* precursor and processed products were identified as 34-kDa and 33-kDa polypeptides labeled in maxicells carrying $TraU^+$ plasmids. Wu et al. (24) have previously reported cell fractionation experiments which show that, like TraF, the 33-kDa polypeptide expressed by the $TraU^+$ plasmid pKI175 is detected in periplasmic preparations. This study, together with similar fractionation experiments (data not shown) and the amino acid sequence encoded by *traU*, suggests that the mature TraU protein is located in the periplasm. It seems likely that TraU is part of a periplasmic complex containing other proteins encoded by the F *tra* region, since the *traF*, *traH*, and *trbB* products also appear to be periplasmic proteins (6, 23, 24). The several hydrophobic amino acid segments in TraU might also sustain TraU association with host- or F-encoded membrane components.

The actual amino-terminal sequence of mature TraU protein remains to be determined biochemically. Signal peptidase I removal of the amino terminus would seem likely to occur by cleavage between Ala-22 and Asp-23, giving rise to an M_r 34,275 *traU* product. It is worth noting, however, that the TraU sequence Ser-24–Ala-25–Cys-26 also resembles the signal peptidase II recognition sequences of some known lipoproteins (7). While lipoprotein processing would be expected to result in a membrane-associated product (26), we do not exclude the possibility that lipid modification of TraU can occur or that the *traU* product might undergo more than one form of processing. For example, Hayashi et al. (7) have reported that only a small fraction of penicillin-binding protein 3 becomes lipid modified during processing.

Both the size of the *traU* product we have identified in maxicells and the size of the *traU* nucleotide sequence are in reasonable agreement with the 38-kDa TraU product size first suggested by lambda transducing phage product analyses (10–12). Recent *tra* operon maps (9, 22), however, were based on a 20-kDa product size, since the preliminary data we obtained through comparison of pKI182 and pKI175 maxicell products were misleading. Reconciliation of other *tra* product data with the accommodation of a 990-base-pair *traU* gene has resulted in a revision of this segment of the map that has recently been confirmed by DNA sequencing. As shown in Fig. 1, *traN* crosses a more distal *Eco*RV site, and a single gene (*trbC*) is responsible for two polypeptide products (originally named TrbD and TrbC) now known to have a precursor-product relationship (S. Maneewannakul, K. Maneewannakul, and D. Moore, unpublished data).

In the DNA sequence we determined, the methionine start codon for *traU* is part of an ATGA sequence that also includes the stop codon for *traW* translation. This overlapping configuration appears to be typical of many *tra* gene sequences and indicates that translation of *traU* is coupled to translation of *traW*. Lack of translational continuity could explain the poor *traU* protein expression levels we observed in maxicells carrying pKI182, pKI145, and other plasmids of similar construction. In contrast, the *tra* operon *Sma*I fragment inserted into the vector *kan* gene in pKI175 places a stop codon for the *kan* reading frame immediately upstream of the translational start sequence for *traW*, and the products of *traW*, *traU*, and the more distal *tra* genes carried by pKI175 appear to be expressed quite well in maxicells.

Although Miki et al. (15) categorized *traU* as an F-pilus assembly gene, our analysis of *traU* mutant strains indicates that an intact TraU protein is essential for DNA transfer but not completely essential for the elaboration of F-pilus filaments. In construction of the mutations *traU347* and *traU348*, we disrupted *traU* by deletion of 372 nucleotides from the gene sequence and insertion of a kanamycin resistance gene at the deletion site. Unlike *traU526*, these mutations had a drastic effect on the transfer frequencies of F derivatives determined in either broth or filter matings. This demonstrated an almost absolute requirement for *traU* in F conjugation. However, the effect of F-pilus synthesis was much less dramatic, and the number of pili expressed by *traU347* and *traU348* strains remained approximately 20% of the wild-type level. It was also of interest that insertion of the *kan* gene and its promoter into *traU* did not appear to significantly disrupt expression of other *tra* operon genes. Even in the case of *traU348*, in which the *kan* promoter and gene are oriented opposite to the direction of *tra* transcription, a coresident plasmid carrying only *traU* could complement the mutation and restore transfer and phage sensitivity properties.

Although the 80% reduction in F-piliation might suffice to explain the inability of *traU347* and *traU348* strains to plaque both RNA and DNA pilus-specific phages, the similar degree of phage resistance exhibited by hosts carrying the F *lac traU526* amber mutation suggests that *traU* mutants may be defective in another aspect of phage infection. Under the conditions used for electron microscopy, pili were produced from the *traU526* strain at about 50% of the wild-type level, in numbers equivalent to those expressed by other derivatives that plated the phages with wild-type efficiency. We did not detect any obvious abnormality in the structure of these pili. F *lac traU526* pilus filaments were observed to react with F-pilus antiserum; pilin subunits in the membranes of all of the *traU* mutant strains we constructed were also found to react normally with JEL93, a monoclonal antibody specific for the acetylated pilin amino terminus (4; C. Hamilton, unpublished data). F *lac traU526* pili were also capable of adsorbing and effecting release of f2 and R17 RNA.

However, the rate at which RNA phages reached the RNase-sensitive ejection stage was considerably slower in F *lac traU526* cultures. Although our methods did not quantitatively assess F-pilus-specific phage adsorption, the number of pili observed, together with the fact that both RNA and DNA phage infections were impeded by *traU* mutations, suggests that TraU function might be required at a postadsorption stage. While we found that *traU526* pili did retract in response to heat treatment, it is still possible that abnormal F-pilin assembly and disassembly reactions reduced the rate at which phages reached the cell surface in these strains. Alternatively, release and transport of phage nucleic acid through the cell envelope might be hindered by the absence of TraU.

F *lac traU* mutants were not included in earlier investigations that tested the capacity of piliate transfer-deficient strains to form stable mating aggregates (14) or to synthesize replacement strand DNA in response to the mating signal (8). Despite their relatively resistant pilus-specific phage profile, it is now clear that *traU* mutants are also piliate. It will therefore be of significant interest to determine the properties of *traU* mutants with respect to these mating functions.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI14426 (to K.I.-I.) from the National Institutes of Health and grant MT5608 (to D.E.B.) from the Medical Research Council of Canada.

LITERATURE CITED

1. **Bradley, D. E.** 1989. Conjugation system of IncC plasmid RA1, and interaction of RA1 pili with specific RNA phage C-1. Res. Microbiol. **140**:439–446.
2. **Chandler, M., and D. Galas.** 1983. Cointegrate formation mediated by Tn*9*. II. Activity of IS*1* is modulated by external DNA sequences. J. Mol. Biol. **170**:61–91.
3. **Frost, L., W. Paranchych, and N. Willetts.** 1984. DNA sequence of the F *traALE* region that includes the gene for F pilin. J. Bacteriol. **160**:395–401.
4. **Frost, L. S., J. S. Lee, D. G. Scraba, and W. Paranchych.** 1986. Two monoclonal antibodies specific for different epitopes within the amino-terminal region of F pilin. J. Bacteriol. **168**:192–198.
5. **Guyer, M. S., R. R. Reed, J. A. Steitz, and K. B. Low.** 1980. Identification of a sex-factor affinity site in *E. coli* as γδ. Cold Spring Harbor Symp. Quant. Biol. **45**:135–140.
6. **Ham, L. M., N. Firth, and R. Skurray.** 1989. Nucleotide sequence of the F plasmid transfer gene, *traH*: identification of a new gene and a promoter within the transfer operon. Gene **75**:157–165.
7. **Hayashi, S., H. Hara, H. Suzuki, and Y. Hirota.** 1988. Lipid modification of *Escherichia coli* penicillin-binding protein 3. J. Bacteriol. **170**:5392–5395.
8. **Kingsman, A., and N. Willetts.** 1978. The requirements for conjugal DNA synthesis in the donor strain during F *lac* transfer. J. Mol. Biol. **122**:287–300.
9. **Ippen-Ihler, K. A., and E. G. Minkley, Jr.** 1986. The conjugation system of F, the fertility factor of *Escherichia coli*. Annu. Rev. Genet. **20**:593–624.
10. **Ippen-Ihler, K. A., D. Moore, S. Laine, D. A. Johnson, and N. Willetts.** 1984. Synthesis of F-pilin polypeptide in the absence of F *traJ* product. Plasmid **11**:116–129.
11. **Johnson, D. A., and N. S. Willetts.** 1983. Lambda transducing phages carrying transfer genes isolated from an abnormal prophage insertion into the *traY* gene of F. Plasmid **9**:71–85.
12. **Laine, S., D. Moore, P. Kathir, and K. Ippen-Ihler.** 1985. Genes and gene products involved in the synthesis of F-pili, p. 535–553. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
13. **Maneewannakul, S., P. Kathir, D. Moore, L.-A. Le, J. H. Wu, and K. Ippen-Ihler.** 1987. Location of F plasmid transfer operon genes *traC* and *traW* and identification of the *traW* product. J. Bacteriol. **169**:5119–5124.
14. **Manning, P. A., G. Morelli, and M. Achtman.** 1981. *traG* protein of the F sex factor of *Escherichia coli* K-12 and its role in conjugation. Proc. Natl. Acad. Sci. USA **78**:7487–7491.
15. **Miki, T., T. Horiuchi, and N. S. Willetts.** 1978. Identification and characterization of four new *tra* cistrons on the *E. coli* K12 sex factor F. Plasmid **1**:316–323.
16. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Moore, D., B. A. Sowa, and K. Ippen-Ihler.** 1981. The effect of *tra* mutations on the synthesis of the F-pilin membrane polypeptide. Mol. Gen. Genet. **184**:260–264.
18. **Moore, D., B. A. Sowa, and K. Ippen-Ihler.** 1982. A new activity in the F *tra* operon which is required for F-pilin synthesis. Mol. Gen. Genet. **188**:459–464.
19. **Moore, D., J. H. Wu, P. Kathir, C. M. Hamilton, and K. Ippen-Ihler.** 1987. Analysis of transfer genes and gene products within the *traB-traC* region of the *E. coli* fertility factor, F. J. Bacteriol. **169**:3994–4002.
20. **Paranchych, W.** 1975. Attachment, ejection and penetration stages of the RNA phage infectious process, p. 85–111. *In* N. D. Zinder (ed.), RNA phages. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Schandel, K. A., S. Maneewannakul, K. Ippen-Ihler, and R. E. Webster.** 1987. A *traC* mutant that retains sensitivity to f1 bacteriophage but lacks F-pili. J. Bacteriol. **169**:3151–3159.
22. **Willetts, N., and R. Skurray.** 1987. Structure and function of the F factor and mechanism of conjugation, p. 1110–1133. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
23. **Wu, J. H., and K. Ippen-Ihler.** 1989. Nucleotide sequence of *traQ* and adjacent loci in the *Escherichia coli* K-12 F-plasmid transfer operon. J. Bacteriol. **171**:213–221.
24. **Wu, J. H., P. Kathir, and K. Ippen-Ihler.** 1988. The product of the F-plasmid transfer operon gene, *traF*, is a periplasmic protein. J. Bacteriol. **170**:3633–3639.
25. **Wu, J. H., D. Moore, T. Lee, and K. Ippen-Ihler.** 1987. Analysis of *E. coli* K12 F factor transfer genes: *traQ*, *trbA* and *trbB*. Plasmid **18**:54–69.
26. **Yamaguchi, K., Y. Fujio, and M. Inouye.** 1988. A single amino acid determinant of the membrane localization of lipoproteins in *E. coli*. Cell **53**:423–432.