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Analysis of Transfer Genes and Gene Products within the *traB-traC* Region of the *Escherichia coli* Fertility Factor, F

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A series of plasmids that carry overlapping segments of F DNA encoding the genes in the *traB-traC* interval was constructed, and a restriction enzyme map of the region was derived. Plasmids carrying deletions that had been introduced at an *HpaI* site within this interval were also isolated. The ability of these plasmids to complement transfer of F *lac* plasmids carrying mutations in *traB*, *traV*, *traW*, and *traC* was analyzed. The protein products of the plasmids were labeled in UV-irradiated cells and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. These analyses showed that the product of *traV* is a polypeptide that migrates with an apparent molecular weight of 21,000. It was not detected when [^{35}S]methionine was used to label plasmid products, but was readily detected in ^{14}C-amino acid labeling experiments. A 21,500-dalton product appeared to stem from the region assigned to *traP*. A 9,000-dalton product was found to stem from a locus, named *traR*, that is located between *traV* and *traC*. No *traW* activity could be detected from the region of *tra* DNA examined. Our data also indicated that *traC* is located in a more promoter-proximal position than suggested on earlier maps. The plasmids constructed are expected to be useful in studies designed to identify the specific functions of the *traB*, *-P*, *-V*, *-R*, and *-C* products.

The genes required for conjugative transfer mediated by the *Escherichia coli* K-12 fertility factor are clustered over a 33.3-kilobase (kb) region of the F plasmid. The characterization of F transfer-deficient mutants and F transfer-associated activities has led to the identification of 20 gene activities from the transfer (*tra*) region that are required for F transfer functions; two additional *tra*-region genes confer F surface exclusion properties. Although a number of the products associated with these genes have been identified (see reference 31 for a summary), the products associated with others remained elusive. Furthermore, analysis of protein products produced by bacteriophages or plasmids carrying segments of *tra* DNA has suggested that additional *tra*-region genes are also present (11, 15, 21, 26). Most of these appear to lie in the less well characterized portions of the *tra* operon that lie in the *traB-traH* interval. To obtain more precise information about these genes and products, we constructed a restriction map of the region and characterized a series of plasmid derivatives that carry overlapping segments of *tra* DNA. We report here the results of our analysis of the *tra* region between *traB* and *traC*. The experiments presented identify the products and positions of three genes that lie between *traB* and *traC* and suggest relocation of the *traC* gene to a more promoter-proximal position. These data, together with additional data that will be presented separately, provide a *tra*-region map that contains considerable new detail (10, 32).

MATERIALS AND METHODS

Bacterial strains and plasmids. The basic bacterial strains used in this study are described in Table 1. Additional plasmids were introduced by transformation. For construction of these derivatives, numerous samples of a single competent culture of each strain were frozen, and these individual aliquots were used as required. Plasmids pBE269 and pSH1 were obtained from M. Achtman; pRS27 and pRS29 were from R. Skurray. These and all plasmids constructed for this study are described in Table 2. Plasmid vectors used were pBR322 (24) and pACYC177 (4).

The EDλ transducing phages were obtained from N. Willetts. F *tra* segments carried by the phages are: *tra(Y)ALE* (EDλ101); *tra(Y)ALEK* (EDλ126); *tra(Y) ALEKBV* (EDλ128); *tra(Y)ALEKBVCW* (EDλ129); *tra(Y)ALEK BVCWUNFQH(G)* (EDλ134); *tra(B)VCW* (EDλ90 and EDλ91); and *tra(B)VCWUNFQ(H)* (EDλ92) as described by Johnson and Willetts (13) and Willetts and McIntire (30). The parental phage is EDλ4. EDλ143 and EDλ144 are derivatives of EDλ91 that carry the *traV569* and *traW546* amber mutations, respectively; these were also obtained from N. Willetts.

Restriction mapping and cloning. Plasmid DNA was purified by ethidium bromide-cesium chloride centrifugation by the procedure of Davis et al. (6). Restriction enzyme digestion and other DNA manipulations were done by procedures described by Maniatis et al. (16). DNA fragment sizes were calculated by comparison with the positions of λ *c*I857 *Pst*I fragments on 0.5, 1.0, or 1.4% agarose or on 3 or 6% polyacrylamide gels, as appropriate.

Identification of phage and plasmid products. EDλ transducing phage lysates were prepared and tested for appropriate complementing activity as described by Willetts and McIntire (30). Transducing phage products were labeled with [^{35}S]methionine after infection of a UV-irradiated culture of *E. coli* XK1800 as described previously (11, 20). The same procedure was used for ^{14}C labeling of phage products, except that the maltose minimal medium used to grow XK1800 contained only required amino acids and the cells were starved of all amino acids for 1 h before UV irradiation. After infection, a total of 10 μCi of ^{14}C-amino acid mixture was added to 0.5 ml of infected cell culture.

All plasmids were transformed into *E. coli* SE5000, and plasmid products were labeled in this strain by a slight modification of the maxi-cell procedure of Sancar et al. (22). A 10-ml culture of SE5000 containing the plasmid to be tested was grown to an optical density at 550 nm of 0.4 in a

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TABLE 1. Bacterial strains

Strain	Genotype	Source (reference)
ED2149	F⁻ *lacΔU124* Δ(*nadA gal attλ bio*)	N. Willetts (30)
EM1200	F *lac*/XK1200	This laboratory[a]
EM1202	F *lac traB2*/XK1200	This laboratory[a]
EM1205	F *lac traC5*/XK1200	This laboratory[a]
EM12546	F *lac traW546*/XK1200	This laboratory[a]
EM12569	F *lac traV569*/XK1200	This laboratory[a]
EM9000	F *lac*/XK5456	This laboratory[a]
EM9546	F *lac traW546*/XK5456	This laboratory[a]
JC5455	F⁻ *lacΔX74 his trp rpsE tsx ton* (λ)	M. Achtman (3)
JC3051	F⁻ *lacΔX74 his trp rpsL tsx mal* (λ)	M. Achtman (3)
SE5000	F⁻ *araD139 lacΔU169 rpsL relA thi recA56*	R. Young
XK1200	F⁻ *lacΔU124* Δ(*nadA gal attλ bio*) *gyrA*	Nal[r] ED2149
XK1800	F⁻ Δ(*lac-pro*) *gal rpsL ilv pro uvr*	This laboratory (19)
XK5456	F⁻ *lacΔX74 his trp rpsE tsx ton*	λ⁻ JC5455

[a] These strains were constructed in standard matings with standard F *lac* and F *lac tra* mutant donors (3, 17).

glucose minimal medium containing an appropriate antibiotic(s) and all amino acids except cysteine and methionine. The culture was irradiated in a petri plate at 45 J/m² (15 s at 22.5 in. (57.2 cm) from our UV lamp or 3 J/m²/s) and transferred immediately to a foil-wrapped flask containing 5 ml of fresh medium. After 1 h at 37°C, cycloserine (150 μl of a 10-mg/ml solution) was added, and incubation was continued overnight. The cells were then centrifuged, washed with 10 ml of medium, and suspended in 5 ml of fresh medium. A total of 25 μCi of [^{35}S]methionine was then added to a 0.2-ml sample of the culture which was incubated at 37°C for 1 h. After centrifugation, cell pellets were frozen (−20°C) until use. Subsequently, they were suspended in 75 μl of sodium dodecyl sulfate gel sample buffer, and 10- to 20-μl samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The procedure for ^{14}C labeling was identical, except that the minimal medium contained no amino acid supplements, labeling was extended over a 2-h period, and a total of 20 μCi of ^{14}C-labeled amino acid mixture was added to the 0.2-ml sample.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis procedures have been described previously (11, 18, 19). The gels shown contained an exponential gradient of 11 to 15% polyacrylamide. Long gels were run in a cooled chamber with 10 W of constant power. Autoradiography was also as described earlier (18), except that gels of ^{14}C-labeled proteins were treated with Enlightening (New England Nuclear Research Products, Boston, Mass.) and handled ac-

TABLE 2. Plasmids

Plasmid no.[a]	*tra* fragment size (kb) and end(s)	Vector cloning site(s)	Source of *tra* DNA (original reference)
pBE269[b]	1.95 *Pst*I	pBR322 *Pst*I (*amp*)	pSH6 (25)
pKI152	2.52 *Hin*cII	pACYC177 *Hin*cII (*amp*)	pRS27
pKI164	2.55 *Hin*cII	pACYC177 *Hin*cII (*amp*)	pKI202
pKI166	1.15 *Xma*I[c]	pACYC177 *Xma*I (*kan*)	pKI167
pKI167[b]	1.15 *Ava*I	pACYC177 *Xma*I (*kan*)	EDλ92
pKI168	1.35 *Xma*I	pACYC177 *Xma*I (*kan*)	EDλ92
pKI169	2.55 + 6.2 *Ava*I	pACYC177 *Xma*I (*kan*)	pSH1
pKI172	2.55 *Ava*I	pACYC177 *Xma*I (*kan*)	pSH1
pKI202	5.6 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	EDλ92
pKI208[d]	4.45 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	pKI202
pKI210[e]	5.25 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	pKI202
pKI211[e]	5.05 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	pKI202
pKI212[e]	5.1 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	pKI202
pKI213[e]	3.4 *Bam*HI/	pBR322 *Bam*HI/ (*tcy*)	pKI202
pKI215[e]	3.0 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	pKI202
pKI216[e]	2.0 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	pKI202
pKI218[e]	2.1 /*Sal*I	pBR322 /*Sal*I (*tcy*)	pKI202
pKI222	1.15 *Sph*I	pBR322 *Sph*I (*tcy*)	pRS27
pKI223[f]	0.7 *Sph*I/*Ava*I	pBR322 *Sph*I/*Ava*I (*tcy*)	pKI222
pKI225[b]	1.95 *Sph*I	pBR322 *Sph*I (*tcy*)	pRS27
pKI226	5.6 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	EDλ143(*traV569*)
pKI227	5.6 *Bam*HI/*Sal*I	pBR322 *Bam*HI/*Sal*I (*tcy*)	EDλ144(*traW546*)
pKI267	1.95 *Pst*I	pBR322 *Pst*I (*amp*)	pBE269
pSH1	13.8 *Eco*RI	RSF2124 *Eco*RI	pRS26 (2)
pRS27	8.3 + 1.3 *Eco*RI	pSC101 *Eco*RI	F (23)
pRS29	1.3 + 13.8 *Eco*RI	pSC101 *Eco*RI	F (23)

[a] For all pKI plasmids, except as noted, the orientation of the *tra* DNA insert with respect to the promoter for the vector gene indicated corresponds to its original orientation to the *traY-Z* promoter; in pKI152, the *traJ* and *traY* promoters are included in the insert and oriented in the same direction as the *amp* promoter.

[b] In pBE269, pKI167, and pKI225, the orientation of the *tra* insert is opposite to the orientation of the vector gene indicated.

[c] Both 1.15 *Ava*I clones obtained from EDλ92 contained the insert in a reverse orientation to the *kan* promoter. When DNA from one of these (pKI167) was digested with *Xma*I and religated, only 1 of 13 new clones carried the insert in the appropriate orientation for *tra* operon transcription from *kan*. This plasmid (pKI166) appears to contain a small deletion and lacks the *Xma*I (*Sma*I) site at coordinate 8.02; the *Pvu*II site at 7.97 is still present.

[d] *Sph*I deletion of pKI202 (Fig. 2).

[e] BAL 31 deletion from pKI202 *Hpa*I site (Fig. 2).

[f] *Ava*I deletion of pKI222.

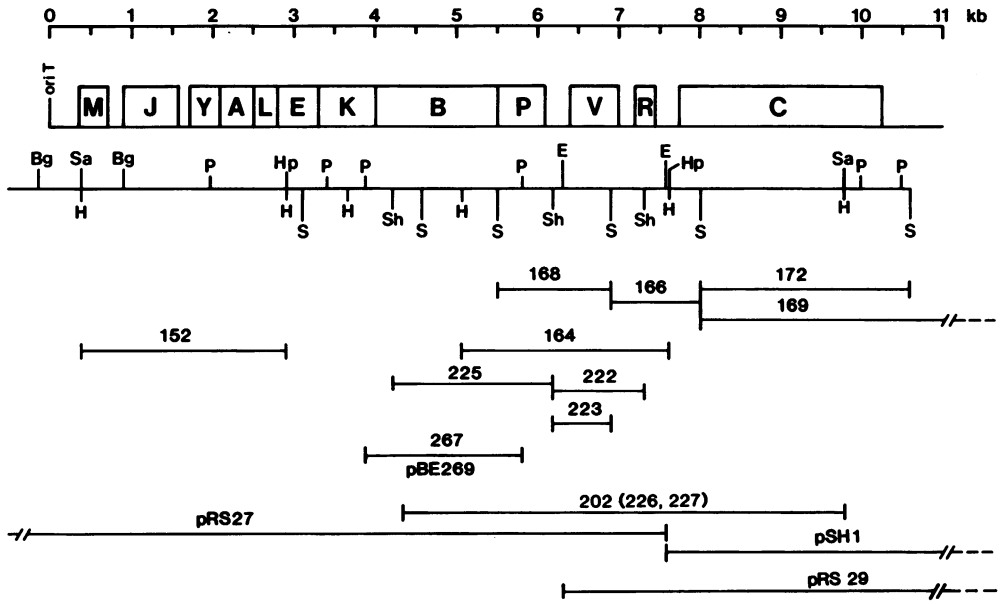

FIG. 1. Map of the F *tra*-region segment extending from the origin of transfer (*oriT*) through *traC*. The position and size of *tra* genes as deduced from DNA sequence (7, 8, 28) or product analysis (this work) are indicated by boxes. Numbered line segments in the bottom portion of the figure delineate the *tra* segments carried by the pKI or other plasmids used in this study. Kilobase coordinates are given at the top of the map. Coordinate 0 is at *oriT* (coordinate 66.7 on the 100-kb map of F [13, 32]; assumed here to be 142 bp to the right of a *Bgl*II site [9]). The positions of restriction sites indicated are for *Bgl*II (Bg), *Eco*RI (E), *Hin*cII (H), *Hpa*I (Hp), *Pst*I (p), *Sal*I (Sa), *Sma*I (S), and *Sph*I (Sh). DNA sequence data place sites between coordinate 0 and the *Pst*I site at bp 3421 (7, 8, 27); sites distal to this region were placed according to restriction fragment sizes estimated to the nearest 50 bp (±50 bp) length. *Eco*RI sites occur at coordinates 6.27 and 7.57; *Sma*I sites are at 3.121, 4.57, 5.52, 6.87, 8.02, and 10.57; *Hin*cII sites occur at 0.389, 2.906, 3.67, 5.07, 7.62, and 9.77.

cording to the directions of the manufacturer for using this product.

Determination of conjugative transfer frequencies. Freshly isolated colonies were picked from selective plates, inoculated into 5 ml of LB medium containing the antibiotics appropriate for plasmid maintenance, and grown overnight. In the morning, cells were centrifuged, suspended at an optical density at 550 nm of 0.3 in fresh LB medium containing no antibiotics, and grown with aeration for about 1 h at 37°C. Viable counts of cultures at this stage were typically 2×10^8. Mixtures containing 0.1 ml of donor culture, 0.1 ml of recipient culture, and 0.2 ml of broth were allowed to mate for 60 min at 37°C, after which the mixture was diluted and transconjugants were selected. The transfer frequency was calculated as the percentage of transconjugant colonies obtained per viable donor cell in the initial mating mixture.

RESULTS

Isolation of plasmid derivatives carrying segments of DNA from the *traB*-(*C*) region. We constructed a variety of plasmids by cloning fragments of F *tra* DNA into the vectors pBR322 and pACYC177. The *tra* fragments carried by these plasmids are delineated in Fig. 1, and the details of plasmid construction are given in Table 2. Except as noted in Table 2 for pKI167 and pKI225, the orientation of the *tra* DNA insert in every pKI plasmid described is such that transcription from the promoter of the vector antibiotic resistance gene that contains the insert proceeds in the *tra* operon direction (left to right in Fig. 1).

The λ *tra* transducing phage EDλ92 (29, 30) served as a convenient source of a 5.6-kb *tra* segment bordered by the lambda *Bam*HI site near the λ *att*::*traB* DNA junction and the *Sal*I site in *traC*. We used pKI202, the pBR322 derivative that carries this wild-type (*traB*)-(*traC*) sequence, as a reference plasmid in many of our experiments. Otherwise identical plasmids, pKI226 and pKI227, were constructed with DNA from the *traV569* and *traW546* amber derivatives EDλ143 and EDλ144, respectively. A series of deletion derivatives of pKI202 were also isolated by *Sph*I digestion (pKI208) or by limited digestion of *Hpa*I-cut pKI202 DNA with BAL 31 (pKI210 to 218). The extent of *tra* DNA remaining in each of the pKI202 derivatives is depicted in Fig. 2.

Mapping of restriction sites. Since it was part of our goal to obtain a more accurate map of gene placement in the region between *traB* and *traC*, a detailed restriction map of the 5.6-kb DNA fragment in pKI202 was constructed (Fig. 2). The position of restriction sites was confirmed through analysis of the pKI202 deletion derivatives and the additional *tra* plasmids cloned.

A less extensive restriction site analysis of other *tra* DNA clones was performed to permit alignment with the *tra* operon map. These data resulted in the restriction map included in Fig. 1. Our fragment sizes were consistent with reports from other laboratories (1, 12, 13, 23, 25, 26, 29, 31). However, consideration of available DNA sequence data and the double-digestion patterns of cloned fragments led us to alter the relative positions of some sites depicted on these earlier maps.

Analysis of λ *tra* transducing phage products. In an initial attempt to identify F *tra* operon products stemming from the *traB-traC* region, we examined the [^{35}S]methionine-labeled products synthesized by λ *tra* transducing phages in UV-irradiated cells. Figure 3A shows the products expressed by the set of phages EDλ101, EDλ126, EDλ128, and EDλ129. These phages derive from EDλ4 and carry *tra* DNA seg-

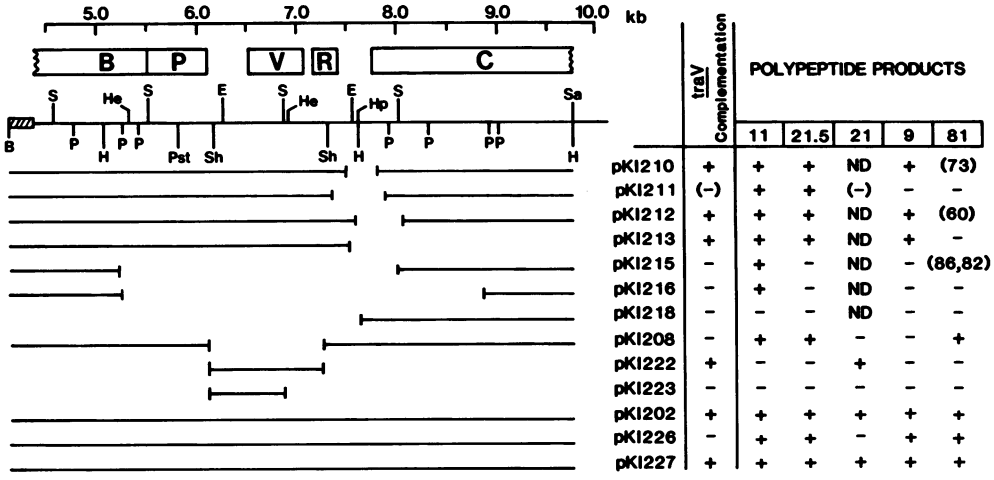

FIG. 2. DNA and protein product analysis of pKI202 and pKI202 derivatives. Lines below the restriction map indicate *tra* DNA retained by the pKI plasmid listed. The results of *traV* complementation tests and the products of these plasmids are summarized on the right; the 21-kDa product was observed only with ^{14}C-amino acid label (ND, ^{14}C analysis not done). Plasmid pKI226 was cloned from DNA carrying the *traV569* mutation: pKI227 was from *traW546* mutant DNA. Boxes indicating approximate gene size and position are as deduced from the detectable products or activities expressed by all pKI plasmids analyzed. The restriction map of the *Bam*HI-*Sal*I fragment carried by pKI202 includes *tra* DNA sites for *Eco*RI (E), *Hae*II (He), *Hin*cII (H), *Hpa*II (Hp), *Pst*I (Pst), *Pvu*II (P), *Sal*I (Sa), *Sph*I (Sh), *Sma*I (S), and a lambda DNA (hatched segment) *Bam*HI (B) site. The approximately 250 bp of lambda DNA also includes the *Ava*I and *Nco*I sites at λ bp 27887 and 27868 (5), but no *Eco*RV, *Kpn*I, *Nco*I, *Pvu*I, *Sst*I, *Xba*I, or *Xho*I sites were associated with the *tra* DNA, and all *tra Ava*I sites were also *Sma*I (*Xma*I) sites. Kilobase coordinates are as in Fig. 1.

ments of increasing length that extend from the same *traY* endpoint (13). Comparison of these products established the position of a number of *tra* proteins on our gels. Apparent molecular weights (MW_a) of the bands indicated were: TraA, 14,000; TraE, 19,500; TraK, 24,000; TraB, 60,000; TraC, 92,000. These values are in reasonable agreement with those reported elsewhere (1, 14) and with the TraA and TraE molecular weights of 13,200 and 21,200 predicted by DNA sequencing (8). A few other polypeptide bands were expressed by only a subset of the phages. Most notably, EDλ128 and EDλ129, which both carry the *tra* region between *traB* and *traC*, expressed an MW_a 21,500 polypeptide. In an effort to determine whether this or another polypeptide difference might reflect expression of the *traV*, *traW*, or another *tra* gene encoded by these phages, we also examined the products of the *tra(B)VCW* phages EDλ90 and EDλ91 and of derivatives of EDλ91 that carried either of the amber mutations *traV569* (EDλ143) or *traW546* (EDλ144). While TraC was obvious among the products of these phages, we were unable to attribute any polypeptide band to *traV* or *traW* through these analyses (data not shown).

Analysis of *tra* activities expressed by cloned derivatives. The ability of plasmids to complement available *tra* mutations was tested by constructing a series of strains carrying these plasmids together with F *lac traB2*, F *lac traV569*, F *lac traC5*, or F *lac traW546*. The sensitivity of these strains to the male phages f1, f2, and Qβ and their capacity to transfer the mutant F *lac* plasmid were then tested. Transfer complementation results are shown in Table 3. We also examined plasmid polypeptide products labeled in maxicells. Representative autoradiograms of sodium dodecyl sulfate-polyacrylamide gels on which [^{35}S]methionine-labeled products were fractionated are shown in Fig. 3. We typically analyzed labeled products of transducing phage EDλ134 and plasmid pKI202 on each gel to provide a standard for the positions of *tra* polypeptides. The results of these experiments can be summarized as follows.

(i) *traB*. Plasmid pBE269 was previously reported to

TABLE 3. Complementation of F *lac tra* mutants

Coresident plasmid	Transfer frequency[a] of F *lac* plasmid bearing the mutation:			
	traB2	*traV569*	*traC5*	*traW546*
None	$<4 \times 10^{-4}$	$<5 \times 10^{-4}$	$<4 \times 10^{-4}$	0.08 (0.002)[b]
pBE269	2.6			
pBR322	$<5 \times 10^{-4}$		$<7 \times 10^{-4}$	
pKI168		$<5 \times 10^{-4}$		
pKI169			0.008	
pKI172			0.003	(0.008)[b]
pACYC177				(0.004)[b]
pKI202		5.8	$<3 \times 10^{-4}$	0.16
pKI208		$<5 \times 10^{-4}$		0.17
pKI210		9.0		0.15
pKI211		0.12		0.20
pKI212		4.0		0.26
pKI213		5.0		0.28
pKI215		$<5 \times 10^{-4}$		0.15
pKI216		$<5 \times 10^{-4}$		0.04
pKI222		16.0		0.29
pKI223		$<5 \times 10^{-4}$		
pKI225	0.029			
pKI226		$<5 \times 10^{-4}$		0.12
pKI227		13.0		0.13
pKI267	46.6			
pRS27		11.0		
pRS29[c]		0.25	0.64	6.2 (0.43)[b]
pSH1			10.0	16.0 (0.97)[b]

[a] Percentage of transconjugants per donor cell added to the mating mixture. Except as noted, donor strains were derivatives of the F *lac tra* mutant donors EM1202 (*traB2*), EM12569 (*traV569*), EM1205 (*traC5*), and EM12546 (*traW546*) that carry the plasmid indicated in the first column. The wild-type F *lac* transfer frequency from strain EM1200 was 55.

[b] Values in parentheses were obtained by using derivatives of the donor EM9546. F transfer frequencies were lower from this host background (F *lac* transfer frequency from EM9000 was 14), but these strains were tested since transfer of F *lac traW546* was less leaky.

[c] Since plasmid pRS29 typically gives low complementation levels, we interpret these values as $TraV^+$ $TraC^+$ $TraW^+$. Low transfer levels were also reported and similarly interpreted by Miki et al. (17).

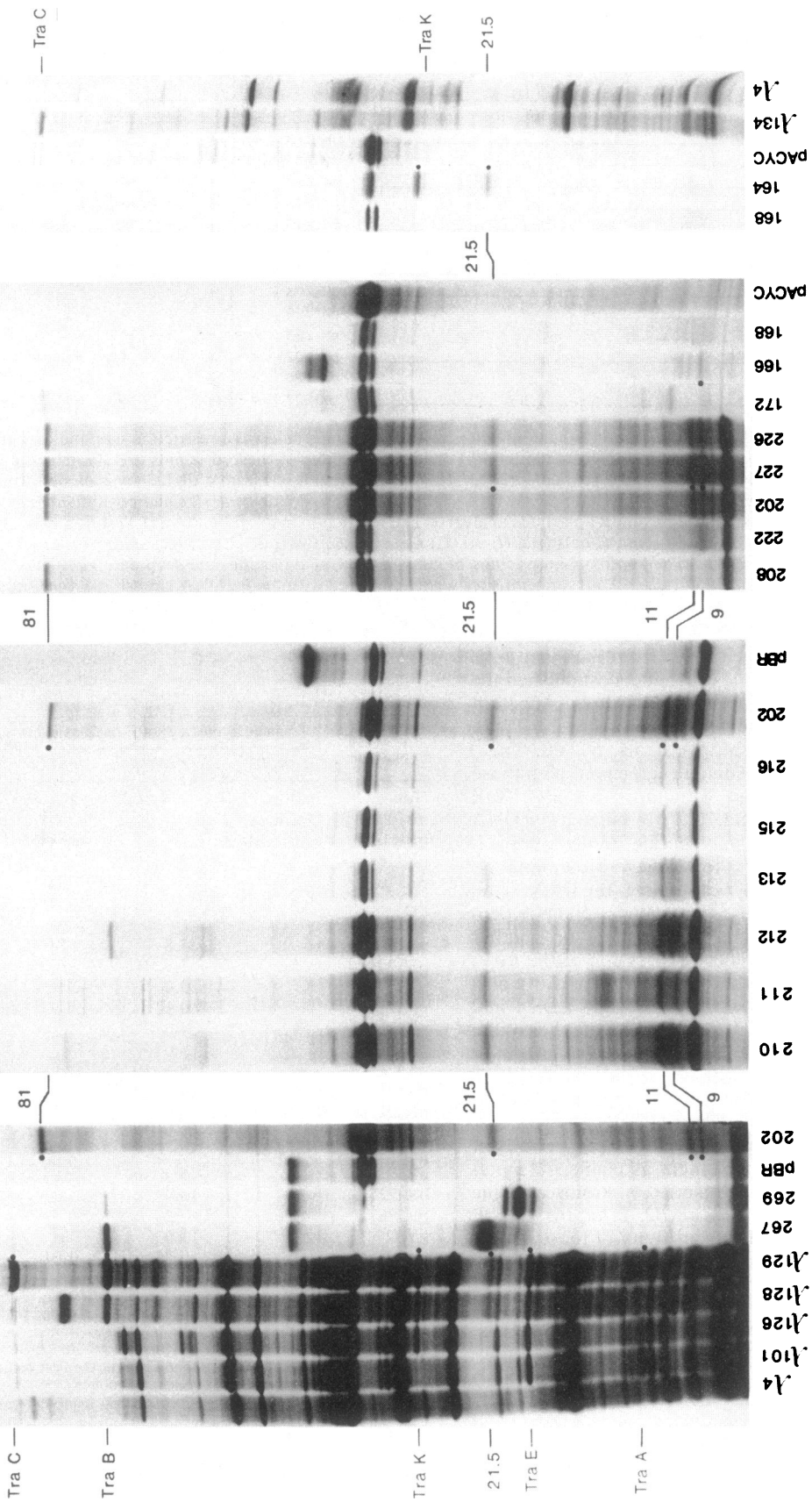

FIG. 3. [^{35}S]methionine-labeled polypeptides expressed by λ transducing phages and plasmids carrying *tra* segments. λ transducing phage products were from cells infected with EDλ4 (parental phage), EDλ101 [*tra(Y)ALE*], EDλ126 [*tra(Y)ALEK*], EDλ128 [*tra(Y)ALEKBV(C)*], EDλ129 [*tra(Y)ALEKBVCW*], and EDλ134 [*tra(Y)ALEKBVCWUNFQH(G)*] as indicated. Other samples are from cells carrying various pK1 plasmids (indicated by number), pBE269 (269), or the vector pBR322 (pBR) or pACYC177 (pACYC). Markers at the left and right of the figure and small dots placed along the EDλ129 lane indicate the positions at which the phage *traC*, *traB*, *traK*, *traE*, *traA*, and 21,500-dalton products migrate. The positions of the four (MW_a 81,000, 21,500, 11,000, and 9,000) products expressed by pKI202 are marked at the edge between each gel segment and by dots placed along sample 202 lanes. The two products of pKI164 discussed are also marked by dots.

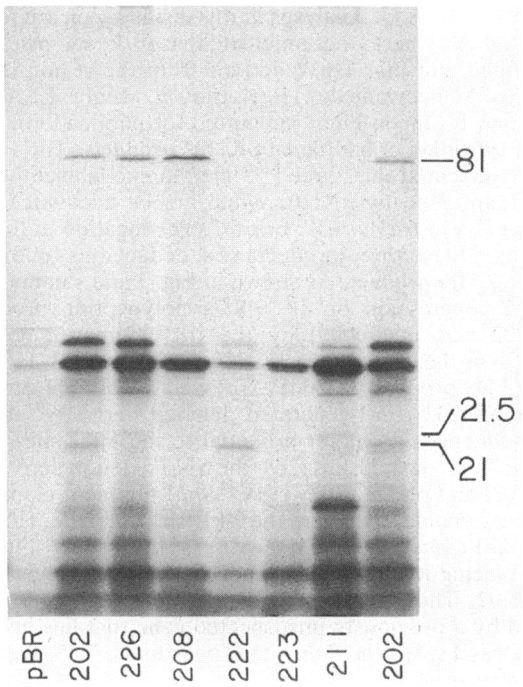

FIG. 4. Analysis of ^{14}C-amino acid-labeled polypeptides expressed by TraV$^+$ and TraV$^-$ plasmids. Numbers below each lane indicate the pKI number of the plasmid present in the strain analyzed. Numbers on the right side indicate the approximate size (kilodaltons) and position of three pKI202 products. Note that the 21-kDa product is also expressed by pKI222, but not by any TraV$^-$ derivative. The autoradiogram shown is from a short (14-cm) gel that did not resolve the 11- and 9-kDa pKI202 products.

complement F *lac traB* donors inefficiently; this plasmid was used in the original identification of TraB (1, 25, 26). We also found that while pBE269 exhibited low complementing activity for transfer of F *lac traB2* (Table 3), the pBE269/F *lac traB* strain remained resistant to male specific phages. However, our restriction enzyme analysis indicated that, in pBE269, the orientation of *traB* to the promoter of the *amp* gene into which it is inserted is the reverse of its original orientation to the *traY-Z* promoter. Plasmid pKI267, which contains the same DNA fragment reoriented to allow *amp* promoter expression of the *tra* operon strand, restored the F *lac traB* donor strain to male phage sensitivity and to a transfer proficiency typical of our F *lac* control (Table 3). Plasmids pBE269 and pKI267 both expressed a polypeptide that comigrated with the MW$_a$ 60,000 TraB protein encoded by the TraB$^+$ phages EDλ128 and EDλ129 (Fig. 3A). As predicted by the map orientation and complementation results, TraB protein is expressed much more efficiently by pKI267 than by pBE269.

No clone of the *Sph*I fragment from the *traB* region was obtained in the orientation in which the pBR322 *tcy* promoter would express *traB* DNA in the *tra* operon direction. Seven clones, like plasmid pKI225, carried the fragment in the reverse orientation. Plasmid pKI225 was unable to complement F *lac traB* mutants to give transfer (Table 3) or male phage sensitivity and did not express the TraB polypeptide. We suspect that translation of *traB* is initiated at a point between the *Pst*I and *Sph*I sites at *tra* coordinates 3.87 and 4.22 and that an *Sph*I fragment insertion in the forward orientation may result in a fusion product that is detrimental to cell growth.

(ii) *traV*. The characteristics of clones carrying the *traV* region are summarized in Fig. 2. Previous evidence placed the F coordinate 73.4 (coordinate 6.67 in Fig. 1 and 2) in *traV* (13) and located the *traV* gene within the small *Eco*RI f15 fragment in this region (17). As expected, pKI202 and pKI227 could complement an F *lac traV569* mutant, while pKI226, which carries the analogous DNA fragment cloned from a *traV569* amber mutant DNA source, could not. Plasmid pKI208, an *Sph*I deletion of pKI202, lacked *traV* activity. Since the corresponding *Sph*I clone, pKI222, was able to complement F *lac traV569*, *traV* lies within the *Sph*I fragment carried by this plasmid. However, pKI223, an *Ava*I deletion of pKI222, was unable to complement *traV*. This suggested that the *Sma*I (*Ava*I) site at coordinate 6.87 is within the *traV* gene.

A comparison of pKI202 and pBR322 products suggested that the 5.6-kb DNA insert in pKI202 results in expression of four novel [^{35}S]methionine-labeled polypeptides which migrate with MW$_a$s of 81,000, 21,500, 11,000, and 9,000, respectively (Fig. 2 and 3). However, all four of these products were still expressed by the *traV569* amber derivative pKI226. Similarly, comparison of the [^{35}S]methionine-labeled proteins expressed by pKI222, pKI223, and the pKI202 deletion derivatives revealed no product expressed in a pattern that correlated with *traV569*-complementing activity. Therefore, we considered the possibility that the methionine labeling of *traV* product was poor and examined products labeled with ^{14}C-amino acids. A ^{14}C-labeled MW$_a$ 21,000 polypeptide that is synthesized by the TraV$^+$ plasmids pKI202 and pKI222 is not expressed by the TraV$^-$ derivatives, pKI226 (*traV569*), pKI208, and pKI223 (Fig. 4). We concluded that the 21-kilodalton (kDa) ^{14}C-labeled protein is the product of *traV*.

It should be mentioned that the pKI202 deletion, pKI211, exhibited somewhat anomalous behavior. This plasmid did not express the 21-kDa polypeptide at a detectable level, and its presence resulted in only limited transfer of F *lac traV569* (Table 3). Since the *Sph*I site at 7.32, as well as DNA proximal to this site, is still present in pKI211 (Fig. 2), it should, like pKI222, carry an intact *traV* gene. One possible explanation is that the 211 deletion affects the stability of the mRNA *traV* sequence.

(iii) *traC*. Since the *Sal*I site endpoint of the F DNA in pKI202 was previously placed within *traC* (12), this plasmid was not expected to complement *traC*. Indeed, the transfer frequency of an F *lac traC5*/pKI202 donor was less than 3×10^{-4}, and pKI202 did not express the 92-kDa *traC* product (Fig. 3). It seemed reasonable to presume, however, that the 81-kDa polypeptide expressed by pKI202 represented a product of the truncated *traC* gene. Analysis of deletion derivatives of pKI202 (summarized in Fig. 2) supported this interpretation. Synthesis of the 81-kDa polypeptide was unaffected by the *Sph*I deletion in pKI208. However, none of the deletion derivatives created by digestion from the *Hpa*I site expressed the 81-kDa polypeptide, although several expressed large fusion protein products (note pKI202, pKI210, pKI212, Fig. 3B). Plasmid pKI213, which lacks the *Eco*RI site at *tra* coordinate 7.57 as well as all *tra* DNA distal to it, expressed no vestige of the 81-kDa product, although it was able to express all other [^{35}S]methionine-labeled pKI202 *tra* products (21.5, 11, and 9 kDa). These data indicated that the 81-kDa fusion polypeptide stemmed from the *traC* region. If the 81-kDa fusion polypeptide expressed by pKI202 is assumed to have a *traC*-encoded amino terminus, how-

ever, then *traC* must begin close to the 7.57 *Eco*RI site, since the 350-base-pair (bp) deletion in pKI210 altered the polypeptide produced. This suggested that the *Sma*I and *Pvu*II sites at coordinates 8.02 and 8.32 (and possibly even the *Hpa*I site at coordinate 7.62) were located within the *traC* gene.

Analysis of other plasmids (Fig. 1) also supported this interpretation. Since plasmid pSH1, which contains the entire F *Eco*RI fragment f1, can express *traC* (1, 2) (Table 3), the *traC* gene N terminus must lie to the right of the *Eco*RI site at coordinate 7.57. However, the *Sma*I fragment clones, pKI172 and pKI169, were unable to complement *traC5* or to express the TraC protein. The large fusion polypeptide expressed by pKI172 is shown in Fig. 3C; pKI169 expressed a polypeptide of the same size (data not shown). Since the *tra* DNA in pKI169 includes the 2.55-kb *Sma*I fragment carried by pKI172 together with the adjacent, distal 6.2-kDa *Sma*I fragment it must include the *traC* carboxy-terminal region. The absence of an intact TraC product again suggests that the *Sma*I site at coordinate 8.02 is within the amino-terminal region of *traC*.

All these data indicate that *traC* lies in a more *tra* promoter-proximal position than suggested on earlier maps. Somewhat less than 2.5 kb should be needed to encode the approximately 92,000-dalton TraC protein. Thus, while the carboxy-terminal region of *traC* must lie distal to the *Sal*I site at coordinate 9.77, the *Sma*I site at coordinate 10.57 is unlikely to be located within the *traC* gene.

It should also be noted that the 81-kDa polypeptide was synthesized by pKI227 as well as pKI202 (Fig. 2 and 3C). Thus, if *traW* was to overlap the *traC* gene, the *traW546* amber mutation must either lie distal to the *Sal*I site at coordinate 9.77 or disrupt a different reading frame from that which is translated to the *traC* protein.

(iv) *traW*. The *traW* gene was originally placed at the *Eco*RI site immediately to the left of *traC* (17). However, our results were inconsistent with this placement. Like Johnson et al. (12) who reported that a clone of the entire *Sal*I *tra(M)JALEKBV(C)* fragment failed to complement *traW*, we were unable to detect *traW* activity from clones carrying the region between *traB* and *traC*. Phage EDλ128 could not complement *traW* (13) despite the fact that our product analysis (Fig. 3A) revealed a large MW_a 75,000 protein that appeared to be a truncated product from *traC*. This suggested that the *tra* DNA carried on this phage includes a large portion of the *traC* gene in addition to the *traALEKBV* genes it complements. Similarly, although pKI202 contains a *tra(B)-tra(C)* region that extends to the *traC Sal*I site, neither pKI202 nor any of its derivatives were able to complement transfer of F *lac traW546* (Table 3). F *lac traW546* transfer frequencies from strains containing pKI202 or pKI227 showed no essential difference, despite the derivation of pKI227 from *traW546* DNA. Our *Sma*I fragment clone, pKI172, also failed to complement the F *lac traW* mutant.

However, we found that pSH1 was able to complement *traW546*, indicating that *traW* does derive from within the large *Eco*RI f1 fragment. This conflicts with the result of Miki et al. (17) who reported that plasmid pRS8 (which includes the f1 fragment together with additional distal F DNA) enhanced F *lac traW* transfer by recombination rather than complementation. The results reported here are thus consistent either with a *traW* location that overlaps *traC* as suggested by the Johnson and Willetts (13) map or with a location that is promoter distal to *traC*. We did find, however, that the $TraV^+C^+W^+$ transducing phage EDλ91 carries a segment of *tra* DNA that extends almost 1 kb distal to the *Sma*I site at 10.57. Analyses of this distal region are reported in detail elsewhere; these indicate that *traW* and *traC* can be separated and that TraW derives from the region distal to *traC* (S. Maneewannakul, P. Kathir, D. Moore, L.-A. Le, J. Wu, and K. Ippen-Ihler, submitted for publication).

Identification of additional pKI202 products. The analyses above indicated that three [^{35}S]methionine-labeled polypeptides expressed by pKI202 could not be accounted for by expression of *traV*, *traW*, or *traC*. The location of the DNA encoding these three products was deduced as follows.

The 9-kDa product. As shown in Fig. 3 and summarized in Fig. 2, expression of the 9-kDa polypeptide product of pKI202 was unaffected by the *traV* amber mutation in pKI226 or the deletions in pKI210, -212, and -213. However, the 9-kDa product was not expressed by pKI211 or by any other pKI202 *Hpa*I-generated deletion derivative that had lost sequences promoter proximal to the *Sph*I site at coordinate 7.32. Plasmids pKI208 (the *Sph*I deletion derivative of pKI202) and pKI222 (the $TraV^+$ *Sph*I fragment clone) were similarly unable to express the 9-kDa polypeptide. However, the *Sma*I clone, pKI166, did express the product (Fig. 1 and 3C), placing its coding sequence between coordinates 6.87 and 8.02. Thus, the 9-kDa polypeptide appears to be encoded by a previously unsuspected gene that lies just distal to *traV* and spans the *Sph*I site at coordinate 7.32. We named this gene *traR*.

The 21.5-kDa product. The 21.5-kDa [^{35}S]methionine-labeled product expressed by pKI202 comigrated with a product that was also expressed by transducing phages that carry the *traB-C* region (EDλ128 and EDλ129; Fig. 3A). As summarized in Fig. 2, the pKI202 deletion derivatives pKI208 and pKI213 still expressed the 21.5-kDa polypeptide, indicating that sequences promoter distal to the *Sph*I site at coordinate 6.17 were not required to encode this product. Since the deletion derivatives pKI215 and pKI216 failed to express the polypeptide, the 21.5-kDa product appeared to stem from *tra* DNA within a region delimited by the *Hin*cII site at coordinate 5.07 (still present in pKI215 and pKI216) and the *Sph*I site at 6.17. Analysis of other plasmids (Fig. 1 and 3D) showed that indeed, plasmid pKI164, which carries the implicated *Hin*cII fragment, does express the 21.5-kDa polypeptide. However, neither pKI168 nor pKI267 expressed this protein, suggesting that both the *Sma*I site at coordinate 5.52 and the *Pst*I site at coordinate 5.82 are within its coding region. Thus, the 21.5-kDa polypeptide appears to stem from the *tra* region previously assigned to *traP*.

The existence of *traP* was originally postulated to account for the detection of a 23.5-kDa polypeptide (protein 6e) in an analysis of F *Eco*RI fragment f6 products (25, 31); because there is no F *traP* mutant, no complementation assay for *traP* activity is available. In earlier analyses, however, protein 6e appeared to migrate just below the 24-kDa *traK* product and to comigrate with the *traJ* product. We therefore carefully examined the corresponding region of our gels for a similar protein. The only plasmid which appeared to express a polypeptide close to this size was pKI164. This novel pKI164 band migrated slightly slower than TraK (Fig. 3D; the position of the *traK* product expressed by EDλ134 is marked). No *tra* insert polypeptide of similar size was detected among pKI202 or pKI168 products even when ^{14}C-amino-acid-labeled proteins were examined (data not shown). Although a vector (*amp*) polypeptide is present in an approximately appropriate position, this band is just above the pKI164 24-kDa product and should not have obscured resolution of "protein 6e" if it migrated in the

expected position. Thus, the interpretation most consistent with our results is that the 24-kDa polypeptide product of pKI164 stems from an *amp-tra* fusion product and that the DNA region assigned to *traP* encodes the 21.5-kDa polypeptide.

The 11-kDa product. The 11-kDa polypeptide was encoded by all pKI202 deletion derivatives except pKI218 in which an extensive deletion removed the λ-*tra* DNA junction and λ *Bam*HI site (Fig. 2 and 3). Since neither pKI267 nor pKI164 expressed a similar protein, the 11-kDa product may represent a fusion product derived from the junction of pBR322 *tcy* and λ *int* DNA sequences at the pKI202 *Bam*HI site.

DISCUSSION

Our data make possible the identification of three gene products that stem from the *traB-traC* interval: an MW_a 21,500 polypeptide from *traP*, an MW_a 21,000 polypeptide from *traV*, and a small, MW_a 9000 product from a new locus we called *traR*. These product molecular weight estimates, together with our restriction analysis of the cloned DNA segments that encode these proteins, permit the location of these genes to be approximated as shown in Fig. 1. On this map, the *traV*, *traR*, and *traC* loci have been arbitrarily spaced within the regions known to delimit them. However, *tra* coordinate 6.87 must lie within *traV*, coordinate 7.32 must be within *traR*, and *traC* must begin between coordinates 7.57 and 8.02. As the estimated size of the *traV* and *traR* genes does not saturate the *traP-traC* interval, it is possible that an additional gene activity remains undetected. Alternatively, since Manning et al. (16a) detected polymerase-binding sites in this region, regulatory regions may exist in DNA sequences between or surrounding *traV* and *traR*. The data presented indicate that *traW* activity does not stem from the *traV-traC* interval; this is consistent with other data that place the *traC* gene proximal to the *Sma*I site at *tra* coordinate 10.57 and *traW* immediately distal to that site (Maneewannakul et al., submitted).

Since plasmid pKI168 did not express the 21.5-kDa product we detect from the *traP* region, *traP* is assumed in Fig. 1 to include the *Sma*I site at coordinate 5.52. This site is 2.14 kb from the end of *traE*, an interval only slightly smaller than that we would have assigned to *traK* and *traB* on the basis of our molecular size estimates for TraK (24 kDa) and TraB (60 kDa). Since such estimates may be misleading, we shortened *traB* to fit the available interval. However, it is possible that some overlap between reading frames in this region could exist.

Alternative hypotheses are that *traP* is actually located distal to coordinate 5.52, but expressed at an undetectable level from pKI168, or that the 21.5-kDa product we observed stems from a start site within *traB* that is not normally expressed from the F *tra* operon. However, we did not detect the 23.5-kDa (protein 6e) product which led to the original postulation of the *traP* locus (26, 31), and the 21.5-kDa product we did observe does appear to stem from the *tra* map interval previously assigned to *traP*. It thus seems most appropriate to retain the map position of *traP* but to suggest that *traP* encodes the protein that migrates with an MW_a of 21,500 on our gels. Our data do not shed light on the origin of protein 6e. It is conceivable that differences in sample or gel procedures have caused a difference in the migration pattern of the *traP* product and that protein 6e is the same polypeptide that migrates at 21.5 kDa on our gels. We note, however, that Thompson and Achtman (26) did report a result with one of their deletion derivatives, pBE257, that suggested that protein 6e derived from between *traK* and *traB*. Although considerations of space caused them to place a separate 6e coding sequence distal to *traB*, it is possible that protein 6e actually did stem from the *traK-B* region. Perhaps its appearance reflected processing or breakdown of the 24-kDa TraK protein.

Analysis of *traB*, *traV*, and *traC* mutants has suggested that the products of these genes function in F pilus assembly and are components of a membrane protein complex which may also serve as a passage during conjugal DNA transfer (10, 32). Whether the *traP* and *traR* products are also essential to F piliation and transfer is not yet known. The clones derived in this study should be useful for introducing and assessing the effect of mutations in these loci on F transfer properties. We also expect the mapping and product analysis reported here to be useful in identifying appropriate *tra* operon reading frames during DNA sequence analysis of the *traB-traC* region. Similarly, the *tra* segments we cloned will aid in construction of strains that overproduce individual *tra* products and in the eventual purification and characterization of their biochemical activities.

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LITERATURE CITED

1. **Achtman, M., P. A. Manning, C. Edelbluth, and P. Herrlich.** 1979. Export without proteolytic processing of inner and outer membrane proteins encoded by F sex factor *tra* cistrons in *Escherichia coli* minicells. Proc. Natl. Acad. Sci. USA **76:** 4837–4841.
2. **Achtman, M., R. A. Skurray, R. Thompson, R. Helmuth, S. Hall, L. Beutin, and A. J. Clark.** 1978. Assignment of *tra* cistrons to *Eco*RI fragments of F sex factor DNA. J. Bacteriol. **133:**1383–1392.
3. **Achtman, M., N. Willetts, and A. J. Clark.** 1971. Beginning a genetic analysis of conjugational transfer determined by the F factor in *Escherichia coli* by isolation and characterization of transfer deficient mutants. J. Bacteriol. **106:**529–538.
4. **Chang, A., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the p15A cryptic mini plasmid. J. Bacteriol **134:**1141–1156.
5. **Daniels, D. L., J. L. Schroeder, F. R. Blattner, W. Szybalski, and F. Sanger.** 1983. Complete annotated lambda sequence, p. 519–676. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II: appendix. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Davis, R., D. Botstein, and J. Roth.** 1980. A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Fowler, T., L. Taylor, and R. Thompson.** 1983. The control region of the F plasmid transfer operon: DNA sequence of the *traJ* and *traY* genes and characterization of the *traY-Z* promoter. Gene **26:**79–89.
8. **Frost, L. S., 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.
9. **Horowitz, B., and R. C. Deonier.** 1985. Formation of Δ*tra* F′ plasmids: specific recombination at *oriT*. J. Mol. Biol. **186:** 267–274.
10. **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.

11. **Ippen-Ihler, K., D. Moore, S. Laine, D. A. Johnson, and N. S. Willetts.** 1984. Synthesis of F-pilin polypeptide in the absence of F *traJ* product. Plasmid **11**:116–129.
12. **Johnson, D., R. Everett, and N. Willetts.** 1981. Cloning of F DNA fragments carrying the origin of transfer *oriT* and the fertility inhibition gene *finP*. J. Mol. Biol. **153**:187–202.
13. **Johnson, D. A., and N. S. Willetts.** 1983. λ transducing phages carrying transfer genes isolated from an abnormal prophage insertion into the *traY* gene of F. Plasmid **9**:71–85.
14. **Kennedy, N., L. Beutin, M. Achtman, R. Skurray, U. Rahmsdorf, and P. Herrlich.** 1977. Conjugation proteins encoded by the F sex factor. Nature (London) **270**:580–585.
15. **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.
16. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning; a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16a. **Manning, P. A., G. Morelli, and C. Fisseau.** 1984. RNA-polymerase binding sites within the *tra* region of the *Escherichia coli* K-12. Gene **27**:121–123.
17. **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.
18. **Moore, D., B. A. Sowa, and K. Ippen-Ihler.** 1981. Location of an F-pilin pool in the inner membrane. J. Bacteriol. **146**:251–259.
19. **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.
20. **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.
21. **Ray, A., K.-C. Cheah, and R. Skurray.** 1986. An F-derived conjugative cosmid: analysis of *tra* polypeptides in cosmid-infected cells. Plasmid **16**:90–100.
22. **Sancar, A., A. Hack, and W. Rupp.** 1979. Simple method for identification of plasmid coded proteins. J. Bacteriol. **137**: 692–693.
23. **Skurray, R. A., H. Nagaishi, and A. J. Clark.** 1976. Molecular cloning of DNA from F sex factor of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **73**:64–68.
24. **Sutcliffe, J.** 1979. Complete nucleotide sequence of the *E. coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
25. **Thompson, R., and M. Achtman.** 1978. The control region of the F sex factor DNA transfer cistrons: restriction mapping and DNA cloning. Mol. Gen. Genet. **165**:295–304.
26. **Thompson, R., and M. Achtman.** 1979. The control region of the F sex factor DNA transfer cistrons: physical mapping by deletion analysis. Mol. Gen. Genet. **169**:49–57.
27. **Thompson, R., and L. Taylor.** 1982. Promoter mapping and DNA sequencing of the F plasmid transfer genes *traM* and *traJ*. Mol. Gen. Genet. **188**:513–518.
28. **Thompson, R., L. Taylor, K. Kelly, R. Everett, and N. Willetts.** 1984. The F plasmid origin of transfer: DNA sequence of wild-type and mutant origins and location of origin-specific nicks. EMBO J. **3**:1175–1180.
29. **Willetts, N., and G. Johnson.** 1979. *Sma*I cleavage map of the transfer region of the *E. coli* K12 sex factor F. Genet. Res. **34**:195–202.
30. **Willetts, N. S., and S. McIntire.** 1978. Isolation and characterization of λ*tra* transducing phages from EDFL223 (F*lac traB*::EDλ4) J. Mol. Biol. **126**:525–549.
31. **Willetts, N., and R. Skurray.** 1980. The conjugation system of F-like plasmids. Annu. Rev. Genet. **14**:41–76.
32. **Willetts, N., and R. Skurray.** 1987. Structure and function of the F factor and mechanism of conjugation, p. 1110–1133. *In* F. C. Neidhart, 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.