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Assignment of *tra* Cistrons to *Eco*RI Fragments of F Sex Factor DNA

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We describe here the cloning of single *Eco*RI fragments from the *tra* region of F DNA using ColE1::Tn*3* as vector. These plasmids, as well as the series of Skurray et al. (Proc. Natl. Acad. Sci. U.S.A. **73**:64-68, 1976), have been used to refine the map positions of *tra* cistrons on the F factor as well as to define a new DNA transfer cistron, *traM*. The current map of the *tra* cistrons is presented. None of the known *tra* cistrons, with the exception of *traG*, straddles an *Eco*RI site. The *Eco*RI site at 82 kilobases splits the *traG* cistron into two portions, an operator-proximal portion necessary for F pilus synthesis and an operator distal portion involved in conjugation itself. The operon structure of the *tra* cistrons was reevaluated, and we found that *traI* is at least partially independent of transcription of the *traA* to *traD* operon.

Bacterial sex factors are prevalent in gram-negative organisms and are considered to be largely responsible for the widespread occurrence of colicinogenic and/or antibiotic-resistant strains (for review, see 14). Numerous, unrelated classes of sex factors have been distinguished, although to date only F-like sex factors have been analyzed in any detail (1, 39).

The F sex factor, the archetype of the FI compatibility group (12), has been analyzed previously by DNA heteroduplex techniques (13, 28), by genetic analysis (1, 39), and by *Eco*RI endonuclease digestion (26a, 32, 36). F DNA can be isolated as a covalently closed circle 94.5 kilobases (kb) long (approximately 62×10^6 megadaltons) (28). A comparison of the genetic properties of deletion mutants with their DNA structure, as determined by heteroduplex analysis, has allowed the coarse assignment of physical locations for certain *tra* (DNA transfer) cistrons (13, 28) and for *pif* (inhibition of female-specific phage multiplication) cistrons (5). Analysis of chimeric plasmids carrying only certain *Eco*RI fragments of F DNA has confirmed the approximate locations for the *tra* and *pif* cistrons (7, 32), as well as having provided a more precise definition of the location of the *frp* (F replication) and *inc* (incompatibility) cistrons (17, 23, 27, 31, 32, 37). Studies with such chimeras have also provided confirmation that cistrons involved in surface exclusion (42; poor recipient ability in conjugation) and fertility inhibition by FinOP$^+$ plasmids (44) mapped in or near the *tra* region of F (7, 32).

We report here the results of a detailed genetic analysis which has allowed *tra* cistrons to be assigned to defined *Eco*RI fragments of F DNA. (A preliminary account of this assignment of *tra* genes has been presented [R. A. Skurray, H. Nagaishi, and A. J. Clark, 2nd Int. Pili Conf., Atlantic City, N.J., 7-9 May 1976] and will be published [Skurray, Nagaishi, and Clark, *in* J. Ou and E. Raizen, ed., Pili, in press).

MATERIALS AND METHODS

Bacterial strains. All bacterial strains were *Escherichia coli* K-12 derivatives. Complementation tests were conducted with plasmid-carrying derivatives of the suppressor-free (Su$^-$) *lacΔX74 rpsL* strain, JC3272 (3). F*lac* derivatives of JC3272 have been previously described (3, 41). Newly constructed strains were purified twice by single-colony isolation and were stored at −30°C in 30% glycerol, 1% Bacto-peptone. Spectinomycin-resistant (Spc^r) or nalidixic acid-resistant (Nal^r) mutants of strain JC3272 were used to detect DNA transfer of F*lac* and the chimeric plasmids. M12 bacteriophage, which adsorbs to the side of the F pilus, was from laboratory stocks.

Plasmids. The transfer-deficient (*tra*) mutants of the *lacI* F*lac* element JCFL0 have been previously described (3, 4). The pRS series of chimeras consists of plasmid pSC101 carrying *Eco*RI fragments of F DNA (7, 32; Skurray, Nagaishi, and Clark, in preparation). The pSH series of plasmids, first described here, are penicillin resistant and consist of a ColE1::Tn*3* plasmid, RSF2124 (34), carrying single *Eco*RI fragments from the *tra* region of F DNA (see below). The Tn*3* insertion in RSF2124 results in somewhat decreased mobilization by F factors (see 38).

Isolation of plasmid DNA. Supercoiled DNA was

purified from Triton X-100 cleared lysates (9) of plasmid-bearing strains by a single or by two successive CsCl-ethidium bromide centrifugations. pRS plasmid DNA was isolated from late-logarithmic-phase cells (yield of approximately 50 μg from 500 ml of culture). The DNA of pSH plasmids was isolated from mid-log-phase cells which had been treated with 200 μg of chloramphenicol per ml for 18 h (8) (yield of approximately 500 μg from 2 liters of culture). DNA preparations were routinely checked by agarose gel electrophoresis before and after digestion with *Eco*RI.

Endonuclease digestion and gel electrophoresis. Endonuclease *Eco*RI was prepared from strain 1100 (RY5, R1-19) and was used as described by Yoshimori (Ph.D. thesis, University of California, San Francisco, 1971). *Sal* I and *Hin*dIII were from New England Biolabs and were used as described by Chater and Wilde (6) and Murray and Murray (24), respectively. Vertical slab gel electrophoresis was performed with 1 or 1.6% agarose (Seakem) in 40 mM tris(hydroxymethyl)aminomethane-acetate, 20 mM sodium acetate, 2 mM ethylenediaminetetraacetate, pH 8.2 (19). Gels were stained with 0.5 μg of ethidium bromide per ml and were photographed under UV light.

Transformation. Plasmid DNA isolated from strain AB1157, carrying the pRS plasmid series, or from strain C600, carrying the pSH plasmid series, was introduced into the desired strain by transformation, as described by Cohen et al. (10).

Construction of pSH plasmids. The cloning vector was RSF2124, and the source of F DNA fragments was either pRS27 (for fragments f6 and f15) or pRS26 (for fragments f15, f1, f17, f19, and f2). *Eco*RI-generated fragments from these plasmids and the vector were joined with T4 DNA ligase (a gift of V. Pirotta) as described by Tanaka and Weisblum (35).

In the first experiment, strain C600 was transformed with the ligated DNA mixture, and penicillin-resistant transformants were screened for lack of colicin production (insertion of DNA at the *Eco*RI site in ColE1::Tn*3* leads to inactivation of colicin production). These were found at a frequency of 2%. DNA from these clones was isolated, digested with *Eco*RI, and examined by agarose gel electrophoresis. Seven clones were examined and yielded two hybrids each containing ColE1::Tn*3* and f15 and two each containing ColE1::Tn*3* and f17.

In the second experiment, strain JC3272 was used as the recipient for transformation. Pen^r colicin-nonproducing transformants, containing and expressing *tra* genes, were identified by qualitative complementation tests. Transfer-defective F*lac* elements carrying the suppressible mutant allele *traJ90*, *traC5*, or *traD14* (3, 4) were introduced from a Su$^+$ host into the transformants, and clones in which complementation yielded an M12-sensitive phenotype (i.e., production of F pili) were identified by replica-plating.

Additional qualitative complementation tests showed that all chimeric plasmids which carried the same *Eco*RI fragment (two carrying f1, seven carrying f2, and six carrying f6) had identical biological properties, with the exception of one of the plasmids carrying f6 which was found to be unstable. One representative from each group was chosen for further analysis and was named after the *Eco*RI fragment of F DNA which it carried (pSH6 for f6, etc.).

Stability of chimeric plasmids. Strains carrying pRS30 readily lose the ability to synthesize F pili when maintained on agar slants at 4°C although tetracycline resistance is retained. Approximately 50% of pRS26 transformants complement *tra* mutants poorly in comparison with the rest of the transformants. Such problems were much less serious with the smaller chimeric plasmids. Approximately 1% of pRS27-carrying derivatives of JC3272 lose all *tra* cistron complementing activity; the comparable value for pRS29-, pRS31-, and pSH6-carrying strains is below 0.1%. Our experience with the other pSH chimeras has been less extensive. Despite these problems, purified cultures maintained at −30°C in 30% glycerol have been stable for over 6 months. We often include tetracycline when inoculating from the glycerol cultures of cells carrying pRS plasmids. This precaution ensures that all the cells which grow carry the chimeric plasmid, but on occasions when tetracycline was not included no problems with plasmid segregation were encountered.

DNA transfer ability. Plasmid DNA transfer was measured after incubation for 40 min at 37°C in mating mixtures carrying 0.2 ml of the donor culture and 1.8 ml of the recipient culture, both grown as previously described (3).

Qualitative complementation tests. Permanent heterozygote colonies carrying an F*lac tra* mutant and a pRS or pSH plasmid were tested by replica-plating for sensitivity to M12 bacteriophages and for the ability to transfer the F*lac*$^+$ element by conjugation. M12 sensitivity was detected on eosin-methylene blue lactose plates spread with 10^{11} M12 plaque-forming units; sensitivity manifested itself by the red appearance of the colonies under reflected light after 4 to 7 h at 37°C.

Quantitative complementation tests. The DNA transfer ability of cultures carrying an F*lac* element and a chimeric plasmid was quantitated in liquid matings under standardized conditions as described above. In addition, the donor culture used was streaked to yield single colonies which were then tested to determine that at least 95% were Lac$^+$, resistant to tetracycline or penicillin, and, where appropriate, showing complementation. The Lac$^+$ exconjugant colonies derived from the recipient were picked and patched in grids of 100 and were tested similarly.

RESULTS

Cloning of new chimeric plasmids. The isolation of six chimeric plasmids, the pRS series, in which pSC101 was used as vector for cloning *Eco*RI generated segments of F DNA has been described (32). An additional seven chimeric plasmids have also been isolated by similar methods (7; Skurray, Nagaishi, and Clark, in preparation). All but one of these are multifragment chimeras carrying several contiguous *Eco*RI fragments of F DNA. For the analysis described here, chimeric plasmids carrying only single *Eco*RI fragments of the *tra* region

were desirable; for other experiments in which larger amounts of isolated DNA were needed, a chloramphenicol-amplifiable cloning vector derived from ColE1 seemed appropriate. Preliminary studies with pSC101 chimeras indicated that F *Eco*RI fragments f6, f15, f1, f17, f19, and f2 spanned the entire *tra* region. Therefore, we have cloned all these fragments (except f19) separately using ColE1::Tn*3* (RSF2124) as cloning vector (the pSH series; see Materials and Methods and Fig. 1). *Eco*RI fragments f7, f5, and f3 have also been cloned separately onto RSF2124 (34) and have been reported elsewhere (30). The F DNA carried by these various chimeras is depicted in Fig. 2, together with the revised physical map of F cistrons determined from the results presented below.

Order of fragments f17 and f19. Fragments f17 and f19 have been shown to lie between f1 and f2 (26a), but the order has not been determined. To assist the assignment of *tra* genes discussed below, we deduced the order of f17 and f19 relative to f2 from partial digests of pRS31 DNA using the enzyme *Eco*RI.

The two possible orientations of the F DNA fragments in pRS31 relative to pSC101 are shown in Fig. 3A and 3B, in which the molecules are drawn after cutting at the *Sal* I site; the *Sal* I site is in pSC101 DNA 0.66 kb from the *Eco*RI site (18). It can be seen that the presence of two partial digestion fragments of 1.56 and 0.90 kb would be characteristic of orientation B with f17 and f19 nearest the *Sal* I site on pSC101 DNA. In addition, the alternatives for the order of f17 and f19 relative to f2 (shown in Fig. 3B and 3C) could be distinguished by the size of the third small partial digestion fragment. Linear pRS31 DNA was generated by the action of *Sal* I; the progress of the *Eco*RI digestion of this linear DNA is shown in the gel in Fig. 3 (slots A-E). Three partially digested fragments with sizes of 1.60, 1.39, and 0.93 kb can be identified; these sizes are only consistent with the arrangement diagrammed in Fig. 3B. Thus, the order of the F DNA fragments is f17, f19, f2.

Assignment of *tra* cistrons to the *Eco*RI fragment of F. Prior genetic analyses of *tra* cistrons were difficult because of the incompatibility barrier which prevents cells from stably carrying two F elements. This barrier encoded for by cistrons on *Eco*RI f5 of F (17, 23, 27, 31, 32, 37) does not exist between F and the chimeric plasmids, and complementation can be determined in stable heterozygote cells.

Complementation analysis of the *tra* cistrons by use of all 13 of the pRS plasmids, which span 90% of the F factor (7; Skurray, Nagaishi, and Clark *in* J. Ou and E. Raizen, ed., Pili, in press)

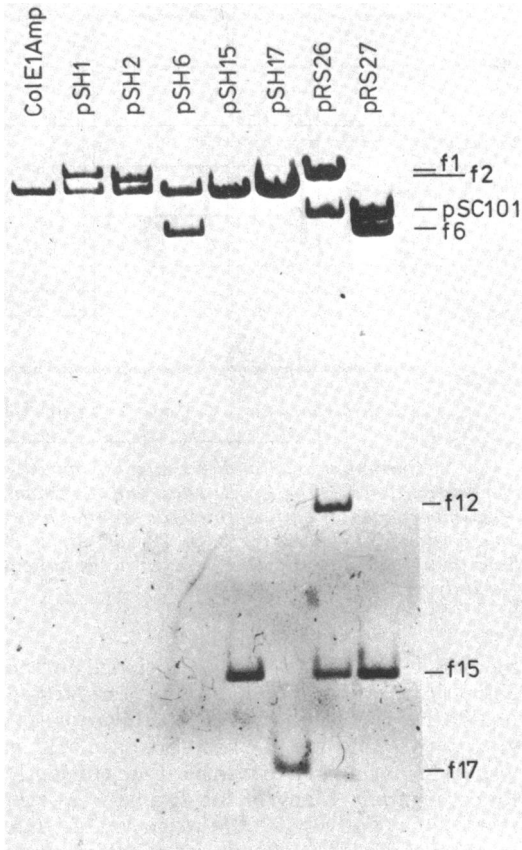

FIG. 1. *EcoRI digestion of pSH and pRS plasmids. The DNAs indicated were digested with EcoRI and run on a 1% agarose slab gel. Note that fragments f1 and f2 are not resolved in the pRS26 track. Fragment f19 is not visible under these conditions, but other experiments in which a layer of 10% polyacrylamide was used under a slab of 1% agarose demonstrated that f19 was present in pRS26 and pRS31 and absent from all of the pSH plasmids.*

showed that the entire *tra* operon is encoded on fragments f6, f15, f1, f17, f19, and f2. In this report we describe in detail complementation analyses with both pRS and pSH plasmids which carry these fragments. In some crosses, one of a number of defined amber-suppressible *tra* mutants of F*lac* was introduced, by conjugation, from a suppressing (Su^+) strain into a nonsuppressing (Su^-) strain carrying a chimeric plasmid. In other crosses, the chimeric plasmid under test was introduced by transformation into a Su^- strain carrying a *tra* mutant of F*lac*. The resulting strains stably carried both F*lac* and the chimeric plasmid, although they were

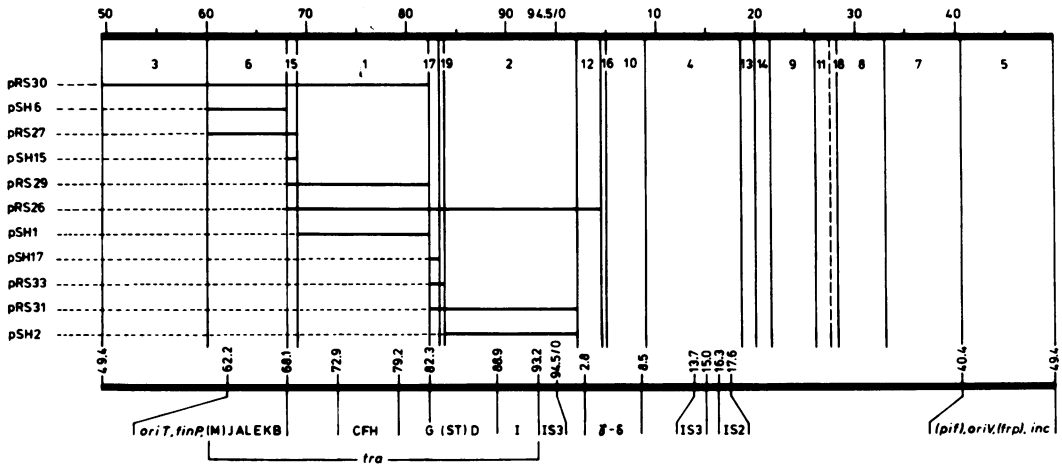

FIG. 2. *Current map of the F sex factor. Kilobase coordinates are shown above the heavy horizontal lines. The vertical lines define EcoRI sites, and the fragment numbers are given between these lines. The EcoRI fragments carried by various chimeras are shown by continuous horizontal lines. The map incorporates data from a variety of sources (7, 13, 20, 22, 26a, 27, 32, 37, 42, 44; Helinski, personal communication) as well as from the results presented here. Parentheses indicate that the enclosed cistrons have not been mapped as precisely as the other cistrons.*

merodiploid for that F DNA included in the chimeric plasmid. These strains were tested qualitatively by replica-plating methods for sensitivity to F pilus-specific bacteriophage M12 as well as for the ability to transfer F*lac* efficiently by conjugation. Many of the strains were also tested quantitatively for the efficiency of DNA transfer of the F*lac* element and of the chimeric plasmid. Strains carrying identical plasmids but constructed by one or the other of the two methods gave similar results, and the qualitative and quantitative results for each strain were in full agreement.

The qualitative results (data not shown) allowed the following conclusions to be made: (i) *Eco*RI fragment f6 carries *traJ, traA, traL, traE, traK,* and *traB*; (ii) f1 carries *traC, traF,* and *traH*; and (iii) f2 carries *traD* and *traI* (Fig. 2). Quantitative results confirming these assignments are presented in Table 1. pSH6 and pRS27 share only f6 but promote efficient transfer of F*lac* mutants carrying mutations in the cistrons of group (i) above. We can distinguish complementation from recombination since complementation results in transfer of F*lac* elements which are still Tra^-, whereas recombination results in transfer of Tra^+ F*lac* elements. The transfer promoted by f6 is due to complementation, rather than recombination, since most of the F*lac* elements transferred without cotransfer of the chimeric plasmid were still mutant (Tra^-) in subsequent tests.

pSH1 and pRS29 possess only f1 in common, and both stimulated DNA transfer from *traC, traF,* and *traH* mutants of F*lac*. In all except one case, the transfer was due to complementation. Such a high level of recombination was observed between pSH1 and JCFL27 (*traH27*) that we could not detect any complementation. Together with the genetic order of *traC, traF, traH,* and *traG* (20, 22) and with the data on *traG* discussed below, these results assign *traC, traF,* and *traH* to f1. In contrast to the low level of DNA transfer, complementation of *traC, traF,* and *traH* mutants to M12 sensitivity (i.e., F pilus synthesis) by pRS29 was efficient. We suspect that the partial *traG* gene product expressed by pRS29 and pSH1 (see below) may inhibit DNA transfer by negative complementation. Consistent with this, pRS26, which synthesizes a complete *traG* protein, gave efficient complementation of *traC, traF,* and *traH* mutations (Table 1).

pRS31 and pSH2 share only *Eco*RI f2; both complement *traD* and *traI* mutations, and thus both *traD* and *traI* must be on *Eco*RI fragment f2. As discussed below, pRS31 also carries and expresses the surface exclusion cistrons *traS* and *traT* (M. Achtman, S. Schwuchow, B. Kusecek, and N. Willetts, manuscript in preparation).

***traG* cistron straddles an *Eco*RI site.** The data in Table 1 indicate that the *traG81* mutation was not complemented for DNA transfer by the chimeric plasmids tested, except for pRS26 in which *Eco*RI f1, f17, f19, and f2 are physically linked (the apparently efficient transfer in the

pSH1 × JCFL81 cross was due to recombination). Four other *traG* mutants were analyzed, and none of the additional four mutants was complemented for DNA transfer by either pRS29 (f15, f1) or pRS31 (f17, f19, f2). This preliminary indication that *traG* straddles an *Eco*RI site between fragments f1 and f17 is supported by the results in Table 2. Three *traG* mutations (*traG24, traG81,* and *traG100*) yielded recombinants with pRS29 but only Tra$^+$ revertants from cells carrying pRS31. Two other mutations (*traG98, traG101*) yielded recombinants with pRS31 but not with pRS29. Finally, when pSH1, pSH17, and pSH2 were tested, *traG81* recombined only with pSH1 and *traG98* only with pSH17. These results demonstrate that part of *traG* is on fragment f1 and part is on f17.

It has previously been observed that some *traG* mutations affect both DNA and F piliation, whereas others prevent DNA transfer but still allow F pilus synthesis (3, 4); one of the hypotheses presented was that the *traG* product is bifunctional (4). The following three observations confirm this hypothesis. (i) Nonpiliated *traG* mutants (*traG24, traG81*) were complemented for F pilus production by plasmids carrying f1 (pRS29, pSH2) but not for DNA transfer. (ii) pRS30 carries the promoter-proximal portion of the *tra* operon up to the *Eco*RI site in *traG*. Cells carrying pRS30 are sensitive to male-specific DNA bacteriophages (32; Skurray, Nagaishi, and Clark, *in* J. Ou and E. Raizen, ed., Pili, in press) and synthesize F pili in equal numbers to (F*lac*)$^+$ cells (Fig. 4). (iii) Both of the *traG* mutations tested which affect F pilia-

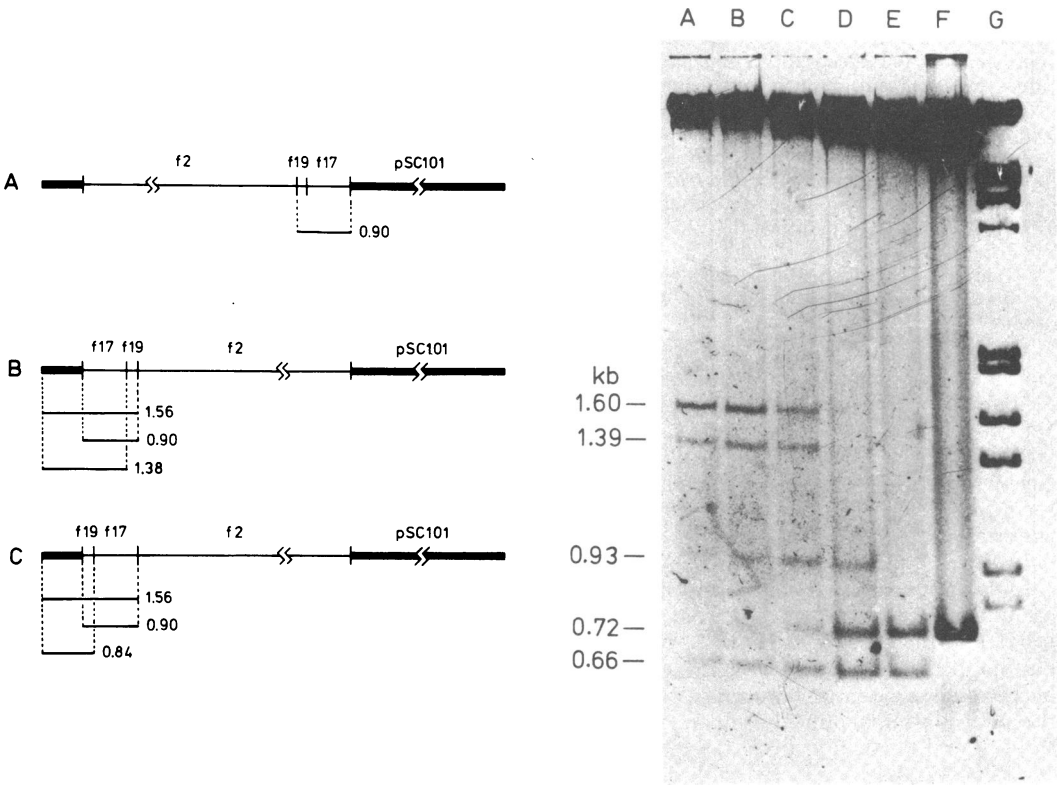

FIG. 3. *Possible arrangements for the EcoRI fragments of pRS31. The molecules are drawn after cutting at the Sal I site in pSC101; the smaller fragments are drawn to scale. Horizontal lines show partial digestion fragments and are labeled with their predicted sizes in kilobases. The right-hand panel is a 1.6% agarose slab gel showing the progress of EcoRI digestion of pRS31 DNA that has been predigested with Sal I. Slots A–E show samples at 2, 4, 10, 20, and 60 min after starting the reaction. The digestion is complete in 60 min (slot E), but at earlier times (slots A–D) three partial digestion products are seen and these are labeled with their estimated sizes in kilobases (1.60, 1.39, and 0.93 kb). Slot F shows an EcoRI digest of pRS31 and allows identification of the 0.72-kb fragment as f17. The 0.66-kb fragment comes from pSC101 DNA. Note that fragment f19 is not visible under these conditions. Slot G is phage λ DNA digested with HindIII and EcoRI as a molecular-weight marker (24).*

TABLE 1. *Complementation tests*

tra mutation (F*lac* mutant)	Transfer efficiency[a] with plasmid: pSH6 (f6)[b]	pRS27 (f6, f15)	pRS29 (f15, f1)	pSH1 (f1)	pRS26 (f15, f1, f17, f19, f2, f12)	pRS31 (f17, f19, f2)	pSH2 (f2)
traM102 (JCFL102)	*25*	*148*	0.0002	0.002	0.0003	0.0008	
traJ90 (JCFL90)	*18*	*62*	—[c]	—[c]		—[c]	—[c]
traA1 (JCFL1)	*2*	*41*	—[c]	—[c]		—[c]	—[c]
traL311 (EDFL2)	*64*	*96*					
traE18 (JCFL18)	*5*	*42*	—[c]	—[c]		—[c]	—[c]
traK105 (JCFL105)	*14*	*41*	0.0007	0.001	0.0008		
traB71 (JCFL71)	*34*	*45*	0.0006	≤0.00007	≤0.00006	—[d]	—[d]
traC5 (JCFL5)	≤0.0001	≤0.00005	*0.9*	*9.1*	*56*	—[c]	—[c]
traF13 (JCFL13)	—[c]	—[c]	*1.9*	*18*	*29*	—[c]	—[c]
traH27 (JCFL27)	—[d]	—[d]	*0.9*	1.1	*45*	0.0005	—[d]
traG81 (JCFL81)	—[d]	—[d]	0.002	2.6	*45*	0.0006	0.005
traD14 (JCFL14)	—[c]	—[c]	—[c]	—[c]	*64*	*44*	*2*
traI41 (JCFL41)					*52*	*17*	*23*

[a] The numbers presented are the transfer efficiency per 100 donor cells introduced into the mating mixture from donor cells carrying both the F*lac tra* mutant (horizontal row) and the chimeric plasmid (vertical row) indicated. Cells carrying a *tra*$^+$ F*lac* element and any one chimeric plasmid showed a transfer efficiency of approximately 100, except for those containing pRS29 where the values were occasionally approximately 5. All values in italics result from complementation. The values with JCFL81 (carrying *traG81*) and JCFL27 (carrying *traH27*) × pSH1 were the result of recombination. Other values are considered to demonstrate lack of complementation and are generally similar to the values for spontaneous transfer of the F*lac* mutants from Su$^-$ cells (3, 4, 41). An additional four *traB*, two *traH*, and one *traI* mutations have been tested quantitatively. The results were equivalent to *traB71*, *traH27*, and *traI41*, respectively, except for *traB2*, which is an amber mutation and gave 10-fold poorer complementation than the missense mutations *traB45*, *traB62*, *traB68*, or *traB71*. A blank space indicates that this combination has not been tested.

[b] F *Eco*RI fragments.

[c] Qualitative complementation tests yielded a negative result.

[d] Qualitative complementation tests with a different *tra* mutation in the same cistron yielded a negative result.

tion map to the left of the *Eco*RI site between f1 and f17, whereas two of the three *traG* mutations which allow F pilus synthesis map to its right (Table 2). Thus, the promoter-distal portion of *traG* is needed for DNA transfer but not for F pilus synthesis, and we can conclude that the *traG* protein is bifunctional, with independent roles in F pilus synthesis and DNA transfer.

Location of cistrons involved in surface exclusion. *traD* is efficiently expressed by pSH2 and must be located on f2. Willetts (42) has mapped a site called *traS* between *traG* and *traD* which is essential for surface exclusion. Of the pRS plasmids, only those carrying at least f17, f19, and f2 (such as pRS31) express surface exclusion (7, 32). A genetic analysis to be described elsewhere (Achtman et al., manuscript in preparation) has subdivided the *traS* site into two surface exclusion cistrons, *traS* and *traT*, and has demonstrated that both are carried and expressed by pRS31. However, neither pRS33 (f17, f19) nor pSH17 (f17) nor pSH2 (f2) expressed detectable surface exclusion (data not shown). In addition, none of the latter three plasmids was able to exert immunity to lethal zygosis (33) (data not shown), a property expressed by chimeric plasmids carrying f17, f19, and f2 (7, 32). Thus, the location of cistrons coding for surface exclusion and immunity to lethal zygosis is on the multifragment region defined by f17, f19, and f2 but cannot currently be defined more precisely.

***traI* cistron and the transfer operon.** The *traI* cistron and its location in the *tra* operon were originally defined with JCFL65 used as a *traI* reference mutant. We have now found (data not shown) that JCFL65 carries several *tra* mutations in different cistrons and have therefore reinvestigated the mutations originally assigned to *traI* and the relation of *traI* to the *tra* operon.

The *traI* cistron is now defined by the reference mutant JCFL41, carrying *traI41*. JCFL41 is complemented by pRS31 (Table 1). We have isolated two mutant plasmids (called pBE1 and pBE2) after in vitro mutagenesis of pRS31 DNA (21) which do not complement JCFL41. As shown in Table 3, they also fail to complement JCFL40, which was previously concluded to carry a *traI* mutation, *traI40* (43). Both pRS31 mutants are still *traD*$^+$ (Table 3) and surface

TABLE 2. *Recombination with traG mutations*[a]

F*lac* *traG* mutation	Chimeric plasmids (*Eco*RI fragments)				
	pSH1 (f1)	pRS29 (f15, f1)	pSH17 (f17)	pRS31 (f17, f19, f2)	pSH2 (f2)
traG24[b, c]		20		≤6	
traG81	25,000	20	≤0.5	0.4	≤1
traG100		30		1	
traG98	≤0.5	≤0.2	20	70	≤0.4
traG101		≤0.3		40	

[a] Donor cells carrying the F*lac* mutant and the chimeric plasmid indicated were tested as in Table 1. The values represent the number of Tra$^+$ Lac$^+$ exconjugants obtained per 10^6 donor cells. Between 10 and 200 Lac$^+$ exconjugant colonies (as many as were available) were screened in each test for the ability to transfer. Values of 1 or lower are considered to represent reversion, whereas values of 20 and higher indicate recombination. In the latter case, most or all of the transferred F*lac* elements were Tra$^+$.

[b] *traG24* is quite leaky, and the relatively high number of Tra$^-$ Lac$^+$ exconjugants obtained rendered this test insensitive. The value of ≤6 probably represents a lack of recombination.

[c] *traG24* and *traG81* result in resistance to M12, whereas *traG100*, *traG98*, and *traG101* do not affect F piliation.

exclusion positive (data not shown). Therefore the mutations in pBE1 and pBE2 are assigned mutation designations *traI215* and *traI224*. To reinvestigate the question of whether *traI* is part of the transfer operon, we tested polar Mu-1 insertions in *traD* for complementation by pRS31 and by the pRS31 *traI* mutants. We found that pBE1 and pBE2, the two *traI* mutants of pRS31, complement the polar Mu-1 insertions 5 to 10% as efficiently as their *traI*$^+$ parent pRS31 (Table 3). These results suggest polarity and that *traI* is in the *tra* operon. However, the fact that some complementation is observed indicates that the polarity is not absolute and that an inefficient promoter may exist between *traD* and *traI*.

***traM* cistron.** An F*lac* mutant, JCFL102, originally thought to carry a frameshift *traI* mutation is now reclassified as carrying a mutation in a new cistron, *traM*. JCFL102 is only complemented by chimeric plasmids carrying f6 (Table 1). Two mutants (pBE3 and pBE4) have been isolated by in vitro mutagenesis (21) or pRS27 (f6, f15) DNA which do not complement JCFL102 (data not shown). These two mutants do complement mutations in all other known cistrons on f6 (*traJ*, *traA*, *traL*, *traE*, *traK*, *traB*, and *finP*) and are mobilized by F factors (i.e., *oriT*$^+$). Thus, JCFL102 and these two additional mutants provide a reference set of *traM* mutations. JCFL102 codes for F pilus production (4, 43). *traM* has been mapped between *traJ* and *finP* (our unpublished data) and must lie outside the *tra* operon.

The *finP* cistron. Skurray et al. (32) have shown that the FinOP$^+$ plasmid R1 inhibits F pilus production by the chimeric plasmid pRS30. They concluded that *finP* was expressed by pRS30 and probably maps on *Eco*RI fragment f6 in accordance with the location determined by Willetts et al. (44). We have shown that both pRS27 and pSH6 can supply the *finP* product such that cells carrying these chimeric plasmids plus the *finO*$^+$ *finP* sex factor R6-5*drd*50 (29) plus the *finP301* mutant of F*lac*, EDFL51, (15) are repressed for F pilus production. Thus, *finP* maps on f6 and is expressed by chimeric plasmids carrying f6.

Transfer origin. Crisona and Clark (11) demonstrated that chimeric plasmids which carry f6 and f15 were mobilized by F-like sex factors. They interpreted these data to mean that a site called *oriT* (40) may exist on f6 which allows specific mobilization by F-like sex factors of that DNA (7, 16). Since neither of the vectors used for cloning (pSC101 and RSF2124) is itself

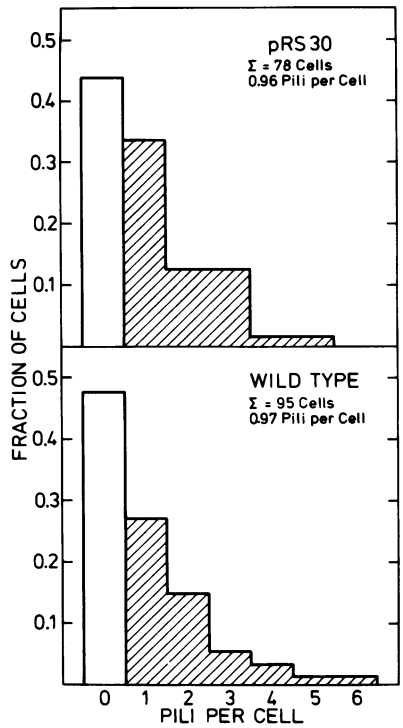

FIG. 4. *Frequency distribution of cell-bound F pili. pRS30 and Flac (JCFL0)-carrying cells were examined in a Siemens Elmiskop Ia electron microscope at a magnification of 28,000 ×. Pili were visualized by adsorption of M12 bacteriophage and staining with phosphotungstic acid (2%, wt/vol, pH 7.9). The data are from two independent determinations for each strain.*

TABLE 3. *Complementation tests with traI mutants*

F*lac tra* mutation	Chimeric plasmid							
	pRS31		Mutants of pRS31[a]				None	
			pBE1		pBE2			
	Transfer efficiency[b]	Tra$^+$ exconjugants[c]	Transfer efficiency	Tra$^+$ exconjugants	Transfer efficiency	Tra$^+$ exconjugants	Transfer efficiency	Tra$^+$ exconjugants
tra$^+$	100	—[d]	110	—	111	—		
traI40	6.8	0.15	0.04	0.72	0.02	0.53	0.007	0.005
traI41	24.3	0.02	0.003	0.02	0.02	0.50	0.005	0.01
traD39	5.1		36	0.02	18	0.02	0.03	0.002
traD298::Mu[e]	7.6	0.03	0.44	0.03	0.54	0.01	0.003	1.0
traD299::Mu[e]	17.1	0.03	1.1	0.03	1.1	0.005	0.00005	—

[a] pBE1 and pBE2 are mutants of pRS31 which are defined as carrying *traI215* and *traI224*, respectively, on the basis of the results presented here.

[b] Tests were performed as described in Table 1.

[c] Tra$^+$ exconjugants refers to the fraction of Tra$^+$ clones among the transferred F*lac* elements; 30 to 600 colonies were screened in different experiments.

[d] A dash indicates that the results would not be relevant; a blank space indicates that the test was not performed.

[e] The F*lac* element MPFL2 which carries the mutation *traD254::Mu* has been described (20). The mutation *traD253::Mu* carried by MPFL9 is caused by a Mu-1 insertion mapping in or promoter-proximal to *traD* and is thus similar to *traD254::Mu*.

efficiently mobilized by F*lac* from Rec$^+$ cells, we could readily test whether f6 does code for mobilization. We found that pRS27 (f6, f15) and pSH6 (f6) are both efficiently mobilized by F*lac*. Approximately 90% of the Lac$^+$ exconjugants from matings with either (F*lac*, pSH6)$^+$ or (F*lac*, pRS27)$^+$ donors also carry the chimeric plasmid. In contrast, RSF2124, pRS29, pRS31, pSH1, and pSH2 are cotransferred at an efficiency of less than or equal to 1%. Our data thus agree with the conclusion of Crisona and Clark (11) that f6 carries a site, presumably *oriT*, which allows mobilization.

DISCUSSION

Genetic analyses of the *tra* cistrons of F have been conducted independently by two groups, Ohtsubo and co-workers (25, 26) and Achtman, Willetts, and Clark (3, 4, 43); the cistrons defined by each group have been tentatively correlated with one another (43). The physical location of Ohtsubo's six *tra* cistrons was defined by DNA heteroduplex analysis of deletions of F8 (F*gal*) (13, 28). The correlations between Ohtsubo's cistrons and our cistrons (43) have been used (13, 20, 32) as indirect evidence for the approximate map locations of most of the 13 *tra* cistrons then known; *traK*, *traB*, and *traH* were not mapped by these correlations. The results presented here are the first direct demonstration of the physical locations of the *tra* cistrons defined by Achtman, Willetts, and Clark. Conclusions not previously reached are that *traK* and *traB* map counterclockwise of 68 kb, *traH* maps counterclockwise of 82 kb, and *traG* straddles the *Eco*RI site at 82 kb. The map (Fig. 2) of the F*tra* region incorporating these results with those from Davidson's group (13, 28) demonstrates that currently only the *tra* region located on f6 approaches saturation with known cistrons. Fragment f15 is part of a 5-kb segment between *traB* and *traC* with no known cistrons. Although the individual cistrons on f1 and f2 may be quite large, it is at least as likely that the current total of 16 cistrons is a strong underestimate of the total number of cistrons within the approximately 30-kb-long *tra* region.

traM is defined here for the first time and is a DNA transfer cistron mapping far away from the other DNA transfer cistrons. Thus, the clustering, on the map of F, of regulatory cistrons (*finP* and *traJ*), F pilus cistrons (*traA* through to part of *traG*), DNA transfer cistrons (part of *traG*, *traD*, and *traI*), and surface exclusion cistrons (*traS* and *traT*) now suffers from one exception.

The *tra* operon promoter would be expected to map on f6 between its positive control gene, *traJ*, and the first cistron, *traA* (20). Hence, the chimeric plasmids pRS29, pRS26, pRS31, pSH1, and pSH2 are presumably dependent on a promoter within the cloning vector for the transcription of *tra* cistrons. We have found biological function of F DNA with those chimeras, indicating that the vectors used can supply a promoter. Slight differences do exist in the effi-

ciency of complementation of *tra* mutations on F*lac* by the pRS and pSH series of chimeric plasmids. Thus, qualitative tests revealed that complementation by the pRS series resulted in stronger sensitivity to M12 than was observed upon complementation by the pSH series (unpublished data).

The results presented here allow the use of the chimeric plasmids as a convenient mapping kit for *tra* mutations. These plasmids are compatible with F or F-like sex factors, and qualitative complementation tests are both unambiguous and simple to perform. We have recently introduced genetically defined *tra* mutations into the plasmids pRS27, pRS29, and pRS31 (22a) and have used these mutants to define the protein gene products of 12 of the *tra* cistrons (2, 22a). Thus, this set of chimeric plasmids should considerably simplify future analyses of conjugation.

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