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Characterization of the F transfer cistron, *traL*

By N. S. WILLETTS
M.R.C. Molecular Genetics Unit, Department of Molecular Biology, University of Edinburgh

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SUMMARY

The properties of two transfer-deficient F*lac* mutants (in *Escherichia coli* K12) carrying mutations in the cistron *traL* are described. The *traL* product is needed for conjugational DNA transfer and for pilus formation, but not for surface exclusion; it is not plasmid-specific. *traL* maps between *traA* and *traE*; one of the *traL* mutations is apparently polar on *traA*. The properties of Tra$^+$ revertants of this mutation suggest that the *traL* product is directly required for F-specific phage infection.

1. INTRODUCTION

In their analysis of the transfer system of the F prime factor F8 (an F*gal*), Ohtsubo, Nishimura & Hirota (1970) allocated three transfer-deficient mutants to 'group H'. These mutants complemented all well-defined transfer-deficient R100-1 mutants, but not those of 'group X' which seemed to carry mutations affecting several transfer cistrons. One of these group H mutants, F8-N32, was tested by Willetts & Achtman (1972) in their analysis of the transfer system of F*lac*. It was found to carry a mutation in a cistron different to the cistrons *traA* through *traK* identified on F*lac*. This cistron was subsequently called *traL* (Willetts, 1972). The two other group H mutants of Ohtsubo *et al.* (1970) may also carry *traL* mutations, but this has not so far been determined.

This paper describes experiments designed to characterize the *traL* cistron further, by determination of surface exclusion, plasmid specificity and genetic location. Also, it shows that JCFL32, a transfer-deficient F*lac* mutant which could not be classified by Achtman, Willetts & Clark (1972) and Willetts & Achtman (1972), probably carries a *traL* mutation which is polar on *traA*.

2. MATERIALS AND METHODS

Bacterial strains. These are described in Table 1, and also in the footnotes to Tables 2 and 3. JE3512, carrying F8-N32, was kindly supplied by E. Ohtsubo. For convenient reference, the mutation it carries has been designated *traL311*.

Bacteriophage strains and techniques. The sources and techniques for f1, MS2, M12, Qβ and T6 have been described previously (Achtman, Willetts & Clark, 1971, 1972).

Media. These have been described previously (Willetts & Finnegan, 1970) except that Oxoid nutrient plates, rather than L plates, were used.

Mating conditions. The techniques described by Finnegan & Willetts (1971) were used. For determination of the transfer ability of a plasmid, a 30 min mating period was allowed.

Mutagenesis. 10 ml of an exponential phase broth culture containing 2×10^8 cells/ml was centrifuged, and the cells resuspended in 5 ml phosphate buffer. 0·05 ml of ethyl methanesulphonate (EMS) was added, the mixture shaken, and incubated at 37 °C for 30 min. The mutagenized culture was then diluted 100-fold into L broth and incubated overnight to allow growth and mutant segregation.

Table 1. *Bacterial strains*

Strain number	Plasmid	Lac	His	Trp	Spc	Str	T6	Other
ED24	.	−	+	+	R	S	R	.
ED256	F8-N32	−	−	−	S	R	R	Lys⁻
ED2100	EDFL2	+/−	−	−	S	R	R	Lys⁻ Gal⁻
JC3272	.	−	−	−	S	R	R	Lys⁻ Gal⁻
JC5462	.	−	−	+	R	R	R	.
JC6259	JCFL32	+/−	−	−	S	R	R	Lys⁻ Gal⁻
JC6583	F*lac*	+/−	+	−	S	S	S	.
JE3512	F8-N32	−	+	+	S	R	?	Thr⁻ Leu⁻

ED24, JC3272, JC5462 and JC6583 are closely related (Achtman *et al.* 1972; Willetts & Achtman, 1972). ED256, ED2100 and JC6259 are all derivatives of JC3272.

3. RESULTS

Construction of EDFL2. In order to determine the properties corresponding to the *traL311* mutation carried by F8-N32, using the same (mainly Gal$^+$) strains used for analysis of transfer-deficient F*lac* mutants, an F*lac traL311* mutant was required. This was constructed by recombination between F8-N32 and wild-type F*lac*.

0·1 ml of an exponential phase broth culture of JC6583 (T6^S Spc^S, carrying F*lac*) was mixed with 0·1 ml of a shaken overnight broth culture (F$^-$ phenocopy) of ED256 (T6^R Spc^S, carrying F8-N32) diluted 1:10 with fresh broth. After 45 min incubation to allow F*lac* transfer to ED256, 0·2 ml of a UV-irradiated T6 suspension (10^{11} particles/ml) was added and the mixture incubated for 20 min to kill the donor strain, JC6583. 0·6 ml broth was added, and the mixture incubated for a further 60 min to allow recombination between the two F prime factors. 1·0 ml of an exponential phase culture of the recipient strain ED24 (Lac$^-$ T6^R Spc^R Str^S) was then added, and the mixture incubated for 45 min. This allowed transfer from ED256 cells both of wild-type F*lac*, and of any F*lac traL311* recombinants (via complementation by *traL*$^+$ alleles expected to be present on other F prime elements in the same cell). 0·25 ml of the mating mixture was diluted to 1·0 ml with fresh broth, and incubated for 45 min to permit expression of the *tra*$^+$ cistrons and pilus formation by cells carrying wild-type F*lac*. 1·0 ml of the F-specific phage MS2 (10^{12} pfu/ml) was then added, and the mixture incubated for 30 min; this killed cells of

ED24 carrying wild-type F*lac*, but not those carrying F*lac traL311* which do not make pili. Excess MS2 phages were removed by Millipore filtration and washing, and the mixture was plated on medium selective for Lac$^+$[Spc^R] progeny.

Table 2. *P1 transductional complementation*

P1 donor strain	Plasmid	Deletion end-point†	Plasmid in transductional recipient*	
			F8-N32	EDFL2
ED256	F8-N32	.	< 1	< 2
ED2100	EDFL2	.	< 1	< 1
JC6259	JCFL32	.	NT	< 1
KI813	.	*traK*$^+$ *traB*$^-$	200	NT
KI819	.	*traK*$^+$ *traB*$^-$	400	NT
KI527	.	*traE*$^+$ *traK*$^-$	420	240
KI805	.	*traA*$^+$ *traE*$^-$	< 1	1
KI838	.	*traJ*$^+$ *traA*$^-$	< 1	< 3
KI846	.	*traJ*$^+$ *traA*$^-$	NT	< 3

* The figures give the % complementation, compared to donor strains carrying wild-type F8, F*lac*, or an integrated F, as appropriate. NT signifies not tested. The strains and methods for P1 transductional complementation and for mapping using the Hfr deletion strains with KI numbers were as described by Willetts & Achtman (1972) and Ippen *et al.* (1972), respectively, except that for complementation of EDFL2, the final recipient was ED24 rather than JC5465 (its His$^-$ Trp$^-$ ancestor), and for complementation of F8-N32, the final recipient was ED365 (a Gal$^-$ P1^R derivative of JC5455, the P1^S ancestor of JC5465).

† The map order of this part of the transfer region is *traJ traA traE traK traB* (Ippen *et al.* 1972.)

The resulting clones were patched on the same selective medium and grown overnight, replicated to nutrient plates and incubated 6–8 hr., and then replica-plate mated with the Lac$^-$ Str^R strain JC5462, selecting Lac$^+$[Str^R] progeny. Tra$^-$ clones detected by this procedure (about 1 % of the total Lac$^+$[Spc^R] progeny) were purified, and the plasmid in one (designated EDFL2) was tested further to confirm that it was an F*lac traL311* element. For this purpose EDFL2 was transferred from ED24 to JC3272 by a variant of the complementation technique described by Achtman *et al.* (1972); a transient population of Tra$^+$ F*his*/EDFL2 heterozygous cells served as the donor culture. The resulting strain was numbered ED2100.

The properties of EDFL2. As expected from the F-specific phase-resistant properties of JE3512 (Ohtsubo *et al.* 1970), ED2100 was resistant to the DNA phage f1 and to the RNA phages M12 and Qβ.

Complementation tests using the conjugational (Achtman *et al.* 1972) or transductional (Willetts & Achtman, 1972) methods showed that EDFL2 complemented mutants in all eleven transfer cistrons *traA* through *traK*. It did not, however, complement F8-N32 in reciprocal transductional complementation tests (Table 2), indicating that it carried the *traL311* mutation. One feature of these tests was the high efficiency of complementation by wild-type F*lac*: 93 Lac$^+$[Spc^R] progeny were obtained per 10^6 P1 grown on JC3273 (the F*lac* derivative of JC3272). This level is

Table 3. *Suppressibility of* traL311 *and* tra-32

Parental strain	Suppressor	Transfer ability	
		EDFL2	JCFL32
JC6256	Su^-	$< 5 \times 10^{-5}$	3×10^{-3}
JC6255	Su_I^+	$< 5 \times 10^{-5}$	4×10^{-4}
M267	Su_{II}^+	$< 5 \times 10^{-5}$	4×10^{-3}
JC6650	Su_{III}^+	$< 6 \times 10^{-5}$	6×10^{-4}
M315	Su_{ochre}^+	$< 7 \times 10^{-5}$	8×10^{-3}
M279	Su_{UGA}^+	0·10	0·15

The parental strains and the suppressor loci which they carry have been described by Achtman *et al.* (1972), except for M267 which is a Su_{II}^+ derivative of JC6256. Transfer ability was measured in a 30 min mating with JC3272 selecting $Lac^+[Str^R]$ progeny. The results are expressed as the number of progeny per 100 donor cells.

similar to that shown by *traD* mutants and about twenty-fold higher than for mutants in other cistrons (Willetts & Achtman, 1972).

EDFL2 was transferred (via complementation with F*his*) to strains carrying various suppressor loci, and the quantitative transfer abilities of these derivatives were measured (Table 3; JCFL32 is discussed below). *traL311* was not suppressed by Su_I^+, Su_{II}^+, Su_{III}^+ or Su_{ochre}^+ alleles, but was suppressed, though rather poorly, by a Su_{UGA}^+ allele. It is therefore presumed to be a UGA nonsense mutation. Four other transfer-deficient F*lac* mutants were suppressed by Su_{UGA}^+ to give similar low levels of transfer (Achtman *et al.* 1972).

Ohtsubo (1970) and Ippen, Achtman & Willetts (1972) have presented maps of the transfer regions of F8 and F*lac* respectively, but *traL* was included in neither of these maps. *traL* was therefore mapped by P1 transductional complementation, using the methods and Hfr deletion strains described by Ippen *et al.* (1972). The deletion strain KI527 (and strains with shorter deletions) retained both *traL* and *traE*, whereas the deletion strain KI805 (and strains with longer deletions) had lost both of these cistrons (Table 2). *traL* is therefore located on one side or the other of *traE*; no deletion strain was available to determine the order of *traE* and *traL* (but see below).

Surface exclusion. The surface exclusion indices of JC3272 derivatives carrying either F8-N32 or EDFL2 were measured in crosses with the Hfr strain KL98, as described by Achtman *et al.* (1971). These indices were 410 and 830 respectively, similar to the index of a strain carrying a wild-type F*lac* element. *traL311* therefore has no effect on surface exclusion.

Complementation by F-like plasmids. Previous experiments have shown that products able to replace those of the eleven F transfer cistrons *traA* through *traK* can all be supplied by ColV2 and ColVB*trp*, and most by R100-1 and R1-19 (Willetts, 1971; Alfaro & Willetts, 1972). However, R100-1 and R1-19 could not supply products corresponding to those of the F*lac traI* or *traJ* cistrons; neither could they supply *traA* products giving, as does that of F*lac*, high efficiencies of plating of F-specific RNA phages (*op. cit.*).

These experiments have been extended to include *traL* (Table 4; JCFL32 is

discussed below). All four F-like plasmids could supply a *traL* product allowing transfer of EDFL2, and cells carrying EDFL2 together with R100-1 or R1-19 showed high efficiencies of plating of RNA phages. The *traL* product is therefore not plasmid-specific.

Table 4. *Plasmid specificities of* traL311 *and* tra-32

(*a*) *Transfer complementation*

Plasmid*	Lac$^+$ progeny per 100 donor cells: EDFL2	JCFL32
ColV2	42	NT
ColVB*trp*	105	NT
R100-1	115	100
R1-19	120	195
R64-11	$2{\cdot}4 \times 10^{-2}$	NT
ColIb*drd*	$1{\cdot}7 \times 10^{-1}$	NT

(*b*) *RNA phage plating efficiencies*

	Relative efficiency of plating†: R100–1		R1–19	
F*lac* plasmid	M12	Qβ	M12	Qβ
none	1	3	20	3
F*lac*	85	95	45	50
EDFL2	50	55	80	85
JCFL32	4	7	25	15

* Transfer complementation by ColV2 was measured in transient populations of heterozygous cells, and by other plasmids in stable derivatives (Alfaro & Willetts, 1972). The plasmids, strains and techniques, are also detailed there. NT signifies not tested.

† The host strain was JC3272. The efficiencies of plating of M12 and Qβ on the F*lac* derivative of JC3272 were taken as 100 %.

Complementation by I-like plasmids. Neither ColIb*drd* nor R64-11 could supply a *traL* product which would allow transfer of EDFL2 (Table 4). This extends previous observations that these plasmids could not supply products able to replace those of the F transfer cistrons *traA* through *traK*, and supports the hypothesis that the transfer systems of F-like and I-like plasmids are genetically dissimilar (Willetts, 1970; Alfaro & Willetts, 1972).

The properties of JCFL32. The mutation carried by the transfer-deficient F*lac* mutant JCFL32 was not classified by Achtman *et al.* (1972) and Willetts & Achtman (1972), since although it gave no complementation of F*lac traA1* or F*lac traA25* in P1 transductional complementation tests, it gave intermediate levels of complementation with F*lac traA1* (which is amber-suppressible) in the more sensitive conjugational complementation tests. Quantitative conjugational complementation experiments have since shown that JCFL32 complements F*lac traA1* 0·014 times as well as does wild-type F*his*. In addition, P1 transductional complementation tests

using P1 grown on JC6259 (which carries JCFL32) as donor, and ED2100 (which carries F*lac traL311*) as recipient, showed that there was no complementation between these mutants (Table 2). JCFL32 therefore seems to carry a *traL* mutation.

The nature of the mutation carried by JCFL32 was investigated by transferring JCFL32 to strains carrying various suppressor loci. It was found to carry a poorly suppressed UGA nonsense mutation (Table 3). JCFL32 showed a fairly high background level of transfer from Su^- strains, but UGA mutations are frequently found to be leaky (Model, Webster & Zinder, 1969). The high level of transfer from a Su^- strain, together with the qualitative nature of the replica-plate mating technique used, was probably responsible for previous misclassification of *tra-32* as a non-suppressible mutation (Achtman *et al.* 1972).

Experiments measuring the properties of cells carrying both JCFL32 and either R100-1 or R1-19 showed that although these cells transferred JCFL32 at wild-type level, they plated F-specific RNA phages with much reduced efficiencies (Table 4). This behaviour is similar to that of *traA* mutants (Willetts, 1971; Alfaro & Willetts, 1972), but not the *traL* mutant (see Table 4 and above).

Cells carrying JCFL32 therefore lack the *traL* product and, to a large extent, the *traA* product. The two simplest explanations for this are that either the UGA mutation *tra-32* is a polar mutation in *traA* or (more likely) *traL*, both cistrons being part of the same operon, or that JCFL32 carries both a UGA *traL* mutation and a leaky *traA* mutation. Attempts were made to distinguish between these two possibilities by isolating and characterizing Tra^+ revertants of JCFL32.

Tra^+ revertants of JCFL32. Revertants were selected in matings between JC6259 (Spc^S, carrying JCFL32) and ED24 ($Lac^- Spc^R Str^S$), selecting Lac^+ [Spc^R] progeny. These were patched, and their transfer abilities measured in replica-plate matings with JC5462 ($Lac^- Str^R$), selecting Lac^+[Str^R] progeny.

No spontaneous Tra^+ revertants were found (i.e. $< 0{\cdot}5\,\%$), probably because these would form only a small proportion of the progeny resulting from the background level of transfer ($8 \times 10^{-4}\,\%$). Therefore, three cultures of JC6259 grown from separate single colonies were first subjected to EMS mutagenesis. After this treatment, about 40% of the Lac^+ progeny of ED24 were found to be Tra^+ revertants. Representative revertant clones were picked and purified; in particular, an effort was made to pick clones showing an intermediate level of transfer which might be $TraL^+$ but still carry a leaky *traA* mutation.

The properties of three representative revertants are shown in Table 5. All transferred very efficiently, at frequencies similar to, or slightly less than, wild-type F*lac*. No revertants were found transferring at levels less than 35 % but higher than the level of transfer of JCFL32 itself. It therefore seems unlikely that JCFL32 carries two separate *tra* mutations.

Although the revertants transferred efficiently, they were relatively resistant to all the F-specific phages tested (Table 5). Cells carrying EDFL3 or EDFL4 gave very few or no plaques, and cells carrying EDFL5 gave a reduced number of smaller, fainter plaques. No fully F-specific phage-sensitive revertant was found amongst six Tra^+ revertants tested. In the case of R100-1, a similar Tra^+ partially F-specific

phage-resistant phenotype has been ascribed to a difference in the *traA* product of R100-1 (Willetts, 1971). In an attempt to determine whether the JCFL32 revertants specified altered *traA* products, R100-1 derivatives were made, and the efficiencies with which they plated M12 were measured (Table 5). In all three cases, M12 was plated with high efficiency, indicating that EDFL3, EDFL4 and EDFL5 all specify *traA* products indistinguishable from that of wild-type F*lac*. The simplest interpretation of this result is that *tra*-32 is a mutation in *traL* rather than *traA*, although the reverse is not rigorously excluded.

Table 5. *Tra*$^+$ *revertants of JCFL32*

Revertant plasmid number	Progeny* per 100 donor cells	Relative e.o.p.†‡			Relative e.o.p. of M12 on R100–1 derivative†
		f1	M12	Qβ	
EDFL3	95	10	5	0.5	60
EDFL4	35	< 0·1	< 0·2	< 0·5	50
EDFL5	135	80	35	20	65

* Transfer ability was measured in 30 min matings between the plasmid derivatives of ED24 and JC3272, selecting Lac$^+$[Str^R] progeny.

† The host strain was JC3272. The efficiency of plating on the F*lac* derivative of JC3272 was taken as 100 %.

‡ The plaques formed on the EDFL3 and EDFL5 derivatives of JC3272 were smaller and fainter than those found on the F*lac* derivative of JC3272.

The following explanation for the properties of JCFL32 and its revertants is therefore proposed. JCFL32 carries a single UGA nonsense mutation in *traL* which is polar on *traA*. This accounts for the reduced level of the *traA* product in cells carrying JCFL32, as shown both by complementation experiments and by the lowered efficiency of plating of M12 on their R100-1 derivatives. Reversion of this mutation simultaneously restores a *traL* product and relieves the polarity effect on *traA*. A wild-type *traA*$^+$ product is specified by the revertants, accounting for the high efficiency of plating of M12 on their R100-1 derivatives. However, the revertants so far examined are not true revertants, but pseudo-revertants with an altered amino acid in the *traL* product at the point of the original mutation to UGA. These altered *traL* products can be utilized for synthesis of pili which allow high levels of conjugational DNA transfer, but do not allow normal infection by F-specific phages. As in the case of F*lac traL311*, the *traL* product of R100-1 can substitute for the altered F *traL* products, allowing high levels of infection by M12.

4. DISCUSSION

In summary, the properties of F*lac traL* mutants showed that the *traL* product is needed for DNA transfer and pilus formation, but not for surface exclusion. It is not plasmid-specific, being indistinguishable from the *traL* products of the F-like plasmids ColV2, ColVB*trp*, R100-1 and R1-19. Deletion mapping experiments showed that *traL* maps next to *traE*: since the presumptive *traL* mutation carried

by JCFL32 was polar on *traA* (1·4 % complementation) but not on *traE* (60 % complementation), the order is probably *traA traL traE*.

The relative resistance to F-specific phages of cells carrying Tra^+ revertants of JCFL32 suggests that the *traL* product, as well as the *traA* product (Willetts, 1971), is intimately connected with F-specific phage infection. Experiments are in progress to determine which step(s) of F-specific phage infection are affected, and to test the possibility that *traL* is the cistron which specifies the F pilin subunit protein.

An apparent contradiction in the present results in that whereas the polarity of the presumptive *traL32* mutation on *traA* suggests that *traA* and *traL* are in the same operon and that *traL* is transcribed before *traA*, the $traA^+$ $traL^-$ genotype of the Hfr deletion strain KI805 suggests that *traA* is transcribed before *traL*. If in fact *traA* is transcribed first, the effect of *traL32* on *traA* could be antipolarity (Yanofsky & Ito, 1967), rather than polarity. If, however, *traL* is transcribed first, then KI805 could appear $traA^+$ $traL^-$ because (i) an efficient internal promoter allows the 30 % complementation of F*lac traA1* observed by Ippen *et al.* 1972, or (ii) $traA^+$ is transcribed as a result of 'readthrough' from a λ or a chromosomal cistron to which it has been fused by the deletion carried by KI805. Against this, KI805 is λ $Q^-R^-L^-J^+$ $att^+_{PB'}$. (D. J. Finnegan & N. S. Willetts, unpublished experiments) and transcription of the λ prophage cistrons L through J takes place in the opposite direction, or (iii) the deletion carried by KI805 does not extend into the *traA traL* operon, but a separate *traL* mutation is present. Experiments in progress to elucidate the operon structure of the transfer cistrons should resolve this point.

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