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The genetic locations of *traO*, *finP* and *tra-4* on the *E. coli* K12 sex factor F

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SUMMARY

Using a series of Hfr and F prime deletion strains, the F transfer inhibition gene *finP* has been mapped between *ori* and *traJ*. Marker rescue experiments with the Hfr deletion strains further showed that *traO*, the site of action of the transfer inhibitor, is located immediately to the left of, or possibly within, *traJ*, and that the polar mutation *tra-4* lies in *traK*.

1. INTRODUCTION

Two classes of F mutants resistant to transfer inhibition by R factors such as R100 have been characterized (Finnegan & Willetts, 1971). One class carries recessive mutations in a gene designated *finP* (formerly *traP*); both the F *finP* product and the R100 *finO* product are required for inhibition of F transfer. *finO* is our new designation for the gene previously written *i*, *fi*, or *fin* (Gasson & Willetts, 1975). The other class carries *cis*-dominant mutations at a locus designated *traO*, and these prevent the *finO* and *finP* products from inhibiting synthesis of the *traJ* product and thence of the other *tra* products (Finnegan & Willetts, 1973).

Previously, *finP* has been approximately located on the genetic map of F, between the ϕ_{II}^{R} locus and *traK* (Finnegan & Willetts, 1972), and *traO* has been shown to be very close to *traJ* (Finnegan & Willetts, 1973). In this paper we present more precise mapping data for these loci, and also for *tra-4*, an amber mutation that prevents expression of several genes in the transfer operon (Willetts & Achtman, 1972).

2. MATERIALS AND METHODS

(i) *Bacterial strains.* The properties of the F^- host strains are given in Table 1. The Hfr deletion strains all have the phenotype $Lac^-Gal^-T6^R$; determination of the deletion end points is described by Ippen-Ihler, Achtman & Willetts (1972) and Willetts (1972, 1974*a*).

(ii) *F prime elements.* The F*lac* elements JCFLO and JCFL4 have been described (Achtman, Willetts & Clark, 1971). EDFL50 is a recombinant derivative of JCFLO carrying both *traJ90* and *traO304* (Finnegan & Willetts, 1973). Derivatives of JCFLO carrying deletions extending into F were obtained as temperature-

Table 1. *Bacterial strains*

Strain number	*Lac*	*Gal*	*His*	*Trp*	*Str*	*Spc*	*T6*	Other	Derivation
ED24	−	+	+	+	S	R	R	.	Finnegan & Willetts (1971)
ED2149	−	−	+	+	S	S	R	Bio⁻Nia⁻	From W3110
ED2194	−	+	+	+	S	R	R	Nal^R	Nal^R from ED24
ED3818	−	−	−	−	R	S	R	Lys⁻Nal^R	Nal^R from JC3272
JC3272	−	−	−	−	R	S	R	Lys⁻	Achtman, Willetts & Clark (1971)
JC5462	−	−	−	+	R	R	R	.	Willetts & Achtman (1972)
JC6255	−	+	+	−	S	S	S	Su_I^+	Achtman, Willetts & Clark (1971)
JC7133	−	+	+	+	R	S	R	RecA⁻Arg⁻Ura⁻	Guyer & Clark (1975)
M174	−	−	+	−	S	S	S	.	Willetts (1974*a*)

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resistant survivors of JCFLO (λcI857) cointegrates where λ had been inserted between *traI* and *lac* (S. McIntire & N. S. Willetts, unpublished data).

The F*gal* element F100 was *supE*$^+$ *att*$^+_\lambda$ λ$^-$ (Willetts, 1974*a*). pJC59, pJC61 and pJC62 are F*arg* elements carrying deletions extending into F, isolated in matings between the Hfr strain JC182 and the RecA$^-$ recipient strain JC7133 (Guyer & Clark, 1975).

(iii) *Media.* These have been described (Finnegan & Willetts, 1971).

(iv) *Marker rescue of* tra$^+$ *alleles.* An exponential culture, 0·1 ml, of JC6255 carrying JCFL4 or EDFL50 was mixed with 0·1 ml of a similar culture of an Hfr deletion strain (all those used had lost surface exclusion). After incubation at 37 °C for 45 min, 0·2 ml T6 (10^{11} pfu/ml, treated with 3000 ergs/mm^2 ultraviolet light) was added and incubation continued for 20 min to kill the donor cells. After adding 0·6 ml L broth, incubation was continued for a further 60 min to allow recombination between the F prime element and the chromosomal F segment. F*lac tra*$^+$ recombinants were then selected by adding 1 ml of an exponential culture of ED24, mating for 60 min, and plating on medium selective for Lac$^+$ [Spc^R] progeny. Since some transfer under these conditions is always due to complementation of F*lac tra*$^-$ elements, the Lac$^+$ [Spc^R] progeny were patched and replica plate-mated with JC5462, selecting Lac$^+$ [Str^R] progeny, to determine the proportion of the clones that carried F*lac tra*$^+$ recombinants.

(v) *Determination of the* FinP *phenotype.* Retransfer of JCFLO or F100 from (R100)$^+$ derivatives of the Hfr or F-prime deletion strains was measured as described by Finnegan & Willetts (1973). It should be noted that it is not necessary to measure retransfer of F *finP*$^-$ mutants in these transient heterozygote experiments since transfer inhibition is slow to be established by an incoming *finP*$^+$ gene (Finnegan & Willetts, 1971; Willetts, 1974*b*).

3. RESULTS

(i) *Mapping* traO. In these experiments, appropriate Hfr deletion strains were tested to determine whether they retained *traO*$^+$. It is impossible to do this by any phenotypic test since *traO* mutations are dominant and *traO*$^+$ functions only in *cis*; furthermore, marker rescue of *traO*$^+$ would be very difficult to measure directly. Advantage was therefore taken of the finding that *traO* is closely linked to *traJ* (Finnegan & Willetts, 1973) to determine whether marker rescue of *traJ*$^+$ from an Hfr deletion strain gave co-rescue of *traO*$^+$. For this, EDFL50 (F*lac traO304 traJ90*) was transferred to two *ori*$^+$ TraJ$^-$ strains (with deletions possibly ending within *traJ*) and to an *inc*$^+$ *ori*$^-$ strain (expected to have lost both *traO* and *traJ*). F*lac traJ*$^+$ recombinants were identified as described in Materials and Methods.

These were then tested to determine whether *traO*$^+$ had been coinherited. This was done by replica plate-mating the Tra$^+$ clones with JC3272 (R100)$^+$ to construct (F*lac traJ*$^+$, R100)$^+$ derivatives; these were in turn replica plate-mated with ED2194, selecting Lac$^+$ [Nal^R] progeny, to identify any transferring at low frequency and hence carrying *traO*$^+$.

Of the three Hfr deletion strains, only KI815 gave F*lac traJ*$^+$ recombinants, and of these a large proportion (33/40) had also inherited *traO*$^+$ (Table 2). That this proportion is not even higher, considering the close proximity of *traJ90* and *traO304* (Finnegan & Willetts, 1973), is perhaps due to localized negative interference, since a cross-over between the end-point of the KI815 deletion and *traJ90* is demanded here. Three of the putative F*lac traJ*$^+$ *traO*$^+$ recombinants were purified, and their donor abilities were shown in quantitative crosses to be reduced 500-fold when R100 was present; the donor abilities of three putative F*lac traJ*$^+$ *traO304* recombinants, on the other hand, were not affected by R100. This confirmed the genotypes of the recombinants, and we conclude that KI815 retains *traJ90*$^+$ and *traO304*$^+$ and so carries a deletion ending within *traJ*. *traO* is therefore either within, or to the left, of *traJ*. The other two Hfr deletion strains, even that retaining *ori*, must have suffered longer deletions removing both of these loci.

Table 2. *Marker rescue of* traJ *and* traO

Hfr deletion strain	Deletion end-point +	Deletion end-point −	Lac$^+$[Spc^R] progeny: Frequency (%)	Lac$^+$[Spc^R] progeny: Tra$^+$/total tested	Lac$^+$[Spc^R] progeny: *traO*$^+$/total Tra$^+$ tested
KI815	*ori*	*traJ*	0·66	72/400	33/40
KI825	*ori*	*traJ*	0·38	0/400	—
KI844	*inc*	*ori*	0·53	0/400	—

JC6255 (EDFL50)$^+$ was the donor strain in these experiments measuring marker rescue of *traJ90*$^+$ and co-rescue of *traO304*$^+$.

(ii) *Mapping* finP. For this, attempts were made to determine the *finP* genotype of a series of Hfr deletion strains, by using their (R100)$^+$ derivatives as intermediates in experiments measuring F100 retransfer. Those retaining *finP* should give low frequencies of retransfer while those that are *finP*$^-$ should give high frequencies. As pointed out in Materials and Methods, the *finP*$^+$ gene of F100 does not affect these experiments. The major difficulty is to determine how many cells of the Hfr deletion strain have received F100 in the initial mating. The integrated *inc*$^+$ F factor segment precluded the use of Gal$^+$/Gal$^-$ sectored colonies as a measure of this, and two less satisfactory measures were therefore adopted. Measure (i) calculated F100 retransfer per 100 primary donor cells (Table 3, column 4), and assumed that the recipient abilities of the Hfr deletion strains in the initial mating were similar. Measure (ii) calculated F100 retransfer per 100 Gal$^+$[T6^R Trp$^+$] derivatives formed in the initial mating (Table 3, column 5), and assumed efficient formation of these, possibly by integration of F100 into the chromosome to escape incompatibility.

Taken together, the results (Table 3) allowed the Hfr deletion strains to be divided into three groups. Firstly, those giving low retransfer frequencies of 0·005–0·02 (measure (i)) or 0·4–1·1 (measure (ii)), were taken to be *finP*$^+$. This group includes the known FinP$^+$ strain KI704 (Finnegan & Willetts, 1972), KI527 and KI846 which are expected to be *finP*$^+$ since their deletions end within

the transfer operon, and KI815. KI815 was shown above to carry a deletion ending within *traJ*, and *finP* must therefore be to the left of *traJ*. Secondly, those giving high retransfer frequencies of 0·14–1·1 (measure (i)) and 13–116 (measure (ii)), were taken to be *finP*$^-$. This group includes the known *finP*$^-$ strain KI49 (Finnegan & Willetts, 1972), four strains with deletions ending between *inc* and *ori*, and KI825. KI825 is *ori*$^+$ and was shown above to have lost *traO* and *traJ*. *finP* must therefore be to the right of *ori*, giving the order *ori finP traJ*. The third group of Hfr deletion strains included three other strains (KI137, KI731 and KI772) with deletions ending between *inc* and *ori*; these gave intermediate retransfer frequencies of 0·03–0·1 (measure (i)) and 4·4–7·0 (measure (ii)), for reasons that remain unclear. However, since the first two groups of strains serve to locate *finP* between *ori* and *traJ*, these three other *inc*$^+$*ori*$^-$ deletion strains should also be *finP*$^-$.

Table 3. *Mapping* finP *using Hfr deletions*

Hfr deletion strain	Deletion end-point +	Deletion end-point −	F100 retransfer (i) per 100 primary donor cells	F100 retransfer (ii) per 100 Gal$^+$ intermediate cells
KI704	*traH*	*traG*	0·006	0·9
KI527	*traE*	*traK*	0·020	1·0
KI846	*traJ*	*traA*	0·005	0·4
KI815	*ori*	*traJ*	0·013	1·1
KI825	*ori*	*traJ*	0·14	13
KI137	*inc*	*ori*	0·03	7·0
KI609	*inc*	*ori*	0·39	33
KI731	*inc*	*ori*	0·10	4·4
KI772	*inc*	*ori*	0·05	5·1
KI837	*inc*	*ori*	0·32	55
KI844	*inc*	*ori*	1·1	116
KI848	*inc*	*ori*	0·87	48
KI49	ϕ_{II}	*inc*	0·42	14

The primary donor strain was M174 (F100)$^+$, and the final recipient strain was JC3272.

Confirmatory evidence for the location of *finP* was sought using a series of F*lac* deletion strains derived from F*lac* (λcI857) cointegrates. Two of these (EDFL171 and EDFL181) carrying deletions ending within the transfer operon, were used as *finP*$^+$ controls, and 42 deletions extending beyond *traJ* were screened in the hope of finding one that was *ori*$^+$ *finP*$^-$ (Table 4). However, although F100 retransfer from all 42 strains such as ED2149 (R100, EDFL173)$^+$ took place at high frequencies, showing them to be *finP*$^-$, the F*lac* deletions were themselves always transferred at low frequencies from the Tra$^+$ heterozygous intermediate cells, proving them to be *ori*$^-$. These results therefore merely confirm that both *ori* and *finP* are located to the right of *inc*. Either *ori* and *finP* must be very close, or there is a preferred site(s) for the end of deletions between *inc* and *ori*.

Three F*arg* elements carrying deletions of part of the F factor were also tested to see if they retained *finP*. One, pJC59, carried a deletion ending within the

transfer operon and was therefore expected to be *finP*+. The other two, pJC61 and pJC62, carry longer deletions that have removed all the *tra* genes and also *ori*; they may retain all, or almost all, of the proximally transferred portion of the Hfr chromosome from which they were derived, and their deletions may therefore end within or immediately to the left of *ori* (Guyer & Clark, 1975, and personal communication).

Table 4. *Mapping* finP *using F prime deletions*

F prime plasmid in intermediate	Deletion end-point +	Deletion end-point −	F100 retransfer (%)*	F*lac* transfer or retransfer (%)*
EDFL181	*traH*	*traG*	0·065	0·094
EDFL171†	*traD*	*traI*	0·034	0·072
EDFL173‡	*inc*	*ori*	43	0·037
pJC59	*traC*	*traF*	.	0·3
pJC61	*inc*	*ori*	.	5·0
pJC62	*inc*	*ori*	.	4·6

In the first group of experiments the primary donor strain was M174(F100)+, the intermediate host strain was ED2149, and the final recipient was JC3272. In the second group, the primary donor strain was JC6255(JCFLO)+, the intermediate host strain was JC7133, and the final recipient was JC3818 (giving a [Nal^R] contraselection).

* Expressed per 100 heterozygous intermediate cells, measured as sectored colonies on lactose-tetrazolium agar.

† ED2149(EDFL171)+ was sensitive to the F-specific phages f1, f2 and Qβ, while its (R100)+ derivative was resistant; this confirmed that EDFL171 is *finP*+.

‡ Forty-one other deletions past *traJ*, isolated from 10 independent JCFLO(λcI857) cointegrates with λ located between *traI* and *lac*, were also found to be *finP*− *ori*−.

Retransfer of JCFLO from strains carrying R100 and either pJC61 or pJC62 took place at frequencies about 20-fold greater than from the strain carrying R100 and pJC59 (Table 4). The overall low frequencies are possibly due to the *recA*− genotype of the intermediate strains. We deduce that while pJC59 is *finP*+, as expected, both pJC61 and pJC62 are *finP*−. These results therefore again confirm that both *ori* and *finP* are located to the right of *inc*. Furthermore, if the deletions carried by pJC61 and/or pJC62 end within or immediately to the left of *ori*, the order must be *inc ori finP*, as found using the Hfr deletion strains.

(iii) *Mapping* tra-4. The amber mutation *tra-4* prevents the expression, either completely or partially, of *traK*, *traB*, *traC*, *traF*, *traH*, *traG* and *traS* (Achtman, Willetts & Clark, 1972; Willetts & Achtman, 1972). It has therefore been suggested that it is a polar mutation in *traK* that reduces expression of genes downstream from it in the transfer operon (Willetts & Achtman, 1972; Ippen-Ihler *et al.* 1972). If this is true, then *tra-4* should map at *traK*. We have accordingly used marker rescue techniques to determine which of a series of Hfr deletion strains retain *tra-4*+. For this, JCFL4 (F*lac tra-4*) was transferred to the Hfr deletion strains, and F*lac tra*+ recombinants were identified as described in Materials and Methods. The results are shown in Table 5.

Hfr deletion strains carrying deletions ending to the right of *traB* gave about 5 % F*lac tra*$^+$ recombinants. This showed that they retained *tra-4*$^+$, and also that some residual complementation was occurring. The three *traK*$^+$ *traB*$^-$ Hfr deletion strains gave decreased levels of F*lac* retransfer, and approximately 45 % of these F*lac* elements were *tra*$^+$ recombinants. Again, these strains must be *tra-4*$^+$, but there is now almost no residual complementation. Only one strain with a deletion end-point possibly within *traK* was available, KI527, and this, together with strains carrying longer deletions, proved to have lost *tra-4*$^+$.

Table 5. *Marker rescue of* tra-4

Hfr deletion strain	Deletion end-point +	Deletion end-point −	Lac$^+$ progeny (%)	Tra$^+$ clones/ total tested
KI431	*traG*	*traS*	0·13	3/147
KI704	*traH*	*traG*	2·4	7/100
KI816	*traF*	*traH*	4·9	4/99
KI540	*traC*	*traF*	2·4	9/100
KI817	*traB*	*traC*	0·83	6/94
KI801	*traK*	*traB*	$7{\cdot}0 \times 10^{-2}$	32/82
KI813	*traK*	*traB*	0·14	79/259
KI819	*traK*	*traB*	$4{\cdot}7 \times 10^{-2}$	70/106
KI527	*traE*	*traK*	$< 10^{-3}$	.
KI805	*traA*	*traL*	$< 10^{-4}$	.
KI544	—	ϕ_{Π}	$< 10^{-4}$	.

The primary donor strain was JC6255 (JCFL4)$^+$; the procedure followed is described in Materials and Methods.

tra-4 is therefore located between the end-points of the deletions carried by KI527 (*traE*$^+$ *traK*$^-$) and by KI801, KI813 and KI819 (*traK*$^+$ *traB*$^-$), and we conclude that it is in fact located within *traK*.

Two other mutations, *tra-28* (UGA-suppressible) and *tra-29* (ochre-suppressible), were similar to *tra-4* in reducing expression of the same group of *tra* genes, though to lesser extents (Achtman, Willetts & Clark, 1972; Willetts & Achtman, 1972). Marker rescue experiments similar to those described above showed that the three *traK*$^+$ *traB*$^-$ Hfr deletion strains retained *tra-28*$^+$, while the *traE*$^+$ *traK*$^-$ strain had lost it; *tra-28* is therefore probably a weakly polar mutation also located in *traK*. *tra-29* was too leaky to be mapped by these methods.

4. DISCUSSION

The results presented in this paper are summarized in Fig. 1 as a map of a part of the F factor showing the positions of *traO*, *finP* and *tra-4*, and also the extents of some of the Hfr and F prime deletions. It is perhaps interesting that the two regulatory genes *finP* and *traJ* lie together. It has recently been found that the F deletion carried by pJC62 extends to F coordinate 62·2 kb, while that of pJC61 is

longer, extending to 52·7 kb (M. Guyer & A. J. Clark, personal communication): *finP*, together with *ori* and all the *tra* genes, must therefore be located to the right of the 62·2 kb point.

The position of *traO304* with respect to *finP* and *traJ90* was not formally determined. However, the extremely low frequency of recombination between *traO304* and *traJ90* demonstrates the very close proximity of these two markers (Finnegan & Willetts, 1973), and suggests that *traO*, like *traJ*, is located to the right of *finP*.

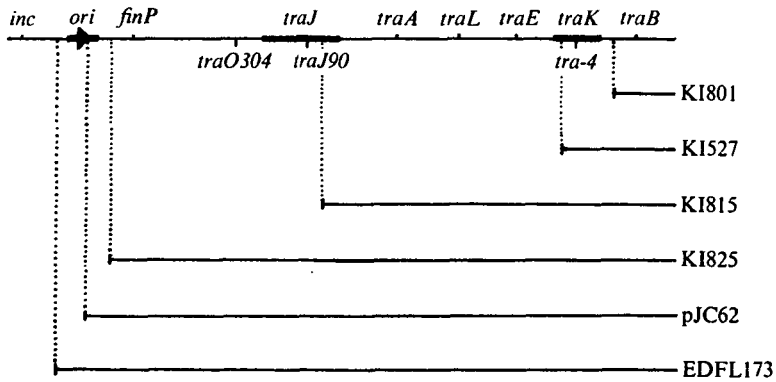

Fig. 1. *The extents of some F deletions.* Only a part of the F map is shown; the order of the transfer genes not shown is *traB traC traF traH traG traS traD traI*. Genes are shown as points except for *ori*, *traJ* and *traK* which are given lengths. *traO304* is assumed to be to the left of *traJ*, and the pJC62 deletion to end within *ori* (see text).

Furthermore, *traO* is probably the site at which the *finO* and *finP* products act to inhibit synthesis of the *traJ* product (Finnegan & Willetts, 1973; Willetts, 1974*b*). *traO* would therefore be expected to lie immediately to the left of *traJ*, if the *finO* and *finP* products prevent initiation of the transcription or translation of *traJ*, or possibly within *traJ* to the left or right of *traJ90*, if they give premature termination of *traJ* transcription. In the figure, the former alternative is assumed; if correct, this shows that *traJ*, like the genes of the transfer operon, is transcribed from left to right as drawn.

Assuming that no unidentified gene(s) are located between *traE* and *traK*, *tra-4* (and *tra-28*) must lie in *traK*. Since all the *tra* genes, excepting *traJ* but including *traD* and *traI*, form a single operon (Helmuth & Achtman, 1976), *tra-4* would be expected to reduce expression of *traD* and *traI* as well as of *traK* through *traS*. That this has not been observed, using relatively qualitative techniques (Achtman *et al.* 1972), is perhaps related to the incompleteness of *tra-4* polarity demonstrated above, coupled with a requirement for only small quantities of the *traD* and *traI* products for transfer.

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