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Transfer-positive J-independent revertants of the F factor in *Escherichia coli* K12

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SUMMARY

Cells carrying *traJ*$^-$ mutants of F are transfer-deficient and are good recipients in conjugation (Achtman, Willets & Clark, 1972). In addition, the *traJ* gene product is involved in pilus- and in plasmid-specificity (Willetts, 1971). J-independent mutants were isolated as revertants of a *traJ*$^-$ mutant; they still carry the *traJ*$^-$ mutation but also carry at least one other mutation which results in transfer in the absence of the *traJ* gene product. J-independent transfer of these mutants is not inhibited by the R100 repressor. Various models are presented which can account for the properties of *traJ*$^-$ mutants and of these J-independent revertants.

Cells carrying an F element are proficient in both transfer (Tra$^+$)† and surface exclusion (Sex$^+$). Both these properties are inhibited in cells carrying, in addition to F, an F-like R factor such as R100, and such (F, R)$^+$ cells are poor donors (Tra$^-$) and good recipients (Sex)$^-$ in conjugation (Watanabe & Fukasawa, 1962; Willetts & Finnegan, 1970). The mechanism of transfer inhibition was postulated (Egawa & Hirota, 1962) to be due to a repressor synthesized by the R factor which inhibits transfer by both F and R. Mutants of R such as R100–1 have been isolated (Egawa & Hirota, 1962) which inhibit neither their own transfer nor that of F. In addition, they do not inhibit surface exclusion by F (Willetts & Finnegan, 1970). Nishimura *et al.* (1967) have postulated that these mutants do not synthesize a functional repressor.

The question arises as to how the repression is exerted. Attempts to answer this question are now appropriate since recent genetic analyses have shown by complementation analysis that F carries at least eleven *tra* cistrons needed for conjugational transfer (Ohtsubo, Nishimura & Hirota, 1970; Achtman *et al.* 1972; Willetts

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† Abbreviations and nomenclature are those proposed by Achtman, Willetts & Clark (1971), Demerec *et al.* (1966), and Taylor (1970). Tra$^+$ cultures are transfer-proficient and act as good donors in conjugation while Tra$^-$ cultures are poor donors. Sex$^+$ cultures are surface-exclusion proficient and act as *poor* recipients in conjugation while Sex$^-$ cultures are *good* recipients. F*lac* mutants have been assigned a unique number in the series JCFLn; JCFL0 is the parental F*lac* element (Achtman *et al.* 1971).

	ϕ_{II}^{r} ... *traJ*	*traA*	*traE*	*traK*	*traB*	*traC*	*traF*	*traH*	*traG*	*traD*	*traI* ... *lac*
F-pili	−	−	−	−	−	−	−	−	+/−	+	+
Surface exclusion	−	+	+	+	+	+	+	+	+	+	+
Transfer from cells carrying R100–1	−	+	+	+	+	+	+	+	+	+	−
F-specific M12 sensitivity of cells carrying R100–1	−	−	+	+	+	+	+	+	+	+	+

Fig. 1. Genetic map and properties of *tra* cistrons on F*lac*. The map is adapted from Ippen-Ihler *et al.* (1972). Conclusions about F-piliation and surface exclusion come from Achtman *et al.* (1972) and about properties of (R100–1, F*lac*$^+$) heterozygote cells from Willetts (1971). If there are any multicistronic operons among these cistrons, then their transcription proceeds from left to right. A surface exclusion cistron, *traS*, is postulated to lie between *traG* and *traD* (N. S. Willetts, *personal communication*).

& Achtman, 1972) and have defined by sequential deletion analysis a linear map for these cistrons (Ohtsubo, 1970; Ippen-Ihler, Achtman & Willetts, 1972). Fig. 1 presents the map together with a summary of some of the pertinent properties of the 11 cistrons. The biochemical defects in these mutants are not known but mutations in eight cistrons result in the lack of synthesis of F-pili (Achtman *et al.* 1971, 1972; Willetts & Achtman, 1972). Since the *traG*, *traD* and *traI* gene products are not needed for F-pilus synthesis (Achtman *et al.* 1971, 1972; Willetts & Achtman, 1972) it is possible that they code for enzymes which are involved directly in DNA transfer.

F-like R factors carry some *tra* cistrons which complement F *tra*$^-$ mutations, and Ohtsubo *et al.* (1970) were able to demonstrate intercistronic complementation between *tra*$^-$ mutants of the R100 factor and *tra*$^-$ mutants of the F factor assigned to six separate cistrons. In addition, Willetts (1971) showed that most (F, R)$^+$ cells carrying both R100–1 and any one F*lac tra*$^-$ mutant had the same properties as (F*lac*)$^+$ cells: they transferred the F*lac* element efficiently and were sensitive to male-specific phage M12 ((R100–1)$^+$ cells are relatively insensitive to M12 (Nishimura *et al.* 1967)). However, (F,R)$^+$ cells carrying R100–1 and an F*lac traA*$^-$ mutant were relatively insensitive to M12, although they did transfer the *traA*$^-$ mutant efficiently. Thus, *traA*, although not necessarily coding for an F-pilus structural protein, is probably involved in specifying the F type of pilus (Willetts, 1971). Presumably, R makes a *traA* product as well, but one with different specificities. (F, R)$^+$ cells carrying both R100–1 and an F*lac traI*$^-$ mutant did not transfer the *traI*$^-$ mutant efficiently. Thus, although an R100–1 analogue of the F *traI*$^-$ product may be presumed to exist (Ohtsubo *et al.* 1970), it must act specifically on R DNA and cannot substitute for the F *traI* gene product. These facts make it possible to assay *traA* and *traI* function in F mutants by measuring their M12 sensitivity and their transfer efficiency from (F, R)$^+$ cells carrying R100–1.

N. S. Willetts (*personal communication*) has also shown that some of the Hfr deletion mutants (used in the mapping of the *tra* cistrons (Ippen-Ihler *et al.* 1972)) which have deletion mutations extending from the right (Fig. 1) into *traD* are

Sex+ while deletion mutants carrying longer deletions extending into *traG* or farther are Sex−. On the basis of this finding, he has postulated the existence of an otherwise undefined cistron, *traS*, which is involved in surface exclusion and which may or may not be involved in transfer.

traJ− mutants of F*lac* are Tra− and do not synthesize F-pili. In addition, they are Sex− (Achtman *et al.* 1971, 1972). Cells carrying R100–1 and an F*lac traJ*− mutant do not transfer the *traJ*− mutant efficiently and are relatively insensitive to M12 phage (Willetts, 1971). The simplest explanation of these observations is that in *traJ*− cells, the *traA*, *traI*, and *traS* proteins are either not made or do not function. Finnegan & Willetts (1971) have postulated on these grounds that the inhibition of transfer exerted by R100 and other F-like factors is due to inhibition of either the synthesis or function of the *traJ* gene product. This model, attractive for its simplicity, now raises the question of what the *traJ* gene product (J protein*) does. However, regardless of the mechanism of action of J protein, the model predicts that should J-independent mutants be isolatable, then they should be resistant to repression by R100. This report describes the isolation of such J-independent mutants.

1. MATERIALS AND METHODS

Unless otherwise specified here, the materials and methods used have been described previously (Achtman *et al.* 1971). Mating ability was measured in 45 min matings at 42 °C while surface exclusion was measured in 60 min matings at 37 °C. All incubations for colony growth were at 37 °C. L (Luria) broth and L agar (Luria & Burrous, 1957) were used as complex media, while the adaptation of M9 medium (Adams, 1959) described by Willetts & Finnegan (1970) was used as minimal medium. M12 phage was obtained from P. H. Hofschneider *via* N. Willetts.

Bacterial strains. Table 1 lists the properties of some of the strains used in this analysis. JC6255 is a Mal+ Str^S conjugational recombinant of a P1·(Su+ His+) transductant of JC6589, and JC6256 is a Mal+ Str^S conjugational recombinant of a His+ Su− transductant of JC6589. Thus, these two strains are very closely related. The J-independent mutants, isolated in strain JC5455, were transferred to JC6255 and JC6256 by selecting Lac+ [His+] progeny. JC5455 is also derived from JC6589 but in several different steps (Achtman *et al.* 1971).

Mutant isolation procedures. All incubations were conducted at 37 °C and without agitation unless specified. An L broth culture inoculated from a single Lac+ colony of M135 (T6^S, carrries JCFL90) was incubated overnight. The next day, a 1:20 dilution in fresh broth was shaken until the cells had reached a concentration of approximately 10^9/ml and the cells were then centrifuged and resuspended in 1/10 volume of sterile distilled water. Nitrosoguanidine (*N*-methyl-*N*-nitroso-*N'*-nitroguanidine) solution was added to a final concentration of 50 μg/ml in 0·1 M citrate buffer at pH 5·5. The mixture was incubated for 15 to 30 min in different experiments.

* The *traJ* gene product must be a protein as amber suppressible *traJ*− mutants have been isolated (Achtman *et al.* 1972).

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Table 1. *Properties of bacterial strains*

No.	Plasmid carried	Lac*	His	Trp	Lys	Met	T6	Su_I†	Source‡
JC3272	F^-	−	−	−	−	+	R	−	From JC6589
JC5455	F^-	−	−	−	+	+	R	−	From JC6589
JC6255	F^-	−	+	−	+	+	S	+	From JC6589
JC6256	F^-	−	+	−	+	+	S	−	From JC6589
JE255	R100–1	−	+	+	+	−	?	?	Y. Hirota
ED21	R100	−	−	−	−	+	R	−	JC3272
ED24	F^-	−	+	+	+	+	R	−	JC5455
KL16	Hfr	+	+	+	+	+	S	?	K. B. Low
M135	JCFL90	+/−	+	−	+	+	S	−	JC6256

* All Lac^- strains, except JE255, carry the *lac* deletion × 74.
† The Su_I^- strains used here do not suppress amber, ochre, or UGA mutations.
‡ Strain derivations are given in Achtman *et al.* (1971).

The mutagenized cells were diluted 1:500 in fresh broth and 0·5 ml aliquots were immediately dispensed to 75 tubes. As the frequency of spontaneous mutants should be low compared to the frequency of mutants which arose because of nitrosoguanidine treatment, mutants isolated from different tubes are probably not sibs. The tubes were incubated for 2 h to allow segregation of mutant clones and to return the cells to exponential growth. 0·5 ml of an exponential culture of strain JC3272 ($T6^R$, F^-) was added to each tube and the tubes were incubated overnight to allow mating and infectious spread of Tra^+ revertant F*lac* elements. As described in the text, such treatment results not only in the transfer of Tra^+ revertants but also of Tra^- F*lac* elements. Therefore, the cultures were subjected on the next day to a second mating to select against Tra^- elements. 1:20 dilutions of the cultures which had been incubated overnight were made in 0·2 ml of fresh broth and these dilutions shaken at 37 °C for 3 h to restore the cells to exponential growth. 0·2 ml of high-titre T6 suspension (3×10^{11} pfu/ml), treated with sufficient ultraviolet light to decrease the phage titre by three orders of magnitude, was added to each tube and 20–30 min incubation allowed for killing of the $T6^S$ donor cells. Such treatment results in a drop of 4 to 5 orders of magnitude in the numbers of $T6^S$ cells and does not affect $T6^R$ recipient cells. A second mating was initiated by adding 0·1 ml of an exponential culture of strain JC5455 (F^-, $T6^R$, Spc^R, Lac^-) and the mating mixtures were incubated a further 1·5 h. 3 ml of molten 0·7 % agar was added to each tube and the contents poured on minimal agar plates selective for Lac^+ [Spc^R] colonies.

Replica-plating tests. Lac^+ [Spc^R] colonies from the mutant isolation procedures were transferred with sterile toothpicks in grids of 100 to fresh minimal agar plates and the plates were incubated overnight. The patched colonies were tested for transfer ability, surface exclusion, and sensitivity to transfer inhibition by the R100 repressor as follows. The colonies were replica-plated to L plates which were incubated for 5–6 h to obtain exponentially growing cells. The L plates were themselves replica-plated to minimal agar plates spread with 0·1 ml of a tester culture. F^- and F^- R^+ tester cultures were tenfold concentrated suspensions in buffer of

a 24 h shaking broth culture. The F^- strain used was JC3272, which is also Lac^- and Str^R, and Lac^+ [Str^R] progeny were selected as a measure of transfer ability. The F^- R^+ strain used was ED21, an $(R100)^+$ derivative of JC3272, and again Lac^+ [Str^R] progeny were selected.

Hfr tester cultures were 50-fold concentrated suspensions in buffer of broth-grown exponential cultures. The Hfr strain used was KL16, which is Spc^S, and transfer from KL16 to the replica-plated colonies (which reflects surface exclusion) was measured by selecting His^+ [Lac^+ Spc^R] progeny. The plates were examined after 24 and 48 h. Repression by the R100 repressor was measured by using the 48 h plates carrying Lac^+ progeny of JC3272 and ED21. These were themselves replica-plated, as above, onto L plates for exponential growth and the L plates were then, in turn, replicated against a tester culture of the F^- Spc^R strain, ED24. Lac^+ [His^+ Trp^+ Spc^R] progeny were selected.

2. RESULTS

Experimental approach. The *Flac* mutant JCFL90, which carries the amber mutation *traJ90*, was chosen for the attempted isolation of J-independent mutants. Unlike revertants of a mis-sense mutant, any JCFL90 Tra^+ revertants in which *traJ90* is still present and functioning physiologically as an amber mutation cannot be due to a mutation within *traJ* which restores J protein function. This becomes obvious when we consider that second-site mutations located in *traJ* distal in the reading frame to *traJ90* cannot affect protein truncation caused by the amber mutation while any mutations within the *traJ90* UAG codon itself would change it to a non-amber codon. It seems highly unlikely that a proximal mutation could change the protein conformation sufficiently to restore activity to a truncated protein. Thus the very isolation of any partial revertants of JCFL90 in which *traJ90* was still present and suppressible by an amber suppressor would indicate that they carry second-site J-independent mutations located outside *traJ*. These could then be tested for repressibility by R100.

Isolation of revertants. Su^- cells carrying JCFL90 transfer it at a frequency of less than 10^{-4} % (Achtman *et al.* 1971) but the few *Flac* elements transferred, even after treatment with nitrosoguanidine, are still Tra^-. Presumably this is due to suppressor mutations and also because of leakiness. However, it was possible to devise a technique which used two successive matings in a variation of the fluctuation test (Luria & Delbrück, 1943) and which did yield revertant mutants. Each experiment involved a mating between a mutagenized $T6^S$ Su^- $(JCFL90)^+$ culture and a $T6^R$ Su^- F^- culture followed by incubation and growth to allow infectious spread of any Tra^+ revertant *Flac* elements. The mutagenized donor cells were then killed by treatment with T6 phage. *Flac* transfer from these cultures should now come primarily from the progeny derived from the F^- parent. The cultures were therefore mated a second time using a Spc^R F^- strain, JC5455, as recipient and Lac^+ derivatives of JC5455 were selected on minimal agar. Seventy-five independent cultures were used per experiment and the dilutions used were such that

Table 2. *Isolation of* Tra$^+$ *revertants*

Expt	Numbers of cultures* (Lac$^+$ [Spc^R] colonies per culture)			
	0	1–10	11–50	100–10000
1	10	51	8	6
2	3	30	23	19

* Tra$^+$ revertants of JCFL90 (carries *traJ90*) were selected after mutagenesis as described in Materials and Methods. After two separate sequential matings with F$^-$ strains, Lac$^+$ [Spc^R] colonies derived by F*lac* transfer via the first F$^-$ strain to the second were selected by plating the final mating mixture on the appropriate minimal agar plates. The results tabulated here summarize the numbers of Lac$^+$ colonies per plate (and thus per culture) observed in two separate experiments of 75 cultures each. In Expt 1, 15 of the cultures contained full Tra$^+$ revertants. Of these, two also contained partial revertants. Four other cultures contained a mixture of non-revertants and partial revertants. In experiment 2, 34 of the cultures contained full Tra$^+$ revertants, and 6 others contained partial revertants.

most cultures did not receive a Tra$^+$ revertant F*lac* element. Thus, even an only partially reverted mutant would not be masked by infectious spread of full revertants.

The experiment was performed twice with the results given in Table 2. As expected from the principles of the fluctuation test (Luria & Delbrück, 1943) there was great variability in the numbers of (F*lac*)$^+$ progeny per culture derived from the second F$^+$ recipient, with some cultures containing no Lac$^+$ progeny, others containing thousands, and the rest containing intermediate levels, Where possible, ten colonies from each culture were patched and tested by replica-plating for *lac*$^+$ transfer ability in the presence and absence of R100 and also for recipient ability in matings against an Hfr strain. The transfer measurements in the absence of R100 indicated whether or not the colonies contained Tra$^+$ revertants, while the transfer measurements in the presence of R100 indicated whether or not transfer of the Tra$^+$ revertants was still inhibited by the R100 repressor. Recipient ability was of interest since *traJ*$^-$ mutants are surface-exclusion deficient.

Again, as expected, most of the cultures with few Lac$^+$ progeny contained only F*lac tra*$^-$ mutants while cultures with many Lac$^+$ progeny contained full revertants. To the limits of resolution of replica-plate matings, these had fully regained transfer ability and surface exclusion and were as inhibited by the R100 repressor as a wild-type F*lac* element. Forty-nine of the 150 cultures examined contained such full revertants. However, 12 cultures contained partial revertants. These showed transfer levels intermediate between those characteristic of JCFL0 and JCFL90 and these were resistant to inhibition by R100. Their surface exclusion properties will be discussed later. A single partial revertant from each of these twelve cultures was purified by single colony isolation.

Quantitative measurements of transfer ability. The twelve partial revertants were transferred into the F$^-$ Lac$^-$ strains JC6255 and JC6256, which are Su_I^+ and Su$^-$, respectively, and are otherwise isogenic. R100 was then transferred into these

Table 3. *Quantitative measurements of transfer ability**

Episome no.	Description	Su⁻ derivative R⁻ (1)	Su⁻ derivative (R100)⁺ (2)	Su_I^+ derivative R⁻ (3)	Su_I^+ derivative (R100)⁺ (4)
JCFL0	*tra*⁺	(100)	0·1	(100)	0·2
JCFL121	*tra*⁺ full revertant	86	0·3	55	0·3
JCFL90	*traJ90*	0·00006	0·0003	17	0·008
JCFL118	Partial revertant	4	8	56	3
JCFL119		0·8	2	17	1·5
JCFL122		0·05	0·07	22	0·1
JCFL123		0·3	0·7	15	0·7
JCFL124		0·005	0·3	3	0·7
JCFL125		0·07	0·2	25	0·3
JCFL126		0·04	0·06	20	0·1
JCFL127		0·06	0·1	23	0·7
JCFL128		0·05	0·05	20	0·2
JCFL129		0·5	1·2	22	0·7
JCFL130		0·6	1·0	17	0·6
JCFL131		0·2	1·0	18	0·6

* The values shown are the geometric means of the results of two or more matings against the F⁻ strain JC3272, expressed as the number of Lac⁺ [Str^R] progeny per Lac⁺ donor cell introduced into the mating mixture. In a few cases, only one mating measurement was performed. Usually, the results from different experiments on the same strain were the same within a factor of two. Su_I^+ derivatives were derived from JC6255 and the results were normalized to a value of 100 for a parallel mating with the (JCFL0)⁺ derivative of that strain used as donor. The actual geometric mean of five experiments was 111 %. Su⁻ derivatives were derived from JC6256 and the results were similarly normalized to a value of 100 for a parallel mating with the (JCFLO)⁺ derivative of that strain. The actual geometric mean of six experiments was 99 %. All matings were conducted at 42 °C and were interrupted by T6 treatment.

derivatives. The R⁻ and R100 derivatives were tested quantitatively for transfer ability. For controls, strains carrying JCFL0 (the parental *tra*⁺ F*lac* element), JCFL90 itself, or JCFL121 (one of the full revertants described above) were also tested. The results are shown in Table 3.

The results in column 1 of Table 3 confirm that all twelve are in fact partial revertants since they transfer more efficiently than JCFL90 and less efficiently than either JCFL0 or the full revertant JCFL121. However, the results in column 3, compared with those of column 1, show that all twelve *still* carry an amber mutation, suppressible in a Su_I^+ host. Furthermore, except for JCFL118 and JCFL124, separately discussed below, all partial revertants are suppressed to the same quantitative level (within the limits of error of such measurements) as JCFL90. Therefore, these 10 mutants still carry the suppressible *traJ90* mutation and by the arguments presented above have presumably acquired a second mutation located outside *traJ*. This conclusion has not yet been confirmed by genetic studies as a linkage system is not available for F.

A comparison of columns 1 and 2 shows further that the unsuppressed transfer level of all 10 mutants is similar in the presence and absence of R100, unlike the

behaviour of JCFL0 or JCFL121 the transfer of which is reduced 1000-fold and 300-fold respectively in the presence of R100. This is not true for the suppressed behaviour of the 10 mutants: a comparison of columns 4 and 3 shows that in a Su_I^+ (R100)$^+$ host, transfer is reduced either to a level similar to that of JCFL0 in the same host or to that of the unsuppressed level of that mutant, whichever is greater.

Thus the R100 repressor inhibits the transfer promoted by the J protein, when restored by suppression of *traJ90*, but not the transfer promoted by the J-independent mutations. This provides direct confirmation of the model proposed by Finnegan & Willetts (1971) that the R repressor acts by inhibiting J protein synthesis or function.

On quantitative grounds, it is not entirely clear whether JCFL118 and JCFL124 are J-independent mutants as well. Both carry amber mutations and both promote transfer which is not inhibited by the R repressor, However, JCFL118 is suppressed by Su_I^+ three times as efficiently as JCFL90. Possibly, this represents a more than additive response when some J protein is restored to cells whose spontaneous J-independent transfer levels are already as high as 4%. JCFL124 is more poorly suppressed than JCFL90 (and in addition shows a very low transfer level from a Su^- strain). It is possible that JCFL124 carries an independent and leaky *tra*$^-$ mutation induced by the nitrosoguanidine treatment, especially since R100 and (R100–1) complement some of the transfer deficiency.

Reversion of other properties. As mentioned above, *traJ*$^-$ mutants seem to lack the *traA* and *traI* functions and are Sex^- as well. Therefore the twelve partial revertants were examined to see whether any had regained these properties. *traA* and *traI* function were measured by examining the M12 plaque-forming ability and *lac*$^+$ transfer ability, respectively, of Su^- (F, R)$^+$ cells carrying both a J-independent mutant and R100–1. Surface exclusion was measured in quantitative matings between the Hfr strain KL16 and exponential cultures of the J-independent mutants (JC5455 derivatives were used so that His^+ recombinants could be selected). The results are presented in Table 4.

(F, R)$^+$ cells carrying R100–1 and any one of most of the mutants did not transfer the J-independent mutant efficiently (especially when compared with the spontaneous transfer level in the absence of R100–1). It is not clear whether the slightly increased transfer in the presence of R100–1 over that manifested in its absence in the cases of JCFL118, 122, 125, 126, 127, 128, and 131 signifies any regained *traI* function. The case of JCFL124 cannot be evaluated as that mutant may be carrying another *tra* mutation (see above) which could be complemented by R100–1 giving rise to the high transfer level. However, it is clear that none of the mutants have regained large amounts of *traI* function.

The situation is clearly different for surface exclusion and *traA* function. Three mutants (JCFL118, JCFL119 and JCFL124) show restoration of high levels of *traA* function and it seems likely that many of the other mutants (see particularly JCFL123, JCFL126, JCFL129, JCFL130, and JCFL131) produced more *traA* protein than their ancestor, JCFL90. Furthermore, JCFL119 and JCFL124 had also regained significant levels of surface exclusion.

Table 4. tra*A*, tra*S* *and* traI *function.*

Episome no.	Description	Spontaneous transfer level*	*traI* function†	*traA* function‡	Surface exclusion§
JCFL0	*tra*$^+$	(100)	(100)	(100)	(100)
JCFL121	*tra*$^+$ full revertant	86	ND	ND	61, 79
JCFL90	*traJ90*	0·00006	0·01	3, 1, 1, ⩽ 0·4	0·2, 0·5
JCFL118	Partial revertant	4	10	119, 90, 34, 45, 59	0·6, 2
JCFL119		0·8	0·6	44, 42, 80, 44	9, 17
JCFL122		0·05	0·6	5, 4, 10	0·2
JCFL123		0·3	0·08	9, 13, 5	0·2
JCFL124		0·005	2	12, 46, 54, 54	8
JCFL125		0·07	0·2	7, 3, 1, ⩽ 0·4	0·2
JCFL126		0·04	0·3	8, 14, 16, 9	0·5
JCFL127		0·06	0·4	7, 5, 6	0·4
JCFL128		0·05	0·2	3, ⩽ 0·6	0·6
JCFL129		0·5	0·4	11, 17, 2	0·5
JCFL130		0·6	0·1	6, 10, 18, ⩽ 0·4	0·5
JCFL131		0·2	0·5	11, 6, 16	0·7

* Data from Table 3, column (1). Transfer from JC6256 derivatives.

† Transfer from (F*lac*, R100–1)$^+$ derivatives of JC6256. Expressed and normalized as in footnote to Table 3. Transfer levels in this column only demonstrate regained *traI* function if they are significantly higher than the spontaneous transfer levels.

‡ As measured by duplicate plaque assays on (F*lac*, R100–1)$^+$ derivatives of JC6256. Each value represents the results of a different experiment.

§ From crosses between KL16 and JC5455 derivatives of the F*lac* elements. Measured and expressed as previously described (Willetts & Finnegan, 1970). The numbers were first expressed as the reciprocal of the ratios between the number of His$^+$ [Spc^r] colonies generated in a test mating of KL16 × any one strain and the number generated in a parallel mating of KL16 × JC5455. They were then normalized to a value of 100 for the results expressed in this fashion of a parallel mating between KL16 and the (JCFL0)$^+$ derivative of JC5455.

3. DISCUSSION

Twelve mutants have been isolated as partial revertants of a *traJ*$^-$ F*lac* element. These all still carry an amber-suppressible transfer mutation which in 10 out of 12 cases is suppressed in a Su_I^+ host to the same quantitative level as their *traJ90*-carrying parent. As all the 26 other amber Tra$^-$ mutants isolated (Achtman *et al.* 1971) are suppressed to distinctly different levels in a Su_I^+ host, this is presumptive evidence that these revertants still carry a *traJ90* mutation. By the arguments presented earlier, these mutants must then carry a second and J-independent mutation, probably outside the *traJ* cistron.

In addition, these 12 mutants are no longer repressible by the R100 repressor even though the selection was only for partial reversion of transfer-proficiency. The easiest explanation is that the model presented by Finnegan & Willetts (1971) is correct, namely that the R100 repressor exerts its effect by acting on the synthesis or function of J protein.

However, the existence of J-independent mutants does not elucidate what the function of J protein is. Briefly summarized, *traJ*$^-$ mutants carry pleiotropically negative mutations that result in lack of conjugational DNA transfer, lack of sex piliation, and apparently lack of *traA*, *traI* and *traS* (postulated) functions. Three alternative and testable models may be devised to explain these properties:

(*a*) The J protein is an enzyme whose enzymic product is then further separately modified enzymically by the *traA*, *traI*, and *traS* gene products. Then *traJ*$^-$ mutants would deprive these three gene products of substrate and would be phenotypically *traA*$^-$, *traI*$^-$ and *traS*.$^-$

(*b*) The J protein interacts directly with the *traA*, *traI*, and *traS* gene products. The interaction could be one of combining with each separately or with all together to form three bi-enzyme complexes or a single multi-enzyme complex or could even be the modification of the *traA*, *traI* and *traS* gene products from inactive proto-enzymes into the functional forms. *traJ*$^-$ mutants would not contain active *traA*, *traI* and *traS* gene products.

(*c*) The J protein acts to regulate the production (at the transcriptional or translational level) of the *traA*, *traI* and *traS* gene products. Since *traJ*$^-$ mutants are recessive (Achtman *et al.* 1972), this model demands that *traJ* be a positive control gene, perhaps even regulating all the *tra* cistrons. Under this model, *traJ* mutants would not synthesize any *traA*, *traI*, or *traS* gene products.

It seems somewhat unlikely that the need for an enzymic product (model *a*) can be abolished by mutation. However, such mutations have previously been described (Barbour *et al.* 1970; Hofnung & Schwartz, 1971) as a result of which a new enzyme or enzyme pathway has become functional. Similarly, mutants have also been described which apparently synthesize a new structural component of an enzyme complex (Kemper & Margolin, 1969). Finally, mutants in which a positively controlled regulation is no longer needed have also been isolated, both in λ (Butler & Echols, 1970; Hopkins, 1970; Court & Sato, 1969) and in the *E. coli ara* (Englesberg *et al.* 1969) and *mal* (Hofnung & Schwartz, 1971) operons. Thus, the isolation alone of these J-independent mutants cannot distinguish between these models. However, their properties should distinguish between them and these are currently under investigation.

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