Genetic Analysis of Deletions of R100-1 that are Both Transfer-deficient and Tetracycline-sensitive

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(Received 6 August 1975)

SUMMARY

The extent of the deletions of five Tet^s Tra⁻ mutants of R100-1 was determined by complementation experiments with wild-type and *tra* mutants of *Flac*. The presence or absence of the origin of transfer on the mutants was also investigated. Using the results, a tentative map of this region of the R factor was drawn: it was essentially similar to the analogous region of the *E. coli* K12 F factor, except that *tet* was located between *traJ* and *traA*.

Some of the deletions had removed the promoter for the transfer operon. This allowed detection of the transcription of *traC* and distal genes from a weak, *traJ*-independent promoter. This is probably the Is2 promoter, since R100-1 carries an Is2 insertion sequence located immediately to the left of *traC* in the correct orientation. Since neither the transfer operon promoter nor the Is2 promoter seemed to be required for transcription of *traI*, it was concluded that, unlike the F factor, this was located in a separate operon.

INTRODUCTION

Amongst a series of spontaneous tetracycline-sensitive (Tet^s) mutants of R100-1, a high proportion (about 50 %) were found to be transfer-deficient (Foster, 1975). We have examined some of these putative Tet^s Tra⁻ R100-1 deletion mutants further to determine which transfer genes have been lost. For this, complementation between the mutants and a series of *Flac tra* point mutants (Achtman, Willetts & Clark, 1972) was measured. It has been shown that such complementation is possible, although with limitations (Ohtsubo, Nishimura & Hirota, 1970; Willetts, 1971). The presence or absence of the origin of transfer (Willetts, 1972) on the Tet^s Tra⁻ R100-1 mutants was also determined, and their levels of surface exclusion were measured. The gene(s) for surface exclusion by R100-1 is co-controlled with the transfer genes (Willetts & Maule, 1974), and is probably located in the transfer region.

These studies have enabled us to infer the probable extent of the deletions carried by the Tet^s Tra⁻ mutants. Furthermore, removal of the promoter for the transfer operon by some of the deletions allowed us to demonstrate the presence of a second, weak promoter within the *tra* operon, able to transcribe *traC* and distal genes.

METHODS

Bacterial host strains. The strains used were JC3272 (*lac his trp lys str-r*), ED2030 (*lac trp spe-r recA*), DU1000 (*lac his trp lys str-r nal-r*), DU1004 (*pro met rif-r*), and DU1025 (*lac his str-r rif-r*). Strain ED2030 is a *gal his⁺ recA56* derivative of JC5455, and DU1000 is a spontaneous *Nal^r* mutant of JC3272; the derivations of JC3272 and JC5455 were given by Achtman, Willetts & Clark (1971). Strains DU1004 and DU1025 are spontaneous *Rif^r* mutants of J5-3 (Clowes & Hayes, 1968) and *E. coli* 236 (from the culture collection of the Genetics Department, Trinity College, Dublin), respectively.

Plasmids. The Tet^s R factor mutants were derived from R100-1, a 'derepressed' mutant of R100 (Egawa & Hirota, 1962), which confers resistance to tetracycline (Tet), chloramphenicol (Chl), streptomycin (Str) and sulphonamides (Sul). The mutants were isolated by penicillin selection in non-mutagenized cultures in the presence of tetracycline; each culture was started from an independent clone (Foster, 1975). pDU3 is a Tra⁺ Tet^s point mutant, while pDU101, pDU103, pDU106, pDU111 and pDU113 are Tet^s Tra⁻ mutants; pDU111* indicates that pDU111 is integrated into the host chromosome. pDU5 is a Chl^r revertant of R100-99, a Chl^s Tet^s double mutant derived from R100-1 (Hashimoto & Hirota, 1966), and pDU100 is a Chl^s point mutant of R100-1 (Foster & Howe, 1973).

The wild-type and amber-suppressible transfer-deficient *Flac* elements have been described (Achtman *et al.*, 1971, 1972; Willetts & Achtman, 1972).

Media. Nutrient broth and nutrient agar were those of Foster & Walsh (1974). Minimal salts agar contained (g/l): K₂HPO₄, 7; KH₂PO₄, 3; MgSO₄, 0.2; (NH₄)₂SO₄, 1; Difco Bacto agar, 20. MacConkey agar contained 40 g Oxoid peptone, 5 g NaCl, 10 g sodium taurocholate, 10 g lactose, 0.01 % neutral red and 2 g Difco Bacto agar per litre, pH 7.0. Sugars were added to a final concentration of 1 %, L-amino acids to 20 µg/ml, tetracycline, chloramphenicol and nalidixic acid to 20 µg/ml, rifampicin to 40 µg/ml and spectinomycin to 10 µg/ml.

Tetracycline, chloramphenicol, nalidixic acid, rifampicin and spectinomycin were gifts from Lederle Laboratories, Parke Davis, Winthrop, Ciba and Upjohn respectively.

Conjugational complementation tests. The Tet^s Tra⁻ R100-1 deletion mutants were transduced into the *recA* strain ED2030, using P1kc and selecting for Chl^r progeny. These derivatives were then used as recipients in matings with Su⁺ donor strains carrying amber-suppressible *Flac tra* mutants and Lac⁺ Chl^r progeny were selected. The strains carrying both R100-1 and *Flac* elements were purified on MacConkey agar containing chloramphenicol. *Flac tra* derivatives of ED2030 (R⁻) used in control matings were selected and purified on MacConkey agar containing spectinomycin.

pDU111* could not be transduced to ED2030 (see below); it was therefore transduced to DU1000, and *Flac tra* derivatives of this strain were constructed, selecting Lac⁺ Chl^r [Nal^r] progeny.

Cultures were prepared for mating by diluting overnight broth cultures 1:10 in fresh broth and incubating at 37 °C without shaking for 4 h. The plasmid-containing donor culture (0.5 ml) was then mixed with 4.5 ml of a culture of the recipient strain DU1000 (or DU1025 for donor strains carrying pDU111*), and incubated at 37 °C for 30 min. Suitable dilutions were then spread on media selective for Lac⁺ [Nal^r] or Chl^r [Nal^r] progeny (or [Rif^r] progeny of DU1025).

Origin of transfer. The method used to determine whether the Tet^s Tra⁻ R100-1 mutants carried the putative R100-1 origin of transfer was based upon that of Willetts (1972). A 0.1 ml portion of an exponential culture of DU1004 (*pro met nal-s*) carrying pDU100

(Tet^r Chl^s) was mixed with 0.1 ml of a shaken overnight (R⁻ phenocopy) culture of ED2030 (RecA⁻ Nal^s) carrying the Tet^s Tra⁻ R100-1 mutant, and the mixture incubated for 40 min. Broth (0.8 ml) was added, followed by incubation for a further 40 min to allow expression of the transfer genes in the resultant ED2030 heterozygotes. At this stage the number of ED2030 cells which had received pDU100 was assayed by plating on medium selective for Tet^r [Pro⁺ Met⁺] progeny of this strain. One ml of an exponential culture of DU1000 (Nal^r) was then added, and after 30 min incubation, dilutions were plated on medium selective for Chl^r [Nal^r] progeny.

Surface exclusion indices. For measurement of the levels of surface exclusion by the Tet^s Tra⁻ R100-1 mutants, derivatives of DU1004 were constructed by transduction. One ml of exponential cultures of these strains (Rif^r) was mixed with 1 ml of a similar culture of DU1000 (Rif^s) carrying pDU100 (Tet^r Chl^s). After 60 min, dilutions were plated on medium selective for Tet^r (His⁺ Trp⁺ Lys⁺ Rif^r) progeny. For pDU111*, which was probably integrated into the chromosome of DU1000 (Nal^r), surface exclusion levels were measured by transferring pDU100 from DU1004 (Nal^s) and selecting Tet^r [Pro⁺ Met⁺ Nal^r] progeny.

RESULTS

The Tet^s Tra⁻ R100-1 mutants

A series of spontaneous Tet^s mutants of R100-1 was isolated by Foster (1975) and many of these were found to be completely transfer-deficient and resistant to the male specific phages MS2 and M13. They retained other properties of the R100-1 parent such as the Chl^r Str^r Sul^r markers, and incompatibility towards other group FII plasmids. They could be transduced by P1kc to both Rec⁺ and RecA⁻ strains of *E. coli*, and are therefore presumed to retain their autonomous plasmid state. R100-1 mutants of this type are pDU101, pDU103, pDU106 and pDU113.

pDU111 is a further Tet^s Tra⁻ R100-1 mutant, but which in addition seems to have suffered loss of a function(s) affecting replication. When first isolated, it was readily lost from cultures grown in the absence of chloramphenicol (99 % of the cells were Chl^s after overnight growth in broth). However, after several months' storage on a Dorset Egg slope, a stable variant, pDU111* was isolated. pDU111* was transduced at a 100-fold lower frequency than the other Tet^s Tra⁻ R100-1 mutants to a Rec⁺ recipient, and not at all to a RecA⁻ recipient; in addition, it was compatible with pDU100 (a Chl^s Tet^r mutant of R100-1). pDU111* is therefore presumed to be integrated into the host chromosome since it has lost both replication and incompatibility gene(s) and can be inherited only as a result of recombination.

Conjugational complementation tests

RecA⁻ strains carrying both a Tet^s Tra⁻ R100-1 mutant and an *Flac tra⁻* element were constructed, and their donor abilities measured, as described in Methods. Most of the transfer gene products of F and R100-1 are interchangeable (Ohtsubo *et al.*, 1970; Willetts, 1971); however, the products of the *traI* and *traJ* genes are plasmid-specific, since R100-1 gives only low levels of complementation with *traI* or *traJ* mutants of F (Willetts, 1971). In addition, R100-1 will not initiate transfer from the F origin of transfer (Reeves & Willetts, 1974), and it has been hypothesized that this is due to the different specificities of the *traI* products (Willetts, 1972). The *traJ* product is required for expression of all the other F transfer genes, including *traI* (Finnegan & Willetts, 1973), which are located in a single operon (Helmuth & Achtman, 1974).

Table 1. *Complementation tests with Flac tra⁻ elements*

The Su⁺ donor strains used as sources of the *Flac* elements were as follows: *Flac*, strain jc6582; *Flac traA1*, jc6608; *Flac traC5*, jc6563; *Flac traF13*, jc6616; *Flac traH80*, m80; *Flac traG81*, m102; *Flac traD83*, m97. They have been described by Achtman *et al.* (1971, 1972).

R factor	<i>Flac</i>		<i>Flac traA1</i>		<i>Flac traC5</i>		<i>Flac traF13</i>		<i>Flac traH80</i>		<i>Flac traG81</i>		<i>Flac traD83</i>	
	R	F	R	F	R	F	R	F	R	F	R	F	R	F
pDU101	$< 7 \times 10^{-5}$	—	—	$< 1 \times 10^{-4}$	—	$< 4 \times 10^{-5}$	—	$< 1 \times 10^{-4}$	—	$< 1 \times 10^{-4}$	—	$< 1 \times 10^{-4}$	—	5×10^{-5}
pDU103	$< 6 \times 10^{-5}$	30	$< 3 \times 10^{-4}$	$< 3 \times 10^{-4}$	5.3×10^{-3}	9.7×10^{-4}	0.52	0.45	0.33	0.35	0.18	0.14	4.9	2.7
pDU106	$< 4 \times 10^{-5}$	25	$< 3 \times 10^{-4}$	$< 3 \times 10^{-4}$	1.7×10^{-3}	7.5×10^{-4}	0.86	1.1	0.23	0.19	0.76	0.74	4.6	2.7
pDU111*	$< 10^{-4}$	0.17	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 5 \times 10^{-5}$	$< 5 \times 10^{-5}$	$< 5 \times 10^{-5}$	$< 5 \times 10^{-5}$	$< 4 \times 10^{-5}$	$< 4 \times 10^{-5}$	$< 4 \times 10^{-4}$	$< 4 \times 10^{-4}$
pDU113	1×10^{-4}	21	$< 1 \times 10^{-4}$	$< 1 \times 10^{-4}$	$< 4 \times 10^{-5}$	1.7×10^{-3}	0.01	0.08	$< 1 \times 10^{-5}$	0.04	$< 1 \times 10^{-4}$	0.31	$< 6 \times 10^{-5}$	0.67
R100-1	21	39	1.6×10^{-3}	1×10^{-3}	1.9×10^{-3}	7.5×10^{-3}	0.01	1.6	0.001	0.1	0.01	0.7	0.04	4.4
			17	3.5	13	4.2	13	4.0	20	3.2	23	5.1	28	4.1

R, R100-1 transfer (%) measured by selecting for Chl^r [Nal^r] progeny; F, *Flac* transfer (%) measured by selecting for Lac⁺ [Nal^r] progeny.

Firstly, transfer of the Tet^s Tra⁻ R100-1 mutants in the presence of wild-type *Flac* was measured (Table 1). Two of the R100-1 mutants (pDU101 and pDU103) were transferred at high levels similar to that of the *Flac* element itself. Two further mutants (pDU106 and pDU113) were transferred at 100-fold lower levels, while pDU111* was never transferred, consistent with a chromosomal location for this element. The two Tet^s Tra⁻ R100-1 mutants transferring at high frequency in the presence of wild-type *Flac* must be *traJ*⁺ and *traI*⁺ and carry *ori* (the R100-1 origin of transfer sequence), while the two transferring at low frequency must have lost at least one of these loci.

Complementation experiments with *Flac* elements mutant in several non-plasmid-specific genes were then performed (Table 1). Complementation in, and transfer from, the *recA* host used was relatively poor, compared with the approximately 100% values found in Rec⁺ strains (Willetts, 1971). We interpret zero levels of transfer of the *Flac tra*⁻ mutants to mean that the R factor deletion has removed all or part of the analogous *tra* gene, and low or very low levels to indicate that the gene is still present. As expected, the levels of transfer of the R100-1 deletion mutants were the same as those of the *Flac tra*⁻ elements for pDU101 and pDU103 (shown above to be *traJ*⁺ *traI*⁺ *ori*⁺), about 100-fold lower for pDU113 (which has lost *traJ*; see below), and zero for pDU111* and pDU106 (*ori*⁻; see below).

pDU106 appeared to have suffered the longest deletion, since it gave no complementation with any of the *Flac tra*⁻ mutants tested. pDU101, pDU103 and pDU111* were similar to each other in having lost *traA* but retaining *traC* and later genes in the transfer operon, while pDU113 retained *traA* as well.

However, although pDU113 retained *traA*, the very low level of complementation indicated a very low level of expression. A likely explanation for this is that pDU113 has lost *traJ*, the product of which is required for expression of the transfer operon (Finnegan & Willetts, 1973); only a low residual level of expression of *traA* can therefore occur.

Although the deletions carried by pDU101, pDU103 and pDU111*, which include *traA*, should have totally removed the promoter for the transfer operon (*p_A*) located between *tet* and *traA*, the genes *traC*, *traF*, *traH*, *traG* and *traD* were expressed at levels sufficient to give approximately 0.1, 20, 5, 10 and 50% complementation of the appropriate *Flac tra*⁻ mutants, respectively (taking complementation by R100-1 as 100%). Similar levels of complementation for these genes in the *tra* operon were also given by pDU113. Since the use of a RecA⁻ host should have prevented recombination between homologous regions of F and R100-1 (no recombinants were in fact found) and therefore any expression of an R100-1 *tra* gene from an F promoter, we propose that a weak promoter is located to the left of *traC*. This would allow partial expression of *traC* and later genes in the *tra* operon. The different levels of retransfer of the *traC*⁻, *traF*⁻, *traH*⁻, *traG*⁻ and *traD*⁻ mutants of *Flac* might be inversely related to the amounts of these gene products required for transfer, and perhaps also to the quantitative abilities of the R100-1 products to replace those of *Flac*.

The origin of transfer

According to the previous section, pDU101 and pDU103 should retain the R100-1 origin of transfer, while pDU106 and pDU113 might have lost this locus. The presence or absence of the origin sequence on these four R100-1 mutants was therefore determined directly. For this, a Tra⁺ Tet^s Chl^s R factor (pDU100) was transferred into the ED2030 (RecA⁻) derivatives carrying the Tra⁻ Tet^s Chl^r R100-1 mutants, and the transfer of chloramphenicol resistance from the resultant Tra⁺ heterozygotes was measured (Table 2).

The three R100-1 mutants pDU101, pDU103 and pDU113 were all transferred at

Table 2. *Origin of transfer and surface exclusion experiments*

R100-1 mutant	Chl ^r Nal ^r progeny/100 heterozygotes†	Surface exclusion index‡
pDU101	2.4	9.3
pDU103	8.7	5.9
pDU106	3.1×10^{-4}	0.79
pDU111*	NT	2.0
pDU113	0.17	6.4
pDU3	NT	43
pDU5	NT	51

† The numbers represent the frequencies of transfer of the Tet^s Tra⁻ Chl^r R100-1 mutants per 100 heterozygous cells also carrying the Tet^r Tra⁺ Chl^s element pDU100.

‡ The ratio of the number of Tet^r progeny of DU1004 (DU1000 in the case of pDU111*) to the number obtained from its derivatives carrying a Tet^s Tra⁻ R100-1 mutant, measured in crosses with a donor strain carrying the Tet^r element pDU100.

NT, not tested.

significant levels, confirming that all these carry the origin sequence. pDU106, however, was transferred at a very low frequency, suggesting that it had lost the origin sequence.

Surface exclusion

R100-1 determines a surface exclusion system (Sf_{XIV}) different from that of F (Sf_{XI}); like that of F, however, it is inhibited during transfer inhibition or in stationary-phase cells, and the gene(s) responsible may therefore form part of the transfer operon (Willettts & Maule, 1974). The surface exclusion gene (*traS*) of F has been located between *traG* and *traD* (Willettts, 1974).

The surface exclusion indices of cells carrying the Tet^s Tra⁻ R100-1 mutants were measured in matings with a donor strain carrying pDU100 (Tet^r) (Table 2). Control experiments showed that the Tet^s Tra⁺ R100-1 point mutants pDU3 and pDU5 had indices of about 50, similar to the levels found previously for R100-1. The four Tet^s Tra⁻ R100-1 mutants carrying deletions ending before *traC* all had indices of 2 to 9, suggesting that they retained *traS* but expressed it at a reduced level from the weak promoter located to the left of *traC*. pDU106 showed no surface exclusion, consistent with a location for the R100-1 surface exclusion genes similar to that of *traS* on F, and with the previous results which suggest that pDU106 carries a deletion removing both p_A and the weak promoter, and possibly all the *tra* genes including *traS*.

DISCUSSION

Perhaps the most interesting of our findings is that a weak promoter is located within the R100-1 transfer region to the left of *traC*. Hu *et al.* (1975) showed that R100-1 carries an Is2 sequence inserted at co-ordinates 67.5 to 68.9 kb within the *tra* region of R100-1, in the orientation such that it should be able to initiate constitutive transcription of the later *tra* genes. This location on R100-1 corresponds to co-ordinate 71.2 kb on F (Sharp, Cohen & Davidson, 1973), which does not carry a similar Is2 insertion, while *traC* is known to be located to the right of 72.9 kb (Sharp *et al.*, 1972). It is therefore most probable that the expression of *traC* through *traD* that we have observed when the promoter for the transfer operon (p_A) is deleted together with the *tet* gene, is due to transcription from the Is2 promoter (p_{Is2}). Assuming that pDU113 is *traJ*⁻, this transcription was, as expected, independent of the presence of the *traJ* product.

Fig. 1. Deletion map of the transfer region of R100-1. Broken lines indicate that it is not known whether the deletion covers this region. The dotted lines show transcription from p_A and p_{Is2} respectively. The order of *traC*, *traF*, *traH*, *traG*, *traS* and *traD* was not determined in these studies, and is assumed to be the same as for F (Ippen-Ihler *et al.*, 1972).

Expression of the later *tra* genes from p_{Is2} will also explain the complementation properties of R100-1 '*traX*⁻' mutants (Ohtsubo *et al.*, 1970) and of R100-1 mutants with λ prophage inserted into *traA* (W. Dempsey and N. S. Willetts, unpublished data). In both cases, reduced levels of complementation with *Flac* elements mutant in later genes of the *tra* operon are observed, and no complementation with those mutant in genes located before *traC*.

Since pDU101 and pDU103 are transferred at high levels in the presence of wild-type *Flac*, even though they have lost p_A , the necessary R100 *traI* product must be obtained by transcription from a promoter other than p_A . This is unlikely to be p_{Is2} , since although *traC* to *traD* are expressed from p_{Is2} , *traI* is apparently not. Thus pDU113 should retain *traI* if it is at the end of the transfer operon distal to *traD*, but its low transfer in the presence of wild-type *Flac* suggests that this *traI* gene is not expressed. Furthermore, the low (1 %) level of transfer of R100 from a cell carrying R100 and *Flac traO*⁻ (Finnegan & Willetts, 1973), shows that expression of the R100 *traI* gene is dependent upon expression of the R100 *traJ* gene (this being the immediate site of action of transfer inhibition), whereas expression from p_{Is2} is *traJ*-independent. We conclude that, unlike the *traI* gene of F, which is the last member of the transfer operon (Helmuth & Achtman, 1974), the *traI* gene of R100 is in a separate operon transcribed by a separate promoter. The location of *traI* on R100 may not be near to *traD*, as in F, but elsewhere; this remains to be determined.

A tentative map of the region of R100-1 studied is presented in Fig. 1, summarizing the extents of the deletions carried by the five Tet^s Tra⁻ R100-1 mutants. The location of *tet* near to *traA* is consistent with the electron-microscope heteroduplex mapping studies of Sharp *et al.* (1972) and the genetic mapping studies of Yoshikawa (1974) and W. Dempsey and N. S. Willetts (unpublished data).

T.J.F. thanks the National Science Council and the Medical Research Council of Ireland for grants.

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