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Transcriptional organization of the Tra2 region controlling conjugational transfer of the narrow host range *Pseudomonas aeruginosa* plasmid R91-5

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SUMMARY

The transcriptional organization of the Tra2 region controlling conjugation by the narrow host range plasmid R91–5 of *Pseudomonas aeruginosa* has been determined. This region of 15·2 kilobase pairs of DNA encodes ten cistrons and specifies the synthesis and functionality of sex pili. The order of the cistrons was previously shown to be *traW*, (*S*, *Z*), *U*, (*V*, *R*), *Q*, *T*, *Y*, *X* (those within parentheses could not be ordered with respect to each other). The organization was determined by complementation tests for the restoration of plasmid transfer between transposon Tn*7* or Tn*501* induced insertion mutants and point mutants representative of the ten cistrons. Polarity mutations induced by Tn*7* permitted the identification of three operons. Cistrons *traW*, *Z*, *S*, *U*, *V*, *R* and *Q* formed a large operon with transcription postulated to be in that order. The second consisted of *traT* and *traY* also in that order and the third consisted of only one cistron, *traX*. Polarity was not observed with Tn*501* insertions as they still permitted full expression of cistrons distal to the site of insertion with respect to the proposed promoter. Despite the unexpected behaviour of this transposon it nevertheless led to the identification of two previously unidentified cistrons, *traO* and *traP* being between *traZ* and *S* and *traR* and *V*, respectively.

1. INTRODUCTION

R91-5 is a derepressed mutant of the narrow host range IncP-10 plasmid R91 of *P. aeruginosa* which is being studied with the view to determining the genetic basis of its restricted host range (Moore & Krishnapillai 1982*a*, *b*). A restriction endonuclease map of it has been constructed which has facilitated the physical and genetic mapping of genes controlling its replication and its conjugal transfer, including its origin and direction of transfer (*oriT*). Two unlinked regions, Tra1 encompassing 4·5 kb of DNA and involved in conjugal DNA metabolism and Tra2 of 15·2 kb controlling the synthesis and functionality of sex pili have been identified. The latter region is also responsible for sensitivity to donor specific phages. The generation of deletions, transposon Tn*7* and Tn*501* insertions affecting

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transfer (*tra*) and the cloning of some of the *tra* DNA were instrumental in defining the limits of Tra1 and Tra2. Complementation tests performed with transient heterozygotes constructed by phage F116L transduction of one mutant plasmid into cells carrying another mutant plasmid has led to the identification of ten cistrons, namely *traX*, *Y*, *T*, *Q*, *R*, *V*, *U*, *S*, *Z* and *W* (Carrigan & Krishnapillai, 1979). Further complementation tests between mutants representative of these ten cistrons controlling sex pili synthesis and functionality with various deletion mutants has allocated these cistrons to Tra2 (Moore & Krishnapillai, 1982*b*). The property of polarity inducing mutations by transposable elements (Kleckner, 1981) provided the opportunity to test complementation between representative mutants of the *tra* cistrons and the insertion mutants to determine the transcriptional organization of the cistrons within Tra2. This paper reports the analysis of such data.

2. MATERIALS AND METHODS

(i) *Media*

Nutrient agar and nutrient broth have been described previously (Carrigan & Krishnapillai, 1979). Carbenicillin (Cb, Beecham Research Laboratories) was used at a concentration of 500 μg/ml in blood agar base (Oxoid). Rifampin as Rifadin (Rif, Lepetit Pharmaceuticals Ltd) was used at a concentration of 200 μg/ml in blood agar base.

(ii) *Bacteria, bacteriophages and plasmids*

The *P. aeruginosa* bacterial strains PAO5 *trp-54 rif-5 fon* [resistance to phage F116L]) and PAO8 (*met-28 ilv-202 str-1*) have been described (Moore & Krishnapillai, 1982*a*). The transducing phage used was F116L (Krishnapillai, 1971). R91-5 and the reference mutants representative of the ten cistrons in Tra2 have been described (Carrigan & Krishnapillai, 1979). The isolation and genetic and physical characterization of the Tra$^-$ Tn*7* and Tn*501* insertion mutants have been described (Moore, 1981). The mapping of the insertions by restriction enzyme fragment analysis was as described in Moore & Krishnapillai (1982*a*). All the Tn*7* mutants have the transposon inserted in the α orientation, i.e. the end closest to the *Eco*RI site is the clockwise end of the inserted transposon. pMO1152 has a double insertion of Tn*7* mapping at 17·4 and 45·3 kb co-ordinates (Moore & Krishnapillai, 1982*a*). For the Tn501 mutants, pMO852, 503 and 881 have the transposon inserted in the α orientation, i.e. the end closest to the *Hin*dIII site is the clockwise end of the inserted transposon. The rest of the Tn*501* mutants have the transposon inserted in the β orientation.

(iii) *Complementation tests*

Complementation for plasmid transfer proficiency was assessed by transfer from transient heterozygotes harboring two Tra$^-$ plasmid mutants, constructed by phage F116L transduction, to a phage resistant final recipient (Carrigan & Krishnapillai, 1979; Moore & Krishnapillai, 1982*b*).

3. RESULTS

Complementation tests: determination of the transcriptional organization of the Tra2 region of R91-5

To determine whether the insertion of Tn*501* or Tn*7* into the Tra genes was polar on promoter distal genes a number of insertion mutants were used in complementation tests with reference mutants from the ten *tra* cistrons. Polarity would be indicated if more than one cistron was affected in the insertion mutants. If polarity was found, then because the order and approximate map positions of the *tra* cistrons are known as well as the places of insertion of the transposons, it should be possible to determine the direction of transcription and the cistrons that make up any one transcriptional unit.

The results of the complementation tests are shown in Tables 1 and 2. It can be seen that some insertion mutants affect more than one cistron. As restriction enzyme fragment analysis of the insertion mutants showed no detectable deletions, the inability of some of the insertion mutants to complement more than one cistron suggests polarity on cistrons distal to the site of insertion with respect to the promoter such that the genetic expression of cistrons downstream is reduced.

The data from the Tn*7* insertions permit an evaluation of the transcriptional organization of the Tra2 region. Insertions in the cistrons *traR*, *V*, *U*, *S* and *W* were polar on cistrons to the left of the insertion up to *traQ* (see Fig. 1). For example, pMO1008 had a Tn*7* insertion at co-ordinate 19·15, in *traU*. This mutant failed to complement not only *traU* mutants but also mutations in *traQ*, *traR* and *traV*. Similarly insertions into *traV* (pMO1011 and pMO1152) also failed to complement *traR* and *traQ* mutants and an insertion into *traR* (pMO1004) failed to complement *traQ* mutants. These data suggest that the cistrons *traW*, *Z*, *S*, *U*, *V*, *R* and *Q* comprise a single transcriptional unit transcribed from the *traW* end of the group of cistrons. Polar mutations within this group of cistrons appear not to effect the expression of *traT*, *Y* and *X*. It is therefore likely that these cistrons are in a different operon or operons. The insertion at co-ordinate 14·1 (pMO1019) inactivates both *traY* and *traT* and is almost certainly inserted into *traT*, hence the inactivation of *traY* is interpreted as being due to polarity, thus suggesting that *traY* and *traT* make up a second operon. The polar insertion into *traT* did not affect the expression of *traX* and so it is likely that *traX* is in a third operon. The data from the Tn*7* insertions therefore suggest that there are three operons in the Tra2 region. The first is transcribed from *traW* through *traZ*, *S*, *U*, *V*, *R* and *Q*. The second, transcribed in the same direction, consisting of *traT* and *traY* and the third which consists of only *traX*. The direction of transcription of *traX* is unknown.

The complementation data for the Tn*501* insertion mutants provided little evidence of polarity. Although some insertion mutants failed to complement reference mutants from two cistrons there was no pattern of polarity which was consistent within the Tn*501* insertions or compared with the polar effects of Tn*7*. In the cases in which two cistrons were affected the insertion was in one cistron and the second cistron affected was promoter distal to the insertion (as determined from the Tn*7* insertions). Therefore in this respect the data are consistent with that

Table 1. *Complementation of reference Tra⁻ mutants by Tn7 insertion mutants**

Insertion mutant	Site of insertion (kb co-ordinate)	Reference Tra⁻ mutants									
		traX pMO200	*traY* pMO337	*traT* pMO313	*traQ* pMO207	*traR* pMO237	*traV* pMO329	*traU* pMO226	*traS* pMO334	*traZ* pMO209	*traW* pMO316
pMO1019	14·1	34	8	0·1	34	42	168	49	165	37	106
pMO1004	17·1	30	40	167	9	1	53	65	297	84	129
pMO1152	17·4	20	13	166	7	2	0	26	173	76	54
pMO1011	17·6	35	44	145	2	0	1	16	298	31	170
pMO1008	19·15	21	26	90	8	2	0	0	214	44	137
pMO1016	19·85	76	75	217	23	32	12	6	3	19	111
pMO1010	22·65	26	56	82	16	6	7	6	17	0·2	4

* Transient heterozygotes were constructed by F116L transduction of the reference Tra⁻ mutants into PA08 strains containing the insertion mutants and transfer of Cb^r was measured out of the double into PA05. The figures in the table are the efficiencies of complementation, calculated as the percentage of transfer using the various reference *tra* mutants, compared with the transfer using R91-5 as the complementing plasmid.

Table 2. *Complementation of reference Tra⁻ mutants by Tn501 insertion mutants**

Insertion mutant	Site of insertion (kb co-ordinate)	Reference Tra⁻ mutants									
		traX pMO200	*traY* pMO337	*traT* pMO313	*traQ* pMO207	*traR* pMO237	*traV* pMO329	*traU* pMO226	*traS* pMO334	*traZ* pMO209	*traW* pMO316
pMO852	12·35	134	0	382	64	67	159	142	510	96	176
pMO875	15·0	435	77	0	137	128	203	139	532	139	316
pMO503	17·5	667	129	214	109	100	95	109	538	105	267
pMO1103	17·0	520	233	311	92	3	82	111	466	113	457
pMO872	18·0	513	232	352	49	21	0·5	145	926	142	259
pMO874	18·65	310	190	189	26	36	7	0	238	71	136
pMO497	20·9	166	106	137	129	106	83	57	83	43	89
pMO881	21·65	167	112	147	130	94	183	118	112	0	23
pMO1079	23·2	609	129	491	201	140	277	89	298	3	3

* See legend to Table 1 for details

from the Tn*7* insertions. However, the reason for the polarity not extending along the length of the proposed operon is not known.

The complementation analysis of the insertion mutants also demonstrates the order of the cistrons which could not be ordered by the deletion and cloning analysis, namely that for *traR*, *traV* and *traS*, *traZ* (Moore & Krishnapllai, 1982*b*). A Tn*7* insertion at co-ordinate 17·1 (pmO1004) abolished the function of *traR* but not *traV* whilst insertions at co-ordinates 17·4 and 17·6 (pMO1152 and pMO1011 respectively) abolished the function of both *traR* and *traV*. The Tn*501* insertion at co-ordinate 17·0 (pMO1103) abolished the function of *traR* whereas the insertion at co-ordinate 18·0 (pMO872) affected *traV* (as well as *traR*). It can therefore be seen that *traV* is to the right of *traR* (see Fig. 1) and hence is earlier in the transcriptional unit. The Tn*7* insertion at co-ordinate 19·85 abolished the function of *traS* but not *traZ*. No Tn*501* insertions affected *traS*; however, two affected *traZ*. These mapped at co-ordinates 21·65 and 23·2 (pMO881 and pMO1079 respectively). It is therefore deduced that *traZ* is clockwise of *traS*. The order of all cistrons is *traX*, *Y*, *T*, *Q*, *R*, *V*, *U*, *S*, *Z* and *W*.

Amongst the Tn*501* insertion mutants used in the complementation tests there were two which complemented reference mutants from all ten cistrons that have so far been identified. These two mutants, pMO497 and pMO503, were clean Tra$^-$ ($< 10^{-6}$ Cb^r/donor) and mapped within the Tra2 region. pMO497 carried an insertion at co-ordinate 20·9 and pMO503 had an insertion at co-ordinate 17·5. As they produced a clean Tra$^-$ phenotype and yet complemented all known *tra* cistrons they must represent mutations in as yet unidentified cistrons. The Tn*501* insertion in pMO497 appears to have mutated a *tra* cistron between *traZ* and *traS* whereas the cistron inactivated in pMO503 appears to be between *traR* and *traV* and these have been designated *traO* and *traP*, respectively.

4. DISCUSSION

The interpretation of the complementation data is compatible with the idea that the Tra2 region R91-5 has three transcriptional units or operons as identified by Tn*7* polarity mutations (see Fig. 1). The first consists of *traW*, *Z*, *S*, *U*, *V*, *R* and *Q* and is transcribed from the *traW* end. The second is transcribed in the same direction and consists of *traT* and *traY*. The cistron *traX* is in another unit but no data is available that would allow the direction of transcription to be determined.

However there are some apparent exceptions to the overall interpretation of these complementation data. For example the Tn*7* mutation in pMO1016, which has an insertion in *traS*, in addition to displaying polarity towards the *traU*, *V*, *R* and *Q* cistrons appears also to reduce the expression of the *traZ* cistron. Similar exceptions have also been observed with phage mu-induced polarity mutations in similar complementation tests in the analysis of the transcriptional organization of the *tra* cistrons of the F plasmid of *E. coli* (Helmuth & Achtman, 1975). The reason(s) for these exceptions are not understood but they perhaps reflect the differential synthesis of the translational products of the individual cistrons and their complex interaction prior to the assembly of the conjugational machinery.

It is also possible to rule out the interpretation of the data in terms of plasmid recombination to give wild-type plasmids since it has previously been shown that this is not the case in this system (Carrigan & Krishnappillai, 1979).

The complementation pattern of the Tn*501* insertion mutants showed that insertion of this transposon was not polar in either orientation of insertion. This was unusual as polar mutations are usually expected of insertions of transposable genetic elements into transcriptional units (Kleckner, 1981). The reason(s) for this property of Tn*501* are unknown. Although Tn*501* insertions failed to induce polarity mutations they nevertheless led to the identification of two new cistrons, namely *traO* (between *traZ* and *traS*) and *traP* (between *traR* and *traV*).

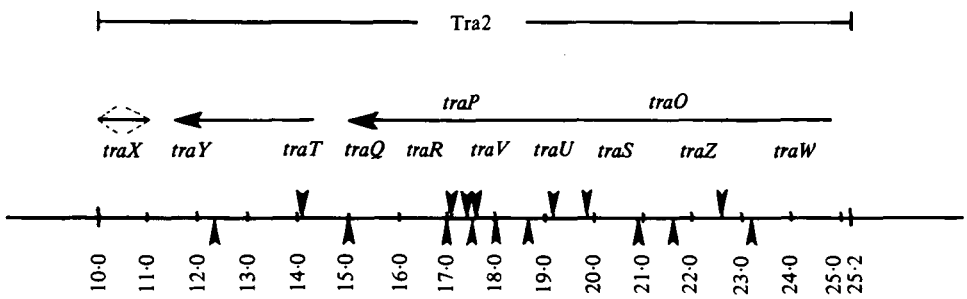

Fig. 1. Proposed transcriptional organization of the Tra2 region of R91-5. The extent of the Tra2 region is shown. Numbers refer to kilobase coordinates on the restriction endonuclease map (Moore & Krishnapillai, 1982*b*). The cistrons shown below the arrows are those defined by Tn*7* polarity mutations and the arrows show the direction of transcription. The dotted arrow heads above *traX* show that the direction of transcription is unknown. Also indicated are the location of the two new cistrons identified in this study by Tn*501* insertions. They are placed above the arrow as their location within the proposed transcriptional unit is unknown. Vertical arrows above the line indicate Tn*7* insertions and those below indicate Tn*501* insertions.

The genetic and physical organization of the *tra* genes of the narrow host range plasmid R91-5 of *P. aeruginosa* (Moore & Krishnapillai, 1982*a*, *b*; this paper) can be compared with that of the narrow host range F plasmid of *E. coli* K12) (Willetts & Skurray, 1980). In F about 34 kilobase pairs of continuous DNA control sex pili synthesis and functionality, stabilization of mating aggregates and conjugal DNA metabolism. The products of 12 cistrons are required for the synthesis of pili and thus for sensitivity to donor specific phages. Six cistrons control conjugal DNA metabolism whereas two specify the stabilization of mating aggregates. All but one of these cistrons are in one very large operon with two others being contiguous but outside this operon. The direction of transcription of these two and the large operon is the same. The similar direction of transcription of the cistrons required for F-mediated conjugation in *E. coli* is also apparent with R91-5-mediated conjugation in *P. aeruginosa*. Again a very large number of cistrons, namely 12, appear to be involved with R91-5 sex pili synthesis and functionality and the stabilization of mating aggregates (Moore & Krishnapillai, 1982*a*; this paper). However the genetic organization of the genes for conjugation of these two

respective narrow host range plasmids differ in two important respects. Firstly unlike the grouping together of the genes for sex pili synthesis and functionality with those for conjugal DNA metabolism in F, two separate, unlinked regions control these processes in R91-5, Tra1 for conjugal DNA metabolism and Tra2 for sex pili synthesis and functionality (Moore & Krishnapillai, 1982*a*). Furthermore, structurally the sex pili of F are morphologically thick and flexible (Bradley, 1980) whereas those of R91-5 are thick and rigid (Bradley, 1980; 1983). Secondly, the total amount of DNA required for both functions in R91-5 is 19·7 kilobase pairs (Moore & Krishnapillai, 1982*a*,*b*). This implies either that conjugation mediated by R91-5 in *P. aeruginosa* requires much less genetic information encoded by the plasmid or that the *P. aeruginosa* host contributes considerably more such information than does *E. coli* K12 to F. Two *E. coli* host transcriptional control factors, *sfrA* and *sfrB*, have been identified which modulate F transcription (Beutin *et al.* 1981) and it remains to be seen whether similar and additional host genes of *P. aeruginosa* regulate the expression of the conjugational genes of R91-5.

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