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Tn*7* and Tn*501* Insertions into *Pseudomonas aeruginosa* Plasmid R91-5: Mapping of Two Transfer Regions

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We constructed a restriction endonuclease map of the *Pseudomonas aeruginosa* narrow-host-range plasmid R91-5. Insertions of transposons Tn*7* and Tn*501* into the plasmid DNA were characterized physically and genetically. The distribution of sites of insertion showed some regional specificity for the insertion of these transposons, especially Tn*501*. The insertion of Tn*7* was unusual in that all 42 of 43 insertions were in the same orientation. By relating phenotypic changes to the site of insertion, the Tn*1* transposon that was already present on R91-5 and coded for carbenicillin resistance was mapped, and its orientation was determined. Two major transfer regions were identified. We believe that Tra1 is involved in conjugal DNA metabolism, whereas Tra2 is involved mainly in production of the sex pili.

We are interested in understanding the genetic basis of plasmid transfer in *Pseudomonas aeruginosa*. To this end, we studied IncP-10, narrow-host-range plasmid R91-5, a derivative of R91 that was derepressed for transfer. R91-5 transfers at 100% efficiency (11), and its DNA can be isolated readily to allow restriction enzyme analysis. R91-5 has been used to map the chromosome of *P. aeruginosa* strain PAT (30) and is currently being used to extend the genetic map of the chromosome of strain PAO (Krishnapillai et al., Genetics, in press; R. Crockett and A. F. Morgan, personal communication). A Tn*501* derivative of R91-5 has been used to generate Hfr-like strains of *Pseudomonas putida*, which have been used to map the *P. putida* chromosome (H. Dean and A. F. Morgan, manuscript in preparation).

Many transfer-deficient mutants (Tra^-) of R91-5 have been isolated and characterized. It has been shown that sensitivity to donor-specific phages (Dps), inhibition of phage G101 [Phi(G101)], and entry exclusion (Eex) are integral parts of the transfer system (8). A transductional complementation system has been developed for the analysis of Tra^- mutants, and 10 cistrons that affect transfer have been identified (9). The mutagenic effects of transposition of transposons Tn*7* and Tn*501* into R91-5 have been investigated (22). It has been found that the frequency of mutation to carbenicillin sensitivity is similar for these two transposons; however, the frequency of induction of Tra^- mutants by recent transposition events is very different for Tn*7* and Tn*501*. It has been concluded that these transposons have different specificities of insertion. In the course of these studies, a large collection of transposon derivatives of R91-5 was assembled. In this paper we describe the physical characterization and mapping of these transposon derivatives by restriction fragment analysis.

The transfer genes of IncP-1 plasmids, such as RP4, have been mapped by generating mutants containing transposon Tn*7* and mapping their insertion sites by restriction fragment analysis (4, 5). With the availability of the large collection of transposon derivatives of R91-5 (22), the method described above can be used to analyze the genetic organization of the plasmid. In this paper we report the construction of a restriction endonuclease map of R91-5 and the identification and mapping of two major transfer regions and the carbenicillin resistance gene (*bla*) by correlating phenotypic changes to the plasmid to the location of insertion of the transposons.

MATERIALS AND METHODS

Bacteria, bacteriophage, and R plasmids. The bacteria, bacteriophage, and R plasmids used are shown in Table 1.

Media and techniques. The media used have been described previously (8, 22). The DNA extraction and purification, restriction endonuclease digestion, and agarose gel electrophoresis techniques used have also been described previously (28), as have the transposition techniques used (22).

Characterization of mutants. The method used to determine sensitivity to donor-specific phages has been described previously (8). To determine plasmid transfer frequencies (Cb^r transfer), we performed plate matings by plating 0.1 ml of donors (diluted appropriately) and 0.1 ml of recipients directly onto a selective medium (nutrient agar containing carbenicillin). For Tc^r transfers, the matings were first performed on

TABLE 1. Bacterial strains, plasmids, and phages used

Strain, plasmid, or phage	Relevant characteristics[a]	Reference
P. aeruginosa strains		
PAO1	*cml-2* prototroph	18
PAO5	*trp-54 rif-5 fon-1*	9
PAO8	*met-28 ilv-202 str-1*	18
PAO1670	*pur-136 leu-8 cml-3 rif-1*	10
Plasmids		
R91-5	Cb Tra^+ Dps^+ Phi (G101) EexP-10 IncP-10	8
pVS1	Hg Su Tra^-	27
RP4-TnC2[b]	Cb Nm/Km Tc Tp Sm Tra^+	5
pMO872[c]	Cb Tra^- Dps^-	This study
pMO505[d]	Cb Tra^-	This study
pKT212	Sm Cm Tc	3
Phage PRD1	Donor specific for R18 and R91-5	25

[a] Host chromosomal designations are according to Bachmann et al. (2). *fon-1*, Resistance to phage F116L. Plasmid phenotype symbols are according to Novick et al. (24).

[b] The TnC transposon is now designated Tn7 (5).

[c] Tra^- mutant of R91-5 induced by Tn*501*. The insertion maps in the Tra2 region at 18.0 kb.

[d] Tra^- mutant of R91-5 induced by Tn*501*. The insertion maps in the Tra1 region at 0 kb.

nutrient agar for 2 h, the plates were washed off, and samples were plated onto a selective medium.

Frequency of mobilization of pKT212. The frequency of mobilization of pKT212 was determined in essentially the same way as the plasmid transfer frequencies (see above). The only difference was that selection was for transfer of markers on the mobilized plasmid (e.g., Tc^r) rather than the conjugative plasmid.

Transformation of plasmid DNA. The transformation method used was essentially the method described by Sinclair and Morgan (26).

Determination of mating aggregate formation. To determine whether strains carrying the plasmid insertion mutants could form stable mating aggregates (23) with recipient strains, we mixed cells of donor and recipient strains together on a microscope slide and covered them with a cover slip. We used a Leitz Ortholux II Zernike phase-contrast microscope to visualize and photograph the cells.

RESULTS

Restriction endonuclease map of R91-5. To map the sites of Tn*7* and Tn*501* insertions, we had to first construct a restriction endonuclease map of R91-5. To do this, R91-5 DNA was digested separately with 16 restriction endonucleases, each of which recognized a different hexanucleotide sequence. The number of fragments produced by these restriction enzymes ranged from 3 for *Bgl*II, *Hpa*I, and *Xba*I up to 20 for *Bam*HI. For restriction mapping we chose the enzymes which produced fewer fragments because the fewer the sites, the easier they were to map. Six enzymes (*Bgl*II, *Eco*RI, *Hin*dIII, *Hpa*I, *Kpn*I, and *Xba*I) were used to digest R91-5 DNA both singly and in various combinations as double digestions. We analyzed the data generated from these digestions essentially as done by Grinsted et al. (14) for RP1, and we also used the data from an *Eco*RI partial digestion to order *Eco*RI fragments G and H; in this way we unequivocally deduced the positions of all 30 restriction sites. Figure 1 shows the resulting restriction endonuclease map of R91-5.

Mapping the sites of insertion of Tn*7* and Tn*501*. The site of insertion and the orientation of the transposon in a transposon insertion derivative of R91-5 were determined by analyzing the results of *Eco*RI and *Hin*dIII digestions (4). The Tn*501* insertion sites were mapped easily because Tn*501* had *Eco*RI sites at each end and these sites are very close to the termini (14). Therefore, after digestion with *Eco*RI, there were only two sites at which Tn*501* could have been inserted. Digestion with *Hin*dIII (one site on Tn*501* [13]) permitted the identification of the site at which the transposon was inserted and also identified the orientation of the transposon. Interpretation of digests of Tn*7* derivatives was slightly more complex, as the *Hin*dIII and *Eco*RI sites were not close to the ends of the transposon or distributed symmetrically. Therefore, after digestion with one enzyme, there were four possible interpretations of the data with regard to the site of insertion. Digestion with the second enzyme generally defined the actual site of insertion. In a few cases we were uncertain about the site of insertion after *Hin*dIII and *Eco*RI digestions. In these instances digestion with another enzyme (*Bgl*II, *Hpa*I, *Kpn*I, or *Xba*I) indicated the actual site of insertion. The map of Tn*7* which we deduced for the purpose of mapping was the same as the map of Hernalsteens et al. (16), except that the distance from the unique *Eco*RI site to the farthest terminus was 9 rather than 10 kilobases (kb).

The transposon insertion derivatives that we mapped were not a random group; rather, they

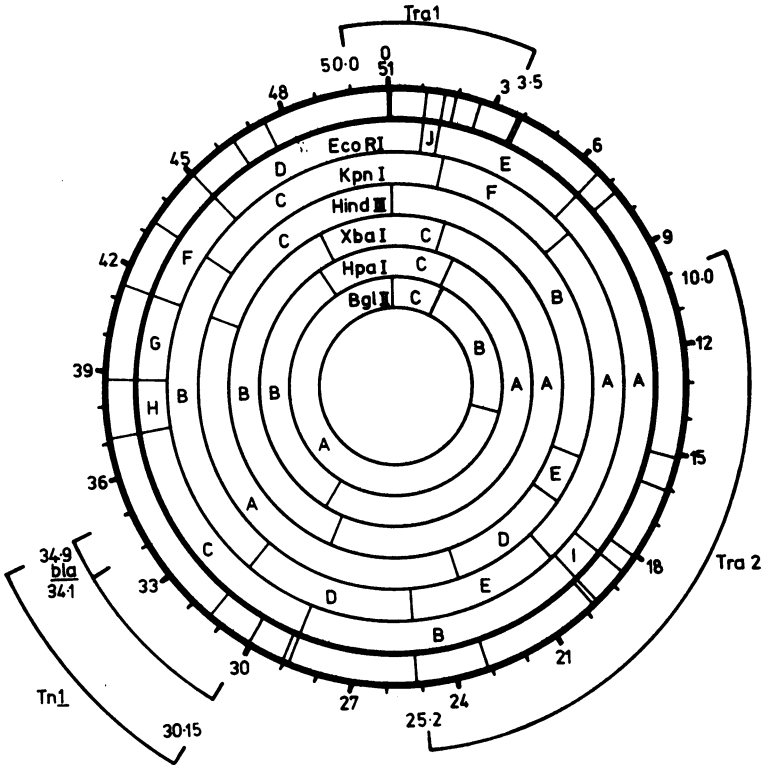

FIG. 1. Physical and genetic map of R91-5. The inner circles show the restriction endonuclease cleavage sites. These sites were orientated with respect to the *Bgl*II site at coordinate 0, and the numbers are the distances (in kilobases) from that site. The outer circle is the composite map of all sites, and the inner circles are the maps for each enzyme separately. The fragments are labeled alphabetically in decreasing order of size, with A being the largest fragment. Also shown are the locations of the two transfer regions and the location of Tn*1*.

were mainly selected plasmids with mutations that were of interest in the study of plasmid transfer. All of the Tn*7*-induced mutants and approximately one-half of the Tn*501*-induced mutants were chosen from a previously described collection of mutants (22). A total of 74 Tn*501* insertions and 43 Tn*7* insertions were mapped. Of these, seven Tn*501* insertions and five Tn*7* insertions had inactivated the R91-5 gene coding for carbenicillin resistance but had not affected plasmid transfer. All of the insertions that produced a Cb^s Tra^+ phenotype mapped between coordinates 34.1 and 34.9 (Fig. 1). The structural gene for the Tem-2 β-lactamase, which confers carbenicillin resistance on R91-5-carrying cells (19), or its promoter or both must fall at least in part between those coordinates. Within R91-5, the gene for β-lactamase is carried on transposon Tn*1* (20). By relating the known physical structure of the closely related

TABLE 2. Mobilization of pKT212 by R91-5 Tra^- insertion mutants[a]

Plasmids in donor	No. tested in donor[b]	Class	Transfer frequency[c]	
			Cb^r	Tc^r
R91-5, pKT212		Wild type	6×10^{-1}	3×10^{-1}
R91-5 mutant, pKT212	9	Tra1	$10^{-4} - <10^{-8}$	2×10^{-1}
R91-5 mutant, pKT212	4	Tra2	$10^{-8} - <10^{-8}$	10^{-8}

[a] Strains containing two plasmids were constructed by transforming strain PAO5 carrying different R91-5 insertion mutants with pKT212. From these double-plasmid donors transfer out of pKT212 and transfer out of the R91-5 mutants were measured independently. The recipient was strain PAO1. A total of 11 of the mutants were Tn*501* induced, and the rest were Tn*7* induced.

[b] The number of different R91-5 insertion mutants tested.

[c] Number of transconjugants per donor.

transposon Tn*3* (15) to the position of the β-lactamase gene and the *Eco*RI sites in R91-5, it was possible to determine the orientation of the Tn*1* element within R91-5. The *bla* gene of Tn*3* is at the extreme right end (as defined by Heffron et al. [15]) of the transposon, and the transposon extends 3.95 kb from the *bla* gene to the left end. In addition, there are no *Eco*RI sites within the transposon. Therefore, the left end of Tn*1* must be anticlockwise from the β-lactamase gene in R91-5 at approximately coordinate 30.15 (Fig. 1), since in the other orientation two *Eco*RI sites would map in the region supposedly covered by the transposon.

Krishnapillai mapped four Tn*501* insertions which not only affected carbenicillin resistance but also caused a 10^2- to 10^4-fold reduction in the transfer frequency of the plasmid (22). These insertions mapped within and at the edges of the β-lactamase gene.

The majority of the insertion mutants were in a third group, whose members were affected in plasmid transfer but remained carbenicillin resistant. The insertions of these mutants mapped into the two main regions, one between coordinates 50 and 3.5 (4.5 kb) and the other between coordinates 10.0 and 25.2 (15.2 kb) (Fig. 1). Therefore, it appeared that there were two distinct regions that coded for plasmid transfer.

The Tn*7* mutants were unusual in that all insertions except one were in the same orientation. We do not know whether the insertion in the exceptional mutant was actually a rare insertion in the opposite orientation or whether the region immediately around and including Tn*7* had undergone an inversion event either during or after Tn*7* transposition. If such an inversion did not extend far beyond Tn*7*, it would not have been detected by the restriction fragment analysis. We found that Tn*501* was inserted in both orientations, although one orientation was three times more prevalent than the other.

The collection of Tn*7*-induced mutants was also unusual in that it had a large proportion (28%) of double insertions of the transposon. In all of the double-insertion plasmids tested, the Tn*7* elements were in the same orientation and the two copies of Tn*7* were widely spaced on opposite sides of the plasmid. For example, the pairs were at coordinates 9.45 and 43.5, 10.9 and 45.1, 16.2 and 35.15, 16.3 and 44.7, 17.4 and 45.3, 17.6 and 31.05, 18.05 and 36.0, 18.5 and 45.45, and 18.95 and 36.15.

We selected most insertion mutants for study on the basis of their phenotypic characteristics (Cb^r, Tra, Dps). Because we investigated a nonrandom group of R91-5 derivatives, it was difficult to evaluate whether there was any regional specificity for the insertion of Tn*7* and Tn*501* into R91-5. However, there was a group of 31 Tn*501*-induced mutants which we selected only on the basis of being Tra^-. Potentially, the sites of insertion in these derivatives could have been anywhere within the two Tra regions. When we investigated the distribution of the sites of insertion, we found that 21 sites mapped in the small Tra region between coordinates 50 and 0.5, a region only 1.5 kb long, whereas only 10 sites mapped in the large 15.2-kb region between coordinates 10.0 and 25.2. Clearly then, there was regional specificity for the insertion of Tn*501*. In addition to this broad regional specificity, there were also more defined sites of insertional hotspots for both Tn*7* and Tn*501*. These hotspots were sites at which more than one insertion mapped (for example, the site at coordinate 25.25, where three Tn*501* insertions mapped, and the site at coordinate 0.5, where four Tn*7* insertions mapped). It has been shown that such hotspots and regional specificity of insertion cannot be determined by the recognition of a simple sequence arrangement (12). Tu and Cohen (29) have suggested that insertional hotspots for Tn*3* may result from the combined effects of adenine-thymine richness and the homology of the recipient genome and the terminal sequences of Tn*3*. This hypothesis may also explain the regional specificity of Tn*501* insertion. It has been shown (7) that the terminal sequences of Tn*501* and Tn*3* are homologous and that the hotspots of Tn*501* insertion into RP1 are close to the termini of Tn*801*, a Tn*1*-like element (13). To determine whether Tn*501* had any preference for insertion close to the Tn*1* element in R91-5, we mapped the sites of insertion of nine Tra^+ Tn*501* derivatives chosen at random. None of these sites mapped close to the termini of Tn*1*. However, they did show regional specificity in insertion, as they all mapped between coordinates 41.5 and 49, with five mapping in the 1-kb region between coordinates 45 and 46. It is known that there are other large regions of the plasmid which are not essential for plasmid replication (Moore and Krishnapillai, submitted for publication), yet in the limited number of insertions investigated none has been mapped in these regions.

Phenotypic characterization of transposon derivatives. To determine the locations of genes that affect certain aspects of transfer and transfer-related phenotypes, we characterized all of the R91-5 derivatives phenotypically. Changes in phenotype were related to the site of Tn*501* or Tn*7* insertion. Initially, the transfer frequencies of all derivatives were determined by plate matings. One of the criteria by which most of the mutants were chosen for physical analysis was their clean Tra^- phenotype ($<10^{-6}$ Cb^r mutants per donor). However, a number of leaky Tra^- ($>10^{-6}$ Cb^r mutants per donor) mutants were

also included in the analysis. The mutations of the clean Tra$^-$ mutants mapped in both Tra regions; Tral mapped between coordinates 50.0 and 3.5, and Tra2 mapped between coordinates 10.0 and 25.2 (Fig. 1). As mentioned above, the mutations of one group of leaky Tra$^-$ mutants mapped close to the *bla* gene, whereas other mutations mapped within Tra2 and on the edge of Tral. There were also two Tn*501*-induced leaky Tra$^-$ mutants whose mutations mapped outside the two major Tra regions, one at coordinate 46 and the other at coordinate 46.05. The mutation of a leaky Tra$^-$ Tn*7* mutant mapped at coordinate 8.5.

We tested sensitivity to the donor-specific phage PRD1 (Dps$^+$) by cross-streaking against a high-titer phage lysate. PRD1 has been shown to absorb to the pili of cells containing plasmid IncP-1 (6). Although cells containing R91-5 have not been shown to have sex pili, it is reasonable to assume that they do and that the site of PRD1 absorption is the pilus. We found that many of the clean Tra$^-$ mutants were resistant to PRD1. By analogy with plasmid F and the F-specific phages which bind to the F pilus (32), we hypothesized that the R91-5 insertion mutants which were resistant to phage PRD1 did not produce functional pili. The Tra$^-$ mutants which retained PRD1 sensitivity may have produced functional pili, but may have been mutant in functions such as conjugal DNA metabolism and mating pair stabilization. The distribution of Dps$^+$ and Dps$^-$ Tra$^-$ mutants was interesting. The mutations of all mutants which were Dps$^-$ mapped in the Tra2 region. This meant that the Tra2 region coded for the synthesis and functionality of the sex pilus. The mutations of the clean Tra$^-$ mutants which retained full sensitivity to PRD1 (Dps$^+$) all mapped in the Tral region, with most tightly clustered into a 1.15-kb region between coordinates 50.3 and 0.45. By analogy with F (1, 31) this group of mutants presumably produced functional pili but were defective in other transfer-related functions, such as conjugal DNA metabolism and mating aggregate stabilization. There was also a class of mutants which was partially sensitive to PRD1. The mutations of clean Tra$^-$ mutants of this class mapped in three regions. There was one group of three Tn*501*-induced mutants containing mutations on the edge of Tra2 around coordinate 12. The mutations of this group were at the edge of the large region in which the mutations of all of the Dps$^-$ mutants mapped. The second group consisted of four Tn*501* mutants which were indistinguishable from each other, and the mutations of these mutants mapped in the Tral region at coordinate 50.2. A third site for mutations of DPs$^{+/-}$ mutants was at the end of the Tral region at coordinate 3.5.

Nature of the transfer-deficient, Dps$^+$ insertion mutants. As mentioned above, Tra$^-$ Dps$^+$ mutants probably have intact, functional pili. To confirm this, we investigated these mutants to determine whether they could mobilize a resident nonconjugative plasmid. We found that R91-5 could efficiently mobilize the naturally nonconjugative IncP-4 plasmid RSF1010 and its in vitro-constructed derivatives pKT210 and pKT212 (3). Strains carrying the Tra$^-$ Dps$^+$ insertion mutants were transformed with pKT212 DNA. From the strains carrying both plasmids, transfers out of pKT212 and the R91-5 mutant were measured independently (Table 2). Transfer of the R91-5 mutant showed that pKT212 did not complement the transfer defect. We found that the Tra$^-$ Dps$^+$ mutants and the mutants whose mutations mapped at coordinate 50.2, which retained partial sensitivity to PRD1, all mobilized pKT212 at frequencies comparable to those of R91-5. This indicated that cells harboring these mutants of R91-5 synthesized normal, functional pili and could form stable mating aggregates. Therefore, it is likely that these derivatives of R91-5 were mutant in some aspect of conjugal DNA metabolism. Such mutations may affect the origin of transfer of enzymes required for processing DNA (e.g., analogous to the hypothesized nicking enzymes of F produced by *traM* and *traI* [32]). Obviously, pKT212 mobilization does not require the gene product(s) encoded by the mutated Tra genes in these R91-5 derivatives. This is analogous to the mobilization of ColE1 by F plasmid, which does not require functional *traM*, *traI*, or *traZ* genes (32). The other partially PRD1-sensitive Tra$^-$ mutants whose mutations mapped around coordinate 12 did not mobilize pKT212 with high efficiency. This inability to mobilize pKT212, plus the fact that plating of PRD1 was affected to some extent, may have meant that the sex pili in the strains containing these plasmids were nonfunctional. Alternatively, the transposon may have been inserted into a gene that affected mating aggregate stabilization (e.g., *traN* of F plasmid [23]) into a gene that coded for a DNA-processing enzyme which was required both for R91-5 transfer and pKT212 mobilization.

To determine whether the partially PRD1-sensitive Tra$^-$ mutants whose mutations mapped around coordinate 12 could form stable mating aggregates, cells carrying the plasmid mutants were mixed with an R$^-$ recipient and examined with a phase-contrast microscope. Control crosses showed that crosses in which stable mating aggregate formation was expected were clearly distinguishable from crosses in which mating aggregate formation was not expected. We found (Fig. 2) that the mutant plas-

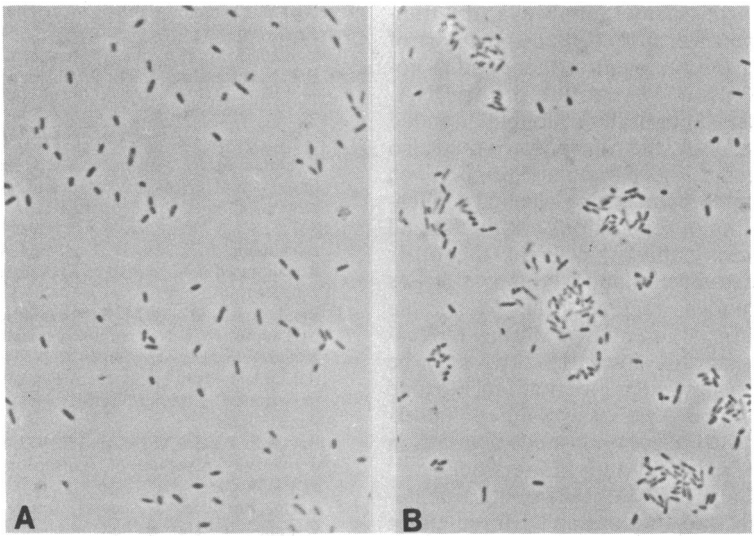

FIG. 2. Photomicrographs of mating mixtures. (A) PAO8(pMO872) × PAO5. There was no clumping of cells into mating aggregates. Similar photomicrographs were obtained with other mutants in the Tra2 region and with control slides of strain PAO5 alone. (B) PAO8(pMO505) × PAO5. Mating aggregates are obvious. Similar observations were made with all crosses in which the donor strain contained a plasmid insertion mutant in the Tra1 region. Control crosses [PAO8(R91-5) × PAO5] gave similar results.

mid-containing cells did not form aggregates with recipient cells; hence, we concluded that either pilus synthesis or mating aggregate stabilization was defective in these mutants.

DISCUSSION

Our restriction enzyme fragment analysis of R91-5 showed that the enzyme cleavage sites are distributed randomly throughout the molecule. Mapping the sites of insertion of Tn*7* and Tn*501* showed that there is some regional specificity for insertion, particularly for Tn*501*. Such specificity explains why Krishnapillai (22) found that only 0.23% of the R91-5 plasmids which recently received Tn*501* were Tra$^-$, even though about 38% of the plasmid genome is involved in plasmid transfer (see above). Most Tn*501* transposition events are outside the Tra regions in nonessential regions of unknown function. In contrast, Tn*7* appeared to have some preference for insertion into the Tra regions, with 59% of recent transposition events including Tra$^-$ mutations (22). The different specificities of insertion of Tn*501* and Tn*7* indicate the usefulness of employing more than one transposon to generate mutants.

Of the Tn*7*-induced mutants that were investigated, 28% contained two copies of Tn*7*. Double insertions of Tn*3* do occur, and they occur at a frequency which is consistent with the hypothesis that each insertion is an independent event (29). The double insertions described above occurred at a far higher frequency in the selected group screened than would be consistent with the hypothesis that the transposition of each copy is an independent event (Tn*7* transposition frequency under the conditions used was approximately 10^{-3} cell transpositions per generation). The origin of these mutants is not clear. It is not known whether the two copies of Tn*7* transposed into R91-5 at the same time or whether one copy was transposed first and then transposed again to a second site in the plasmid molecule. There is some evidence that the latter may be the case because some of the mutants were tested for transfer activity immediately after isolation and then again some time later, after the DNA was isolated. Initially, some mutants had a leaky Tra$^-$ phenotype, and when they were checked later, they had a clean Tra$^-$ phenotype. In these cases Tn*7* may have been inserted initially into a nonessential, ancillary Tra gene to produce a leaky Tra$^-$ phenotype, and subsequently the Tn*7* was transposed into an essential Tra gene to abolish all Tra activity.

Another interesting feature of Tn*7* transposition was the observation that all but one of the insertions were inserted into R91-5 in the same orientation. No other transposon has been reported to insert with such orientation specificity. However, a similar finding has been reported for the transposition of Tn*7* into RP4 (4). This difference between Tn*7* and other transposons may indicate differences in the mechanism of transposition, and therefore it is interesting to speculate how such specificity may arise. To

insert in the same orientation always, the transposon must be able to recognize some sort of polarity within the recipient replicon; such polarity could be the 3'-5' structure of the DNA. Because of the antiparallel, double-stranded structure of the DNA, the transposon would also have to recognize one particular strand. Such recognition could occur during plasmid replication, where within a replication fork one strand is replicated continuously, whereas the other strand replicates discontinuously and at stages has single-stranded regions (21). Thus, the strands could be differentiated during replication, and it is at this stage that Tn*7* may be transposed in a particular orientation into one strand. Such a model would explain our observations only if the recipient plasmids in question replicate unidirectionally, not bidirectionally.

We found that Tn*501* inserts in both orientations, although one orientation is three times more prevalent than the other. The preponderance of insertions in a particular orientation may have been due to a bias in the selection of the mutants used in this study. For example, if the insertion of Tn*501* in one orientation was strongly polar whereas insertion in the other orientation was not, then the fact that only selected mutants were investigated (mainly clean Tra^-) may have biased the sample toward those insertions in which Tn*501* was in the strongly polar orientation, hence abolishing the functions of a number of promoter-distal genes within an operon rather than nonpolar insertions, which would only abolish the function of the gene into which they inserted.

By correlating the phenotypic changes produced by transposon insertion into various regions, we mapped the positions and extents of two major transfer regions. We concluded that Tra1 is involved exclusively with conjugal DNA metabolism and that Tra2 codes for the synthesis and functionality of sex pili. It was interesting that two distinct regions controlled the different functional aspects of plasmid transfer. This is in contrast to F, in which most of the *tra* genes are in a single large operon covering approximately 30 kb of DNA (32). The distribution of *tra* into more than one region has been found in RP4 (5) and in the Ti plasmid (17). The growing body of data concerning the transfer genes of various plasmids indicates that the organization of the transfer genes of the very well-characterized plasmid F is not typical of all plasmids; therefore, to understand plasmid transfer systems, it will be necessary to study a variety of plasmids from different bacterial species.

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