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SHORT PAPER

Genome organization of the *Pseudomonas aeruginosa* narrow host range plasmid R91-5 determined by deletion and cloning analysis

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SUMMARY

By physical and genetic analysis of deletion mutants of the narrow host range IncP-10 *P. aeruginosa* conjugative plasmid R91-5 it has been shown that the phenotypes related to its transfer, namely the inhibition of the replication of the phage G101, entry exclusion and the fertility inhibition of the wide host range plasmid R18 map at kilobase coordinates 19·7–20·7, 18·5–19·7, 28·8–30·15 and/or 34·9–36·15, respectively. These locations have been confirmed by cloning restriction enzyme generated DNA fragments of R91-5 into a small broad host range, multicopy non-conjugative plasmid.

1. INTRODUCTION

The IncP-10 narrow host range plasmid R91-5 of *P. aeruginosa* has been studied in detail in order to understand the genetic basis of its conjugational transfer (Moore & Krishnapillai, 1982*a*, *b*). Two unlinked regions, Tra1 a 4·5 kb segment of DNA involved in conjugal DNA metabolism and Tra2, a 15·2 kb segment controlling the synthesis and functionality of sex pili, have been identified. As expected, Tra2 is responsible for sensitivity to donor specific phages (Dps). Polarity mutations induced by Tn*7* have identified three operons within Tra2 and Tn*501* insertions have defined two more cistrons within it (Moore & Krishnapillai, 1985). We have previously reported a relationship between *tra* and two other properties of R91-5, namely its ability to inhibit the replication of the *P. aeruginosa* phage G101 (Phi(G101)) and expression of plasmid entry exclusion (Eex) (Carrigan & Krishnapillai, 1979). The plasmid furthermore fertility inhibits the transfer of the promiscuous plasmid R18 (Fi(R18)) (Carrigan & Krishnapillai, 1979). Here we identify the location of these functions on the R91-5 genome by using deletion mutants and by cloning restriction enzyme generated fragments.

2. MATERIALS AND METHODS

(i) *Phenotypic characterization of plasmids*

The characterization with respect to Tra, Dps, Phi(G101) and Eex have been described (Krishnapillai, 1979). Fi(R18) was assayed by constructing double plasmid (R18, R91-5 or deletion or chimeric plasmid) containing cells and testing the transfer out of R18 from such cells by selection for Cb^r or Tc^r transconjugants (the latter for R18, R91-5).

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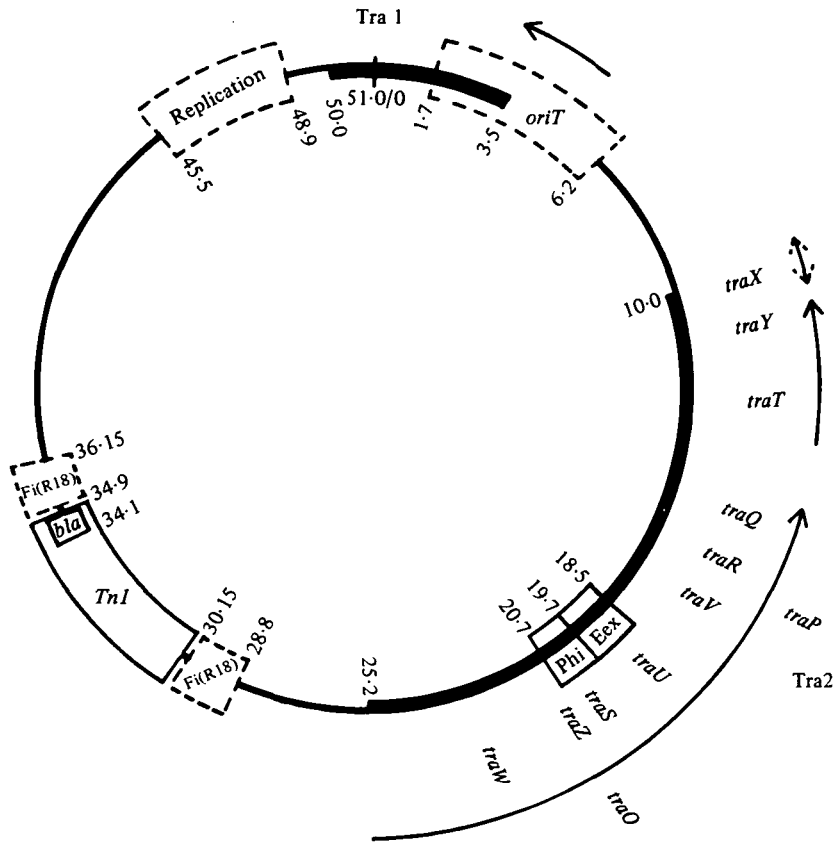

Fig. 1. Physical and genetic organization of R91-5 derived from previous (Moore & Krishnapillai, 1982*a*, *b*) and the present work. The regions covered by the thick lines show the extent of the Tra1 and Tra2 regions. The *tra* cistrons are shown in their map positions determined from the analysis of insertion and deletion mutants and the chimeric plasmids. The boxes surrounded by broken lines indicate the region within which the functions map. They do not necessarily imply that the genes coding for the functions cover the whole region indicated by the box. The arrow above *oriT* (origin of plasmid transfer) shows the direction of transfer. The arrows in Tra2 indicate the presence of three transcriptional units identified by polarity mutations induced by Tn*7* insertions and their direction of transcription (Moore & Krishnapillai, 1985). The dotted arrow heads above *traX* imply that the direction of transcription is unknown. Also shown are two new *tra* cistrons identified by Tn*501* insertions and their map location but because this transposon does not induce polarity mutations their precise location within the transcriptional units are not known and thus are placed outside the arrows (Moore & Krishnapillai, 1985). The locations of the plasmid replication region and the Tn*1* transposon (with the location of the *b1a* gene encoding beta-lactamase) are also shown.

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(ii) *In vitro derived deletions of R91-5 and the cloning of R91-5 restriction enzyme generated fragments*

These were described previously (Moore & Krishnapillai, 1982*b*).

3. RESULTS AND DISCUSSION

With the availability of the set of deletion mutants of R91-5 (Moore & Krishnapillai, 1982*b*) it was possible to determine the genetic and physical relationship between Tra, Dps, Phi(G101), Eex and Fi(R18). Having determined the physical end points of the deletions by restriction enzyme fragment analysis (Moore & Krishnapillai, 1982*b*) the mutants were tested for retention or loss of the above phenotypic properties. By a correlation of retention of these genetic properties with the retention of the appropriate length of plasmid DNA it was possible to deduce the location of the genetic functions on the genome. It was found that the region encoding Phi(G101)$^+$ was between coordinates 19·7–20·7 kb, and Eex$^+$ between 18·5–19·7 kb. Fi(R18)$^+$ was between 28·8–30·15 kb and/or 34·9–36·15 kb straddling the location, 30·15–34·1 kb, of the Tn*1* transposon. These locations were then confirmed by the genetic analysis of recombinant plasmids, constructed by the cloning of R91-5 DNA fragments in the multicopy, wide host range vector pKT210 (Bagdasarian *et al.* 1979; Moore & Krishnapillai, 1982*b*), for the expression of these phenotypes. These results are summarized in the Figure. It had previously been postulated that the *traS* cistron of R91-5 may play an important role in the expression of Phi(G101) (Carrigan & Krishnapillai, 1979). This has been confirmed here and elsewhere (Moore & Krishnapillai, 1985) by showing that both Phi(G101) and *traS* map in the region between coordinates 19·7–20·7 (Fig. 1). Therefore the mapping data is consistent with the proposal that *traS* (at least in part) codes for the Phi(G101) phenotype. The location of Eex at coordinates 18·5–19·7 coincides with the *tra* cistron *traU* (Fig. 1). However *traU* cannot be the gene encoding entry exclusion since a transposon Tn*501* insertion at coordinate 18·65 leads to a Tra$^-$ phenotype whilst retaining Eex$^+$ (Moore & Krishnapillai, 1985).

The overall genetic and physical organization of R91-5 is unlike that of the IncFI plasmid F of *E. coli* K12. In F about 34 kb of continuous DNA controls the synthesis and functionality of sex pili, stable mating aggregate formation and conjugal DNA metabolism with no obvious separation of the major classes of function (Willetts and Skurray, 1980). However in R91-5 there are two unlinked regions, Tra1 of 4·5 kb which controls conjugal DNA metabolism and Tra2 of 15·2 kb controlling sex pili synthesis and functionality and stable mating aggregate formation (Moore & Krishnapillai, 1982*a*, *b*; see figure). In F one very large operon encompasses all the *tra* cistrons except *traM* and *traJ* (Willetts & Skurray, 1980) whereas Tra2 of R91-5 has three operons (Moore & Krishnapillai, 1985) plus at least one for Tra1 (the transcriptional organization of this region has not yet been determined). The origin of transfer (*oriT*) of F is outside the *tra* genes (Willetts & Skurray, 1980) whereas *oriT* of R91-5 overlaps with Tra1 (Moore & Krishnapillai, 1982*b*; figure). Furthermore the location of the inhibition of the plating of the phage G101 by R91-5 in *P. aeruginosa* is about in the middle of Tra2 (Fig. 1) whereas the homologous system *pif* of F is outside the *tra* genes (Willetts & Skurray, 1980).

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