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Genetic analysis of a transposon carrying toluene degrading genes on a TOL plasmid pWW0

Masataka Tsuda and Tetsuo Iino

Laboratory of Genetics, Department of Biology, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, Japan

Summary. Toluene degrading (*xyl*) genes on a *Pseudomonas* TOL plasmid pWW0 are located within a 39-kb DNA portion. The 56-kb region including these *xyl* genes and its 17-kb derivative with a deletion of the internal 39-kb portion transposed to various sites on target replicons such as pACYC184 and R388 in *Escherichia coli recA* strains. Thus the 56- and 17-kb regions were designated Tn*4651* and Tn*4652*, respectively. Genetic analysis of Tn*4652* demonstrated that its transposition occurs by a two-step process, namely, cointegrate formation and its subsequent resolution. The presence in *cis* of DNA sequences of no more than 150 bp at both ends of Tn*4652* was prerequisite for cointegrate formation, and this step was mediated by a *trans*-acting factor, transposase, which was encoded in a 3.0-kb segment at one end of the transposon. Cointegrate resolution took place site-specifically within a 200-bp fragment, which was situated 10 kb away from the transposase gene. Based on the stability of cointegrates formed by various mini-Tn*4652* derivatives, it was shown that the cointegrate resolution requires two *trans*-acting factors encoded within 1.0- and 1.2-kb fragments that encompass the recombination site involved in the resolution.

Key words: TOL plasmid – Toluene degrading genes – Transposon – Tn*4651* – Tn*4652*

Introduction

The ability of *Pseudomonas putida* mt-2 to catabolize toluene and its related compounds depends on the expression of *xyl* genes on an 115-kb TOL plasmid pWW0 (Williams and Murray 1974; Kunz and Chapman 1981; Harayama and Don 1985). All the *xyl* genes are located within a 39-kb segment bounded by direct repeats of a 1.4-kb sequence (Fig. 1); this segment has been known to be excised occasionally by reciprocal recombination between the direct repeats (Williams and Murray 1974; Bayley et al. 1977; Downing et al. 1979; Meulien et al. 1981). The DNA regions including the 39-kb segment have also been demonstrated to be inserted into other replicons such as the *Pseudomonas* chromosomes and other plasmids, although the size of the insertions was variable ranging from 56 to 104 kb (White and Dunn 1977; Nakazawa et al. 1978; Jacoby et al. 1978; Chakrabarty et al. 1978; Franklin and Williams 1980; Chatterjee and Chakrabarty 1982; Furukawa et al. 1985; Jeenes and Williams 1982; Meulien and Broda 1982; Lehrbach et al. 1982). Among these insertions, the 56-kb region (Fig. 1) has been presumed to be a transposable element, as suggested by several experiments in *Pseudomonas* (Jeenes and Wiliams 1982; Cane and Williams 1982, 1986). However, independence of the host *recA* function for the insertion has remained to be examined.

In this study, genetic analysis of the 56-kb TOL region and its internal deletant (Fig. 1) in *Escherichia coli recA* strains led us to conclude that the 56-kb region is a transposon. Analysis of its various deletion mutants also showed that its transposition occurs by a two-step process, that is, cointegrate formation and its resolution, and we have mapped the sites and genes involved in these two steps.

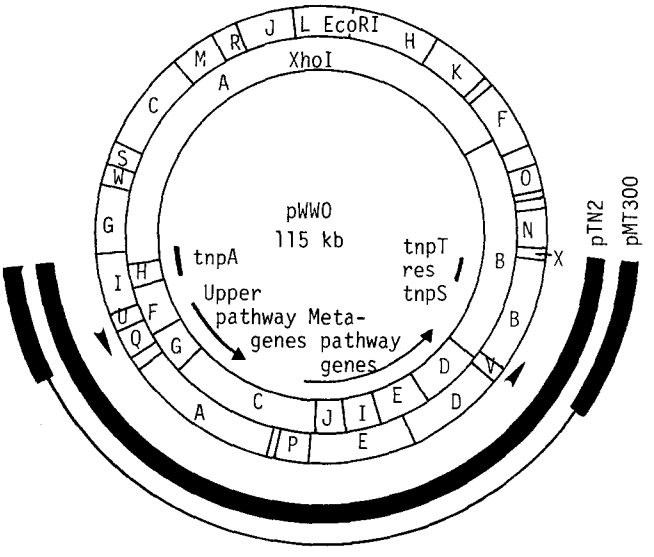

Fig. 1. Physical and functional map of pWW0. Locations of the restriction sites and the toluene degrading genes are taken from Lehrbach et al. (1982) and Harayama and Don (1985). Location of the factors involved in Tn*4652* transposition is also presented (see text for details). The *arrowhead* outside the map represents the 1.4-kb sequence (Meulien et al. 1981). *Bold lines* outside the map indicate the TOL-derived segments present on pTN2 and pMT300, and the *thin line* indicates the deleted segment on pMT300 (Nakazawa et al. 1980; this study)

Offprint requests to: M. Tsuda

Table 1. Plasmids used in this study

Plasmid	Relevant phenotype[a]	Deviation and reference
pTN2	Tra$^+$ Ap^r Km^r Tc^r Tol$^+$	RP4::Tn*4651* (Fig. 1); Nakazawa et al. (1980)
pMT300	Tra$^+$ Ap^r Km^r Tc^r	RP4::Tn*4652* (Fig. 1); this study
Rtsl::Tn*1725*	Tra$^+$ Rep^{ts} Km^r Cm^r	Ubben and Schmitt (1986)
R388	Tra$^+$ Tp^r Su^r	Ward and Grinsted (1982)
pBR322	Ap^r Tc^r	Bolivar et al. (1977)
pACYC184	Cm^r Tc^r	Chang and Cohen (1978)
pUC4K	Ap^r Km^r	Vieira and Messing (1982)
pCM1	Ap^r Cm^r	Close and Rodriguez (1982)
pMT252	Tc^r	pACYC184 Δ (*Sca*I-*Pvu*II)[b] (Fig. 2); this study
pMT258	Cm^r	pACYC184 Δ (*Xba*I-*Sal*I) (Fig. 2); this study
pMT362	Ap^r	pBR322*tet*::Tn*4652*[c,d]; this study
pMT372	Tc^r	pMT252::Tn*4652*[c]; this study
pMT382	Cm^r	pMT258::Tn*4652*[c]; this study
pMT425	Ap^r	pBR322 derivative; the *Hin*dIII-*Eco*RV fragment of pBR322 was replaced by the leftmost *Dra*I-*Hin*dIII fragment of Tn*4652* (Fig. 4); this study
pMT569	Ap^r	pBR322 derivative; the *Eco*RI-*Eco*RV fragment of pBR322 was replaced by the 2.2-kb *Xba*I-*Sma*I fragment of Tn*4652* (Fig. 5); this study

[a] Abbreviations are according to Novick et al. (1976) except Tol, *m*-toluate utilization
[b] *Pvu*II site at coordinate 0.58 on the pACYC184 map
[c] These plasmids were obtained by the mating-out system using R388::Tn*4652* as the donor replicon
[d] Tn*4652* is inserted between the *Bam*HI and *Sph*I sites on pBR322 in the orientation so that the coordinates of pBR322 (Bolivar et al. 1977) and of Tn*4652* (Fig. 3) are in the opposite direction

Materials and methods

Media. L broth and L broth agar have been described previously (Tsuda et al. 1981). Minimal agar used was M9 salt medium (Miller 1972) supplemented with 5 mM *m*-toluate and 1.5% agar. Final concentrations of antibiotics added to media are as follows: ampicillin (Ap), 50 μg/ml; chloramphenicol (Cm), 30 μg/ml; kanamycin (Km), 50 μg/ml; nalidixic acid (Nal), 20 μg/ml; streptomycin (Sm), 200 μg/ml; sulfathiazole (Su), 800 μg/ml; tetracycline (Tc), 10 μg/ml; and trimethoprim (Tp), 800 μg/ml.

Bacterial strains and plasmids. KT2440 is a *P. putida* strain defective in its restriction system (Bagdasarian et al. 1981). All the experiments on transposition were performed using *E. coli recA* strains, HB101 (*hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44*) (Maniatis et al. 1982), DH1 (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1?*) (Maniatis et al. 1982), and DH1::Tn*1725*, a DH1 derivative that carries a chromosomal copy of Tn*1725*. This last strain was isolated from DH1 (Rtsl::Tn*1725*) by the procedure described by Ubben and Schmitt (1986).

Plasmids used are listed in Table 1. pMT300 is a derivative of pTN2 with the internal 39-kb TOL DNA portion deleted by reciprocal recombination between the direct repeats of the 1.4-kb sequence (Fig. 1). The strain carrying pMT300 was found among spontaneous mutants of KT2440 (pTN2) unable to utilize *m*-toluate.

DNA manipulations. Large-scale isolation of plasmid DNA was according to the alkali lysis method of Maniatis et al. (1982) followed by isopycnic centrifugation in CsCl/ethidium bromide gradients. Rapid, small-scale preparation of plasmid DNA was by the method of Machida et al. (1982). Established procedures (Maniatis et al. 1982) were employed for digestion of plasmid DNA with restriction endonucleases, filling-in of cohesive ends by Klenow fragment of *E. coli* DNA polymerase I and/or T4 DNA polymerase, ligation of DNA fragments, and transformation. Synthetic oligonucleotide linkers including the restriction sites for *Bgl*II and *Xho*I were CAGATCTG and CCTCGAGG, respectively.

Conjugation. Aliquots of 0.75 ml of overnight broth cultures of donor and recipient strains were mixed in an Eppendorf tube and centrifuged. This concentrated suspension of bacteria was spotted onto a sterilized membrane filter on an L broth agar plate, incubated for 3–4 h at 37° C, and resuspended in 1 ml L broth. The diluted suspension of the bacteria was spread on appropriate selective plates.

*Assay of Tn*4652 *transposition.* Because Tn*4652* has no selectable marker, the pUC4K-derived Km^r determinant (Vieira and Messing 1982) was inserted in vitro into Tn*4652* and its deletants. A derivative of DH1 containing two plasmids was constructed: one was pACYC184 (or its deletant) carrying the Tn*4652* derivative, and the other was a conjugal plasmid R388, which has been considered to have no transposable element (de la Cruz and Grinsted 1982). The resulting strain was used as the donor to mate with HB101. The transposition frequency was expressed as the number of the Km^r Sm^r transconjugants per R388 transconjugant. (R388 was transferred at the frequency of $1.0–5.0 \times 10^{-1}$ per donor cell.) In the same mating, mobilization of the donor replicon was also examined by selecting for Cm^r or Tc^r transconjugants. Mobilization of pACYC184 itself was at the frequency less than 5×10^{-9}. When the mini-Tn*4652* was defective in transposition, the pBR322 derivative carrying part of Tn*4652* was further introduced into the donor strain.

Plasmids in the transconjugants were characterized by (i) conjugation and (ii) small-scale plasmid preparation followed by transformation, digestion with restriction endonucleases, and agarose gel electrophoresis.

Tn1725 mutagenesis. Mutagenesis of pMT415 with Tn*1725* was carried out according to the procedure described by Ubben and Schmitt (1986) using DHl::Tn*1725* (pMT415).

Results

*Transposition of Tn*4651 *and Tn*4652 *onto pACYC184*

Plasmids pTN2 and pMT300 are RP4 derivatives with insertions of the 56- and 17-kb TOL sequences, respectively (Fig. 1). DHl(pACYC184) (pTN2) and DHl(pACYC184) (pMT300) were mated with HB101, and Cm^r transconjugants were obtained in both matings at a frequency of approximately 5×10^{-6} per Km^r transconjugant. Cleared lysates prepared from each Cm^r transconjugant usually contained three species of plasmids. Two of them were the parental RP4 derivative used (pTN2 or pMT300) and a pACYC184 derivative which had acquired part of the RP4 derivative (see below). The largest third plasmid was assumed to be a fusion product of the other two plasmids because prolonged cultivation of each transconjugant resulted in decrease in the amount of the largest plasmid and, in association with this event, increase in those of the others. In about one fifth of the transconjugants in each mating, the pACYC184 derivatives had insertions of Tn*1*, a transposon resident on RP4 itself (Hedges and Jacob 1974). In the remaining transconjugants in the matings using pTN2 and pMT300, the pACYC184 derivatives had at various sites insertions of the 56- and 17-kb TOL sequences, respectively. The distribution of representative 17-kb inserts is depicted in Fig. 2. We designated the two transposable regions Tn*4651* and Tn*4652*, respectively (Lederberg 1987).

The Tn*4652* insert on pACYC184 was able to transpose as a discrete unit (Fig. 3) to other replicons such as R388 (see below) and pBR322 (data not shown), whereas the Tn*4651* insert on pACYC184 segregated at high frequency a deletant that was identical to Tn*4652*. Because of this instability of Tn*4651* on pACYC184, Tn*4652* was used for further analysis.

*Transposition of Tn*4652 *from pACYC184 onto R388*

Hereafter in this study, transposition of the Tn*4652* derivatives was examined using pACYC184 as the donor replicon in the mating-out experiments with R388 as the target replicon.

Tn*4652-332* has an insertion of the Km^r determinant at the unique *Bgl*II site (Fig. 3). Conjugation using the donor strain containing R388 and pACYC184::Tn*4652-332* (pMT332) yielded both Km^r and Cm^r transconjugants at frequencies of approximatley 1.0×10^{-4} per R388 transfer. Of the Km^r transconjugants, 5%–10% carried R388::Tn*4652-332* alone. The remaining Km^r and all the Cm^r transconjugants inherited all of the unselected drug-resistance markers specified by pMT332, and carried three plasmids, two of which were pMT332 and R388::Tn*4652-332*. Restriction analysis showed that the third plasmid was a cointegrate of R388 and pACYC184 which had two directly repeated copies of Tn*4652-332*, one at each junction. This cointegrate was unstable so that it gradually resolved into pMT332 and R388::Tn*4652-332* after introduction into other Rec$^-$ strains by transformation and conjugation.

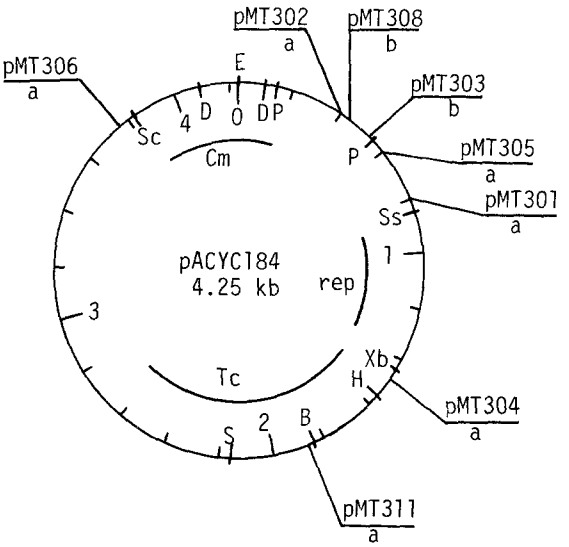

Fig. 2. Distribution of Tn*4652* insertions on pACYC184. Physical and functional map of pACYC184 is taken essentially from Chang and Cohen (1978) with slight modification based on our analysis (this study). The orientation of insertion is denoted by (*a*) when the coordinates of pACYC184 and of Tn*4652* (Fig. 3) are in the same direction and by (*b*) when in the opposite direction. Abbreviations for restriction sites employed are as follows: A, *Aat*II; B, *Bam*HI; Bg, *Bgl*II; D, *Dra*I; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn*I; N, *Nru*I; P, *Pvu*II; Ps, *Pst*I; S, *Sal*I; Sc, *Sca*I; Sm, *Sma*I; Ss, *Sst*II; St, *Stu*I; V, *Eco*RV; X, *Xho*I; Xb, *Xba*I

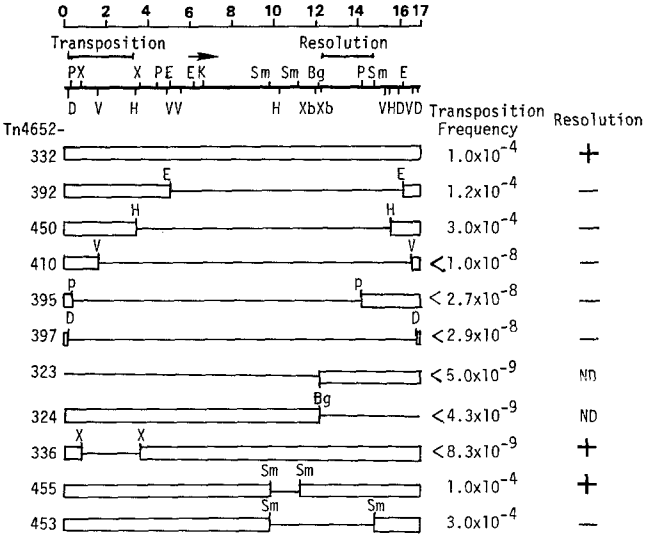

Fig. 3. Physical map of Tn*4652* and transposition of its derivatives. The physical map of Tn*4652* is shown at the *top*. The *arrow* indicates the 1.4-kb sequence (Meulien et al. 1981). The *open box* and *thin line* represent the segments present and absent, respectively, in the mini-transposon. Derivation of plasmids carrying the transposons are as follows: Tn*4652-332*, *-324*, *-336*, *-455*, and *-453* from pMT304; Tn*4652-323* from pMT311; Tn*4652-392*, *-395*, and *-397* from pMT372; and Tn*4652-450* and *-410* from pMT382. Except Tn*4652-323* and *-324*, the modified transposons have insertions of the pUC4K-derived Km^r determinant (Vieira and Messing 1982) at the unique *Bgl*II site in Tn*4652-332* and between the deleted segments in the others. Symbols + and — denote the presence and absence, respectively, of resolution of the cointegrates in the transconjugants in the mating-out system. When mini-Tn*4652* was defective in transposition, the transconjugants were obtained by introducing the complementing plasmid pMT415 (see text and Fig. 4) into the donor strain

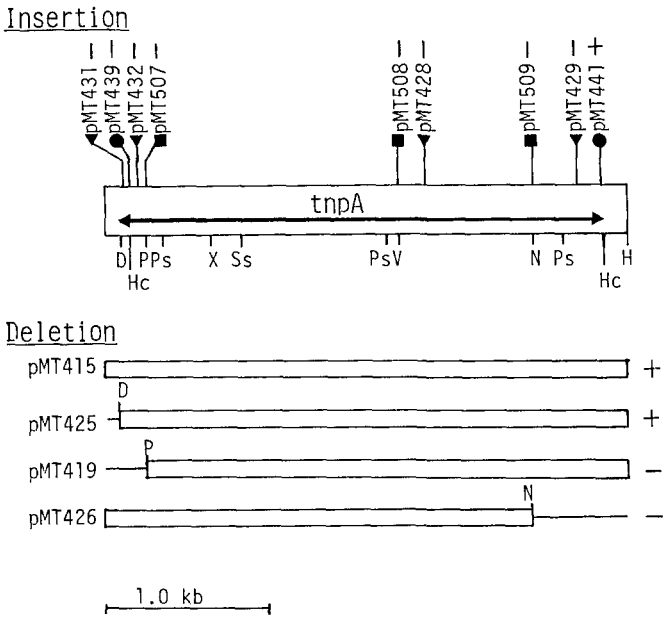

Fig. 4. Location of transposase gene on Tn*4652*. The leftmost 3.2-kb fragment of Tn*4652* is shown. Plasmids depicted in the figure were introduced into the donor strain employed in the mating-out system, and their ability to complement the transposition of defective Tn*4652-410, -395, -397,* and *-336* was examined. Symbols + and − denote that transposition of these four derivatives occured at the frequencies of $1.0–5.0 \times 10^{-4}$ and less than 1.0×10^{-8}, respectively. Plasmid pMT415 is a derivative of pMT362 lacking the DNA segment from the *Hin*dIII site on the pBR322 portion to the leftmost *Hin*dIII site on the Tn*4652* portion (Fig. 3), and pMT419 and pMT426 are deletants of pMT415. Plasmids at the *top* of the figure were mutants of pMT415 with the insertions of Tn*1725* (▼), the pCMl-derived Cm^r determinant (Close and Rodriguez 1982) (●), and the *Bgl*II linker (■)

Factors necessary for cointegration

Removal of the outermost *Eco*RI and *Hin*dIII sites (Fig. 3) had no significant effect on the transposition frequencies. Deletions covering part of the left-hand 3.2 kb (in Tn*4652-410, -395, -397,* and *-336*) abolished the transposition. All of these four derivatives restored transposability to the normal frequencies in the presence of pMT415, a pBR322 derivative carrying the 3.2-kb segment described above (Fig. 4), indicating that this segment encodes a factor which is effective in *trans* for transposition. This factor is conventionally named transposase by analogy with those in other transposons (Heffron 1983).

Various mutants isolated from pMT415 were examined for their ability to complement the transposition defect of the mini-Tn*4652* derivatives (Fig. 4). This analysis led to the more definite localization of the endpoints of the transposase gene (*tnpA*): one between the outermost *Dra*I site and the Tn*1725* insertion site in pMT431, and the other between the Tn*1725* insertion site in pMT429 and its closest *Hin*cII site (Fig. 4).

Deletants lacking either end of Tn*4652* (Tn*4652-323* and *−324*; Fig. 3) were not able to transpose even in the presence of pMT415. This observation, in conjunction with the transposability of Tn*4652-397* in the presence of pMT415, indicates that the presence in *cis* of both external 150-bp DNA segments from the outermost *Dra*I sites to the termini is prerequisite for transposition (Fig. 3).

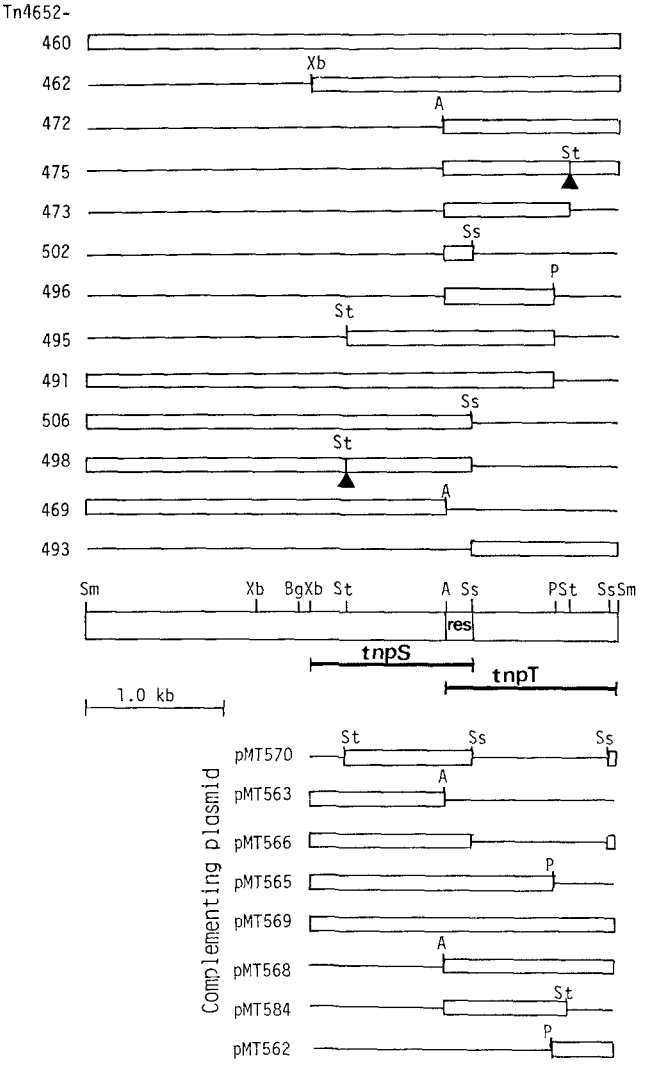

Fig. 5. Genetic organization of factors for cointegrate resolution. The derivatives of Tn*4652-460* loaded on the pACYC184 replicon are shown above the restriction map of the 3.5-kb *Sma*I fragment, and the deletion derivatives of pMT569, a pBR322-based hybrid plasmid, are shown below the map. The *open box* and *thin line* indicate the DNA segments present and absent, respectively, on the plasmids, and the DNA structures outside the 3.5-kb segment are not depicted in the figure for simplicity. Symbol ▲ represents insertion of the *Xho*I linker

Factors necessary for cointegrate resolution

The Km^r transconjugants that also received the pACYC184 replicon in the mating-out system were classified into two groups, depending on the mini-Tn*4652* derivatives employed (Fig. 3). One group were the transconjugants obtained by using Tn*4652-336* and *-455*. They contained, like those obtained by using Tn*4652-332* (see above), three species of plasmids, i.e., the donor replicon, R388::mini-Tn*4652*, and the unstable cointegrate. In the other group, each transconjugant carried only the mini-Tn*4652*-mediated cointegrate which was stable enough to show no structural alteration even after introduction into other Rec$^-$ strains. This stability of the cointegrate is in good agreement with the observation that more than 99.5% of the Km^r transconjugants inherited the unselected pACYC184-specified markers. Taking into account the stability and instability

Table 2. Resolution of cointegrates mediated by mini-Tn*4652*[a]

Cointegrate mediated by	Complementing plasmid (pMT)								
	None	570	563	566	565	569	568	584	562
Tn*4652-460*	+	+	+	+	+	+	+	+	+
Tn*4652-462*	+	+	+	+	+	+	+	+	+
Tn*4652-472*	−	−	−	+	+	+	−	−	−
Tn*4652-475*	−	−	−	−	−	+	−	−	−
Tn*4652-473*	−	−	−	−	−	+	−	−	−
Tn*4652-502*	−	−	−	−	−	+	−	−	−
Tn*4652-496*	−	−	−	−	−	+	−	−	−
Tn*4652-495*	−	−	−	−	−	+	−	−	−
Tn*4652-491*	−	−	−	−	−	+	+	−	−
Tn*4652-506*	−	−	−	−	−	+	+	−	−
Tn*4652-498*	−	−	−	−	−	+	−	−	−
Tn*4652-469*	−	−	−	−	−	−	−	−	−
Tn*4652-493*	−	−	−	−	−	−	−	−	−

+, resolution of cointegrate; −, no resolution of cointegrate

[a] Cointegrate of R388 and pACYC184 (or its deletant) connected by two copies of mini-Tn*4652*

of the cointegrates mediated by Tn*4652-453* and Tn*4652-455*, respectively (Fig. 3), we inserted the 3.5-kb *Sma*I fragment present on Tn*4652-455* into the unique *Sma*I site (in the Km^r determinant) on Tn*4652-397*. The cointegrate formed by the resulting transposon was found to be unstable. These analyses led us to conclude that transposition of Tn*4652* occurs by a two-step process, that is, cointegrate formation and its resolution, and that all the factors necessary for resolution are located within the 3.5-kb fragment.

The 3.5-kb fragment was also inserted at the *Sma*I site (in the Km^r determinant) on Tn*4652-450* to obtain Tn*4652-460*, which was in turn used to construct various mutants (Fig. 5). The Tn*4652-460* derivatives with deletions in the right-hand 2.2-kb *Xba*I-*Sma*I fragment gave rise to stable cointegrates in the transconjugants in the mating-out system (Table 2). Among these conitegrates, all those retaining the central 0.2-kb *Aat*II-*Sst*II fragment (Fig. 5) were resolved into the two final products in the presence of pMT569, a pBR322 derivative carrying the 2.2-kb fragment. These results show that the *cis*-acting site and *trans*-acting factor(s) for cointegrate resolution are located within the 0.2- and 2.2-kb fragments, respectively.

Deletants of pMT569 (Fig. 5) were next examined for their ability to resolve the stable cointegrates described above (Table 2). Plasmids pMT565 and pMT566, but neither pMT563 nor pMT570, were able to resolve the cointegrate containing Tn*4652-472*, indicating that the internal 1.0-kb *Xba*I-*Sst*II fragment encodes *trans*-acting factor(s) for resolution of the cointegrate (Fig. 5). This ability of pMT565 and pMT566 to promote resolution was not exerted on the cointegrate formed by Tn*4652-475, -473,* or *-502* (Table 2). The ability of pMT568 and the inability of pMT584 and pMT562 to resolve the cointegrates formed by Tn*4652-491* and *-506* indicate that the right-hand 1.2-kb *Aat*II-*Sma*I fragment also encodes the *trans*-acting factor(s) for resolution of the two species of cointegrates (Fig. 5). pMT568 did not however promote the resolution of the cointegrate formed by either Tn*4652-495* or *-498*, derivatives of Tn*4652-491* and *-506*, respectively, which lack their unique *Stu*I site.

Based on these results, it is inferred that resolution of the Tn*4652*-mediated cointegrate occurs at the *cis*-acting site, named *res* by analogy with that in the Tn*3* family of transposons (Heffron 1983), within the 0.2-kb fragment in the presence of at least two *trans*-acting factors whose genes, named *tnpS* and *tnpT*, are located within the 1.0-kb *Xba*I-*Sst*II and the 1.2-kb *Aat*II-*Sma*I fragments, respectively (Fig. 5).

Discussion

The proposal that the 56- and 17-kb regions on plasmid pWW0 are transposons (Jeenes and Williams 1982; Cane and Williams 1982, 1986) has been definitely supported in this study using *E. coli recA* strains, and we have designated the two regions Tn*4651* and Tn*4652*, respectively.

Our genetic analysis on Tn*4652* showed that (i) its transposition takes place by a two-step process, i.e., cointegrate formation and its resolution, (ii) DNA sequences of less than 150-bp at both ends are required in *cis* for transposition (Fig. 3), and (iii) the gene encoding the transposase, which can function efficiently in *trans*, is situated in the 3.0-kb segment at one end (Fig. 4). These properties of Tn*4652* resemble those of the Tn*3* family of transposons (Heffron 1983).

Striking differences between Tn*4652* and the Tn*3* family (Heffron 1983) were however observed in three aspects of cointegrate resolution. First, the Tn*4652*-specified resolution activity was, compared with those of the Tn*3* family (de la Cruz and Grinsted 1982), considerably weaker so that, in the mating using DHl (R388) (pMT332) as the donor strain, 90%–95% of the Km^r transconjugants inherited the pMT332 replicon and still carried the unstable cointegrate even after 1 week in culture. Secondly, in contrast to the localization of *tnpR* and *res* in close proximity to *tnpA* in the Tn*3* family (Heffron 1983), the transposase gene and the 2.2-kb fragment encoding the factors for cointegrate resolution were separated by a 9 kb DNA segment in Tn*4652* (Fig. 1). Thirdly, it was inferred that resolution of the Tn*4652*-mediated cointegrate requires two *trans*-acting factors (Fig. 5). In vitro biochemical analysis of resolution of the cointegrates formed by the Tn*3* family has demonstrated that (i) the resolvase is the only protein required for resolution (Newman and Grindley 1984; Halford et al.

1985; Rogowsky and Schmitt 1985), and (ii) the resolvase of Tn*1000*, a transposon belonging to the Tn*3* family, consists of two functional domains: one for recognizing the specific sequences at *res*, and the other for catalysing the site-specific recombination reaction (Abdel-Meguid et al. 1984; Newman and Grindley 1984). Therefore, it may be possible that the two domains in the *tnpR* product in the Tn*3* family are separated into the *tnpS* and *tnpT* products in Tn*4652*. Alternatively only one of the two products may be directly involved in the resolution reaction and the other product may regulate either the gene expression of the catalytic product or its enzymatic activity.

This study was mainly concerned with Tn*4652*, which was derived from Tn*4651* by reciprocal recombination between the 1.4-kb direct repeats. Our results showed that (i) transposition of Tn*4651* and Tn*4652* from RP4 to pACYC184 occured at the similar frequencies, and (ii) both Tn*4651*- and Tn*4652*-mediated cointegrates of RP4 and pACYC184 resolved into the final products at the similar rates. It is therefore unlikely that Tn*4651* encodes additional and/or alternative factors specific for its transposition in the some 10-kb spacer region between the upper and lower *xyl* operons (Harayama and Don 1985), although we cannot conclusively rule out such an architecture for Tn*4651*.

The size of the pWW0-derived DNA regions inserted into other replicons has been shown to have wide variety (Lehrbach et al. 1982). Our results presented in this study clearly demonstrate that the 56-kb region is a transposon. A study of the transposability of other regions is now in progress to understand the genesis of the pWW0 genome.

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