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Genetic and Molecular Characterization of Tn*21*, a Multiple Resistance Transposon from R100.1

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The transposon Tn*21* has been transposed from R100.1 to plasmid pACYC184 and, from the resulting recombinants, to plasmid R388. The sites of insertion and the orientation of the element in several pACYC184::Tn*21* recombinants have been examined. Restriction enzyme analysis of these recombinants has resulted in a detailed map of Tn*21*; this is compared with the published maps of the relevant part of R100.1. Heteroduplex analysis has shown short inverted repeat sequences at the ends of the element. With various in vitro-generated deletion mutants of Tn*21*, the internal gene necessary for transposition (*tnpA*) was localized within the terminal 4.3 kilobases of the right-hand end of the element. Genetic analysis of transposition of Tn*21* suggests that the process proceeds via cointegrates. Since the end products of transposition are simple recombinants of the element and the recipient replicon, Tn*21* must contain a gene that codes for a resolvase type of activity (*tnpR* gene).

Transposable elements are specific DNA sequences that can insert more or less indiscriminately into other DNA sequences. It is now generally accepted that these elements play a very important role in evolution (18). In this context, the r-determinant regions of the IncFII plasmids R1, R100, and R6 have been extensively studied and indicate how plasmids coding for resistance to several antibiotics can arise (19). Two of the largest transposable elements from this source have hardly been studied at all; these are Tn*4* (a 24-kilobase (kb) portion of the r-determinant region of R1; 19) and Tn*21* (20 kb of R100; 20). The two are very closely related, the differences being that Tn*4* contains Tn*3* as part of its structure and Tn*21* contains a 1.45-kb insertion (9).

We are interested in the origin and evolution of transposons. We have learned that Tn*21* is closely related to transposons Tn*501* and Tn*1721* (J. Grinsted, F. de la Cruz, J. Altenbuchner, and R. Schmitt, submitted for publication) and want to discover the mechanism by which Tn*21* picked up its resistance genes. This paper describes the basic genetic and physical characteristics of Tn*21*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains and plasmids used are shown in Tables 1 and 2, respectively.

Growth of bacteria. All growth was at 37°C. The liquid medium used was nutrient broth (Oxoid Ltd.); solid medium was usually nutrient broth solidified with 1.5% agar. Where indicated, minimal medium (14) solidified with 1.5% agar was used; this was supplemented with the required L-amino acids (20 μg/ml) or with Casamino Acids (Difco Laboratories, 0.5%) plus L-tryptophan (20 μg/ml). The concentrations of drugs (and abbreviations) used were: ampicillin (Ap), 100 μg/ml; chloramphenicol (Cm), 25 μg/ml; nalidixic acid (Nx), 25 μg/ml; kanamycin sulfate (Km), 50 μg/ml; sulfadimine (Su), 500 μg/ml in minimal medium; trimethoprim lactate (Tp), 25 μg/ml in minimal medium; tetracycline hydrochloride (Tc), 10 μg/ml; streptomycin sulfate (Sm), 10 μg/ml for selection of R100.1, 20 μg/ml for selection of pACYC184::Tn*21* or R388::Tn*21* recombinants, and 200 μg/ml for selection of UB1637; spectinomycin hydrochloride (Sp), 40 μg/ml; and mercury (Hg), either 12 μg of $HgCl_2$ per ml or 150 μg of Mercurochrome per ml. Superscripts r or s to the abbreviations denote resistance or sensitivity to that drug.

Transfer of plasmids. Conjugation between bacteria was usually carried out by cross-streaking strains on nutrient agar plates and incubating overnight. The mixed growth was then scraped off the plate and suspended in broth, and suitable dilutions were plated on selective agar. Transformation was carried out essentially as described by Cohen et al. (10). DNA extracted for restriction enzyme analysis (see below) was suitable for this purpose.

Isolation of pACYC184::Tn*21* recombinants. About 10^7 bacteria of strain UB6191 were spread on nutrient agar containing streptomycin (40 μg/ml). UB6191 is resistant to about 10 μg/ml of this drug. Above this concentration, the efficiency of plating falls to about 10^{-4} at 40 μg/ml. Thus, plating on this concentration of streptomycin is enriching for bacteria containing pACYC184::Tn*21* recombinants, since pACYC184 is a high-copy number plasmid, and the level of resistance to streptomycin increases with the gene copy number

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TABLE 1. Strains used for genetic experiments

Strain[a]	Plasmids carried[b]
UB1637[c]	None
UB5201[d]	None
UB6191	R100.1, pACYC184
UB6195	R388, pUB818, pUB2401
UB6196	R388, pUB818, pACYC184
UB6197	R388, pUB818, pUB2406
UB6198	R388, pUB818, pUB2407
UB6200	R388, pUB2401
UB6201	R388, pUB2406
UB6202	R388, pUB2407
UB6207	R388, pUB818, pUB2413
UB6208	R388, pUB818, pUB2414
UB6345	R388, pUB2402

[a] All strains, except UB1637, were UB5201 strains containing different plasmids. They were constructed by mating in R100.1 and R388 and by transforming with the other plasmids, using standard methods.

[b] Characteristics of the plasmids are shown in Table 2.

[c] UB1637 is a *recA56* derivative of JC3272 (1); it is *his lys trp recA56 strA* (*rpsL*).

[d] UB5201 is a *recA56* derivative of UB281 (4); it is *pro met recA56 nalA*.

up to about 50 μg/ml (24). About 10^3 colonies grew. These were resuspended together in broth and allowed to grow and then plasmid DNA was isolated from the mixture and used to make UB5201 Sm^r (20 μg/ml). Such transformants contained pACYC184::Tn*21* recombinants (see below).

DNA preparation and manipulation. The method of preparation of DNA depended on its intended use: for estimating the size of supercoiled plasmids, lysis with sodium dodecyl sulfate followed by a 15-min clearing spin in an Eppendorf 5412 centrifuge (3) was used; for restriction enzyme analysis, plasmid DNA was prepared from overnight cultures (10 ml) essentially by a scaled-down version of the method described by Grinsted et al. (13); and for large-scale preparations, CsCl-ethidium bromide gradients were used, the method being essentially that described by Humphreys et al. (16). Digestion with restriction endonucleases and electrophoresis were as described by Grinsted et al. (13); and heteroduplex analysis was as described by Bennett et al. (5). For the generation of in vitro recombinants, DNA from CsCl-ethidium bromide gradients was used. Ligation of digests (using T4 DNA ligase from Boehringer Mannheim Corp.) was carried out at DNA concentrations favoring inter- or intramolecular annealing (11), depending on the experiment (see Fig. 3).

RESULTS

Analysis of pACYC184::Tn*21* recombinants. Recombinants of pACYC184 and Tn*21* were isolated as described above. About 600 were tested: all were Sm^r/Sp^r Su^r Hg^r Km^s (the latter indicating that the donor plasmid, R100.1, was not present), and of these 35% were Tc^r Cm^r, 40% were Cm^r Tc^s, and 25% were Cm^s Tc^r. These results show that insertion of Tn*21* sometimes inactivated genes on pACYC184 (Cm Tc). In all cases examined, transformants containing the recombinant plasmids contained only a single plasmid with a size of 24 kb. The size of pACYC184 is 4.1 kb, suggesting that Tn*21* is about 20 kb. Digests of such plasmids with *Eco*RI contained eight common fragments of 5.6, 4.2, 1.8, 1.6, 1.3, 0.3, 0.25, and 0.25 kb, corresponding to the *Eco*RI fragments G, I, K, L, M, N, O, and P of the parent plasmid R100.1, which account for a contiguous segment of 15.2 kb within the r-determinant region of R100.1 (9). In addition to these fragments, two fragments of variable size were also always present; the sum of their sizes was always 8.8 kb. These fragments must be the junction fragments from the outermost *Eco*RI sites of Tn*21* to the single *Eco*RI site in pACYC184. Since the size of pACYC184 is 4.1 kb (7), the sum of the external segments of Tn*21* from the outermost *Eco*RI

TABLE 2. Plasmids used

Plasmid[a]	Relevant markers[b]	Derivation
R100.1	Cm Tc Sm/Sp Su Hg Tra^+ *drd*	Reference 17
R388	Tp Su Tra^+	Reference 17
pACYC184	Cm Tc Mob^- (R388)	Reference 7
pUB818	Ap Tc Mob^- (R388)	Grinsted et al., submitted for publication
pUB2401	Cm Sm/Sp Su Hg	pACYC184(*tet*::Tn*21*); Fig. 1
pUB2402	Tc Sm/Sp Su Hg	pACYC184(*cat*::Tn*21*); Fig. 1
pUB2403	Tc Sm/Sp Su Hg	pACYC184(*cat*::Tn*21*); Fig. 1
pUB2404	Tc Cm Sm/Sp Su Hg	pACYC184::Tn*21*; Fig. 1
pUB2406	Cm	*Eco*RI deletion of pUB2401; Fig. 2
pUB2407	Tc Hg	*Bam*HI deletion of pUB2402; Fig. 2
pUB2413	Cm	*Bam*HI deletion of pUB2401; Fig. 2
pUB2414	Cm Hg	*Bam*HI deletion of pUB2404; Fig. 2

[a] R100.1 and R388 were obtained from laboratory stocks and were transferred to suitable strains by conjugation; pACYC184 was obtained from D. Sherratt. pUB818 is *tnpA* derivative of pJOE105, a Tn*1721* recombinant with a derivative of pBR322 (22). These and all the rest of the plasmids were maintained in strain UB5201.

[b] Abbreviations are as recommended by Novick et al. (21).

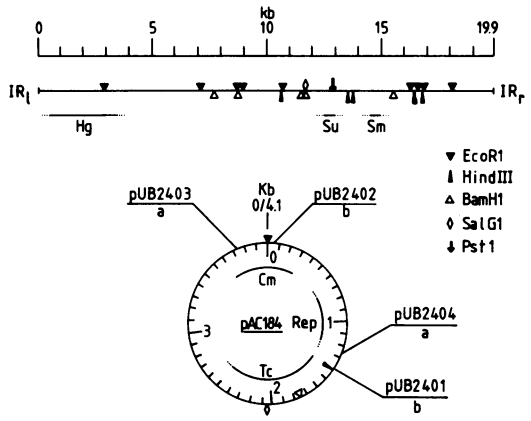

FIG. 1. Restriction enzyme map of Tn*21* and sites of insertion of four pACYC184::Tn*21* recombinants. The restriction enzyme maps of the four recombinants were constructed by double digestions with the different enzymes. The preliminary maps obtained were corroborated by the construction and restriction analysis of the various deletion derivatives (Fig. 2, data not shown). In each of the recombinants, a 19.9-kb segment of DNA corresponding to that of Tn*21* had been inserted into pACYC184. The resulting map of Tn*21* is shown at the top, defining a coordinate system for the transposon. The localization of the ends of the element with respect to the internal sites is described in the text; these ends are inverted repeat sequences (Fig. 3) and have been designated IR$_l$ and IR$_r$. The sites of insertion of Tn*21* into the pACYC184 vector in the four recombinants analyzed are shown at the bottom; the orientation of insertion is denoted by (a) when the coordinates of the element and of pACYC184 are in the same direction and by (b) when in the opposite direction. The positions of the resistance genes and the replication region (*rep*) in the restriction map of Tn*21* and in pACYC184 were already known (6, 7).

sites to the termini must be 4.7 kb (8.8 minus 4.1). This gives a total length for Tn*21* of 19.9 kb (15.2 plus 4.7), which agrees well with the sizes of the recombinant molecules (see above). Four recombinants (pUB2401 to pUB2404) were examined in detail (Fig. 1 and 2). The extent of Tn*21* beyond its outermost *Eco*RI sites (i.e., how the sum of 4.7 kb is distributed between its two ends) was indicated by two of these recombinants: in pUB2401, the distance between the *Eco*RI site in the *mer* operon of Tn*21* and the external *Hin*dIII site is 2.9 kb, showing that the distance from this *Eco*RI site to the terminus of the element cannot be more than this; and in pUB2402, the outermost *Eco*RI site at the other end of the element to the external *Eco*RI site is 1.9 kb, showing that the distance from this site within the element to the terminus cannot be more than this distance. Since the sum of these distances (2.9 kb and 1.9 kb) is close to the estimated size of the two external segments of the transposon (4.7 kb; see above), they must be close to the distances from the outermost *Eco*RI

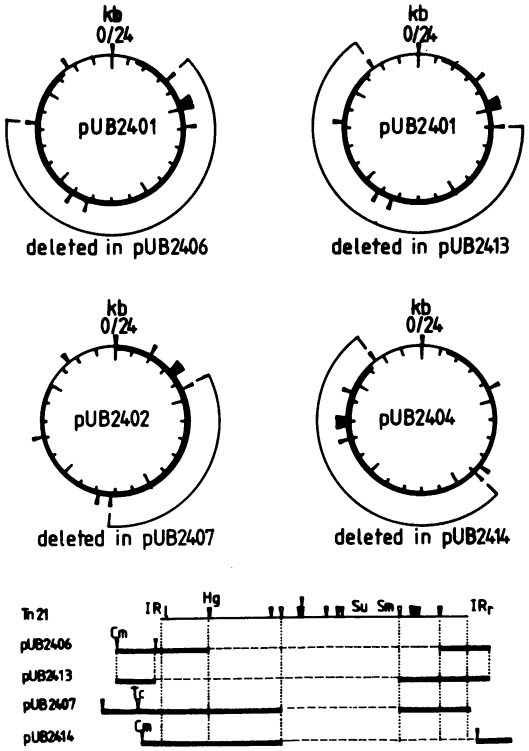

FIG. 2. Generation of derivatives of pACYC184::Tn*21* recombinants. Recombinants were digested with *Eco*RI or *Bam*HI, then ligated and used to transform strain UB5201 to either Cm^r or Tc^r. Plasmid pUB2406 resulted from *Eco*RI digestion of pUB2401 followed by transformation to Cm^r, and pUB2407 resulted from digestion with *Bam*HI, followed by transformation to Tc^r. In these two cases, two fragments had to be ligated so that the ends of Tn*21* were correctly oriented. This was simply achieved because of the presence in pACYC184 of an *Eco*RI site in the Cm gene and a *Bam*HI site in the Tc gene. Thus, selection of Cm^r or Tc^r after digestion with *Eco*RI or *Bam*HI required that the fragments were ligated in the correct orientation so that the genes that code for resistance were reformed. The plasmids pUB2413 and pUB2414 were formed by *Bam*HI digestion followed by transformation to Tc^r of pUB2401 and pUB2404, respectively. In these two cases, the reactions simply involved removal of *Bam*HI fragments and circularization of the remaining fragment. The circular maps shown on top indicate the regions of the original plasmids that have been deleted (not all of the restriction enzyme sites are shown on these maps). The linearized maps of the deletants shown below start at the *Eco*RI site of pACYC184 and have been oriented with respect to Tn*21* to show directly which parts of the element remain (solid lines).

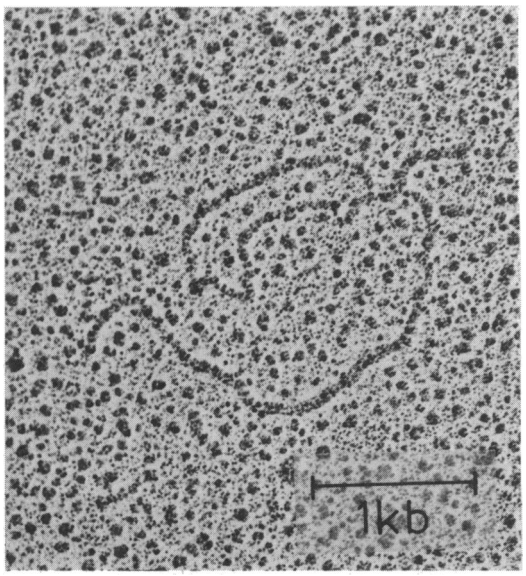

FIG. 3. Heteroduplex formation between plasmid DNA obtained from plasmids pUB2406 and pACYC184, both digested with *Sal*GI. DNAs were digested to completion with endonuclease *Sal*GI (which produces a single cut in each of the molecules; see Fig. 1) separately. Approximately 0.2 μg of DNA from each digestion were mixed together in a buffer of 0.1 M NaOH–20 mM EDTA (final concentration) and incubated at room temperature for 15 min. Then the solution was neutralized with 2 M Tris-chloride (pH 7.5), and formamide added to 45% (vol/vol). This final solution (550 μl) was incubated for 210 min at 30°C. The DNA was prepared for electron microscopic examination as described by Bennett et al. (5). Four different molecules were measured. The length of the short arm of the double-stranded DNA was estimated to be 0.51 ± 0.07 kb, using the overall length of pACYC184 (4.1 kb) as the internal standard.

sites to termini of the element. This assignment was confirmed by a heteroduplex of pUB2406 (a derivative of pUB2401; Fig. 2) with pACYC184, both of which had been digested with *Sal*GI (Fig. 3). This shows that the site of insertion of the Tn*21* was 0.5 kb from the *Sal*GI site; the distance of the *Sal*GI site to the *Eco*RI site in the *mer* operon in pUB2401 (the parent of pUB2406) is 3.5 kb (Fig. 2), indicating that the distance from this outermost *Eco*RI site to the terminus of the element is 3.0 kb. This is consistent with the assignment of 2.9 kb (see above). The detailed restriction enzyme map of Tn*21* derived from the data from the various recombinants is shown in Fig. 1. The heteroduplex shown in Fig. 3 also shows that Tn*21* is flanked by short inverted repeat sequences.

Transposition of Tn*21* from pACYC184::Tn*21* recombinants. Tn*21* transposed from pACYC184::Tn*21* recombinants to the conjugal plasmid R388 (strains UB6200 and UB6345, Table 3, UB2401 and pUB2402 are such recombinants, Fig. 1). In all cases examained, the genes coding for Hg^r, Sp^r, and Su^r cotransposed (Table 3, footnote *f*).

An intermediate in the transposition of Tn*3* is a cointegrate of donor and recipient replicons; this is then resolved to give recombinant plus the original donor (3, 15). If the recipient is a conjugal plasmid, the cointegrate can be transferred to another cell. This would result in the mobilization of all of the genes on the donor replicon, even if the donor plasmid was not normally mobilizable. The plasmid pACYC184 was not itself mobilized by R388 (e.g., strain UB6196, Table 4); however, the pACYC184 sequences in pACYC184::Tn*21* recombinants were mobilized by R388 (Table 3, Cm^r of pUB2401 was mobilized in UB6200 and Tc^r of pUB2402 was mobilized in UB6345). Thus, cointegrates are formed during the transposition of Tn*21*. The transposition frequency in these strains was at least 50 times the mobilization frequency (Table 3, this ratio is called the resolution effect). This implies that the cointegrates can be resolved. (Since

TABLE 3. Genetic characterization of Tn*21* transposition[a]

Donor strain[b]	Donor plasmid[c]	Frequency of following transconjugants:			
		Sp^r/Tp^r[d]	Hg^r/Tp^r[d]	Tc^r/Tp^r[e]	Cm^r/Tp^r[e]
UB6200	pUB2401	4.5×10^{-4}[f]	4.5×10^{-4}[f]		6.7×10^{-6}
UB6201	pUB2406				0 ($<1.0 \times 10^{-8}$)
UB6345	pUB2402	3.5×10^{-3}	3.0×10^{-3}	5.0×10^{-5}	
UB6202	pUB2407		6.0×10^{-4}	9.0×10^{-6}	

[a] Donor strains were mated with UB1637 as described in Materials and Methods. Dilutions of the mating mixtures were plated in minimal agar supplemented with lysine, histidine, and tryptophan plus the relevant drug.
[b] See Table 1 for description.
[c] See Table 2 for description.
[d] Values are a measure of the frequency of transposition.
[e] Values are a measure of the frequency of conduction of the different pACYC184 derivatives; that is, the frequency of transfer of fused replicons (8).
[f] Sp^r Colonies (10^3) were replicated onto Hg plates and Su plates, and Hg^r colonies (10^3) were replicated onto Sp and Su plates. All of the Sp^r colonies were also Hg^r and Su^r and vice versa.

they can be resolved, they are only transient and have little opportunity of being transferred, giving a lower frequency for mobilization than for overall transposition.) All experiments were carried out in *recA* strains, implying that some other recombination function is involved in resolution. It seems likely that, as with Tn*3* (15), Tn*21* contains its own "resolvase" function, coded for by a *tnpR* gene.

Localization of transposon-encoded genes necessary for the transposition of Tn*21*. Removal of the DNA between the outermost *Bam*HI sites of Tn*21* removes the genes encoding Sp^r and Su^r, but leaves those responsible for Hg^r (Fig. 1). The plasmid pUB2407 contains such a deleted Tn*21* (Fig. 2). This element (called Tn*810*) can still transpose, as shown by the Hg^r transconjugants observed when strain UB6202 was donor (Table 3). Furthermore, the Tc gene of pACYC184 on pUB2407 was mobilized (strain UB6202, Table 3), showing that cointegrate intermediates were formed. Resolution of these cointegrates could occur (the resolution effect was greater than 50; Table 3), indicating that Tn*810* probably contains a *tnpR* gene.

Removal of the DNA between the outermost *Eco*RI sites of Tn*21* gave the plasmid pUB2406 (Fig. 2). The remainder of Tn*21* in this plasmid does not contain a selectable marker, so direct transposition cannot be assayed. However, if it can transpose, it should mobilize the Cm gene of pACYC184 carried by pUB2406. There was no such mobilization (strain UB6201, Table 3), implying that this deletion has removed or disrupted a gene required for transposition (*tnpA* gene). Thus, 2.9 kb of the left-hand end of Tn*21* plus 1.8 kb of the right-hand end (as in pUB2406) are not sufficient for transposition. However, 7.8 kb of the left-hand end plus 4.4 kb of the right-hand end (as in the *Bam*HI-generated deletant in pUB2407, see above) contain all of the genes required for transposition of Tn*21*.

The 7.8 kb of the left-hand end and the 4.4 kb of the right-hand end of Tn*21* were cloned separately to see which is responsible for transposition functions. The resulting plasmids are pUB2414, which contains the left-hand end, and pUB2413, which contains the right-hand end (Fig. 2). Since the Tn*21* sequences in these plasmids contain only one of the ends of the element, they cannot themselves transpose. They were tested by examining their ability to complement a transposition-defective (*tnpA*) mutant of Tn*1721* (which codes for Tc^r). This was part of the plasmid pUB818 (Table 2, footnote *a*); this plasmid plus a plasmid containing the Tn*21* sequences plus R388 were put in the same cell, and the transposition of the defective Tn*1721* to R388 was assayed by mating out and selecting for Tc^r transconjugants (Table 4). Tn*21* and Tn*1721* are closely related (Grinsted et al., submitted for publication), and Tn*21* itself can complement transposition of the defective Tn*1721* (Table 4, strain UB6195, Tn*21* is part of pUB2401). (Without a complementing element there was, of course, no transposition [for instance, strain UB6196, Table 4].) The derivative of Tn*21* carried on pUB2406 (generated by deletion with *Eco*RI) did not transpose independently (Table 3) and also did not complement the defect in the mutant of Tn*1721* (strain UB6197, Table 4). The left-hand 7.8 kb of Tn*21* (in pUB2414) also did not complement transposition (strain UB6208, Table 4). However, in the presence of the right-hand 4.4 kb of Tn*21* (in plasmid pUB2413), transposition of the *tnpA* mutant of Tn*1721* did occur (strain UB6207, Table 4). Apart from pACYC184 sequences (and pACYC184 did not complement transposition; see strain UB6196, Table 4), the only sequence in common between pUB2401, pUB2407, and pUB2413 (the three plasmids that complemented the transposition defect in pUB818, Table 4) is

TABLE 4. Complementation of transposition of a *tnpA* mutant of Tn*1721* by derivatives of pACYC184::Tn*21* recombinants[a]

Donor strain	Complementing plasmid	Tc^r/Tp^r transconjugants[b]
UB6196	pACYC184	$0 (<1 \times 10^{-8})$
UB6195	pUB2401	3×10^{-5}
UB6197	pUB2406	$0 (<5 \times 10^{-7})$
UB6198	pUB2407	4×10^{-4}
UB6207	pUB2413	1×10^{-5}
UB6208	pUB2414	$0 (<5 \times 10^{-7})$

[a] Donor strains and strain UB1637 were mated as described in Materials and Methods. Dilutions of the mixtures were plated on Sm plus either Tp or Tc.

[b] In all matings in which transconjugants were obtained, 12 well-isolated colonies were analyzed for the restriction pattern of their plasmid DNA. Plasmid pUB818 produces three *Eco*RI fragments of 5.4, 4.2, and 3.7 kb. The 3.7-kb fragment contains the pJOE100 portion of the plasmid and, thus, the Ap^r gene; the other two fragments comprise the Tn*1721* derivative. Except when pUB2407 was used as the complementing plasmid, all of the transconjugants produced *Eco*RI restriction patterns consistent with their being recombinants of R388 with the Tn*1721* deletion, that is, the 5.4- and 4.2-kb fragments of the transposon plus two extra fragments of variable size corresponding to R388. However, plasmid pUB2407 is Tc^r and can be mobilized by R388 (see Table 3). Restriction analysis demonstrated that only 2 of 12 of the resulting colonies contained the bands expected for the R388-deleted Tn*1721* recombinants. The rest were R388::pUB2407 cointegrates or the resulting resolution products. The true frequency of complementation would then be approximately 7×10^{-5}.

the 4.4 kb of the right-hand end of Tn*21*. Hence, the *tnpA* gene(s) must be part of this sequence. Since the deletion of Tn*21* in pUB2406 did not complement transposition, the gene must either be between coordinates 15.5 and 18.1 of the map shown in Fig. 1, or it must span the outermost *Eco*RI site at coordinates 18.1. If the *tnpA* gene of Tn*21* is of a similar size to that of Tn*3* (3 kb, reference 15), the latter would be the case (see below).

DISCUSSION

The transposition of Tn*21* involves the transfer en bloc of the antibiotic resistance markers Hg, Su, and Sm/Sp (Table 3) contained within a contiguous stretch of DNA of about 20 kb, originally present in the plasmid R100.1. We have not emphasized the results that led us to the physical map of Tn*21* (Fig. 1), since the corresponding region of R100.1 has been studied previously (6, 9). However, our results do not agree with the location of the *Bam*HI sites reported by Foster et al. (12). In fact, the position of the sites shown in Fig. 1 solves the problem these authors had in trying to reconcile their *Bam*HI restriction enzyme data from Tn*801* insertions into the *mer* operon of R100.1, the known relative location of the IS*1* sequence and *Eco*RI map of the plasmid. Using the sites shown in Fig. 1, their results suggest that the *mer* operon starts at coordinate 0.4 kb in the Tn*21* map of Fig. 1 (2.5 kb to the left of the first *Eco*RI site) and ends at coordinate 3.4 (0.5 kb to the right of the same site); this gives space in R100.1 for the IS*1* sequence (which starts 2.9 kb to the left of the *Eco*RI site) and explains why insertions within the *Eco*RI fragment I can give a Hg^s phenotype. The map of Tn*21* shown in Fig. 1 can be compared with that of Tn*2603* (25); the restriction maps of both transposons are identical except for an insertion of 0.7 kb in Tn*2603*, which contains the gene coding for resistance to oxacillin.

There is a short inverted repeat sequence at the ends of Tn*21* (Fig. 3). Since the products of the *tnpA* genes of Tn*21*, Tn*501*, and Tn*1721* complement one another (Table 4; Grinsted et al., submitted for publication), it would be expected that the inverted repeat sequences of these elements should be very similar (since the *tnpA* products must recognize the ends of the elements). This has been borne out by sequencing studies; the inverted repeat sequences of Tn*501* and Tn*1721* (38 base pairs) are almost identical (2) and that of Tn*21* is homologous to those of Tn*501*/Tn*1721* in 30 of 38 base pairs Z. X. Zheng, M. Chandler, R. Hipskind, M. Clerget, and L. Caro, Nucleic Acids Res., in press).

Complementation of mutants of Tn*1721* by derivatives of Tn*21* (Table 4) indicates that the *tnpA* gene of Tn*21* is located in the 4.4 kb sequence at the right-hand end of the element (coordinates 15.5 to 19.9, Fig. 1). This is supported by the fact that this region of Tn*21* hybridizes with a 4-kb region of Tn*501* and Tn*1721* that is known to code for transposition functions (2; de la Cruz, unpublished results). There seems little doubt from the data presented here (Table 3) that Tn*21* contains a *tnpR* gene and that it is contained in the Tn*21* sequences of Tn*810* (the element generated by deletion with *Bam*HI of Tn*21*). We have found recently that removal of the DNA between the *Hin*dIII sites at coordinates 16.4 and 16.8 results in loss of the resolution activity coded for by Tn*21* (Grinsted and de la Cruz, manuscript in preparation), indicating the location of the *tnpR* gene. The *tnpA* gene of Tn*3* is 3.05 kb (15). If the *tnpA* gene of Tn*21* is also 3 kb it must be located between coordinates 16.8 and 19.9, since this is the only place it can fit in the region 15.5 to 19.9 if there is another gene close to coordinate 16.9. One end of the *tnpA* gene would then be at the end of the element, as is the case with Tn*3* (15). (It should be noted that there are good reasons for thinking that the *tnpA* gene of Tn*3* is related to the *tnpA* genes of Tn*501*/Tn*1721* and Tn*21*; particularly, the inverted repeats of all these elements are related (2; Zheng et al., Nucleic Acids Res., in press).)

The presumptive resolvase of Tn*21* seems much more active than the one in the related transposon Tn*501* when assayed by the same experimental approach (23). However, the resolution effect of the resolvase of Tn*21* (50, Table 3) is still orders of magnitude below that of Tn*3* (5×10^4, Fig. 4 in reference 23). By regulation of the activity of their resolvases, transposons can control the half-life of the intermediate cointegrates. It is tempting to speculate that because of low efficiency of their resolvase functions, transposons such as Tn*21* or Tn*501*, when still forming a cointegrate, have time for secondary rearrangements which, at the end, result in the incorporation of new genes within the transposable unit as in the model proposed by Altenbuchner et al. (2).

In addition to the genes coding for transposition functions, the *mer* operon of Tn*21* is homologous to that of Tn*501* (R. H. Rownd, personal communication; de la Cruz, unpublished results). Tn*21* looks like Tn*501* with about 12 kb of other DNA inserted into its middle. The evolutionary relationship between these two elements and Tn*1721* are dealt with elsewhere (2; Grinsted et al., submitted for publication). A further step in this evolutionary tree could have been the acquisition of an *oxa* gene by Tn*21* to produce Tn*2603* (25).

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