JOURNAL OF BACTERIOLOGY, Aug. 1994, p. 5059–5067
0021-9193/94/$04.00+0

Vol. 176, No. 16

Conduction of pEC22, a Plasmid Coding for MR · *Eco*T22I, Mediated by a Resident Tn*3*-Like Transposon, Tn*5396*

JEFF ELHAI,[1,2]* YUPING CAI,[1]† AND C. PETER WOLK[1,2]

Michigan State University–Department of Energy Plant Research Laboratory[1] and Center for Microbial Ecology,[2] Michigan State University, East Lansing, Michigan 48824

Received 31 January 1994/Accepted 28 May 1994

pEC22 is a small plasmid that encodes the restriction-modification system MR · *Eco*T22I. Restriction and functional analysis of the plasmid identified the positions of genes encoding that system. The plasmid is able to be conducted by conjugal plasmids, a process mediated by a transposon contained within pEC22. This cryptic transposon, called Tn*5396*, was isolated from pEC22 and partially sequenced. The sequence of Tn*5396* is for the most part typical of transposons of the Tn*3* family and is most similar to that of Tn*1000*. The transposon differs from closely related transposons in that it lacks well-conserved sequences in the inverted-repeat region and has an unusually long terminal inverted repeat. Consideration of regions of internal sequence similarity in this and other transposons in the Tn*3* family supports a theory of the mechanism by which the ends of Tn*3*-like transposons may maintain substantial identity between their inverted repeats over the course of evolutionary time.

Conjugal plasmids, such as F, are large enough to accommodate the many genes required by the complex process of conjugation (43). While small plasmids are incapable of self-transmission, two means are known by which they may be transferred by a coresident conjugal plasmid (13). First, the conjugal plasmid may transfer the nonconjugal plasmid by mobilization, in the strict sense of Clark and Warren (13). Mobilization requires the action of Mob proteins, generally encoded by the nonconjugal plasmid, and a nicking site, termed *oriT*, on the nonconjugal plasmid. Mobilization can be a very efficient process, with a rate of transfer comparable to that of self-transmission by conjugal plasmids.

Conduction (13) offers a second means by which nonconjugal plasmids may be transferred. Conduction requires the cointegration of the conjugal and nonconjugal plasmids so that transfer of both takes place in a single event. Cointegration can occur by *rec*-dependent recombination between homologous regions on the two plasmids or by *rec*-independent transposition of an element residing on either plasmid. Most commonly, it is a transposable element on the conjugal plasmid, such as Tn*1000* in the case of F, that mediates conduction, but instances of conduction mediated by transposable elements on the nonconjugal plasmid are known (15, 28). In general, the rate of conduction is much lower than the rate of self-transmission of the conjugal plasmid.

pEC22 (46) is a small plasmid originally isolated from *Escherichia coli* TB22. Like many small, otherwise cryptic plasmids, it encodes a restriction-modification system, in this case *Eco*T22I, an isoschizomer of *Ava*III. We have used cloned methylases to protect DNA from restriction by *Ava*I and *Ava*II upon conjugation into certain cyanobacteria (17). To extend protection to include restriction by *Ava*III, we sought to identify and clone the gene encoding the *Eco*T22I methylase, M · *Eco*T22I. Characterization of pEC22 indicated that most of its sequence consists of a transposable element that is responsible for the conduction of the plasmid by conjugal plasmids.

* Corresponding author. Present address: Dept. of Biological Sciences, Florida International University, University Park Campus, Miami, FL 33199. Phone: (305) 348-3584. Fax: (305) 348-1986. Electronic mail address: Cyano@Servax.Fiu.Edu.

† Present address: Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720

MATERIALS AND METHODS

Strains and plasmids. The strains of *E. coli* and the plasmids used in this work are shown in Table 1. Strain CPB1893 was derived from CPB1293, a spontaneous *mcrA* derivative of W3110 *lacI*^{q}*L8*, by transducing *mcrB1 hsdR2*, linked to Tn*10*, from ER1372, selecting for resistance to fusaric acid and concomitant excision of Tn*10* (29), and then selecting for spontaneous resistance to nalidixic acid. When appropriate, *E. coli* was grown on selective medium with the following concentrations of antibiotics: chloramphenicol, 25 μg/ml; kanamycin sulfate, 50 μg/ml; nalidixic acid, 20 μg/ml; streptomycin sulfate, 20 μg/ml; and tetracycline, 10 μg/ml (all from Sigma Chemical Co., St. Louis, Mo.). Strains ER1372 and WA921(pEC22, S-a) were gifts from Elisabeth Raleigh (New England Biolabs, Beverly, Mass.) and Katsutoshi Mise (National Institute of Hygienic Sciences, Tokyo, Japan), respectively. WA921(pEC22) was obtained from WA921(pEC22, S-a) by screening colonies for loss of resistance to kanamycin (Km^r) conferred by the separate plasmid S-a.

Transposon insertion derivatives of pEC22 were made by infecting WA921(pEC22) with λ1105 (42), carrying Tn*10* element 9 (Tn*10.9*), which confers Km^r. Plasmid DNA from pooled Km^r mutants was isolated and used to transform HB101, DH5α, or WA921 to Km^r. The Km^r determinant in Tn*10.9* is flanked by 78-bp inverted repeats (IRs) of a sequence from IS*10*-R that are identical to the sequences flanking the non-self-transposable tetracycline resistance (Tc^r) determinant, Tn*10.8*, in conjugal plasmid pOX38.8. No other sequence similarity exists between Tn*10.8* and Tn*10.9*. Derivatives of pEC22 marked with Tn*10.9* are collectively called pRL591 and are distinguished by suffixes, as shown in Fig. 1. pRL599 is a derivative of pRL591-DD5, in which the chloramphenicol resistance (Cm^r) marker C.C2 (18), cut out of polylinker L.EHE2 (18) with *Hin*cII, was placed between the two *Mlu*I sites (trimmed with Klenow fragment) that flank the Km^r marker of pRL591-DD5.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Reference or source
E. coli strains		
CPB1293	*lacI*^{q} *mcrA*, derived from W3110 *lacI*^{q}*L8*	This work
CPB1893	Nx^r *mcrA mcrB hsdR2*, derived from CPB1293	This work
DH5α	Nx^r *recA hsdR*	Bethesda Research Laboratories
ED8654	HsdM$^+$ HsdR$^+$	9
ER1372	*hsdR2 mcrB1 zjj-202*::Tn*10*	Elisabeth Raleigh
HB101	Sm^r *recA hsdR*$_B^+$ *hsdS*$_B$	10
RR1	Sm^r *hsdR*$_B^+$ *hsdS*$_B$	8
W3110 *lacI*^{q}*L8*	*lacI*^{q}	11
WA921	*hsdR*	46
Plasmids		
pEC22	Determines M · *Eco*T22I and R · *Eco*T22I	46
pIC20H	Ap^r	30
pKT210	Cm^r Sm^r	5
pOX38.8	Tc^r derivative of F without IS elements, marked with Tn*10* element 8 (Tn*10.8*)	36
pRL443	Ap^r Tc^r Km^s derivative of RP4	19
pRL493	Ap^r Cm^r derivative of pIC20H	This work
pRL591	Set of Tn*10.9* insertion derivatives of pEC22	This work
pRL599	Cm^r insertion derivative of pEC22	This work
pRL602	Deletion derivative of pRL591-W45	This work
pRL603	Deletion derivative of pRL591-DB4	This work
pRL605	Deletion derivative of pRL599	This work
pRL611	Ap^r Cm^r, determines M · *Eco*T22I	This work
pRL615	Deletion derivative of pRL591-HA8	This work
pRL616	Deletion derivative of pRL591-HA8	This work
pRL622	Deletion derivative of pRL591-W45	This work
pRL635	Deletion derivative of pRL591-HC12	This work
pRL636	Deletion derivative of pRL591-HC12/W45	This work
pUC8	Ap^r	39

Plasmid pRL493, a cloning vector, consists of a *Pst*I-*Xho*I fragment carrying the Cm^r determinant from pKT210 placed in the *Sal*I site of pIC20H, with the *Pst*I site connected to the *Sal*I site by means of a short linker taken from pUC8. pRL611 consists of a 1.25-kb *Msp*I fragment carrying M · *Eco*T22I from pEC22 placed in the *Cla*I site of pRL493. pRL602 and pRL622 are deletion derivatives of pRL591-W45 generated by digestion with *Pvu*II or *Stu*I plus *Pvu*II, respectively. pRL603 is an *Nhe*I-*Xba*I-generated deletion derivative of pRL591-DB4. pRL605 is an *Ase*I-generated deletion derivative of pRL599. pRL615 and pRL616 are deletion derivatives of pRL591-HA8 generated by digestion with *Dra*I or *Xmn*I, respectively. pRL635 is an *Eco*RI-generated deletion derivative of pRL591-HC12. pRL636 comprises the DNA between insertions HC12 and W45, connected by Tn*10.9*, and was constructed by recombining in vitro DNA from pRL591-HC12 and pRL591-W45.

Determination of phenotypes. The restriction phenotypes of strains carrying pEC22 or its derivatives were determined by comparing the infectivity of λ_{S232} (a gift from Dale Kaiser, Stanford University) grown on WA921(pEC22, S-a) (and therefore methylated against the restriction function of *Eco*T22I, R · *Eco*T22I) with that of λ_{S232} grown on ED8654 (not methylated against R · *Eco*T22I). Methylation phenotypes were determined by two methods: (i) comparing the infectivity on restriction-competent DH5α(pRL591-DB4) of λ_{S232} grown on a test strain with that of λ_{S232} grown on ED8654, and (ii) assessing the ability of *Eco*T22I or its isoschizomer *Nsi*I to cut the plasmid isolated from the test strain. Tn*10.9* has two *Eco*T22I sites, and pEC22 has at least one such site.

To determine the ability of a conjugal plasmid to mobilize derivatives of pEC22, Rec$^-$ donor strains (HB101 or DH5α) carrying either pRL443 (Ap^r Tc^r) or pOX38.8 (Tc^r) and *rec* recipient strains (DH5α or HB101, in some cases carrying pRL611) were grown overnight, washed and regrown in fresh medium, and combined for mating. Matings were performed in liquid medium, which was shaken slowly, or occasionally on plates. After 2 h, the mixture was diluted and plated on selective medium. In all experiments, the number of recipients gaining the Tc^r marker carried by the conjugal plasmid was comparable to the total number of recipients except when transfer of the Tc^r marker was not observed at all.

DNA sequencing. All fragments reported were sequenced on both strands after subcloning into the multiple cloning site of pUC19 (45). Plasmid preparations isolated by the boiling method (25) were purified through a Magic miniprep column (Promega, Madison, Wis.) and sequenced (34) by means of a model 373A automated DNA sequencer (Applied Biosystems, Inc., Foster City, Calif.), with forward (5′ TGTAAAAC GACGGCCAGT 3′) and reverse (5′ CAGGAAACAGCTAT GACC 3′) M13 primers. Sequences were manipulated with a program, EditBase, kindly provided by Niels Nielson (Purdue University). The BLAST suite of programs (2) was used to compare DNA sequences and translated sequences from pEC22 with those within the GenBank database. Deduced amino acid sequences were aligned with the aid of the University of Wisconsin Genetics Computing Group programs (16).

Nucleotide sequence accession number. The sequences obtained in this work have been deposited in the GenBank database under accession numbers U04360 (Tn*5936*$_t$), U04362 (Tn*5936*$_o$), and U04363 (partial internal sequence of *tnpA* of Tn*5936*).

RESULTS

Characterization of pEC22. pEC22 was characterized initially by restriction analysis, leading to the map shown in Fig. 1. In an effort to identify the gene encoding M · *Eco*T22I, pEC22

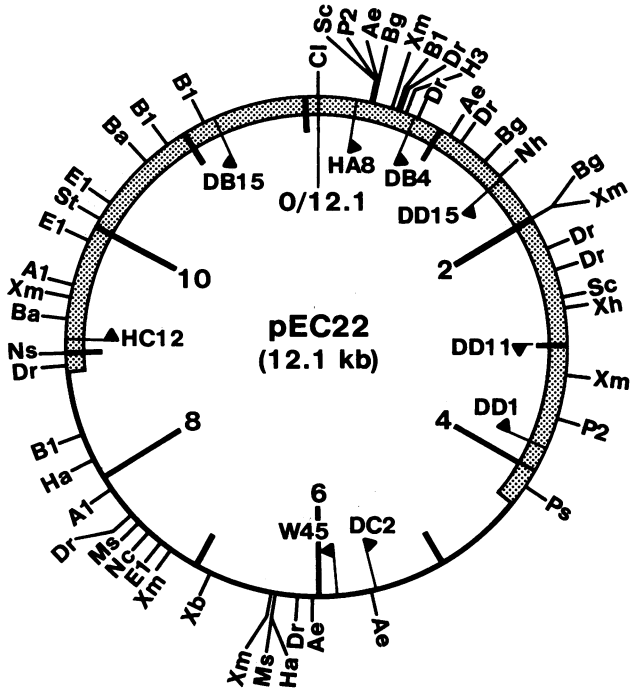

FIG. 1. Restriction map of pEC22. Flags show insertions of Tn*10.9*, with the direction of the flag giving the direction of transcription of the gene conferring resistance to kanamycin. A derivative of pEC22 carrying a specific insertion is called pRL591-X, where X is the name of the insertion. The stippled region corresponds to Tn*5393* (see text). Abbreviations for restriction sites: A1, *Ava*I; Ae, *Ase*I; B1, *Bgl*I; Ba, *Bam*HI; Bg, *Bgl*II; Cl, *Cla*I; Dr, *Dra*I; E1, *Eco*RI; H3, *Hin*dIII; Ha, *Hae*III; Ms, *Msp*I; Nc, *Nco*I; Nh, *Nhe*I; Ns, *Nsi*I; P2, *Pvu*II; Ps, *Pst*I; Sc, *Sca*I; St, *Stu*I; Xb, *Xba*I; Xh, *Xho*I; Xm, *Xmn*I. Not all sites are shown for *Hae*III and *Msp*I, and there may be more sites for *Nsi*I. There are no sites for *Apa*I, *Mlu*I, *Pml*I, *Sal*I, *Sma*I, *Sph*I, or *Sst*I.

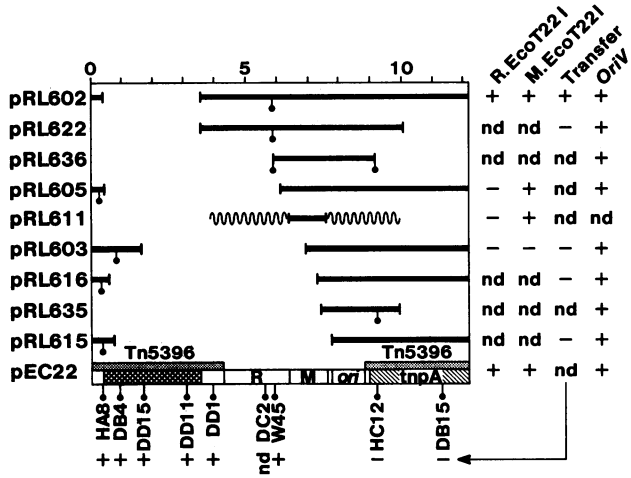

FIG. 2. Functional map of pEC22, based on phenotypes of deletion derivatives (left column) and Tn*10.9* insertion mutants (bottom row). Thick lines show portions of pEC22 retained in each deletion derivative. Lollipops show positions of insertion of Tn*10.9*. Apart from insertions, DNA not derived from pEC22 is represented by wavy lines (of arbitrary length). Restriction and methylation phenotypes were determined as described in Materials and Methods (data not shown). Transfer phenotypes (shown to the right of deletion derivatives and below insertion derivatives) are listed as + if the frequency of transfer (as defined in Table 2) exceeded 10^{-5}, − if it did not, and nd if it was not determined. Possession of *oriV* is indicated as + if the pEC22-derived DNA can propagate itself without the aid of any foreign origin of replication and by − or nd otherwise. The presumptive transposase gene is denoted *tnpA* and is indicated by hatching (see text). The region of Tn*5396* (stippled) proposed to be dispensable for transposition is indicated by cross-hatching.

was subjected to transposon mutagenesis with Tn*10.9*. Since $R^+ M^-$ mutants would undoubtedly be nonviable, we screened first for R^- mutants. The map positions of selected insertions are shown in Fig. 2. Only those insertions clustered around coordinates 5.5 to 5.8 (e.g., DC2 and W45 in Fig. 2) resulted in an R^- phenotype. Methylase activity was unaffected in these mutants.

Analysis of deletion derivatives confirmed the location of the genetic determinant of R · *Eco*T22I and localized the determinant of M · *Eco*T22I to an adjacent region (Fig. 2). A 1.25-kb *Msp*I fragment from this region was subcloned (producing pRL611), and this fragment proved sufficient to confer an M^+ phenotype. Deletion derivatives also aided in the identification of the origin of replication of pEC22. The intersection of all deletion derivatives shown in Fig. 2 delimits the region containing the origin to the 1.4 kb shared by pRL615 and pRL636.

Transfer of pEC22 derivatives by conjugal plasmids. Two regions of pEC22 were found to be required for transfer mediated by conjugal plasmid pOX38.8 or pRL443 (Table 2, Fig. 2, and data not shown). One of the required regions, between coordinate 3.6 and the genes encoding the restriction-modification system, was defined by comparison of two deletion derivatives, pRL602 and pRL603 (Fig. 2). Tn*10.9* insertions HC12 and DB15 define the remaining region required. Deletion or insertion between coordinates 0.4 and 3.6 had no effect on transfer efficiency. Conjugation experiments were often performed with recipient strains carrying pRL611 (M · *Eco*T22I), in case the transfer of genes encoding *Eco*T22I into the recipient affected viability, but this precaution proved to be unnecessary.

Transfer of the marker on the nonconjugal plasmid might also occur via conduction mediated by homologous recombination. To minimize the possibility of recombination-mediated conduction, experiments were performed with Rec^- donors. Such donors also allow distinction between conduction mediated by Tn*3*-type transposons, which are able to form unstable cointegrates in Rec^- hosts (20), and that mediated by composite transposons such as Tn*5*, which are not able to do so (24).

If the Km^r marker carried by pEC22 derivatives had been transferred by mobilization of the derivatives, then the recipient should have gained the conjugal and nonconjugal plasmids as separate molecules. Secondary conjugation into new recipients should then exhibit transfer efficiencies comparable to those of the original conjugation. Efficiencies of secondary transfer of the products of initial conjugal transfer were clearly not the same as those of the parental strains (compare Table 2 with Table 3). Instead, three alternative results were obtained: (i) the cotransferred conjugal plasmid, pOX38.8, lost self-transmissibility (Table 3, lines d and e); (ii) transfer of Km^r was completely linked to transfer of Tc^r on pOX38.8, representing an increase in transfer efficiency of 10,000- to 100,000-fold (Table 3, lines a, b, c, and f; compare with Table 2, experiment 1; and data not shown); or (iii) transfer of Km^r was substantially but incompletely linked to transfer of Tc^r (Table 3, lines g, h, and i).

Since complete or partial linkage of Km^r with the conjugal

TABLE 2. Conjugal transfer of pEC22 derivatives

Transferred plasmid	Mutation[a]	Frequency of transfer[b] of Km^r (no. × 10^{-5})			Frequency of transfer of restriction activity[c]
		Expt 1	Expt 2	Expt 3	
pRL591-DB4	Ω0.8	1.6		15	0/9
pRL591-DD15	Ω1.6	1.7			0/3
pRL591-DD11	Ω3.0	1.6			0/3
pRL591-DD1	Ω3.8	1.3			0/1
pRL591-W45	Ω5.8	1.7	2.2	28	NA
pRL591-DB15	Ω11.1	0.06		0.01	2/9
pRL602	Ω5.8, Δ0.4–3.6			41	
pRL603	Ω0.8, Δ1.6–6.9		0.2	0.04	NA
pRL615	Ω0.3, Δ0.8–7.7		0.03		NA
pRL616	Ω0.3, Δ0.6–7.2		0.1		NA
pRL622	Ω5.8, Δ10.0–3.6		0.3	0.09	

[a] Ω, insertion of Tn*10* element 8 at the designated coordinate of pEC22; Δ, deletion of pEC22 between the designated coordinates.

[b] Frequency is given as the fraction of recipients that gained the Km^r determinant on the pEC22 derivative in relation to those recipients that gained the Tc^r determinant carried on the conjugal plasmid. In experiment 1, the donor strain was DH5α(pRL443, test plasmid) and the recipient strain was HB101(pRL611). In experiment 2, the donor strain was DH5α(pOX38.8, test plasmid) and the recipient strain was HB101. In experiment 3, the donor strain was DH5α(pOX38.8, test plasmid) and the recipient strain was HB101(pRL611).

[c] Frequency is given as the fraction of recipients that are R · *Eco*T22I$^+$ among the recipients that gained the Tc^r determinant on the conjugal plasmid. The denominator indicates the actual number of recipients tested. Results from two similar experiments are pooled. NA, not applicable; assays of restriction activities from recipients of pRL591-W45, pRL603, pRL615, and pRL616 are not informative, since donors carrying these plasmids do not restrict.

TABLE 3. Secondary transfer of a marker originating from pEC22 derivatives

Expt no.	Donor strain[a]	Frequency of transfer[b] of Km^r (no. × 10^{-5})
1[c]		
a	HB101[pRL611, (pOX38.8 + pRL591-DB4)]	100,000 (16)
b	HB101[pRL611, (pOX38.8 + pRL591-DD1)]	100,000 (12)
c	HB101[pRL611, (pOX38.8 + pRL591-DB15)]	100,000 (16)
2[d]		
d	DH5α[pRL611, (pOX38.8 + pRL591-HA8)] isolate 1	—[e]
e	DH5α[pRL611, (pOX38.8 + pRL591-HA8)] isolate 2	—
f	DH5α[pRL611, (pOX38.8 + pRL591-HA8)] isolate 3	100,000 (12)
g	DH5α[pRL611, (pOX38.8 + pRL591-W45)] isolate 1	17,000 (48)
h	DH5α[pRL611, (pOX38.8 + pRL591-W45)] isolate 2	17,000 (12)
i	DH5α[pRL611, (pOX38.8 + pRL591-W45)] isolate 3	25,000 (12)
j	DH5α[pRL611, (pOX38.8 + pRL591-W45)] isolate 4	0 (12)

[a] For experiment 1, donor strains were obtained as Km^r Tc^r colonies from primary conjugation experiments (see Table 2, experiment 1); for experiment 2, they were obtained from a similar experiment with *recA* strain DH5α or HB101 as the donor. The plasmids shown in parentheses indicate those carried by the original donor (conjugal plasmid + pEC22 derivative). Each donor of the secondary conjugation therefore carries pRL611 and any plasmid(s) transferred from the primary conjugation.

[b] Frequency is given as the fraction of recipients that are Km^r among the recipients that gained the Tc^r determinant carried on the conjugal plasmid. The numbers in parentheses indicate the number of Tc^r colonies tested for Km^r.

[c] Recipient strain was CPB1893.

[d] Recipient strain was RR1. Each isolate was taken from a separate Km^r Tc^r colony on the primary conjugation plate.

[e] —, no Tc^r recipients obtained.

plasmid indicated that the marker had become inserted into that plasmid, recipients were tested for the acquisition of a second marker, restriction activity, on the pEC22 derivatives. Those derivatives tested whose Km^r marker had been transferred with relatively high efficiency (>10^{-5}) in the initial conjugation were found not to have passed the ability to restrict incoming phage to exconjugants, suggesting that only a portion of the derivatives had become inserted into the conjugal plasmid or that the gene encoding R · *Eco*T22I had been selectively lost in recipients. In some instances, R · *Eco*T22I activity did accompany the Km^r marker of one derivative (pRL591-DB15) that transferred Km^r with low efficiency.

Trapping and partial sequence of a transposon, Tn*5396*, in pEC22. The results of the conjugation experiments are most easily explained by the presence of a transposable element in pEC22. If the region of pEC22 that was linked to the conjugal plasmid is a transposable element, it should be possible to trap the element on a small plasmid that is amenable to restriction analysis. This was achieved by selecting for transfer of the nonmobilizable plasmid pRL611 from a strain containing this plasmid and conjugal plasmid pOX38.8 that had been modified by transfer with the pEC22 derivative pRL591-DB4 (one of the crosses shown in Table 3, experiment 1). The Cm^r marker of pRL611 was recovered from recipients at a frequency of 3 × 10^{-6} relative to the frequency of transfer of the Tc^r marker of pOX38.8. Plasmids from three such exconjugants were analyzed, and in each case, a plasmid identical to pRL611 except for an insertion of 7.4 kb was found. The insertions were indistinguishable from each other by restriction analysis and corresponded to DNA of pEC22 from coordinate 9.0 (via 0) to 4.3. Insertion occurred at three different sites in pRL611. To elucidate the nature of the inserted DNA, fragments spanning an insertion site in pRL611 were sequenced. In the insertion derivative chosen, the inserted DNA mapped within the *bla* gene, the sequence of which is known (6).

The terminal sequences (Fig. 3) show that the insertion is bounded by 66-bp IRs and flanked by a 5-bp direct repeat derived from the *bla* gene on pRL611. The IRs exhibit considerable sequence similarity to those found in members of the Tn*3* family (Fig. 4), particularly in the first 38 bp, which is the typical length of inverted repeats in Tn*3*-like transposons (36). Sequence similarity is greater to members of the Tn*3*

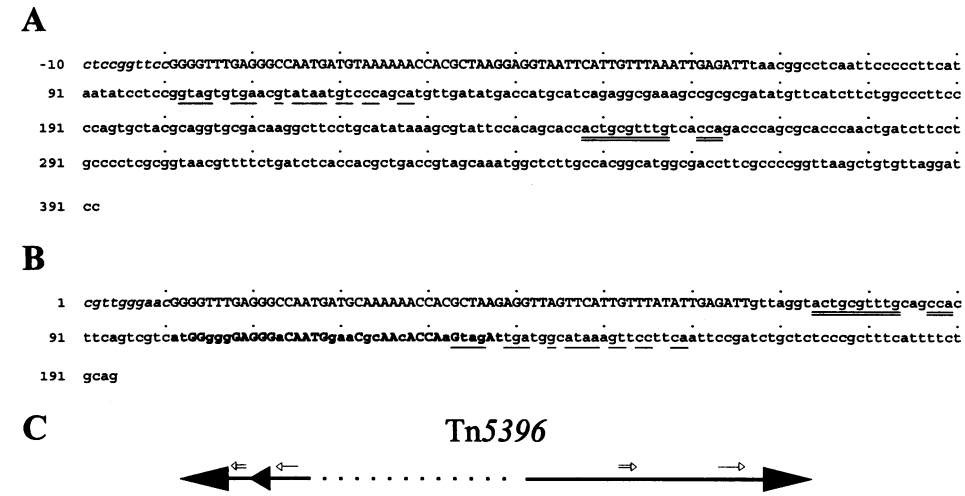

FIG. 3. Sequence of terminal repeats of Tn*5396*. Sequences derived from pRL611, nucleotides 3658 to 3672 in pBR322 coordinates (6), are shown in italic. The terminal IR sequences are given in uppercase letters. A proposed degenerate repetition of the IR (IR_{o2}) is set off in boldface, with uppercase letters indicating bases in common with IR_o. Regions of extensive sequence similarity outside of the IRs are shown by single or double underlines. (A) Sequence of IR_t (transposase-proximal end of transposon) and adjacent sequences (up to the first *Bam*HI site in Tn*5396*). (B) Sequence of IR_o (other end of transposon) and adjacent sequences (up to the *Pst*I site in Tn*5396*). The sequence is of the strand opposite to that given for IR_t. (C) Representation of Tn*5396*, showing the relative positions and orientations of regions of sequence similarity between IR_o (left) and IR_t (right). Heavy arrowheads represent the terminal repeats and the putative secondary terminal repeat. Short single- and double-shafted arrows correspond to single- and double-underlined regions, respectively, in panels A and B.

subgroup than to those of the Tn*21* subgroup (22) or to other transposons within the Tn*3* family. The greatest sequence similarity is with Tn*1000* (also called γδ, a transposable element found naturally on plasmid F and on the *E. coli* chromosome, with the sequence similarity extending on one arm at a reduced level beyond the first 38 bp and throughout the sequenced region (Fig. 4 and data not shown). The transposable element on pEC22 has been placed in the Plasmid Reference Center Prefix Registry (E. Lederberg, Stanford University) and assigned the name Tn*5396*.

Members of the Tn*3* family (with the exception of the incomplete element IS*101*) carry *tnpA*, encoding the transposase, almost always ending within one terminal repeat of the transposon (22). An open reading frame of 118 codons was found that extends from one end of a small region that was sequenced, terminates within one of the IRs of Tn*5396*, and, translated, shows a marked similarity to the C terminus of transposases of the Tn*3* family. We therefore labeled this end IR_t. A 575-bp sequence internal to Tn*5396*, about 1.4 kb from the last determined codon of the open reading frame near IR_t, contains a single open reading frame in the same orientation as that near IR_t and 191 codons in length. The amino acids encoded by these two sequences were compared with those found in transposases of other transposons of the Tn*3* family (Fig. 5). Pairwise comparisons confirm the inference from IR similarities that Tn*5396* lies within the Tn*3* subgroup and is most closely related to Tn*1000*. Although together the regions compared comprise only about 30% of the complete sequence of a typical Tn*3*-type transposase, they appear to sample adequately the variability of the transposase, since values for comparisons based on entire sequences that have been reported previously (4, 22) are very close to those reported here.

IRs within Tn*5396*. A comparison of the sequenced termini of Tn*5396* revealed several regions of similarity apart from the 66-bp IRs (Fig. 6 and Fig. 3). A second degenerate copy of the inverted repeat is present in the IR_o-proximal end of Tn*5396* (Fig. 3B, coordinates 103 to 133, labeled IR_{o2}; see also Fig. 4), and the two termini have two extended regions of sequence similarity. These relationships are shown graphically in Fig. 3C.

An examination of several other transposons in the Tn*3* family (Fig. 6) yielded the following observations. First, several transposons in the Tn*3* family have degenerate IRs beyond the 38-bp termini. Tn*1000* and IS*101* both have extensive regions of imperfect identity between one arm and the inverted sequence of the other, separated by a gap of several base pairs from the 38-bp IR. In many cases, a plausible continuation of the IR is offset in one arm with respect to the other. For example, in Tn*3*, identity between the two arms resumes after a gap in IR_o of 4 bp and in IR_t of 42 bp. There are similarly plausible continuations in Tn*917* and Tn*5393*. While it is hazardous to make connections between isolated regions of identity, it is striking that regions of identity so often appear just past the end of the IR, as if a longer IR had been interrupted.

Second, several transposons have copies or degenerate copies of the IRs elsewhere in the transposon. Complete secondary IRs have been reported for Tn*501* (21) (see also Fig. 6) and Tn*917* (35) and the related transposon Tn*1721* (1). It may be of some significance that, like Tn*5396*, IS*101*, Tn*917*, Tn*1546*, and, arguably, Tn*1000* show degenerate copies (Fig. 6). The possible second copy in the transposase gene-proximal arm of Tn*1546* is of special interest. The genes encoding the transposases in the Tn*3* family generally end at a stop codon at position 34 to 36 of the IRs (see Fig. 4). Tn*1546* is exceptional in that its transposase gene terminates at positions 75 to 77. This triplet, however, lies in the secondary IR (Fig. 6 and coordinates 66 to 79 in Fig. 4) at precisely the position where the stop codon would be expected in a typical Tn*3*-like IR_t.

DISCUSSION

The known functions of pEC22, i.e., genes encoding the *Eco*T22I restriction-modification system and the origin of replication, occupy a 4.7-kb region of the plasmid. Several

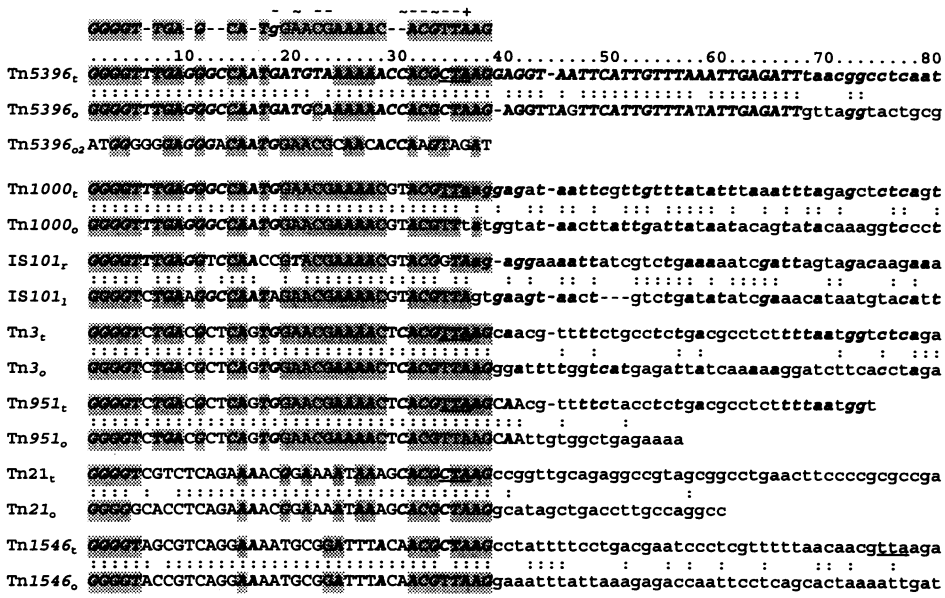

FIG. 4. Comparison of IRs of several transposons in the Tn*3* family. The letter t following the name of the transposon designates the *tnpA*-proximal end of the transposon, and the letter o refers to the other end. IR_{o2} refers to the degenerate IR on the *tnpA*-distal end of Tn*5396* (Fig. 3). IS*101* lacks a *tnpA* gene, and its ends are arbitrarily designated r and l. Bases given in uppercase letters signify those bases contained within the IR. Bases in boldface italics are those that match corresponding bases of Tn*5396*. A consensus IR for members of the Tn*3* subgroup (22, 36), represented by Tn*1000*, IS*101*, Tn*3*, and Tn*951* but not including Tn*5396*, is shown at the top of the figure. Tn*4556* is also included in this subgroup by Amemura-Maekawa and Ohtsubo (3) but not by others (22, 36). Tn*21* is representative of the Tn*21* subgroup (22), and Tn*1546* is a transposon within the Tn*3* family but outside either subgroup. Shading indicates that the base at that position matches that of the consensus for the Tn*3* subgroup. Symbols above the consensus sequence refer to the ability of IRs derived from Tn*3* mutated at that base pair to support transposition (33): +, greater than 50% of wild-type IR transposition activity; ~, 20 to 50% of wild-type activity; −, less than 3% of wild-type activity. Underlined triplets indicate the stop codon for *tnpA*. IR sequences (besides those of Tn*5396*) were obtained from GenBank under the following accession numbers: Tn*1000*, X60200; IS*101*, X01654; Tn*3*, V00613; Tn*951*, M25019 and M25021; Tn*4556*, M29297; Tn*21*, X04891; and Tn*1546*, M97297.

findings point to the conclusion that the remaining 7.4 kb comprises a cryptic transposon. First, pEC22 derivatives could be transferred by conjugal plasmids so long as two separable regions were preserved. From mutational analysis, one such region evidently lies between coordinate 3.8 (the site of Tn*10.9* insertion DD1) and coordinate 4.3 (the end of the segment of pEC22 that is inserted into other plasmids). We suggest that this region is required because it contains one end of the transposon. A second required region is defined by two Tn*10.9* insertions, DB15 and HC12, that are spaced 2.0 kb apart. If we assume that the presumptive transposase identified by sequencing is comparable in size to other transposases of the Tn*3* family, then both DB15 and HC12 insertions would lie within *tnpA*, and so this second required region would bear the gene that encodes the transposase. Undoubtedly, a third region, IR_t, is also required for transfer, but we did not obtain mutants affected in IR_t that would not also be affected in *tnpA*.

Second, the recipient gained pEC22 DNA linked to the conjugal plasmid. In some cases, the evidence for such acquisition is indirect, namely, the conjugal plasmid lost the ability to transmit itself, presumably because the transposon inserted itself into a gene required for transmission. When the selected marker lay within the 7.4-kb region, pEC22 DNA outside of this region was not present in the modified conjugal plasmid and the marker was stably maintained, indicating normal transposition. When the selected marker lay outside of the 7.4-kb region, the marker was not stably maintained on the conjugal plasmid, consistent with the idea that normal resolution of the cointegrate structure in the recipient separates the marker from the conjugal plasmid.

Third, a constant 7.4-kb region of pEC22 could be trapped, inserted into different sites on another plasmid. Transposition of the region onto a small, well-characterized plasmid enabled us to map the ends of the transposon. The high transferability of insertion derivatives pRL591-DB4 and pRL591-W45 and of pRL602, an insertion-plus-deletion derivative of pEC22, can now be explained, since the deletion neither invades the region identified by Tn*10.9* insertions as being required for transposition nor removes either end of the transposon. The transpos-

Tn*5396*	100				
Tn*1000*	83	100			
Tn*3*	61	61	100		
Tn*21*	30	31	30	100	
Tn*917*	26	26	26	32	100
	Tn*5396* (191) (118)	Tn*1000* 290-478 925-1041	Tn*3* 253-443 899-1016	Tn*21* 233-428 875-988	Tn*917* 239-430 864-973

FIG. 5. Amino acid sequence identity between transposases of the Tn*3* family. The fraction of identical amino acid residues is shown as a percentage in pairwise comparisons between two regions within representative transposases of the Tn*3* family. Comparisons between transposons within the Tn*3* subgroup are shaded. The two regions correspond to 191 amino acids in the middle of the transposase and 118 amino acids at the C terminus. The corresponding regions in the amino acid sequences of particular transposases are shown in the bottom row. Aligned sequences were scored by counting each gap as a single mismatch. No comparison gave more than five mismatches. Sequences were obtained from GenBank under the accession numbers shown in the legend to Fig. 4 except for Tn*917* (M11180).

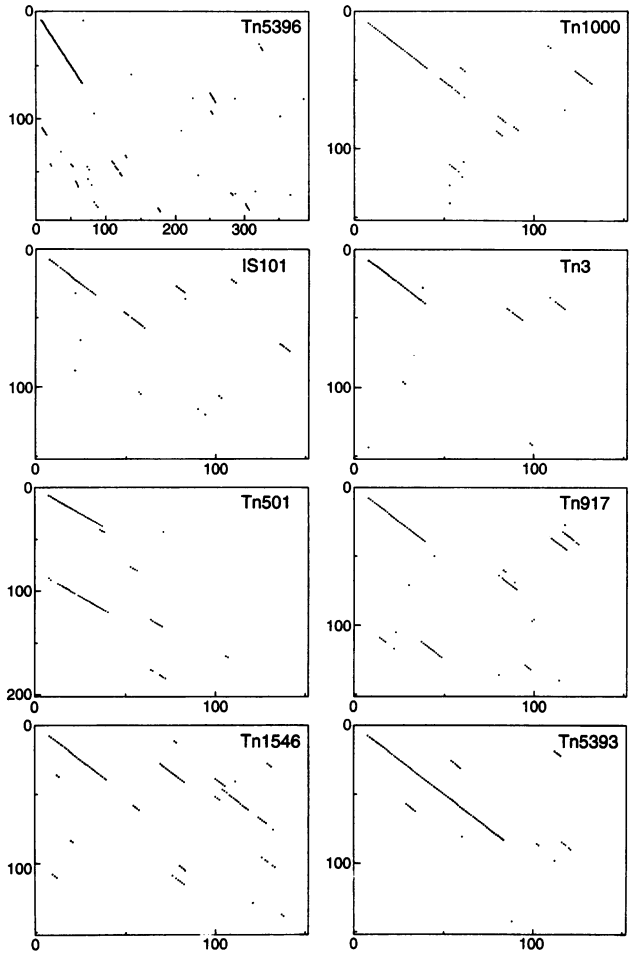

FIG. 6. Pairwise comparisons of termini of transposons within the Tn*3* family. Each panel shows a comparison of a transposase-proximal terminus (horizontal axis) and the other terminus (vertical axis) of a transposon, except in the case of IS*101*, where the horizontal axis is IS*101*$_r$ and the vertical axis is IS*101*$_l$. Each dot represents a positive result (at least 10 matches) from a comparison between a 15-base region on one terminus and a 15-base region on the other, each region centered around a reference base. Numbers on the axes indicate the distance (in bases) of reference bases from the terminus. Any single dot may be the result of random congruence of sequences. A diagonal series of dots indicates an extensive region of sequence similarity. For example, three of the minor diagonals in the Tn*5396* panel correspond to the features shown in Fig. 3: IR_{o2} (starting at 9 horizontal and 109 vertical), single-underlined sequences (starting at 109 horizontal and 140 vertical), and double-underlined sequences (starting at 250 horizontal and 76 vertical). Sequences were obtained from GenBank under the accession numbers shown in the legends to Fig. 4 and 5 except for Tn*501* (Z00027) and Tn*5393* (M96392).

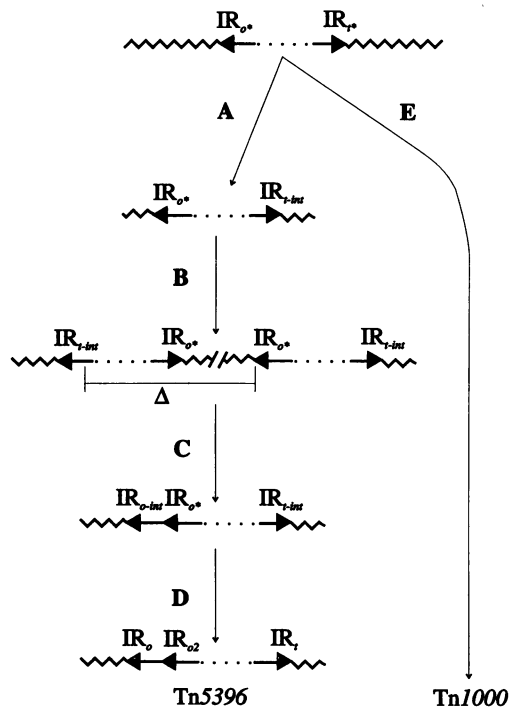

FIG. 7. Proposed evolutionary relationship between Tn*5396* and Tn*1000*. The two transposons are presumed to have arisen from a common ancestor that had IRs similar to IR_{o2} (Fig. 4), here labeled IR_{o*} and IR_{t*}. The shading of the arrowheads represents the degree of divergence of the IR sequence from that of IR_{0*} and IR_{t*}. The jagged lines represent sequences outside of the transposon. (A) Mutation of IR_{t*} to a sequence ($IR_{t\text{-}int}$) similar to IR_t of Tn*5396* makes the two IRs nonidentical. (B) An intramolecular transposition places a second copy of the transposon on the replicon in antiparallel orientation. (C) Deletion removes most of the second copy of the transposon, leaving a duplication of $IR_{t\text{-}int}$ plus adjacent sequences. One IR is renamed $IR_{o\text{-}int}$ to reflect the loss of the nearby gene encoding the transposase. (D) Without selection for function, IR_{o*} degenerates to its present form (IR_{o2} in Fig. 4). Mutations in $IR_{o\text{-}int}$ and $IR_{t\text{-}int}$ produce the current sequences of IR_o and IR_t of Tn*5396*. (E) Similar divergence of termini, duplication, and deletion leads to Tn*1000*.

able region thus defined, Tn*5396*, carries no known function apart from transposition itself. It does not confer resistance to ampicillin, chloramphenicol, kanamycin, streptomycin, or tetracycline (46) (data not shown).

The transfer of pEC22 mediated by Tn*5396* is a rare instance of conductance by means of a transposable element borne by a nonconjugal plasmid. pNTP16 is another plasmid that is conducted by its own transposon, but it also carries a *mob*/*oriT* system that mediates mobilization by conjugal plasmid R64 (28). Considering the limited coding capacity, outside of Tn*5396*, that is not accounted for by MR · *Eco*T22I and *oriV*, it is not likely that pEC22 possesses an intact *mob* region. While conductance cannot achieve the maximal rates of transfer achieved by *mob*/*oriT*, the latter depends for its effectiveness on the specific nature of the conjugal system (41). The lower rates of transmission afforded by transposition should normally be available regardless of the conjugal plasmid.

Analysis of the IR sequences of Tn*5396* (Fig. 4) and a partial amino acid sequence of its presumptive transposase (Fig. 5) clearly places the transposon within the Tn*3* subgroup of the Tn*3* family (3, 22, 36). Even though the transposon is quite similar to other members of its subgroup, Tn*5396* exhibits two obvious differences that set it apart. First, the transposon deviates in seven bases within the IR (Fig. 4, bases 19 through 23, 28, and 34) that are otherwise absolutely conserved within the subgroup. Site-specific mutation of two of these sites in both IRs of Tn*3* reduces transposition frequency by more than 97% (33). Transposases within the Tn*3* subgroup evidently have different substrate requirements, a fact already established by the inability of Tn*3* transposase to support transposition of Tn*1000* (36). The transposases of Tn*5396* and Tn*1000*, however, show considerably greater sequence similarity than those of Tn*3* and Tn*1000* (Fig. 5). It would be interesting to see

if the former two transposases also have different substrate requirements. Such a result would aid in defining the regions of the transposase that interact with the IRs.

The second distinguishing characteristic of Tn*5396* is its long IR sequence. We believe, however, that this difference is not as exceptional as it may at first appear. Although the majority of known transposons in the Tn*3* family have IRs of 38 bp, IRs of other lengths have been described (12, 14, 31, 38). Furthermore, the sequence comparisons presented in Fig. 6 indicate that many other members of the Tn*3* family may have cryptic extensions to the known regions of inverted similarity between their two arms.

How did these long IRs come about, and why is it that the IRs of a transposon, regardless of IR length, bear more similarity to each other than to the homologous IRs of different transposons? Functional constraints might account for the parallel evolution of termini within the 38 bp sufficient for transposition (25). Perhaps there are subtle effects on transposition, thus far undetected, determined by sequences beyond the minimal IR, but unless this is true, it is difficult to imagine a mechanism to explain the similarity between termini that does not appeal to sequence duplication at the termini, generated most plausibly by transposition.

Intermolecular transposition of one element into a second element readily explains the occurrence of secondary IRs in many transposons, such as Tn*501* (21), Tn*951* (31), and Tn*4653* (38). In these instances, the internal element is sufficiently different from the external element to avoid immunity (22), which acts to reduce the incidence of secondary transposition of a transposon to nearby targets.

Intramolecular transposition by heterologous transposons more readily explains the extensive secondary inverted repeats of Tn*917* (35) and Tn*1721* (1). In the former case, there is an internal 73-bp imperfect duplication of IR_o extended inwards by an additional 35 bp and in parallel orientation. In the latter case, there is a 1,750-bp duplication (beyond the region shown in Fig. 6) of IR_t and adjacent sequences, also in parallel orientation. The secondary repeats in both transposons can be accounted for by intramolecular transposition, not affected by immunity (7, 39), followed by aberrant resolution of the duplication. An extension of this process, as outlined below, can lead to sequence identity between IR_t and IR_o.

Figure 7 illustrates a series of events that can account for the structures observed in Tn*5396* and other Tn*3*-like transposons. Other authors have proposed similar ideas to explain how a new gene is recruited into a transposon (1). An intramolecular transposition event places a second copy of the transposon antiparallel to the first (39), yielding a composite transposon whose outer IRs are initially identical and derived from IR_t of the original transposon. Deletion events progressively reduce the size of the transposon, and superfluous termini are eventually lost by deletion or mutation. In this view, long IRs would be interpreted as an intermediate stage in the process of transposon formation and indicative of a relatively recent origin, while older transposons have IRs reduced to the minimum required for function. Likewise, alternative (1) or cryptic homologous IRs (Fig. 3 and 6) may represent the molecular remains of primordial termini superseded by the termini resulting from intramolecular transposition events or of the termini inserted from those events. Some cryptic IRs, especially those with nearly intact inside-facing ends, although incapable of supporting transposition, may still be functional in increasing (27) or broadening the level of immunity against further transposition.

IR_{o2} (Fig. 3 and 4) would appear to be a relic of an IR closer in time than IR_o to the ancestor that it has in common with the IRs of Tn*1000*. This degenerate IR exhibits the GAACG sequence (coordinates 19 to 23) common to all members of the Tn*3* subgroup except Tn*5396* but also exhibits the ACCA sequence (coordinates 28 to 31) peculiar to Tn*5396*. Figure 7 shows one evolutionary route that can account for the present IR structure of Tn*5396* and Tn*1000*. Whether or not Tn*5396* actually followed the described history, the figure illustrates the kinds of events—intramolecular transposition in inverted orientation followed by deletion—that can explain how the termini of transposons remain substantially identical despite integral changes within the transposon. A mechanism of this type would have to operate much more frequently than the acquisition of new functions (22, 23) in order to account for the observation that the termini of transposons in general are more similar to each other than they are to the termini of related transposons.

ACKNOWLEDGMENTS

We thank Katsutoshi Mise for pEC22, Elisabeth Raleigh for ER1372, Niels Nielson for software to manipulate and analyze DNA, and an anonymous reviewer for a thorough reading of the manuscript.

This work was supported by the Center for Microbial Ecology under N.S.F. grant BIR 9120006 and by the U.S. Department of Energy under grant DE-FG02-90ER20021.

REFERENCES

1. **Allmeier, H., B. Cresnar, M. Greck, and R. Schmitt.** 1992. Complete nucleotide sequence of Tn*1721*: gene organization and a novel gene product with features of a chemotaxis protein. Gene **111**:1–20.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
3. **Amemura-Maekawa, J., and E. Ohtsubo.** 1991. Functional analysis of the two domains in the terminal inverted repeat sequence required for transposition of Tn*3*. Gene **103**:11–16.
4. **Arthur, M., C. Molinas, F. Depardieu, and P. Courvalin.** 1993. Characterization of Tn*1546*, a Tn*3*-related transposon conferring glycopeptide resistance by synthesis of dipsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J. Bacteriol. **175**:117–127.
5. **Bagdasarian, M., R. Lurz, B. Rückert, F. C. H. Franklin, M. M. Bagdasarian, J. Frey, and K. N. Timmis.** 1981. Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in *Pseudomonas*. Gene **16**:237–247.
6. **Balbás, P., X. Soberón, E. Merino, M. Zurita, H. Lomeli, F. Valle, N. Flores, and F. Bolivar.** 1987. Plasmid vector pBR322 and its special-purpose derivatives—a review. Gene **50**:3–40.
7. **Bishop, R., and D. J. Sherratt.** 1984. Transposon Tn*1* intramolecular transposition. Mol. Gen. Genet. **196**:117–122.
8. **Bolivar, F., R. Rodriguez, P. J. Greene, M. Betlach, H. L. Heyneker, H. W. Boyer, J. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. Gene **2**:95–113.
9. **Borck, K., J. D. Beggs, W. J. Brammar, A. S. Hopkins, and N. E. Murray.** 1976. The construction in vitro of transducing derivatives of phage lambda. Mol. Gen. Genet. **146**:199–207.
10. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
11. **Brent, R., and M. Ptashne.** 1981. Mechanism of action of the *lexA* gene product. Proc. Natl. Acad. Sci. USA **78**:4204–4208.
12. **Chiou, C.-S., and A. L. Jones.** 1993. Nucleotide sequence analysis of a transposon (Tn*5393*) carrying streptomycin resistance genes in *Erwinia amylovora* and other gram-negative bacteria. J. Bacteriol. **175**:732–740.
13. **Clark, A. J., and G. J. Warren.** 1979. Conjugal transmission of plasmids. Annu. Rev. Genet. **13**:99–125.
14. **Cornelis, G., H. Sommer, and H. Saedler.** 1981. Transposon Tn*951* (TnLac) is defective and related to Tn*3*. Mol. Gen. Genet. **184**:241–248.

15. **Deragon, J.-M., P. Corriveau, and G. Gingras.** 1990. Plasmid from photosynthetic bacterium *Ectothiorhodospira* sp. carries a transposable streptomycin resistance gene. Plasmid **23:**226–236.
16. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
17. **Elhai, J., and C. P. Wolk.** 1988. Conjugal transfer of DNA to cyanobacteria. Methods Enzymol. **167:**747–754.
18. **Elhai, J., and C. P. Wolk.** 1988. A versatile class of positive-selection vectors based on the nonviability of palindrome-containing plasmids that allows cloning into long polylinkers. Gene **68:**119–138.
19. **Elhai, J., and C. P. Wolk.** Unpublished data.
20. **Gill, R., F. Heffron, G. Dougan, and S. Falkow.** 1978. Analysis of sequences transposed by complementation of two classes of transposition-deficient mutants of Tn*3*. J. Bacteriol. **136:**742–756.
21. **Grinsted, J., and N. L. Brown.** 1984. A Tn*21* terminal sequence within Tn*501*: complementation of *tnpA* gene function and transposon evolution. Mol. Gen. Genet. **197:**497–502.
22. **Grinsted, J., F. De La Cruz, and R. Schmitt.** 1990. The Tn*21* subgroup of bacterial transposable elements. Plasmid **24:**163–189.
23. **Hall, R. M., D. E. Brookes, and H. W. Stokes.** 1991. Site-specific insertion of genes into integrons: role of the 59-base element and determination of the recombination cross-over point. Mol. Microbiol. **5:**1941–1959.
24. **Hirschel, B. J., D. J. Galas, and M. Chandler.** 1982. Cointegrate formation by Tn*5*, but not transposition, is dependent on *recA*. Proc. Natl. Acad. Sci. USA **79:**4530–4534.
25. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114:**193–197.
26. **Huang, C.-J., F. Heffron, J.-S. Twu, R. H. Schloemer, and C.-H. Lee.** 1986. Analysis of Tn*3* sequences required for transposition and immunity. Gene **41:**23–31.
27. **Kans, J. A., and M. J. Casadaban.** 1989. Nucleotide sequences required for Tn*3* transposition immunity. J. Bacteriol. **171:**1904–1914.
28. **Lambert, C. M., H. Hyde, and P. Strike.** 1987. Conjugal mobility of the multicopy plasmids NTP1 and NTP16. Plasmid **18:**99–110.
29. **Maloy, S., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145:**1110–1112.
30. **Marsh, J. L., M. Erfle, and E. J. Wykes.** 1984. The pIC plasmid and phage vectors with versatile cloning sites for recombination selection by insertional inactivation. Gene **32:**481–485.
31. **Michiels, T., and G. Cornelis.** 1984. Detection and characterization of Tn*2501*, a transposon included within the lactose transposon Tn*951*. J. Bacteriol. **158:**866–871.
32. **Misra, T. K., N. L. Brown, D. C. Fritzinger, R. D. Pridmore, W. M. Barnes, L. Haberstroh, and S. Silver.** 1984. Mercuric ion-resistance operons of plasmid R100 and transposon Tn*501*: the beginning of the operon including the regulatory region and the first two structural genes. Proc. Natl. Acad. Sci. USA **81:**5975–5979.
33. **Nissley, D. V., F. G. Lindh, and M. A. Fennewald.** 1990. Mutational analysis of the inverted repeats of Tn*3*. J. Mol. Biol. **213:**671–676.
34. **Sanger, F. S., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
35. **Shaw, J. H., and D. B. Clewell.** 1985. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B resistance transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **164:**782–796.
36. **Sherratt, D.** 1989. Tn*3* and related transposable elements: site-specific recombination and transposition, p. 163–184. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
37. **Siemieniak, D. R., J. L. Slightom, and S.-T. Chung.** 1990. Nucleotide sequence of *Streptomyces fradiae* transposable element Tn*4556*: a class-II transposon related to Tn*3*. Gene **86:**1–9.
38. **Tsuda, M., K.-I. Minegishi, and T. Iino.** 1989. Toluene transposons Tn*4651* and Tn*4653* are class II transposons. J. Bacteriol. **171:**1386–1393.
39. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
40. **Wang, G., X. Xu, J.-M. Chen, D. E. Berg, and C. M. Berg.** 1994. Inversions and deletions generated by a mini-γδ (Tn*1000*) transposon. J. Bacteriol. **176:**1332–1338.
41. **Warren, G. J., A. J. Twigg, and D. J. Sherratt.** 1978. ColE1 plasmid mobility and relaxation complex. Nature (London) **174:**259–261.
42. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32:**369–379.
43. **Willetts, N., and R. Skurray.** 1980. The conjugation system of F-like plasmids. Annu. Rev. Genet. **14:**41–76.
44. **Wrighton, C. J., and P. Strike.** 1987. A pathway for the evolution of the plasmid NTP16 involving the novel kanamycin resistance transposon Tn*4352*. Plasmid **17:**37–45.
45. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
46. **Yoshida, Y., and K. Mise.** 1986. Occurrence of small Hsd plasmids in *Salmonella typhi*, *Shigella boydii*, and *Escherichia coli*. J. Bacteriol. **165:**357–362.