

NOTES

Tn1403, a Multiple-Antibiotic Resistance Transposon Made Up of Three Distinct Transposons[∇]

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Transposon Tn1403 from a clinical *Pseudomonas* strain is composed of three transposons, including Tn5393c. A related transposon Tn1404* from a plant-associated *Pseudomonas* strain lacks Tn5393 but includes a transposon carrying the *tet*(C) tetracycline resistance determinant. These compound transposons illustrate the role of preexisting transposons in generating clusters of antibiotic resistance genes.

Many clinical and environmental isolates of gram-negative bacteria that are resistant to a number of different antibiotics carry several antibiotic resistance genes clustered in large multiple-antibiotic resistance regions. The role of class 1 integrons and associated gene cassettes in forming such clusters is now well understood (3), but the role of other transposons and diversity-generating elements has received less attention. Recently, we determined the structure of a multiple-antibiotic resistance region that has six resistance genes, of which only one is in a gene cassette and one (*sulI*) is in the 3'-conserved segment (3'-CS) of the class 1 integron. Three further resistance genes are within or derived from known transposons, and one is associated with the novel insertion element CR1 (8, 9). Other examples of complex arrangements can be found in the recently reported sequences of several large plasmids or genomic islands. Where one transposon lies within another, they can move together as long as the transposition functions of the backbone transposon are not disrupted, and this piggy-backing route is used by class 1 integrons, most of which are defective transposons that need transposition proteins to be supplied in *trans*.

Tn1403 was recovered from a multiply antibiotic resistant clinical strain of *Pseudomonas aeruginosa* isolated in the United States in 1973-1974 (5, 13), and it confers resistance to carbenicillin, chloramphenicol, streptomycin, and spectinomycin (5). Genes conferring resistance to these antibiotics are found in an array of three gene cassettes, *blaP1-cmlA1-aadA1*, located within an In4-type class 1 integron, In28, located within Tn1403 (10). In28 has lost the *sulI* gene normally found in the 3'-CS, explaining the susceptibility to sulfonamides. However, the region to the right of In28 has been reported to contain a further *aphC*-type gene conferring resistance to streptomycin (13). Here, we have sequenced the remainder of Tn1403 and

identified within it a further transposon, Tn5393c, which carries the *strA-strB* gene. The structure of Tn1403 was also compared to those of other class II transposons.

Structure of Tn1403. Tn1403, which had been transposed into the IncW plasmid R388 (5), was obtained from G. Jacoby. The region to the right of In28 was sequenced using cloned EcoRI and HindIII fragments (10) as substrates. The region to the left of In28, which contains no EcoRI or HindIII sites, and the region to the right of the EcoRI site in the right-hand 38-bp inverted repeat (IR) were sequenced directly using R388::Tn1403 as the substrate or using PCR-amplified fragments purified using a QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's protocols. The sequence was determined on both strands. Automated sequencing, assembly, and analysis of sequences were carried out as described previously (9).

Tn1403 was found to be 19.63 kb long and is bounded by 38-bp IR and flanked by a 4-bp duplication of the target sequence within the *trwC* gene of R388. It is made up of three distinct entities (Fig. 1A), the transposon backbone of 7,884 bp, In28 (6.27 kb), which was sequenced previously (10), and a 5,470-bp version of Tn5393, known as Tn5393c, which lacks the IS found in Tn5393. Tn5393c is located in the right arm of the backbone (Fig. 1A). Tn5393 is a 6,705-bp transposon made up of a transposition module and a module carrying the *strA* and *strB* genes with an insertion sequence, IS1133, located between the modules (1). The Tn5393 relative in Tn1403 lacks the IS1133 element but is otherwise over 99.5% identical to the original sequence (GenBank accession no. M95402) and to Tn5393c (GenBank accession no. AF262622) (4).

The Tn1403 backbone transposon. The 3.74-kb region to the left of In28 was found to contain IR_{tmp}, the *tnpA* and *tnpR* genes, encoding the TnpA transposase and TnpR resolvase, and most of the *res* site. Because In28 is located within *resI* (10, 13), part of the *resI* subsite lies to the right of In28. The closest relatives of this *tnp* module (94% or 93% identical over the full length) are found in Tn1013 (GenBank accession no. AM261760), which is related to the Tn1403 backbone along its full length (Fig. 1C), and Tn4656, a catabolic transposon en-

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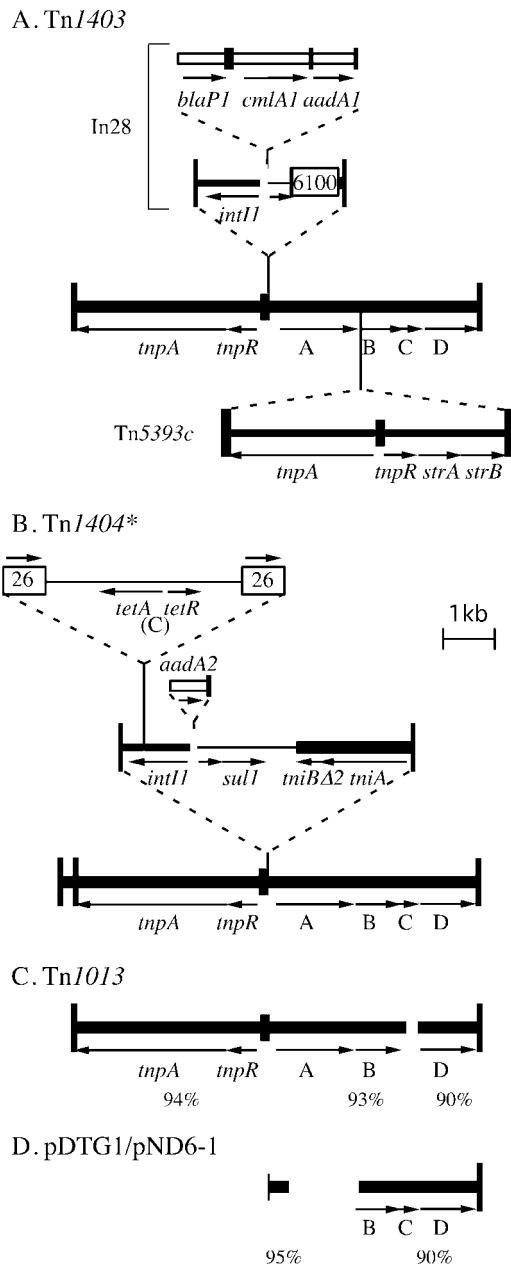


FIG. 1. Structure of *Tn1403* and related transposons. A. The structure of *Tn1403* is derived from the previously determined sequence of In28 (10) and the sequence determined here. B. The structure of *Tn1404** was deduced by joining the partial sequences available in GenBank accession numbers AF157797 to AF157801 using available sequences as follows. AF157801 was linked to AF157800 using the sequence of the transposition module of *Tn1403* reported here. AF157800 was linked to AF157799 using the sequence of the pSC101 *tet(C)* region in X01654, and the gap in the 5'-CS between AF157799 and AF157798 was filled from AF313472. The end of the *aadA2* gene cassette was from U12338, and the 3'-CS and *tni* region were from AJ223604, which has an In0-like segment that does not include IS1326. C. *Tn1013*, from GenBank accession no. AM261760, is between 90 and 94% identical to *Tn1403* with a deletion of 78 bp in *orfC*. D. Other regions related to the *Tn1403* backbone. pDTG1 and pND6-1, which are identical to one another in this region, are from GenBank accession numbers AY208917 and AY491307. High identity with *Tn1403* extends from between *resI* and *resII* (bp 179 in Fig. 2) to the outer end of *Tn1403*, indicating that they may be derived from a transposon related to *Tn1403*. Tall vertical bars represent the IR, and medium

TABLE 1. Relationships between transposon transposition modules^a

Transposon	% Identity with:						
	<i>Tn1013</i>	<i>Tn4656</i>	<i>Tn4653</i>	<i>Tn501</i>	<i>Tn1721</i>	<i>Tn21</i>	<i>Tn1696</i>
<i>Tn1403</i>	94.6	93.2	91.9	90.6	90.5	73.3	73.2
<i>Tn1013</i>		92.7	91.9	91.3	91.3	73.2	72.8
<i>Tn4656</i>			92.1	90.8	90.5	73.1	72.9
<i>Tn4653</i>				94.1	93.3	73.6	73.0
<i>Tn501</i>					95.6	74.2	73.9
<i>Tn1721</i>						73.6	73.2
<i>Tn21</i>							81.5

^a The numbers represent the percent nucleotide identities between the transposition modules. For the GenBank accession numbers, see the legend to Fig. 2.

coding xylene and toluene degradation determinants (GenBank accession no. AB062597) (12). Other close relatives are listed in Table 1. However, the sequence of short regions (GenBank accession no. AF157801 and AF157800) from either end of the transposition module of a transposon called *Tn1404* (11), but here named *Tn1404** to avoid confusion with the previously known *Tn1404* (7), are identical to the *Tn1403* sequence, indicating that *Tn1404** contains the same transposition module. Potentially characteristic differences defining two evolutionary groups are found in the short region between the initiation codon of the *tnpR* gene and the *res* site (Fig. 2). *Tn1403*, *Tn4656*, and *Tn1013* have related sequences, and *Tn501*, *Tn4653*, and *Tn1721* have a sequence that is significantly diverged from the *Tn1403* segment. The equivalent regions from the more distantly related class II transposons *Tn21* and *Tn1696* are shorter.

The 9,628-bp region to the right of In28 in *Tn1403*, with the *Tn5393* and one copy of the flanking duplication removed, differed at only four positions from the sequence of the right arm of *Tn1404** (GenBank accession number AF157797). The products of *orfA* and *orfC* were previously identified as homologues of a sulfate permease and a DksA-like DnaK suppressor protein, respectively (11). The OrfB protein is related to a series of proteins annotated as universal stress proteins (UspA). Close relatives of this DNA sequence were found in *Tn1013* and in plasmids pND6-1 and pDTG1 (GenBank accession numbers AY208917 and AF491307) (2, 6), which are identical to one another in this region. However, in pDTG1 and pND6-1 the sulfate permease gene (*orfA*) is missing (Fig. 1D).

Relationship to *Tn1404.** The closest relative of *Tn1403* is the partially sequenced transposon *Tn1404** isolated from a tetracycline-resistant *Pseudomonas* strain from a Michigan apple orchard (11). The structure of *Tn1404** (Fig. 1B) was deduced from the available sequence mapping (11) using sequence determined here and standard sequences to fill the gaps. *Tn1404** contains a class 1 integron in the same location

vertical bars represent the *res* sites of transposons. Open vertical bars represent the *attI* site of class 1 integrons, and cassettes are shown as an open box with a short vertical bar (representing the 59-bp) at one end. ISs are shown as open boxes with the IS number within. Genes are indicated by arrows with gene names below or A, B, C, and D indicating *orfA* to *orfD*.



FIG. 2. Comparison of the region between *tnpR* and *res* in class II transposons. Sequences are from this study for Tn1403 and from GenBank accession no. AB062597 for Tn4656, AM261760 for Tn1013, X61367 for Tn1721, Z00027 for Tn501, AJ344068 for Tn4653, AF071413 for Tn21, U12338 for Tn1696, and X90708 for Tn4378. The start codons and potential alternate start codons for the *tnpR* gene are underlined, and the crossover position within *resI* is in bold. Positions of bases duplicated by insertion of a class 1 integron or Tn5053 are boxed.

as In28 but it has an In5-type backbone lacking IS1326, in contrast to the In4-type backbone of In28. The cassette arrays also differ. Tn1404* lacks Tn5393c but contains a transposon consisting of the *tet(C)* tetracycline resistance determinant flanked by two copies of IS26 (11). This transposon lies within the *intI1* gene of the integron. These two compound transposons illustrate the role of preexisting transposons in generating different clusters of antibiotic resistance genes within the same backbone, adding to the diversity generated by variability in integron-associated gene cassettes.

Nucleotide sequence accession number. The 12.9 kb of sequence reported in this paper has been added to GenBank accession no. AF313472 to give AF313472.2.

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