Embedded elements in the IncP\(\beta\) plasmids R772 and R906 can be mobilized and can serve as a source of diverse and novel elements

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IncP plasmids are important contributors to bacterial adaptation. Their phenotypic diversity is due largely to accessory regions located in one or two specific parts of the plasmid. The accessory regions are themselves diverse, as judged from sequenced plasmids mostly isolated from non-clinical sources. To further understand the diversity, evolutionary history and functional attributes of the accessory regions, we compared R906 and R772, focusing on the oriV–trfA accessory region. These IncP\(\beta\) plasmids were from porcine and clinical sources, respectively. We found that the accessory regions formed potentially mobile elements, Tn510 (from R906) and Tn511 (from R772), that differed internally but had identical borders. Both elements appeared to have evolved from a TnAO22-like mer transposon that had inserted into an ancestral IncP\(\beta\) plasmid and then accrued additional transposable elements and genes from various proteobacteria. Structural comparisons suggested that Tn510 (and a descendent in pB10), Tn511 and the mer element in pJP4 represent three lineages that evolved from the same widely dispersed IncP\(\beta\) carrier.

Functional studies on Tn511 revealed that its mer module is inactive due to a merT mutation, and that its aphAI region is prone to deletion. More significantly, we showed that by providing a suitable transposase gene in trans, the defective Tn510 and Tn511 could transpose intact or in part, and could also generate new elements (stable cointegrates and novel transposons). The ingredients for assisted transposition events similar to those observed here occur in natural microcosms, providing non-self-mobile elements with avenues for dispersal to new replicons and for structural diversification. This work provides an experimental demonstration of how the complex embedded elements uncovered in IncP plasmids and in other plasmid families may have been generated.

**INTRODUCTION**

Plasmids of the broad-host-range incompatibility group P (also denoted IncP-1) were first detected in *Pseudomonas aeruginosa* and enteric bacteria from clinical sources (Thomas & Smith, 1987). They are considered to be potent mediators of horizontal gene transfer, since they promote both inter- and intra-species conjugation and can replicate in taxonomically disparate proteobacteria (Adamczyk & Jagura-Burdzy, 2003; Krishnapillai, 1988; Thomas, 2000). Until the last decade, significant sequence data were available only for plasmids RP1/RK2 (the so-called ‘Birmingham’ plasmids) (Pansegrau et al., 1994) and R751 (Thorsted et al., 1998), which, respectively, serve as the prototype plasmids of the IncP\(\alpha\) and IncP\(\beta\) subgroups. Currently, 10 or more IncP\(\beta\) plasmids have been fully sequenced as have several representatives of four other IncP subgroups (Bahl et al., 2009; Schlüter et al., 2007; Sen et al., 2010). Each subgroup contains phylogenetically distinguishable plasmid lineages (Bahl et al., 2009; Kamachi et al., 2006). The newer collection of IncP plasmids, in contrast to the early one (Smith & Thomas, 1989), has been drawn mainly from non-clinical sources (e.g. water, activated sludge and soil) and from diverse hosts, some unknown (Bahl et al., 2009; Schlüter et al., 2007). The ubiquitous distribution of the IncP plasmids underscores their importance in horizontal gene transfer and has led to efforts to define and better understand plasmid population dynamics and factors that affect them (De Gelder et al., 2005, 2007).

Almost all of the IncP plasmids studied encode traits such as resistance to various antibiotics and antimicrobials, and degradation of complex compounds and xenobiotics, many of which are clinically and environmentally relevant (Bahl et al., 2009; Schlüter et al., 2007). In some instances, the

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Abbreviations: DR, direct repeat; IR, inverted repeat; TE, transposable element.

The GenBank/EMBL/DDBJ accession numbers for the Tn510 and Tn511 sequences of R906 and R772 are DQ471307 and EU287476, respectively.
traits are encoded by class I or class II transposons that are demonstrably self-mobile (Schlüter et al., 2007; Villarroel et al., 1983). Such modular elements represent acquired accessory genes that have persisted on the plasmid because of a selective advantage to the bacterial host. Knowledge of the plasmid DNA sequences has been crucial in revealing the true nature and complex organization of the accessory genes, which include combinations of functional modular elements as well as defective and degraded structures and incidental (orphan) genes. In some instances, the entire accessory unit is phenotypically cryptic (Schlüter et al., 2007).

The accessory genes are typically confined to two regions of the plasmid (Trefault et al., 2004; Villarroel et al., 1983), thereby interrupting a conserved plasmid ‘backbone’ that was postulated to be contiguous in the IncP plasmid progenitor (Smith & Thomas, 1987, 1989; Smith et al., 1993) and is recognizable as such in some extant members [e.g. pBP136 (Kamachi et al., 2006) and pJP4 (Trefault et al., 2004)]. The two regions are situated between the replicative functions, oriV and trfA, and the conjugation functions, tra (Tra 1) and trb (Tra 2). The accessory genes that occur in the two regions are not necessarily located at the same site in the backbone, so that plasmids can be distinguished phylogenetically based on the position and type of insert that is present (Kamachi et al., 2006; Sota et al., 2007). Of relevance to this study are the IncPβ plasmids pB10 (Schlüter et al., 2003), pJP4 (Trefault et al., 2004), R772 (Coetzee, 1978) and R906 (Terakado & Mitsuhashi, 1974). The first two of these have been fully sequenced, and molecular maps, supported by limited sequence data, are available for R772 (~61 kb) and R906 (~58 kb) (Smith & Thomas, 1987, 1989; Smith et al., 1993).

Thorsted et al. (1998) suggested that accessory genes may target the oriV–trfA and tra–trb regions because each region contains an array of conserved 20 bp inverted repeats (IRs) proposed to facilitate insertion of DNA segments. Although attractive, no experimental evidence to support this view was obtained in a study on the targeting of Tn21Km to the oriV–trfA region of pBP136 (Sota et al., 2007). Instead, plasmid fitness of the insertion derivatives appeared to be a predominant factor. This finding accorded with the preponderance of Tn21-like elements found naturally in the oriV–trfA region of IncP plasmids generally (Schlüter et al., 2007) and with the observed conservation of the plasmid backbone. A different contributing factor accounts for the predominant insertion of Tn5053/Tn402-like elements in the tra–trb region (Schlüter et al., 2007). This interaction stems from the transpositional dependence of these elements on a target-encoded resolvase (ParA) that assists oriented and directed insertion into the cognate res (resolution) sequence located in the tra–trb region (Kamali-Moghaddam & Sundström, 2000; Minakhina et al., 1999; Petrovskij & Stanisich, 2010).

The main focus of this study is R772 (IncPβ), obtained from a clinical Proteus mirabilis strain isolated in the USA in 1974 (Coetzee, 1978). The molecular map of R772 (Hille et al., 1983; Smith & Thomas, 1987) indicates that its accessory genes occur in the oriV–trfA and tra–trb regions and are distinctive. Those in the oriV–trfA region are responsible for the sole (kanamycin)-resistance phenotype of R772 (Hille et al., 1983), and also include a suspected cryptic Tn501-like mercury(II)(mer)-resistance determinant (Smith & Thomas, 1987). The position and sequence of the mer-associated IR of the cryptic element have been determined (Smith et al., 1993) and are identical to those of an intact Tn501-like element in pB10 and pJP4, suggesting that all three plasmids are derived from the same ancestral plasmid (Schlüter et al., 2003). R906 is apparently also a member of this group that is similar to pB10, based on comparative restriction-enzyme profiling (Smith & Thomas, 1987; Schlüter et al., 2003). Its accessory genes also occur in the oriV–trfA and tra–trb regions and are responsible for the multiresistance phenotype of R906. This plasmid was isolated in Japan during 1969–1972 from a porcine Bordetella bronchiseptica strain (Hedges et al., 1974; Terakado & Mitsuhashi, 1974).

Here, we report the sequence and functional attributes of the accessory genes in the oriV–trfA regions of R772 and R906. Our aim was to determine the diversity and evolutionary history of the genes and their propensity for lateral transfer. Our findings support the view (Schlüter et al., 2003, 2007) that the oriV–trfA accessory region is a mosaic of transposable elements (TEs), and that the regions in R772, R906, pJP4 and pB10 are phylogenetically related. We also observed, as have others (Schlüter et al., 2007), that the earlier accrued elements may lack mobility functions due to deletion mutations. Few studies have systematically assessed the ability of the in situ accessory unit, and its parts, to relocate. Our findings demonstrate that relocation is possible and that the R772 unit is especially dynamic. Since accessory genes occur in other families of plasmids and are components of bacterial genomes (e.g. Kung et al., 2010), our observations have broader applicability.

**METHODS**

**Strains, plasmids and media.** The auxotrophic Escherichia coli K-12 derivatives used were DH5α (recA1 Res− Mod+ NalR) (Hanahan, 1983), LT101 (recA13 Res− Mod− RifR) (Palombo et al., 1989), UB281 (Rec− NalR) (Hedges et al., 1973) and its recombinant-deficient derivative UB5201 (Sanchez et al., 1982). The plasmids used are listed in Table 1. R772-1 has a mutation in inpa of Tn5403 (inpaAso) that was created by BamHI cleavage of the purified plasmid, treatment with T4 DNA polymerase and religation. The introduced frameshift mutation is at nucleotide 2570 of inpa of Tn5403 (inpaAso). Additional plasmids that were generated in vivo are described in the text and in Table 4 (i.e. pBR322 with inserts of Tn510, Tn511, Tn511-2, Tn511-3 or Tn5403). Bacterial strains were grown at 37 °C in nutrient agar (NA) and nutrient broth (NB), as described previously (Palombo et al., 1989). NA was supplemented with the required antimicrobial agents at the following concentrations (µg ml−1): oxacillin (Oxa) 200; chloramphenicol (Cm), gentamicin sulphate (Gm), kanamycin sulphate (Km), mercuric chloride (Hg) and tetracycline hydrochloride (Tc) 10; ampicillin (Ap) and rifampicin (Rif) 100; nalidixic acid (Nal) 8; streptomycin sulphate (Sm) 5.
Conjugation procedures, transposition assay and analysis of transconjugants. Exponential-phase cultures in NB were employed in conjugation experiments performed by the quantitative filter method (Palombo et al., 1989). The donor strains (derivatives of DH5α) contained three plasmids that were sequentially introduced by transformation (for non-conjugative plasmids) or conjugation (for self-transferable plasmids), as described previously (Petrovski & Stanisich, 2010). LT101 (Rif<sup>R</sup>) served as the recipient. The transposition assay involved the formation of cointegrates between the conjugative plasmid [i.e. R772, R772-1 (both Km<sup>R</sup>) or R906 (Hg<sup>R</sup>)] and the target plasmid, pBR322 (Tc<sup>R</sup> Ap<sup>R</sup>), in the presence of a third plasmid (Cm<sup>R</sup>) carrying a functional or non-functional transposase (tpnA<sub>21</sub>) gene. Since pBR322 is non-conjugative and non-mobilizable (it lacks mob genes), the transconjugants that are recovered on Tc-selective agar medium have inherited transpositional cointegrates. Consequently, they display the resistance profile of the two component plasmids except in instances where transposition involved the pBR322 amp gene. Resolution of cointegrates in the recipient was detected by recovering the pBR322 component (i.e. pBR322::Tn or pBR322<sup>amp·</sup>::Tn). This was done by extracting DNA from Tc<sup>R</sup> Ap<sup>R</sup> or Tc<sup>R</sup> Ap<sup>R</sup> transconjugants and transforming it into <i>E. coli</i> DH5α via Tc<sup>R</sup> selection. The resistance profile of the transformants indicated that all had inherited the resolved pBR322 component and not an unresolved cointegrate. In the case of pBR322<sup>amp·</sup>::Tn plasmids, the insertion site and orientation of the transposon within <i>amp</i> were determined in sequencing reactions using primers SP1 (5′-CATTCGCTTTATCCGAG-CAACTCCAT-3′), SP2 (5′-GGAACCGCTATTCGACTGCAG-3′) and SP3 (5′-TGATTCCGATCATGAGAC-3′). The term ‘α-orientation’ refers to transposition insertions in which the maps of pBR322 and the transposon are similarly oriented; ‘β-orientation’ refers to insertion in the opposite orientable. The stable cointegrate pVS1650 was formed during transposition of Tn510 from R906 to pBR322 (Table 4) and contains one complete copy of Tn510 and also a Tn510 remnant. The structure of pVS1650 was determined from the Sau3AI restriction profile and from analysis of the DNA sequences at each Tn510 junction in pVS1670 (Fig. 3). The sequence data were obtained using primers SP1 and SP3, and showed that Tn510 was inserted at nucleotide 3319 of the pBR322 amp gene and was flanked by 5 bp direct repeats (DRs) (AGATA). It also showed that one copy of Tn510 in pVS1650 had sustained an extensive deletion (from nucleotide 194 in ‘tpnA<sub>21</sub>’ to IRm1; Figs 1 and 3), thereby abolishing the TnpR<sup>res</sup> resolution system.

DNA techniques and sequence determination. DNA manipulations and cloning were performed using standard methods (Sambrok et al., 1989). The DNA sequences of Tn510 and Tn511 were determined using suitable overlapping fragments that were cloned directly from R906 and R772 into pBluescript KS<sup>+</sup>. The fragments were sequenced at the Agenomics Sequencing Facility (Plant Biotechnology, La Trobe University, Australia) on an Applied Biosystems ABI373 DNA analyser. The DNA extension products were generated by cycle sequencing reactions employing AmpliTaq DNA polymerase and pUC/M13 forward and reverse primers (Sambrok et al., 1989). The sequence data were compiled and analysed using the suites of programs provided by National Center for Biotechnology Information (NCBI; National Library of Medicine, Bethesda, MD USA) and Expasy (Swiss Institute of Bioinformatics, Geneva). PCR amplification of DNA (Petrovski & Stanisich, 2010) employed primers SP9 (5′-GAATTCCTTCAGAG-CAACTCCAT-3′) and SP10 (5′-GGATCCCGATCATGAGAC-3′) to amplify <i>soeD</i><sub>772</sub> and SP32 (5′-GGATCCGATCATGAGAC-3′) and SP33 (5′-GGATCCGATCATGAGAC-3′) to detect Tn511-A (Km<sup>R</sup>) mutants of R772.

RNA extraction and RT-PCR. Exponential-phase cultures were grown in 10 ml NB to OD<sub>600</sub> = 0.3, mixed with 500 μl phenol:chloroform (5:95) and the cells were pelleted and resuspended in 500 μl ice-cold buffer [10 mM KCl, 5 mM MgCl<sub>2</sub>, 10 mM Tris/HCl (pH 7.5)]. An equal volume of hot phenol {one part phenol and five parts buffer (5:95)} was added and the solution was incubated at 95°C for 1 min to lyse the cells. After centrifugation, nucleic acids in the supernatant were extracted with 300 μl phenol:chloroform (1:1)

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### Table 1. Plasmids used in this study

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Relevant characteristics</th>
<th>Reference or source</th>
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<tbody>
<tr>
<td>R772</td>
<td>IncP&lt;sup&gt;β&lt;/sup&gt; conjugative plasmid; Tn511&lt;sup&gt;+&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Coetzee (1978)</td>
</tr>
<tr>
<td>R772-1</td>
<td>R772 with ttpA&lt;sub&gt;5403&lt;/sub&gt; mutation in Tn511</td>
<td>This study</td>
</tr>
<tr>
<td>R906</td>
<td>IncP&lt;sup&gt;β&lt;/sup&gt; conjugative plasmid; Tn510&lt;sup&gt;+&lt;/sup&gt; Hg&lt;sup&gt;R&lt;/sup&gt; Oxa&lt;sup&gt;R&lt;/sup&gt; Sm&lt;sup&gt;R&lt;/sup&gt; Su&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Hedges et al. (1974)</td>
</tr>
<tr>
<td>pBR1MCS-5</td>
<td>Broad-host-range vector; Gm&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Kovach et al. (1995)</td>
</tr>
<tr>
<td>pBR322</td>
<td>&lt;i&gt;E. coli&lt;/i&gt; vector; Ap&lt;sup&gt;R&lt;/sup&gt; Tc&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Bolivar et al. (1977)</td>
</tr>
<tr>
<td>pBluescript KS+</td>
<td>&lt;i&gt;E. coli&lt;/i&gt; vector; Ap&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pUB307</td>
<td>Inc&lt;sup&gt;P&lt;/sup&gt; conjugative plasmid (Tc&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt;)</td>
<td>Bennett et al. (1977)</td>
</tr>
<tr>
<td>pUB2401</td>
<td>pACYC184 tet::Tn21; the plasmid (Cm&lt;sup&gt;R&lt;/sup&gt;) carries Tn21 (Hg&lt;sup&gt;R&lt;/sup&gt; Sm&lt;sup&gt;R&lt;/sup&gt; Ap&lt;sup&gt;R&lt;/sup&gt;)</td>
<td>de la Cruz &amp; Grinsted (1982)</td>
</tr>
<tr>
<td>pVS1650</td>
<td>Stable R906–pBR322 cointegrate; carries Tn510 and a Tn510 remnant; Hg&lt;sup&gt;R&lt;/sup&gt; Oxa&lt;sup&gt;R&lt;/sup&gt; Sm&lt;sup&gt;R&lt;/sup&gt; Su&lt;sup&gt;R&lt;/sup&gt; Tc&lt;sup&gt;R&lt;/sup&gt; Ap&lt;sup&gt;S&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pVS1659</td>
<td>pBluescript KS+ with R906 EcoRI–HindIII clone encoding &lt;i&gt;merTP510&lt;/i&gt; Ap&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pVS1660</td>
<td>&lt;i&gt;Ban&lt;/i&gt;HI deletion of pUB2401; lacks most of Tn21 but retains ttpA&lt;sub&gt;321&lt;/sub&gt; Cm&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pVS1661</td>
<td>Sphi–&lt;i&gt;EcoRV&lt;/i&gt; deletion of pVS1660; lacks all of Tn21; Cm&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pVS1670</td>
<td>SstI-ligation derivative of pVS1650; contains pBR322 and flanking Tn510 sequences; lacks most of R906; Tc&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pVS1683</td>
<td>pBluescript KS+ with R772 EcoRI–HindIII clone encoding &lt;i&gt;merTP511&lt;/i&gt; Ap&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pVS1692</td>
<td>pBRMCS-5 with R906 EcoRI–HindIII clone encoding &lt;i&gt;merTP511&lt;/i&gt; Gm&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pVS1719</td>
<td>pBR322::Tn511; Tc&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
</tbody>
</table>
then twice with chloroform alone, and precipitated, on ice, with 2-propanol. After centrifugation for 15 min at 13 000 r.p.m. (17 000 g), the nucleic acid pellet was washed with 70% ethanol, dried and dissolved in 30 µl diethyl-pyrocarbonate-treated single-distilled H2O (DEPC–dH2O) and treated with 1 U DNase I (Promega). The RNA was extracted and precipitated, as described above, resuspended in DEPC-dH2O and quantified spectrophotometrically. RT-PCR was performed in two steps. Reverse transcription was performed using 2 µg template RNA annealed to primer SP8 (5′-CGTAAGACATCTCGGCAAG-3′) in a reaction mixture containing 200 U Moloney murine leukemia virus reverse transcriptase (Promega), the supplied reaction buffer, 1.25 mM reaction volume of 15 µl. After incubation at 42 °C for 1 h, the reaction was terminated by heating (95 °C for 5 min) and the reverse-transcription products were subjected to PCR amplification using Taq DNA polymerase (Petrovski & Stanisch, 2010) and primers SP8 and SP13 (5′-GTCTGACAAAACCGTAG-3′).

**RESULTS AND DISCUSSION**

The **oriV–trfA** regions of R772 and R906 contain defective derivatives of the same **mer** transposon

We determined the DNA sequences of R906 and R772 from a PstI site near oriV to a position about 260 bp beyond a HindIII site near trfA (Fig. 1). On analysis, each sequence revealed the presence of a defective, nested transposon embedded within plasmid sequences. The transposon in R906 was named Tn510 (13 336 bp); that in R772 was named Tn511 (18 769 bp). Both transposons were clearly related, since their respective 38 bp IR termini (IRt1 and IRm1) are identical and differ only in a few bases from those of Tn21 (Liebert et al., 1999) (Fig. 2a). The 5 bp DR sequences that flank Tn510 and Tn511 were also identical (i.e. TGGCCT), and are each part of longer sequences recognizable as IncP backbone (i.e. the 40 bp from the PstI site to IRt1 and the 262 bp beyond IRm1) (Fig. 1). A simple explanation of these features is that Tn510 and Tn511, although different in size, are both derived from a progenitor transposon that entered a common ancestor of R906 and R772 by transpositional insertion. From the parts of the Hg2+-resistance (**mer**) and transposition (**tnp**) modules that have been retained, the progenitor is most like the Tn21/Tn501-family transposon TnAO22 (Ng et al., 2009) (Fig. 2b, c), and will be ascribed this name to aid discussion.

Specifically, the type 6 **mer** modules (**merRTPADEorf2**) of Tn510 and Tn511 are of identical length and sequence (>99%) to those of TnAO22 and Tn501 (Liebert et al., 1997) (Table 2). The **tnp** modules are incomplete. They too are near identical to **tnp** of TnAO22, and more similar to

### Table 2. Features and coding sequences of Tn510 and Tn511, and their closest relatives

<table>
<thead>
<tr>
<th>Feature and/or gene sequence</th>
<th>Tn coordinates (nucleotides)†</th>
<th>Percentage nucleotide identity to the most closely related sequences‡</th>
<th>GenBank accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn510</td>
<td>Tn511</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRt1-tnpAI§</td>
<td>1–273</td>
<td>100% to pAO22 from Achromobacter sp. and pMOL30 from Cupriavidus metallidurans</td>
<td>EU696790; CP000354</td>
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<tr>
<td>IS1071</td>
<td>274–3477</td>
<td>100% to pIP4 from Ralstonia eutropha and pTSA from Comamonas testosteroni</td>
<td>AF311437; CP000541</td>
</tr>
<tr>
<td>‘tnpA’-like</td>
<td>NA</td>
<td>100% to pCNBl from Comamonas sp. and to Ralstonia pickettii</td>
<td>CP001644; AB266144</td>
</tr>
<tr>
<td>IRt2-tnpA2</td>
<td>NA</td>
<td>99% to pMOL28 from C. metallidurans and a fosmid clone from an unknown organism</td>
<td>AB266144; AJ877225</td>
</tr>
<tr>
<td>‘Tn5393c’</td>
<td>3478–7836</td>
<td>99% to pPRAS2 from Aeromonas salmonicida and pPSR1 from Pseudomonas syringae</td>
<td>AF262622; AY342395</td>
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<tr>
<td>Tn5403 socD-like</td>
<td>3421–7083</td>
<td>99% to Klebsiella pneumoniae and pEC-L46 from E. coli</td>
<td>X75779; GU371929</td>
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<tr>
<td>Tn5403 socD-like</td>
<td>7442–8560</td>
<td>71% to Pantoea vagans C9-1 and 66% to Erwinia bilingiae Eb66111</td>
<td>CP002206; FP326843</td>
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<tr>
<td>aprotA1</td>
<td>NA</td>
<td>98% to pSe-Kan from Salmonella enterica and pRts1 from Proteus vulgaris</td>
<td>GQ426885; AP004237</td>
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<tr>
<td>IRt3-tpnA3*R</td>
<td>10 562–14 252</td>
<td>99% to pMOL28 from C. metallidurans and a fosmid clone from an unknown organism</td>
<td>AB266144; AJ877225</td>
</tr>
<tr>
<td>tnpA1R-orf2-mer-IRm1</td>
<td>7837–13 346</td>
<td>99% to pAO22 from Achromobacter sp. and pMOL28 from C. metallidurans</td>
<td>EU696790; X90708</td>
</tr>
</tbody>
</table>

†The sequences of Tn510 and Tn511 are available under GenBank accession numbers DQ471307 and EU287476, respectively. NA, Not applicable.

‡The sequence of Tn510 is identical to the corresponding parts of a Tn510 derivative in pB10 (accession number AJ564903).

§A ‘ symbol indicates the part of the gene (or feature) that is missing (i.e. the 3’ end and/or the 5’ end).

||These sequences are the best of only several database entries with high identity. All other sequences listed in Table 2 had high identity to numerous database entries for different plasmids and/or bacteria.
the module in Tn21 (~97% identity) than to that in Tn501 (~70% identity). Instead of the expected trnAR-res components, Tn511 contains a 3′ remnant of the transposase gene (i.e. the 207 bp ‘trnA1’) adjacent to IRS. In Tn510, the wild-type trnA1 has been disrupted and the central portion deleted. Consequently, the 3′ remnant occurs near IRS (i.e. the 239 bp ‘trnA1’), and the 5′ remnant (i.e. the 264 bp ‘trnA1’), together with the resolvase gene (trnR) and res sequence, occurs near the mer module. It appears, therefore, that the TnAO22 trn module underwent significant changes during its evolution into Tn510 and Tn511 (discussed below), whereas the mer module remained largely unaffected. The latter finding was unexpected, since HgII-resistance is a trait of R906 but not of R772, which is suspected to carry a potentially dysfunctional merA (Smith & Thomas, 1989). We investigated the basis of this phenotypic difference using E. coli K-12 as the host.

R772 confers sensitivity to HgII because the MerT import protein encoded by Tn511 is defective

The mer modules of Tn510 (from R906) and Tn511 (from R772) were found to be identical, except for single-base differences in merT (351 bp), which encodes an HgII-transport protein, and merA (1686 bp), which encodes the HgII reductase (Barkay et al., 2003). The differences in merT511 (T→A at nucleotide 32) and merA511 (A→T at nucleotide 1661) result in predicted amino acid substitutions (i.e. Leu11→Pro in MerT511, Lys554→Met in MerA511). Either of the mutated genes could be responsible for the HgS phenotype of R772, since a merA or merT mutation can result in HgII sensitivity (Hamlett et al., 1992). To individually assess the functions of merT511 and merA511, we took advantage of the fact that a different phenotype, HgII hypersensitivity, arises when wild-type merT is expressed in the absence of wild-type merA (whether or not merR is present) (Lund & Brown, 1987). We therefore cloned merTP from Tn510 and Tn511 and used disk-diffusion assays to study the phenotypic effect of the genes when present alone or in strains with R906 (which has a functional merA and merT) or R772 (which has a potentially dysfunctional merA).

The data obtained (Table 3) confirm that R906, and not R772, confers HgII resistance (lines 1–3). They also showed that the R906 merTP510 genes cause HgII hypersensitivity when merA906 is absent (line 4), and that hypersensitivity was counteracted when merA906 was present. The effect was partial (line 7) when HgII import was elevated, and complete (line 9) when import was lower (i.e. when the high- and low-copy-number pBlue-merTP510 and pBBG-merTP510 plasmids were present, respectively). Significantly, the cloned R772 merTP510 genes failed to confer HgII hypersensitivity (compare lines 4 and 5). Since merP510 and merP511 are identical, this observation provides genetic evidence that merT511 is defective. In contrast, the R772 merA511 gene, despite a missense mutation, appears to be functional, since strains with the active MerTP510 import system and with either merA510 (in R906)
or merA_{511} (in R772) are phenotypically similar (compare lines 7 and 10 and lines 9 and 12). Our conclusion that MerT_{511} is defective is also consistent with the basal level sensitivity (i.e. a 3 mm zone) exhibited by the strain with merTP_{511} and merA_{511} (line 11). The changed residue in MerT_{511} (Leu_{11}A Pro) is near the first putative transmembrane helix of the protein (residues 12–34) (Barkay et al., 2003). Consequently, it may alter the membrane topology or function of MerT_{511}, thereby affecting critical residues in the helix, such as the vicinal Cys_{24} and Cys_{25}, which are required for the Hg II resistance and/or hypersensitivity phenotypes (Hobman & Brown, 1996).

Tn_{510} in R906 and Tn_{511} in R772 contain different nested components

Although Tn_{510} and Tn_{511} are clearly derived from the same TnAO22 base element, they differ in other structural components. Tn_{510} contains part of Tn_{5393c} (L’Abée-Lund & Sørum, 2000): the streptomycin-resistance (strAB) arm is present but the 3′ end of mpA_{5393} and the associated IRT are missing (Fig. 1). Tn_{510} also contains IS_{1071}; however, this is not flanked by the 5 bp DRs typically associated with its transposition (Sota et al., 2006). Since the sequences of the Tn_{510} components directly abut each other, we conclude that TnAO22 first inherited Tn_{5393c},
which then inherited IS1071 (Fig. 2b). If the respective insertions occurred towards the 5’ end of tnpA1 and the 3’ end of tnpA5393c, a leftward IS1071-mediated deletion would account for the missing sequences (i.e. tnpA’-IR5393c, ‘tnpA1’ and the left DR of IS1071).

Tn511 is structurally more complex than Tn510. In addition to the TnAO22 base element, remnants of two other Tn21-like elements are present that differ in sequence identity. The larger remnant (IR3-tnpA3*-tnpR) probably originated from a Tn21-like element (84–89 % identity) that inserted into tnpA1 of TnAO22, and was followed by TnpR-mediated resolution at the heterologous res sites. Consequently, IR3-tnpA3*-tnpR forms part of a hybrid transposon that has, as its other arm, res-mer-IRm1 (95–99 % identity to Tn21) (Fig. 1). The second Tn21-like remnant is IRt2-’tnpA2 (99 % identity). This remnant abuts Tn5403, a Tn3-related transposon (Rinkel et al., 1994a). Significantly, Tn5403 lacks its typical 5 bp DRs (Rinkel et al., 1994a), suggesting that it was responsible for deleting the remainder of tnpA2. Since Tn5403 is the sole intact element in Tn511, we assume that it is the most recently acquired entity.

The remaining components in Tn511 include three ‘orphan’ genes. One is an IS1071-like remnant gene, ‘tnpA’, that is in a similar position to the intact tnpA1071 in Tn510 but is only distantly related (69 %); a sequence identical to ‘tnpA’ occurs in an IS1071-like element in pCNB1 (IncPβ) (Ma et al., 2007). The other ‘orphan’ genes occur in a 3478 bp region that abuts Tn5403 on its right. The region is not related to reported sequences, except for aphA1 and a socD-like gene (Table 2). The former encodes an aminoglycoside 3’-phosphotransferase responsible for the KmR phenotype of R772. The socD511 product is 46 % identical to an amino acid oxidase (SocD) involved in santhopine catabolism in Agrobacterium tumefaciens C58 (Baek et al., 2005) and to putative fructosyl amino acid oxidases in other soil-dwelling bacteria (Table 2). It is possible that socD511 is functional, since its transcription from R772 in E. coli DH5α was detected by RT-PCR (see Methods) (data not shown). R772 is to our knowledge the first example of an IncP plasmid to carry a socD-like gene.

A possible model that explains the structural origin of Tn511 is depicted in Fig. 2(c).

### Table 3. Hg²⁺-resistance phenotype of E. coli strains carrying cloned merTP genes from Tn510 and Tn511

<table>
<thead>
<tr>
<th>Line number cited in text</th>
<th>Plasmids in E. coli DH5α</th>
<th>Zone diameter † (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IncPβ (Tn)</td>
<td>Coresident plasmid*</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>None or empty vector</td>
</tr>
<tr>
<td>2</td>
<td>R906 (Tn510)</td>
<td>None or empty vector</td>
</tr>
<tr>
<td>3</td>
<td>R772 (Tn511)</td>
<td>None or empty vector</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>pBlue-merTP510</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>pBlue-merTP511</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>pBBG-merTP510</td>
</tr>
<tr>
<td>7</td>
<td>R906 (Tn510)</td>
<td>pBlue-merTP510</td>
</tr>
<tr>
<td>8</td>
<td>R906 (Tn510)</td>
<td>pBlue-merTP511</td>
</tr>
<tr>
<td>9</td>
<td>R906 (Tn510)</td>
<td>pBBG-merTP510</td>
</tr>
<tr>
<td>10</td>
<td>R772 (Tn511)</td>
<td>pBlue-merTP510</td>
</tr>
<tr>
<td>11</td>
<td>R772 (Tn511)</td>
<td>pBlue-merTP511</td>
</tr>
<tr>
<td>12</td>
<td>R772 (Tn511)</td>
<td>pBBG-merTP510</td>
</tr>
</tbody>
</table>

*The empty vectors were pBlue (=pBluescriptKS+) and pBBG (=pBBR1MCS-5). Derivatives of these were pVS1659 and pVS1692 (both with merTP510) and pVS1683 (merTP511).
†The results shown are from a single disk-diffusion assay. The data from independent assays displayed the same trends, although the diameter of zones of clearing may have differed (± 1 mm).

Based on in silico findings we concluded that Tn510 and Tn511 are defective transposons, since they lack a complete tnpA gene (Fig. 1). They do, however, have intact tnpR-res loci (near merE) and terminal IRs. Thus, whilst appearing to be permanently embedded structures, we thought that Tn510 and Tn511 might relocate if a cognate tnpA gene (e.g. tnpA21 from Tn21) was provided in trans, as might smaller sections of Tn511 if its internal IRt2 and IRt3 sequences formed compatible termini with IRm1. The intact IS1071 and Tn5403 elements were potentially self-mobile. These various suppositions were tested in conjugation experiments designed to detect transposition of Tn510 (Hg²⁺, Sm²⁺), Tn511 (Km²⁺) and its components, in the presence and absence of tnpA21 [on pVS1661 (Cm²⁺)]. The non-conjugative plasmid pBR322

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**The provision of tnpA21 in trans enables transposition of Tn510, Tn511 and the components Tn511-2 and Tn511-3**

Based on in silico findings we concluded that Tn510 and Tn511 are defective transposons, since they lack a complete tnpA gene (Fig. 1). They do, however, have intact tnpR-res loci (near merE) and terminal IRs. Thus, whilst appearing to be permanently embedded structures, we thought that Tn510 and Tn511 might relocate if a cognate tnpA gene (e.g. tnpA21 from Tn21) was provided in trans, as might smaller sections of Tn511 if its internal IRt2 and IRt3 sequences formed compatible termini with IRm1. The intact IS1071 and Tn5403 elements were potentially self-mobile. These various suppositions were tested in conjugation experiments designed to detect transposition of Tn510 (Hg²⁺, Sm²⁺), Tn511 (Km²⁺) and its components, in the presence and absence of tnpA21 [on pVS1661 (Cm²⁺)]. The non-conjugative plasmid pBR322
Independent and TnpA<sub>21</sub>-assisted transposition of Tn510, Tn511 and their component elements to the target plasmid pBR322

<table>
<thead>
<tr>
<th>Line number cited in text</th>
<th>Plasmids in donor* with target plasmid pBR322</th>
<th>Transconjugants†</th>
<th>Transposition derivative (number)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IncP plasmid (Tn)</td>
<td>Genotype of helper plasmid</td>
<td>Transposition frequency</td>
</tr>
<tr>
<td>1</td>
<td>R906 (Tn510)</td>
<td>tnpA&lt;sub&gt;21&lt;/sub&gt;−</td>
<td>&lt;5.0 × 10&lt;sup&gt;−8&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>R906 (Tn510)</td>
<td>tnpA&lt;sub&gt;21&lt;/sub&gt;−</td>
<td>7.0 × 10&lt;sup&gt;−6&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>R772 (Tn511)</td>
<td>tnpA&lt;sub&gt;21&lt;/sub&gt;−</td>
<td>2.5 × 10&lt;sup&gt;−6&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>R772-1 (Tn511)</td>
<td>tnpA&lt;sub&gt;21&lt;/sub&gt;−</td>
<td>2.0 × 10&lt;sup&gt;−7&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>R772-1 (Tn511)</td>
<td>tnpA&lt;sub&gt;21&lt;/sub&gt;−</td>
<td>8.8 × 10&lt;sup&gt;−7&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Donors were derivatives of E. coli DH5<sup>x</sup> carrying a conjugative plasmid [R906 (H<sup>R</sup>), R772 (Km<sup>R</sup>) or R772-1 (with a defective Tn5403 in Tn511)], together with pBR322 (Tc<sup>R</sup>Ap<sup>R</sup>) and either a tnpA<sub>21</sub>− plasmid (pVS1661) or its tnpA<sub>21</sub>− derivative (pVS1660) (both Cm<sup>R</sup>). The recipient was LT101 (Rif<sup>R</sup>).

†Transconjugants were isolated on NA containing Rif<sup>R</sup> or H<sup>R</sup>II or Km<sup>R</sup> [to detect transfer of R906 and R772 (or R772-1), respectively] or Tc (to detect conductual transfer of pBR322). The transposition frequency represents the ratio of Tc<sup>R</sup> to H<sup>R</sup> transconjugants per R906<sup>R</sup> donor, or Tc<sup>R</sup> to Km<sup>R</sup> transconjugants per R772<sup>−</sup> (or R772-1<sup>−</sup>) donor. The mean transfer frequencies of R906 and R772 (or R772-1) were 4.0 × 10<sup>−1</sup> and 4.2 × 10<sup>−1</sup>, respectively. Experiments were performed at least three times using independently constructed donors.

‡The pBR322 transpositional derivatives present in transconjugants were isolated and characterized as described in Methods. The transconjugants studied were all randomly chosen from amongst the Tc<sup>R</sup>Ap<sup>R</sup> subgroup or Tc<sup>R</sup>Ap<sup>S</sup> subgroup.

(Tc<sup>R</sup> Ap<sup>R</sup>) served as the transpositional target. Its transfer was monitored via the incidence of Tc<sup>R</sup> transconjugants and is dependent on the formation of transpositional cointegrates (see Methods). These can be resolved in the recipient by the cognate TnpR-res system, presuming that it is functional.

The data obtained (Table 4) show that despite efficient conjugal transfer of R906 and R772 (~4.0 × 10<sup>−1</sup> per donor; see footnote), only the R772<sup>−</sup> donor, and not that with R906, elicited transfer of pBR322 (lines 1 and 3). The R772-mediated events were attributed to Tn5403, since none occurred in the corresponding experiment involving R772-1, a tnpA<sub>5403</sub> mutant (line 4). Confirmation of Tn5403 transposition was obtained by analysing four Tc<sup>R</sup> transconjugants (see Methods). All contained resolved pBR322::Tn5403 plasmids in which the insert was at a different site. In one of these (from a Tc<sup>R</sup> Ap<sup>S</sup> transconjugant), Tn5403 was in amp<sup>R</sup> of pBR322 (at nucleotide 4181), where it was flanked by 5 bp DRs (CATTT) and was able to relocate to pUB307 when tested further (data not shown). We concluded that Tn5403 of Tn511 is self-mobile and that its transposition to random target sites involves the formation of a cointegrate, a feature previously attributed to this element (Hille et al., 1983; Rinkel et al., 1994a). The failure of R906 to similarly transfer pBR322 (line 1) demonstrated that IS1071 of Tn510 is not self-mobile in this assay system, despite its reported ability to form transpositional cointegrates (Sota et al., 2006; Wyndham et al., 1994), and neither is Tn510 itself.

Line 2 of Table 4 shows that tnpA<sub>21</sub> facilitated transfer of pBR322 by R906, presumably by restoring transposition of Tn510. This conclusion was confirmed by analysing four Tc<sup>R</sup> Ap<sup>S</sup> transconjugants, three of which contained resolved pBR322amp::Tn510 plasmids whose Tn510 insertion sites and orientation in amp<sup>R</sup> were determined (i.e. at nucleotides 3710 and 4062 in β-orientation, at nucleotide 3530 in α-orientation). The fourth plasmid (pVS1650) was an unusual R906-pBR322 cointegrate that had formed by Tn510 insertion into amp<sup>R</sup>, but had not resolved because most of the second copy of Tn510 was absent (see Methods) (Fig. 3). The structural features of pVS1650 are interesting, as the pBR322 replicon is fortuitously enclosed by IRm1 and IRt1 termini, forming the novel pBR322-Tn510 transposon Tn510-2 (Fig. 3). Although not formally tested, Tn510-2, like Tn510, should be capable of tnpA<sub>21</sub>-assisted transposition. The IRm1 and IRt1 termini of Tn510 were identified in all four plasmids studied and in each case they were flanked by different 5 bp DRs, consistent with transposition of a Tn21/Tn501-like element.

We also studied tnpA<sub>21</sub>-assisted transposition of Tn511 by using the Tn5403-inactive plasmid R772-1. Transposition was detected (line 5), and the resulting products in 19 Tc<sup>R</sup> transconjugants were analysed further. All had recoverable pBR322::Tn plasmids that contained different parts of Tn511. Those from 10 Tc<sup>R</sup> Km<sup>R</sup> transconjugants were resolved pBR322::Tn511-3 plasmids. All were of similar size (~12.5 kb) and sequence analysis of four confirmed the presence of Tn511-3 (i.e. at nucleotides 2431, 2510
and 3162 in z-orientation, and at nucleotide 3143 in β-orientation). In each instance, the Tn511-3 insert was bounded by IRt3 and IRm1, and flanked by 5 bp DRs. Larger plasmids (~22.5 kb) were recovered from all nine TcR KmR transconjugants. One was identified as a pBR322::Tn511 plasmid and eight as pBR322::Tn511-2 plasmids, based on the presence or absence of the Ncol site located near IRt1 (Fig. 1). The latter group was further divisible into four (via HindIII profile), suggesting differences in either the position or the orientation of the inserted Tn511-2.

These various observations provide experimental evidence of bona fide transposition of Tn510 and Tn511, and of their components (Tn511-2, Tn511-3 and Tn5403), from their in situ locations in R906 or R772. With the exception of Tn5403, transposition of these otherwise immobile elements was assisted by tnpA21 supplied in trans. This feature is expected to extend to Tn510-2, a novel transposon that contains the pBR322 replicon. Our failure to detect transposition of IS1071 is consistent with a recent study (Sota et al., 2006) which showed that transposition is host-dependent and does not occur in E. coli as previously suspected.

### The aphAI region of Tn511 is inherently unstable

The DNA sequence of Tn511 revealed that aphAI is flanked by similarly oriented regions of homology of >2 kb in length (i.e. tnpA2 and tnpA3*) (Fig. 1). These regions are potential substrates for the host recombination (RecA) system, and could result in deletion of the intervening ~10 kb region containing Tn5403, socD and aphAI. To test this possibility, PCRs were performed using primers SP32 and SP33 (Fig. 1) and template DNA from Rec+ and Rec− strains that had been grown for ~25 generations in the absence of kanamycin. The strains carried either R772 or pBR322::Tn511 (pVS1719). PCR products were obtained only from the Rec+ sources and were of ~2.5 kb in size (Fig. 4), consistent with the presence of RecA-generated plasmid deletants involving ’tnpA2 and tnpA3*. The greater product yield from the pBR322::Tn511 sample was attributed to the increased chance of deletions due to the smaller size and high copy number of this plasmid compared with R772. Indeed, ~30% of the colonies grown from the Rec+ pBR322::Tn511+ culture were KmR, whereas no segregants were detected (<1%) from the Rec+ R772+ culture. The extracted plasmids from four KmR segregants were all smaller (~10 kb) than pBR322::Tn511 and differed from it in HindIII and EcoRI profiles, consistent with loss of the Tn5403-socD-aphAI segment (Fig. 1). These observations demonstrate the emergence of another new element from Tn511, denoted Tn511-4 (Fig. 1), that, like Tn511-3, has no associated phenotype. They also imply that the maintenance of R772 in its clinical strain (Coetzee, 1978), and since then in laboratory strains, was due to aminoglycoside selection pressure. Indeed, the latter may be a strict requirement, since expression of aphAI imposes a cost burden on its bacterial host (Kim et al., 2006).

### Hypothetical genesis of Tn510 and Tn511 and their evolutionary potential

The DNA sequences of Tn510 (from R906) and Tn511 (from R772) and the experimental features of these elements provide clues to their origins. A simple scenario is that Tn510 and Tn511 evolved mainly by sequential acquisition of TEs and accompanying DNA rearrangements. In the primary event, TnAO22 inserted into the IncPβ oriV-trfA region, forming the extant 5 bp target duplication. Other TEs were then acquired, altering TnAO22 internally and establishing the Tn510 and Tn511 lineages (Fig. 2). A derivative of Tn510 occurs in pB10(IncPB) (Schlüter et al., 2003). Its sequence and location are identical to those of Tn510, except that IS1071 is disrupted by a Tn1721-like element. A third lineage is represented by the element in pJP4 (Schlüter et al., 2003; Trefault et al., 2004): it retains the mer-IRm1 end of TnAO22 but the IRt end and adjacent backbone sequences have been replaced by the acquired TEs. An alternative scenario is that TnAO22 evolved prior to its transposition into the same oriV-trfA site by a tnpA-assisted process (Table 4). This scenario is unlikely, since target specificity is not a feature of Tn21/Tn501-like elements related to TnAO22 (Grinsted et al., 1990), although they favour the IncPβ oriV-trfA region (Sota et al., 2007).

The various components of Tn510 and Tn511, except for the regions flanking socD and aphAI, are recognizable genes or remnant TEs. We speculate that all may have been

---

**Fig. 4.** Instability of the Tn5403-socD-aphAI region of Tn511 in E. coli. Plasmid DNA isolated from RecA+ (UB281) and RecA− (UB5201) strains carrying either pBR322::Tn511 (pVS1719) or R772 was PCR-amplified using primers SP32 and SP33, and the reaction products were separated by electrophoresis on an agarose gel. Lanes: 1, λ HindIII-digested marker DNA; 2, pVS1719+ strain; 3, R772+ strain; 4, pVS1719+ strain; 5, R772+ strain. The arrow indicates the position of a faint band that occurs in lane 5.
acquired from environmental bacteria given the ecology of the components. TnAO22 was detected in an *Achromo-
bacter* sp. (Ng et al., 2009); however, the broader family of Tn21/Tn501-like elements is common in Gram-negative
bacteria (Grinsted et al., 1990; Liebert et al., 1999; Yurieva et al., 1997). Tn5393 is typically associated with phyto-
pathogens [e.g. *Erwinia* and *Pseudomonas* species (Chiov & Jones, 1993; Sundin et al., 1995)], and IS1071
with pollutant-degrading bacteria [e.g. *Comamonas* and *Pseudo-
monas* species (Di Gioia et al., 1998; Wyndham et al., 1994)], including those that support IS1071 transposition
(Sota et al., 2006). Tn5403 was detected in aquatic enterob-
bacteria [i.e. *Klebsiella* spp. and *Enterobacter* agglomerans
(Rinkel et al., 1994a, b)], and soCD genes occur in members of the
*Rhizobiaceae* (Baek et al., 2005). The aphaI gene may have been from bacteria associated with humans or animals
(Vakulenko & Mobashery, 2003), and is possibly a TE
remnant. Alternatively, from the Tn511 aphaI–soCD region may
have been inherited en bloc by an unknown capture
process. An agrobacterial/rhizobial source is possible, since kanamycin resistance occurs amongst these bacteria
(Brockman & Bezdicek, 1989; Cole & Elkan, 1979; F.
Taner & V. A. Stanisch, unpublished data).

All of the above-mentioned bacteria could have been
accessed by the ancestral IncPβ plasmid, since the IncP
family in general has a broad host range (Sen et al., 2010;
Thomas & Smith, 1987) and occurs in diverse habitats
(Bahl et al., 2009; Smalla et al., 2000). Tn510 and Tn511
have added interest, since R906 and R772 were isolated
in the 1970s from vertebrate sources, yet their components,
and Tn5403 and soCD31, are similar to those in environmentally sourced IncP plasmids. Some of the com-
ponents (e.g. Tn5393 and Tn5403) appear to be dissemi-
nating more widely and have been found in complexes
with other TEs or resistance genes in bacteria from humans
(Boyd et al., 2004; L’Abée-Lund & Sørum, 2000;
Mantengoli & Rossolini, 2005; Ruppé et al., 2009). The
compositions of Tn510 and Tn511 thus lend support to the
opinion that gene flow from environmental microcosms
can affect bacteria present in humans (the source of R772)
and animals (the source of R906) (Davies, 1994; Mindlin et al., 2006; Schlüter et al., 2007).

The genesis models (Fig. 2) also posit that the various
components were acquired at different times, possibly as
intact TEs, and were subject to mutational change. A
missense mutation in merT31 (Table 3) accounts for the
HgII sensitivity of R772, despite its full-length mer module.
Deletion mutations caused either by the TEs or by
homology (RecA)-dependent processes are also evident.
TE-mediated events are expected to leave the TE intact,
whilst removing the DR and adjoining DNA on one flank
(Petrovski & Stanisch, 2010; Wang et al., 1994). This type
of event explains why Tn5403 and IS1071 each border a
remnant gene and lack recognizable DRs, even though
these are reinstated on subsequent transposition, as
observed for Tn5403 (Table 4). Presumably, both elements
could continue to initiate deletions, producing new
variants. RecA-dependent deletions were not recognizable in
Tn510 and Tn511; however, such events involving the Tn511 ‘tnpA2 and tnpA3’ sequences account for the
kanamycin-sensitive derivative Tn511-4 (Figs 1 and 4). A
similar process in the Tn510 derivative of pB10 could result in
tetracycline-sensitive segregants.

To this point we have focused on the evolution of Tn510
and Tn511 in their IncPβ carrier. Other evolutionary
consequences attended transposition of the elements when
assisted in trans by tnpA of Tn21. The TnpA21 transposase
is relatively non-specific (Grinsted et al., 1990) and was
able to recognize IRt1 and IRm1, which differ from the
Tn21 termini, as well as the internal IRt2 and IRt3, which
do not (Fig. 2a). Consequently, transposition of Tn510 and
Tn511 was detected, and also that of Tn511-2 and Tn511-3
(Fig. 1, Table 4). Similar events enabling defective elements
to escape from their carrier replicon could presumably
occur during natural encounters of IncP plasmids and
Tn21/Tn501-like transposons in the diverse habitats in
which both occur (Smalla et al., 2000). One of the Tn510
transposition events resulted in the formation of an R906-
pBR322 cointegrate that was stabilized by the absence of a
complete second copy of Tn510 (Table 4, Fig. 3). A similar
stable cointegrate was formed between R772 and pTi in
*Agrobacterium*, involving Tn5403 as the initiating element
(Hille et al., 1983). Such cointegrates are presumably due
to errors in the transposition process and may be relatively
common. They enable the transfer of ‘host-limited’ genes
(e.g. pBR322) to the pantheon of IncP hosts, where they
may remain linked to the IncP replicon or have opportu-
nities to separate from it, as predicted for the hybrid
transposon Tn510-2 (Fig. 3).

In overview, this study of Tn510 and Tn511 shows how
complex embedded structures may have arisen. We focused
on the functional attributes of these elements to show that
they are not genetic dead-ends but can contribute to
horizontal gene transfer in perhaps unanticipated ways.
Similar elements, varying in structural complexity, occur
in other IncP plasmids (e.g. Tn4321 of R751; Partridge &
Hall, 2003; Thorsted et al., 1998) and indeed beyond the
IncP family [e.g. the transposons in pWW53 (IncP-7);
Yano et al., 2007]. Their occurrence reinforces the impor-
tance of accessory genes in plasmid diversity and, via the
potentially advantageous encoded properties, to niche-
based adaptation of the bacterial host.

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Transposition of nested defective transposons


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