A comparison of the β-lactamase elements detected on three classes of large plasmids together with the chromosomes of penicillin-resistant Staphylococcus aureus revealed substantial physical and genetic relatedness. In most cases, β-lactamase production could be associated with the presence of a DNA segment of approximately 6.7 kb. Analysis showed that the plasmid-borne determinants constitute nearly identical transposons or transposon-like elements. An element indistinguishable from one of these, Tn4002, which is carried by the pSK1 family of plasmids in clinical isolates from Australian hospitals, was also identified on the staphylococcal chromosome and is implicated in an evolutionary cycle of transposition between chromosomal and extrachromosomal sites in Australian strains of multiresistant S. aureus.

INTRODUCTION

Penicillin resistance (Pc') is often mediated by a β-lactamase (penicillinase) which inactivates the antibiotic through hydrolysis of the β-lactam ring. The enzymes responsible for Pc' in Staphylococcus aureus have been historically associated with large plasmids (about 30 kb) which additionally encode resistance to the heavy metals including arsenate (Asa), arsenite (Asi), cadmium (Cd) and mercury (Hg) (Peyru et al., 1969; Shalita et al., 1980). Murphy & Novick (1979) suggested that the Pc' determinant of these plasmids may reside on a transposon, designated Tn552, which can translocate from a chromosomal to a plasmid site.

In recent years, Pc' in S. aureus isolates from the USA and Europe has been attributed either to β-lactamase/heavy metal resistance plasmids or to the presence of a family of self-transmissible plasmids which also encode resistance to the aminoglycosides gentamicin (Gm), tobramycin (Tm) and kanamycin (Km) and to quaternary ammonium compounds (Qa) and ethidium bromide (Eb) (Goering & Ruff, 1983; Witte & Dünhaupt, 1984). The Pc' region of one of these plasmids, pCRG1600, was shown to lie within a 6-7 kb transposon, designated Tn4201, which is capable of undergoing rec-independent translocation between plasmids and the chromosome (Weber & Goering, 1988).

Plasmids similar or identical to the β-lactamase/heavy metal resistance plasmids have been identified in Australian isolates of S. aureus from the mid-1940s; however, since the early 1970s, Pc' has been found to be predominantly determined by the chromosome (Lyon et al., 1983; Gillespie et al., 1985, 1986b). In recent years, a determinant for Pc' has been detected on some members of a unique family of Eb'Qa' and acriflavine resistance (Ac') plasmids which often additionally encode Gm'Tm'Km' via Tn4001 and/or trimethoprim resistance (Tp') through the carriage of a unique dihydrofolate reductase determinant (dfrA) on the transposon Tn4003 (Gillespie & Skurray, 1986; Lyon & Skurray, 1987; Lyon et al., 1987; Young et al., 1987; Skurray
Table 1. Characteristics of *S. aureus* plasmids and strains

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Year of isolation</th>
<th>Resistance to*</th>
<th>Size (kb)</th>
<th>β-Lactamase production</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSK1</td>
<td>1980</td>
<td>GmTmKmAcEbQaTp</td>
<td>28.4</td>
<td>–</td>
<td>Lyon et al. (1987)</td>
</tr>
<tr>
<td>pSK4</td>
<td>1981</td>
<td>GmTmKmAcEbQaTpPc</td>
<td>35.1</td>
<td>+</td>
<td>Gillespie et al. (1987b)</td>
</tr>
<tr>
<td>pSK8</td>
<td>1981</td>
<td>GmTmKmAcEbQaTpPc</td>
<td>35.1</td>
<td>+</td>
<td>Gillespie et al. (1987b)</td>
</tr>
<tr>
<td>pSK11</td>
<td>1980</td>
<td>GmTmKmTpPc</td>
<td>41.8</td>
<td>+</td>
<td>Gillespie et al. (1987b)</td>
</tr>
<tr>
<td>pSK15</td>
<td>1982</td>
<td>GmTmKmAcEbQaPc</td>
<td>31.1</td>
<td>+</td>
<td>Gillespie et al. (1987b)</td>
</tr>
<tr>
<td>pSK16</td>
<td>1982</td>
<td>GmTmKmAcEbQaTpPc</td>
<td>35.1</td>
<td>+</td>
<td>Gillespie et al. (1987b)</td>
</tr>
<tr>
<td>pSK17</td>
<td>1981</td>
<td>GmTmKmAcEbQaPc</td>
<td>36.4</td>
<td>+</td>
<td>Gillespie et al. (1987b)</td>
</tr>
<tr>
<td>pSK41</td>
<td>1977</td>
<td>GmTmKmNmPmEbQa</td>
<td>47.8</td>
<td>–</td>
<td>Lyon et al. (1987)</td>
</tr>
<tr>
<td>pUW3626</td>
<td>1981</td>
<td>GmTmKmNmPmEbQaPc</td>
<td>54.4</td>
<td>+</td>
<td>Lyon et al. (1987)</td>
</tr>
<tr>
<td>pI524</td>
<td>1953</td>
<td>PcAsaAsiCdHg</td>
<td>31.8</td>
<td>+</td>
<td>Gillespie et al. (1985)</td>
</tr>
<tr>
<td>pSK74</td>
<td>1953</td>
<td>PcAsaAsiCdHg</td>
<td>31.8</td>
<td>+</td>
<td>Gillespie et al. (1985)</td>
</tr>
</tbody>
</table>

Strain

- SK1655: 1965 SmTc – Gillespie et al. (1987a)
- SK1660: 1976 PcMcSmTcEmCdHg + Gillespie et al. (1987b)
- SK1717: 1977 PcMcSmTcEmGmTmKmNmAkCdHg + Gillespie et al. (1987b)
- SK1734: 1978 PcMcSmTcEmGmTmKmNmCmCdHg + Gillespie et al. (1987b)
- SK1903: 1978 PcSmTcGmTmKmCd + Gillespie et al. (1987b)
- SK460: 1981 PcMcSmTcEmGmTmKmNmAkCdHg + Gillespie et al. (1987b)
- SK1589: 1981 PcMcSmTcEmCmCdHg + Gillespie et al. (1987b)
- SK1591: 1981 PcSmTcEm + Gillespie et al. (1986a)

* Ac, acriflavine; Ak, amikacin; Asa, arsenate; Asi, arsenite; Cd, cadmium; Cm, chloramphenicol; Eb, ethidium bromide; Em, erythromycin; Gm, gentamicin; Hg, mercury; Km, kanamycin; Mc, methicillin; Nm, neomycin; Pc, penicillin; Pm, paromomycin; Qa, quaternary ammonium compounds; Sm, streptomycin; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim.

et al., 1988). We have investigated the nature of the Pe' determinants in Australian isolates of *S. aureus* and in this paper we report our findings, compare these determinants with Tn552 and a Tn4201-like element from the self-transmissible plasmid pUW3626, and provide evidence for the evolutionary spread of Pe' determinants between different plasmids and the chromosome.

**METHODS**

**Bacterial plasmids and strains.** These are listed in Table 1, together with relevant characteristics. We are grateful to G. K. Best, Medical College of Georgia, USA, M. L. Cohen, Center for Infectious Diseases, Atlanta, Georgia, USA, and R. P. Novick, Public Health Research Institute of the City of New York, USA, for providing strains containing plasmids pSK41, pUW3626 and pI524, respectively.

**General procedures.** Standard culture media and methods for the determination of β-lactamase production and resistance to antimicrobials and heavy metals were as previously described (Lyon et al., 1983; Gillespie et al., 1985).

**DNA manipulations and analysis.** The isolation of purified plasmid DNA and whole-cell DNA, digestion with restriction endonucleases, agarose gel electrophoresis, the estimation of DNA fragment sizes, and all recombinant DNA techniques were done as reported previously (Lyon et al., 1983, 1984a, 1987). The isolation and radiolabelling of DNA probes, DNA–DNA hybridization, and examination of heteroduplexed DNA by electron microscopy were done essentially as described in earlier papers (Lyon et al., 1984b, 1987; Gillespie et al., 1986b).

**RESULTS AND DISCUSSION**

**β-Lactamase transposons on *S. aureus* plasmids**

The 35.1 kb plasmid pSK4 (Lyon et al., 1983; Gillespie et al., 1987b), in addition to encoding Ac'Eb'Qa', Gm'Tm'Km' and Tp', was shown to encode Pe' via the production of a β-lactamase. Comparative restriction analysis revealed that pSK4 differed from the 28.4 kb Ac'Eb'Qa', Gm'Tm'Km' and Tp' plasmid pSK1 only through the possession of an additional 6.7 kb insert of DNA lying between the Tp' and Ac'Eb'Qa' determinants (drfA and qacA, respectively) (Fig. 1). Another five plasmids (pSK8, pSK11, pSK15, pSK16 and pSK17) were
Q-Lactamase determinants of S. aureus

found to have insertions of identical size and restriction pattern at demonstrably different sites and in the opposite orientation to that in pSK4 (Fig. 1). One of the plasmids, pSK11, was shown to possess two such insertions, the first at an identical site to pSK8 and the second within qacA, resulting in an Ac'Eb'Qa' plasmid phenotype.

Although the different sites of insertion exhibited some degree of regional specificity, the orientation independence of the insert, together with demonstrated insertional inactivation, suggested that the 6.7 kb Pcr region on these plasmids might be transposable. In agreement with this, genetic evidence for the translocation of a 6.7 kb Pcr element from chromosome to plasmid was obtained by the detection of two insert-bearing derivatives of pSK1 following storage of strains of S. aureus bearing pSK1 and a chromosomal Pcr determinant. One of the resultant plasmids, pSK40, was indistinguishable from pSK4, whereas the other, pSK12, had suffered a 1.1 kb deletion immediately to the left of the 6.7 kb DNA insert (Fig. 1).

In a manner similar to that detailed above, heteroduplexes were generated between the self-transmissible plasmid pUW3626, which is highly related to pCRG1600 (Goering et al., 1985) and probably carries a Pcr element similar to Tn4201 on that plasmid (Weber & Goering, 1988), and the β-lactamase-negative plasmid pSK41, which lacks a Tn4201-like element but is otherwise identical to pUW3626 (Lyon et al., 1987). Heteroduplexes between these two plasmids exhibited a 6.5 ± 0.06 kb single-stranded DNA segment flanked by terminal inverted repeats of less than 80 bp; a total of three molecules formed between pSK1 and pSK4 were examined as were six molecules with a similar transposon-like structure formed in heteroduplexes between pSK1 and pSK8 (data not shown). Together, the genetic and physical data indicated that the Pcr determinant detected on members of the pSK1 family of Ac'Eb'Qa' plasmids comprised a transposable element, which we have designated Tn4002 (Gillespie & Skurray, 1986; Lyon & Skurray, 1987).

In order to conduct further comparisons both between these elements and with the β-lactamase transposon Tn552, detailed restriction maps of the plasmids carrying these β-lactamase elements were prepared.

Tn552 resides on the 31.8 kb heavy-metal-resistance plasmid pI524 (Murphy & Novick, 1979), which we have shown by extensive restriction and hybridization analysis to be equivalent
Fig. 2. Electron micrographs of heteroduplexes between (a) pSK1 and pSK4 and (b) pSK41 and pUW3626. Plasmid DNA was cleaved with EcoRI and the ends of the heteroduplexed fragments are designated A and B. In (a), ends A and B correspond to the EcoRI sites at coordinates 13-55 and 25.35 kb of pSK4, while in (b) ends A and B denote the EcoRI sites at pUW3626 map coordinates 42.8 and 54.4 kb (see Fig. 3). The arrow marked C indicates the single-stranded loop and short double-stranded stem of the Pc' region of (a) pSK4 (Tn4002) and (b) pUW3626.

The β-lactamase region of pI524/pSK74 was found to share substantial restriction similarity with the β-lactamase regions of pSK4 (Tn4002) and pUW3626 (Fig. 3). The blaI and blaZ segments on these plasmids appeared indistinguishable, and hybridization analysis using the bla-specific 0.8 kb HindIII-XbaI fragment derived from pSK74 as a 32P-labelled probe supported this result (data not presented). That the 0.8 kb HindIII-XbaI fragment includes sequences of the β-lactamase structural gene (blaZ) has been determined by Tn5 mutagenesis of cloned fragments of pSK4 in our laboratory, and by the nucleotide sequencing of the blaZ gene from the heavy-metal-resistance plasmid pI258 (McLaughlin et al., 1981; Wang & Novick,
Fig. 3. Comparative restriction maps of the β-lactamase regions of pI524/pSK74, pSK4 and pUW3626. Solid lines represent adjacent sequences on the plasmids; restriction sites are indicated by B (BglII), E (EcoRI), H (HindIII), Ha (HaeIII), Hh (HpalI), Hh (HpalII), Pv (PvuII), S (Saul), V (EcoRV) and X (XhoI). Map coordinates are expressed in kb and correlate with the published maps of pI524/pSK74 (Gillespie et al., 1986b), pSK4 (Gillespie & Skurray, 1986) and pUW3626 (Lyon et al., 1987). The invertible region (inv) of pI524/pSK74 is shown in the (+) and (−) orientations, with the inverted repeats (IRL and IRR) designated for the (+) orientation. The short inverted repeats of the β-lactamase elements on pSK4 (Tn4002) and pUW3626, as detected by our heteroduplex analysis, are indicated by the open boxes; it remains to be established if there are repeat structures at the ends of Tn552 on pI524/pSK74. The regions coding for the β-lactamase structural gene, bluZ (Wang & Novick, 1987), and for negative control of β-lactamase production (blaZ) are also shown; the exact location and extent of blaZ has not been reported. Shown at the bottom are the fragments derived from a subclone of pSK74 and used as hybridization probes to detect inv- and blaZ-specific sequences (see text and Fig. 4).
1987); the blaZ gene on the latter plasmid is equivalent to that encoded on pSK4, pSK74 and pUW3626 on the basis of restriction mapping and DNA hybridization (unpublished data). Some variance was detected in the remainder of the β-lactamase region, with pI524/pSK74 and pSK4 sharing seven of the eight restriction sites determined in the in0 segment, and pUW3626 possessing five of these sites (Fig. 3). In both Tn4002 on pSK4 and the Pc element on pUW3626, inv appeared to be incapable of inversion, existing solely in the negative orientation of the inversion, a consequence, perhaps, of the absence of IRg. Recently, Weber & Goering (1988) have reported a restriction analysis of the Pc transposon Tn4201 from the self-transmissible plasmid pCRG1600. Comparison of restriction maps indicates that Tn4201 is related to Tn4002 and to the Pc regions of pI524/pSK74 and pUW3626; from the arrangement of restriction sites, Tn4201 also carries inv in the negative orientation (Webber & Goering, 1988).

Restriction endonuclease, DNA hybridization and homoduplex/heteroduplex analyses have demonstrated that other members of the pSK1 plasmid family (viz. pSK8, pSK11, pSK12, pSK15, pSK16, pSK17 and pSK40) also carry Tn4002 (data not presented). Similar studies on heavy-metal-resistance plasmids isolated from S. aureus strains collected in Australia between 1947 and 1965 (Gillespie et al., 1985), while revealing the existence of Pc elements with homologous bla and inv segments, showed that inv in these elements was often capable of inversion or was maintained in the (+) orientation (M. Gillespie & R. Skurray, unpublished). In this respect, the Pc elements detected on Australian heavy-metal-resistance plasmids mirrored those found elsewhere during this period (Murphy & Novick, 1979; Shalita et al., 1980).

Detection of Tn4002 on S. aureus chromosomes

The majority of Pc S. aureus collected in Australia since the early 1970s were found to possess chromosomally-encoded β-lactamase (Gillespie et al., 1985) which, in some instances, was shown to be Tn4002 on the basis of translocation to a resident plasmid (see above). Such strains were therefore examined for the presence of chromosomal sequences homologous to Tn4002, using hybridization probes for bla and inv consisting of the 0-8 kb HindIII-XbaI and 1-65 kb BglI-PvuII fragments of pSK74, respectively (Fig. 3).

Hybridization of HaeIII-digested whole-cell DNA from six of seven S. aureus strains exhibiting chromosomal β-lactamase production with the bla-specific probe revealed a 2-55 kb HaeIII fragment of identical size to that detected in digests of heavy-metal-resistance plasmids, pSK1 family plasmids and self-transmissible plasmids encoding Pc, thereby suggesting that homologous determinants are responsible for both plasmid and chromosomal Pc; in the remaining isolate, SK1903, an 8-7 kb HaeIII fragment hybridized with the probe (data not presented). However, bla-specific hybridization with HindIII digests of whole-cell DNA from these strains revealed that a 4-5 kb HindIII fragment hybridized with the probe in strains which possessed bla sequences on the 2-55 kb HaeIII fragment (Fig. 4a, lanes D, E, F, H, I and J), whereas SK1903 (Fig. 4a, lane G) carried bla sequences on a 1-3 kb HindIII fragment. Therefore, neither of the chromosomal HindIII fragments with bla homology was equivalent to the bla-containing HindIII fragments of pSK74 (Fig. 4a, lane A), pSK4 or pUW3626, indicating that such plasmids had probably not integrated, in their entirety, into the genome. Further support for this notion resulted from the finding that a mercury-resistance region, highly related to that found on β-lactamase/heavy-metal-resistance plasmids, physically maps on the chromosome in strains such as SK460, SK1589, SK1660, SK1717, and SK1734 (Table 1) not in association with other β-lactamase/heavy-metal plasmid sequences but with the determinants for methicillin and tetracycline resistance (Gillespie et al., 1987a; Matthews et al., 1987; M. Gillespie & R. Skurray, unpublished data).

To determine if the chromosomal β-lactamase regions also possessed an invertible region and therefore represented a complete Pc element such as Tn552 or Tn4002, appropriately digested whole-cell DNA was hybridized with the inv-specific probe. The six chromosomal strains which exhibited similar HaeIII and HindIII fragments when hybridized with the bla-specific probe were found to possess an equivalent 8-1 kb HindIII fragment with homology to inv; this fragment did not correspond in size with the inv-specific HindIII fragments of pSK74, pSK4 or pUW3626 (data not presented). As with the bla sequences, the chromosomal inv sequences are
seemingly contiguous with genomic and not integrated plasmid DNA. Two strains, SK1717 and SK1734, each demonstrated an additional HindIII fragment with inv sequence homology, thereby suggesting that the chromosomes of these strains possess two copies of inv; the \( \beta \)-lactamase producing and non-producing isolates, SK1903 and SK1655, respectively (Fig. 4a, lanes G and C), failed to hybridize with the inv-specific probe, indicating that neither strain carries inv sequences. Hence, although SK1903 possesses bla sequences, it does not carry an element equivalent to Tn4002.

Having established the presence of both bla and inv sequences on the chromosomes of several Pcr S. aureus, whole-cell DNA was digested with BglII and HindIII and hybridized with the inv-specific probe to confirm the contiguity of the two regions (as shown in Fig. 3) and to determine the orientation(s) of the invertible region in each case. A BglII–HindIII fragment of 4.5 kb hybridized with this probe in those six isolates which carried chromosomal \( \beta \)-lactamase and exhibited inv sequence homology (Fig. 4b, lanes D, E, F, H, I and J). This fragment was equivalent in size to that produced by Tn4002 in pSK4 (Fig. 4b, lane B) and to that resulting from the (−) orientation of inv in pSK74 (Fig. 4b, lane A); the 4.8 kb BglII–HindIII fragment of pSK74 with inv homology resulted from the (+) orientation of inv (Fig. 4b, lane A). Strains SK1717 and SK1734 (Fig. 4b, lanes E and F) each demonstrated an additional 3.2 kb fragment with inv sequence homology which presumably resulted from the extra copy of inv on the chromosomes of these strains.

The finding that the chromosomal inv in the (−) orientation is located adjacent to the bla locus, together with the evidence presented above indicating that this \( \beta \)-lactamase region is not associated with an integrated plasmid, suggests that the chromosomes of a number of the Pcr S. aureus strains isolated in Australia since 1976 (Table 1) possess copies of a Pcr element equivalent to Tn4002. Such evidence reinforces the idea that the presence of Tn4002 on the pSK1 family of plasmids in clinical isolates may have resulted from the transposition of this element from the chromosome, an event equivalent to the transposition we have detected with laboratory-derived insertions of Tn4002 into pSK1.
The chromosomal $\beta$-lactamase determinant described by Asheshov (1969) has also been shown to be transposable and is designated Tn552 (Shalita et al., 1980). This designation results from the observation that a 6-1 kb DNA segment specifying $\beta$-lactamase production transposed from its chromosomal location in staphylococcal strain 9789 to the resident Pc-resistance plasmid pI9789, thereby constructing the Pc-resistance plasmid pRN4115 (Asheshov, 1969; Murphy & Novick, 1979). Tn552 was subsequently found to be identical to the pI524 $\beta$-lactamase region, which included the bla determinant and inv as a reversibly invertible segment (Murphy & Novick, 1979; Shalita et al., 1980). However, from the reported size of Tn552 (6-1 kb) and that for the $\beta$-lactamase region of pI524 (6-8 kb) a discrepancy of 0-7 kb (the size of one of the IRs of inv) is evident (Murphy & Novick, 1979). Comparison of the reported restriction endonuclease fragment sizes of pI9789 (Shalita et al., 1980) indicates that this plasmid contains a 0-7 kb DNA segment near the position at which inv would be located following transposition of Tn552. This led to the suggestion that pI9789 carries one of the IRs of inv (Shalita et al., 1980) and it is therefore possible that transposition of Tn552 to a site immediately adjacent to this IR sequence in pI9789 could result in a $\beta$-lactamase region equivalent to that of pI524. Alternatively, such a structure might have arisen by recombination between the IR located on Tn552 and that proposed to be present on pI9789. Such IR-mediated recombination/translocation might also explain the site-specificity of the Tn552 insertion to this plasmid (Murphy & Novick, 1979). If this argument is correct, Tn552 would contain only one IR sequence of inv, thereby making the three transposons Tn552, Tn4002 and Tn4201 very close relatives. It would also then be likely that the inv of Tn552 on the chromosome of strain 9789 is in the (−) orientation, as with inv on Tn4002, Tn4201 and pUW3626, since the second IR (IR$_k$) is essential for reversible inversion to take place. IR$_k$ has recently been demonstrated to encode a DNA invertase, Bin, related to the Hin family of site-specific recombinases; bin may also be implicated in the transposition of these Pc-resistance elements (Rowland & Dyke, 1988).

**Evolution of $\beta$-lactamase transposons in S. aureus**

The preceding evidence suggests an interesting scenario for the evolution of Pc-resistance elements in Australian S. aureus strains over the last 40 years. Resistance to penicillin in the majority of isolates during the first 20 years of antibiotic therapy was mediated by heavy-metal-resistance plasmids carrying elements such as Tn552 (Gillespie et al., 1985; M. Gillespie & R. Skurray, unpublished). The 1970s saw the emergence of strains exhibiting chromosomal Pc-resistance, presumably as a result of the transposition of Tn552-like elements to the chromosome rather than the integration of entire $\beta$-lactamase/heavy-metal-resistance plasmids. In the strains we have examined, the latter is improbable given the failure to detect contiguous plasmid sequences at the chromosomal $\beta$-lactamase locus and the inability of chromosomal inv sequences to undergo reversible inversion. Within the same decade two new families of plasmids became associated with Pc-resistance elements, as the self-transmissible plasmids in isolates from the USA gained elements such as Tn4201, perhaps through cohabitation with compatible heavy-metal-resistance plasmids (Gray et al., 1983), and the pSK1 family of plasmids in multi-resistant Australian isolates almost certainly acquired Tn4002 from a chromosomal site. The underlying reasons for this evolutionary course are obscure, but are undoubtedly a response to changing selection pressures in the hospital environment and may well be linked to antibiotic therapy practices of the periods in question.

The Pc-resistance elements Tn552, Tn4002 and Tn4201 have presumably diverged from a common ancestor and further analysis should shed light on the molecular rearrangements involved in this process. Of particular interest for future study is the specificity of insertion exhibited by these elements. Tn552 (Murphy & Novick, 1979) and Tn4201 (Weber & Goering, 1988) both show specificity of insertion for particular regions of plasmid vectors, or in the case of the latter, the chromosome. With Tn552 the specificity is for the invertible region IR, and, as pointed out above, such an insertion may result from IR-specific recombination/translocation rather than conventional transposition. In contrast to these elements, Tn4002 was shown to be inserted at demonstrably different sites within a 600 bp region of the pSK1 family of plasmids but, like Tn4201, occupied an identical chromosomal site in six distinct strains. Such evidence suggests
that Tn4002 had translocated from a single chromosomal site to the co-resident pSK1 plasmid on a number of occasions. The two copies of Tn4002 in pSK11 may reflect secondary insertion when the preferred region is occupied, or may alternatively represent intramolecular transposition. The fact that a transposable element with such apparently complex specificity is compatible with at least three radically different plasmid vectors, together with the chromosome, suggests an intimate evolutionary development of all of these components.

We wish to thank Linda Messerotti for technical assistance. This work was supported in part by a Project Grant from the National Health and Medical Research Council (Australia). M. T. G. and B. R. L. were recipients of Commonwealth Postgraduate Research Awards.

REFERENCES


