Structural and Evolutionary Relationships of β -Lactamase Transposons from *Staphylococcus aureus*

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(Received 15 July 1988)

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^r) is often mediated by a β -lactamase (penicillinase) which inactivates the antibiotic through hydrolysis of the β -lactam ring. The enzymes responsible for Pc^r 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^r 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^r 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 & Dünnhaupt, 1984). The Pc^r 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^r 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^r has been detected on some members of a unique family of Eb^rQa^r and acriflavine resistance (Ac^r) plasmids which often additionally encode $Gm^rTm^rKm^r$ via Tn4001 and/or trimethoprim resistance (Tp^r) through the carriage of a unique dihydrofolate reductase determinant (*dhfrA*) on the transposon Tn4003 (Gillespie & Skurray, 1986; Lyon & Skurray, 1987; Lyon *et al.*, 1987; Young *et al.*, 1987; Skurray

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Table 1. *Characteristics of S. aureus plasmids and strains*

	Year of isolation	Resistance to*	Size (kb)	β -Lactamase production	Reference
Plasmid					
pSK1	1980	GmTmKmAcEbQaTp	28.4	—	Lyon <i>et al.</i> (1987)
pSK4	1981	GmTmKmAcEbQaTpPc	35.1	+	Gillespie <i>et al.</i> (1987b)
pSK8	1981	GmTmKmAcEbQaTpPc	35.1	+	Gillespie <i>et al.</i> (1987b)
pSK11	1980	GmTmKmTpPc	41.8	+	Gillespie <i>et al.</i> (1987b)
pSK15	1982	GmTmKmAcEbQaPc	31.1	+	Gillespie <i>et al.</i> (1987b)
pSK16	1982	GmTmKmAcEbQaTpPc	35.1	+	Gillespie <i>et al.</i> (1987b)
pSK17	1981	GmTmKmAcEbQaPc	36.4	+	Gillespie <i>et al.</i> (1987b)
pSK41	1977	GmTmKmNmPmEbQa	47.8	—	Lyon <i>et al.</i> (1987)
pUW3626	1981	GmTmKmNmPmEbQaPc	54.4	+	Lyon <i>et al.</i> (1987)
pI524		PcAsaAsiCdHg	31.8	+	Murphy & Novick (1979)
pSK74	1953	PcAsaAsiCdHg	31.8	+	Gillespie <i>et al.</i> (1985)
Strain					
SK1655	1965	SmTc		—	Gillespie <i>et al.</i> (1987a)
SK1660	1976	PcMcSmTcEmCdHg		+	Gillespie <i>et al.</i> (1987b)
SK1717	1977	PcMcSmTcEmGmTmKmNmAkCdHg		+	Gillespie <i>et al.</i> (1987b)
SK1734	1978	PcMcSmTcEmGmTmKmNmCmCdHg		+	Gillespie <i>et al.</i> (1987b)
SK1903	1978	PcSmTcGmTmKmCd		+	Gillespie <i>et al.</i> (1987b)
SK460	1981	PcMcSmTcEmGmTmKmNmAkCdHg		+	Gillespie <i>et al.</i> (1987b)
SK1589	1981	PcMcSmTcEmCmCdHg		+	Gillespie <i>et al.</i> (1987b)
SK1591	1981	PcSmTcEm		+	Gillespie <i>et al.</i> (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 Pc^r 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 Pc^r 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 $Ac^rEb^rQa^r$, $Gm^rTm^rKm^r$ and Tp^r , was shown to encode Pc^r via the production of a β -lactamase. Comparative restriction analysis revealed that pSK4 differed from the 28.4 kb $Ac^rEb^rQa^r$, $Gm^rTm^rKm^r$ and Tp^r plasmid pSK1 only through the possession of an additional 6.7 kb insert of DNA lying between the Tp^r and $Ac^rEb^rQa^r$ determinants (*dfrA* and *qacA*, respectively) (Fig. 1). Another five plasmids (pSK8, pSK11, pSK15, pSK16 and pSK17) were

Fig. 1. Comparative restriction maps of pSK1 and Pc^r plasmid pSK4. Restriction endonuclease sites are indicated by B (*Bgl*II) and E (*Eco*RI). The sites of insertion and relative orientations (indicated by the arrowheads) of the 6.7 kb Pc^r transposon Tn4002 in the plasmids pSK4, pSK8, pSK11, pSK12, pSK15, pSK16, pSK17 and pSK40 are indicated on the map of the prototype Pc^r plasmid pSK1. Regions encoding Tp^r (*dfrA*), $Ac^rEb^rQa^r$ (*qacA*) and the transposon encoding Gm^r , Tm^r and Km^r (Tn4001) are also indicated.

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 $Ac^sEb^sQa^s$ 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 Pc^r region on these plasmids might be transposable. In agreement with this, genetic evidence for the translocation of a 6.7 kb Pc^r 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 Pc^r 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).

Heteroduplex analysis of pSK4 and pSK1 (Fig. 2a), revealed 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 Pc^r determinant detected on members of the pSK1 family of $Ac^rEb^rQa^r$ plasmids comprised a transposable element, which we have designated Tn4002 (Gillespie & Skurray, 1986; Lyon & Skurray, 1987).

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 Pc^r 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 6.6 ± 0.5 kb structure composed of a single-stranded loop with inverted repeat sequences of less than 80 bp (results from six molecules) (Fig. 2b). Hence, the Pc^r transposon Tn4002 and the Tn4201-like element on pUW3626 possess several common characteristics. 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 *Eco*RI and the ends of the heteroduplexed fragments are designated A and B. In (a), ends A and B correspond to the *Eco*RI sites at coordinates 13.55 and 25.35 kb of pSK4, while in (b) ends A and B denote the *Eco*RI 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*^r region of (a) pSK4 (Tn4002) and (b) pUW3626.

to pSK 74, a plasmid isolated from Australian isolates of *S. aureus* (Gillespie *et al.*, 1985). The β -lactamase region of pI524/pSK 74 consists of two segments (Fig. 3). The first is composed of the β -lactamase structural gene (*blaZ*) and the gene for negative control of β -lactamase production (*blaI*), and the second, *inv*, comprises a 2.2 kb region of DNA flanked by 650 bp inverted repeat sequences (*IR*_L and *IR*_R) which can undergo reversible *rec*-independent inversion (Murphy & Novick, 1979). The orientation of *inv* can be determined from the position of asymmetrically located restriction sites, and analysis of pI524/pSK 74 has revealed the presence of subpopulations of molecules bearing *inv* in both the positive and negative orientations (see Murphy & Novick, 1979).

The β -lactamase region of pI524/pSK 74 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 *Hind*III-*Xba*I fragment derived from pSK 74 as a ³²P-labelled probe supported this result (data not presented). That the 0.8 kb *Hind*III-*Xba*I 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 p1524/pSK74, pSK4 and pUW3626. Solid lines represent adjacent sequences on the plasmids; restriction sites are indicated by B (*Bgl*II), E (*Eco*RI), H (*Hind*III), Ha (*Hae*III), H₁ (*Hpa*I), H₁₁ (*Hpa*II), S (*Sal*I), V (*Eco*RV) and X (*Xba*I). Map coordinates are expressed in kb and correlate with the published maps of p1524/pSK74 (Gillespie *et al.*, 1986b), pSK4 (Gillespie & Skurray, 1986) and pUW3626 (Lyon *et al.*, 1987). The invertible region (*inv*) of p1524/pSK74 is shown in the (+) and (-) orientations, with the inverted repeats (IR_L and IR_R) designated for the (+) and (-) 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 p1524/pSK74. The regions coding for the β -lactamase structural gene, *bla*Z (Wang & Novick, 1987), and for negative control of β -lactamase production (*bla*I) are also shown; the exact location and extent of *bla*I 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 *bla*-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 *inv* segment, and pUW3626 possessing five of these sites (Fig. 3). In both Tn4002 on pSK4 and the Pc^r 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 IR_R . Recently, Weber & Goering (1988) have reported a restriction analysis of the Pc^r transposon Tn4201 from the self-transmissible plasmid pCRG1600. Comparison of restriction maps indicates that Tn4201 is related to Tn4002 and to the Pc^r regions of pI524/pSK74 and pUW3626; from the arrangement of restriction sites, Tn4201 also carries *inv* in the negative orientation (Weber & 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^r 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^r 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^r *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 *Hind*III-*Xba*I and 1.65 kb *Bgl*II-*Pvu*II fragments of pSK74, respectively (Fig. 3).

Hybridization of *Hae*III-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 *Hae*III fragment of identical size to that detected in digests of heavy-metal-resistance plasmids, pSK1 family plasmids and self-transmissible plasmids encoding Pc^r , thereby suggesting that homologous determinants are responsible for both plasmid and chromosomal Pc^r ; in the remaining isolate, SK1903, an 8.7 kb *Hae*III fragment hybridized with the probe (data not presented). However, *bla*-specific hybridization with *Hind*III digests of whole-cell DNA from these strains revealed that a 4.5 kb *Hind*III fragment hybridized with the probe in strains which possessed *bla* sequences on the 2.55 kb *Hae*III 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 *Hind*III fragment. Therefore, neither of the chromosomal *Hind*III fragments with *bla* homology was equivalent to the *bla*-containing *Hind*III 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^r 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 *Hae*III and *Hind*III fragments when hybridized with the *bla*-specific probe were found to possess an equivalent 8.1 kb *Hind*III fragment with homology to *inv*; this fragment did not correspond in size with the *inv*-specific *Hind*III fragments of pSK74, pSK4 or pUW3626 (data not presented). As with the *bla* sequences, the chromosomal *inv* sequences are

Fig. 4. (a) Autoradiograph following hybridization of *Hind*III-cleaved *S. aureus* plasmid and whole-cell DNA with a specific probe for *bla* sequences comprising the 0.8 kb *Hind*III-*Xba*I fragment of pSK74. Lanes: A, pSK74; B, —; C, SK1655; D, SK1660; E, SK1717; F, SK1734; G, SK1903; H, SK460; I, SK1589; J, SK1591. Molecular size markers (*Hind*III-cleaved λ phage DNA) are shown at left. (b) Autoradiograph following hybridization of *Bgl*II- and *Hind*III-cleaved *S. aureus* plasmid and whole-cell DNA with a specific probe for *inv* sequences comprising the 1.65 kb *Bgl*II-*Pvu*II fragment of pSK74. Lanes: A, pSK74; B, pSK4; C, SK1655; D, SK1660; E, SK1717; F, SK1734; G, SK1903; H, SK460; I, SK1589; J, SK1591. Molecular size markers (*Hind*III-cleaved λ phage DNA) are shown at left.

seemingly contiguous with genomic and not integrated plasmid DNA. Two strains, SK1717 and SK1734, each demonstrated an additional *Hind*III fragment with *inv* sequence homology, thereby suggesting that the chromosomes of these strains possess two copies of *inv*; the β -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 Pc^r *S. aureus*, whole-cell DNA was digested with *Bgl*II and *Hind*III 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 *Bgl*II-*Hind*III fragment of 4.5 kb hybridized with this probe in those six isolates which carried chromosomal β -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 *Bgl*II-*Hind*III 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 β -lactamase region is not associated with an integrated plasmid, suggests that the chromosomes of a number of the Pc^r *S. aureus* strains isolated in Australia since 1976 (Table 1) possess copies of a Pc^r 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 β -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 β -lactamase production transposed from its chromosomal location in staphylococcal strain 9789 to the resident Pc^s plasmid pI9789, thereby constructing the Pc^r plasmid pRN4115 (Asheshov, 1969; Murphy & Novick, 1979). Tn552 was subsequently found to be identical to the pI524 β -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 β -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 β -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_R) is essential for reversible inversion to take place. IR_R 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^r elements (Rowland & Dyke, 1988).

Evolution of β -lactamase transposons in S. aureus

The preceding evidence suggests an interesting scenario for the evolution of Pc^r 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^r , presumably as a result of the transposition of Tn552-like elements to the chromosome rather than the integration of entire β -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 β -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^r 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 multiresistant 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^r 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 pSK1 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.

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