Tn554 inserts in methicillin-resistant *Staphylococcus aureus* from Australia and England: comparison with an American methicillin-resistant group

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We have compared methicillin-resistant (Mc^r) *Staphylococcus aureus* isolates from Australia, the UK and the USA with regard to chromosomal inserts of the macrolides–lincosamides–streptogramin B (MLS)-resistance transposon Tn554. The American isolates were known to have a distinctive Tn554 insert, designated insert 6, which was closely associated epidemiologically with the methicillin-resistance phenotype. Southern blots of DNA from Australian and London, UK Mc^r isolates were hybridized with a range of probes related to Tn554. The isolates had similar or identical Tn554 inserts, and we consider them to be a single group, designated 'Australondon'. Australondon isolates were compared in detail with a deletion mutant, ANS62, that had lost the methicillin-resistance determinant *mec*, plus other resistance determinants resident in the *mec* region of the chromosome, and with an American Mc^r isolate containing Tn554 insert 6. The Australondon isolates had three Tn554 inserts. Sequence analysis with the polymerase chain reaction showed that all of these inserts differed from classical Tn554 in that the 3'-terminal residues of the transposons were reverse complements of the usual GATGTA. One of the Australondon inserts, designated 6B, closely resembled Tn554 insert 6 in the sequence of its left flanking chromosomal DNA. This insert was found to abut the deletion from the *mec* region which results in strain ANS62. We infer that Tn554 insert 6B is part of the *mec* region of the chromosome in Australondon isolates, supporting the idea that insert 6 of the American isolates is also part of this chromosomal region.

Introduction

In the past decade, hospitals in New Jersey, USA (Tillotson *et al.*, 1989), eastern Australia (Pavillard *et al.*, 1982; Lyon *et al.*, 1983) and London, UK (Cookson *et al.*, 1986; Cookson & Phillips, 1988) have suffered outbreaks of infection by methicillin-resistant (Mc^r) *Staphylococcus*

aureus. The predominant isolates within each location are sufficiently similar to each other to suggest regional spread of a single, or of a few closely related, strain(s). We refer to these sets of isolates as groups. The New Jersey group was identified during a survey for macrolides–lincosamides–streptogramin B (MLS)-resistant staphylococci, and was characterized by Southern blotting DNA from representative isolates with probes related to MLS determinants (Tillotson *et al.*, 1989). This group was MLS^r due to *ermA* genes situated in copies of the classical *ermA*-containing chromosomal transposon, Tn554. Tn554 is a relatively site-specific transposon that, in experiments with derivatives of the laboratory *S. aureus* strain 8325, was found to insert into a single (primary) chromosomal attachment site, *att554* (Krolewski *et al.*, 1981; Murphy & Löfdahl, 1984; Murphy,

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Abbreviations: MLS, macrolides–lincosamides–streptogramin B; PCR, polymerase chain reaction; EMRSA, epidemic methicillin-resistant *S. aureus*; ΔANS46/62, the DNA deleted from strain ANS46 yielding strain ANS62.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number M32312.

1989). However, we have shown that contemporary clinical isolates often have secondary Tn554 inserts, as defined by the presence of inserts that differ substantially from the primary insert in flanking chromosomal DNA restriction maps (Thakker-Varia *et al.*, 1987; Tillotson *et al.*, 1989). We have designated a series of different secondary inserts by the numbers 2 to 8, in distinction to the primary insert, assigned the number 1 (Tillotson *et al.*, 1989). Three of the inserts relevant to the present work are shown in Fig. 1.

The New Jersey Mc^r outbreak group contained, in addition to the primary insert, secondary insert #6 in chromosomal attachment site *att155*, not detected in any Mc^s *S. aureus* strains. Earlier studies had shown that Mc^r *S. aureus* isolates contain chromosomal DNA, amounting to 30 kb or more, which is also absent from Mc^s strains (Beck *et al.*, 1986; Matsushashi *et al.*, 1986; Matthews *et al.*, 1987). This DNA contains the Mc^r determinant *mec* and determinants for various other resistances (Skinner *et al.*, 1988; Skurray *et al.*, 1988; Ubukata *et al.*, 1989). It was proposed that Tn554 insert 6 of the New Jersey Mc^r group might likewise be part of the *mec* region (Tillotson *et al.*, 1989).

The Australian and London Mc^r isolates were known to resemble the New Jersey group in having chromosomal MLS^r determinant(s) (Townsend *et al.*, 1983, 1987; Matthews *et al.*, 1987). To explore further the possibility of a physical relationship between Tn554 inserts and the *mec* complex of resistance genes, we have characterized the Tn554 elements carried by the Australian and London Mc^r groups. A variant (ANS62) of the Australian Mc^r strain ANS46 that had become Mc^s because of deletion of part of its *mec* region was especially informative (Matthews *et al.*, 1987). The Australian and London Mc^r groups were found to resemble each other and to differ from the New Jersey group by harbouring three Tn554 inserts, all of which had distinctive transposon-chromosomal junction sequences. One of these inserts, designated 6B, was of special interest. It closely resembled New Jersey Tn554 insert 6 with regard to the left arm of its attachment site and it was indeed found to be in the *mec* region.

Methods

Bacterial strains. *S. aureus* isolates (Table 1) were checked for antibiotic susceptibilities (Barry & Thornsberry, 1980; Jenssen *et al.*, 1987) just prior to DNA preparation. The plasmid pEM715 carrying a cloned copy of Tn554 was provided by E. Murphy (Public Health Research Institute, New York, USA), in *S. aureus* strain RN4932 (Thakker-Varia *et al.*, 1987).

DNA methods. Total cellular DNA was isolated after lysing cells with lysozyme, lysostaphin and SDS (Tillotson *et al.*, 1989); proteinase K

Table 1. *Bacterial strains*

Designation	Origin	Description, and source or reference
N61	London, UK	EMRSA, pT181(+) (this work)
N62		EMRSA, pT181(-) (this work)
ANS46	Melbourne, Australia	Prototype Australian epidemic Mc ^r isolate (Matthews <i>et al.</i> , 1987)
ANS62		Mc ^s derivative of ANS46 (Matthews <i>et al.</i> , 1987)
R155*	New Jersey, USA	Prototype New Jersey Mc ^r isolate, with a primary Tn554 insert (in <i>att554</i>), and insert 6 (in <i>att155</i>) (Tillotson <i>et al.</i> , 1989)
WJ137		Prototype New Jersey Mc ^s isolate, with a primary Tn554 insert (in <i>att554</i>), and insert 2 (in <i>att137</i>) (Thakker-Varia <i>et al.</i> , 1987)

* Previously designated R31, this isolate is indistinguishable in our hands from isolate 155, the strain after which *att155* was named (Tillotson *et al.*, 1989). In the interest of simplicity we renamed it R155.

(200 µg ml⁻¹) was substituted for pronase. Southern blot analysis was done as previously described (Thakker-Varia *et al.*, 1987; Tillotson *et al.*, 1989) except that DNA segment probes were sometimes labelled by random primer extension (Feinberg & Vogelstein, 1986). Most oligonucleotide probes were designed to have apparent *T_m* values of 46–48 °C, and hybridizations were done at *T_m* minus 3–5 °C (Berent *et al.*, 1986). Filters hybridized with labelled DNA segments were washed twice with 6 × SSC (1 × SSC is 0.15 M-NaCl, 15 mM-sodium citrate, pH 7.0), twice with 1 × SSC and twice with 0.1 × SSC, at room temperature for 20 min. Filters hybridized with labelled oligonucleotides were washed with 90 mM-Tris/HCl (pH 8.0) containing 6 mM-EDTA, 0.9 M-NaCl and 0.1% SDS, four times for 10 min at room temperature, and once for 1 min at the hybridization temperature (Berent *et al.*, 1985).

Polymerase chain reaction (PCR) amplification of junctional regions was done with Amplitaq (Cetus Corp) according to the supplier's recommended protocol, except that two reactions were run, and primer ratios were adjusted to 20:1 or 1:20 to facilitate sequencing (Innis *et al.*, 1988; Mihovilovic & Lee, 1989). Reactions contained ≈ 1 µg DNA and 1.5 mM-MgCl₂; 30 cycles were run in a Perkin-Elmer thermal cycler, at 95 °C for 1 min, 37 °C for 2 min, and 60 °C for 2 min.

Sequencing was done by a dideoxynucleotide method (Sanger *et al.*, 1977) using a T7 DNA polymerase kit (Pharmacia). For the PCR products, we used each low concentration PCR primer alternately as sequencing primer. For sequencing the cloned MA13 segment of ANS46 DNA, we used as primer oligonucleotide TEM for sequencing out to approximately 150 residues beyond the end of Tn554, and oligonucleotide T53 as primer for sequencing back on the other strand (see Fig. 1).

Results

Comparison of Australian and London Mc^r isolates

The results in this and following sections were obtained with a series of probes which are either cloned DNA

Fig. 1. Physical map of Tn554 inserts found in New Jersey strains, and junction oligonucleotides used to compare variants of these in Australondon strains. At the top we show plasmid pEM715 (Thakker-Varia *et al.*, 1987), used as source of Tn554 segment probes, with *Fnu*4HI (F) sites bounding the segments used. The wavy lines represent sequence from the pT181 vector (Khan & Novick, 1983). Below, we diagram the primary insert (#1) and secondary inserts (#2, #6) found previously (Murphy & Löfdahl, 1984; Thakker-Varia *et al.*, 1987; Tillotson *et al.*, 1989). Junction chromosomal sites for *Xho*I (Xh), *Eco*RV (R5), *Pst*I (P), *Eco*RI (R), *Hind*III (H) and *Cla*I (C) that are within 7 kb of transposon boundaries, as well as relevant sites (Murphy *et al.*, 1985) within the transposon, are shown. H40 and H57 designate cloned junctional fragments from insert 6 (Tillotson *et al.*, 1989). The bold vertical lines indicate the boundaries of Tn554. We follow the convention of E. Murphy and colleagues (Murphy & Löfdahl, 1984; Murphy *et al.*, 1985; Murphy, 1989) here and elsewhere with regard to orientation of the transposon; the major coding strand is presented 5' to 3', left to right. An expanded map of insert boundaries is shown in the lower half of the figure. Oligonucleotides which were used in the comparative analysis of the Australondon and New Jersey strains are shown, with Australondon-specific ones in parentheses. Precise sizes and locations of oligonucleotide sequences are given in Table 2.

fragments of Tn554 inserts (Fig. 1) or oligonucleotides corresponding to Tn554 or Tn554 attachment sites (Fig. 1 and Table 2).

Eight recent Mc^rMLS^r isolates from London Hospitals were compared with the Australian Mc^r strain ANS46 and the New Jersey Mc^r strain R155. Total cellular DNA was digested with *Eco*RI, *Hind*III, or *Hind*III plus *Pst*I. Southern blots were probed with cloned Tn554 primary insert fragments C and D from plasmid pEM715 (which contain also some sequence from the vector pT181), and with junction fragment H40 of insert 6 (Fig. 1). Five of the London isolates contained Tn554, and with the exception of one feature (the absence of integrated plasmid pT181, as described below) were indistinguishable from each other and from the Australian Mc^r isolate ANS46 (data not shown). These five London isolates had antibiotic susceptibility

patterns, including the 'inducible-constitutive' MLS^r phenotype, characteristic of eastern Australian isolates of Mc^r *S. aureus* (Townsend *et al.*, 1983) and of a group of epidemic Mc^r isolates from London (termed EMRSA, following Cookson & Phillips, 1988) (Townsend *et al.*, 1987). We consider these five London isolates to be EMRSA. These results confirm that the EMRSA and the Australian epidemic strains are closely related (Townsend *et al.*, 1987), and we shall refer to them as the Australondon group.

The feature of the initial Southern blot results that distinguished two of the five London EMRSA isolates from ANS46 was the absence of a chromosomal copy of the pT181-like plasmid (Khan & Novick, 1983) in the *mec* region (Gillespie *et al.*, 1986; Skinner *et al.*, 1988); these two EMRSA isolates failed to hybridize with probes containing pT181 sequences. However, they were

resistant to tetracycline and minocycline (albeit slightly less so than the other three EMRSA), probably due to the presence of *tetM*, located elsewhere on the chromosome (Lyon & Skurray, 1987; Cookson & Phillips, 1988). Results for ANS62 were the same as those for the EMRSA isolates lacking pT181; i.e. *tetM* appears to remain and is therefore, as expected, not in the DNA segment (' Δ ANS46/62') deleted from ANS46 to give strain ANS62.

Southern hybridization analysis of Tn554 inserts of Australondon Mc^r isolates: comparison with a Mc^s variant and with New Jersey isolates

The six strains listed in Table 1 were chosen for more detailed analysis. Chromosomal DNA samples were digested with six restriction endonucleases, singly and in pairs, as indicated in the legend to Fig. 1.

Southern blots were probed sequentially with Tn554-related oligonucleotides (Fig. 1 and Table 2), and with fragments from Tn554 (Fig. 1). We show representative Southern hybridization patterns in Figs 2 and 3. These illustrate the complexity of hybridization patterns obtained from Australondon DNA when relatively non-specific probes are used (Fig. 2*a*), and the clearer picture which emerges when more specific probes are used (Figs 2*b*, *c* and 3). The complexity of the patterns relates to the presence of three Tn554 inserts in different attachment sites in the chromosome: #1 is indistinguishable from the primary insert in *att554*; #2B resembles insert 2 (Thakker-Varia *et al.*, 1987); and #6B (6B' in ANS62, as described below) resembles insert 6 (Tillotson *et al.*, 1989).

Oligonucleotide probe design

To detect junction restriction fragments, filters were hybridized with oligonucleotides corresponding to left and right regions of Tn554 (see Fig. 1 and Table 2). Oligonucleotide T6B, corresponding to the 5'-terminal 21 residues of the transposon, was used to detect left junction fragments of the Australondon inserts. Oligonucleotide TEM was used to detect right junction fragments, since oligonucleotide T4 (which corresponds more closely to T6B as a right terminal transposon probe) did not recognize DNA from Australondon isolates.

Oligonucleotides of higher specificity were designed to facilitate assignment of hybridizing fragments to a particular Tn554 insert class. We initially used oligonucleotides spanning transposon-chromosomal junctions as determined for American strains (Fig. 1): T20 and T22 for the primary insert, T21 and T23 for insert 6, and T32 and T48 for insert 2. As expected from the

Table 2. Oligonucleotide probes: hybridization with Australondon DNA

Oligonucleotides*	Australondon inserts†			
	1	2B	6B	6B'
Tn554				
T6B +21 to +1 (L end)	+	+	+	+
T9 +129 to +112 (L penultimate)	+	+	+	+
TEM -82 to -65 (R penultimate)	+	+	+	+
T4 -15 to -1 (R end)	-	-	-	-
T54 -19 to -1 (R end)‡	+	+	+	+
att554, insert 1				
T20 +4 to -13 (L junction)	+	-	-	-
T22 -9 to +7 (R junction)	-	-	-	-
T2 +79 to +61 (R arm)	+	-	-	-
att137, insert 2				
T32 -11 to +6 (L junction)	-	+	-	-
T48 -13 to +6 (R junction)	-	-	-	-
T50 +29 to +44 (R arm)	-	+	-	-
att155, insert 6				
T41 -65 to -85 (L arm)	-	-	+	+
T27 -40 to -24 (L arm)	-	-	+	+
T37 -26 to -10 (L arm)	-	-	+	+
T21 +5 to -12 (L junction)	-	-	-	-
T23 -9 to +8 (R junction)	-	-	-	-
T55 +1 to +15 (R arm)	-	-	-	-
T26 +52 to +33 (R arm)	-	-	-	-
att46, insert 6B				
T53A -11 to +6 (L junction)	-	-	+	+
T53 +111 to +94 (R arm)	-	-	+	-

* Residue numbers indicate nucleotide positions to the left (negative numbers) or right (positive numbers) of the relevant transposon-chromosome junction. The left column of numbers specifies the 5', and the right column the 3', ends of the oligonucleotides. The numbering convention is illustrated in Figs 4 and 5 for several oligonucleotides. Sequences may be found as follows: Tn554, Murphy *et al.* (1985); *att554* and insert 1, Murphy & Löfdahl (1984); *att137* and insert 2, Dubin *et al.* (1990); *att155* and insert 6, Tillotson *et al.* (1989); *att46* and insert 6B, present work, Figs 4 and 5.

† Strong hybridization (+) indicates no mismatches, or at most one terminal mismatch, between target and probe under the hybridization conditions used.

‡ Right transposon end except with reverse complement of the usual GATGTA extreme terminal hexanucleotide.

results for right transposon-terminal oligonucleotide T4, the right junction oligonucleotides failed to recognize any of the Australondon inserts. Unexpectedly, the oligonucleotide corresponding to the left junction of insert 6, T21, failed to recognize Australondon insert 6B. However, using oligonucleotides TEM and T2 (Fig. 1), a 161-nucleotide product spanning the right junction of the primary insert of one of the London strains (N56) was generated by PCR. Similarly, we used Tn554 oligonucleotide T9 and left *att155* oligonucleotide T27 (Fig. 1) to generate a 169-nucleotide product spanning the left junction of ANS46 insert 6B. Both PCR products were then sequenced.

The extent to which the Australondon junction sequences differ from their American homologues is

Fig. 2. *EcoRI* digests of chromosomal DNA. Here and in Fig. 3 we show blots hybridized with probes of least specificity on the left, so that the left-most patterns give an overall picture, and the other patterns identify more precisely certain of the bands. Patterns represent filters hybridized sequentially with different probes, as indicated for (a), (b) and (c). The end lanes show bands from *HindIII* digests of bacteriophage λ used as size standards: 23 kb, 9.4 kb, 6.6 kb, 4.4 kb, and (for Fig. 3) 2.3 kb and 2.0 kb. The other lanes (1–6) show, in order, digests of DNA from London isolates N61 and N62; Australian strains ANS46 and ANS62; and New Jersey isolates R155 and WJ137 (described in Table 1). Bands are identified as to source. For Tn554 inserts we indicate insert number and whether left (L) or right (R) junction. Bands from Ψ Tn554 (see text) are designated ' Ψ '. The streaks in lane 6 lacking identifying labels are artifacts from the adjacent marker lane.

Fig. 3. *PstI* digests. The plan is as for Fig. 2. Bands are identified as in that Figure, all being from right junctions.

shown in Fig. 4. At the left junction of insert 6B, the seven residues of the chromosome adjacent to Tn554 were found to differ from those of insert 6 in two positions, one of which converts the otherwise conserved GATGTA core of the attachment site (Murphy, 1989) to

GATATA. Interestingly, this is one of few core variants found to retain full activity in experiments with *in vitro* core mutants (E. Murphy, personal communication). The right junction of the primary insert of strain N56 was found to differ from standard versions of this junction

Fig. 4. Novel junctions of Australondon Tn554 inserts. Sequences obtained for Australondon inserts by PCR are compared with previously determined sequences for similar inserts. The upper two lines show the left junction regions of New Jersey insert 6 (Tillotson *et al.*, 1989) and Australondon insert 6B. The lower two lines show the right junction regions of the classical primary insert (Murphy & Löfdahl, 1984) and the Australondon primary insert; asterisks indicate identity. We show the strand corresponding to the main coding strand of Tn554. Residues involved in junction fragment translocation are in **bold** type; chromosomal residues are in upper case and transposon residues in lower case. Dashed arrows represent oligonucleotide probes, with arrowheads at the 3' ends.

Fig. 5. Sequence of right adjacent chromosomal DNA of insert 6B (right arm of *att46*) of ANS46. We show the sequence beginning at the first nucleotide beyond the transposon, and compare it with the corresponding sequence for *att155* (Tillotson *et al.*, 1989). Oligonucleotides are designated as for Fig. 4. The *EcoRV* site common to *att46* and *att155* is overlined.

(Murphy & Löfdahl, 1984) in that the 3'-hexanucleotide of the transposon was replaced by the reverse complement (TACATC) of the standard 3'-hexanucleotide GATGTA. These results permitted generation of oligonucleotides specific for the left junction of insert 6B (oligonucleotide T53A) and the right transposon terminus of the Australondon primary insert (oligonucleotide T54) (Figs 1 and 4).

Oligonucleotide T53A recognized the left junctions of insert 6B in all the Australondon isolates, and also the left junction of the corresponding insert (6B') of strain ANS62. T54 recognized all three Tn554 inserts of all Australondon isolates, and of strain ANS62; thus the transposons of all these inserts have reverse complements of the standard GATGTA at their 3'-termini.

A third Australondon-specific oligonucleotide was based on sequence data for the right chromosomal arm of insert 6B, obtained from cloned fragment MA13 (Matthews *et al.*, 1990). This fragment is the leftmost of those cloned from the *mec* region of ANS46 that contain

DNA from ΔANS46/62. Examination of its restriction pattern suggested that it also contained DNA from the right portion of a Tn554 insert. Confirmation of this was given when it was found to hybridize with right penultimate Tn554 oligonucleotide TEM. TEM was then used as primer for sequencing the right end of the transposon, and adjacent chromosomal DNA (i.e. the right arm of the attachment site, designated *att46*). The presence of the reverse complement of GATGTA at the transposon terminus was confirmed (data not shown). We show in Fig. 5 the sequence determined for 120 residues of *att46* beyond this terminus, and compare it with the corresponding region of the attachment site (*att155*) of New Jersey insert 6. Homology between the two stretches was only 70%, which permitted design of an oligonucleotide probe (T53) specific for the right chromosomal arm of insert 6B. This oligonucleotide recognized insert 6B in all the Australondon isolates examined; it failed to recognize any other inserts, including insert 6B' of ANS62.

Hybridization patterns for ANS62, the deletion mutant of ANS46, are shown in Fig. 2, lanes 4. These lanes lack the bands arising from Ψ Tn554 (Fig. 2*a, b*). Further, the ANS62 patterns include a band, designated 6B'R, not present in wild-type Australon don isolates, and lack *Eco*RI fragment 6BR, as reflected in the *Eco*RI

The third Australondon Tn554 insert was designated 6B on the basis of the high similarity of the sequence of its left flanking DNA to that of insert 6 out to residue 85 (Fig. 4 and Table 2). Other hybridization data (not

shown) indicated that the left flanking chromosomal DNA sequences of these two inserts were highly similar for up to 0.5 kb from the transposon, and then diverged (Fig. 6). In contrast, the right flanking chromosomal DNA of these two inserts diverged significantly beginning a few nucleotides from the transposon (Figs 5 and 6).

The restriction maps of the left flanking DNA of inserts 6B and 6B' were the same, whereas those of corresponding right flanking DNA were completely different from each other, apart from an apparently fortuitous concordance of *Hind*III sites (Fig. 6, lowest two lines).

Discussion

Multiplicity of Tn554 inserts in the Australondon group

The occurrence of three Tn554 inserts in the Australondon isolates is not unprecedented (Tillotson *et al.*, 1989). Whether the accretion of Tn554 inserts confers an advantage on a strain is unknown. However, it seems likely that the three copies of Tn554 confer upon Australondon strains the higher basal (uninduced) level of MLS^r (compared to classical inducible strains) characteristic of this group (Townsend *et al.*, 1983).

Ancestry of Australondon Tn554

The different terminal 3'-hexanucleotide (TACATC) of the three Tn554 elements in the Australondon group, compared with those of isolates characterized earlier, can be explained by junction fragment translocation that occurs during Tn554 transposition (Murphy, 1989; Dubin *et al.*, 1990). The 3'-terminal six or seven nucleotides of a transposon in a particular site constitute a 'copy' of the six or seven chromosomal nucleotides 5' to the parental transposon (i.e. the core of the parental attachment site). Tn554 inserts preferentially in a single orientation, designated (+), in its primary chromosomal site, and transposition occurs preferentially into this site, with insertion occurring just 3' of the attachment site core, GATGTA. This, coupled with junction translocation (not conventional target duplication), explains the preponderance of inserts with 'copies' of the GATGTA core at the 3'-ends of the transposons, at least in laboratory strains (Murphy & Löfdahl, 1984; Murphy, 1989) derived from the original Wisconsin isolate (Weisblum & Demohn, 1969). On the other hand, Tn554 readily inserts in (−) orientation in plasmids (Murphy & Löfdahl, 1984) and can do so, albeit rarely, in the chromosome of laboratory strains (Murphy & Löfdahl, 1984; Murphy, 1989). Such (−) insertions occur immedi-

ately 5' of the attachment site core. Thus, for a (−) insert in a primary site, this core is seen as the reverse complement, TACATC, of the usual GATGTA. The inserts of the Australondon group must have arisen by transposition from one or more (−) inserts in a primary, or primary-like, site. The nature of the transposon donor is open to speculation, but interesting candidates are Western Australian strains with Tn554 inserts in conjugative plasmids (Townsend *et al.*, 1986).

*Tn554 inserts 6B and 6, and their physical relationship to *mec**

Perhaps our most important findings pertain to physical linkage between particular Tn554 inserts and *mec*. The present findings show that this linkage exists for insert 6B of the Australondon group. There is good evidence that the only difference between Australian isolate ANS46 and ANS62 is the absence from ANS62 of about 40 kb of contiguous DNA, ΔANS46/62, containing *mec* and resistance determinants for tetracycline, cadmium and mercury (Matthews *et al.*, 1987; Inglis *et al.*, 1988; Skinner *et al.*, 1988). The difference between the right junction fragments of ANS46 insert 6B and ANS62 insert 6B' shows that insert 6B is at or near a boundary of ΔANS46/62. We have established that this is the left boundary (as conventionally drawn; see Matthews *et al.*, 1987) by demonstrating that a portion of insert 6B is included in clone MA13 from ANS46; this clone, in turn, had been shown to contain DNA from the left portion of ΔANS46/62 (Matthews *et al.*, 1990).

An *Eco*RV site 16–21 residues 3' to the transposon of insert 6B (Fig. 5) is missing from insert 6B' (Fig. 6). In addition, the transposon-terminal oligonucleotide T54 hybridized with insert 6B' as well as with insert 6B (Table 2), indicating that the left deletion boundary in ANS46 is within 21 residues of the transposon. It is quite possible that the two boundaries correspond precisely, which of course would directly implicate the transposon in the deletion event.

The similarity between the right flanking DNA of inserts 6 and 6B (i.e. the right arms of *att*155 and *att*46) is low compared to the striking similarity between the respective left arms. This implies common ancestry for the left, but not the right, arms, and suggests that during the divergence of the Australondon and New Jersey lineages the arms of an ancestral attachment site (*att*155-like or *att*46-like, whichever is older) must have become separated.

Several lines of evidence suggest that insert 6 is in the *mec* region of the New Jersey lineage: (a) the epidemiological association between the insert and Mc^r (Tillotson *et al.*, 1989); (b) the similarity between the left flanking DNA of inserts 6 and 6B; and (c) the fact that

insert 6B is in the *mec* region. Further work is in progress better to define the structure of this variant of *mec* region DNA and its relationship with the Australondon version.

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