Physical mapping of the mec region of an Australian methicillin-resistant Staphylococcus aureus lineage and a closely related American strain

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Methicillin-resistant (Mc') staphylococci contain chromosomal DNA that is absent from Mc' cells. This extra DNA harbours the methicillin resistance determinant mec and often other resistance determinants. The mec region can differ substantially in structure among different isolates. We present studies on the mec region of a group of Staphylococcus aureus isolates prevalent in Australia and London. Southern hybridization analyses of a prototype Australian isolate, ANS46, and an isogenic Mc' deletion mutant, ANS62, allowed the physical map of the region to be extended to 55 kb. The DNA corresponding to the deletion, which includes mec and resistance determinants for mercury, cadmium (Cd) and tetracycline, amounted to 41 kb. It was bounded precisely at one end by the macrolides-lincosamides-streptogramin B (MLS)-resistance transposon, Tn554. Near the other end was an element with homology to Tn554, +Tn554, which carried the Cd' determinant. The mec region of an American Mc' isolate, R35, was found to be virtually the same as that of ANS46, except that it lacked Tn554. Another class of American Mc' isolates, prevalent since 1987, differs markedly from ANS46 in mec region organization. However, this other American class also contains an insertion of Tn554 in the mec region, and the attachment site for this insertion was found to have significant homology to attachment sites for the Tn554 and +Tn554 insertions in the mec region of the Australian strain. These results suggest possible roles of Tn554 and Tn554-like elements in the evolutionary variation of the mec region.

Introduction

In Staphylococcus aureus, the structural gene for methicillin resistance, mec, resides in a region of the chromosome that is of considerable interest. The mec region can harbour various resistance determinants in addition to mec, the number, nature and arrangement of which can vary significantly among different isolates (Ubukata et al., 1989; Matthews et al., 1990; Dubin et al., 1991). mec is part of a DNA insertion of 20 kb or more that has no allelic counterpart in chromosomes of typical Mc' S. aureus isolates. This DNA is thought to have arisen by transfer from a different organism (Matthews et al., 1987; Beck et al., 1986; Song et al., 1987; Skinner et al., 1988; Dubin, 1990). In the prototype Australian isolate ANS46, which has been extensively characterized (Matthews et al., 1987, 1990), mec is associated with mer, tet, and cad genes and four copies of the insertion sequence IS257 (Fig. 1).

The staphylococcal transposon Tn554 encodes macrolides-lincosamides-streptogramin B (MLS) and spectinomycin (Sp) resistance (reviewed in Murphy, 1990). Tn554 is relatively site-specific, entering only the primary chromosomal attachment site in laboratory S. aureus strain 8325 (Murphy et al., 1981). However, clinical isolates often have secondary chromosomal insertions of Tn554 (Thakker-Varia et al., 1987; Dubin, 1990). A new group of Mc' S. aureus isolates causing an outbreak in New Jersey in 1987 (the New Jersey group) was found to contain a characteristic secondary insertion designated class 6 (Tillotson et al., 1989). A group of isolates endemic in Australia and England for about 10
Fig. 1. Maps of the mec regions of Australian strain ANS46 and New Jersey strain R35. All sites for EcoRI (R), PstI (P), Smal (S), XbaI (Xb), and Xhol (Xh), and selected sites for BglII (Bg), ClaI (C), EcoRV (R5) and HindIII (H) are shown. The XhoI site in parentheses was partially resistant to cleavage. IS257 insertions are designated 1-4 (Matthews et al., 1990) and Tn554 insertion 6B is designated Tn554.6B. Orientations of transposase reading frames of Tn554 and ψTn554 are shown by arrows. Below the ANS46 map we depict cloned fragments MA13 and MA14 with selected restriction sites, and general locations for Hg and Cd resistance determinants (Skinner et al., 1990). The location of the merA gene as inferred from the restriction map of Laddaga et al. (1987) is indicated by a. The positions of several oligonucleotide probes (T prefixes) hybridizing with ANS46 DNA are shown. Those from T53, T41, T45A, and T45B were used in mapping a substantial portion of the region. The positions of several oligonucleotide probes (T prefixes) hybridizing with ANS46 DNA are shown. Those from T53, T41, T45A, and T45B were used in mapping a substantial portion of the region. The positions of several oligonucleotide probes (T prefixes) hybridizing with ANS46 DNA are shown. Those from T53, T41, T45A, and T45B were used in mapping a substantial portion of the region.
Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sp</th>
<th>MLS</th>
<th>Hg</th>
<th>Tc</th>
<th>Mc</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>WJ137</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>R155</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>ANS46</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>ANS62</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>(R)†</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>R35</td>
<td>S</td>
<td>R‡</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: Sp, spectinomycin; MLS, macrolides-lincosamides-streptogramin B; Hg, mercury; Tc, tetracycline; Mc, methicillin; Cd, cadmium.
† Decreased Tc due to the absence of tetA, but presence of another Tc determinant.
‡ MLS due to ermA in the plasmid pNE131.

in strain R35 that is homologous to att46. When an att site is occupied, DNA to the left (or right) of the transposon is referred to as the left (or right) att site arm. This terminology is extended to unoccupied att sites by reference to the position of the predicted transposon-mediated cleavage of the site.

The designations insertion 6B' and att46' for ANS62 are special cases. As explained in experiments now to be described, these are the same as insertion 6B and att46 except for the fact that AANS46/62 abuts the insertion.

The term pseudotransposon for $Tn554 reflects the fact that a portion of this element (about half) was found to be homologous to Tn554, while the rest lacked homology. Further, DNA adjacent to the left end of $Tn554 (orientation as in Fig. 1) proved to be homologous to DNA adjacent to Tn554 insertion 6 of strain R155.

Results

General organization of the Australondon mec region, mapping rationale, and comparison to New Jersey strain R35

Fig. 1 shows a physical map covering 55 kb of the mec region of the ANS46 chromosome. DNA in the central portion of this map amounting to approximately 35 kb has been characterized in detail (Matthews et al., 1990; Inglis et al., 1990). This 35 kb stretch extends between the regions of the leftmost and rightmost cloned fragments from AANS46/62, clones MA13 and MA14. In addition to mec, this 35 kb region contains an Hg resistance operon and an integrated copy of the Tc' plasmid pT181, both flanked by copies of the insertion sequence IS257 (Skinner et al., 1988; Matthews et al., 1990).

The clone MA13 includes part of Tn554 insertion 6B (Chikramane et al., 1991). As shown below, MA14 includes part of what we refer to as the pseudotransposon ψTn554, which hybridized with some of the probes derived from Tn554. Thus, Tn554-related probes helped refine the map of mec-associated DNA covered by MA13 and MA14, and permitted extension of the map beyond the cloned segments. These probes are summarized in Figs 1 and 2, and Table 2.

Fig. 3 shows Southern hybridization patterns illustrating relationships between DNA contained in clones MA13 and MA14, Tn554 insertion 6B, and the ψTn554 insertion. We ran, in parallel, samples of a HindIII plus PstI digest of an MA13-containing plasmid (lane 1), a HindIII plus PstI digest of chromosomal DNA from an Australondon strain (lane 2), and a HindIII digest of an MA14-containing plasmid (lane 3). (a) shows ethidium bromide-stained bands; patterns are as expected from earlier work (see Fig. 3 legend and Matthews et al., 1990).

Fig. 3(b) shows a blot hybridized with oligonucleotide TEM. This probe is specific for Tn554, and hybridizes with right junction fragments of the three Tn554 insertions of the Australondon chromosome (Chikramane et al., 1991). The 4-6 kb PstI/HindIII band from Tn554 insertion 6B comigrates with a band from MA13, showing that MA13 includes the right junction of Tn554 insertion 6B (Fig. 1). MA14 did not hybridize with the TEM probe (Fig. 3), nor did it hybridize with...
Tn554 cloned fragment probes A–C (Fig. 2 and Table 2) (data not presented). Thus, this clone must lack homology to Tn554, and the portion of ψTn554 included in MA14 (the left half as drawn in Fig. 1, the right half as drawn in Fig. 2) also lacks homology to Tn554.

Fig. 3c presents results with an oligonucleotide probe, T26, based on the sequence of the right flanking chromosomal DNA of Tn554 insertion 6 (i.e. the right arm of attachment site att155 as it occurs in the 1987 New Jersey outbreak group (Tillotson et al., 1989). T26 failed to hybridize with MA13 (lane 1), confirming the finding (Chikramane et al., 1991) that the right flanking chromosomal DNA of insertion 6B (i.e., the right arm of the attachment site in ANS46 referred to as att46) differs from that of att155. However, oligonucleotide T26 did hybridize with an MA14 HindIII fragment of approximately 2.4 kb (lane 3). A similar fragment had previously been found in New Jersey strain R35 (Tillotson et al., 1989). The 2-4 kb HindIII fragments from ANS46 and R35 also hybridized with an EcoRV/HindIII fragment of clone H40 specific for the right adjacent chromosomal DNA of insertion 6 of strain R155 (Table 2 and Fig. 2). This HindIII fragment was absent from ANS62 (data not shown). Thus, the mec regions of ANS46 and R35 carry a substantial length of DNA similar to the right arm of att155. Sequence analysis (S. G. Chikramane & D. T. Dubin, unpublished results) has shown that both the T26 and H40 probes hybridize to DNA that abuts ψTn554 (on the left as drawn in Fig. 1 and on the right as drawn in Fig. 2). Thus this DNA can be considered to be an arm of a putative att site, ‘attψTn554’, which is partially homologous to att155. The 2-4 kb HindIII fragment of MA14 corresponds to a junction fragment of ψTn554 (approx. coordinates 39-5 to 42 kb in Fig. 1).

To further explore the relationship between R35 and ANS46 we used a series of probes spaced along the ANS46 map in Southern hybridizations with DNA digested with the nine endonucleases cited in the legend to Fig. 1. Several of these probes are oligonucleotides, to the SaII/EcoRI fragment of MA14, and to H40. Dashed lines indicate that the precise extent of homologous sequence is not known. For R35 we interrupt the map over the region presumed to correspond to the (absent) Tn554 insertion.
Table 2. Probes derived from Tn554 elements used for mapping mec-associated DNA

<table>
<thead>
<tr>
<th>Tn554-related elements† (prototype strain)</th>
<th>Insertion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>6 (R155)</td>
</tr>
<tr>
<td>Left att155</td>
<td></td>
</tr>
<tr>
<td>T41</td>
<td>+</td>
</tr>
<tr>
<td>H57 EcoRI/ClaI segment</td>
<td>+</td>
</tr>
<tr>
<td>Tn554 (internal)</td>
<td></td>
</tr>
<tr>
<td>T6B (left end)</td>
<td>+</td>
</tr>
<tr>
<td>Fragment C</td>
<td>+</td>
</tr>
<tr>
<td>Fragment B</td>
<td>+</td>
</tr>
<tr>
<td>Fragment A</td>
<td>+</td>
</tr>
<tr>
<td>Fragment D</td>
<td>+</td>
</tr>
<tr>
<td>TEM (right penultimate)</td>
<td>+</td>
</tr>
<tr>
<td>T54 (Australondon right end)</td>
<td>-</td>
</tr>
<tr>
<td>ψTn554</td>
<td></td>
</tr>
<tr>
<td>T59</td>
<td>-</td>
</tr>
<tr>
<td>MA14 EcoRI/SalI segment</td>
<td></td>
</tr>
<tr>
<td>Right att155</td>
<td></td>
</tr>
<tr>
<td>T26</td>
<td>+</td>
</tr>
<tr>
<td>H40 EcoRI/HindIII segment</td>
<td>+</td>
</tr>
<tr>
<td>Right att46</td>
<td></td>
</tr>
<tr>
<td>T53</td>
<td>-</td>
</tr>
<tr>
<td>MA13 HindIII/EcoRI segment†</td>
<td></td>
</tr>
</tbody>
</table>

* Oligonucleotide probes (T prefixes) have been described previously (Chikramane et al., 1991; see also Fig. 1) except for T59. This is the reverse complement of GAATTCGTCTAGGC, which corresponds to Tn554 residues 2212 to 2229 (Murphy et al., 1985), except that the underlined residue, which is a C in Tn554, was converted to a T to create an XbaI site (see Results). DNA fragment probes are shown in Figs 1 and 2.

† Tn554 and ψTn554 insertions include adjacent att arms. Hybridization (+) or lack of it (−) of the insertions and of att35 to the listed probes is shown.

† Around the 10 kb coordinate on the map of Fig. 1.

T43B are from the mec sequence (Song et al., 1987); and TMA14 is based on sequencing (S. G. Chikramane & D. T. Dubin, unpublished results) of MA14. All of these probes hybridized strongly with ANS46 and R35 and yielded the same Southern hybridization patterns with both strains (data not shown).

Southern hybridization using Tn554 insertion-related probes: extension of the map through and beyond MA13 and MA14

The presence in ANS46 of two other copies of Tn554 (the primary insertion and a secondary insertion, 2B), in addition to Tn554 6B and ψTn554 (Chikramane et al., 1991), led to complex Southern hybridization patterns with some Tn554-related probes. To facilitate interpretation, in most experiments four strains were processed in parallel with ANS46. ANS62, the Mc deletion mutant of ANS46, indicated whether a particular ANS46 band was all or partly within ΔANS46/62 and hence could be considered part of the mec region. WJ137, a recent New Jersey Mc isolate, provided markers for Tn554 insertions 1 and 2B (Thakker-Varia et al., 1987). New Jersey isolate R35 proved especially valuable in that its mec region closely resembled that of ANS46, but it lacked Tn554. Finally, it was of interest to compare New Jersey outbreak prototype strain R155 with an Australondon strain.

As an example, Fig. 4 shows hybridizations of EcoRI-digested DNA. The complexity of patterns obtained for ANS46 with an internal Tn554 probe (B) which spans the single EcoRI site of the transposon is shown in Fig. 4(a), lane 5. The complexity is due to the presence of three Tn554 insertions, as well as ψTn554. This last element also has a single EcoRI site, and yields two EcoRI fragments (4·6 and ca 25 kb) which hybridize with probe B. The R35 sample illustrates the utility of this strain. The fragments of R35 that hybridize with internal Tn554-related probes such as probe B can be attributed to the pseudotransposon. Two hybridizing bands from R35 (Fig. 4a, lane 6) migrated with the 4·6 and ≈25 kb
bands of ANS46, indicating that the latter bands arose from \( \psi \text{Tn554} \).

ANS62 lacked bands corresponding to the pseudo-transposon (Fig. 4a, lane 4), showing that this element is part of \( \Delta \text{ANS46/62} \). In contrast, ANS62 retained bands corresponding to Tn554 insertion 6B. We designate the 6B equivalent in ANS62 insertion 6B'. The band corresponding to the right EcoRI fragment (6B'R, lane 4) migrated differently from its counterpart in ANS46, reflecting the fact that insertion 6B' is near the \( \Delta \text{ANS46/62} \) boundary (Chikramane et al., 1991).

Fig. 4(b) illustrates the homology between the DNA flanking the left end of insertion 6B of the Australondon lineage (i.e. the left arm of \( \text{att46} \)), and the left arm of \( \text{att155} \). Oligonucleotide T41, based on the \( \text{att155} \) sequence of strain R155 (Tillotson et al., 1989), recognized EcoRI fragments in ANS46 DNA (6BL, lane 5) and ANS62 DNA (6B'L, lane 4), as well, of course, as a fragment in R155 DNA (6L, lane 3).

The comigration of 6B'L with 6BL (Fig. 4b, lanes 4 and 5) is in accord with our previous mapping (Chikramane et al., 1991), and reflects the fact that the deletion does not extend leftwards into Tn554 insertion 6B. The marked difference in migration between the 6B'L/6BL bands, and that from R155 (6L), reflects the fact that sequences near \( \text{att46} \) and \( \text{att155} \) become divergent beginning a few hundred residues leftwards of the insertion sites. The \( \approx 7 \) kb band generated in R35 DNA by probe T41 (Fig. 4b, lane 6) indicates the presence in this strain of DNA homologous to left arms of \( \text{att155} \) and \( \text{att46} \), but the band size differs from those of the other strains (lanes 3, 4 and 5) because R35 lacks

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**Fig. 3.** Southern blot analysis of fragments MA13 and MA14. *BglII* fragments of ANS46 DNA were cloned into the *BamHI* site of pGEM-1 (Skinner et al., 1988; Matthews et al., 1990). The end lanes of each panel show markers from a *HindIII* digest of bacteriophage \( \lambda \). (a) shows ethidium bromide-induced fluorescence patterns. Lane 1 shows a *HindIII* plus *PstI* digest of the MA 13-containing plasmid. The major bands are a 3.6 kb *HindIII/PstI* product that includes the 2.9 kb vector moiety, a 4.6 kb *PstI/HindIII* product corresponding to a right junction fragment of insertion 6B, and a 2.8 kb *PstI/HindIII* product just downstream (the latter two as diagrammed in Fig. 1). Lane 2 shows a *HindIII* plus *PstI* digest of DNA from a prototype London isolate of the Australondon lineage (Chikramane et al., 1991). Lane 3 shows a *HindIII* digest of the MA14-containing plasmid. The major bands are a 4.3 kb product that includes the vector moiety, and 2.4 and 2.0 kb products corresponding to those diagrammed in Fig. 1. (b) and (c) represent Southern blots of the separation of (a) and of a parallel separation, hybridized with Tn554-related oligonucleotides TEM (b) or T26 (c) (see Table 2). Designations 1, 2B and 6B, and \( \psi \) refer to right junction fragments of Tn554 insertions or of the \( \psi \text{Tn554} \) insertion, respectively.
Tn554 insertions. This R35 band corresponds to a locus similar to *att*46; we designate the locus *att*35 and discuss it in greater detail in connection with Fig. 5.

Fig. 4(c) shows the same blot hybridized to a probe containing plasmid pT181 DNA. This probe hybridized to large fragments in ANS46 and R35 DNA (pT181, lanes 5 and 6). The pT181-hybridizing bands proved to be the same EcoRI fragment as hybridized to Tn554-related probes (a). This was shown by using the array of probes described above. The large EcoRI fragment of ANS46 and R35 thus contains the integrated copy of pT181, mec, and most of ψTn554 (Fig. 1). The faster migrating bands in ANS62 and ANS46 (Fig. 4c, lanes 4 and 5) are probably due to the presence of a Cm' plasmid resembling pSK2 (Lyon & Skurray, 1987) that contains replication DNA homologous to that of pT181 (Projan et al., 1985).

The experiment of Fig. 5 shows ClaI digests probed to illustrate some properties of left ends (orientation as in Fig. 2) of Tn554-related elements in the mec region, and of the left arms of corresponding attachment sites. A filter was hybridized with probe T6B (a), an oligonucleotide based on the left end of Tn554 and which also recognizes ψTn554 (Table 2); and then (b) with the 0.5 kb EcoRI/ClaI fragment of H57 from the New Jersey strain R155, which contains only sequence to the left of the *att*155 insertion site (Fig. 2). A similar filter was hybridized with the entire 1-4 kb EcoRI/HindIII H57 fragment illustrated in Fig. 2 (c). This last probe recognized all the bands shown in Fig. 5(a) and (b), but also hybridized to a 0.8 kb fragment in R35 (Fig. 5c, lane 6). This agrees with the previous conclusion that the left end of the mapped region of R35 is similar to that of ANS46, except that it lacks a Tn554 insertion. The absence of the insertion would bring the ClaI site of the right arm of the putative *att*35 site to within 0.8 kb of the ClaI site of the left arm (see Fig. 1). The resulting ClaI fragment would hybridize with the 1-4 kb H57 fragment but not the 0.5 kb EcoRI/ClaI fragment derived from it, or with oligonucleotide T6B. Thus, R35 appears to contain an unoccupied Tn554 *att* site.

Verification of this inference was provided by a
Fig. 5. Southern blot analysis of Clai digests. For (a) and (b), a blot was hybridized sequentially with oligonucleotide T6B and the 0.5 kb EcoRI/Clai fragment of cloned insertion 6 left junction fragment H57 (Fig. 2). A parallel blot was hybridized with the entire 1.4 kb H57 fragment (c). Lanes are as for Fig. 4, and bands are identified as for that figure (all being left as drawn in Fig. 2), with the following elaborations: 1* designates a restriction fragment length polymorph of the primary insertion of WJ137; ψ* designates a band arising due to partial resistance of the left-adjacent Clai site (Fig. 2) of the R35 pseudotransposon insertion; and att designations are applied to bands containing solely attachment site arm moieties.

Southern blot of a Clai/EcoRV digest of R35 DNA, probed with att155-specific oligonucleotide T41. This probe should hybridize with a 130 bp fragment from att35. A Clai cleavage point occurs 113 residues to the left of the putative att35 cleavage site (as determined for att155; see Tillotson et al., 1989) and an EcoRV site occurs 19 residues to the right (as determined for att46; Chikramane et al., 1991). Such a fragment was observed, but hybridization of R35 blots with an oligonucleotide, GTGGGGATATAACCCC, that should recognize att46 (see Chikramane et al., 1991) yielded faint bands, suggesting that att35 and att46 are similar but not identical.

All hybridizing chromosomal segments in Fig. 5(b) correspond to attachment site arm (as opposed to transposon) sequences. The designation att46' refers to the hypothetical attachment site of ANS62 insertion 6B', the right arm of which is quite different from the right arm of att46 (i.e. the right adjacent chromosomal DNA of insertion 6B of ANS46). The results of Fig. 5(b) show that the similarity between the left arm of att155 and the left arms of att46, att46' and att35 extend substantially beyond their shared Clai sites. All hybridized with the 0.5 kb EcoRI/Clai fragment of strain R155 clone H57 (Fig. 2).

Fig. 5 demonstrates the only difference noted between the R35 and ANS46 mec regions besides those related to insertion 6B. The Clai site adjacent to the ψTn554 element in R35 (but not that in ANS46) was partially resistant to digestion (Fig. 5a and c, lane 6). This resistance was unaffected by prolonging the digestion time or by increasing the concentration of enzyme. The Clai site in question may be methylatable. Perhaps in R35 it is partially methylated while in ANS46 it is unmethylated.

Hybridization properties of ψTn554

Fig. 2 presents maps of Tn554 insertions 6 of R155, 6B of strain ANS46, and 6B' of ANS62, and the analogous chromosomal region of strain R35. These are compared to the ψTn554 insertion of ANS46 and R35. The ψTn554
Table 3. Hybridization analysis of the left end of the ANS46/ANS62 deletion

<table>
<thead>
<tr>
<th>Oligonucleotide probes</th>
<th>Insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (ACG)</td>
</tr>
<tr>
<td>T56 TACTCTTGATAATACATC/A</td>
<td>+</td>
</tr>
<tr>
<td>T57 CTCTTGATAATACATC/AC</td>
<td>+</td>
</tr>
<tr>
<td>T58 TCTTGATAATACATC/ACC</td>
<td>+</td>
</tr>
</tbody>
</table>

insertion is shown in opposite orientation to that of Fig. 1, to illustrate the similarity between the pseudotransposon and \( \psi \)Tn554. In particular, the spacing between the internal \( HpaI \), \( EcoRI \) and \( XbaI \) sites of Tn554 was indistinguishable from that of corresponding sites in \( \psi \)Tn554. However, there was a discrete difference between these regions of pseudotransposon and transposon, namely, the second \( \psi \)Tn554 \( XbaI \) site apparently coincident with the \( EcoRI \) site. Examination of the sequence of Tn554 (Murphy et al., 1985) revealed the string CCTAGA just to the right of the \( EcoRI \) site. We tested the idea that the corresponding sequence in \( \psi \)Tn554 was TCTAGA (the \( XbaI \) recognition sequence) using oligonucleotide T59 (Table 2). T59 hybridized with \( \psi \)Tn554 (data not shown), implying that the \( EcoRI \) site of \( \psi \)Tn554 does correspond to that of Tn554. Also, internal fragments of \( \psi \)Tn554 hybridized to 1-2 and 2 kb internal \( Fnu4HI/EcoRI \) fragments B1 and B2 of Tn554 (Fig. 2) as follows: \( \psi \)Tn554 fragments leftwards of the \( EcoRI/XbaI \) locus hybridized with probe B1 but not B2, while fragments to the right of this reference point hybridized with probe B2 but not B1.

The relationship between Tn554 and \( \psi \)Tn554 was examined with other probes summarized in Table 2 and Fig. 2. There was extensive sequence similarity between the left 2-3 kb of \( \psi \)Tn554 and the corresponding part of Tn554 (Fig. 2). No homology was detected between the right portions of the two elements.

Left boundary of the ANS46/ANS62 deletion

It was inferred from earlier results (Chikramane et al., 1991) that the left boundary of \( \Delta \)ANS46/62 was within 21 residues of the right end of Tn554 insertion 6B. This was based on the facts that insertions 6B of ANS46 and 6B' of ANS62 both hybridize with the right transposon-terminal oligonucleotide T54 (Table 2), and that ANS62 lacks an EcoRV site that occurs at residues 16-21 rightwards of ANS46 insertion 6B (Fig. 2 and Chikramane et al., 1991). To localize more precisely the left boundary of the deletion, DNA from ANS46 and ANS62 was hybridized with a set of overlapping oligonucleotides corresponding to the right transposon–chromosome junction of insertion 6B (Table 3). A single 3' terminal mismatch did not prevent hybridization (see the T56 insertion 2B result), but two 3' terminal mismatches did (T57 insertion 2B). The results for insertion 6B' of ANS62 indicated that the chromosomal sequence directly to the right of the transposon differs from the corresponding sequence for insertion 6B at the first residue beyond the transposon. We infer that the left boundary of the deletion begins precisely at the end of the Tn554 sequence of insertion 6B.

Right boundary of the ANS46/ANS62 deletion

The location of the right boundary of \( \Delta \)ANS46/62 was studied with oligonucleotide T6B, which corresponds to the left end of \( \psi \)Tn554 (Fig. 2). To map more precisely the site of hybridization of this oligonucleotide, PCR was used to amplify the DNA between T6B and oligonucleotide T59 (Fig. 2). Major discrete products running at \( \approx 1-2 \) kb resulted when R35 or ANS46 DNA was used as template (Fig. 6a, lanes 1-4). The \( \approx 2-2 \) kb PCR product from ANS46 DNA (Fig. 6a, lane 2) probably arises from false priming by T59 in the region of the \( EcoRI \) site of Tn554. The corresponding region of Tn554, but not \( \psi \)Tn554, contains a HindIII site (Fig. 2), and the \( \approx 2-2 \) kb product was virtually undetectable when ANS46 template DNA was treated with HindIII prior to amplification (Fig. 6a, lane 1). The PCR product from the pseudotransposon was \( \approx 0-1 \) kb smaller than the \( XhoI/XbaI \) junction fragment, placing the left end of \( \psi \)Tn554 \( \approx 0-1 \) kb from the \( XhoI \) site (Fig. 2). More precise values...
Fig. 6. PCR sizing of terminal and junction fragments of $\psi Tn554$. For (a) we subjected DNA samples to PCR amplification using as primers oligonucleotide T59 and an oligonucleotide corresponding to T6B, oriented towards T59 (Fig. 2 and Table 2). Samples (10%) of the products were subjected to electrophoresis through a 1.2% agarose gel and stained with ethidium bromide. Lanes: 1, ANS46 DNA previously treated with HindIII; 2, intact ANS46 DNA; 3, R35 DNA previously treated with HindIII; 4, intact R35 DNA; 5, 1 kb ladder. For (b), 1% portions of two of the PCR products were digested with HpaI, and subjected to electrophoresis as for (a). HpaI plus XhoI digests of chromosomal DNA preparations were run in parallel. After blotting, the filter was hybridized with Tn554 probe B1 (Fig. 2). Lanes: 1, '1 kb ladder'; 2 and 3, HpaI-treated PCR product of HindIII-digested ANS46 and R35 DNA, respectively; 4-6, HpaI/XhoI digests of ANS62, ANS46, and R35 chromosomal DNA, respectively. Sizes of pertinent marker bands are in bp; a 617 bp band arising from an internal HpaI/XhoI fragment of Tn554 (Murphy et al., 1985) was also used as a size marker. Relevant experimental bands are designated by arrows: namely, the HpaI fragments of the PCR products, which were estimated to be 820 and 410 bp; and the HpaI/XhoI junction fragments of $\psi Tn554$, which were estimated to be 900 bp.

were obtained by comparing HpaI digests of the PCR products with XhoI/HpaI digests of chromosomal DNA (Fig. 6b). The difference between the appropriate PCR fragments and corresponding chromosomal fragments was 80 bp, indicating that the XhoI site is located this distance (± 10 bp) from the pseudotransposon.

The chromosomal restriction sites near the right junction of insertion 6B' of ANS62 are a mirror image of those near the left junction of $\psi Tn554$ (as drawn in Fig. 2). Thus, when both regions are viewed in the same orientation relative to the chromosome (Fig. 1), the restriction patterns are essentially the same. In particular, the right junction Rsal/XhoI fragment of insertion 6B' is ≈160 bp (Fig. 2). Since the rightmost Rsal site in Tn554 is 80 bp from the end (Murphy et al., 1987), the XhoI site adjacent to insertion 6B' of ANS62 (Fig. 2) must be ≈80 bp beyond the transposon. These results indicate that the chromosomal sequences rightwards from the XhoI site flanking insertion 6B' and leftwards from the XhoI site flanking $\psi Tn554$ (Fig. 2) are similar. Thus, the right boundary of ΔANS46/62 is located between the right end of $\psi Tn554$ (as drawn in Fig. 1) and the chromosomal XhoI site, and extends no further than ≈80 bp past the pseudotransposon.

**Discussion**

**General features of the mec region of ANS46 and comparison to New Jersey Mc' lineages**

The present studies have mapped all the sites for five restriction endonucleases and many of the sites for another seven over a stretch of 55 kb of chromosomal DNA corresponding to the mec region of the prototype Australian Mc' isolate ANS46 (Figs 1 and 2). In addition to mec, this region contains determinants for spectinomycin and MLS-resistance (in Tn554), Hg', Tcr (in pT181), and Cd' (in a novel element, $\psi Tn554$). The mec region of the New Jersey isolate R155 differs substantially from that of ANS46. It lacks the integrated plasmid pT181, the pseudotransposon $\psi Tn554$ (with its Cd' determinant) and the Hg' locus present in ANS46. However, the homologies between the left arms of att155 and att46, and between the right arms of att155...
and att\(\psi\)Tn554, imply that strains R155 and ANS46 have evolved from a common Mc\(^c\) ancestor. Complex rearrangements in the regions flanking the attachment sites must have occurred during the divergence of the two lineages.

The relationship between the Australondon group and New Jersey Mc\(^c\) isolate R35 appears to be much closer. Our initial hybridization results led us to suggest that R35 contained the equivalent of att155, but that sequences different from Tn554 were inserted in this attachment site (Tillotson et al., 1989). The present work shows that this 'insertion' constitutes more than 30 kb of mec region DNA, and that the att155 right arm occurs in the wrong orientation relative to the left. It is simpler to view the mec region of R35 as a virtual copy of that of ANS46, without a Tn554 insertion in its version (att35) of att46. Also, one can view the region of R35 corresponding to the right arm of att155 as an arm of att\(\psi\)Tn554. We propose that R35 and the Australondon strains share a relatively recent common ancestry, and that integration of the Tn554 insertions into the DNA of an ANS46 ancestor occurred in Australia or England after the geographical separation of the Australondon and R35 lineages.

Strain R35 was collected in a 1987 survey of two New Jersey hospitals, and was the only one of its kind among 35 Mc\(^c\) S. aureus isolates characterized (most being similar to strain R155) (Tillotson et al., 1989). This seemed to belie the apparent ability of Australondon strains to spread and displace other Mc\(^c\) strains (Pavillard et al., 1982; Cooopson & Phillips, 1988). Interestingly, a more recent (1990) survey showed that one of these New Jersey hospitals, R35-like Mc\(^c\) strains now predominate, although the original New Jersey outbreak (R155-like) strains continue to predominate at the other hospital (D. T. Dubin & W. D. Jenssen, unpublished results).

The ANS46/ANS62 deletion

The deletion of mec DNA in ANS62 was shown here to be 41 kb (Fig. 1), which is in accord with a recent estimate (≈ 50 kb) based on field inversion gel electrophoresis (Inglis et al., 1990). The proximity of the left boundary of ANS46/62 to the right boundary of insertion 6B led earlier to the suggestion that the transposon might have played a role in generating the deletion (Dubin, 1990). The present work, which demonstrates the precise coincidence of these boundaries, strengthens this idea. The fact that the right boundary of the deletion is now shown to be at or near an end of \(\psi\)Tn544 suggests that the pseudotransposon was also involved in the deletion event. The exact nature of this event remains to be determined.

Strain ANS62 was recovered by screening ≈ 3000 colonies for methicillin sensitivity after treatment of ANS46 with acriflavine. The ANS46/ANS62 type of deletion is probably not a rare event, and indeed may occur even more frequently with other types of stress, for example ultraviolet irradiation (P. R. Matthews & P. R. Stewart, unpublished observations). Similar types of deletion involving the mec region can occur upon storage, or under conditions of starvation (Matthews et al., 1990; Inglis et al., 1990). The Tn554 and \(\psi\)Tn554 insertions of ANS46 DNA as well as the copies of IS257 may contribute to the instability and the evolutionary variation of this region.

Nature of \(\psi\)Tn554

The portion of Tn554 to which \(\psi\)Tn554 shows extended sequence similarity (roughly the left half as drawn in Fig. 2) is that which encodes the Tn554 transposases (Murphy et al., 1985). There is good evidence that the other region of \(\psi\)Tn554 contains the C\(^d\) determinant that is present in ANS46. A probe containing sequences from the cad\(^A\) gene of plasmid pIS24 hybridized with the 10 kb XbaI fragment of ANS46 corresponding to coordinates 34-44 kb of Fig. 1 (Matthews et al., 1990). This, coupled with the present results showing that the left portion of MA14 (orientation as for Fig. 1) corresponds to an arm of \(att\psi\)Tn554, supports the placement of the C\(^d\) determinant of ANS46, as shown in Fig. 1, in the EcoRV/Sall fragment of MA14. DNA from all of the C\(^d\) strains listed in Table 1 hybridized to this fragment, whereas DNA from the C\(^d\) strain R155, like ANS62, did not (data not shown). Ongoing sequence analysis will identify the C\(^d\) gene of \(\psi\)Tn554 and will determine if reading frames corresponding to Tn554 transposases remain intact. This will help ascertain whether \(\psi\)Tn554 is a mobile element and what its evolutionary relationship to Tn554 might be.

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