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Insertional Inactivation of Staphylococcal Methicillin Resistance by Tn*551*

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Transposon Tn*551* was translocated into the chromosome of a methicillin-resistant (*mec*) strain of *Staphylococcus aureus* by heat inactivation of a thermosensitive plasmid carrying Tn*551* and selection for erythromycin-resistant (Em^r) survivors. Two independent chromosomal insertions of Tn*551* were obtained which reduced the level of the methicillin resistance by a factor of 50 to 100, making the strains phenotypically methicillin sensitive (Mec^s). Each of the Tn*551* insertions was on the largest fragment produced by *Eco*RI digestion of the chromosomal DNA of these strains. The integration sites lie about 1 kilobase apart. These Mec^s strains reverted to Mec^r at frequencies of 2.4×10^{-8} and 3.6×10^{-5}, respectively. The majority of Mec^r revertants still were Em^r; only a few lost the Em^r phenotype concomitantly with reversion to the Mec^r phenotype. Hybridization data with labeled Tn*551* showed complex rearrangements and deletions in the region of the insertion. These two Tn*551* insertions do not lie on the same linkage group, II, as the *mec* determinant. The phenotypic expression of methicillin resistance, therefore, is also dependent upon a chromosomal genetic marker not physically linked to the *mec* determinant.

Methicillin, a semisynthetic penicillin, is resistant to the action of β-lactamase. The genetic determinant of methicillin resistance (*mec*) in *Staphylococcus aureus* is located on the chromosome (7, 8, 19, 24) as the last known marker at one end of the genetic cluster of group II (16), with closest linkage to *pur-102* (9). Genetic transfer of *mec* by transduction or by transformation is independent of the host *recA* function (2, 18). The behavior of *mec* in cotransductional studies (22, 23) supports the hypothesis that it resides on an inserted DNA sequence in *S. aureus* and that there is no allelic equivalent in methicillin-sensitive (Mec^s) strains. The nature of methicillin resistance observed in *S. aureus* is not yet fully understood. There is no known methicillin-inactivating enzyme. The peptidoglycan synthesis involved in cell division is reported to be methicillin resistant (20), and a major shift occurring in the methicillin affinity of penicillin-binding proteins has also been reported (3). Moreover, Mec^r strains show cells with a heterogenous distribution of methicillin resistance level within the population (6). The nature of this heterogeneity is not yet understood.

To examine further the genetic and biochemical nature of methicillin resistance, mutants affecting *mec* were constructed by using the transposable element Tn*551*, which codes for *ermB* and can insert into various sites on the DNA (14, 15). A thermosensitive plasmid carrying Tn*551* (12) was introduced into a strain harboring *mec*. Erythromycin-resistant (Em^r) colonies resulting from the insertion of Tn*551* into the host chromosome were selected at the nonpermissive temperature. Two independent mutants were selected, in which the Tn*551* insertion had either reduced or abolished the expression of *mec*. A preliminary characterization of these insertion mutants is presented in this report.

MATERIALS AND METHODS

Organisms. The *S. aureus* strains used in this study, their genotypes, and their origins are listed in Table 1. They are all derivatives of the NCTC 8325 strain. The *mec* determinant in strains BB294 and BB295 stems from *S. aureus* E142, isolated from a patient, which has been transduced into an NCTC 8325 background. For generalized transductions, phage 80α was used.

Media. *S. aureus* was grown in tryptic soy broth (Difco Laboratories) or in L-broth (5 g of NaCl, 5 g of yeast extract [Difco], and 10 g of tryptone [Difco] per liter). For phage propagation, 5 mM $CaCl_2$ was added. Agar plates were solidified with 1.5% Difco agar; soft agar for overlays was solidified with 0.6% Difco agar. Purine (Pur) autotrophs were selected on complete defined medium (16). Resistance to antibiotics was screened with novobiocin (10 μg/ml), erythromycin (20 μg/ml), methicillin (6.5 μg/ml), or Cd^+ (10^{-4} M) in L-agar. Minimal inhibitory concentrations (MICs) of methicillin were determined by serial twofold dilutions of the drug in Mueller-Hinton broth (Difco) at 30°C.

Growth conditions. Phage propagation by the agar overlay method, growth of strains harboring the tem-

TABLE 1. Bacterial strains used and their characteristics

Strain	Genotype	Relevant phenotype	Origin
BB255	Same as NCTC 8325		This laboratory; strain NCTC 8325(pI524) cured by ethidium bromide from its plasmid and selected as penicillinase-negative colony
BB294	Same as NCTC 8325, *mec nov-142*	Mec^r Nov^r	This laboratory, by transduction of strain BB255 with phage ϕ11 grown on strain E142 (7) and selection for Mec^r (to give strain NCTC 8325 *mec* [BB270]) and subsequent transduction of strain BB270 with phage 80α grown on strain NCTC 8325 *nov-142 uraA141 hisG15 purA102 pig-131* (ISP86; P. Pattee)
BB295	Same as NCTC 8325, *mec purA102 nov-142*	Mec^r Pur$^-$ Nov^r	This laboratory, by transduction of strain BB270 with phage 80α grown on strain ISP86
RN2906	Same as NCTC 8325-4(pI258 *blaI401 repA36*)	Em^r Cd^r	R. P. Novick (12)
BB307	Same as NCTC 8325, *mec nov-142* (pI258 *blaI401 repA36*)	Mec^r Nov^r Em^r Cd^r	This study, by transduction of strain BB294 with phage 80α grown on strain RN2906
BB303	Same as NCTC 8325-4(pBB2)	Em^r	This laboratory, by transformation of strain NCTC 8325-4 with the largest *Eco*RI fragment of ϕ11de which harbors Tn*551*
BB308	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^s Nov^r Em^r	This study; Em^r Mec^s isolate of strain BB307 selected at 43°C
BB309	Same as NCTC 8325, *mec nov-142* Ω2004(*chr*::Tn*551*)	Mec^s Nov^r Em^r	This study; Em^r Mec^s isolate of strain BB307 selected at 43°C
BB311	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^r Nov^r Em^s	This study; Mec^r Em^s revertant of strain BB308
BB314	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^r Nov^r Em^s	This study; Mec^r Em^s revertant of strain BB308
BB315	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^r Nov^r Em^s	This study; Mec^r Em^s revertant of strain BB308
BB316	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^r Nov^r Em^s	This study; Mec^r Em^s revertant of strain BB308
BB318	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^r Nov^r Em^r	This study; Mec^r Em^r revertant of strain BB308
BB319	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^r Nov^r Em^r	This study; Mec^r Em^r revertant of strain BB308
BB320	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^r Nov^r Em^r	This study; Mec^r Em^r revertant of strain BB308
BB321	Same as NCTC 8325, *mec nov-142* Ω2003(*chr*::Tn*551*)	Mec^r Nov^r Em^r	This study; Mec^r Em^r revertant of strain BB308
BB323	Same as NCTC 8325, *mec nov-142* Ω2004(*chr*::Tn*551*)	Mec^r Nov^r Em^s	This study; Mec^r Em^s revertant of strain BB309
BB326	Same as NCTC 8325, *mec nov-142* Ω2004(*chr*::Tn*551*)	Mec^r Nov^r Em^s	This study; Mec^r Em^s revertant of strain BB309
BB327	Same as NCTC 8325, *mec nov-142* Ω2004(*chr*::Tn*551*)	Mec^r Nov^r Em^s	This study; Mec^r Em^s revertant of strain BB309
BB312	Same as NCTC 8325, *mec nov-142* Ω2004(*chr*::Tn*551*)	Mec^r Nov^r Em^r	This study; Mec^r Em^r revertant of strain BB309
RN2584	Same as NCTC 8325, *thy-101 pur-102 pig-131*	Thy$^-$ Pur$^-$	R. P. Novick

perature-sensitive plasmid pRN3208, and growth in presence of methicillin took place at 30°C. Where not otherwise mentioned, the growth temperature was 37°C.

Preparation of DNA. For the preparation of total cell DNA (chromosomal and plasmid), a 50-ml overnight culture in L-broth was lysed with lysostaphin (Becton, Dickinson & Co.) and sodium dodecyl sulfate (10). The pronase step was omitted. After three phenol extractions and five ether extractions of the lysate, the

DNA was spooled out on glass rods and dissolved in TNE (10 mM Tris [pH8], 10 mM NaCl, 1 mM EDTA). The DNA thus obtained was pure enough for cleavage by restriction enzymes. Plasmid DNA was isolated by the same method from 6-liter cultures. A cleared lysate was prepared (4), and the plasmid DNA was separated from the chromosomal DNA in CsCl density gradients.

Restriction, electrophoresis, and transfer of DNA to Millipore filters. Restriction endonucleases *Eco*RI, *Bgl*II, *Pst*I, and *Hpa*I were obtained from Boehringer Mannheim Corp. and were used according to the recommendations of the manufacturer. Analytical and preparative gel electrophoresis and extraction of DNA fragments from agarose gels were performed as described previously (1). DNA was transferred to nitrocellulose filters (Millipore Corp.) by the method of Southern (21). For the calibration of gels, a *Hin*dIII digest of bacteriophage λ (25) or an *Hpa*I digest of pI524 (11) was used.

Radioactive labeling of DNA. DNA was labeled with ^{32}P by nick translation (17). [α-^{32}P]dCTP was obtained from Amersham Corp.

DNA-DNA filter hybridization. Heat-denatured ^{32}P-labeled plasmid DNA was hybridized overnight at 65°C to nitrocellulose filters containing the restricted DNA in 0.2% sodium dodecyl sulfate–3× salt solution (1× salt solution is 0.3 M NaCl, 18 mM $NaH_2PO_4 \cdot H_2O$, 20 mM NaOH, and 2 mM EDTA). The filters were washed for 5 min at 65°C in 3× salt solution–0.2% sodium dodecyl sulfate and then twice for 30 min each at 42°C in 2× salt solution. The filters were dried, and autoradiographs were prepared on Agfa RP2 films.

RESULTS

Inactivation of methicillin resistance by Tn*551*. A plasmid temperature sensitive for replication and carrying the transposon Tn*551* coding for erythromycin resistance was transduced with phage 80α, grown on strain RN2906, into the Mec^r recipient strain BB294. Selection was made for the plasmid on either erythromycin- or cadmium-containing plates at the permissive temperature. The transduction efficiency was 5.6×10^{-8} with selection for erythromycin resistance and 3.2×10^{-7} with selection for cadmium resistance. Twelve of these transductants were purified on selective agar and checked for loss of erythromycin and cadmium resistance when incubated at 42°C. Translocation of Tn*551* was obtained by growing these strains on erythromycin-containing plates at the nonpermissive temperature. An overnight culture with 5×10^4 to 5×10^5 CFU was spread per plate and incubated at 42°C. Two hundred to two thousand colonies appeared after 2 days, and they were replica plated onto methicillin-containing plates. Tn*551* can integrate into the chromosome at various sites, though preferentially at certain hot spots (15); integration of the total plasmid occurs less frequently. If Tn*551* integrates into a DNA fragment that is responsible for the regulation of or is a structural gene of methicillin resistance, it will affect the expression of the methicillin resistance. Only 2 of the 12 strains tested yielded colonies arising at a frequency of ≤0.5% showing weak or no growth on methicillin. The absence of cadmium resistance indicated that only the transposon and not the total plasmid had integrated into the chromosome. From a single colony of each strain, strains BB308 and BB309 were purified for further analysis.

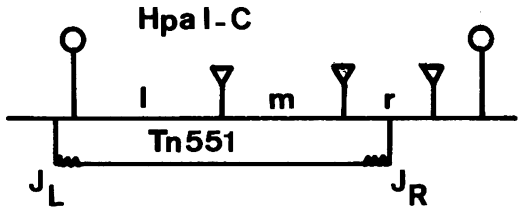


FIG. 1. Restriction cleavage map of Tn*551* (13). The DNA fragment isolated from pBB2 and used for hybridization experiments corresponds to the *Hpa*I fragment C of pI258 shown here. The *Bgl*II fragments of Tn*551* are designated as follows: *l*, left-hand fragment; *m*, middle part; *r*, right-hand fragment. Symbols: ⚲, *Hpa*I cleavage site; ⅄, *Bgl*II cleavage site.

Characterization of the mutant strains BB308 and BB309. Strains BB308 and BB309 were Em^r and showed no growth after 24 h at 30°C on plates containing 6.5 μg of methicillin per ml. They had become phenotypically Mec^s. Tn*551* had presumably integrated into a DNA fragment responsible for the expression of the methicillin resistance. After incubation of the methicillin plates for 48 h, Mec^r revertants appeared. For determination of the reversion rate of strains BB308 and BB309 to Mec^r, exponentially growing cultures were sonicated with a Branson sonifier with an energy output sufficient to disrupt cell agglomerations. Decreasing concentrations were plated onto methicillin-containing plates. Mec^r revertants were picked onto methicillin plates and replica plated onto erythromycin plates to follow the loss of Tn*551*. Direct plating was not successful, since too many methicillin-tolerant cells survived on the plates. The spontaneous reversion rate to Mec^r was about 4.2×10^{-8} for strain BB308 and 3.6×10^{-5} for strain BB309. Surprisingly, the reversion was not exclusively due to the loss of Tn*551*. Only 55% of the BB308 Mec^r revertants had lost erythromycin resistance (118 colonies were tested), and 2% of the 414 colonies of strain BB309 tested were Mec^r Em^s. The reversion might reflect an internal rearrangement or transposition of Tn*551*, since Tn*551* excision occurred only in a few cases. A secondary mutation of the chromosome might also have restored methicillin resistance.

Hybridization with radioactively labeled Tn*551*. The insertion site of Tn*551* in strains BB308 and BB309 and in some of their Mec^r revertants was determined by hybridizing radioactively labeled Tn*551* to *Eco*RI and *Bgl*II digests of chromosomal DNA. As a source of Tn*551* DNA, a *Hpa*I fragment of pBB2 corresponding to *Hpa*I fragment C of pI258 was used. It carries almost the complete 5.2-kilobase (kb) Tn*551* (13). Figure 1 shows a map of the cleavage sites surrounding Tn*551* (5) and the DNA fragments isolated for hybridization experiments. With *Hpa*I-C as a probe, the 265 base pairs of Tn*551* extending to the left of J_L were missing, and plasmid DNA of about 1.4 kb at the right-hand side of J_R, unrelated to Tn*551*, was carried along. This additional DNA was of no relevance. Control experiments (data not shown) with the isogenic strain BB294 did not reveal any sequences hybridizing to *Hpa*I-C.

Tn*551* DNA has no *Eco*RI site, and the presence of Tn*551* sequences on the chromosomal DNA was first determined in an *Eco*RI digest. Tn*551* had integrated into the largest *Eco*RI fragment of the chromosome of strains BB308 and BB309 (Fig. 2). Also, in the few Mec^r revertants, Tn*551* was still on the largest *Eco*RI fragment. Two Mec^r Em^s revertants from strain BB308 had lost all of the Tn*551* DNA; these are represented here by strain BB323. All Mec^r Em^s revertants from strain BB308 retained a part of the Tn*551* DNA.

To check for rearrangements within the chromosomal or transposon DNA, hybridizations with subunits of Tn*551* were made by using fragments from a *Bgl*II digest of *Hpa*I-C. *Bgl*II divides Tn*551* into three subunits, one carrying the left end, one carrying the middle part, and one carrying the right end. Each of these fragments was hybridized separately to *Bgl*II digests of strains BB308 and BB309 and their revertants. Figure 3A shows a summary of these hybridizations. *Hpa*I-C, carrying Tn*551*, was hybridized to the *Bgl*II digests of the chromosomal DNA. Figure 3B shows which subunit of Tn*551* had hybridized to each band (the single hybridizations are not shown). Tn*551* had inserted at two different sites into strains BB308 and BB309. The left end was linked to a 8.5-kb *Bgl*II fragment in strain BB308 and to a 9.5-kb *Bgl*II fragment in strain BB309 (Fig. 3). The middle part was constant. The size of the right end is larger than the resolving power of the gel and could not be determined precisely. An *Hpa*I digest of the chromosome, hybridized to *Hpa*I-C of Tn*551* (Fig. 4), showed that the right end of Tn*551* had hybridized to a 4.8-kb *Hpa*I fragment in strain BB309 and to a 6.1-kb *Hpa*I fragment in strain BB308. The Tn*551* integration sites in strains BB308 and BB309 lie about 1 to 1.3 kb apart, assuming that no deletion within Tn*551* had taken place after its insertion into the chromosomal DNA. This finding is corroborated by the fact that each insertion behaves in a distinguishable way, each producing Mec^r revertants at a characteristic rate.

Various patterns were produced by hybridizing Tn*551* to DNA digests of the Mec^r revertants. One representative of each pattern found is shown in Fig. 3. In strain BB320, a Mec^r Em^r revertant of strain BB308, the Tn*551* insertion remained unchanged. A secondary mutation or rearrangement on a DNA fragment outside Tn*551*, but involved in the expression of the Mec^r phenotype, had restored methicillin resistance without affecting Tn*551*. Strain BB321 had the same pattern as strain BB320, but its hybridization was weaker. In addition, there was a larger band produced by a deletion within Tn*551* which fused the left- and right-hand fragments. In strain BB319 the middle part and the right end had been entirely deleted, and the left-hand fragment remained, now fused to a large fragment. All of these strains still expressed the Em^r phenotype. The next four patterns are those of strains BB311, BB314, BB315, and BB316, which had lost erythromycin resistance. Strain BB316, apparently still identical to its parent strain BB308 in its Tn*551* integration site, must have acquired a secondary mutation reversing Mec^s to Mec^r, which also inactivated the expression of the Em^r phenotype. A weaker hybridization signal and a smaller left-hand fragment in strain BB315 indicates that a part of the left-hand fragment had been deleted. In strain BB311, the deletion involving the left end of Tn*551* extended itself beyond a *Bgl*II site on the chromosomal DNA, so that the remaining piece of the left end was fused to a larger DNA fragment. Two representatives of the BB314 pattern were found. In these, the middle part was fused to a larger fragment, due to a deletion from the left-hand fragment and a part of the middle fragment, and the right end seemed to be more stable and did not change its position. All three Em^r Mec^r revertants of strain BB309 were found to be of the same type, represented here by strain BB312. They were indistinguishable from their parent. Restoration of methicillin resistance was probably due to an event outside of the Tn*551* insertion site. Two of the Mec^r Em^s revertants seemed to have lost almost the complete Tn*551*. There was, however, a very faint hybridization signal visible, and a *Bgl*II fragment hybridized weakly with the left end. This pattern is represented here by strain BB323. In the other two types of Mec^r Em^s revertants, one strain, BB326, was found with a smaller left-hand fragment, and three strains were found which had the left-hand fragment fused to the middle part;

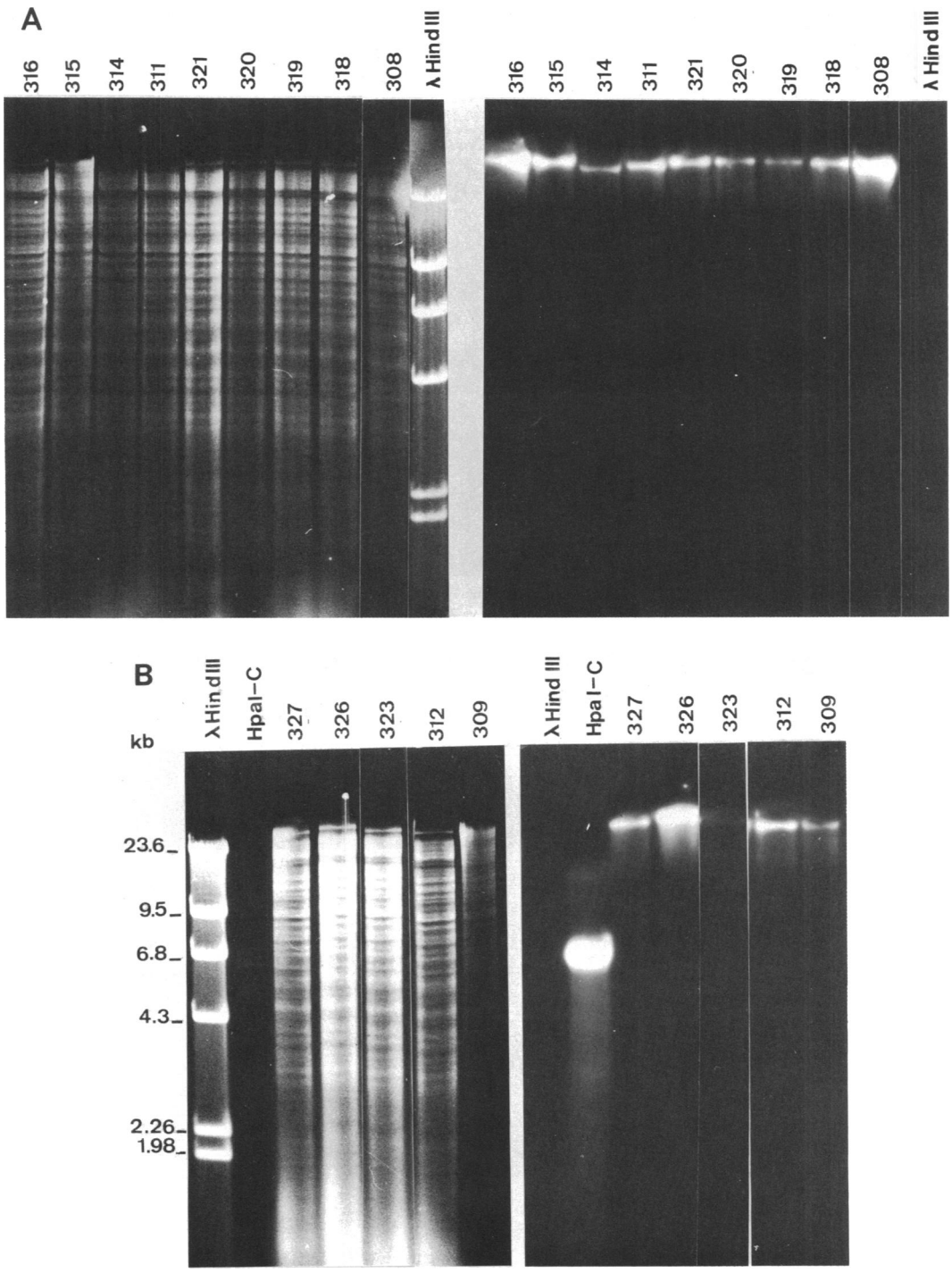
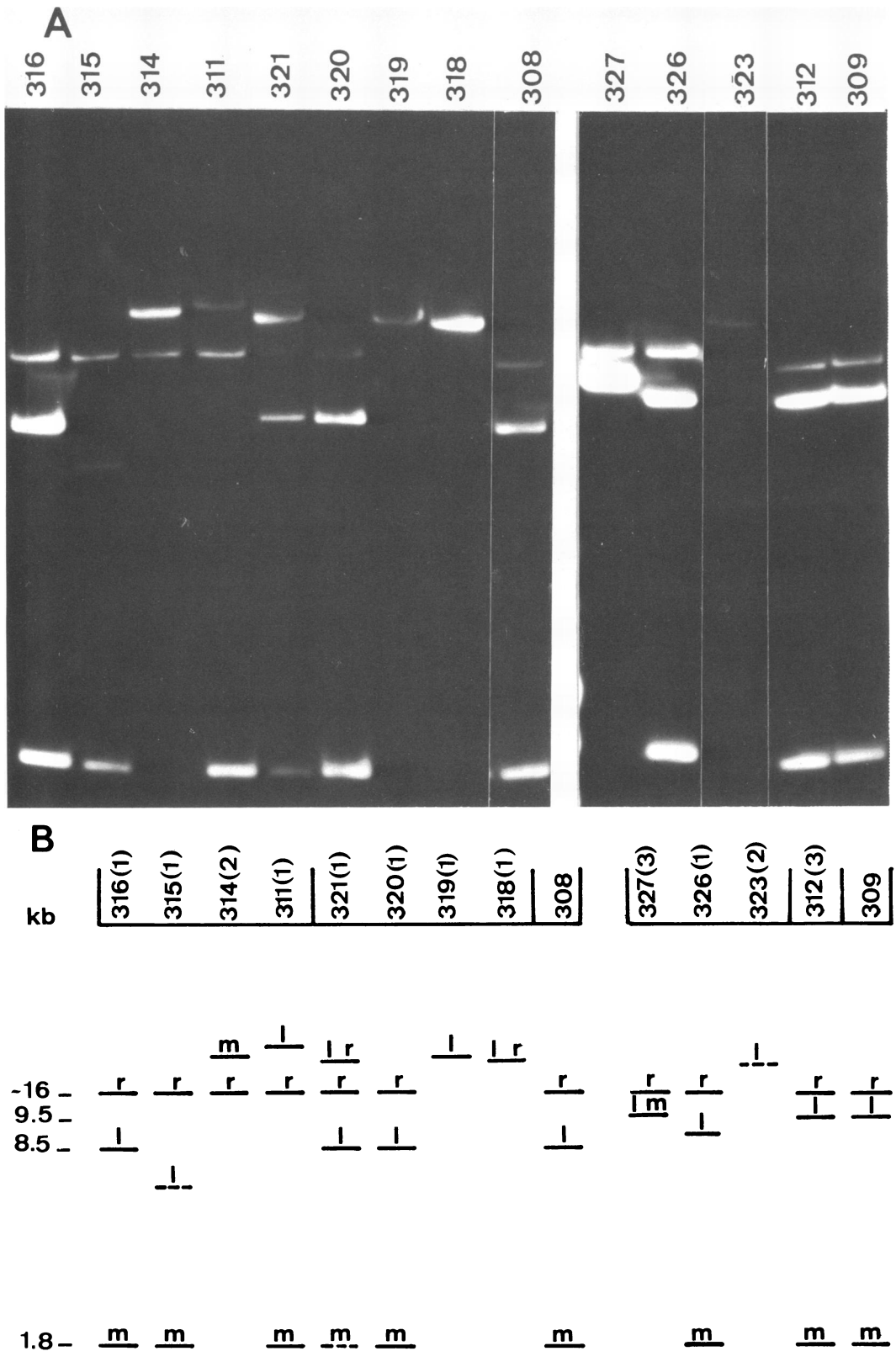


FIG. 2. *Eco*RI digests of chromosomal DNA hybridized to radioactively labeled Tn*551*. Strain BB308 and its Mec^r revertants (A) and strain BB309 and its Mec^r revertants (B) were hybridized to Tn*551*; *Eco*RI digests, gel electrophoresis, transfer of DNA to nitrocellulose membranes, and hybridization with labeled *Hpa*I fragment C carrying Tn*551* were performed as described in the text. The left side of each panel shows the gel with the *Eco*RI digests, and the right side shows an autoradiograph of Tn*551* hybridized to these bands. The numbers indicate the strains used.


A
316
315
314
311
321
320
319
318
308
327
326
323
312
309
B
kb
316(1)
315(1)
314(2)
311(1)
321(1)
320(1)
319(1)
318(1)
308
327(3)
326(1)
323(2)
312(3)
309
-16
9.5
8.5
1.8
r
m
l

the latter are represented here by strain BB327. In these revertants, the right end seemed also to be stable.

Methicillin MICs. The MICs of methicillin in strains BB308 and BB309 and in their different types of revertants were determined in liquid medium to see whether any correlation could be made with the various reversion patterns, the rearrangements, or the loss of Tn*551* fragments. However, the MICs of the revertants were all high, ranging between 250 and 1,000 μg/ml, with no recognizable pattern (Table 2). The MICs for strains BB308 and BB309 could not be determined in broth. The values given in Table 2 are too high, due to the high spontaneous reversion rates of strains BB308 and BB309 to Mec^r.

Cotransduction experiments with phage 80α. Tn*551* was first thought to have integrated into the *mec* determinant, which is the last known marker at one end of chromosomal linkage group II. Generalized transduction experiments with phage 80α grown on strains BB308 and BB309 were therefore performed, using a Mec^s strain, BB255, and an isogenic Mec^r strain, BB270, as acceptor strains. Selection was made for erythromycin resistance. The Tn*551* insertions inactivated the expression of the Mec^r phenotype in all Em^r transductants of strain BB270 (Table 3). When grown on methicillin plates, these transductants segregated Mec^r revertants after 2 days. Transductants from strain BB309 as a donor had a higher reversion rate than transductants from strain BB308. These transductants reflect the reversion rate of their parents.

It was expected that the *mec* determinant would be cotransduced with the Em^r phenotype. Transductants obtained with strain BB255 as a Mec^s acceptor strain, however, never produced Mec^r revertant colonies. Cotransduction of novobiocin resistance with erythromycin resistance was also not observed, and selection of Nov^r transductants in strains BB255 and BB270 was unsuccessful since all Nov^r transductants

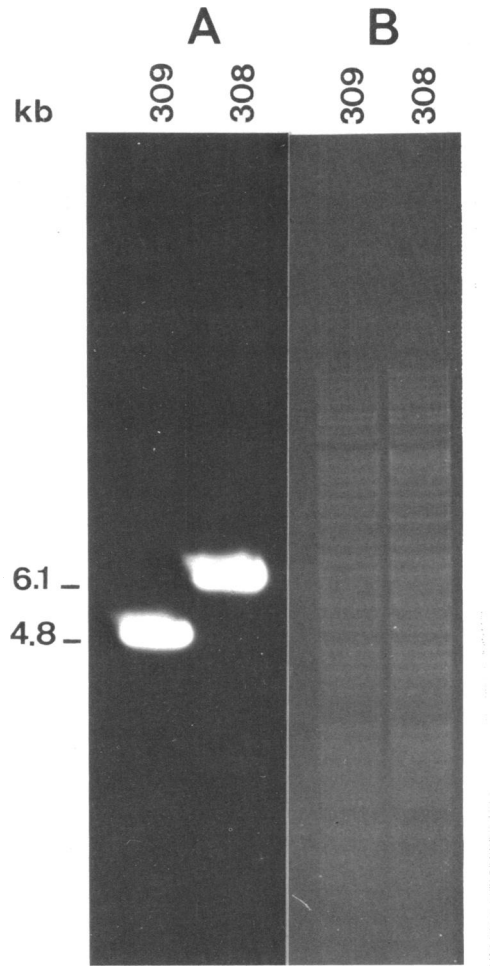


FIG. 4. Hybridization of Tn*551* to *Hpa*I digests of chromosomal DNA of strains BB308 and BB309. (A) Autoradiograph of the hybridization. (B) *Hpa*I digests. Digests, gel electrophoresis, transfer of DNA to nitrocellulose membranes, and hybridization with labeled Tn*551* DNA were performed as described in the text.

FIG. 3. Hybridization of Tn*551* to *Bgl*II digests of chromosomal DNA. Digests of DNA, transfer of DNA to nitrocellulose membranes, and hybridization to radioactively labeled Tn*551* DNA were performed as described in the text. (A) *Hpa*I-C carrying the Tn*551* transposon was hybridized to *Bgl*II digests of chromosomal DNA. The strain number is given over each lane. Strains BB311, BB314, BB315, and BB316 are Em^s Mec^r revertants of strain BB308; strains BB323, BB326, and BB327 are Em^s Mec^r revertants of strain BB309; strains BB318, BB319, BB320, and BB321 are Em^r Mec^r revertants of strain BB308; strain BB312 is an Em^r Mec^r revertant of strain BB309. The uppermost fragments of strains BB327 and BB326 seem, on this gel, to be larger than the corresponding *r* fragments of strains BB312 and BB309. This is due to an artifact, namely, uneven migration of the bands and different positions within the slots, in this particular gel. From the single-hybridization experiments (data not shown), their sizes were confirmed to be identical. (B) Schematic representation of the hybridization pattern shown in (A). By separately hybridizing to *Bgl*II digests each of the three subunits produced by *Bgl*II from *Hpa*I-C, the bands hybridizing with Tn*551* could be attributed to either the left-hand fragment (*l*), the middle part (*m*), or the right-hand fragment (*r*). The strain number is given over each lane; numbers within parenthesis are the number of strains isolated showing the same hybridization pattern.

TABLE 2. Methicillin MICs

Strain	Relevant genotype and Tn*551* insertion	Phenotype[a] on plates		MIC (μg/ml) of methicillin
		Em (20 μg/ml)	Mec (6.5 μg/ml)	
BB255		s	s	2
BB294	*mec*	s	r	500
BB308	*mec* Ω2003	r	s	4[b]
BB309	*mec* Ω2004	r	s	32[b]
BB311	*mec* Ω2003	s	r	500
BB314	*mec* Ω2003	s	r	500
BB315	*mec* Ω2003	s	r	1,000
BB316	*mec* Ω2003	s	r	250
BB318	*mec* Ω2003	r	r	1,000
BB319	*mec* Ω2003	r	r	1,000
BB320	*mec* Ω2003	r	r	250
BB321	*mec* Ω2003	r	r	1,000
BB323	*mec* Ω2004	s	r	1,000
BB326	*mec* Ω2004	s	r	1,000
BB327	*mec* Ω2004	s	r	500
BB312	*mec* Ω2004	r	r	500

[a] s, Sensitive; r, resistant.
[b] These values are too high due to the growth of Mec^r revertants (see text).

induced a phage that lysed the transductants. The cotransduction rate of methicillin sensitivity and novobiocin resistance has been reported to be extremely low (1 to 5%) (9); assuming that Tn*551* had integrated into *mec*, too few transductants were tested to check for linkage. No cotransduction of Em^r was observed with Pur$^+$ autotrophy, even though Mec^s cotransduces with Pur$^+$ at a frequency of 15% (22). All Pur$^+$ transductants (155 from strain BB308 and 50 from strain BB309) into the Mec^r Pur$^-$ Nov^r strain BB295 remained Mec^r and had not become Em^r. The reciprocal experiment, selection for erythromycin resistance in this acceptor strain, produced no Pur$^+$ autotrophs, but again the expression of *mec* was abolished. Linkage of Em^r with Pur$^+$ was also attempted with strain RN2584 (Mec^s Pur$^-$) as acceptor, but there was no cotransduction.

Transformation allows linkages to be seen over longer distances of DNA. Cotransformation of Mec^r with Nov^r was observed with BB308 or BB309 DNA, but no linkage with Em^r could be demonstrated. The *mec* determinant is chromosomal. Ω2003 and Ω2004 were, however, not on linkage group II. Further transduction experiments revealed a cotransduction frequency of Ω2003 and Ω2004 of 45% with *trpE85* and of 4.2 with *lys-115* (manuscript in preparation).

DISCUSSION

Tn*551* integrated at a specific site of the chromosome of a Mec^r strain, reducing the expression of the Mec^r phenotype. Two independent insertions of Tn*551* were isolated. Both of them mapped in the same region of the chromosome, on the largest *Eco*RI fragment, and they were about 1 kb apart. Phenotypically, they were distinguishable by their different reversion rates to Mec^r. Both Tn*551* insertions

TABLE 3. Cotransduction of erythromycin resistance with various markers of linkage group II[a]

Donor strain	Selection marker	Acceptor strain	Transduction efficiency	No. of transductants tested	No. of cotransductants that were:			
					Mec^r	Em^r	Pur$^+$	Nov^r
BB308	Em^r	BB255	8.5×10^{-6}	409	0	409	ND[b]	0
BB308	Em^r	BB270	6.0×10^{-5}	525	0[c]	525	ND	0
BB308	Em^r	RN2584	1.3×10^{-5}	257	0	257	0	ND
BB308	Em^r	BB295	1.9×10^{-5}	366	0[c]	366	0	ND
BB308	Novrd	BB255	5.1×10^{-6}	4	0	0	ND	4
BB308	Nov^r	BB270	7.4×10^{-6}	5	5	0	ND	5
BB308	Pur$^+$	BB295	2.1×10^{-5}	155	155	0	155	ND
BB309	Em^r	BB255	1.2×10^{-5}	356	0	356	ND	0
BB309	Em^r	BB270	6.4×10^{-5}	521	0[c]	521	ND	0
BB309	Em^r	RN2584	1.1×10^{-5}	71	0	71	0	ND
BB309	Em^r	BB295	1.0×10^{-5}	100	0[c]	100	0	ND
BB309	Nov^r	BB255	6.0×10^{-6}	3	0	0	ND	3
BB309	Nov^r	BB270	5.1×10^{-5}	7	7	0	ND	7
BB309	Pur$^+$	BB295	2.0×10^{-6}	50	50	0	50	ND

[a] The transduction experiments were carried out as described in the text with phage 80α.
[b] ND, Not determined.
[c] Mec^r revertants were coming up after 2 days of incubation.
[d] Nov^r transductants were surrounded by a lysis zone seen clearly against a background of slight residual growth. Upon picking of these Nov^r transductants on novobiocin plates for replica plating, most of them lysed, probably due to a bacteriophage.

inserted into a different linkage group of the staphylococcal chromosome than that of the *mec* determinant. The DNA region where Tn*551* had integrated seemed to be very unstable, generating different patterns of deletions and rearrangements affecting and extending preferentially into the left-hand part of Tn*551*. It might be the neighborhood of the Tn*551* insertion sites which induces these rearrangements or deletions. Only two complete excisions of Tn*551* were observed in the Mec^r revertants. It would be of interest to isolate more revertants to determine the precise origins and ends of these rearrangements by finer restriction mapping. These events might reflect the variability of the expression of the levels of methicillin resistance within a population of cells. In conclusion, there seems to be an additional function, coded by the chromosome which influences the level of methicillin resistance, which is located on a different linkage group than the *mec* determinant. Work on mapping the Tn*551* insertions more precisely by transduction is in progress.

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LITERATURE CITED

1. **Bächi, B.** 1980. Physical mapping of the *Bgl*I, *Bgl*II, *Pst*I and *Eco*RI restriction fragments of staphylococcal phage ϕ11 DNA. Mol. Gen. Genet. **180**:391–398.
2. **Cohen, S., H. M. Sweeney, and S. K. Basu.** 1977. Mutations of prophage ϕ11 that impair the transducibility of their *Staphylococcus aureus* lysogens for methicillin resistance. J. Bacteriol. **129**:237–245.
3. **Hartman, B., and A. Tomaz.** 1981. Altered penicillin-binding proteins in methicillin-resistant strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **19**:726–735.
4. **Humphreys, G. O., G. A. Willshaw, and E. S. Anderson.** 1975. A simple method for the preparation of large quantities of pure plasmid DNA. Biochim. Biophys. Acta **383**:457–463.
5. **Kahn, S. A., and R. P. Novick.** 1980. Terminal nucleotide sequences of Tn*551*, a transposon specifying erythromycin resistance in *Staphylococcus aureus*: homology with Tn*3*. Plasmid **4**:148–154.
6. **Kayser, F. H., E. J. Benner, R. Troy, and P. D. Hoeprich.** 1971. Mode of resistance against β-lactam antibiotics in staphylococci. Ann. N.Y. Acad. Sci. **182**:106–117.
7. **Kayser, F. H., J. Wüst, and P. Corrodi.** 1972. Transduction and elimination of resistance determinants in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **2**:217–223.
8. **Kayser, F. H., J. Wüst, and P. Santanam.** 1976. Genetic and molecular characterization of resistance determinants in methicillin resistant *Staphylococcus aureus*. J. Med. Microbiol. **9**:137–148.
9. **Kuhl, S. A., P. A. Pattee, and J. N. Baldwin.** 1978. Chromosomal map location of the methicillin resistance determinant in *Staphylococcus aureus*. J. Bacteriol. **135**:460–465.
10. **Lindberg, M., J.-E. Sjöström, and T. Johansson.** 1972. Transformation of chromosomal and plasmid characters in *Staphylococcus aureus*. J. Bacteriol. **109**:844–847.
11. **Murphy, E., and R. P. Novick.** 1979. Physical mapping of *Staphylococcus aureus* penicillinase plasmid pI524: characterization of an invertible region. Molec. Gen. Genet. **175**:19–30.
12. **Novick, R. P.** 1974. Studies on plasmid replication. III. Isolation and characterization of replication defective mutants. Molec. Gen. Genet. **135**:131–147.
13. **Novick, R. P., I. Edelman, P. D. Latta, E. C. Swanson, and P. Pattee.** 1979. Translocatable elements in *Staphylococcus aureus*. Contrib. Microbiol. Immunol. **6**:41–55.
14. **Novick, R. P., I. Edelman, M. D. Schwesinger, A. D. Gruss, E. C. Swanson, and P. A. Pattee.** 1979. Genetic translocation in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U.S.A. **76**:400–404.
15. **Pattee, P. A.** 1981. Distribution of Tn*551* insertion sites responsible for auxotrophy on the *Staphylococcus aureus* chromosome. J. Bacteriol. **145**:479–488.
16. **Pattee, P. A., and D. S. Neveln.** 1975. Transformation analysis of three linkage groups in *Staphylococcus aureus*. J. Bacteriol. **124**:201–211.
17. **Rigby, P. W., J. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick-translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
18. **Shafer, W. M., and J. J. Iandolo.** 1980. Transduction of staphylococcal enterotoxin B synthesis: establishment of the toxin gene in a recombination-deficient mutant. Infect. Immun. **27**:280–282.
19. **Sjöström, J.-E., S. Löfdahl, and L. Phillipson.** 1975. Transformation reveals a chromosomal locus of the gene(s) for methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **123**:905–915.
20. **Smith, P. F., and B. J. Wilkinson.** 1981. Differential methicillin susceptibilities of peptidoglycan syntheses in methicillin-resistant *Staphylococcus aureus*. J. Bacteriol. **148**:610–617.
21. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
22. **Stewart, G. C., and E. D. Rosenblum.** 1980. Genetic behavior of the methicillin resistance determinant in *Staphylococcus aureus*. J. Bacteriol. **144**:1200–1202.
23. **Stewart, G. C., and E. D. Rosenblum.** 1981. Transduction versus transformation of methicillin resistance in *Staphylococcus aureus*. Curr. Microbiol. **5**:227–230.
24. **Stiffler, P. W., H. M. Sweeney, and S. Cohen.** 1973. Absence of circular plasmid deoxyribonucleic acid attributable to a genetic determinant for methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **116**:771–777.
25. **Thomas, M., and R. W. Davis.** 1975. Studies on the cleavage of bacteriophage lambda DNA with *Eco*RI restriction endonuclease. J. Mol. Biol. **91**:315–328.