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Genetic Behavior of the Methicillin Resistance Determinant in *Staphylococcus aureus*

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The cotransformation frequency of *mecC5* with *pur-102* using *Staphylococcus aureus* C5 deoxyribonucleic acid was found to be approximately 45%. However, in cotransduction studies, there was a 15% cotransduction of purine prototrophy and methicillin sensitivity but, in the reciprocal cross, no purine-prototrophic plus Mec^r cotransductants were obtained (frequency less than 0.06%). The data support the hypothesis that the *mec* determinant resides on an inserted deoxyribonucleic acid sequence in *S. aureus* and that there is no allelic equivalent in sensitive cells.

Staphylococcal methicillin resistance is, perhaps, the most enigmatic of all bacterial antibiotic resistances. The mechanism by which *Staphylococcus aureus* expresses its intrinsic form of penicillin resistance is unknown, and the nature of the genetic determinant for methicillin resistance (*mec*) is largely unknown. Reliable studies of methicillin-resistant (Mec^r) strains strongly support a chromosomal location for *mec* (1, 5, 9, 11), and Kuhl et al. (4) have mapped the *mec* determinant of the Mec^r strain DU4916 (3) and of 20 Mec^r clinical isolates within a specific linkage group on the staphylococcal chromosome, with closest linkage to *pur-102*. Genetic transfer of *mec* by transduction (2, 8) and by transformation (9) has been demonstrated to be independent of host recombination functions and has prompted speculation that *mec* may comprise part of a transposable genetic element. The data we present here support the hypothesis that *mec* resides on an inserted DNA sequence in *S. aureus* and that Mec^s strains do not possess an allelic equivalent to *mec*.

Transformation experiments (Table 1) confirm the linkage of *mec* and *pur-102*. When primary selection was for Pur$^+$ and transformants were scored for Mec^r, we obtained 46% cotransformation for *mecC5*, which is in good agreement with the 40% cotransformation that Kuhl et al. (4) observed for *mec-4916*. The data in Table 1 also confirm our previous observation (10) that only a fraction of *mec* recombinants survive primary selection on methicillin-containing media. The *pur*$^+$ *mec* cotransformants represent a subset of the totality of *mec* transformants, yet fewer Mec^r transformants were obtained by direct selection (363 versus 426).

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Cotransduction of *mec* and *pur*$^+$ was attempted using the Mec^r strains C5 and DU4916 as donors, and with primary selection for purine prototrophy (Table 2). No Mec^r cotransductants were ever observed, although several thousand Pur$^+$ were screened. These results were surprising for two reasons. The cotransformation frequency of 46% suggests that the two determinants should be contransducible. Furthermore, Kuhl et al. (4) observed a 1 to 5% cotransductional frequency between Mec^s and novobiocin resistance in *S. aureus* clinical isolates, although the *nov-142* locus maps further from *mec* than does *pur-102*. To determine whether Mec^s, as opposed to Mec^r, could be cotransduced with Pur$^+$, we used as an additional recipient strain GS2005, a *mecC5* transformant of GS860 which retained its auxotrophic *pur-102* marker. Donors and recipients in the transductional crosses were thus isogenic except at the *mec* and *pur* loci. The initial selection was for Pur$^+$ with the transductants replica-plated to methicillin-containing media (Table 3).

No Mec^r cotransductants were obtained, confirming our previous observation. However, Mec^s cotransduced with Pur$^+$ at a frequency of 15%. It should be noted that methicillin resistance is a very stable trait and no spontaneous loss was detected.

Because of very low transduction frequencies obtained with primary selection on methicillin, cotransduction studies of *pur-102* and *mec* with *mec* being the primary selective marker were not pursued. However, no Pur$^+$ cotransductants were obtained from 25 randomly selected Mec^r transductants of GS860 (with strain C5 as the donor strain).

It appears that although methicillin sensitivity and Pur$^+$ can readily be cotransduced, the methicillin resistance determinant cannot be co-

TABLE 1. *Transformation of methicillin resistance: direct versus indirect selection*[a]

Primary selection	No. of transformants[b]	% Mec^r cotransformation (no. of Mec^r colonies)
Pur$^+$	926	46(426)
Mec^r	363	

[a] Donor was Mec^r strain C5 (1); the genotype of the recipient, strain GS860, was 8325-4 *pyr-141 hisG15 nov-142 pur-102*. Transformation methods are those of Pattee and Neveln (6). The Pur$^+$ transformants were selected on CDS medium (6) lacking the adenine and guanine supplements and with the amino acid mixture replaced with 0.7% Casamino Acids (technical grade; Difco). After allowing 48 h at 37°C for expression, the transformants were replica-plated to brain heart infusion agar (Difco) containing 5% NaCl (final concentration) and 6.25 μg of methicillin (Bristol Laboratories) per ml. Methicillin-containing plates were incubated for 48 h at 30°C.

[b] Total number of colonies recovered from 0.8 ml of transformation suspension plated on appropriate selective media. Input per ml of transformation suspension was 20 μg of C5 DNA and 2.5×10^9 colony-forming units of strain GS860.

TABLE 2. *Cotransduction of purine prototrophy and methicillin resistance*[a]

Transducing phage (donor strain)	MOI	Pur$^+$ transduction frequency[b]	Mec^r cotransduction (%)
80α (C5)	0.3	2.80×10^{-6}	<0.02
29 (DU4916)	0.5	1.26×10^{-6}	<0.21

[a] Transduction experiments were carried out as described previously (7) at the given multiplicities of infection (MOI) with strain GS860 as recipient. Selective conditions are described in Table 1.

[b] The number of transductants obtained per plaque-forming unit of phage.

transduced with Pur$^+$. Mec^r and Mec^s do not, therefore, map as allelic equivalents in *S. aureus*.

A hypothesis to account for these observations proposes that the methicillin resistance determinant is part of a foreign piece of DNA which has become inserted into the chromosome (adjacent to the *pur* locus) of a staphylococcal cell by an illegitimate recombinational event. Because of the foreign and inserted nature of this DNA, there would be no corresponding Mec^s allele in wild-type strains. Genetic transfer of this foreign DNA would be possible because of the flanking staphylococcal DNA sequences which would provide the sequence homology required for the recombinational events. With transformation, the length of the donor DNA would not be a limiting factor, and *mec* and its flanking staphylococcal sequences would often be present on the same DNA fragment with *pur-102*. With transduction, however, there is the limitation of DNA length introduced into the cell. In cotransduction of methicillin resistance with purine prototrophy, the DNA fragment must contain both determinants plus sequences adjacent to *mec* to provide homology for recombination. This length of DNA might constitute more than a "phage headful" and, therefore, could not be transduced as an intact fragment. As a consequence of this, each marker would be individually transduced, but no cotransduction of the two loci would be possible.

TABLE 3. *Cotransduction of purine prototrophy with methicillin resistance or methicillin sensitivity*

Recipient	Transducing phage (donor strain)[a]	MOI[b]	Pur$^+$ transduction frequency[c]	Mec^r or Mec^s cotransduction frequency (%)[d]
GS860	80α (GS2001)	0.12	2.55×10^{-6}	<0.06
GS2005	80α (GS4)	0.15	1.37×10^{-6}	15.5

[a] Strain GS4 is Pur$^+$ Mec^s; strain GS2001 is Pur$^+$ Mec^r (10).

[b] MOI, Multiplicity of infection.

[c] The number of transductants obtained per plaque-forming unit of phage.

[d] Selection was on purine-deficient medium containing 5 μg of methicillin per ml, and the plates were incubated for up to 96 h at 30°C before being considered negative for growth.

This model would account for certain observations which have been made concerning the genetic behavior of the *mec* determinant. Methicillin-sensitive strains have not been demonstrated to mutate spontaneously to methicillin resistance because there would be no sensitive allele at which the mutational event could occur. The low frequency of transduction of methicillin resistance and the requirement for UV irradiation of the transducing phage lysate to get reasonable transduction frequencies can be explained. The *mec* DNA, because of its nonhomologous nature, requires the presence of flanking staphylococcal sequences to provide the homology required for chromosomal integration. However, headful restraints result in these flanking sequences being relatively short on the transduced DNA fragment. Thus the probability of a crossover event occurring is reduced. By stimulating recombination with UV irradiation of the DNA, the probability of a crossover occurring in the short flanking sequences is increased.

The low rates of transduction of methicillin resistance seen in vitro would be accounted for by the reduction in homology necessary for the recombinational events of integration and by the inefficient expression of the resistance at the time of primary selection (Table 1; 10).

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LITERATURE CITED

1. **Cohen, S., and H. M. Sweeney.** 1970. Transduction of methicillin resistance in *Staphylococcus aureus* dependent on an unusual specificity of the recipient strain. J. Bacteriol. **104**:1158–1167.
2. **Cohen, S., H. M. Sweeney, and S. K. Basu.** 1977. Mutations in prophage ϕ11 that impair the transducibility of their *Staphylococcus aureus* lysogens for methicillin resistance. J. Bacteriol. **129**:237–245.
3. **Dornbush, K., H. O. Hallander, and F. Löfquist.** 1969. Extrachromosomal control of methicillin resistance and toxin production in *Staphylococcus aureus*. J. Bacteriol. **98**:351–358.
4. **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.
5. **Lacey, R. W., and J. Grinsted.** 1973. Genetic analysis of methicillin-resistant strains of *Staphylococcus aureus*: evidence for their evolution from a single clone. J. Med. Microbiol. **6**:511–526.
6. **Pattee, P. A., and D. S. Neveln.** 1975. Transformation analysis of three linkage groups in *Staphylococcus aureus*. J. Bacteriol. **124**:201–211.
7. **Rubin, S. J., and E. D. Rosenblum.** 1971. Effects of the recipient strain and ultraviolet irradiation on transduction kinetics of the penicillinase plasmid of *Staphylococcus aureus*. J. Bacteriol. **108**:1200–1204.
8. **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.
9. **Sjöström, J.-E., S. Löfdahl, and L. Philipson.** 1975. Transformation reveals a chromosomal locus of the gene(s) for methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **123**:905–915.
10. **Stewart, G. C., and E. D. Rosenblum.** 1980. Transduction of methicillin resistance in *Staphylococcus aureus*: recipient effectiveness and beta-lactamase production. Antimicrob. Agents Chemother. **18**:424–432.
11. **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.