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# Novel Type V Staphylococcal Cassette Chromosome *mec* Driven by a Novel Cassette Chromosome Recombinase, *ccrC*

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**Staphylococcal cassette chromosome *mec* (SCC*mec*) is a mobile genetic element composed of the *mec* gene complex, which encodes methicillin resistance, and the *ccr* gene complex, which encodes the recombinases responsible for its mobility. The *mec* gene complex has been classified into four classes, and the *ccr* gene complex has been classified into three allotypes. Different combinations of *mec* gene complex classes and *ccr* gene complex types have so far defined four types of SCC*mec* elements. Now we introduce the fifth allotype of SCC*mec*, which was found on the chromosome of a community-acquired methicillin-resistant *Staphylococcus aureus* strain (strain WIS [WBG8318]) isolated in Australia. The element shared the same chromosomal integration site with the four extant types of SCC*mec* and the characteristic nucleotide sequences at the chromosome-SCC*mec* junction regions. The novel SCC*mec* carried *mecA* bracketed by IS*431* (IS*431-mecA*-Δ*mecR1*-IS*431*), which is designated the class C2 *mec* gene complex; and instead of *ccrA* and *ccrB* genes, it carried a single copy of a gene homologue that encoded cassette chromosome recombinase. Since the open reading frame (ORF) was found to encode an enzyme which catalyzes the precise excision as well as site- and orientation-specific integration of the element, we designated the ORF cassette chromosome recombinase C (*ccrC*), and we designated the element type V SCC*mec*. Type V SCC*mec* is a small SCC*mec* element (28 kb) and does not carry any antibiotic resistance genes besides *mecA*. Unlike the extant SCC*mec* types, it carries a set of foreign genes encoding a restriction-modification system that might play a role in the stabilization of the element on the chromosome.**

The spread of antibiotic resistance among *Staphylococcus aureus* strains is of great concern in the treatment of staphylococcal infections, since *S. aureus* has quickly acquired resistance to all antibiotics introduced for clinical use. The first clinical isolate of methicillin-resistant *S. aureus* (MRSA) was reported in 1961, only 1 year after the introduction of methicillin (18). MRSA produces penicillin-binding protein 2′ (PBP 2′), which has a reduced affinity for β-lactam antibiotics (11, 34, 42). The *mecA* gene, which encodes PBP 2′, and its regulatory genes, *mecI* and *mecR1*, were cloned and sequenced in the 1980s (27, 38). Those genes are located on the chromosome, and they have become widely distributed among many staphylococcal species (1, 12, 14, 23, 35, 37, 40, 41).

In the last few years, understanding of the genetic basis for methicillin resistance has advanced significantly. MRSA is produced when methicillin-susceptible *S. aureus* (MSSA) acquires a genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*).

SCC*mec* is a genomic island (Gisland) that is inserted at the 3′ end of *orfX* and that is located near the replication origin of *S. aureus* (3, 24). Since our discovery of the first SCC*mec* element from pre-MRSA strain N315 in 1999, several types of SCC*mec* elements have been identified by determining their entire nucleotide sequences (16, 17, 26, 39). Pre-MRSA is a *mecA* gene-carrying MSSA strain in which *mecA* gene expression is strongly repressed by the presence of an intact *mecI* gene. The SCC*mec* element contains the *mec* gene complex (the *mecA* gene and its regulators) and the *ccr* gene complex, which encodes site-specific recombinases responsible for the mobility of SCC*mec* (22).

Four classes of the *mec* gene complex have been identified by PCR, using chromosomal DNA from methicillin-resistant coagulase-negative staphylococci as templates. The different *mec* gene complexes are structured as follows: class A, IS*431-mecA-mecR1-mecI*; class B, IS*431-mecA*-Δ*mecR1*-IS*1272*; class C, IS*431-mecA*-Δ*mecR1*-IS*431*; and class D, IS*431-mecA*-Δ*mecR1* (21).

The *ccr* gene complex contains two site-specific recombinase genes, *ccrA* and *ccrB*, which are responsible for the mobility of SCC*mec* (16, 22). There are four allotypes in each of the *ccrA* and *ccrB* genes: *ccrA1*, *ccrA2*, *ccrA3*, and *ccrA4* for *ccrA* and *ccrB1*, *ccrB2*, *ccrB3*, and *ccrB4* for *ccrB*. SCC*mec* is classified into allotypes according to the combination of the *mec* gene complex class and the *ccr* gene complex type that it possesses (16, 26), as follows: type I SCC*mec*, class B *mec* gene complex and type 1 *ccr* gene complex; type II SCC*mec*, class A *mec* gene complex and type 2 *ccr* gene complex; type III SCC*mec*, class A *mec* gene complex and type 3 *ccr* gene complex; and type IV SCC*mec*, class B *mec* gene complex and type 2 *ccr* gene complex. The region other than the *mec* and *ccr* gene complexes is designated the J (junkyard) region. Each SCC*mec* type is further classified into subtypes on the basis of the J-region sequence (13).

SCC is a basic mobile genetic element that serves as the vehicle for gene exchange among staphylococcal species; it has been reported in some coagulase-negative staphylococci as well as in *S. aureus* (15, 20, 25). SCC*mec* is a member of the

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TABLE 1. Bacterial strains and plasmids used in this study

| Strains or plasmid | Description | Reference(s) or source |
|---|---|---|
| Strains | | |
| MRSA | | |
| WIS [WBG8318] | C-MRSA strain isolated in Australia | 31 (provided by W. B. Grubb) |
| 81/0342 | NORSA strain isolated in Australia | 32 (provided by J. D. Turnidge) |
| 91/2574 | NORSA strain isolated in Australia | 32 (provided by J. D. Turnidge) |
| N315 | Pre-MRSA strain isolated in Japan in 1982 | 12 |
| N315ex | SCC*mec* excised from strain N315 | 22 |
| Plasmids | | |
| pYT3 | Temperature-sensitive shuttle vector (tetracycline resistant) | 9a |
| pSR2 | pYT3 into which the *ccrA2* and *ccrB2* genes were cloned | 22 |
| $pSR5_w$ | pYT3 into which $ccrC_{Wis}$ was cloned | This study |
| $pSR5_E$ | pYT3 into which $ccrC_{0342}$ was cloned | This study |
| pYTattII | pYT3 into which *attII* (the presumptive attachment-sequence in type II SCC*mec*) was cloned | 22 |
| pYTattV | pYT3 into which *attV* (the presumptive attachment sequence in type V SCC*mec*) was cloned | This study |
| pSR2attII | pYTattII into which the *ccrA2* and *ccrB2* were genes cloned | 22 |
| $pSR5_E$attV | pYTattV into which $ccrC_{0342}$ was cloned | This study |

SCC family, the members of which specialize as carriers of methicillin resistance.

Both $SCC_{12263}$ (found in *S. hominis* GIFU12263) and $SCC_{476}$ (found in MSSA strain 476 [the seventh *S. aureus* strain whose whole genome sequence is being determined]) carried *ccrA* and *ccrB* genes, but they did not carry *mecA* or any other antibiotic resistance gene (20) (http://www.sanger.ac.uk/Projects/S_aureus/). SCC*cap1* encodes a capsule gene cluster that confers a mucoid appearance because of overexpression of the capsule in *S. aureus* and is also a member of the SCC family (25).

MRSA has been a major causative agent of nosocomial infections (2). Recently, however, MRSA has become increasingly isolated from patients with community-acquired infections (4, 5, 9, 28, 30, 31). The SCC*mec* typing system that we described above has turned out to be an important marker for distinguishing these two categories of MRSA. Namely, by using the SCC*mec* typing system, we have provided strong evidence for the independent derivation of health care-associated MRSA (H-MRSA) and community-acquired MRSA (C-MRSA) clones (32).

The majority of H-MRSA strains carry one of the three types of SCC*mec* (type I, II, or III) (6, 16), whereas well-defined American C-MRSA and nonmultiresistant oxacillin-resistant *S. aureus* (NORSA) strains carry type IV SCC*mec* (32). Then, Vandenesch et al. (43) reported that majority of C-MRSA strains in France also carry type IV SCC*mec*. Type IV SCC*mec* is a small element that does not carry antibiotic resistance genes other than *mecA* and has multiple subtypes. The extreme heterogeneity of the chromosome genotypes in C-MRSA strains suggests that type IV SCC*mec* is highly transmissible. However, we have also noted several strains whose SCC*mec* elements are nontypeable (32). We propose that clarifying the unknown structures of these SCC*mec* elements is indispensable for increasing the typeability of strains for SCC*mec*-

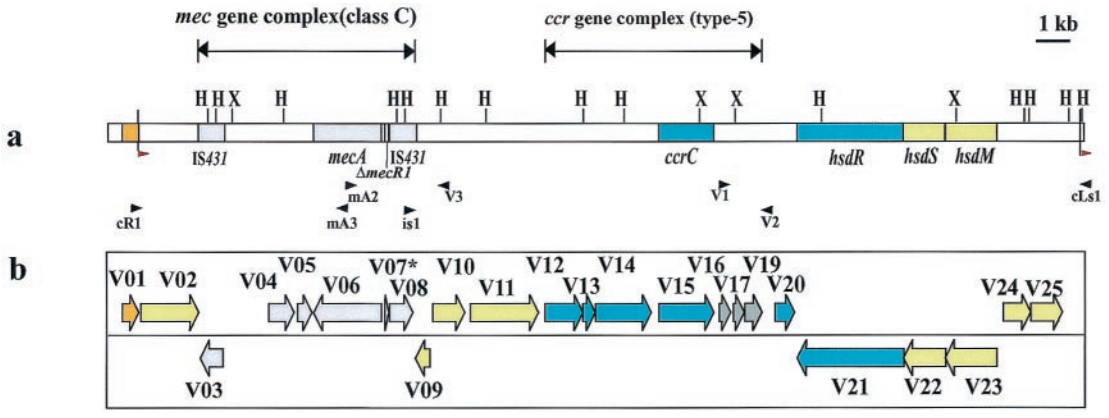


FIG. 1. Genetic structure of the type V SCC*mec* element of strain WIS. The structure of the type V SCC*mec* element illustrated is based on the nucleotide sequence deposited in the DDBJ/EMBL/GenBank databases under accession no. AB121219. (a) Essential structure of type V SCC*mec*. The locations of the essential genes are illustrated. The colors of the essential genes are based on those for the ORFs described for panel b. The HindIII (H) and XbaI (X) restriction sites are indicated. Black arrowheads indicate primer-specific locations. The nucleotide sequences of primers cR1, mA2, mA3, is1, and cLs1 have been described previously (17, 26). The nucleotide sequences of primers V1, V2, and V3 are listed in Table 2. The locations of direct repeats are indicated by red arrowheads. (b) ORFs in and around type V SCC*mec*. The ORFs corresponding to sequences more than 100 bp are indicated by arrows, which also indicate the directions of the ORFs. Light gray arrows, ORFs conserved in the five types of SCC*mec* elements with identities of more than 99%; gray arrows, ORFs conserved in the five types of SCC*mec* elements with identities of 48.1 to 93.4%; blue arrows, ORFs commonly found in both the type V SCC*mec* element and the J region of type III SCC*mec*; yellow arrows, ORFs unique to type V SCC*mec*; orange arrow, *orfX*.

TABLE 2. Primers used in this study

| Genetic element(s) or purpose and primer | Nucleotide sequence | Expected product size (kb) | Reference or source |
|---|---|---|---|
| Type V SCC*mec* of WIS | | | |
| V1 | 5′-TACCACTTTTACCACTTAGCTTT-3′ | | This study |
| V2 | 5′-ATGAGGCTTTAACATTTCCATCA-3′ | | This study |
| is1 | 5′-ACATTAGATATTTGGTTGCGT-3′ | | 21 |
| V3 | 5′-TATCATTACACTCTTGAGTCTCT-3′ | | This study |
| mA2 | 5′-AACGTTGTAACCACCCCAAGA-3′ | | 21 |
| mA3 | 5′-TGCTATCCACCCTCAAACAGG-3′ | | 21 |
| Detection of *ccrC* | | | |
| γF | 5′-CGTCTATTACAAGATGTTAAGGATAAT-3′ | 0.52 | This study |
| γR | 5′-CCTTTATAGACTGGATTATTCAAAATAT-3′ | | This study |
| Construction of recombinant plasmids with *ccrC* | | | |
| Vcc1 | 5′-AAAAGGATCCAAGTTGTTTGCTTAGCGTCATTA-3′ | 1.93 | This study |
| Vcc2 | 5′-AAAAGGATCCTAGTACTCATATGATTAAGTGGT-3′ | | This study |
| *att*SCC-V (*att*SCC of type V SCC*mec*) | | | |
| mVR2 | 5′-AAAAAGTCGACTACCGTCGATATCAATTGCTTTTT-3′ | 0.90 | This study |
| mVL2 | 5′-AAAAAGTCGACTGGAGACGTAGTATAAATATAGCT-3′ | | This study |
| Detection of precise excision and closed circular DNA formation | | | |
| *att*B*scc* (N315) | | | |
| cL1 | 5′-ATTTAATGTCCACCATTTAACA-3′ | 0.28 | 17 |
| cR1 | 5′-AAGAATTGAACCAACGCATGA-3′ | | 17 |
| *att*B*scc* (WIS) | | | |
| cLs1 | 5′-TGCCAATCACAGTTCAATCAATT-3′ | 0.31 | 26 |
| cR1 | 5′-AAGAATTGAACCAACGCATGA-3′ | | 17 |
| *att*SCC (N315) | | | |
| mL1 | 5′-GAATCTTCAGCATGTGATTTA-3′ | 0.46 | 22 |
| mR8 | 5′-ATGAAAGACTGCGGAGGCTAACT-3′ | | 22 |
| *att*SCC (WIS) | | | |
| mVL1 | 5′-TACTTTGGTTTCATATTAATAGCACT-3′ | 0.29 | This study |
| mVR1 | 5′-TCACTAGTGTAATTATCGAATGAT-3′ | | This study |
| Generation of chromosome-SCC*mec* (mini-SCC) junction | | | |
| α | 5′-TTTCACACAGGAAACAGCTATGAC-3′ | | 22 |
| β | 5′-ATCACGATATTGCTTATAAGCA-3′ | | 22 |
| γ | 5′-ATGTTATTAAGCAGATTGCGTCAA-3′ | | This study |

based epidemiology and, most importantly, for obtaining a better understanding of the role of SCC in the evolution of *S. aureus*, including the acquisition of multidrug resistance.

In this study, we determined the entire nucleotide sequence of the unknown element found in Australian C-MRSA strain WIS to characterize this SCC*mec* element and to relate it phylogenetically to other known SCC*mec* types. It turned out to be a distinct type of SCC*mec* with a distinct *ccr* gene homologue and with the *mecA* gene characteristically bracketed by two insertion sequences.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The bacterial strains used in the experiments described here are shown in Table 1. Brain heart infusion (BHI) broth and agar (Becton Dickinson, Sparks, Md.) were used as culture media for *S. aureus*. Tetracycline (Sigma Chemical Co., St. Louis, Mo.), tobramycin (Shionogi Co., Osaka, Japan), and ceftizoxime (Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan) were used where appropriate at the concentrations indicated in the text.

**DNA manipulation and nucleotide sequencing.** The DNA fragments encompassing the entire SCC*mec* nucleotide sequence of strain WIS were amplified by long-range PCR with several sets of primers, as follows: the region from the *orfX* gene to the *mecA* gene was covered by primers cR1 and mA3, and the region from the *mecA* gene to the chromosomal region flanked to the left end of SCC*mec* was amplified by PCR with primers is1 and cLs1. The latter region was later amplified by PCR with primer sets mA2 and V3, is1 and V2, and V1 and cLs1, as indicated in Fig. 1. The PCR products were purified with a QIAquick PCR purification kit (Qiagen, Hilden, Germany), and their nucleotide sequences were determined. The amplification steps for PCR, long-range PCR, and nested PCR were performed as described previously (17).

**Construction of recombinant plasmids and excision assay.** The DNA fragments containing the *ccrC* gene of strain WIS ($ccrC_{WIS}$) and the *ccrC* gene of strain 81/0342 ($ccrC_{0342}$) were amplified by PCR with primers Vcc1 and Vcc2 and

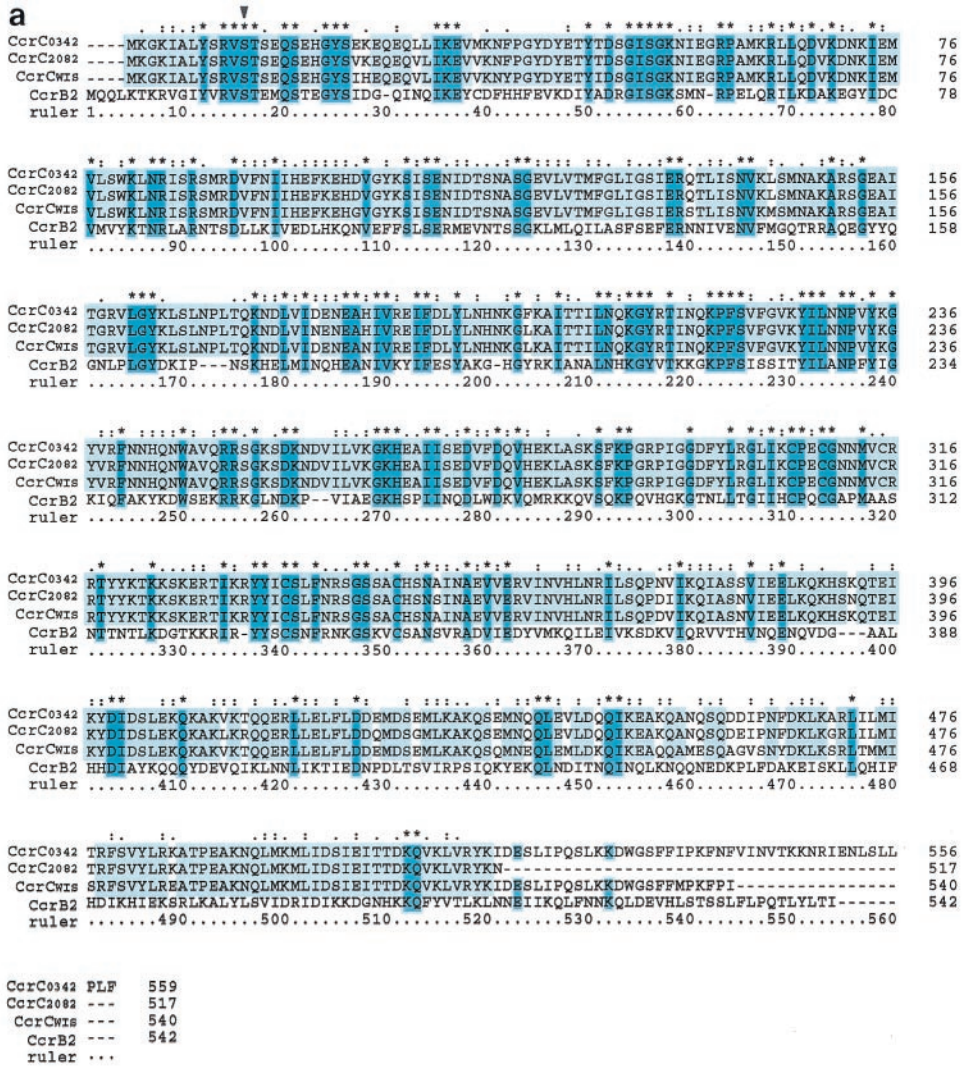


FIG. 2. Characteristics of deduced amino acid sequences of CcrC. (a) The deduced amino acid sequences of the three *ccr* genes were aligned with that of *ccrB2* of strain N315. The alignment was performed by using ClustalX software with the protein weight matrix PAM series. The three Ccr proteins were $CcrC_{WIS}$ (CcrC of WIS), $CcrC_{0342}$ (CcrC of strain 81/0342; the sequence of the strain 91/2674 CcrC was exactly identical to that of $CcrC_{0342}$), and $CcrC_{2082}$ (CcrC of strain 85/2082). The three Ccr proteins were highly homologous. Of the 517 amino acids corresponding to $CcrC_{2082}$, 484 amino acids were conserved in the three Ccr proteins (pale blue) and 30 amino acids were conserved in two of three Ccr proteins. One hundred thirty-eight amino acids were conserved in $CcrC_{0342}$, $Ccr_{WIS}$, and CcrB2 (blue). The consensus sequence shown above the amino acid sequences indicates conserved amino acids, as follows: asterisks, identical or conserved residues in all sequences in the alignment; colons, conserved substitutions; dots, semiconserved substitutions; hyphens, gaps. A black arrowhead indicates the presumptive serine involved in the phosphoseryl linkage conserved in the $NH_2$-terminal catalytic domain of site-specific recombinases of the invertase-resolvase family. (b) Phylogenetic relationships among *ccrA* genes, *ccrB* genes, *ccrC* genes, and two site-specific recombinases. The two site-specific recombinases used were the integrase (*int*) of bacteriophage ϕ FC1 found in *E. faecalis* (1,216 bp; DDBJ/EMBL/GenBank accession no. AF124258) and the site-specific recombinase found in *C. acetobutylicum* ATCC 824 (1,635 bp; DDBJ/EMBL/GenBank accession no.AE007636). The *ccrA* and *ccrB* genes used were as follows: *ccrA1* and *ccrB1**, respectively, found in strain NCTC10442 (DDBJ/EMBL/GenBank databases accession no. AB033763); *ccrA2* and *ccrB2*, respectively, found in strains N315 (DDBJ/EMBL/GenBank databases accession no. D86934) and CA05 (DDBJ/EMBL/GenBank databases accession no. AB063172); *ccrA3* and *ccrB3*, respectively, found in strain 85/2082 (DDBJ/EMBL/GenBank databases accession no. AB037671); and *ccrA4* and *ccrB4**, respectively, found in strain HDE288 (DDBJ/EMBL/GenBank databases accession no. AF411935). Since *ccrB1* of NCTC10442 and *ccrB4* of HDE288 were truncated, we reconstituted large ORFs of 1,626 bp (*ccrB1**) and 1,629 bp (*ccrB4**) by adding an adenine and used them for the comparison. The nucleotide sequences of five *ccrA* genes, five *ccrB* genes, four *ccrC* genes, and two site-specific recombinases were aligned by using the ClustalX program. The phylogenetic tree was generated by the neighbor-joining method by creating 2,000 bootstrap replicates. The tree was visualized with Tree View software, which was obtained from the website http://taxonomy.zoology.gla.ac.uk/rod/treeview.htm. The branch length indicates the distance, which is expressed as the number of substitutions per 100 bases.

digested with BamHI. The fragments were cloned into the BamHI site of temperature-sensitive shuttle vector pYT3 to construct recombinant plasmids $pSR5_W$ and $pSR5_E$, respectively. The fidelity of the cloning was ascertained by determining the nucleotide sequence of the cloned DNA fragment. Recombinant plasmids were introduced into *S. aureus* cells by electroporation, as described previously (22).

For the excision assay, approximately $10^3$ cells of transformants and their parent strains were inoculated into 10 ml of BHI broth containing tetracycline (10 mg/liter), and the culture was incubated at 30°C with shaking. After 20 h, an aliquot of 0.8 ml was transferred to an Eppendorf tube and cells were collected by centrifugation. DNAs were extracted from the cells and used as templates for

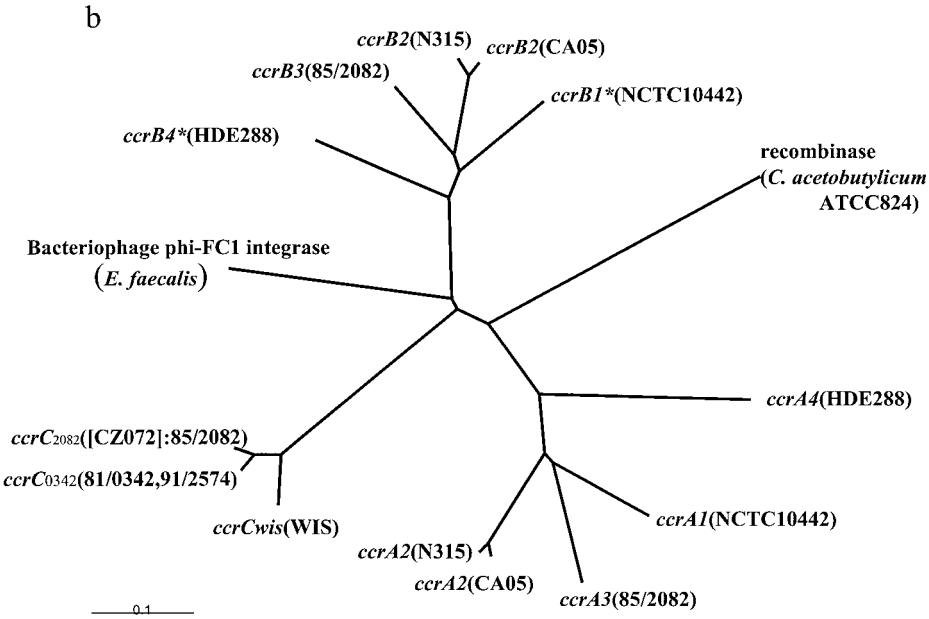


FIG. 2—*Continued.*

subsequent PCR experiments to identify the *att*B*scc* and *att*SCC sequences generated in the cells with the primer sets listed in Table 2.

To investigate the strains from which DNA was excised for the generation of SCC*mec*, cells were further cultivated in BHI broth with tetracycline at 30°C, with one passage per day for 10 days. The cultures were diluted, and approximately $10^3$ cells were inoculated for each passage. The cells were evaluated for the loss of SCC*mec* by replicating them onto agar plates with and without tobramycin at 10 mg/liter (for strain N315) and ceftizoxime at 5 mg/liter (for strain WIS).

**Construction of a recombinant plasmid carrying the *ccrC* gene and *att*SCC formed in WIS(pSR5$_E$) and integration assay.** Recombinant plasmid pSR5$_E$attV, which carried $ccrC_{0342}$ and *att*SCC formed in WIS(pSR5$_E$), was constructed as a model of the closed circular form of type V SCC*mec* (mini-SCC). Briefly, the DNA fragment containing *att*SCC of type V SCC*mec* was amplified by PCR with primers mVR2 and mVL2 (Table 2). DNA extracted from WIS(pSR5$_E$) was used as the template for the amplification of type V *att*SCC. A SalI-digested DNA fragment carrying *att*SCC type V was cloned into pYT3 to produce pYT3attV. Plasmid pSR5$_E$attV was constructed by cloning a BamHI-digested DNA fragment carrying $ccrC_{0342}$ into pYT3attV.

For the integration assay, recombinant plasmids and control plasmids were introduced into N315 by electroporation, and the cells were grown on BHI agar plates containing tetracycline at a concentration of 10 mg/liter at 30°C for 46 to 47 h. The colonies that grew on each tetracycline plate were resuspended in 0.5 ml of BHI broth, spread onto the BHI agar plates with or without tetracycline (10 mg/liter), and incubated at 30°C (permissive temperature for the replication of plasmid pYT3) and 43°C (a temperature nonpermissive for the replication of plasmid pYT3) for 18 h.

**Computer analysis.** Open reading frames (ORFs) of more than 100 bp were identified with the GAMBLER software, and their functions were predicted by a search homology with the BLAST program. All the ORFs in and around type V SCC*mec* were compared to those of the four known types of SCC*mec* (type I in strain NCTC10442 [DDBJ/EMBL/GenBank accession no. AB033763], type II in strain N315 [DDBJ/EMBL/GenBank accession no. D86934], type III in strain 85/2082 [DDBJ/EMBL/GenBank accession no. AB037671], and type IVa in strain CA05 [DDBJ/EMBL/GenBank accession no. AB063172]). The homologies between the nucleotide sequence of the type V SCC*mec* element of strain WIS and those of the type I, II, III, and IVa SCC*mec* elements were studied as described previously (20).

Several types of analyses were carried out with the BLAST program at the website of the National Center for Biotechnology Information (http://www3.ncbi.nlm.nih.gov/BLAST/). The codon usage values for prokaryotes were taken from a database (http:www.kazusa.or.jp/codon). Codon usage was tabulated from international DNA sequence databases (sequence status for the year 2000) (29). The similarity of codon usage was evaluated by codon bias analysis (19).

**Nucleotide sequence accession number.** The sequence of the type V SCC*mec* element of strain WIS has been deposited in the DDBJ/EMBL/GenBank databases under accession no. AB121219.

## RESULTS

**Type V SCC*mec* as a new member of the SCC*mec* family.** Our purpose was to investigate the genetic organization of the unknown element carrying the class C *mec* gene complex that was found in three strains, a C-MRSA strain (strain WIS) and two NORSA strains (strains 81/0342 and 91/2574). For that reason, we selected strain WIS and determined the nucleotide sequence of the region in and around the *mec* gene complex. The overall organization of the element and the strategy used to amplify the DNA fragment by long-range PCR with different sets of primers are shown in Fig. 1a.

The element carried the class C *mec* gene complex, which is composed of a copy of insertion sequence IS*431*, *mecA*, truncated *mecR1* (Δ*mecR1*), and another copy of IS*431* inserted in the opposite direction (21). From the size of Δ*mecR1* and the direction of another copy of IS*431*, it was judged that the element carried the class C2 *mec* gene complex (21).

Neither the *ccrA* gene nor the *ccrB* gene, both of which are responsible for the mobility of SCC*mec*, was found in the element. Instead, a novel ORF sequence, V15, encoding a protein similar to site-specific recombinases was found. The deduced amino acid sequence of V15 showed the highest degree of similarity to that of a type III SCC*mec* ORF, CZ072, with sequence identity of 93.2%.

We examined by PCR whether the novel ORF is commonly found in the other two NORSA strains. By amplifying DNA fragments with the primer set listed in Table 2 and determining the nucleotide sequences of the two DNA fragments, we found that the other two strains also carried identical ORFs. These ORFs were actually highly similar to that of WIS (ORF V15), with a nucleotide sequence identity of 91.4%.

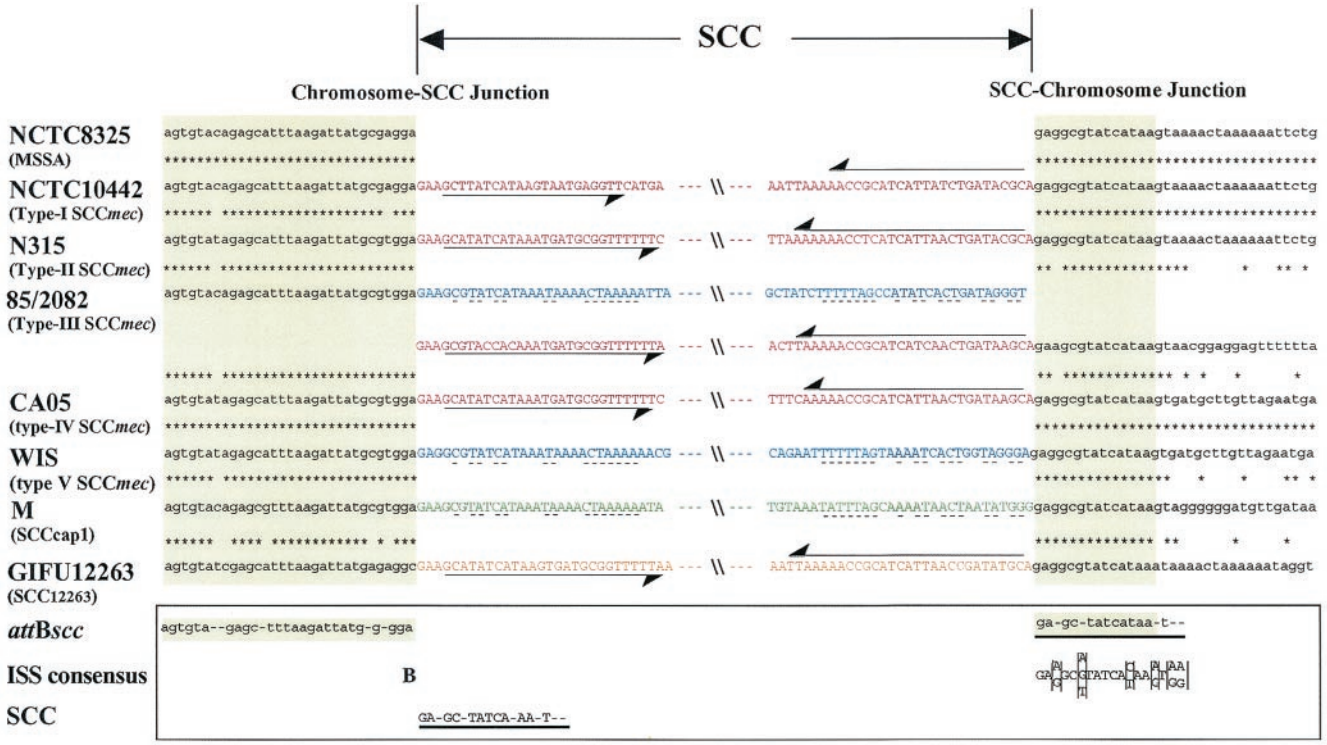


FIG. 3. Chromosome-SCC junction sequences. The nucleotide sequences at the left and right boundaries of the SCC*mec* element of strain WIS are aligned with those of six previously reported SCC elements: type I SCC*mec* of *S. aureus* NCTC10442 (DDBJ/EMBL/GenBank databases accession no. AB033763), type II SCC*mec* of *S. aureus* N315 (DDBJ/EMBL/GenBank databases accession no. D86934), type III SCC*mec* of *S. aureus* 85/2082 (DDBJ/EMBL/GenBank databases accession no. AB037671), type IV SCC*mec* of *S. aureus* CA05 (DDBJ/EMBL/GenBank databases accession no. AB063172), SCC*cap1* of *S. aureus* M (DDBJ/EMBL/GenBank databases accession no. U10927), and $SCC_{12263}$ of *S. hominis* GIFU12263 (DDBJ/EMBL/GenBank databases accession no. AB063171). Two sets of nucleotide sequences are listed for the type III SCC*mec* of *S. aureus* 85/2082. One set is composed of the nucleotide sequence of the left extremity (direct repeat 1) and the nucleotide sequence in the midst of the element flanked by direct repeat 2. The other set is composed of the nucleotide sequence containing direct repeat 2 and the right extremity of the element flanked by direct repeat 3 (15). Thin arrows indicate inverted repeats at both extremities of SCC*mec* elements carrying the *ccrA* and *ccrB* genes. Dotted lines indicate the characteristic nucleotide sequence conserved at both extremities of type V SCC*mec* or SCC elements carrying the *ccrC* gene. The 3′ ends of *orfX* are indicated with light green shading and lowercase bases. The consensus sequences of *att*B*scc* and ISS are boxed. Direct repeat sequences in ISS and the left end of the SCC element are indicated by thick lines. Nucleotide sequences of both extremities of SCC or SCC*mec* elements are colored as follows: red, type I, II, and IV SCC*mec* elements and the region between direct repeat 2 and direct repeat 3 in type III SCC*mec* element; orange, SCC12263; blue, type V SCC*mec* and the region between direct repeat 1 and direct repeat 2 in type III SCC*mec* element; green, SCCcap1.

The deduced amino acid sequences of the four ORFs, ORF V15 of strain WIS, the corresponding ORFs of strains 81/0342 and 91/2574, and ORF CZ072 of strain 85/2082, showed high degrees of similarity, with identities of 93.2 to 96.9%, although their C-terminal portions were dissimilar. Figure 2a shows the alignments of the deduced amino acid sequences of four ORFs: ORF V15, the corresponding ORF of 81/0342, CZ072 of 85/2082, and *ccrB* of N315. All four ORFs have a catalytic motif at the N-terminal domain, which is characteristic of recombinases of the invertase-resolvase family. They were basic proteins with pI values of 9.68 to 9.85. These features are similar to those of the CcrA and CcrB proteins that have been reported previously. We tried to relate the newly found *ccrC* genes phylogenetically to previously reported *ccr* genes: the three types of each of the *ccrA* and *ccrB* genes and another set of *ccrA* and *ccrB* genes (16, 33). In order to investigate the phylogenetic relations of those *ccr* genes, we reconstituted the putative *ccrB1* (ccrB1*) and *ccrB4* (*ccrB4**) genes of 1626 and 1,629 bp, respectively, by adding back an adenine to the deleted point of the truncated *ccrB1* and *ccrB4* genes, since *ccrB1* of NCTC10442 and *ccrB4* of HDE288 are truncated. Figure 2b illustrates the phylogenetic relations of those ORFs, extant Ccr proteins, and other site-specific recombinases such as the integrase of *Enterococcus faecalis* bacteriophage ϕ FC1 and the site-specific recombinase of *Clostridium acetobutylicum* ATCC 824 (Fig. 2b). A phylogenetic tree showed that the four ORFs constitute a novel group of *ccr* genes distinct from the *ccrA* and *ccrB* genes. Accordingly, we designated the ORFs cassette chromosome recombinase C (*ccrC*) as a new putative site-specific recombinase and the elements as type V SCC*mec*. Type V SCC*mec* is defined by the carriage of the class C2 *mec* gene complex and the *ccrC* gene, which is located in the type 5 *ccr* gene complex, as described below.

**The boundaries of type V SCC*mec*.** Both the left and right boundaries of type V SCC*mec* were determined by comparing their nucleotide sequences with those of previously reported SCC*mec* and SCC elements. Determination of the nucleotide sequence of *att*B*scc* on the chromosome and *att*SCC on closed

circular SCC, generated by precise excision of the element, supported the predicted positions of the boundaries. Type V SCC*mec* was integrated at exactly the same nucleotide position at the 3′ end of *orfX* (shown as a yellow square in Fig. 3), where the four types of SCC*mec* were integrated (Fig. 3). Two SCC elements which did not carry the *mecA* gene, SCC*cap1* and $SCC_{12263}$, were also integrated at the same position. SCC*cap1*, which is found in *S. aureus* M, is an element that carries a capsule gene cluster (25). $SCC_{12263}$ was found in *S. hominis* GIFU12263, and it is an element that carries active *ccrA1* and *ccrB1* genes (20). Previously, we could not judge the exact extremities of the SCC*mec* elements only by comparison of the nucleotide sequences of SCC*mec* elements found in *S. aureus* strains. This was possible because of the availability of new data from the nucleotide sequences of the *att*B*scc* elements from strains M and GIFU12263, generated by precise excision of the SCC element, and they were estimated to be at the positions shown in Fig. 3 (20, 25). The integration site sequence for SCC (ISS), which is uniquely present at the 3′ end of *orfX* in MSSA NCTC8325, is conserved in all strains examined so far. The ISS contains the consensus sequence 5′-BGA (A/G)GC(A/G/T)TATCA(C/T)AA(A/G)T(A/G)(A/G)-3′ (where the cutting site is between the B [B signifies A, C, or G] and the G at the 5′ end). The nucleotide sequences of the ISSs and the nucleotide sequences of the left extremities of the elements were nearly identical and constitute directly repeated sequences. These directly repeated sequences at the chromosome-SCC junction were found in all the elements, as shown in Fig. 3. In contrast, the degenerate inverted repeats found at both extremities of the four types of SCC*mec* and $SCC_{12263}$ were not found at the extremities of type V SCC*mec* or SCC*cap1* (Fig. 3).

**Structure of type V SCC*mec*.** Judging from the nucleotide sequences at both extremities of the type V SCC*mec* shown in Fig. 3, type V SCC*mec* was estimated to be 27,624 bp. This is slightly larger than the type IV SCC*mec* elements (21 to 25 kb) but smaller than the type I SCC*mec* element of NCTC10442 (34 kb), the type II SCC*mec* element of N315 (53 kb), and the type III SCC*mec* element of 85/2082 (67 kb).

A total of 23 ORFs larger than 100 bp were found in the type V SCC*mec* element (Fig. 1b and Table 3). No antibiotic resistance gene other than *mecA* was found in the element. Figure 4 compares the nucleotide sequences of the five types of SCC*mec* elements obtained with the BLAST program. Type V SCC*mec* is composed of the regions conserved in other types of SCC*mec* and the regions unique to type V SCC*mec*. The regions in type V SCC*mec* similar to extant SCC*mec* elements are denoted A, B, and C in Fig. 4. Region A contained seven ORFs conserved in all five types of SCC*mec* elements with very high degrees of identity. The identities between region A1 of type V SCC*mec* and the corresponding regions in other SCC*mec* elements were more than 99.7%. Region A1 contained six ORFs in the *mec* gene complex, a transposase for IS*431* (V03), a glycerophosphoryldiester phophodiesterase homologue (V04), a hypothetical protein (V05), *mecA* (V06), and Δ*mecR1* (V07) (shown in light gray in Fig. 1b). Δ*mecR1* (V07) was smaller than Δ*mecR* in the class B *mec* gene complex. Region A2 corresponded to IS*431*, which was found to be closely associated with the deletion point of Δ*mecR1*. This insertion of a copy of IS*431* whose direction is opposite that of IS*431 mec* is characteristic of the class C2 *mec* gene complex. The transposases V03 and V08 encoded by two IS*431* copies were not identical. The transposase V03 was nearly identical to that of IS*431mec* elements of four other types of SCC*mec* elements, whereas the transposase V08 showed slightly lower identity to that of IS431*mec* elements.

Region B represents the region conserved in type I, II, and IV SCC*mec* elements, with nucleotide sequence identities of 79.7 to 82.6%. Two ORFs, V17 and V19, were located in this region of type V SCC*mec* (Table 3). Two ORFs showed high degrees of similarity to the ORFs in the *ccr* gene complex of type I, II, and IV SCC*mec* elements.

The sequence of region C of type V SCC*mec* was homologous only to the corresponding region of the type III SCC*mec* element of strain 85/2082. Two regions of the type V SCC*mec* sequence, C1 and C2, showed high degrees of similarity to the corresponding region of the type III SCC*mec* element of strain 85/2082, with nucleotide sequence identities of 86.6 and 94.4%, respectively. Region C1 contained four ORFs (V12 to V15) whose sequences showed high degrees of similarity to the sequences of CZ072 (*ccrC*), CZ073, CZ074, and CZ075 located downstream of *orfX* in 85/2082. Although the region corresponding to region B was not identified in the type III SCC*mec* element, two sets of three ORFs whose sequences were similar to those of V16, V17, and V19, respectively, and which had amino acid identities ranging from 48.1 to 67.1% were found in the element. The first set of three ORFs (Z011, Z013, and Z014) was located downstream of the *ccrA3* and *ccrB3* genes, and these three ORFs were constituents of the type 3 *ccr* gene complex. The other set of three ORFs (CZ070, CZ069, and CZ068) was located downstream of *ccrC* (CZ072). It was noted that seven ORFs (three ORFs upstream of *ccrC*, *ccrC*, and three ORFs downstream of *ccrC*) were conserved in type V SCC*mec* and the J region of the type III SCC*mec* of 85/2082 (the amino acid identities of the corresponding ORFs were greater than or equal to 48.1%).

Thus, they were unified as a *ccr* gene complex together with the *ccr* gene, similar to the constructions of the type I to IV SCC*mec* elements.

Region C2 of type V SCC*mec* contained two ORFs (V20 and V21) which showed high degrees of similarity to CZ053 and CZ059 (truncated *hsdR*), respectively, in type III SCC*mec*. Although two regions, C1 and C2, were located near each other in type V SCC*mec*, the regions homologous to C1 and C2 in type III SCC*mec* were separated (Fig. 4).

The deduced amino acid sequence of ORF V21 (*hsdR*) showed a high degree of similarity (identity, 98.0%) to that of ORF Z059 (truncated *hsdR*) in the type III SCC*mec* of 85/2082, whereas it showed a very low degree of similarity (identity, less than 21%) to HsdR proteins encoded by *hsdR* genes in the *S. aureus* genome ($hsdR_{aur}$).

The regions other than those described above are unique in type V SCC*mec*. Two ORFs, V22 (*hsdS*) and V23 (*hsdM*), encoding the restriction-modification system were located at the right end of the element. Two type I restriction-modification DNA specificity domains, one of which is known as the target recognition domain and the other of which is the region conserved in the *hsd* subunit, were identified in ORF V22. The deduced amino acid sequence of ORF V23 showed the highest degree of similarity to the putative HsdM protein of *Lactococ-*

TABLE 3. ORFs in and around type V SCC*mec* of WIS with deduced products showing similarities to extant proteins

| ORF[a] | Value for CDS[b] | | | | | |
|---|---|---|---|---|---|---|
| | Starting nucleotide | Ending nucleotide | Size (bp) | Length (amino acids) | Gene | Product |
| V01 | 418 | 897 | 480 | 159 | *orfX* | Conserved hypothetical protein orfX |
| V02 | 952 | 2652 | 1,701 | 566 | | Hypothetical protein |
| V03 | 3367 | 2693 | 675 | 224 | *tnp* | Transposase for IS*431* |
| V04 | 4709 | 5452 | 744 | 247 | | Glycerophosphoryl diester phosphodiesterase homolog |
| V05 | 5549 | 5977 | 429 | 142 | | Conserved hypothetical protein |
| V06 | 8029 | 6023 | 2,007 | 668 | *mecA* | PBP 2′ |
| V07* | 8129 | 8239 | 111 | 36 | Δ*mec R1* | Truncated signal transducer protein MecR1 |
| V08 | 8277 | 8951 | 675 | 224 | *tnp* | Transposase for IS*431* |
| V09 | 9439 | 9011 | 429 | 142 | | Hypothetical protein |
| V10 | 9520 | 10449 | 930 | 309 | | Hypothetical protein |
| V11 | 10611 | 12599 | 1,989 | 662 | | Hypothetical protein |
| V12 | 12794 | 13903 | 1,110 | 369 | | Hypothetical protein |
| V13 | 13896 | 14264 | 369 | 122 | | Hypothetical protein |
| V14 | 14264 | 15907 | 1,644 | 547 | | Hypothetical protein |
| V15 | 16132 | 17754 | 1,623 | 540 | *ccrC* | Cassette chromosome recombinase C |
| V16 | 17915 | 18253 | 339 | 112 | | Hypothetical protein |
| V17 | 18332 | 18658 | 327 | 108 | | Hypothetical protein |
| V19 | 18674 | 19177 | 504 | 167 | | Hypothetical protein |
| V20 | 19563 | 20129 | 567 | 188 | | Hypothetical protein |
| V21 | 23325 | 20206 | 3,120 | 1039 | *hsdR* | Type 1 restriction-modification system endonuclease |
| V22 | 24547 | 23309 | 1,239 | 412 | *hsdS* | Specificity subunit of type I restriction-modification system |
| V23 | 26051 | 24537 | 1,515 | 504 | *hsdM* | Modification subunit of type I restriction-modification system |
| V24 | 26242 | 27054 | 813 | 270 | | Hypothetical protein |
| V25 | 27054 | 27986 | 933 | 310 | | Hypothetical protein |

[a] ORFs in parentheses were located outside of the type V SCC*mec*. Incomplete ORFs that are potentially defective genes or pseudogenes containing frameshift mutations are designated with asterisks.
[b] CDS, coding sequence. The nucleotide positions are given from the nucleotide sequence deposited in the DDBJ/EMBL/GenBank databases under accession no. AB121219, and the sizes were measured from the 5′ (starting nucleotide) to the 3′ (ending nucleotide) direction.
[c] Identity of the amino acid sequence to each ORF.

*cus sakei*, with an identity of 65.3%. On the other hand, both the HsdS (V22) and the HsdM (V23) proteins showed low levels of similarity to the HsdS and HsdM proteins encoded by the corresponding genes in staphylococcal G islands, with identities of less than 26 and 29%, respectively (3, 24). Figure 5 shows the phylogenetic relationships of the HsdR proteins (Fig. 5a), the HsdS proteins (Fig. 5b), and the HsdM proteins (Fig. 5c). Phylogenetic trees clearly showed that three ORFs, V21 (HsdR), V22 (HsdS), and V23 (HsdM), belonged to a group distinct from those found in the *S. aureus* genome or the G island.

The G+C content of type V SCC*mec* was 30.5%. This value is lower than that for *S. aureus* (32.8 to 32.9%).

Further analysis of the G+C content of the third nucleotide in the codon (GC3) revealed that the GC3 values for the ORFs in the region unique to type V SCC*mec* were very low (range, 14.2 to 19.7%; average, 18.6%). When we applied the definition of the criteria that we used for *S. aureus* genome analysis, the ORFs were regarded as possible alien genes; i.e., their GC3 values differed by more than 1.5 standard deviations from the average GC3 values for all ORFs longer than 150 bp in the *S. aureus* genome (3, 24). They were transposases for IS*431* (V02 and V08), *mecA* (V07), Δ*mecR1* (V08), V12, V17, V18, V19, V20, and *hsdS* (V22).

**CcrC-mediated precise excision and closed circular SCC*mec* formation.** To investigate whether CcrC can catalyze the precise excision of type V SCC*mec*, we constructed two recombinant plasmids, $pSR5_W$ and $pSR5_E$, which carried $ccrC_{WIS}$ and $ccrC_{0342}$, respectively, and introduced them into WIS and N315, respectively, by electroporation.

As controls, plasmids pSR2 (a plasmid formerly called pSR that carries the *ccrA2* and *ccrB2* genes) and pYT3 were also each introduced into WIS and N315. After cultivation of the eight purified transformants, WIS($pSR5_W$), WIS($pSR5_E$), WIS (pSR2), WIS(pYT3), N315($pSR5_W$), N315($pSR5_E$), N315(pSR2), and N315(pYT3), as well as recipient strains, for 20 h at 30°C, the DNAs were extracted from the cultures. We tested by PCR whether *att*B*scc* was generated and whether the closed circular form of SCC*mec* was formed in each strain. The strategy and the primer sets used for the detection of *att*B*scc* and the closed circular form of SCC*mec*, which carries *att*SCC, are shown in Fig. 6c and Table 2, respectively.

When we used the chromosomal DNAs of WIS($pSR5_W$) and WIS($pSR5_E$) as templates, a 0.31-kb DNA fragment containing *att*B*scc* and a 0.29-kb DNA fragment containing *att*SCC were successfully amplified, whereas no DNA fragment was amplified with DNAs extracted from WIS and WIS(pYT3) (Fig. 6a). By nucleotide sequencing of the amplified DNA fragments, we have verified that a novel nucleotide sequence, *att*SCC of type V SCC*mec*, is generated by the head-to-tail ligation of both termini and that *att*B*scc* is generated by the precise excision of type V SCC*mec* (Fig. 6b). These results show that CcrC serves as a site-specific recombinase responsible for the precise excision and formation of the closed circular form of type V SCC*mec*. Since no visible DNA fragment was amplified with DNAs extracted from N315($pSR5_W$) and N315

TABLE 3—*Continued*

| Data indicating homology to ORF in SCC*mec* of: | | | | | | | |
|---|---|---|---|---|---|---|---|
| NCTC10442 (type I) | | N315 (type II) | | 85/2082 (type III) | | CA05 (type IV) | |
| % Identity[c] | Corresponding ORF (size [bp]) | % Identity | Corresponding ORF(s) (size [bp]) | % Identity | Corresponding ORF(s) (size [bp]) | % Identity | Corresponding ORF (size [bp]) |
| 100 | *orfX* (480) | 100 | *orfX* (480) | 100 | *orfX* (480) | 100 | *orfX* (480) |
| 99.10 | E040 (675) | 99.10 | N062, N070 (675) | 99.1, 98.7, 98.2, 98.2 | Z035, Z041, Z046, Z058 (675) | 99.10 | Q012 (675) |
| 100 | CE026 (744) | 99.00 | CN039 (633) | 100 | CZ030 (744) | 100 | CQ007 (744) |
| 99.30 | CE025 (429) | 100 | CN038 (429) | 100 | CZ029 (429) | 100 | CQ005 (429) |
| 99.70 | $mecA_{10442}$ (2007) | 99.90 | $mecA_{N315}$ (2007) | 99.60 | $mecA_{2082}$ (2007) | 99.90 | $mecA_{CA05}$ (2007) |
| 100 | $\Delta mecR1_{10442}$ (987) | 100 | $mecR1_{N315}$ (1758) | 88.60 | $\Delta mecR1_{2082}$ (114) | 100 | $\Delta mecR1_{CA05}$ (987) |
| 97.80 | E040 (675) | 97.80 | N062, N070 (675) | 97.8, 97.3, 97.8, 97.8 | Z035, Z041, Z046, Z058 (675) | 97.80 | Q012 (675) |
| | | | | 76.70 | CZ075 (1101) | | |
| | | | | 84.30 | CZ074 (372) | | |
| | | | | 82.10 | CZ073 (1644) | | |
| | | | | 93.60 | CZ072 (1554) | | |
| 54.50 | E031 (351) | 53.60 | N041 (351) | 50.9, 48.6 | Z011 (351), CZ070 (342) | 55.90 | Q007 (351) |
| 91.70 | E032 (327) | 89.30 | N042 (312) | 48.1, 48.1 | Z013 (395), CZ069 (312) | 87.40 | Q008 (312) |
| 91.60 | E033 (510) | 92.80 | N043 (318) | 61.7, 67.1 | Z014 (522), CZ068 (507) | 93.40 | Q009 (510) |
| | | | | 97.20 | CZ053 (323) | | |
| | | | | 98.00 | Z059* (2244) | | |

($pSR5_E$), we could not be certain whether CcrC works on other types of SCC*mec* elements.

In contrast, DNA fragments of 0.28 and 0. 31 kb containing *att*B*scc* were amplified with DNAs extracted from N315(pSR2) and WIS(pSR2), respectively. Furthermore, DNA fragments of 0.46 and 0.29 kb containing *att*SCC were amplified with DNAs extracted from the two strains, respectively. Subsequent nucleotide sequencing showed that the fragments contained *att*B*scc* and *att*SCC. The data indicate that the sets of *ccrA* and *ccrB* genes cause precise excision and formation of the closed circular forms of both type II SCC*mec* and type V SCC*mec*.

**CcrC catalyzes site- and orientation-specific integration of type V SCC*mec*.** To examine whether the putative closed circular form of type V SCC*mec* serves as a substrate for integration similar to that of type II SCC*mec*, we constructed experimental plasmid $pSR5_E$attV, in which $ccrC_{0342}$ and the presumptive attachment sequence of type V SCC*mec* (*attV*) are subcloned, and used the plasmid as a model of the closed circular form of type V SCC*mec* (mini-SCC). Recombinant plasmids pSR2attII (a plasmid formerly called pSRatt that carries *ccr2* genes and the presumptive attachment sequence of type II SCC*mec* [*att*SCC-II]), pYTattII (a plasmid carrying *att*SCC-II), pYTattV (a plasmid that carries the presumptive attachment sequence of type V SCC*mec* [*att*SCC-V]), pSR2, $pSR5_E$, and pYT3 were used as controls. The recombinant plasmids were introduced into N315ex by electroporation, followed by selection on plates with tetracycline at 30°C. A colony of each transformant was respread onto BHI agar plates containing tetracycline (10 mg/liter) and incubated for 18 h at 30 and 43°C (the latter of which is nonpermissive for plasmid replication). The numbers of colonies that grew on each plate were counted. Two strains, N315ex($pSR5_E$attV) and N315ex (pSR2attII), generated significant number of colonies at 43°C compared with the numbers that grew at 30°C (2.2 and 4.4%, respectively), whereas the other strains did not generate colonies when they were grown at 43°C. The result shows that integration of the plasmids into the chromosome occurs when the *att*SCC and the *ccr* genes are present on the plasmids and that CcrC may mediate the integration of $pSR5_E$attV (a model of the closed circular form of type V SCC*mec*) at an efficiency nearly equal to that for the integration of pSR2attII (a model of the closed circular form of type II SCC*mec*) mediated by a set of CcrA and CcrB proteins.

The integration sites of the plasmids and their directions in the chromosome were examined by PCR experiments with primers as follows: cL1 and cR1, whose sequences flank that of the *att*B region on the chromosome of N315ex; α (RV in pUC119) and β (in the *ccrB* gene), whose sequences flank that of the *att*SCC-II region of plasmid pSR2attII; and γ (in the *ccrC* gene), whose sequence flanks the *att*SCC-V region of plasmid $pSR5_E$attV (Fig. 6d and Table 2). The results show that two plasmids, pSR2attII and $pSR5_E$attV, generated plasmid-chromosome integration junctions which were detected by two combinations of primers: primers cL1-β and cR1-α and primers cL1-γ and cR1-α, respectively. By determining the nucleotide sequences of the amplified DNA fragments, we found that the fragment contains sequences which are identical to those of the chromosome-SCC*mec* junction regions of N315 or artificially constructed chromosome (N315)-type V SCC*mec* junction regions.

These results indicate that the recombinant plasmid is integrated in the chromosome only when it carries the *ccrA* and *ccrB* genes and *att*SCC-II or the *ccrC* gene and *att*SCC-*attV* and that *ccrC* mediates the site- and orientation-specific integration of type V SCC*mec* in a way similar to that for a set of *ccrA* and *ccrB* genes.

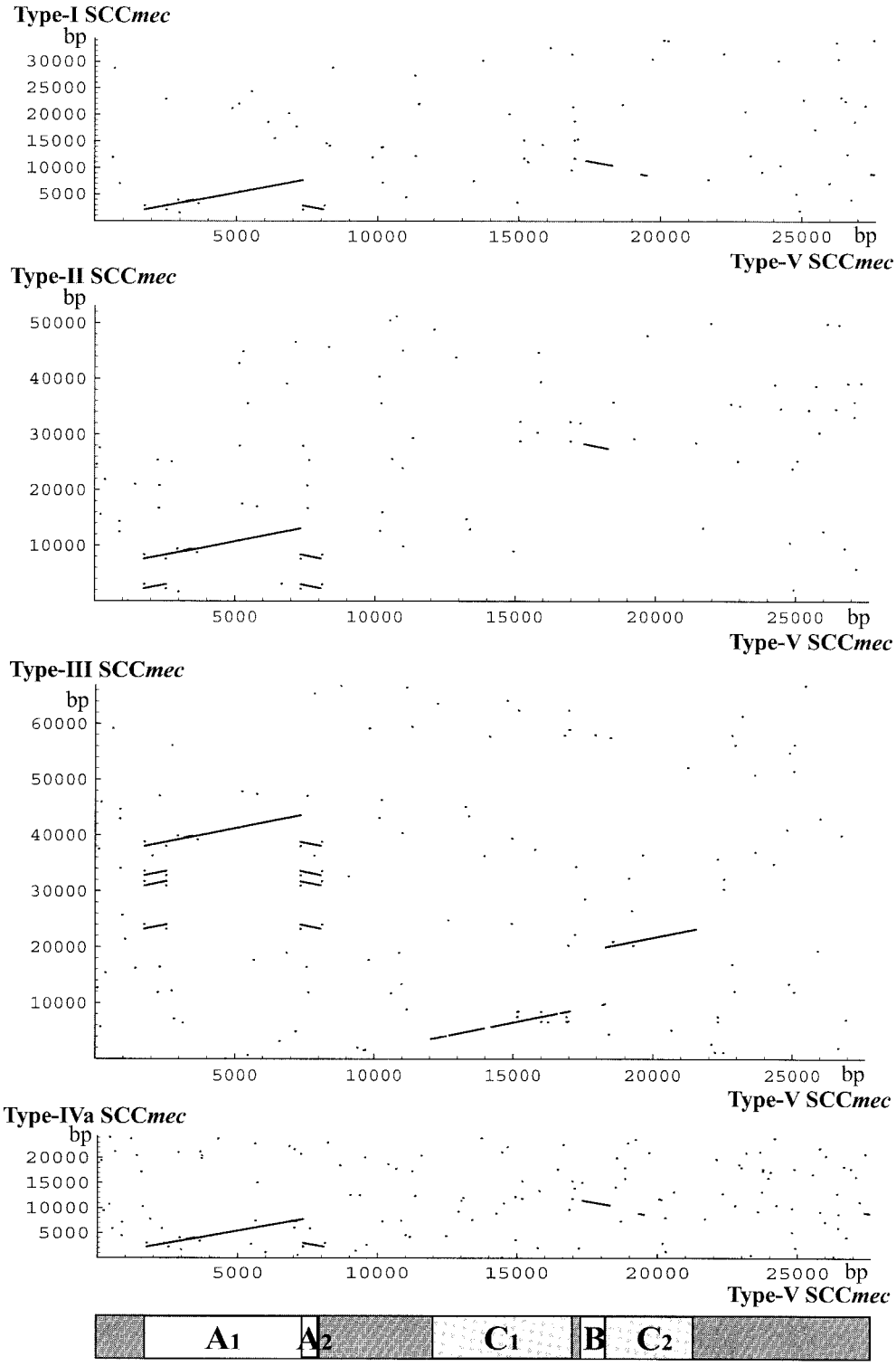


FIG. 4. Homologous regions in nucleotide sequences of the type V SCC*mec* and extant SCC*mec* elements. The nucleotide positions of the type V SCC*mec* element are indicated on the horizontal axes, and those of extant SCC*mec* elements (types I, II, III, and IVa) are indicated on the vertical axes. The following regions of the nucleotide sequences of the SCC*mec* elements were used: type V SCC*mec*, nucleotides 880 to 28503 of the nucleotide sequence with DDBJ/EMBL/GenBank databases accession no. AB121219; type I SCC*mec*, the region from nucleotides 4504 to 38867 of the nucleotide sequence with DDBJ/EMBL/GenBank databases accession no. AB033763; type II SCC*mec*, nucleotides 4687 to 57653 of the nucleotide sequence with DDBJ/EMBL/GenBank databases accession no. D86934; type III SCC*mec*, nucleotides 899 to 67794 of the nucleotide sequence with DDBJ/EMBL/GenBank databases accession no. AB037671; and type IVa SCC*mec*, nucleotides 975 to 25222 of the nucleotide sequence with DDBJ/EMBL/GenBank databases accession no. AB063172. The regions showing the high degrees of similarity between two SCC*mec* elements are indicated with lines. Rising lines signify that homologous regions between two elements are located in the opposite direction. Short homologous regions appear as dots. The regions through A (A1 and A2), B, and C (C1 and C2) were designated by the extent of nucleotide sequence identity described in the text. The locations of the regions in the nucleotide sequence of type V SCC*mec* are as follows: A1, nucleotide positions 1778 to 7341; A2, nucleotide positions 7342 to 8131; B, nucleotide positions, 16964 to 18296; C1, nucleotide positions 12041 to 17040; and C2, nucleotide positions 18309 to 21540.

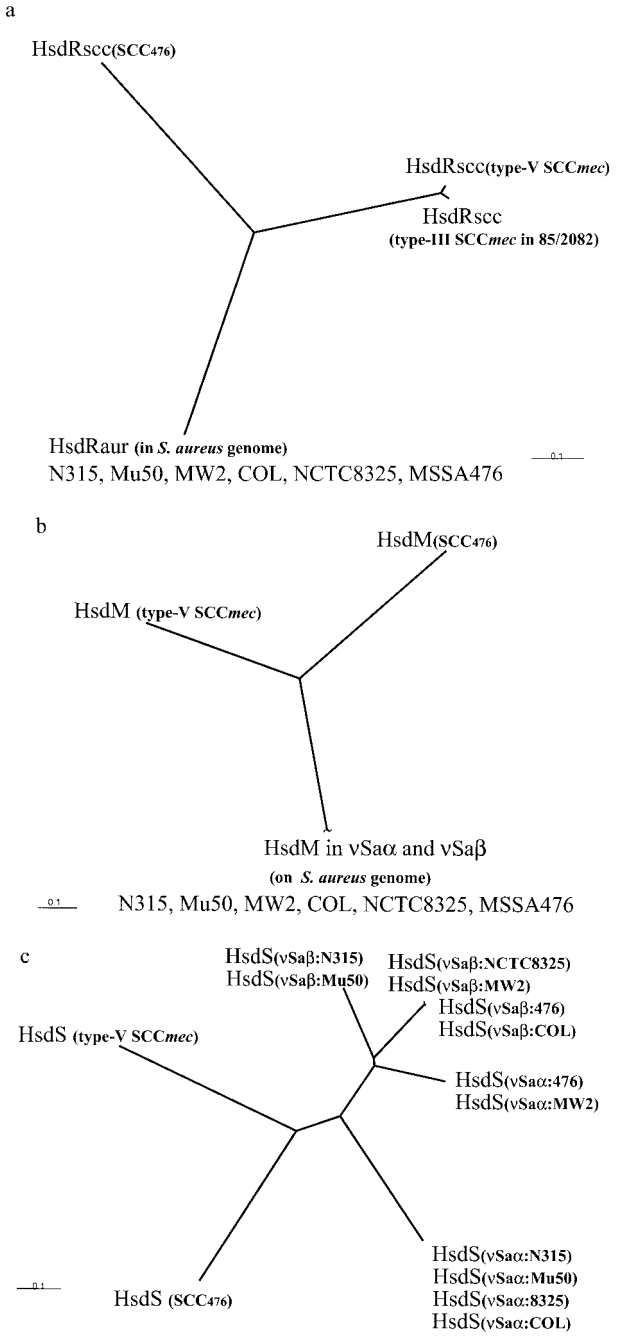


FIG. 5. Phylogenetic relations of the constituents of the *hsd* system. Type V SCC*mec* carries three genes, *hsdR*, *hsdM*, and *hsdS*, which encode a type I restriction-modification system. To look for the derivations of those genes, the phylogenetic relationships among the genes found in the type V SCC*mec* element and previously reported *hsd* genes were investigated by creating phylogenetic trees for the *hsdR* (a), *hsdM* (b), and *hsdS* (c) genes. Phylogenetic trees were generated as described in the legend to Fig. 2b. The nucleotide sequence of the truncated *hsdR* gene in type III SCC*mec* was obtained from the DDBJ/EMBL/GenBank databases (accession no. AB037671). All other nucleotide sequences of the *hsd* genes analyzed here were obtained from websites that provide the whole genome sequences of the following *S. aureus* strains: N315 and MW2 (http://www.bio.nite.go.jp/), Mu50 (http://w3.grt.kyushu-u.ac.jp/VRSA/), MSSA 476 and strain 252 (http://www.sanger.ac.uk/Projects/S_aureus/), COL (http://www.tigr.org/tdb/mdb/mdbinprogress.html), and NCTC8325 (http://www.genome.ou.edu/staph.html).

## DISCUSSION

**Characteristics of a new site-specific recombinase, CcrC.** We demonstrated in this study that type V SCC*mec* carries a recombinase gene, *ccrC*, which can mediate type V SCC*mec* recombination events (integration and excision), whereas a set of *ccrA* and *ccrB* genes was previously found to be required for this function in other types of SCC*mec* elements. It was curious that both *ccrC* gene variants, $ccrC_{WIS}$ (1,623 nucleotides) and $ccrC_{0342}$ (1,680 nucleotides), have been demonstrated to be active in the precise excision function. Approximately one-fourth of the deduced amino acid sequence from the $NH_2$ terminus, which contains the serine residue that catalyzes DNA strand exchange (36), was well conserved among the CcrB variant and two CcrC variants (Fig. 2a). Therefore, a lack of some amino acids in the COOH-terminal domain may not much affect the activities of Ccr proteins. However, when we compared the frequency of precise excision by quantitative PCR, we observed that *att*B*scc* was present in slightly larger amounts in WIS($pSR5_E$) than in WIS($pSR5_W$) (X. X. Ma, unpublished data). That was why we used $ccrC_{0342}$ to construct the recombinant plasmid used for the integration experiment.

Mini-SCC plasmid $pSR5_E$attV was integrated in the N315ex chromosome at exactly the same nucleotide position at which pSR2attII integrated. The data indicate that CcrC recognizes the ISS in a way similar to that in which a set of CcrA and CcrB proteins in combination does.

Interestingly, when *ccr* genes were introduced into host cells carrying preexisting SCC*mec* to test *ccr* gene-mediated excision, the frequency of excision differed depending on the combination of the types of SCC*mec* elements and *ccr* genes. The types of SCC*mec* elements greatly influenced the efficiency of excision mediated by *ccrC*. The recombinase encoded by *ccrC* appeared to be inactive in excising type II SCC*mec* from the N315 chromosome, while it was active in the excision of type V SCC*mec* from the WIS chromosome. It is tempting to speculate that the difference resides in the nucleotide sequences at the extremities of SCC*mec*. Characteristic inverted repeat sequences were located at the extremities of the extant types of SCC*mec* carrying *ccrA* and *ccrB* genes, whereas they were not found at the extremities of type V SCC*mec*. They have also not been found in the extremities of SCC*cap1*, which carries a broken homologue of the *ccrC* gene (25). A similar preference between the types of *ccr* and SCCs was also observed in the integration experiment. The efficiency of integration decreased when the recombinant plasmids carried the *att*SCC sequences of different types of SCC*mec* elements. The efficiency of integration observed with N315ex($pSR5_E$attII) and N315ex(pSR2attV) was smaller than that observed with N315ex($pSR5_E$attV) and N315ex(pSR2attII). Those data suggest that both *ccr* proteins have specificity for the recognition of the nucleotides located at the extremities of the SCC elements.

**Origin of *hsd* system and its role in maintenance of the element.** The restriction-modification system seems to play an important role in the stability of certain regions of the *S. aureus* chromosome (3, 24). A set of *hsdS* and *hsdM* genes is located in each of the two Gislands, *v*Saα and *v*Saβ, in the genomes of all seven *S. aureus* isolates that have been completely sequenced (isolates COL, MRSA 252, MSSA 476, MW2, Mu50,

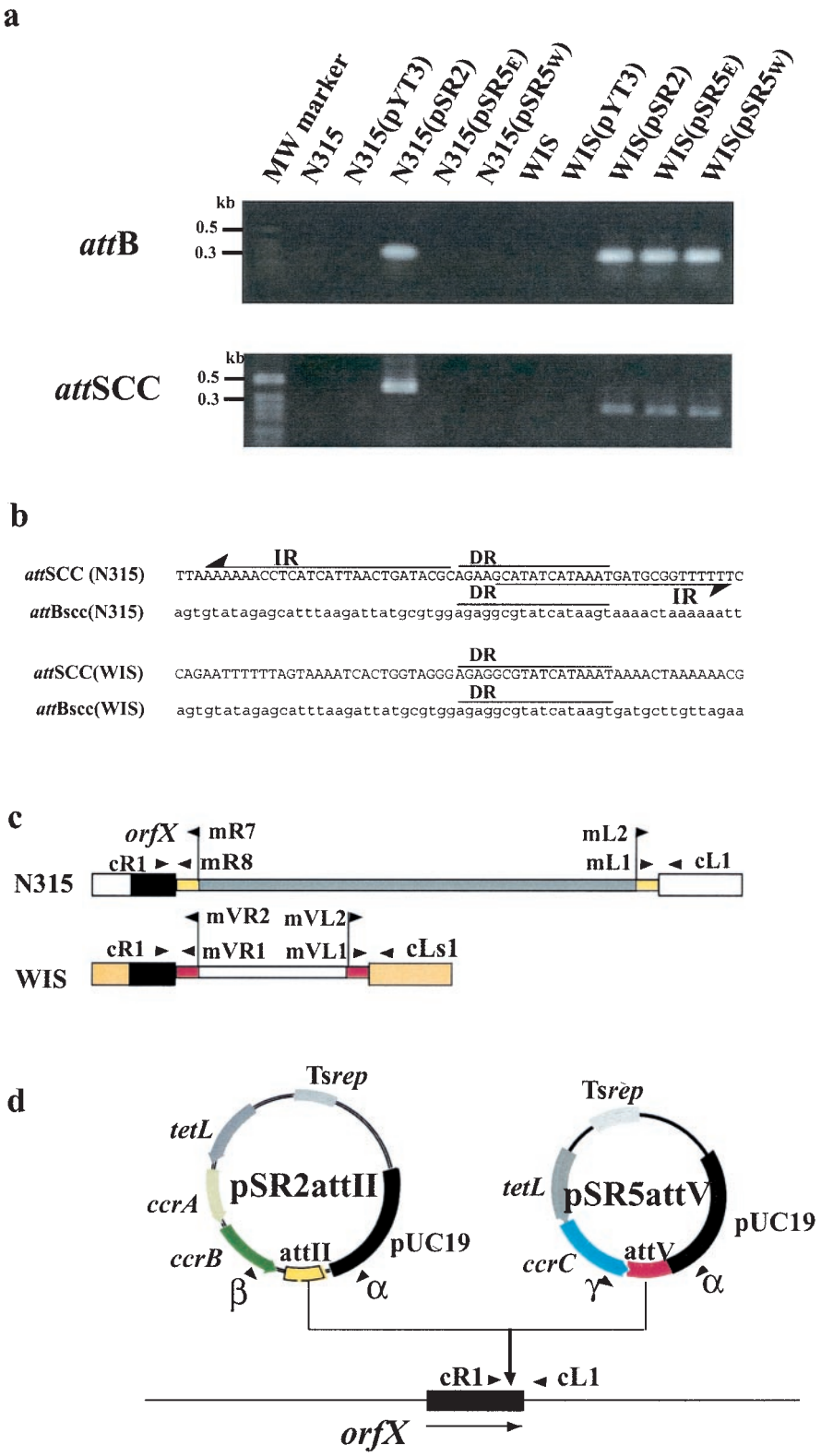


FIG. 6. Precise excision and site- and orientation-specific integration of SCC*mec*. (a) Detection of *ccr*-mediated SCC*mec* excision and appearance of *att*SCC. Template DNAs for PCR were extracted from a culture incubated in BHI broth with tetracycline (10 mg/liter) for 20 h. Four sets of primers were used to detect the precise excision and the closed circular form of SCC*mec* in strain N315 or WIS cells. The locations of the primers used are shown in panel b. For N315, primers cL1 and cR1 were used to detect *att*B*scc* (276 bp), and primers mL1 and mR8 were used to detect *att*SCC (456 bp). For WIS, primers cLs1 and cR1 were used to detect *att*B*scc* (314 bp), and primers mVL1 and mVR1 were used to detect *att*SCC (292bp). Lane MW, molecular size marker (1-kb ladder; Invitrogen, Carlsbad, Calif.), and only the relevant sizes are indicated. (b) Generation of *att*B*scc* and *att*SCC. The nucleotide sequences of *att*B*scc* in the DNA fragments amplified from N315ex and N315(pSR2) and

N315, and NCTC8325). The sequences of the HsdM proteins encoded by the two Gislands are nearly identical to each other, whereas those of the HsdS proteins in the target recognition domain regions differ significantly from each other. The *hsdR*$_{aur}$ gene (the *hsdR* gene commonly found on the genomes of the seven *S. aureus* strains) encodes the restriction function and is found on the *S. aureus* genome at the same locus, which is far from the two Gislands. The sequences of the HsdR proteins of the seven strains were nearly identical. It is speculated that the Gislands, which carry enterotoxins and exotoxin-like genes, are protected from deletional loss by the copresence of the modification function (3).

Unlike the other G islands, the type V SCC*mec* is unique in that it carries a complete set of the genes involved in the type I restriction-modification system, composed of *hsdR*, *hsdS*, and *hsdM*. A complete set of the genes involved in the restriction-modification system is also found on an SCC in MSSA strain 476 (SCC$_{476}$) (http://www.sanger.ac.uk/Projects/S_aureus/). The restriction-modification system encoded by those elements was judged to be distinct from that encoded by the *hsdR*$_{aur}$, *hsdM*, and *hsdS* genes on the *S. aureus* chromosome, according to data from phylogenetic trees as well as codon usage patterns. This may signify that those mobile genetic elements were formed in a species different from *S. aureus* and that *S. aureus* acquired those elements by horizontal transfer. We tried to excise SCC*mec* elements from strain WIS. Although we could not obtain them from either WIS(pSR5$_E$) or WIS(pSR5$_W$), large amounts of DNA fragments containing *attBscc* and *att*SCC were amplified by PCR from cultures of both strains. It is reported that carriage of type II restriction-modification system genes contributes to plasmid stability (7, 8). Handa et al. (10) showed that *Escherichia coli* cells harboring a plasmid that carries the type II restriction-modification system die due to restriction cleavage of the chromosome after they lose the plasmid. This postsegregational killing phenomenon may explain why we could not excise SCC*mec* elements, despite the PCR amplification results indicating that an apparent excision event occurred in cultured cells of strain WIS. The restriction enzymes (encoded by either *hsdRaur* or *hsdRscc*) that remain in the cells after the loss of type V SCC*mec* might have cleaved the chromosome and killed the cells from which the element was excised. Thus, the type I restriction-modification system may serve as a stabilizer of the type V SCC*mec* element integrated in the *S. aureus* chromosome.

**Type V SCC*mec* as an element found in C-MRSA.** MRSA clones are defined by the types of SCC*mec* and the genotypes of the MSSA chromosomes into which the SCC*mec* element is integrated. Multiple MRSA clones carrying type IV SCC*mec* were identified in C-MRSA strains from both the United States and Australia by multilocus sequence typing for chromosome genotyping and the PCR technique for SCC*mec* typing (32). On the basis of that observation, it was proposed that C-MRSA strains have been generated de novo from *S. aureus* populations more diverse than H-MRSA strains (32). The identification of a new type of SCC*mec* in C-MRSA strains in the present study further supports that proposal. Type V SCC*mec* was structurally similar to type IV SCC*mec*, in that it contains *mecA* as the only gene encoding antibiotic resistance. Its size (28 kb) was also comparable to that of type IV SCC*mec* and was much smaller than those of the type I to III SCC*mec* elements (34 to 67 kb) found in H-MRSA strains. Although the type V SCC*mec* element was slightly larger than the smallest subtype of type IV SCC*mec* (type IVb; 21 kb), the only difference was the presence of a restriction-modification system in type V SCC*mec*.

The most unique feature of type V SCC*mec* is the carriage of a type 5 *ccr* gene complex composed of the *ccrC* gene and its surrounding ORFs (Fig. 1). After sequence determination, we realized that the type 5 *ccr* gene complex is also found in SCC elements other than the type V SCC*mec*. SCC*cap1*, which encodes capsule formation but which has no *mec* gene, carried a gene complex similar to the type 5 *ccr* gene complex, although most of the ORFs in SCC*cap1* were truncated. In addition, the Ψ*ccr* gene complex that we previously reported in the type III SCC*mec* element was closely related to the type 5 *ccr* gene complex: ORF CZ072 was a *ccrC* gene homologue, and the sequences of the surrounding ORFs were highly homologous to those of the corresponding ORFs in the type 5 *ccr* gene complex (Table 3). A copy of type 3 *ccr* gene complex and three ISS copies, in addition to a copy of type 5 *ccr* gene complex, are present in the type III SCC*mec* element (13, 15). This configuration now indicates that the long DNA region downstream of *orfX*, previously designated the J3 region of type III SCC*mec*, is in fact an SCC element independent of the rest of the region. We now consider the latter region to constitute a true type III SCC*mec*. If this is the case, the SCC—in which a truncated copy of *hsdR*, the mercury resistance operon, and Tn*554*, in addition to the type 5 *ccr* gene complex, are carried—must have been integrated prior to or in succession with the type III SCC*mec* element.

The class C2 *mec* gene complex, which, along with the type 5 *ccr* gene complex, defines the type V SCC*mec* element, is distributed among coagulase-negative staphylococcal species, especially *S. haemolyticus*. Among 38 *S. haemolyticus* strains

from WIS(pSR2), WIS(pSR5$_W$), and WIS(pSR5$_E$) are indicated *att*B*scc*(N315) and *att*B*scc*(WIS), respectively. The nucleotide sequences of *att*SCC in DNA fragments amplified from N315(pSR2) and from WIS(pSR2), WIS(pSR5$_W$), and WIS(pSR5$_E$) are indicated *att*SCC(N315) and *att*SCC(WIS), respectively, above the nucleotide sequence of *att*B*scc*. (c) Locations of the primers used to detect *att*B*scc* generated from N315 (primers cR1 and cL1) and WIS (primers cR1 and cLs1) and *att*SCC generated from N315 (primers mR8 and mL1) and WIS (primers mVR1 and mVL1). The locations of the primers used to clone *att*SCC-II (primers mR7 and mL2) and *att*SCC-V (primers mVR2 and mVL2) are also illustrated. The drawings are not to scale. (d) Identification of site- and orientation-specific integration of recombinant plasmids (mini-SCCs) into the chromosome. The recombinant plasmids carrying *ccr* gene and *att*SCC of each type, pSR2attII and pSR5$_E$attV, were introduced into N315ex by electroporation as models of SCC*mec* elements. DNAs were extracted from cultures of the transformants grown at 43°C on BHI agar plates containing tetracycline (10 mg/liter). The locations and directions of the four primers (primers α, β, cL1, and cR1) used to detect the integration of plasmid pSR2attII as well as four primers (primers α, γ, cL1, and cR1) used to detect the integration of plasmid pSR5attV are illustrated. We could amplify DNA fragments of the expected sizes by the site- and orientation-specific integration of the plasmids. The expected sizes of the DNA fragments are 1,255 bp (cL1-β) and 735 bp (cR1-α) for pSR2attII and 826 bp (cL1-γ) and 719 bp (cR1-α).

tested, 30 strains carried the class C2 *mec* gene complex and the other 8 strains carried *mec* gene complexes of class A (5 strain), class B (2 strain), or class C1 (1 strain) (21). The deletion point of Δ*mecR1* in the class C2 *mec* gene complex of the type V SCC*mec* element was identical to that in the class C2 *mec* gene complex found in *S. haemolyticus* strain JB16 and *S. epidermidis* strain JK8 (21). The data suggest the horizontal transfer of a certain molecular version of the class C2 *mec* gene complex among staphylococcal species. On the other hand, only 20 of 30 *S. haemolyticus* strains carrying the class C2 *mec* gene complex had the *ccrC* gene. Three other strains carried *ccrA2* and *ccrB2* genes, and the remaining seven did not carry any of the type 1 to 4 *ccr* genes (X. X. Ma, H. Yuzawa, and M. Kapi, unpublished observations). Thus, class C2 may not always be linked with the type 5 *ccr* gene complex, and additional types of SCC*mec* elements may be existent in coagulase-negative staphylococci. We have also reported that many subtypes defined by J1-region polymorphisms exist in the type IV SCC*mec* elements prevalent in C-MRSA strains. Type IV SCC*mec* elements with diverse J1 regions are distributed in more than 30% of community-acquired *S. epidermidis* isolates in Japan (K. Hisata, K. Hiramatsu et al., unpublished data). We think that it is quite likely that the *mec* gene complex and the *ccr* gene complexes (of diverse SCC elements) go through complex recombination and rearrangement processes in the genomes of coagulase-negative staphylococci; thus, novel types of SCC*mec* elements are incessantly generated, and only a fraction of them are transferred to *S. aureus* strains in the community. Thus, we may be witnessing only the tip of the iceberg of the SCC*mec* element diversity displayed by C-MRSA isolates. The type IV and type V SCC*mec* elements that have come onto the scene, however, may be the most refined molecular products and may have high competitive capabilities in terms of their transferability among the cells of staphylococcal species and strains.

## REFERENCES


1. **Archer, G. L., D. M. Niemeyer, J. A. Thanassi, and M. J. Pucci.** 1994. Dissemination among staphylococci of DNA sequences associated with methicillin resistance. Antimicrob. Agents Chemother. **38:**447–454.
2. **Ayliffe, G. A.** 1997. The progressive international spread of methicillin-resistant *Staphylococcus aureus.* Clin. Infect. Dis. **24:**74–79.
3. **Baba, T., F. Takeuchi, M. Kuroda, H. Yuzawa, K. Aoki, A. Oguchi, Y. Nagai, N. Iwama, K. Asano, T. Naimi, H. Kuroda, L. Cui, K. Yamamoto, and K. Hiramatsu.** 2002. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet **359:**1819–1827.
4. **Centers for Disease Control and Prevention.** 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. JAMA **282:**1123–1125.
5. **Chambers, H. F.** 2001. The changing epidemiology of *Staphylococcus aureus.* Emerg. Infect. Dis. **7:**178–182.
6. **Enright, M. C., D. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt.** 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA **99:**7687–7692.
7. **Gerdes, K., P. B. Rasmussen, and S. Molin.** 1986. Unique type of plasmid maintenance function: postsegregational killing of plasmid-free cells. Proc. Natl. Acad. Sci. USA **83:**3116–3120.
8. **Gillen, J. R., D. K. Willis, and A. J. Clark.** 1981. Genetic analysis of the RecE pathway of genetic recombination in *Escherichia coli* K-12. J. Bacteriol. **145:**521–532.
9. **Groom, A. V., D. H. Wolsey, T. S. Naimi, K. Smith, S. Johnson, D. Boxrud, K. A. Moore, and J. E. Cheek.** 2001. Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural American Indian community. JAMA **286:**1201–1205.

9a. **Hanaki, H., K. Kuwahara-Arai, S. Boyle-Vavra, R. S. Daum, H. Labischinski, and K. Hiramatsu.** 1998. Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50. J. Antimicrob. Chemother. **42:**199–209.

10. **Handa, N., A. Ichige, K. Kusano, and I. Kobayashi.** 2000. Cellular responses to postsegregational killing by restriction-modification genes. J. Bacteriol. **182:**2218–2229.
11. **Hartman, B. J., and A. Tomasz.** 1984. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus.* J. Bacteriol. **158:**513–516.
12. **Hiramatsu, K., Asada, E. Suzuki, K. Okonogi, and T. Yokota.** 1992. Molecular cloning and nucleotide sequence determination of the regulator region of *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA). FEBS Lett. **298:**133–136.
13. **Hiramatsu, K., Y. Katayama, H. Yuzawa, and T. Ito.** 2002. Molecular genetics of methicillin-resistant Staphylococcus aureus. Int. J. Med. Microbiol. **292:**67–74.
14. **Hurlimann-Dalel, R. L., C. Ryffel, F. H. Kayser, and B. Berger-Bachi.** 1992. Survey of the methicillin resistance-associated genes *mecA*, *mecR1-mecI*, and *femA-femB* in clinical isolates of methicillin-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **36:**2617–2621.
15. **Ito, T., K. Okuma, X. X. Ma, H. Yuzawa, and K. Hiramatsu.** 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug. Resist. Update **6:**41–52.
16. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **45:**1323–1336.
17. **Ito, T., Y. Katayama, and K. Hiramatsu.** 1999. Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Chemother. **43:**1449–1458.
18. **Jevons, M. P.** 1961. "Celbenin"-resistant staphylococci. Br. Med. J. **124:**124–125.
19. **Karlin, S., J. Mrazek, and A. M. Cambell.** 1998. Codon usages in different gene classes of the *Escherichia coli* genome. Mol. Microbiol. **29:**1341–1355.
20. **Katayama, Y., F. Takeuchi, T. Ito, X. X. Ma, Y. Ui-Mizutani, I. Kobayashi, and K. Hiramatsu.** 2003. Identification in methicillin-susceptible *Staphylococcus hominis* of an active primordial mobile genetic element for the staphylococcal cassette chromosome *mec* of methicillin-resistant *Staphylococcus aureus.* J. Bacteriol. **185:**2711–2722.
21. **Katayama, Y., T. Ito, and K. Hiramatsu.** 2001. Genetic organization of the chromosome region surrounding *mecA* in clinical staphylococcal strains: role of IS*431*-mediated *mecI* deletion in expression of resistance in *mecA*-carrying, low-level methicillin-resistant *Staphylococcus haemolyticus.* Antimicrob. Agents Chemother. **45:**1955–1963.
22. **Katayama, Y., T. Ito, and K. Hiramatsu.** 2000. A new class of genetic element, staphylococcal cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus.* Antimicrob. Agents Chemother. **44:**1549–1555.
23. **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.
24. **Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu.** 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus.* Lancet **357:**1225–1240.
25. **Luong, T. T., S. Ouyang, K. Bush, and C. Y. Lee.** 2002. Type 1 capsule genes of *Staphylococcus aureus* are carried in a staphylococcal cassette chromosome genetic element. J. Bacteriol. **184:**3623–3629.
26. **Ma, X. X., T. Ito, C. Tiensasitorn, M. Jamklang, P. Chongtrakool, S. Boyle-Vavra, R. S. Daum, and K. Hiramatsu.** 2002. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **46:**1147–1152.
27. **Matsuhashi, M., M. D. Song, F. Ishimoto, M. Wachi, M. Doi, M. Inoue, K. Ubukata, N. Yamashita, and M. Konno.** 1986. Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to β-lactam antibiotics in *Staphylococcus aureus.* J. Bacteriol. **167:**975–980.
28. **Naimi, T. S., K. H. LeDell, D. J. Boxrud, A. V. Groom, C. D. Steward, S. K. Johnson, J. M. Besser, C. O'Boyle, R. N. Danila, J. E. Cheek, M. T. Osterholm, K. A. Moore, and K. E. Smith.** 2001. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996–1998. Clin. Infect. Dis. **33:**990–996.
29. **Nakamura, Y., T. Gojobori, and T. Ikemura.** 2000. Codon usage tabulated from international DNA sequence databases: status for the year 2000. Nucleic Acids Res. **28:**292.
30. **Nimmo, G. R., J. Schooneveldt, G. O'Kane, B. McCall, and A. Vickery.** 2000. Community acquisition of gentamicin-sensitive methicillin-resistant *Staphylococcus aureus* in southeast Queensland, Australia. J. Clin. Microbiol. **38:**3926–3931.

31. **O'Brien, F. G., J. W. Pearman, M. Gracey, T. V. Riley, and W. B. Grubb.** 1999. Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. J. Clin. Microbiol. **37:**2858–2862.
32. **Okuma, K., K. Iwakawa, J. D. Turnidge, W. B. Grubb, J. M. Bell, F. G. O'Brien, G. W. Coombs, J. W. Pearman, F. C. Tenover, M. Kapi, C. Tiensasitorn, T. Ito, and K. Hiramatsu.** 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. **40:**4289–4294.
33. **Oliveira, D. C., A. Tomasz, and H. Lencastre.** 2001. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. Microb. Drug Resist. **7:**349–361.
34. **Reynolds, P. E., and D. F. J. Brown.** 1985. Penicillin-binding proteins of beta-lactam resistant strains of *Staphylococcus aureus.* FEBS Lett. **192:**28–32.
35. **Ryffel, C., W. Tesch, I. Birch-Machin, P. E. Reynolds, L. Barberis-Maino, F. H. Kayser, and B. Berger-Bachi.** 1990. Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis.* Gene **94:**137–138.
36. **Sherratt, D.** 1989. Tn*3* and related transposable elements: site-specific recombination and transposition, p. 163–184. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
37. **Sjostrom, J. E., S. Lofdahl, and L. Phillipson.** 1975. Transformation reveals a chromosomal locus of the gene(s) for methicillin resistance in *Staphylococcus aureus.* J. Bacteriol. **123:**905–915.
38. **Song, M. D., M. Wachi, M. Doi, F. Ishino, and M. Matsuhashi.** 1987. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. FEBS Lett. **221:**167–171.
39. **Sousa, M. A., and H. Lencastre.** 2003. Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. J. Clin. Microbiol. **41:**3806–3815.
40. **Suzuki, E., K. Hiramatsu, and T. Yokota.** 1992. Survey of methicillin-resistant clinical strains of coagulase-negative staphylococci for *mecA* gene distribution. Antimicrob. Agents Chemother. **36:**429–434.
41. **Suzuki, E., K. Kuwahara-Arai, J. F. Richardson, and K. Hiramatsu.** 1993. Distribution of *mec* regulator genes in methicillin-resistant *Staphylococcus* clinical strains. Antimicrob. Agents Chemother. **37:**1219–1226.
42. **Utsui, Y., and T. Yokota.** 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **28:**397–403.
43. **Vandenesch, F., T. Naimi, M. C. Enright, G. Lina, G. R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M. E. Reverdy, and J. Etienne.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. **9:**978–984.