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Novel Mobile Variants of Staphylococcal Cassette Chromosome *mec* in *Staphylococcus aureus*

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Staphylococcus aureus **staphylococcal cassette chromosome *mec* type IV (SCC*mec* IV) is associated with virulent community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and frequent horizontal transfer among staphylococci. To gain insight into the mechanism of transfer, we studied the *ccrA*/*B* type 2 recombinase-mediated excision of SCC*mec* IV ($n = 5$ strains) and SCC*mec* II ($n = 2$). In SCC*mec* IV- but not SCC*mec* II-containing strains, spontaneous excision of the cassette was observed. Introduction of *ccrA*/*B* type 2 recombinase genes under control of an *S. aureus* bacterial phage promoter in the different strains yielded excision of SCC*mec* II and multiple excision variants of SCC*mec* IV. Sequencing of the alternatively excised products in SCC*mec* IV strains identified a 100-bp shortened SCC*mec*′ variant and a 5,877-bp, conserved SCC-like element that lacks *mecA* and *ccrA*/*B* recombinases. Excision of the SCC-like element in wild-type *S. aureus* was dependent on the presence of SCC*mec*. The element could be excised separately or as part of a novel composite cassette together with SCC*mec*. The relative abundance of and variety in SCC*mec* IV excisions may contribute to the frequency of horizontal transfer and genetic plasticity in SCC*mec* IV MRSA strains.**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen (2). Methicillin resistance is conferred by the *mecA*-encoded PBP2′, which has a low affinity for beta-lactam antibiotics. *mecA* is located on the staphylococcal cassette chromosome *mec* (SCC*mec*), a large 20- to 65-kb mobile element in *S. aureus* (9). Based on the class of *mecA* gene complex and the type of *ccr* gene complex present, SCC*mec* cassettes are classified as types I to V (6, 7, 12). SCC*mec* type II (SCC*mec* II) carries *ccrA*/*B* type 2 recombinases, and SCC*mec* IV carries *ccrA*/*B* type 2 or type 4 recombinases (depending on the classification scheme used), whereas SCC*mec* V carries a *ccrC* recombinase. After cloning of the *ccrA*/*B* type 2 and *ccrC* promoter-gene complex into SCC*mec* II and V MRSA strains, respectively, chromosomal excision and integration of the SCC*mec* cassette have been observed (6, 9). The *ccr* recombinases are anticipated to cleave DNA in the *attB* site directly upstream of the integration site sequence (ISS) for SCC. The ISS has a putative consensus sequence, GANGCNTATCANAANTNN, which is present at the 3′ end of *orfX* in *S. aureus* (6).

The relatively small SCC*mec* IV may be more frequently transferred among staphylococci and is associated with community-acquired MRSA (14). We have previously described a possible case of in vivo transfer of SCC*mec* IV from *Staphylococcus epidermidis* to *S. aureus* (16). In this study, three strains are described which were consecutively isolated from a single patient. Methicillin-susceptible *Staphylococcus aureus* (MSSA) strain wkz-1 shared among others a unique pulsed-field gel electrophoresis pattern with MRSA strain wkz-2, except for the presence of an SCC*mec* IV cassette. A cassette with the same restriction pattern was found in *S. epidermidis* strain O7.1, which may have served as the SCC*mec* donor strain (16). The mechanism of SCC*mec* transfer, however, is still unknown.

To gain insight into the mechanism of transfer, we further characterized these strains and studied the excision of SCC*mec* IV in these and other MRSA strains. Our data show that chromosomal excision mediated by *ccrA*/*B* type 2-containing recombinases is a dynamic process that is certainly not confined to SCC*mec* IV alone. We provide evidence for the excision and circularization of SCC*mec* IV in wild-type *S. aureus* and *S. epidermidis* strains and the formation and excision of a new composite SCC*mec*.

MATERIALS AND METHODS

Strains. Staphylococcal strains used in this study include MSSA wkz-1, SCC*mec* IV MRSA wkz-2, *S. epidermidis* O7.1 (16), MRSA MR108 (5, 8), MW2 (1), Ca05 (JCSC1968), and 8/6-3P (JCSC1978) (11), and SCC*mec* II MRSA Mu50 and N315 (10). We used *Escherichia coli* DH10β and *S. aureus* strain RN4220 for cloning of the different *ccrA*/*B* constructs in *S. aureus*. *S. aureus* strain WVW 189 was used for the amplification of an *S. aureus* phage repressor promoter. WVW 189 is RN6390 containing pACL 1484 with a phage repressor promoter cloned in its EcoRI/XbaI site, upstream of GFP*uvr*. This phage promoter yields a strong, constant expression of green fluorescent protein in *S. aureus* (15). The primers used in this study are listed in Table 1. Each primer is given a unique code and a number corresponding to the location of the primer as shown in Fig. 2. Depending on the genetic variation in the strains at different primer positions, primers with different codes may share the same primer location number (Table 1).

Cloning. Two different recombinant *ccrA*/*B* type 2 plasmids were constructed in which the *ccrA*/*B* genes were placed either under the control of their endogenous promoter or under the control of a phage repressor promoter. The endogenous −300-bp promoter region and adjacent *ccrA*/*B* genes were amplified by PCR from MRSA strain wkz-2, using primers C1 and C2 (Table 1). To generate the phage repressor *ccrA*/*B* construct, the wkz-2 *ccrA*/*B* genes were amplified using primers C2 and C3 with DNA from strain wkz-2. The phage repressor promoter was obtained from strain WVW 189 by using primers C4 and C5 (15). The endogenous promoter-*ccrA*/*B* fragment was cloned in the EcoRI-SalI site of shuttle vector pSK236, whereas the phage promoter and *ccrA*/*B* genes were

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TABLE 1. Primers used for cloning and excision experiments

Application	Code	Position in Fig. 2	Location	Orientation[a]	Strain(s)[b]	Sequence[c]	TA (°C)[d]
Cloning	C1		*ccr* promoter	F	wkz-2, O7.1	CGGAATTCTGTAGAGTTTGCATCTATCCTGG	68
	C2		*ccrA/B*	R	wkz-2, O7.1	ACGCGTCGACTCTGTTTCTTCGAATCTGCAAAT	74
	C3		*ccrA/B*	F	wkz-2, O7.1	GCTCTAGAATGAAACAACAATCTCTTGC	62
	C4		Phage promoter	F	WVW 189	CGGAATTCCTTGTTTTGAATCAAGTCA	66
	C5		Phage promoter	R	WVW 189	GCTCTAGACCGTTTGATAACTTCATAAT	56
Excision	E1	1	Upstream of SCC-like element	F	wkz-2, MR108	GCATTCAGATTATTGACTGTTGG	58
	E2	2	SCC-like element	R	wkz-2, MR108	CTACCAGCAATACCTCATACC	52
	E3	3	Upstream of SCC*mec*	F	wkz-2, MR108	TTTTGCTGTTTTTATCACCATATTGAA	59
	E4				Ca05	AATTTACCAGACAGCCTGGTGC	61
	E5				JCSC1978, Mu50, N315	ATTTAATGTCCACCATTTAACA	53
	E6	4	SCC*mec*	R	wkz-2, O7.1, Ca05	GTCCTAACAAGCGGTCAACACC	62
	E7				JCSC1978	CATCAAACTTTAAGGGAGAAGC	57
	E8				MW2, Ca05, wkz 2	CCACGTTATGGAGGTGCTCTG	62
	E9				MR108	AACGGTCTGGACGAAGTAAGG	59
	E10				Mu50, N315	**GAATCTTCAGCATGTGATTTA**	52
	E11	5	SCC*mec*	F	All	**ATGAAAGACTGCGGAGGCTAACT**	61
	E12	6	SCC*mec*	R	All	CAGCCGCTTCATAAAGGGATT	61
	E13	7	SCC*mec*	F	wkz-2	GGCTGAAAAAACCGCATCAT	61
	E14	8	*orfX*	R	All	**AAACGACATGAAAATCACCAT**	56

[a] F, forward primer; R, reverse primer.
[b] Strain(s) on which primers were used.
[c] Primer sequences depicted in bold have been described previously Katayama et al. (9).
[d] Annealing temperature was chosen 5°C below the melting temperature of the primer. For primer combinations in the PCR, the lowest TA was chosen.

cloned in the EcoRI-XbaI and XbaI-SalI sites of this vector, respectively. The pSK236 vector confers carbacillin resistance in *E. coli* and chloramphenicol resistance in *S. aureus*. The pSK236 recombinant plasmids were introduced in *E. coli* DH10β and subsequently introduced in the DNA restriction-deficient *S. aureus* strain RN4220 by electroporation. The endogenous *ccrA/B*-promoter construct was transduced into MRSA wkz-2, using phage 11 according to standard procedures. The phage repressor promoter-*ccrA/B* construct was transduced into MSSA wkz-1 and the MRSA strains wkz-2, Ca05, JCSC 1978, MW2, MR108, Mu50, and N315. The inserts of all bacterial clones were confirmed with PCR and sequencing.

PCR. *S. aureus* was grown on Luria-Bertani agar plates (containing 10 mg/liter chloramphenicol when pSK236 derivatives were present) for 16 h at 37°C. DNA lysates were made using standard methods. Chromosomal DNA was purified using a QIAGEN purification kit (Hilden, Germany). Excision of SCC*mec* was measured by PCR, using divergent primers on both outer regions of the cassette that yield a PCR product only upon excision and circularization of the SCC element, analogous to the method described in reference 9. Excision was confirmed using two chromosomal primers flanking the SCC elements to detect the reformed *attB* site upon excision of the cassette (9). Except for PCRs to detect *attB* after SCC excision, which were performed on chromosomal purified DNA, all PCRs were performed on DNA lysates. This was necessary since the circular SCC*mec* was not efficiently purified by either plasmid or chromosomal DNA isolation protocols (data not shown). PCRs that did not yield products specific for SCC circularization, or the reformed *attB* site, were performed again with different template concentrations, using a touchdown PCR program. The touchdown PCR program was as follows: an initial denaturation step for 5 min at 94°C, followed by an initial 10 cycles of three steps consisting of 30 s at 94°C, 1 min at annealing temperature (TA) plus 10°C to TA (Table 1) decreasing 1°C per cycle, and 1 min 30 s at 72°C for extension. These first 10 cycles were followed by another 25 to 35 cycles of three steps consisting of 30 s at 94°C, 1 min at TA (Table 1), and 1 min 30 s at 72°C and a final 5-min extra extension. A PCR strategy according to Oliveira and de Lencastre (12) was performed for subtyping of SCC*mec*.

Sequencing. All PCR-amplified fragments were purified with a QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and sequenced. DNA regions upstream of SCC*mec* were sequenced using an LA PCR in vitro cloning kit (Takara Mirus Bio Corporate Information, Madison, WI) according to the manufacturer's protocol. This kit enables nested PCR amplification of unknown sequences upstream of known sequences. Sequences were analyzed against all available sequences by using the BLAST algorithm. Multilocus sequence typing (MLST) was performed according to Enright et al. (4).

RESULTS

Genetic characterization of strains associated with horizontal transfer of SCC*mec*. MRSA wkz-2 may originate from the transfer of SCC*mec* IV from *S. epidermidis* O7.1 to MSSA wkz-1 (16). Therefore, excision of SCC*mec* was studied primarily in these strains. To this end, first these strains and the SCC*mec* elements carried by them were further genetically characterized. Both *S. aureus* strain wkz-1 and *S. aureus* strain wkz-2 have MLST sequence type 30. The SCC*mec* types of strains wkz-2 and O7.1 were identical according to the multiplex PCR developed by Oliveira and de Lencastre (12). Both cassettes carry *mec* complex B, type 2 *ccrA/B*, and loci A and D. We sequenced the left (400 bp) and right (1,800 bp; in the proximity of *orfX*) extremities of SCC*mec* in both the wkz-2 and O7.1 strains. The DNA sequences were 100% identical between the two strains. These sequences completely matched the outer ends of SCC*mec* IV carried by community-acquired MRSA strain Ca05 (GenBank accession no. AB063172). Subsequently, the *ccrA* and *ccrB* genes of strains wkz-2 and O7.1 were sequenced. As expected, the complete *ccrA/B* DNA sequences were also identical between the strains. On their turn, these *ccrA/B* sequences were indistinguishable from the *ccrA/B* of strain MR108 (GenBank accession no. AB096217) but differed from that of Ca05 (data not shown). When we used the methods described above, the SCC*mec* of *S. epidermidis* O7.1 and MRSA wkz-2 were indistinguishable.

Excision of SCC*mec* in SCC*mec* II and IV MRSA. To address the first step in horizontal transfer of SCC*mec*, we examined

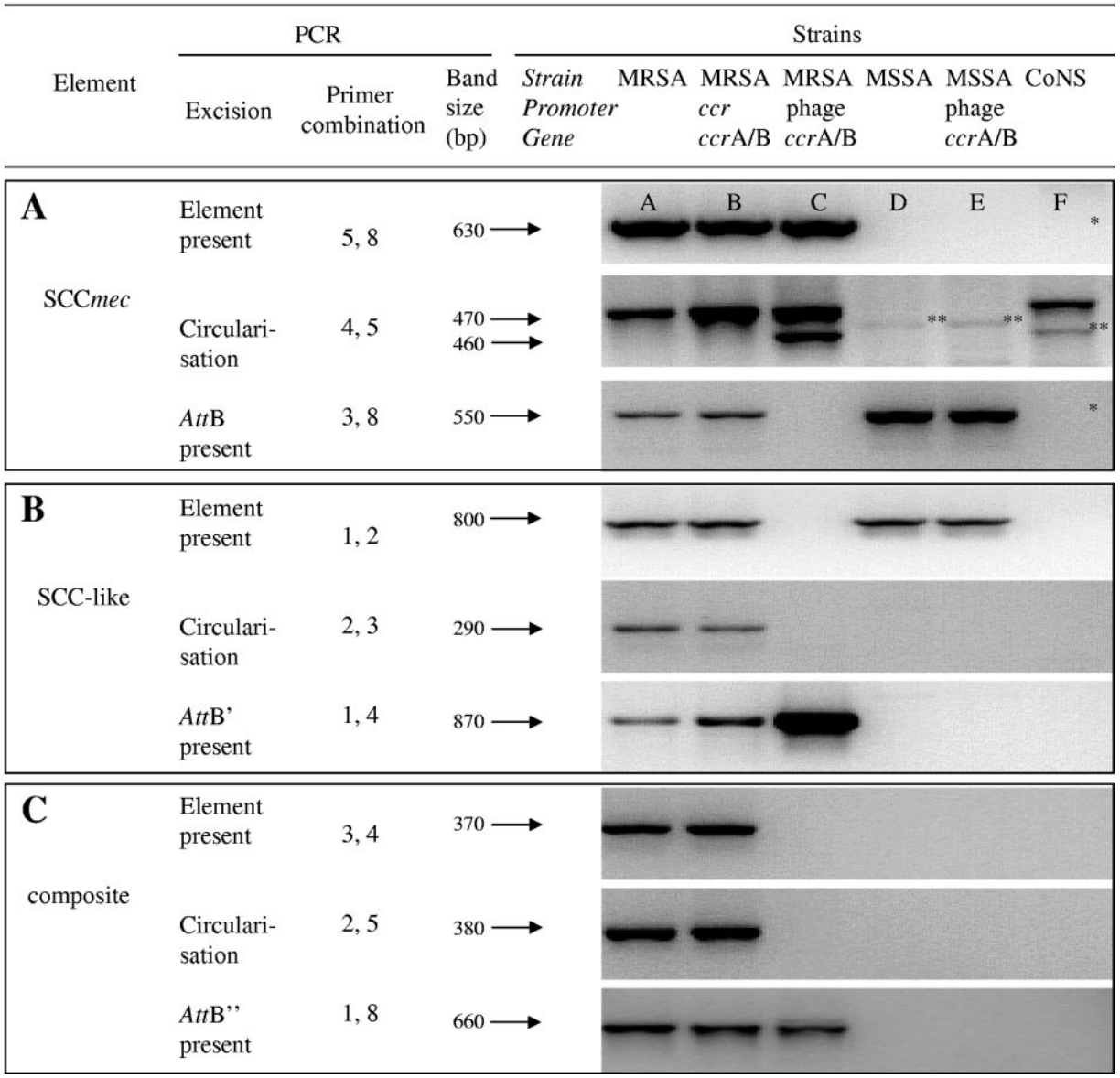

FIG. 1. Excision of three different SCC elements in MRSA strain wkz-2, MSSA strain wkz-1, and coagulase-negative staphylococcus (CoNS) strain O7.1. These strains were possibly involved in the in vivo horizontal transfer of SCC*mec* IV from *S. epidermidis* to *S. aureus* (16). Excision of the SCC elements was measured in wild-type strains and in strains complemented with the *ccrA/B* type 2 recombinase genes under the control of either the endogenous *ccr* promoter or a phage repressor promoter. Excision of the SCC elements was measured by PCR: first, the presence of the element was detected, subsequently the circularization of the element, and finally the reformed *attB* site upon excision of the element. *attB′* and *attB″* are alternative *attB* sites reformed after excision of the SCC-like element and the SCC-like/SCC*mec* composite element, respectively (Fig. 2). All PCR products were confirmed by sequencing. The primer combinations used correspond to the primer position numbers depicted in Fig. 2. Note that the wkz-2 strain complemented with the phage promoter-*ccrA/B* construct lost the SCC-like element and consequently was negative in any PCR using primers located on this element (primers E2 and E3, location numbers 2 and 3 [Fig. 2]). *, No product due to the absence of CoNS O7.1-specific primers outside of SCC*mec*. **, Aspecific bands, as confirmed by sequencing.

SCC*mec* excision in SCC*mec* IV MRSA ($n = 5$), the SCC*mec* IV-positive *S. epidermidis* strain O7.1, and SCC*mec* II MRSA strains ($n = 2$). All these strains carry *ccrA/B* type 2 recombinases. Excision results for the MSSA wkz-1, MRSA wkz-2, and *S. epidermidis* O7.1 strains are displayed in Fig. 1, whereas the excision data for all other strains are summarized in Table 2. A schematic of the different excision products and the location of the different excision primers is given in Fig. 2.

Spontaneous excision of the SCC*mec* IV cassette was detected in MRSA wkz-2 and *S. epidermidis* strain O7.1 (Fig. 1A). In these strains, both the circularization PCR and the *attB* PCR yielded PCR products with the predicted sequence (Fig. 1A, lanes A and F). Excision frequencies in MRSA wkz-2 (1:5,000) and *S. epidermidis* strain O7.1 (1:1,000) were estimated by semiquantitative excision and *mecA* PCR (data not shown).

Excision of SCC*mec* IV was also observed for strains MW2 and Ca05. For the remaining SCC*mec* IV MRSA strains, JCSC 1978 and MR108, circularization of SCC*mec* was not detected

TABLE 2. Excision of SCC elements in different *S. aureus* strains

Strain	Country	Year	Location	SCC*mec* type	Excision[a]			
					SCC*mec*	SCC*mec*′	SCC-like element	Composite
wkz-1	Netherlands	2000	Unknown				—	
O7.1	Netherlands	2000	Unknown	IV	+	—		
MW2	United States	1998	Community	IVa	+	—		
Ca05	United States	1998	Community	IVa	+	+/−		
JCSC1978	United States	1998	Community	IVb	+/−	I		
MR108	Japan	1982	Community	IVc	+/−	I	+	+
wkz-2	Netherlands	2000	Hospital	IV	+	I	+	+
Mu50	Japan	1996	Hospital	II	I	I		
N315	Japan	1982	Hospital	II	I	I		

[a] Excision of four different SCC elements in MRSA SCC*mec* II and SCC*mec* IV strains. Analogous to the excision results shown in more detail in Fig 1, excision was determined by PCRs specific for the presence and circularization of the SCC element and on the reformed *attB* site upon excision of the element. An empty box indicates that the element is not present in the strain. −, No excision. +, Excision in wild-type strains. Both the excision and *att*B PCRs are positive. +/−, Excision in wild-type strains is partly demonstrated. The excision PCR is below the detection limit, and the *attB*-specific PCR is positive. In addition, excision is induced after introduction of the phage promoter-*ccrA*/*B* construct in the strain. I, Inducible excision. Excision is induced after introduction of the phage promoter-*ccrA*/*B* construct in the strain.

whereas the *attB* PCR was positive. In contrast, excision of SCC*mec* II in strains Mu50 and N315 was not observed either by circularization or by reformation of the *attB* site (Table 2).

We constructed several clones with enhanced *ccrA*/*B* activity as a tool to explore *ccrA*/*B*-mediated excision of SCC*mec* in all *S. aureus* strains. In these clones, *ccrA*/*B* type 2 recombinases were placed under the control of either the endogenous *ccr* promoter or a phage promoter.

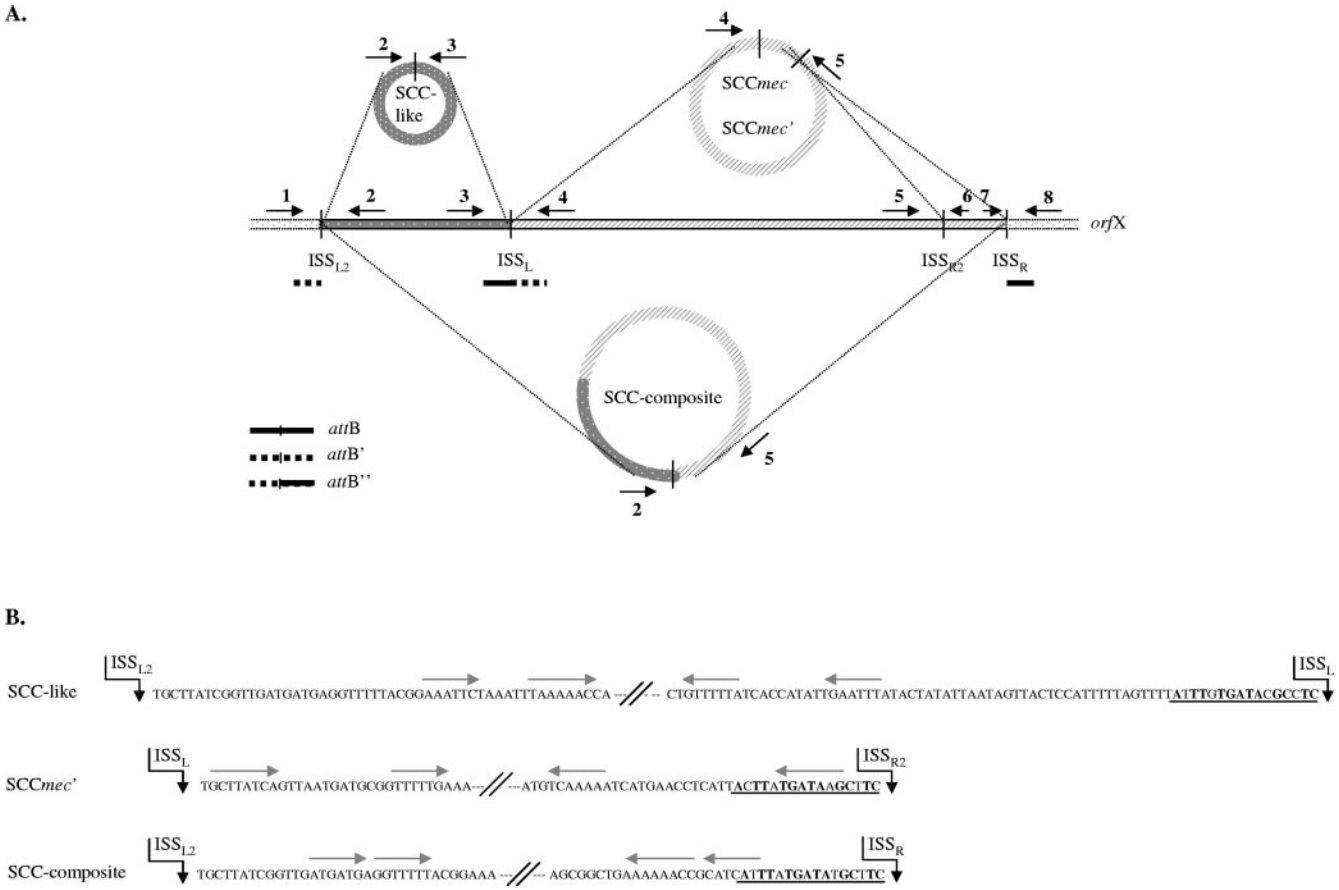

FIG. 2. Diversity of SCC excision products in SCC*mec* IV MRSA. (A) Schematic presentation of different SCC excision products generated by type 2 *ccrA*/*B* recombinases in SCC*mec* IV strains. The arrows indicate the positions of the different primers used to detect excision. Numbers correspond to the primer position numbers in Table 1. Thick and dotted lines represent the chromosomal parts that reunite upon excision of the diverse SCC elements. For SCC*mec*, this site is known as *attB*. Upon excision of the SCC-like element and the composite cassette, alternative *attB* sites were formed that we named *attB*′ and *attB*″, respectively. (B) ISS regions and indirect repeats of SCC excision products. The ISS consensus region is underlined, whereas consensus nucleotides are given in bold. Gray arrows denote indirect repeats. Vertical arrows indicate the positions at which the element is excised, at the 3′ end of the ISS.

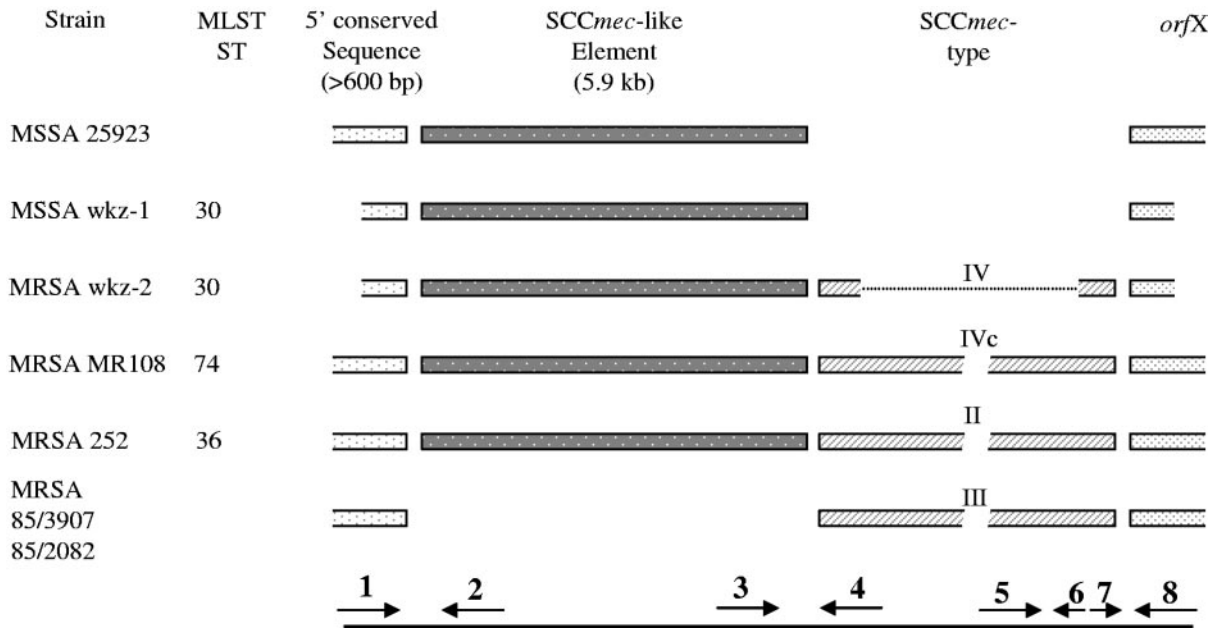

FIG. 3. Schematic presentation of the chromosomal organization of the SCC-like element in different *S. aureus* strains. Arrows indicate the positions of the different primers used to detect excision of the various elements. Numbers correspond to the primer position numbers in Table 1.

In SCC*mec* II strains Mu50 and N315, SCC*mec* excision could be induced after introduction of the phage promoter-*ccrA/B* construct (Table 2). Induced SCC*mec* II excision in strain N315 is in agreement with that described by Katayama et al. (9). Interestingly, two SCC*mec* excision products were observed in strain wkz-2 (Fig. 1A, lane C) and all other MRSA strains complemented with the phage promoter-*ccrA/B* construct, except for strain MW2 (Table 2). Sequencing of excision products and chromosomal remnants in these strains showed that SCC*mec* was cleaved either at the "normal" ISS_R next to *orfX*, or 100 bp upstream at an alternative consensus sequence called ISS_{R2}, yielding a shortened SCC*mec* called SCC*mec'* (Fig. 2). In wild-type strains, excision of SCC*mec'* was detected only in strain Ca05, as determined with primers E4 and E12 (Table 2). Circularization of the 100-bp fragment between the target sites ISS_{R2} and ISS_R was not detected in any of the clones (data not shown).

SCC-like element. Two of the phage promoter-*ccrA/B* clones (MRSA wkz-2 and MRSA MR108) lost their capability to bind primer 3 upstream of SCC*mec* (see Fig. 2 for primer location). The introduction of the *ccrA/B* construct in these strains was accompanied by the absence of amplification when primer 3 was used (Fig. 1A, lane C, Fig. 1C, lane C, and Table 2). We therefore hypothesized that these clones lost the SCC*mec* upstream region due to aberrant *ccr* recombinase-mediated excision. Using an LA PCR in vitro cloning kit, we identified a 5,877-bp SCC-like element present in MRSA wkz-2 and MRSA MR108 wild-type strains, which was lost in these strains upon introduction of the phage promoter-*ccrA/B* construct. The SCC-like element contains ISSs at its extremes, two unknown open reading frames, three transposases, and an element that matches parts of prophage PV83 (7).

Using the BLAST algorithm, we examined the presence of SCC-like elements among published staphylococcal sequences deposited in GenBank. Besides MSSA strain wkz-1 and MRSA strain wkz-2, the SCC-like element is present in the MSSA strain 25923, an SCC*mec* II MRSA strain (MRSA 252), and an SCC*mec* IV MRSA strain (MR108). In all of these strains, a conserved region of at least 600 bp was identified upstream of the SCC-like element. Two SCC*mec* III MRSA strains (85/3907 and 85/2082) lacked the SCC-like element but did contain this 600-bp upstream region (Fig. 3).

Excision of SCC-like element. Is the SCC-like element mobile? To study this, we examined the excision of the SCC-like element separately or in combination with SCC*mec* in different strains. The SCC-like element was excised in MRSA wkz-2 (Fig. 1B, lane A) and MRSA MR108 wild-type strains (Table 2), as determined by a PCR specific for circularization and a PCR on the alternative reformed *attB* site that we called *attB'* (Fig. 2). Interestingly, SCC-like excision was observed neither in the MSSA wkz-1 wild-type strain nor in MSSA wkz-1 strains complemented with the phage promoter-*ccrA/B* construct, although they contained this element (Fig. 1B, lanes D and E). Finally, excision and circularization of a composite element consisting of the SCC-like element and SCC*mec* were observed in MRSA wkz-2 (Fig. 1C, lane A) and MRSA 108 (Table 2), as measured by a PCR specific for circularization and a PCR on the chromosomal remnants called *attB'* (Fig. 2). Excision of the composite cassette at the alternative ISS_{R2} was not detected (data not shown).

In summary, excision of SCC*mec* IV occurred among wild-type MRSA and MRSE strains, whereas SCC*mec* excision could be induced in SCC*mec* II MRSA strains. In addition, four different mobile SCC elements were detected in *S. aureus*. Two excision products, SCC*mec* and SCC*mec'*, were observed in MRSA strain Ca05. Three excision products,

the SCC-like element, SCC*mec*, and a novel composite cassette, were observed in SCC*mec* IV MRSA strains wkz-2 and MR 108 (Fig. 2).

DISCUSSION

Here, we report for the first time *ccrA/B* type 2 mediated excision and circularization of SCC elements among wild-type SCC*mec* type IV strains under normal laboratory conditions. In addition, we observed the excision of several ISS-flanked elements within one MRSA strain, either individually or as part of a composite cassette, which is suggestive of a high genetic plasticity among these strains.

Interestingly, the excision results suggest an optimum length for cassettes to become excised and circularized. Circularization could not be detected for the small, 100-bp fragment at the *orfX* extremity of SCC*mec*, and excision of the 52-kb SCC*mec* II cassette in Mu50 and N315 was inducible only. In contrast, excision of the 6-kb SCC-like element, the 20-kb SCC*mec* IV cassette, and the 26-kb composite cassette could be readily detected in wild-type strains (Fig. 2). Since the SCC*mec* II and SCC*mec* IV strains carried the same *ccrA/B* type and ISSs, this difference in excision efficiency may be due to differences in cassette size rather than differences in recombinase activity or recombinase target sequences. Spontaneous excision of SCC*mec′*, however, is probably a rare phenomenon. In contrast to strains complemented with the phage promoter-*ccrA/B* construct, concomitant excision of SCC*mec* and SCC*mec′* could not be detected in the Ca05 wild-type strain (determined with primer combination 4 and 5) (data not shown). This may be explained by the much lower excision frequency of SCC*mec′* than that of SCC*mec* in the Ca05 wild-type strain, resulting in detectable PCR amplification of the latter element only. Comparison of all ISSs involved in excision in our experiments confirms the putative ISS reported earlier by Ito et al. (6).

The SCC-like element does not contain any known virulence or resistance genes. Nevertheless, its presumed mobility (in the presence of SCC*mec*), together with the observation that an ancient MSSA strain and several virulent epidemic MRSA strains possess this element, suggests that its presence may be beneficial for the bacterial host. We hypothesize that similar to the "train-wagon"-like organization of gene cassettes in integrons (13), several ISS-flanked elements may be coupled to SCC*mec* and get mobilized either as a composite cassette or alone by using the recombinases of the adjacent SCC*mec* cassette. The same mobilization mechanism may apply for the newly described arginine catabolic mobile element in the virulent, community-acquired MRSA strain USA300 (3). Similar to the SCC-like element, this element is likely to be excised by *ccrA/B* recombinases contained in the adjacent SCC element. In addition, it may be possible that SCC excision and reintegration at another ISS position will lead to shuffling of SCC elements, thereby conferring genetic plasticity without the need for horizontal transfer. We could not, however, detect shuffling of SCC elements in our strains (data not shown).

Several lines of evidence suggest that the SCC-like element is subject to horizontal transfer. First, it is flanked by ISS consensus elements. Second, the element is well conserved among several MSSA and MRSA strains of different MLST sequence types and MRSA strains carrying different SCC*mec* cassettes, whereas it is absent in other strains that share the same sequences upstream of the SCC-like element (Fig. 3). Finally, our data show that the SCC-like element can be excised separately from the chromosome. Our data strongly suggest that the excision of the SCC-like element is dependent on the presence of SCC*mec*, as SCC-like excision was undetectable in the MSSA wkz-1 strain. Although it is likely that SCC-like excision depends on SCC*mec* containing *ccrA/B* recombinases, we cannot exclude other mechanisms, as excision of the SCC-like element could not be induced in the MSSA wkz-1 strain after introduction of a phage promoter-*ccrA/B* construct.

Ito et al. already suggested the presence of this SCC-like element in an *S. aureus* MSSA strain 25923 isolated in 1945 (DDBJ/EMBL/GenBank accession no. AB047239) (7). They hypothesized that this element may be an immobile remnant of a functional SCC*mec* that lost its recombinases (and *mec* region) (9). Our data suggest a second possibility: the element may have belonged to a composite SCC cassette or requires *trans* functions from an SCC with recombinases. After loss of the SCC cassette carrying the recombinase genes, the SCC-like element became immobilized in the genome. However, acquisition of an SCC element containing recombinases may mobilize this element again.

Such an event may have occurred in the *S. aureus* wkz-1 strain. We previously showed that wkz-1 and wkz-2 shared a unique genotype among 312 MRSA isolates, as measured by phage typing, their antibiotic resistance profiles, pulsed-field gel electrophoresis, and ribotyping. In addition, it was shown that the wkz-2 and O7.1 strains showed the same rare SCC*mec* restriction pattern (16). We now show that MSSA strain wkz-1 and MRSA strain wkz-2 belong to the same MLST genotype and that the MSSA wkz-1 strain and *S. epidermidis* strain O7.1 shared identical (partial) SCC*mec* sequences. These findings are in line with our earlier observation (16) of the putative in vivo transfer of SCC*mec* from *S. epidermidis* O7.1 to MSSA strain wkz-1, yielding MRSA strain wkz-2. It is therefore likely that the SCC*mec* element is mobile and may have been acquired from *S. epidermidis* O7.1 or another unknown source at some point in time. Excision of the SCC-like element was not detected in the wkz-1 strain, but upon introduction of SCC*mec* in the wkz-2 strain, the SCC-like element became mobile. In addition to its separate excision, the SCC-like element could be coexcised together with SCC*mec* in this strain. Thus, the SCC-like element may also hijack SCC*mec* for its mobilization. Taken together, these findings suggest that horizontal transfer of SCC*mec* created a novel mobile element composed of the SCC-like element and SCC*mec*.

In conclusion, this study identified four different SCC excision variants in SCC*mec* IV strains. The relative abundance of and variety in SCC*mec* IV excisions, exemplified by the formation and excision of a new SCC*mec* IV composite, may contribute to the frequency of horizontal transfer and genetic plasticity in SCC*mec* IV MRSA strains.

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REFERENCES

1. **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.

2. **Chopra, I.** 2003. Antibiotic resistance in Staphylococcus aureus: concerns, causes and cures. Expert Rev. Anti. Infect. Ther. **1:**45–55.
3. **Diep, B. A., S. R. Gill, R. F. Chang, T. H. Phan, J. H. Chen, M. G. Davidson, F. Lin, J. Lin, H. A. Carleton, E. F. Mongodin, G. F. Sensabaugh, and F. Perdreau-Remington.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant Staphylococcus aureus. Lancet **367:**731–739.
4. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
5. **Hiramatsu, K., E. Suzuki, H. Takayama, Y. Katayama, and T. Yokota.** 1990. Role of penicillinase plasmids in the stability of the *mecA* gene in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **34:**600–604.
6. **Ito, T., X. X. Ma, F. Takeuchi, K. Okuma, H. Yuzawa, and K. Hiramatsu.** 2004. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob. Agents Chemother. **48:**2637–2651.
7. **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.
8. **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. Updates **6:**41–52.
9. **Katayama, Y., T. Ito, and K. Hiramatsu.** 2000. A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **44:**1549–1555.
10. **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.
11. **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.
12. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:**2155–2161.
13. **Ploy, M. C., T. Lambert, J. P. Couty, and F. Denis.** 2000. Integrons: an antibiotic resistance gene capture and expression system. Clin. Chem. Lab. Med. **38:**483–487.
14. **Robinson, D. A., and M. C. Enright.** 2004. Multilocus sequence typing and the evolution of methicillin-resistant Staphylococcus aureus. Clin. Microbiol. Infect. **10:**92–97.
15. **Rooijakkers, S. H., W. J. Van Wamel, M. Ruyken, K. P. van Kessel, and J. A. van Strijp.** 2005. Anti-opsonic properties of staphylokinase. Microbes Infect. **7:**476–484.
16. **Wielders, C. L., M. R. Vriens, S. Brisse, L. A. Graaf-Miltenburg, A. Troelstra, A. Fleer, F. J. Schmitz, J. Verhoef, and A. C. Fluit.** 2001. In-vivo transfer of mecA DNA to Staphylococcus aureus. Lancet **357:**1674–1675.