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# Genetic Organization of the *mecA* Region in Methicillin-Susceptible and Methicillin-Resistant Strains of *Staphylococcus sciuri*

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**A homolog of the *Staphylococcus aureus* methicillin resistance gene *mecA* was recently shown to be ubiquitous in independent isolates of the animal species *Staphylococcus sciuri*. The *mecA* gene homolog and regions flanking it were cloned and sequenced from four strains of *S. sciuri*: strain K1 (ATCC 29062), a representative of *S. sciuri* subsp. *sciuri*; two strains (K3 and K8) representing *S. sciuri* subsp. *rodentius*; and strain K11, a representative of *S. sciuri* subsp. *carnaticum*. Strains K1 and K11 were susceptible to methicillin, while strains K3 and K8 showed heterogeneous resistance. The *mecA* genes of strains K1 and K11 and one of the two copies of *mecA* (*mecA1*) present in strain K3 had virtually identical DNA sequences in the *mecA* gene and were similar in genetic organization in the flanking regions. In contrast, the single copy of *mecA* in strain K8 and the second copy of *mecA* (*mecA2*) in strain K3 had *mecA* DNA sequences identical to that of *S. aureus mecA*, and the *mecA* region in these two strains was also similar to that of the same region in the *S. aureus* strain used for comparison. Interestingly, an open reading frame defining an N-terminal truncated polypeptide, NTORF101, with a high degree of homology to a DNA segment in the hypervariable region of methicillin-resistant *S. aureus* (and also similar to the *Escherichia coli* gene *ugpQ*) was also identified downstream of the *mecA* homolog of strain K11, representing *S. sciuri* subsp. *carnaticum*. The *ugpQ*-like gene is not present in methicillin-susceptible strains of *S. aureus*. The presence of such a *ugpQ*-like gene together with the homolog of *mecA* in strain K11 supports the speculation that these genetic elements may be evolutionary relatives and/or precursors of the genetic determinant of methicillin resistance in *S. aureus*.**

It has been established that *mecA* and its product PBP2A are the central determinants of methicillin resistance (12, 13, 16, 17, 21, 29). The 2-kb *mecA* gene and flanking DNA are unique to methicillin-resistant strains of staphylococci and no equivalent locus exists in methicillin-susceptible bacteria (6, 19), indicating that the *mec* determinant was acquired by horizontal gene transfer.

A number of speculations concerning the origin of *mecA* have been proposed (1, 11, 20, 28). In a recent communication, we demonstrated the ubiquitous presence of a *mecA*-like gene in strains of the animal species *Staphylococcus sciuri* (8) and suggested that this species may harbor an evolutionary precursor of the structural gene of PBP2A of methicillin-resistant strains of staphylococci. Cloning and sequencing of the *mecA* homolog from *S. sciuri* K1 revealed an overall 88% similarity in amino acid sequence and 80% identity in DNA sequence to the *mecA* gene of methicillin-resistant *S. aureus* (MRSA). Comparison with sequence information available in the BLAST data bank indicated that the *mecA* homolog of *S. sciuri* was by far the most similar to *mecA* of all known genes (high score of 3,080, with the next-closest score, for the *Enterococcus faecium* PBP5 gene, being 300) (30).

The collection of 134 *S. sciuri* strains that reacted with the *mecA* DNA probe in the initial screen came from a wide variety of ecological sources, were genetically diverse, and contained members of three subspecies: *S. sciuri* subsp. *sciuri*, *S. sciuri* subsp. *rodentius*, and *S. sciuri* subsp. *carnaticum* (8). To test the possibility that one strain in this large and diverse collection contains a variant of *mecA* with even closer sequence similarity (>80% DNA sequence similarity) to the *mecA* of MRSA, we generated *mecA* gene fingerprints from 30 of the most diverse *S. sciuri* isolates in the collection and compared these for DNA sequence diversity of their respective *mecA* homologs. In addition, the *mecA* regions of two *S. sciuri* subsp. *rodentius* strains (K3 and K8) and strain K11 of *S. sciuri* subsp. *carnaticum* were cloned and sequenced to obtain more information on the genetic organization of the entire *mecA* region. As controls, appropriate sequence information was assembled for the *mecA* regions of the MRSA strains BMS-1 (2), BB270 (5), and BB589 (7), and additional sequencing was done on the native *mecA* homolog already identified in *S. sciuri* K1.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are described in Table 1.

**Media and growth conditions.** The bacterial strains of *S. aureus* and *S. sciuri* were grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) with aeration at 37°C. Luria-Bertani medium was used to propagate *Escherichia coli* DH5α, and ampicillin was added at the concentration of 100 μg/ml$^{-1}$ for selection and maintenance of the plasmids listed in Table 1.

**DNA methods and nucleotide sequencing.** All routine DNA manipulations were performed essentially as described in references 25 and 3. Restriction enzymes, calf intestine alkaline phosphatase, and T4 DNA ligase were purchased from New England Biolabs, Inc. (Beverly, Mass.), and used as recommended by the manufacturer. Southern analysis was performed with ECL random prime labeling and detection systems (Amersham Life Science, Arlington Heights, Ill.) as recommended by the manufacturer. DNA sequence was determined by the dideoxy-chain termination method (26) with an automated DNA sequencing

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TABLE 1. Strains and plasmids used in this study

| Strain or plasmid | Relevant characteristics or strain designation (origin)[a] | Origin or reference |
|---|---|---|
| Strains | | |
| *E. coli* DH5α | *recA endA1 gyrA96 thi-1 hsdR17 supE44 relA1* ϕ80 d*lacZ*ΔM15 | Bethesda Research Laboratories |
| *S. aureus* | | |
| COL | Homogeneous Mc$^r$ | Rockefeller University collection |
| BB589 | | 7 |
| *S. sciuri* subsp. *sciuri* | K1 (eastern grey squirrel), K2 (beef tongue), K38 (Holstein cow), K47 (Palestine mole rat), K55 (pilot whale), K56 (Norway rat), K69 (adult human), K74 (horse), K105 (preadolescent child), K132 (howler monkey), K141 (California mouse), K144 (Jersey calf), K156 (hairy-footed hamster), KL056 (opossum), KL064 (southern flying squirrel) | 8 |
| *S. sciuri* subsp. *rodentius* | K3 and K4 (neonatal ward), K8, K9, K27, and K29 (Norway rat), K10 (European red squirrel), K26 (pilot whale) | |
| *S. sciuri* subsp. *carnaticum* | K11 (veal leg, sliced), K30 and K33 (Jersey cattle heifer), K31 (Jersey cattle calf), K61 (pilot whale) | |
| Plasmids | | |
| pBR322 | Cloning vector, Amp$^r$ Tc$^r$ | New England Biolabs |
| pGEM-3Z | Subcloning vector, Amp$^r$ | Promega Corp. |
| pSW-15 | pGEM-3Z/6.7-kb *Hin*dIII fragment from K11 containing K11 *mecA* | This study |
| pSW-16 | pGEM-3Z/5.8-kb *Hin*dIII fragment from K3 containing K3 *mecA1* | This study |
| pSW-17 | pGEM-3Z/4.1-kb *Hin*dIII fragment from K3 containing K3 *mecA2* and N-terminal *mecR1* | This study |
| pSW-18 | pGEM-3Z/4.1-kb *Hin*dIII fragment from K8 containing K8 *mecA* and N-terminal *mecR1* | This study |
| pSW-19 | pGEM-3Z/2.3-kb *Hin*dIII fragment from K3 containing C-terminal *mecR1* and *mecI* | This study |
| pSW-20 | pGEM-3Z/1.5-kb *Hin*dIII fragment from K8 containing C-terminal *mecR1* and *mec1* | This study |
| pCP2 | pGEM-3Z/5.1-kb *Eco*RI fragment from K1 containing K1 *mecA* | 30 |

[a] Mc$^r$, methicillin resistance; Amp$^r$, ampicillin resistance; Tc$^r$, tetracycline resistance.

system (model 377; PE/ABI). Nucleotide and derived amino acid sequences were analyzed with the Wisconsin Genetics Computer Group software.

**Fingerprinting of the *mecA* gene.** A 1.3-kb internal fragment of the *mecA* gene was amplified from *S. aureus* COL and *S. sciuri* strains by PCR (24). The primers SAMECA165 (5′-CGATAATGGTGAAGTAGA-3′) and SAMECA1482 (5′-TATATCTTCACCAACACC-3′) used in the amplification were specific for the corresponding DNA sequence in both *S. aureus mecA* and *S. sciuri* K1 *mecA*; the amplified PCR fragment includes the internal *mecA* region starting at the 164th bp from the initiation codon and ending at the 531st bp from the termination codon (30). Chromosomal DNAs from *S. aureus* COL and *S. sciuri* strains were used as templates in amounts of 10 ng for each reaction. PCR amplification was performed in a DNA thermal cycler (Perkin-Elmer/Cetus) by using the Perkin-Elmer/Cetus PCR reagent kit according to the manufacturer's instructions. The PCR program was as follows: 94°C for 5 min; 30 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 2 min, and 72°C for 5 min; 4°C, hold.

The PCR product was purified with the Wizard PCR Preps DNA purification system (Promega, Madison, Wis.), and then 10 ng of the PCR product was digested with *Mse*I in a volume of 20 μl. Following the digestion, 4 μl of labeling mixture (10 mM Tris hydrochloride [pH 7.4], 10 mM $MgCl_2$, 6 mM dithiothreitol, 0.25 mM dGTP, 0.25 mM dTTP, 0.25 mM dCTP, 10 μCi of [α-$^{32}$P]dATP/μl, 0.4 U of Klenow fragment/μl) was added to the digests, the labeling sample was incubated at room temperature for 10 min, and 8 μl of loading buffer was used to stop the reaction. The plasmid pBR322 was digested with *Hpa*II and labeled with [α-$^{32}$P]dCTP by using a procedure similar to that described above to provide molecular size markers (31). Samples (2 to 4 μl) were applied to a 6% nondenaturing polyacrylamide gel, and electrophoresis was performed at 1,200 V for 2 h 20 min. The gel was transferred to Whatman filter paper, dried with a Gel Dryer (Bio-Rad model 583), and exposed for autoradiography using X-ray film (X-Omat; Kodak) at −70°C for 4 to 12 h.

## RESULTS

**Fingerprints of *mecA* from 30 strains of *S. sciuri*.** The fingerprints obtained by digesting the amplified *mecA* fragments of the 30 selected *S. sciuri* strains with *Mse*I could be grouped into six different patterns (which differed in the number and molecular size of bands). The 16 *S. sciuri* subsp. *sciuri* isolates distributed across four of the six patterns (patterns I through IV), the 5 *S. sciuri* subsp. *carnaticum* strains fell into patterns I, III, and V, and 8 of the 10 *S. sciuri* subsp. *rodentius* strains shared fingerprint pattern V (Fig. 1). The first five fingerprint patterns turned out to be minor variants of pattern I shown by the already sequenced *mecA* in strain K1 (30).

The only substantially different *mecA* fingerprint (pattern VI) was detected in two strains belonging to *S. sciuri* subsp. *rodentius*. One of these, strain K3, carried two copies of *mecA* (8). One of the two *mecA* copies (*mecA1*) produced fingerprint pattern V; this copy is a variant of the *mecA* native to *S. sciuri*. The second copy of *mecA* (*mecA2*) showed the very different fingerprint pattern VI and required the use of pSW-17 plasmid DNA (containing K3 *mecA2*) as template. This second copy of *mecA* in strain K3 and the single copy of *mecA* found in the second *S. sciuri* subsp. *rodentius* strain, K8, were virtually identical in fingerprint pattern to the *mecA* from the MRSA strain COL (Fig. 1).

**Cloning and DNA sequencing of the *S. sciuri mecA* genes and flanking regions.** Strain *S. sciuri* ATCC 29062 (K1), representing subspecies *sciuri*, two strains (K3 and K8) belonging to subspecies *rodentius*, and strain K11, belonging to subspecies *carnaticum*, were selected for further investigation. The *mecA* homologs were localized by *Hin*dIII restriction digestion and Southern blotting (8); the *Hin*dIII fragments containing the *mecA* region from strains K11 (6.7 kb), K3 (5.8 kb for *mecA1* and 4.1 kb for *mecA2*), and K8 (4.1 kb) were each ligated into cloning vector pGEM-3Z to generate the recombinant plasmids pSW-15 (K11 *mecA*), pSW-16 (K3 *mecA1*), pSW-17 (K3

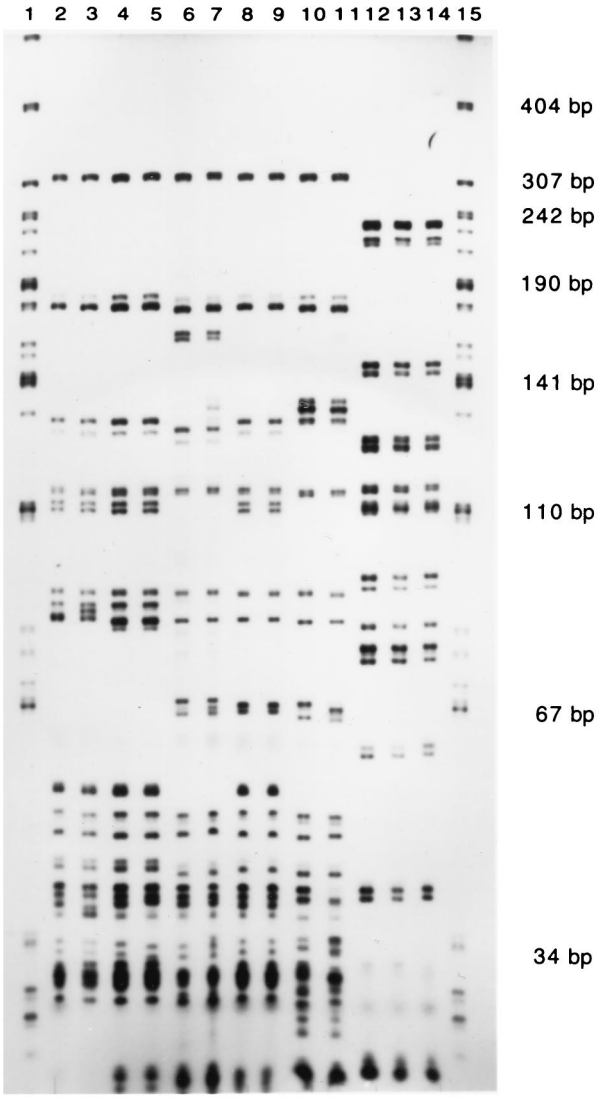


FIG. 1. *Mse*I fingerprints of the *mecA* genes from a selected group of *S. sciuri* strains. Lanes 1 and 15, pBR322 digested with *Hpa*II size markers; lanes 2 and 3, fingerprints of *S. sciuri* K1 and *S. sciuri* subsp. *carnaticum* K11 (pattern I); lanes 4 and 5, fingerprints of *S. sciuri* K47 and K105 (pattern II); lanes 6 and 7, fingerprints of *S. sciuri* K2 and *S. sciuri* subsp. *carnaticum* K61 (pattern III); lanes 8 and 9, fingerprints of *S. sciuri* K165 and KL064 (pattern IV); lanes 10 and 11, fingerprints of *S. sciuri* subsp. *rodentius* K3 (*mecA1*) and *S. sciuri* subsp. *carnaticum* K30 (pattern V); lanes 12 to 14, fingerprints of *S. sciuri* subsp. *rodentius* K3 (*mecA2*) and K8 and *S. aureus* COL (pattern VI).

*mecA2*), and pSW-18 (K8 *mecA*). The upstream *Hin*dIII fragments of K3 *mecA2* (2.3 kb) and K8 *mecA* (1.5 kb) were also cloned into pGEM-3Z to yield plasmids pSW-19 and pSW-20 since *mecI* and part of *mecR1* were expected to be included in this area.

The DNA inserts in the plasmid constructs were sequenced through both strands by the strategy of primer walking. DNA sequences of the *mecA* regions were analyzed for open reading frames (ORFs), and the DNA sequences of pSW-19 and pSW-17 were assembled into a K3 *mecA2* region. The sequences of pSW-18 and pSW-20 were similarly combined to generate the K8 *mecA* region. In addition, further sequencing was performed in the *mecA* region of *S. sciuri* K1 (30). The maps for the *mecA* regions of these strains are shown in Fig. 2.

**Similarities among the *mecA* genes.** K3 *mecA1* and K11 *mecA* were similar to K1 *mecA* (94.5 and 98.2% for DNA sequence; 98.5 and 99.0% for amino acid sequence). In contrast, K3 *mecA2* and K8 *mecA* were almost identical to *S. aureus mecA* (99.3 and 99.9% for DNA sequence; 96.6 and 96.9% for amino acid sequence). The similarity between *S. aureus mecA* and *S. sciuri* K1 *mecA* was 79.5% for DNA sequence and 87.8% for amino acid sequence (30).

**Genetic organization of *mecA* of strain K8 and *mecA2* of strain K3.** DNA sequences flanking the *mecA* genes in strains K8 and K3 showed close similarities to the corresponding areas surrounding *mecA* in the *S. aureus* strain used for comparison (Fig. 2). Intact *mecI* and *mecR1*, with 100% amino acid sequence similarity to the corresponding sequences in *S. aureus*, were identified at the 5′ ends of both K3 *mecA2* (Fig. 2b) and K8 *mecA* (Fig. 2a). Similar genetic organization was also observed at the 3′ ends where ORF142s in K3 *mecA2* and K8 *mecA* were more than 99% homologous to ORF142 located downstream of the *S. aureus mecA* (Fig. 2c; Table 2). These ORFs showed 81% similarity to a protein of unknown function from *Bacillus subtilis* (accession no. 1881333; Table 2). Further downstream of K8 *mecA* and K3 *mecA2*, a polypeptide, truncated by the *Hin*dIII cloning site in the N terminus and designated NTORF101, showed 58.3% similarity and 35.4% identity to the C-terminal part of glycerophosphoryl diester phosphodiesterase encoded by the *E. coli ugpQ* gene (14), and the 44 residues at the C-terminal part of NTORF101 were almost identical to ORF44 in the *S. aureus mecA* region (Fig. 2c). Using several oligonucleotides as primers, we examined the genetic organization beyond the 3′ *Hin*dIII restriction site by comparing the sizes of K3 and K8 PCR products with those of *S. aureus* BB589 PCR fragments. The results indicated that the 3′ flanks of K3 *mecA2* and K8 *mecA* were similar to that of *S. aureus* and that this region contained the entire hypervariable region and IS*431* sequence (Fig. 2a and b). In the K8 *mecA* region, an N-terminally truncated polypeptide (NTORF78) was identified at the 5′ end. NTORF78 was actually the C terminus of the putative transposase in IS*431*, and this IS*431* was confirmed to be intact by PCR. At the 5′ end of the K3 *mecA2* region, we identified a C-terminally truncated peptide, CTORF261, that showed 84% similarity to the xylose repressor of *S. xylosus* (27); amino acid residues 94 to 261 of this ORF were identical to ORF168 at the 5′ end of the *S. aureus mecA* region (Fig. 2b and c).

**Genetic organization of *mecA* of strain K11 and *mecA1* of strain K3.** DNA sequences flanking the *mecA* homologs of strains K11 and K3 had several features in common with those also present flanking the native *mecA* already sequenced from *S. sciuri* K1 (Fig. 2; Table 2). CTORF585, located at the 5′ end of the K11 *mecA* region (in an orientation inverted with respect to *mecA*), showed significant amino acid sequence homology (75%) to a protein of unknown function from *B. subtilis* (14a [accession no. 1181242]) which was nearly identical to the corresponding part of CTORF450 in the *S. sciuri* K1 *mecA* region (Fig. 2d and e). The regions 3′ to K11 *mecA*, K3 *mecA1*, and K1 *mecA* were similar in genetic organization (Fig. 2d to f; Table 2); ORF141 of K11 showed 55% similarity to a protein of unknown function from *Acinetobacter cateoacelicus* (18a [accession no. Y09102]) and was also similar to ORF145 in the *mecA* regions of K3 (*mecA1*) and K1 (98 and 91% amino acid similarity, respectively). K11 ORF503 showed 55% homology to the glutathione reductase of *Nicotiana tabacum* (9) and was identical to the corresponding part of CTORF179 in the K1 *mecA* region and 92% similar to ORF454 in the *mecA1* region of K3.

Together these observations document two distinct types of

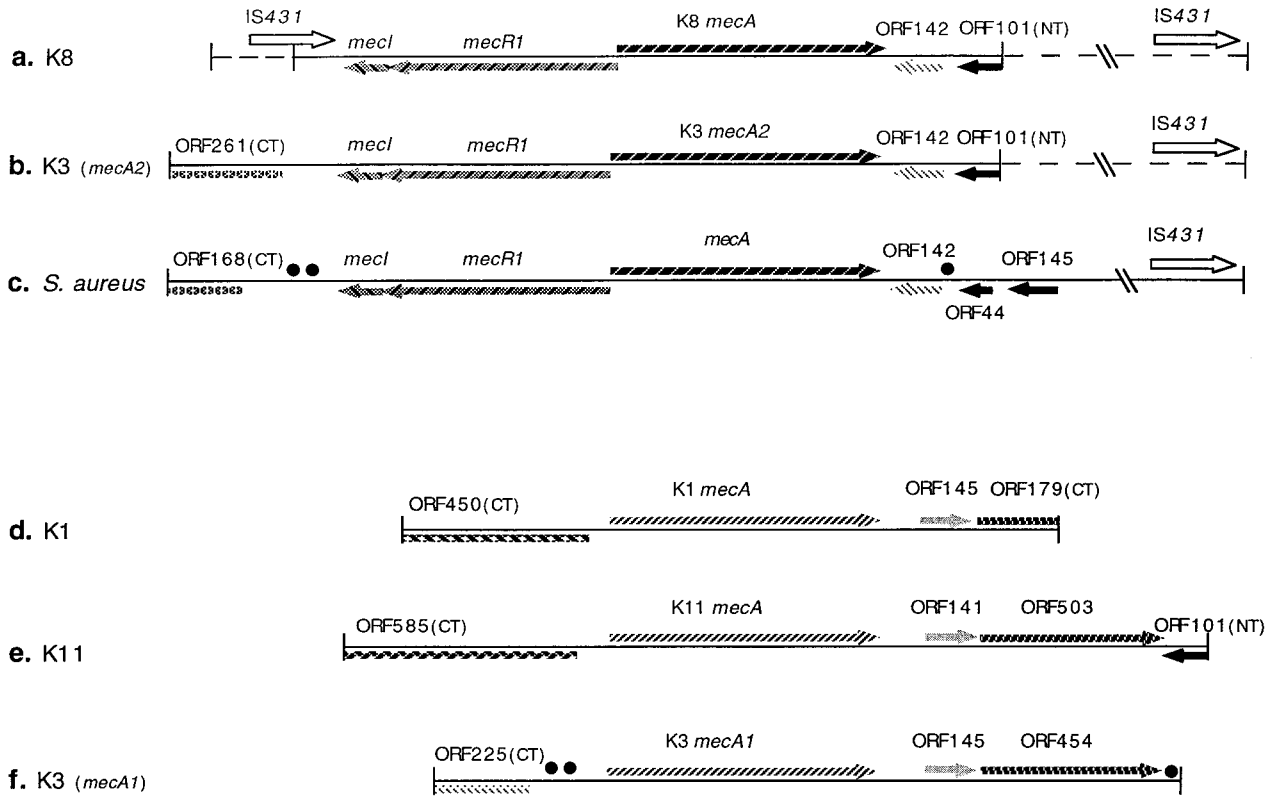


FIG. 2. Genetic organization of the *mecA* regions of *S. sciuri* (K1), *S. sciuri* subsp. *rodentius* (K8 and K3), *S. sciuri* subsp. *carnaticum* (K11), and *S. aureus*. The *S. aureus mecA* region (c) was assembled by using DNA sequences from the database. The direction of gene transcription is shown by the arrows. Identical symbols are used to indicate sequences of significant similarity. Dashed symbols indicate regions determined by PCR analysis. Homologies between short DNA segments are indicated by ● (near ORF454 of K3 and near ORF142 of *S. aureus*) or by ●● (near CTORF225 of K3 and near CTORF168 of *S. aureus*). Accession numbers for these sequences are as follows: K8 (5,596 bp), Y13096; K3 *mecA2* (6,368 bp), Y13095; K11 (6,684 bp), Y13094; K3 *mecA1* (5,806 bp), Y13052; and K1 (5,068 bp), Y09223. To facilitate comparison, relevant DNA sequences of the *mecA* region of *S. aureus* are also provided: *mecRI* (1) (accession no. L14020), *mecA* (23) (accession no. X52593), hypervariable region (22) (accession no. X52594), and IS*431* (4, 5) (accession no. X53818). These sequences were assembled to provide a map for the entire *S. aureus mecA* and flanking region (c [9,047 bp; accession no. Y14051]). Overlaps or gaps at junctions in this map were determined by sequencing the appropriate PCR products of *S. aureus* BB589 (7).

genetic organization in the *mecA* regions of these bacteria: (i) *S. aureus*-type organization shared by *mecA* from strain K8 and *mecA2* of strain K3 and (ii) *S. sciuri*-type organization shared by *mecA* of strains K1 and K11 and *mecA1* of strain K3. Nevertheless, some differences were also noted. For instance, CTORF225 located at the 5′ end of the K3 *mecA1* region was absent from the K11 and K1 genes. CTORF225 showed 50% similarity to the 4-aminobutyrate aminotransferase of *E. coli* (17a [accession no. P50457]).

**Similarities between the genetic organization of the *S. aureus*-type and *S. sciuri*-type *mecA* regions.** Interestingly, some similarities in genetic organization also existed across the two types of *mecA* regions. (i) An N-terminally truncated peptide (NTORF101) was found at the 3′ end of the *S. sciuri*-type *mecA* region in strain K11 (Fig. 2e); this was almost identical to the NTORF101s located in the *S. aureus*-type *mecA* regions of strains K3 and K8 and was also similar to ORF44 of the 3′ *mecA* flank of MRSA (Fig. 2a to c). (ii) At 135 bp upstream of K3 *mecA1*, a DNA fragment of 291 bp (nucleotides 893 to 1183 [Fig. 2f]) was 76.5% identical to a DNA segment downstream of *mecI* of *S. aureus* (nucleotides 938 to 1218 [Fig. 2c]). (iii) A DNA sequence of 47 bp downstream of ORF454 of K3 *mecA1* (nucleotides 5565 to 5611 [Fig. 2f]) showed 81% identity to an area downstream of ORF44 in the *S. aureus mecA* region (nucleotides 6002 to 6048 [Fig. 2c]). (iv) The 306-bp DNA sequence (nucleotides 6378 to 6684 [Fig. 2e and 3b) at the 3′ end of K11 (corresponding to the coding area at the C terminus of K11 ORF503 and the entire region of K11 NTORF101) was 85% identical to a DNA segment in the *S. aureus* hypervariable region (nucleotides 6049 to 6355 [Fig. 2c and 3b]).

## DISCUSSION

The *S. sciuri* K1 *mecA* homolog identified in our previous study is the gene most similar to the *mecA* of methicillin-resistant staphylococcal strains among all known genes (30). Moreover, the *mecA* homolog seems to be native to *S. sciuri* because of its ubiquitous presence in each of a large number of independent natural isolates (8), suggesting that *S. sciuri mecA* may be a close evolutionary relative of the *mecA* gene of *S. aureus*, which encodes PBP2A and is the central genetic determinant of β-lactam antibiotic resistance in that bacterium. A group of 30 *S. sciuri* strains selected for maximum genetic and ecological diversity was used to amplify and fingerprint the resident *mecA* homologs, which were then compared for sequence diversity and similarity to the *S. aureus mecA* gene. This method provides a convenient high-resolution technique for examination of genetic and sequence relatedness, and a difference of less than 5% in DNA sequence is detectable in the form of a different fingerprint pattern (18).

The results of this comparative fingerprinting study showed only a low degree of sequence diversity among the *mecA* ho-

TABLE 2. Amino acid sequence similarity of ORFs in *S. sciuri mecA* regions with polypeptides in the database

| ORF | Similarity with polypeptides in database (%) | Accession no. or reference |
|---|---|---|
| *S. sciuri* subsp. *sciuri* K1 *mecA* region | | |
| CTORF450 | Unknown-function protein of *B. subtilis* (75) | 1181242 |
| ORF145 | Unknown-function protein of *A. cateoacelicus* (55) | Y09102 |
| CTORF179 | Glutathione reductase (55) | 9 |
| *S. sciuri* subsp. *carnaticum* K11 *mecA* region | | |
| CTORF585 | CTORF450 of K1 *mecA* region (100) | Y09223 |
| ORF141 | ORF145 of K1 *mecA* region (91) | Y09223 |
| ORF503 | CTORF179 of K1 *mecA* region (99) | Y09223 |
| NTORF101 | C terminus of *E. coli* UgpQ (58) | 14 |
| *S. sciuri* subsp. *rodentius* K3 *mecA1* region | | |
| CTORF225 | 4-Aminobutyrate aminotransferase of *E. coli* (50) | P50457 |
| ORF145 | ORF145 of K1 *mecA* region (98) | Y09223 |
| ORF454 | CTORF179 of K1 *mecA* region (99) | Y09223 |
| *S. sciuri* subsp. *rodentius* K8 *mecA* region | | |
| ORF142 | Unknown-function protein of *B. subtilis* (81) | 1881333 |
| NTORF101 | NTORF101 of K11 *mecA* region (98) | This study |
| *S. sciuri* subsp. *rodentius* K3 *mecA2* region | | |
| CTORF261 | Xylose repressor of *S. xylosus* (84) | 27 |
| ORF142 | ORF142 of K8 *mecA* region (100) | This study |
| NTORF101 | NTORF101 of K11 *mecA* region (98) | This study |

mologs in spite of the broad genetic diversity of the strains examined, which included members of three different subspecies. In fact, the *mecA* homolog with the highest degree of relative similarity (79.5%) to the structural gene of PBP2A was still the previously sequenced *mecA* of *S. sciuri* K1.

Surprisingly, 2 of the 30 strains examined (K3 and K8, both belonging to the subspecies *rodentius*) contained *mecA* genes virtually identical to that of *S. aureus*. One of the strains (K3) contained two copies of *mecA*: one (*mecA2*) practically identical to the PBP2A determinant of *S. aureus*, and the other (*mecA1*) virtually identical to the *mecA* homolog already sequenced from *S. sciuri* K1, which appears to be, with minor variations, the dominant form of this gene in the species *S. sciuri*. *S. sciuri* strains carrying only this form of *mecA* are susceptible to methicillin, suggesting that this native or "silent" gene does not confer antibiotic resistance to the bacteria. The second strain, K8, seemed to have lost the native *mecA* homolog and carried only the MRSA type of *mecA*.

K3 *mecA2* and K8 *mecA* were almost identical to the *mecA* of MRSA, both in DNA sequence and in genetic organization, and both strains showed heterogeneous methicillin resistance (8). These strains may have acquired a *mecA* gene from *S. aureus* or *S. epidermidis*. Strain K3 was a human-colonizing isolate recovered at a neonatal ward of an African hospital, and strain K8 was isolated from a rodent (8). The appearance of *S. aureus mecA* in these *S. sciuri* isolates suggests that avenues for genetic exchange between these two staphylococcal species exist. The apparent loss of the native *mecA* copy from K8 suggests that some genetic event such as deletion or exchange may have occurred in this strain following, perhaps, the transient coexistence of the two types of *mecA* genes similarly to the case of strain K3, which carries both the *S. aureus*-type and the native *S. sciuri*-type *mecA*.

The genetic organization at the 3′ ends of K3 *mecA2* and K8 *mecA* was very similar to that of the so-called hypervariable region of *S. aureus* BB270 (22), and the entire IS*431* was detected at both the 5′ and 3′ ends of K8 *mecA* and at the 3′ end of *mecA2* of K3, supporting the proposal that these insertion sequence (IS) elements may be involved with the acquisition of *mecA* (5, 22). The organization at the 5′ part of the *mecA* genes in the *S. sciuri* subsp. *rodentius* strains showed two kinds of differences. One is that a second IS*431* copy was located 191 nucleotides downstream of *mecI* in the K8 *mecA* region, i.e., in an area where in the case of most MRSA strains sequences of transposon Tn*554* are located (10). Nevertheless, at least one MRSA isolate has been described in which a second copy of IS*431* was found at a unique *mecR1* deletion junction (20a).

The second difference involved CTORF261 in the K3 *mecA2* region, which had 93 more N-terminal amino acid residues than CTORF168 in the *S. aureus mecA* region, while the rest of the amino acid sequences appeared to be identical. Nevertheless, DNA sequencing of this area revealed several deletions and one substitution within a stretch of 120 nucleotides (Fig. 3a), as well as evidence for rearrangement events possibly caused by IS or transposon insertion, which must have interrupted the translation of this polypeptide at the N terminus.

The genetic organization of the *mecA* regions in the three *S. sciuri* strains harboring native or silent *mecA* showed close similarities, as indicated in Fig. 2 and Table 2. The ORFs at the 3′ ends of all three *mecA* genes and also at the 5′ ends of the K11 and K1 genes were almost identical in both amino acid sequence and organization. Nevertheless, CTORF225 at the 5′ of K3 *mecA1* was different from the CTORF585 of K11 and also from the CTORF450 of K1, which confirms that the subspecies *carnaticum* may be related more closely to the subspecies *S. sciuri* than to the subspecies *rodentius* (15). The polypeptides encoded by the ORFs at the 5′ end of the native *S. sciuri mecA* genes showed no homology to *mecI* and *mecR1*. It is conceivable that the *mecA* gene may have acquired the 5′ regulatory sequences of *mecI-mecR1* by rearrangement of DNA at some later stage during the evolution of this region. It is interesting that the ORFs at the 5′ ends of *S. sciuri mecA* genes are located on the opposite DNA strand with respect to the direction of transcription of the genes. We do not know

**a. DNA alignment of downstream *mecI*.**

```
SA      661 TAGAAATCGTTGCCTTATTAA.CTC....TTTTAAGATATTT..GGACGC 703
SSK3    661 TAGAAATCGTTGCCTTATTAATCTCAAGATTTTTAGATATTTGAGTACGC 710

SA      704 GAAATATTATGATGG.TATAAATTTCCCTTAGCACTCTTTTTTCATTATC 752
SSK3    711 GAAATATTATGATGGTTATAAATTTCCCTTAGCACTCTTTTTTCATTATC 760

SA      753 ATTTATATTT....TATTTTCCATAATTGCCT 780
SSK3    761 ATTTATATTTAAAATATTTTCCATAATTGCCT 792
```

**b. DNA alignment of NTORF101 coding area.**

```
SA     6049 CTATTGAGACAAATGCACCATTTTATCTGCATTGTCTGTAAAGATACCAT 6098
SSK11  6378 CTACTCAGACAAATGCACCATTTTATCTGCGCTATCTGTAAATATGCCAT 6427

SA     6099 CAACTCCCCAATTAGCAAGTTGGTTTGCACGTGCTGGTTTGTTTACAGTC 6148
SSK11  6428 CAACGCCCCAGTTTGCTAATTGGTTTGCGCGTGCTGGTTTGTTTACGGTC 6477

SA     6149 CATACGTTCAATTCATAACCCGCTTCTTTTACCATTTTTACTTTTGCTTT 6198
SSK11  6478 CAAACGTTTAATTCATAACCCGCTTCTTTGACCATTCTTACTTTTGCTTT 6527

SA     6199 AGTAAGTTTGGCATCTTCAGTGTTTACTATTTTAGCATTACAGTAATCTA 6248
SSK11  6528 TGTGAGTTTGGCATCTTCTGTATTCACTATCTTCGCATTGCAATAGTCTA 6577

SA     6249 AAAGTGTTCTCCAGTCTTCACGAAACGAAGTTGTATGGAATATAACTGCT 6298
SSK11  6578 ACAATGTTCTCCAGTCTTCTTTGAATGATGTCGTATGGAAGATGACAGCT 6627

SA     6299 CTGTTATATTATGGCATGATTTCTTCTGCAAGTTTAACAAGCACAACATT 6348
SSK11  6628 CTTTTATATTCTGGCATGATTTCTTCTGCTAGTTTAACGAGTACGACGTT 6677

SA     6349 AAAGCTT 6355
SSK11  6678 AAAGCTT 6684
```

FIG. 3. DNA sequence alignment of short segments in the area outside the *mecA* coding region. (a) DNA sequence at the N terminus of CTORF261 in K3 *mecA2* compared with the corresponding region in *S. aureus*. Bold letters represent the direct repeat which is the likely position for IS insertion. (b) DNA sequence of NTORF101 in K11 compared with the intervening area between ORF142 and ORF145 in *S. aureus*. The nucleotide at position 6239 (C) in the *S. aureus* sequence was replaced with an A residue, creating an extra stop codon and causing interruption of the UgpQ-like polypeptide.

whether this arrangement may be advantageous for acquisition of the *mecI-mecR1* sequence.

Two DNA fragments mapped in the area outside the K3 *mecA1* region (Fig. 2) showed significant sequence similarities to two DNA segments also located outside the *S. aureus mecA* coding region at a location and in an orientation which were very similar in the *S. sciuri* and *S. aureus* strains. One may speculate that these short DNA homologs may represent remnants of DNA sequences in the area outside a hypothetical *mecA* precursor gene remaining after the precursor *mecA* and its flanks underwent the multiple and imprecise insertion, excision, and rearrangement events that eventually led to the emergence of the resistance determinant form of *mecA*.

It is also noteworthy that a 306-bp DNA sequence at the 3′ end of the native *mecA* in *S. sciuri* subsp. *carnaticum* K11 showed 85% identity to a segment of DNA in the *S. aureus mecA* hypervariable region (Fig. 3b). This finding is of considerable significance both because of the high degree of DNA sequence similarity and also because of the importance of the hypervariable region in the transposition of *mec* DNA. It is commonly accepted that the core sequence of *mec* DNA acquired originally by *S. aureus* probably included at least three regions: the *mecA* gene; 1 to 2 kb of 3′ sequence followed by a copy of the IS-like element IS*431*mec (IS*257*); and the 5′ regulatory sequence *mecR1* and *mecI* (1). The DNA sequence located between *mecA* and IS*431* was termed hypervariable because of its considerable length polymorphism among different staphylococcal isolates. Within this region was identified a polypeptide (ORF145) which was homologous to the N terminus of the glycerophosphoryl diester phosphodiesterase (UgpQ) of *E. coli* (except for the minimal direct repeat unit [*dru*] region) (22). As mentioned above, the 3′ flank of *mecA* and the DNA sequence in the hypervariable region are believed to be extraspecies (foreign) DNA for *S. aureus*. For these reasons, our finding of DNA sequences homologous to the hypervariable region in association with the native *mecA* homolog in *S. sciuri* provides further credence to the suggested evolutionary relationship between the native *mecA* of *S. sciuri* and the *mecA* region in *S. aureus*. To obtain more precise information on this important issue, we compared the DNA and deduced amino acid sequences of the 3′ region of *mecA* in strain K11 to the corresponding sequences in *S. aureus* BB270, which we have resequenced. We observed the following: (i) the similarity between the *S. sciuri* and *S. aureus* DNAs in this region was higher than in the *mecA* coding area (85 and 80% respectively [Fig. 3b]); (ii) the NTORF101 in strain K11 *mecA* was almost identical to the NTORF101s in the K3 *mecA2* and K8 *mecA* regions, and the 44 C-terminal residues of NTORF101 were identical to ORF44 of the MRSA strain (Fig. 4d); and (iii) NTORF101 (58% similarity [Fig. 4c]) and ORF44 (65% [Fig. 4b]) were homologous to the C terminus of

**a. Amino acid sequence alignment of ECUGPQ and SAORF145.**

```
UGPQ      9 IVAHRGGGKLAPENTLASIDVGAKY.GHKMIEFDAKLSKDGEIFLLHDDN 57
ORF145    4 VYGHRGLPSKAPENTIASFKAASEVEGINWLELDVAITKDEQLIIIHDDY 53

UGPQ     58 LERTSNGWGVAGELNWQDLLRVDAGSWYSKMFKGEPLPLLSQVAERCREH 107
ORF145   54 LERTTNMSGEITELNYDEIKDASAGSWFGEKFKDEHLPTFDDVVKIANEY 103

UGPQ    108 GMMANIEIKPTTGTGPLTGKMVALAARELWAGMTPPLLSSFEIDALEAAQ 157
ORF145  104 NMNLNVELKGITGPNGL.............ALSKSMVKQVE.EQLTNLN 138

UGPQ    158 QAAPEL 163
ORF145  139 QNQEEL 144
```

**b. Amino acid sequence alignment of ECUGPQ and SAORF44.**

```
UGPQ    203 LKDAGLRILVYTVNKPQRAAELLRWGVDCICTDAIDVIGP 242
ORF44     2 VKEAGYELNVWTVNKPARANQLANWGVDGIFTDNADKMVH 41
```

**c. Amino acid sequence alignment of ECUGPQ and K11NTORF101.**

```
UGPQ    147 SFEIDALEAAQQAAPELPRGLLLD..EWRDDWRELTARLGCVSIHLNHKL 194
ORF101    1 SFNVVLVKLAEEIMPEYKRAVIFHTTSFKEDWRTLLDYCNAKIVNTEDAK 50

UGPQ    195 LNKARVMQLKDAGLRILVYTVNKPQRAAELLRWGVDCICTDAIDVIGP 242
ORF101   51 LTKAKVRMVKEAGYELNVWTVNKPARANQLANWGVDGIFTDSADKMVH 98
```

**d. Multi-sequence alignment of SAORF44, K11NTORF101 and K8NTORF101.**

```
SA
K11    1  SFNVVLVKLA EEIMPQYNRA VIFHTTSFRE DWRTLLDYCN AKIVNTEDAK   50
K8     1  SFNVVLVKLA EEIMPEYKRA VIFHTTSFKE DWRTLLDYCN AKIVNTEDAK   50

SA     1  .......MVK EAGYELNVWT VNKPARANQL ANWGVDGIFT DNADKMVHLS   43
K11   51  LTKAKVKMVK EAGYELNVWT VNKPARANQL ANWGVDGIFT DNADKMVHLS  100
K8    51  LTKAKVRMVK EAGYELNVWT VNKPARANQL ANWGVDGIFT DSADKMVHLS  100

SA    44  Q
K11  101  Q
K8   101  E
```

FIG. 4. Relationship of the *E. coli* UgpQ (ECUGPQ) and *S. aureus* hypervariable regions in ORF145 (SAORF145) and ORF44 (SAORF44) and NTORF101 of strain K11 (K11NTORF101). (a) ORF145 was 57% homologous to the N-terminal part of *E. coli* UgpQ. (b) ORF44 was 65% similar to the C-terminal part of UgpQ. (c) NTORF101 of strain K11 showed 58% similarity to the C terminus of *E. coli* UgpQ. (d) Multisequence alignment of *S. aureus* ORF44, K11 NTORF101, and K8 NTORF101, documenting a high degree of similarity.

*E. coli* UgpQ to a degree similar to that of ORF145 (57% [Fig. 4a]) of the hypervariable region at the N terminus of *E. coli* UgpQ. By an alignment analysis using the Genetics Computer Group program, it could be clearly seen that ORF145 and ORF44 originally must have belonged to an intact UgpQ-like protein (Fig. 4a and b). Through comparison of the DNA sequences, it was possible to identify two point mutations in this sequence: one substitution at 45 nucleotides downstream of ORF145 (Fig. 3b) and one deletion or addition at a position very close to the stop codon of ORF145 (data not shown). These alterations created two extra stop codons and caused a frameshift by which the UgpQ-like polypeptide was truncated into a shorter ORF (Fig. 2c). The existence of a UgpQ-like protein in *S. sciuri* subsp. *carnaticum* and the high similarity of this peptide to the UgpQ-like peptide in the *S. aureus* hypervariable region further strengthen our speculation concerning the evolutionary origin of *mecA*. One may propose that the *S. sciuri* subsp. *carnaticum mecA* region which was the original source of the *mec* determinant became truncated, deleted, and mobilized, presumably by some IS or transposon. The coding sequence eventually emerging as the structural gene of methicillin resistance has undergone extensive modification under the selective pressure of antibiotics, while the linked UgpQ-like peptide may have been retained unchanged between the early and ultimate evolutionary stages of *mecA* because this protein performed a function not related to antibiotic resistance, and it has been retained as a conserved determinant throughout the evolution of the *mec* cluster.

We also analyzed two polypeptides, ORF141 and ORF503 in the intervening region between K11 *mecA* and NTORF101, and compared them with the *mecA* 3′ peptide ORF142. The amino acid sequence of ORF142 showed 43% homology with ORF141 and 50% similarity to the N-terminal portion of ORF503. These homologies are too low to represent an evolutionary relatedness between these ORFs and ORF142 located downstream of *S. aureus mecA*. It may be that the original component of this intervening region was already deleted and then the ORF142 fragment was added to the region.

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