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Recruitment of the *mecA* Gene Homologue of *Staphylococcus sciuri* into a Resistance Determinant and Expression of the Resistant Phenotype in *Staphylococcus aureus*

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Strains of methicillin-resistant *Staphylococcus aureus* (MRSA) have become the most important causative agents of hospital-acquired diseases worldwide. The genetic determinant of resistance, *mecA*, is not a gene native to *S. aureus* but was acquired from an extraspecies source by an unknown mechanism. We recently identified a close homologue of this gene in isolates of *Staphylococcus sciuri*, a taxonomically primitive staphylococcal species recovered most frequently from rodents and primitive mammals. In spite of the close sequence similarity between the *mecA* homologue of *S. sciuri* and the antibiotic resistance determinant *mecA* of *S. aureus*, *S. sciuri* strains were found to be uniformly susceptible to β-lactam antibiotics. In an attempt to activate the apparently "silent" *mecA* gene of *S. sciuri*, a methicillin-resistant derivative, K1M200 (for which the MIC of methicillin is 200 μg/ml), was obtained through stepwise exposure of the parental strain *S. sciuri* K1 (methicillin MIC of 4 μg/ml) to increasing concentrations of methicillin. DNA sequencing of the *mecA* homologue from K1M200 revealed the introduction of a point mutation into the −10 consensus of the promoter: the replacement of a thymine residue at nucleotide 1577 in the susceptible strain K1 by adenine in the resistant strain K1M200, which was accompanied by a drastic increase in transcription rate and the appearance of a new protein that reacted with monoclonal antibody prepared against the penicillin-binding protein 2A (PBP2A), i.e., the gene product of *S. aureus mecA*. Transduction of *mecA* from K1M200 (cloned into a plasmid vector) into a methicillin-susceptible *S. aureus* mutant resulted in a significant increase of methicillin resistance (from a methicillin MIC of 4 μg/ml to 12 and up to 50 μg/ml), the appearance of a low-affinity PBP detectable by the fluorographic assay, and the production of a protein that reacted in a Western blot with monoclonal antibody to PBP2A. Antibiotic resistance and the protein products disappeared upon removal of the plasmid-borne *mecA* homologue. The observations support the proposition that the *mecA* homologue ubiquitous in the antibiotic-susceptible animal species *S. sciuri* may be an evolutionary precursor of the methicillin resistance gene *mecA* of the pathogenic strains of MRSA.

The emergence and worldwide spread of methicillin-resistant *Staphylococcus aureus* (MRSA) between the early 1960s and the late 1990s have begun to pose serious threats to the chemotherapy of staphylococcal diseases worldwide. The genetic determinant of methicillin resistance in MRSA is the acquired gene *mecA,* which encodes the low-affinity penicillin-binding protein 2A (PBP2A), which, according to current theory, can function as a surrogate transpeptidase in the presence of high concentrations of β-lactam antibiotics that inactivate the four high-affinity PBPs native to *S. aureus* (5). The *mecA* gene and the associated large (40- to 60-kb) *mec* element (9, 10, 13, 15, 21, 27) are not native to *S. aureus* but were acquired from an extraspecies source by an unknown mechanism (3, 18). The nature of the extraspecies source, i.e., the evolutionary origin of *mecA* and the formation of the *mec* element, has remained largely a matter of speculation (1, 8, 11, 20, 26).

In a recent effort to track the evolutionary origin of *mecA*, we used a DNA probe internal to this gene in *S. aureus* to screen bacterial isolates belonging to 13 different staphylococcal species for bacteria that would give a positive signal with this DNA probe under hybridization conditions of high stringency. This effort has led to the identification of a close homologue of the *S. aureus mecA* gene in *Staphylococcus sciuri*, a species considered taxonomically the most primitive among staphylococci and found mainly in rodents and primitive mammals (4). Each one of 134 independent and genetically diverse *S. sciuri* isolates was found to carry the *mecA* homologue (4), which, similarly to *mecA* of *S. aureus*, encoded a protein with a putative transglycosylase and transpeptidase domain, the latter showing the conserved motifs and linear structure typical of the penicillin-binding domain of bacterial transpeptidases (30, 31). Overall similarity was 88% on the amino acid level, while even closer similarity (91% identity) was demonstrated within the transpeptidase domains of the *mecA* genes of *S. aureus* and *S. sciuri*.

In methicillin-resistant strains of *S. aureus,* the *mecA* gene provides a unique and broad range of resistance to all β-lactam antibiotics. Surprisingly, strains of *S. sciuri* carrying the structurally similar *mecA* homologue were found to be uniformly susceptible to β-lactam antibiotics, including even penicillin. The contrast between the striking structural similarity of the *S. sciuri mecA* homologue to the *mecA* gene of *S. aureus* and the complete lack of associated antibiotic resistance in the case of *S. sciuri* prompted us to explore possible structural changes in the *S. sciuri mecA* homologue and its transcription in β-lactam-

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Origin or reference
Strains		
Escherichia coli DH5α	*recA endA1 gyrA96 thi-1 hsdR17 supE44 relA1* ϕ80d*lacZ*ΔM15	Bethesda Research Laboratories
Staphylococcus		
K1	*Staphylococcus sciuri* ATCC 29062, methicillin susceptible	4
K1M200	Methicillin-step-selected mutant of K1	This study
RN4220	Restriction-negative mutant of strain 8325-4	R. Novick
COL	Homogeneous Mc^r	Rockefeller University collection
RUSA4	COL Ω551 (*mecA*::Tn*551*) Em^r heterogeneous Mc^s	7
SWTD10	RUSA4/pSTSW-6	This study
SWTD11	RUSA4/pSTSW-8	This study
SWTD22	RUSA4/pSTSW-2C	This study
SWET28	RN4220/pSTSW-6	This study
SWET30	RN4220/pSTSW-8	This study
SWET21	RN4220/pSTSW-2C	This study
SWET33	RN4220/pLCSW-1	This study
SWET34	RN4220/pLCSW-2	This study
SWET55	RN4220/pLCSW-5	This study
MGPET1	RN4220/pLC4, negative control strain for assay of promoter activity	19
MGPET2	RN4220/pro9/10, positive control strain for assay of promoter activity	19
Plasmids		
pSPT181C	Shuttle vector Amp^r Cm^r Tc^r, added 1.2-kb *cat* fragment in plasmid pSPT181	This study
pSTSW-2C	pSPT181C/3,737-bp PCR product of *S. aureus mecA* region	This study
pSTSW-6	pSPT181C/3,460-bp PCR product of *S. sciuri* K1 *mecA* region	This study
pSTSW-8	pSPT181C/3,460-bp PCR product of *S. sciuri* K1M200 *mecA* region	This study
pLC4	Amp^r Cm^r promoterless *xylE* gene	24
pro9/10	pLC4/967-bp promoter region of *pbp2*	19
pLCSW-1	pLC4/514-bp putative promoter region of K1 *mecA*	This study
pLCSW-2	pLC4/514-bp putative promoter region of K1M200 *mecA*	This study
pLCSW-5	pLC4/545-bp putative promoter region of COL *mecA*	This study

resistant mutants isolated in the laboratory. The observations described in this communication suggest that the antibiotic pressure selects for a unique structural change in the regulatory sequence of the *mecA* homologue, converting it to an antibiotic resistance determinant capable of expressing the resistant phenotype even in the genetic background of *S. aureus*.

MATERIALS AND METHODS

Bacterial strain, plasmids, media, and growth conditions. The bacterial strains and plasmids used in this study are described in Table 1. Tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) was employed to grow staphylococcal isolates, and Luria-Bertani medium (Difco) was used to propagate *Escherichia coli* DH5α; ampicillin (100 μg/ml) and chloramphenicol (20 μg/ml) were added to media to ensure maintenance of the plasmids in *E. coli* and staphylococci, respectively.

PAP and susceptibility test. Population analysis profiles (PAPs) were determined by spreading aliquots of overnight cultures at various dilutions onto tryptic soy agar plates containing increasing concentrations of antibiotics. The number of CFU was determined after 48 h of incubation at 37°C or at 30°C for the strains containing thermosensitive plasmids (6). Susceptibility tests were done with paper disks (31) for the following antibiotics (micrograms per disk): ampicillin (10), nafcillin (1), oxacillin (1), cefotaxime (10), vancomycin (30), teicoplanin (30), tetracycline (30), and erythromycin (15).

DNA methods. All routine DNA manipulations were performed essentially as described in the work of Sambrook et al. (22) and Ausubel et al. (2). Introduction of shuttle plasmids into *S. aureus* by electroporation and transduction was described previously (17, 31). DNA sequences were determined by the dideoxy chain termination method (23) with an automated DNA sequencing system (model 377; Perkin-Elmer Applied Biosystems Inc., Foster City, Calif.) at The Rockefeller University Sequencing Facility. Nucleotide and derived amino acid sequences were analyzed with the GCG program (Genetics Computer Group, Inc., Madison, Wis.) and DNAStar software (Lasergene, Madison, Wis.).

PCR. PCR amplification of DNA was performed as described previously (31, 32). High-fidelity PCR with the GeneAmp XL PCR kit (Perkin-Elmer Cetus, Branchburg, N.J.), which includes r*Tth* DNA polymerase XL, was used to reduce sequencing error.

RNA preparation and Northern blot analysis. Northern blotting was performed as previously described (33, 34). The RNA preparation was extracted by use of the FastRNA isolation kit (Bio 101, Vista, Calif.) according to the recommendations of the manufacturer. The PCR-generated DNA probes were radiolabeled with [α-^{32}P]dCTP (Amersham Life Science Inc., Arlington Heights, Ill.) by the random prime method using the Ready to Go labeling kit (Pharmacia, Piscataway, N.J.) and hybridized under high-stringency conditions.

Primer extension analysis. The 5′ ends of transcripts of the *S. sciuri mecA* homologues from strains K1 and K1M200 were determined by primer extension with the oligonucleotide MAK1PE, TTCAATGGCATCAATTGTTTCG, complementary to the DNA sequence in strain K1 between nucleotides (nt) 1730 and 1751 (32). Primer labeling with [γ-^{32}P]ATP, reverse transcription, and primer extension were described previously (34). For each primer extension, 1 to 50 μg of RNA was used. In primer extension experiments, the products of sequencing reactions initiated by the same primer were loaded in parallel lanes on the same gel.

Promoter fusions. The following primers were used to amplify DNA fragments encompassing the region upstream of the *S. sciuri mecA* homologues from strains K1 and K1M200: (i) K1MABI1N, GAAGGATCCTATAGCACCTAACACAG, representing the sequence between nt 1166 and 1191 in strain K1, and (ii) K1MAPHIII, CGAAGCTTACAATCACGATGGCGATGA, the complementary sequence between nt 1654 and 1680 (32). The DNA segment representing the promoter of the *mecA* gene of *S. aureus* was amplified using the following primers: (iii) K8MAPBI, CCAGGATCCATTTGTCGGAATGCCTTAA (corresponding to the sequence between nt 2213 and 2240 in strain K8), and (iv) primer K8MAPHIII, CACAAGCTTCTATTAAAATAAGTGGAAC (complementary to the sequence between nt 2713 and 2758) (32). The PCR products were cloned into plasmid pLC4 to generate recombinant plasmids pLCSW-1 (carrying the promoter for *mecA* from strain K1), pLCSW-2 (carrying the promoter for *mecA* from strain K1M200), and pLCSW-5 (carrying the *S. aureus mecA* promoter). The plasmids were next introduced into strain RN4220 by electroporation to yield strains SWET33, SWET34, and SWET55, representing strains that carried the promoter regions of *mecA* from strains K1 and K1M200 and *S. aureus*, respectively. Catechol 2,3-dioxygenase activity was used to quan-

titate promoter activity using the assay of Sheehan et al. (24), and crude enzyme extracts were prepared as described previously (19). The reaction mixture, consisting of 100 mM potassium phosphate buffer (pH 7.5), 0.2 mM catechol, and 100 to 300 μl of crude extract, was incubated at 37°C for 30 min, and optical density readings were taken at 375 nm at 5-min intervals. One milliunit of activity was defined as that leading to the formation of 1 nmol of 2-hydroxymuconic semialdehyde per min. Specific activity was calculated in milliunits per milligram of protein. Protein concentration was measured using the Bio-Rad D_C protein assay kit (Bio-Rad Laboratories, Hercules, Calif.). The RN4220 strain containing pLC4 (MGPET1) and pro9/10 (MGPET2) were used as the negative and positive controls, respectively.

Introduction of the *S. sciuri mecA* homologues into *S. aureus* mutant RUSA4. The 3,460-bp regions of the *mecA* homologues from strains K1 and K1M200 were PCR amplified with primers K1MABI1N (GAAGGATCCTATAGCACC TAACACAG; sequence between nt 1166 and 1191) and K1MABI2 (TATGGA TCCTACAGATTTGCCTGCATG; complementary sequence between nt 4602 and 4626). The amplified sequences were ligated with shuttle plasmid vector pSPT181C to form pSTSW-6 and pSTSW-8. The recombinant plasmids were introduced into strain RN4220 by electroporation and then transduced into *S. aureus* mutant RUSA4 by phage 80α to yield the transductants SWTD10 and SWTD11. RUSA4 is a derivative of the highly and homogeneously MRSA strain COL in which resistance was inactivated by a Tn*551* insert in the resident *mecA* gene (7, 14). As a control, the plasmid pSTSW-2C, which carries a 3,737-bp segment of the *S. aureus mecA* region (corresponding to sequence between nt 1603 and 5340 in strain K8), was introduced into RUSA4 to give transductant SWTD22.

Membrane purification and analysis of PBPs. Membrane proteins were prepared from bacterial cultures of the late exponential stage (25). Forty or eighty micrograms of each protein extract was labeled with [^{3}H]benzylpenicillin *N*-ethyl-piperidin (NEP) salt (87.4 mCi/mg; Merck, Rahway, N.J.) for 10 min at 30°C after preincubation with nafcillin (20 μg/ml) for 10 min at 30°C, in order to block the appearance of all but the low-affinity PBPs in the fluorogram. The labeling reaction was stopped by addition of an excess of unlabeled benzylpenicillin. Separation of proteins was performed on 8% acrylamide gels at the constant current of 20 mA essentially according to the method of Laemmli (12). Following the separation, the gel was stained with Coomassie blue, and PBPs were detected on the dried gels by fluorography (25).

Analysis of PBP2A and PBP2A-like protein by Western blotting. The amount of membrane protein in each sample was essentially based on the protein concentration measured by use of the Bio-Rad D_C protein assay kit and further confirmed with a Coomassie blue-stained gel. Electrophoresis was performed with the same procedure as PBP analysis. Proteins for immunoblotting were transferred to a Hybond ECL nitrocellulose membrane, and Western blotting was developed using the ECL Western blotting analysis system (Amersham Pharmacia Biotech UK Ltd., Little Chalfont, England) according to the manufacturer. A monoclonal antibody against PBP2A of *S. aureus* (Eli Lilly & Co., Indianapolis, Ind.) was used at a concentration of 1:10,000 as the primary antibody. The secondary antibody was peroxidase-labeled anti-rabbit antibody included in the kit. To block the nonspecific signal of protein A, 3 μg of ChromPure human immunoglobulin G, Fc fragment (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.), per ml was added during the blotting procedure with the primary antibody.

RESULTS

Isolation of a β-lactam antibiotic-resistant step mutant of *S. sciuri*. The β-lactam antibiotic-susceptible strain *S. sciuri* K1 (methicillin MIC of 4 μg/ml) (4, 30) was used as the parental strain to generate the highly methicillin-resistant derivative K1M200. A culture of strain K1 was incubated in growth medium (TSB) containing 4 μg of methicillin per ml until the appearance of a turbid culture that was used as the inoculum for TSB containing 8 μg of the antibiotic per ml. Stepwise exposure to gradually increasing concentrations of methicillin continued up to the isolation of the resistant culture K1M200, which was capable of growing in TSB containing 200 μg of the antibiotic per ml.

Strain K1M200 exhibited homogeneous methicillin resistance, the MIC of methicillin being more than 200 μg/ml (Fig. 1A), and was also resistant to other β-lactam antibiotics such as penicillin G, nafcillin, cefotaxime, and oxacillin. The MICs for the parental strain K1 have increased in mutant K1M200 from 0.1 to 50 μg/ml (penicillin), from 0.75 to 100 μg/ml (nafcillin), from 1 to 200 μg/ml (oxacillin), and from 6 to 400 μg/ml (cefotaxime). Both strains K1 and K1M200 were fully susceptible to vancomycin, teicoplanin, tetracycline, erythromycin, and kanamycin. The antibiotic-resistant phenotype of K1M200 was stable in response to serial culturing in the absence of antibiotic.

Comparison of the DNA sequences of the *mecA* homologues carried by *S. sciuri* strains K1 and K1M200. The 2,605-bp *mecA* region corresponding to the sequence between nt 1166 and 3771 (including 474 bp upstream and 131 bp downstream of the *mecA* gene) was PCR amplified from strains K1 and K1M200 using primers K1MABI1N (AGCTGCTATAGCAC CTAACACAG) and CP2F3C (AATATATGGAGCATGGT ATTTCTATGCAG). Comparison of the DNA sequences of PCR products identified only one difference: a single point mutation that replaced the thymine residue at nt 1577 in the promoter region of strain K1 with an adenine in strain K1M200.

Transcriptional analysis of the *mecA* homologues in strains K1 and K1M200. Primer extension analysis using 50 μg of RNA template from strain K1 identified only a weak signal for the primer extension product located at the position corresponding to the nt 1585 cytosine (lane PE1 in Fig. 2), suggesting that this residue is the transcriptional start (+1) of the *mecA* homologue in strain K1. Two reverse transcriptase (RT) products were generated with RNA from strain K1M200, and these were located at the positions corresponding to the nt 1584 thymine and the nt 1585 cytosine residues, respectively (lane PE2 in Fig. 2). The signal at nt 1584 was much stronger than that at nt 1585, and the amount of RT product produced from 1 μg of K1M200 RNA template was much larger than that generated from 50 μg of RNA from strain K1; one may roughly estimate that the transcription rate of the *mecA* homologue resident in K1M200 was at least a hundredfold higher than that in strain K1 (lanes PE2 to PE4 in Fig. 2). Based on the +1 site of the strain K1 *mecA* homologue, the nucleotides TATATT (nt 1573 to 1578) should be the −10 consensus of the promoter for the *mecA* homologue of strain K1. In K1M200, the −10 consensus sequence was changed from TAT ATT to TATAAT due to the point mutation that resulted in the replacement of thymine with adenine at nt 1577 and a greatly increased rate of transcription of *mecA*.

Determination of the specific activity of the catechol 2,3-deoxygenase produced by the reporter gene indicated that the promoters of strains K1 and K1M200 and the *S. aureus* strain COL carried by the electrotransformants SWET33, SWET34, and SWET55, respectively, generated 330, 9,900, and 18,000 U of enzyme activities at the stationary phase of growth, respectively. No enzyme activity was detectable in early-exponential-growth-phase cultures of SWET33 carrying the promoter of strain K1, while SWET34 and SWET55 carrying the promoters of strain K1M200 and the *S. aureus* strain COL produced 2,600 and 4,500 activity units of catechol 2,3-deoxygenase, respectively, in cultures of comparable cell densities in the early exponential phase of growth (Fig. 3).

Northern blot analysis with a probe internal to the *S. sciuri*

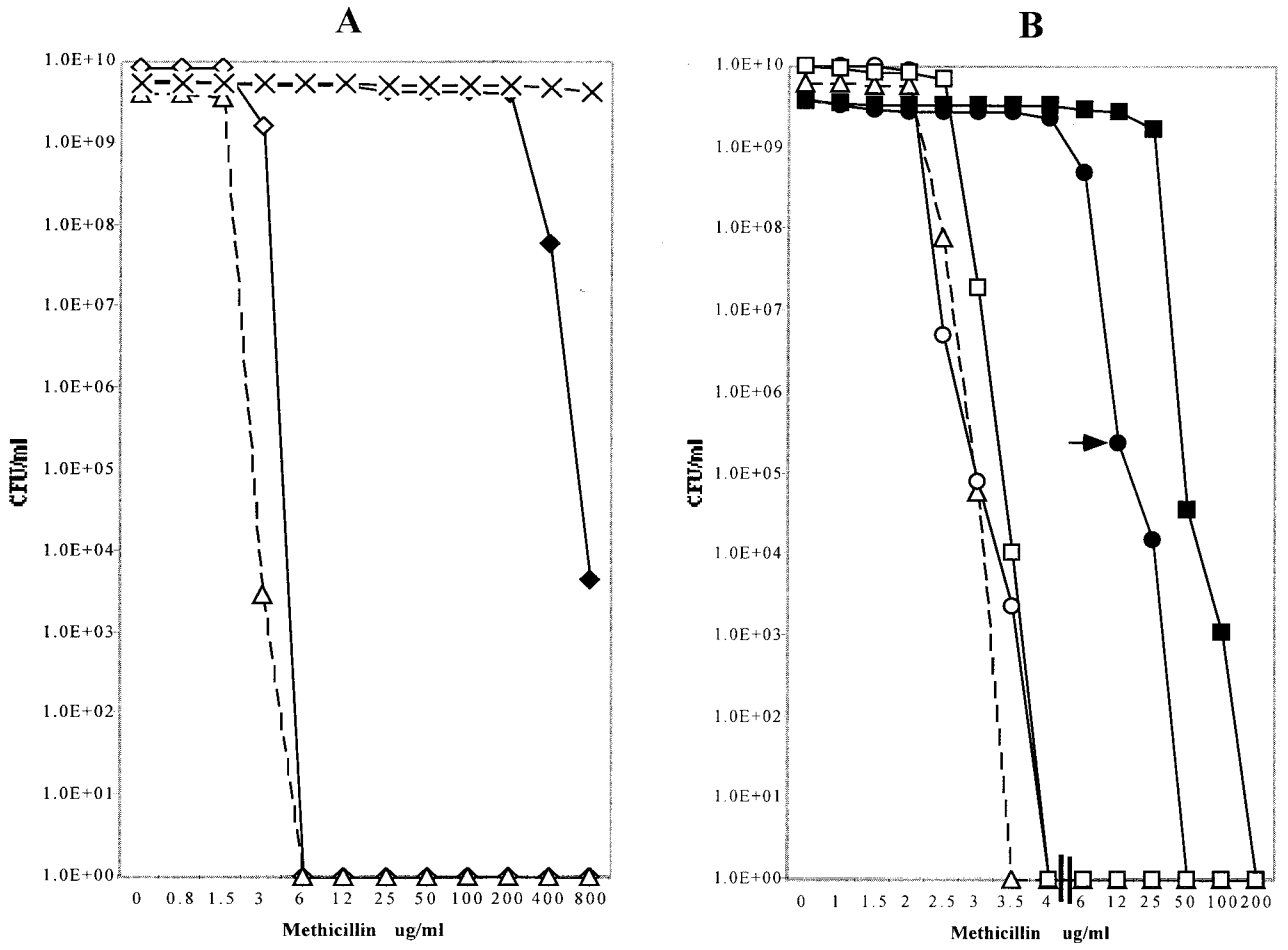

FIG. 1. Methicillin susceptibility profiles of drug-susceptible and drug-resistant isolates of *S. sciuri* and expression of methicillin resistance in transductants of *S. aureus* carrying *mecA* homologues from *S. sciuri*. Bacterial strains were grown and tested for their methicillin resistance phenotype by plating different dilutions of the cultures on agar containing increasing concentrations of methicillin, as described for PAPs in Materials and Methods. (A) MRSA strain COL (dashed lines and X's) and its methicillin-susceptible insertional mutant derivative RUSA4 with the *mecA* gene inactivated by Tn*551* (open triangles and dashed lines) and *S. sciuri* strain K1 (open diamonds and solid lines) and K1M200 (closed diamonds and solid lines). (B) The PAPs of transductants of RUSA4 carrying various *mecA* homologues from *S. sciuri*: transductant SWTD10 with the *mecA* homologue from strain K1 (open triangles and dashed lines); SWTD11 carrying the activated *mecA* homologue from strain K1M200 (solid circles and solid lines); SWTD11S1, a subpopulation of transductant SWTD11 exhibiting an increased resistance level and picked from the agar plate, as indicated by the arrow (solid squares and solid lines); and cultures of SWTD11 (open circles) and SWTD11S1 (open squares), after curing of the *mecA*-carrying plasmids from the bacteria.

mecA gene (nt 3121 to 3613) showed that a band with a molecular size of 2 kb was detectable in 10 μg of total RNA from strain K1M200, which corresponded in size to the transcript of the *S. sciuri mecA* gene; no comparable signal was detectable with strain K1 (data not shown).

Expression of the methicillin-resistant phenotype in *S. aureus* from the *mecA* gene of the antibiotic-resistant strain K1M200 of *S. sciuri*. The *mecA* genes from *S. sciuri* strains K1 and K1M200 were introduced into the background of the *S. aureus* strain RUSA4, a derivative of the highly methicillin-resistant strain COL in which the resident *S. aureus mecA* gene was insertionally inactivated by Tn*551* (7, 14). Figure 1B shows the antibiotic susceptibility profiles of transductant SWTD10 (carrying *mecA* derived from the methicillin-susceptible *S. sciuri* strain K1) and transductant SWTD11 (carrying *mecA* with the promoter mutation derived from the methicillin-resistant *S. sciuri* mutant K1M200). Also shown in Fig. 1B are the antibiotic susceptibility profiles of several control strains. It may be seen that the introduction of *mecA* from strain K1M200 was able to confer a significant degree of methicillin resistance on the *S. aureus* strain used as the transductional recipient. Removal of the plasmid-borne gene resulted in the complete disappearance of resistance. No increase in the methicillin MIC for the recipient strain RUSA4 was detected in transductant SWTD10 carrying the *mecA* gene derived from the methicillin-susceptible *S. sciuri* strain K1.

Production of a PBP2A-like protein in *S. aureus* carrying the *S. sciuri mecA* homologue. To examine the expression of the *S. sciuri mecA* homologues introduced into *S. aureus* by transduction, membrane proteins of the transductants were analyzed by Western blotting and by the PBP fluorographic assay. As controls, membrane proteins from the *S. aureus* strain COL, its insertional mutant RUSA4, and the transductant SWTD22 were used. A single protein band reacting with the monoclonal

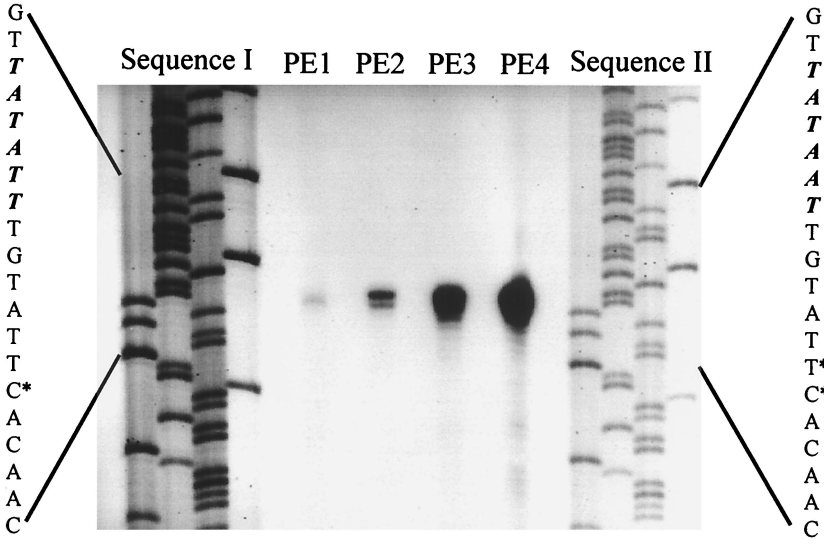

FIG. 2. Mapping of the 5′ ends of the *S. sciuri mecA* transcript by primer extension analysis. Total RNAs from strains K1 and K1M200 were hybridized with an oligonucleotide (MAK1PE) complementary to the *mecA* mRNA of strain K1 and extended by RT. The precise base mapping was done by comparing the migration of the primer extension (PE) product with a parallel sequencing reaction primed by an identical oligonucleotide. The sequence encompassing the initiation start is enlarged on the left and the right of the *mecA* sequences of strains K1 and K1M200. The sequences of the coding strands are shown, and the −10 consensus sequences are indicated by italics. The initiation starts (+1) for *mecA* are indicated by asterisks. The left side of the figure shows the *mecA* sequences of strain K1 obtained using plasmid pSTSW-6 as template. The right side of the figure shows the *mecA* sequences of strain K1M200 obtained using plasmid pSTSW-8 as template. The primer extension products (PE1 through PE4) from left to right are RT cDNAs generated from 50 μg of K1 RNA and 1, 5, and 25 μg of RNAs from strain K1M200, respectively.

antibody against PBP2A was detected in each one of the membrane protein preparations from *S. aureus* strain COL, transductant SWTD22 (carrying the *S. aureus mecA* gene), and transductant SWTD11 (carrying the *S. sciuri mecA* gene from strain K1M200) and also in membrane protein preparations from *S. sciuri* strain K1M200 (Fig. 4). As estimated from the intensity of labeling, the amount of protein produced by K1M200 was about half of that detectable in the *S. aureus* strain COL. No protein reacting with the anti-PBP2A monoclonal antibody was detected in the membrane protein preparations from *S. sciuri* strain K1, the *S. aureus mecA* insertional mutant RUSA4, and the transductant SWTD10, which carried *mecA* from the drug-susceptible *S. sciuri* strain K1.

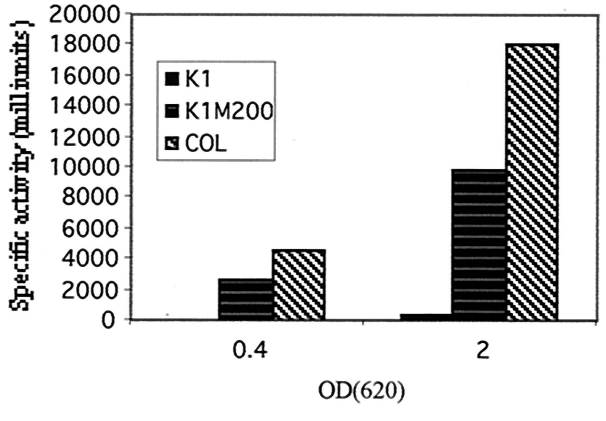

FIG. 3. Increased transcription of the *mecA* homologue in the drug-resistant strain K1M200. The rate of transcription of *mecA* was determined in constructs containing promoter regions from the *S. sciuri* strains K1 and K1M200 and from the MRSA strain COL (left to right, respectively). Crude enzyme extracts were prepared from the cultures at the early exponential growth phase (optical density at 620 nm = 0.4) and the stationary growth phase (optical density at 620 nm = 2.0). Specific enzyme activity is expressed in milliunits per milligram of cellular protein. The control strain with the vector pLC4 gave no XylE activity.

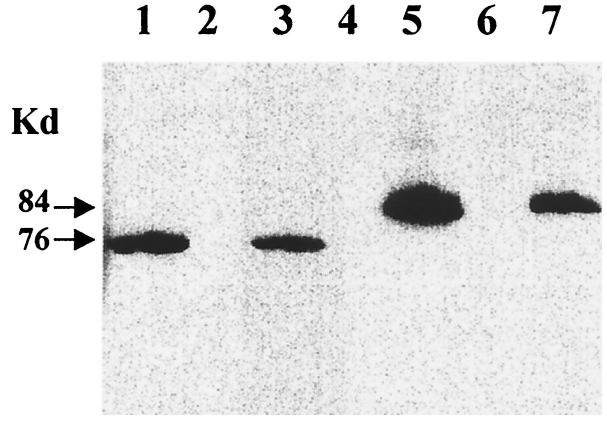

FIG. 4. Detection by Western blotting of a PBP2A-like protein encoded by the *mecA* homologue of *S. sciuri*. Membrane protein extracts were produced and tested by Western blotting for the production of protein that reacts with monoclonal antibody prepared against PBP2A, the gene product of the antibiotic resistance gene of *S. aureus*. The amount of membrane protein used was 30 μg in lanes 1 through 5 and 60 μg in lanes 6 and 7. Lane 1, MRSA strain COL; lane 2, *S. aureus* mutant RUSA4; lane 3, transductant SWTD22; lane 4, transductant SWTD10; lane 5, transductant SWTD11; lane 6, *S. sciuri* strain K1; lane 7, *S. sciuri* strain K1M200.

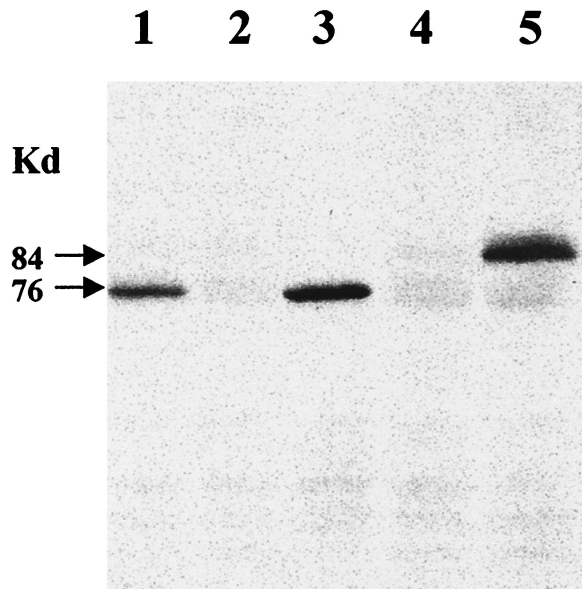

FIG. 5. Detection by the fluorographic penicillin-binding assay of a PBP2A-like protein encoded by the *mecA* homologue of *S. sciuri*. Membrane proteins prepared as described in Materials and Methods were tested under conditions of a competition assay for the presence of a low-affinity PBP using the fluorographic assay. Lanes 1 to 5 contain 30 μg of membrane proteins each prepared from the MRSA strain COL (lane 1), mutant RUSA4 (lane 2), transductant SWTD22 carrying a plasmid-borne copy of the *S. aureus mecA* gene (lane 3), transductant SWTD10 carrying the *mecA* homologue from *S. sciuri* strain K1 (lane 4), and transductant SWTD11 carrying the activated *mecA* homologue of *S. sciuri* strain K1M200 (lane 5). The membrane preparations were preincubated with nafcillin for 10 min, followed by an additional incubation with [^{3}H]benzylpenicillin, and processed for fluorography as described in Materials and Methods.

The membrane protein preparations were also tested by the fluorographic assay to detect low-affinity PBPs under the conditions of a competition assay. Protein preparations from the same constructs that reacted with the monoclonal antibody in the Western blot assay also showed the presence of a low-affinity PBP in the fluorographic assay which had the same apparent molecular size as the protein identified by Western blotting (Fig. 5). Interestingly, both the antibody-reactive protein band and the low-affinity PBP band produced by transductant SWTD11 migrated slower than PBP2A detected by these two assays in the *S. aureus* strain COL and transductant SWTD22.

DISCUSSION

The studies described here were designed to probe the similarities and contrasts that exist between the *mecA* homologues carried by two staphylococcal species, *S. aureus* and *S. sciuri*. In *S. aureus*, the *mecA* gene is acquired from an unknown extraspecies source: it is present only in methicillin-resistant strains, providing these bacteria with blanket resistance against the most important class of antimicrobial agents—the family of β-lactam antibiotics. In *S. sciuri*, a close structural homologue of the *S. aureus mecA* gene appears to be a domestic gene present in each one of the large number of independent isolates examined. Yet, in contrast to the case of *S. aureus*, *S. sciuri* strains carrying the *mecA* gene homologue are uniformly susceptible to all β-lactam antibiotics (4). *S. sciuri* is a staphylococcal species taxonomically remote from *S. aureus,* and it is unlikely that these bacteria have often been exposed to β-lactam antibiotics in their natural habitat, which is the skin of rodents and primitive mammals (4). In an attempt to probe a possible recruitment of the *mecA* homologue of *S. sciuri* as part of a drug resistance mechanism, we tested the effect of selective antibiotic pressure applied to *S. sciuri* in the laboratory on the structure and expression of the *mecA* homologue.

The results of these experiments were quite striking. Comparison of the sequence of the *S. sciuri mecA* homologue of the drug-susceptible strain *S. sciuri* K1 to that of the laboratory-selected methicillin-resistant derivative K1M200 identified a single point mutation introduced into the promoter region of the *mecA* gene of the resistant strain. The replacement of the thymine residue with an adenine at nt 1577 changed the sequence TATATT to TATAAT, which was accompanied by a striking increase in the rate of transcription of the *mecA* gene and the appearance in the resistant cells of a protein product that reacted with a monoclonal antibody prepared against the *S. aureus* gene product PBP2A. The monoclonal antibody has specifically recognized the 38-amino-acid peptide encoded by the DNA sequence between nt 115 and 174 close to the amino terminus of the *S. aureus mecA* gene (29). These findings strongly suggest that the mechanism of β-lactam resistance generated by antibiotic selection in the laboratory mutant *S. sciuri* K1M200 involves, as a genetic determinant of drug resistance, the *mecA* homologue resident in this bacterium. The critical event for the recruitment of this gene to be part of the mechanism of drug resistance appears to be the generation of a more efficient promoter.

In order to further test the relationship between the methicillin-resistant phenotype and the structure of the *S. sciuri mecA* homologues, the *mecA* gene of the drug-susceptible parental strain *S. sciuri* K1 and the *mecA* gene of the drug-resistant mutant strain K1M200 were ligated into plasmid vectors which were then introduced by transduction into the background of *S. aureus*. The *S. aureus* strain selected for this purpose was a methicillin-susceptible derivative of a highly and homogeneously resistant MRSA strain, COL, in which the resident *mecA* gene was inactivated by a Tn*551* insert (14). Previous studies have shown that the genetic background of this strain allows optimal expression of the resistant phenotype (7).

These transduction experiments produced several observations indicating that the *mecA* homologue from the antibiotic-resistant strain of *S. sciuri* can also generate a drug-resistant phenotype in the heterologous background of *S. aureus* and that the mechanism of this resistance is similar to the one operating in MRSA strains, namely, it confers a broad range of resistance to β-lactam antibiotics and is associated with the production of a PBP2A-like protein.

Introduction of the *mecA* homologue from the drug-resistant strain K1M200 into *S. aureus* strain RUSA4 resulted in the increase of the methicillin MIC from 4 μg/ml for the recipient strain to 12 μg/ml for the transductant SWTD11. In addition, the methicillin MIC for a subpopulation of the same transductant (SWTD11S1) increased even more, to 50 μg/ml. Upon removal of the plasmid-borne *mecA* gene, the MICs of the

cured bacteria were reduced back to the level of susceptibility of the recipient strain, indicating that the drug-resistant phenotype depended on the presence of the *S. sciuri mecA* homologue introduced into the *S. aureus* cells. No increase in the MIC was detected for transductants that received the *mecA* homologue derived from the drug-susceptible strain *S. sciuri* K1.

Yet another similar feature of the drug-resistant phenotype in MRSA strains and in the *S. sciuri* strain K1M200 as well as its transductant derivative in *S. aureus* was the broad range of resistance to structurally different β-lactam antibiotics.

Transductants that received the activated *mecA* homologue from the drug-resistant mutant K1M200 (but not transductants that received the "silent" *mecA* homologue from the drug-susceptible strain K1) produced a single low-affinity PBP detectable both by fluorography run under conditions of a competition assay and by Western blotting (Fig. 4 and 5). These observations confirm and extend the validity of the conclusions derived from similar experiments done in the *S. sciuri* background: it seems that the *S. sciuri mecA* homologue encodes a protein that is similar both in antigenicity and in penicillin-binding properties to PBP2A, i.e., the gene product of the *S. aureus* methicillin resistance determinant.

Interestingly, the molecular size of this PBP2-like protein was greater than what would be predicted from the size of the gene and also greater than the size of PBP2A encoded by the *S. aureus mecA* gene. Similar apparent molecular size differences have already been observed in mutant proteins, and they may be related to altered detergent binding properties or incomplete denaturation under the conditions of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (16, 28).

Together, the observations described in this communication provide experimental evidence that the *mecA* homologue which is a native gene in *S. sciuri* with an as yet undefined domestic function can be recruited to become an antibiotic resistance determinant under conditions of drug selection. The critical alteration that makes the silent *mecA* homologue of a drug-susceptible *S. sciuri* strain an effective drug resistance determinant appears to be the replacement of a single nucleotide within the promoter sequence, which results in a drastic increase in the rate of transcription of the gene into a protein that closely resembles the gene product of the *S. aureus mecA* determinant.

The additional observation, namely, that the activated form of the *S. sciuri mecA* gene can replace the *S. aureus mecA* determinant producing a PBP2A-like protein and providing a significant level of broad-range β-lactam resistance to *S. aureus*, supports the proposal that the *mecA* homologue ubiquitous in the animal species of *S. sciuri* may be the evolutionary precursor of the methicillin resistance determinant of MRSA.

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