

The *sbcDC* Locus Mediates Repression of Type 5 Capsule Production as Part of the SOS Response in *Staphylococcus aureus*[∇]

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Most strains of *Staphylococcus aureus* produce one type of capsular polysaccharide that belongs to either type 5 or type 8. The production of these capsules has been shown to be regulated by various regulators. Here we report that the *sbcD* and *sbcC* genes are involved in the repression of type 5 capsule production. Chromosomal deletions in the *sbcDC* genes resulted in increased capsule promoter activity, capsule gene transcripts, and capsule production. The survival rates of the *sbcDC* deletion mutant were reduced upon UV irradiation compared to those for the wild-type strain Newman, suggesting that the genes are involved in DNA repair in *S. aureus*. The two genes were organized as an operon and were expressed very early in the exponential growth phase. A subinhibitory concentration of ciprofloxacin or mitomycin C induced *sbcDC* transcription but repressed the capsule promoter activity, suggesting that the *sbcDC* genes and the capsule genes are part of the SOS regulon. By reporter gene fusion and Northern blotting, we found that *sbcDC* regulated capsule by downregulating *arl* and *mgr*. Further genetic studies indicate that *sbcDC* functions upstream of *arl* and *mgr* in capsule regulation. Collectively, our results indicate that *sbcDC*, upon the SOS response, represses type 5 capsule production through an *arl-mgr* pathway. To our knowledge, this is the first demonstration that an *SbcDC* homolog was involved in transcriptional regulation.

Staphylococcus aureus is a major human opportunistic pathogen responsible for a broad spectrum of infections ranging from food poisoning and superficial skin abscesses to more serious diseases such as pneumonia, meningitis, endocarditis, septicemia, and toxic shock syndrome. The organism is capable of producing an array of virulence factors, including surface-associated adhesions, secreted exoproteins, toxins, and capsular polysaccharides (CP), which contribute to the pathogenesis of the organism (18). Most *S. aureus* strains produce one type of CP belonging to either type 5 or type 8 (CP5 or CP8, respectively) (reviewed in references 16 and 24). The *cap5* and *cap8* genes required for the synthesis of CP5 and CP8, respectively, are organized as an operon in which the polycistronic message is controlled primarily by the promoter located at the beginning of the operon. The *cap5* and *cap8* operons are allelic, sharing common genes that flank the central specific genes (26). The sequences in the promoter regions between the *cap5* and *cap8* operons are nearly identical, indicating that the regulation mechanisms between CP5 and CP8 are similar (12).

The production of CP5 and CP8 is highly regulated by various environmental cues, such as carbon dioxide, iron concentration, in vivo growth, and specific nutrients (16, 24). We have shown previously that global regulators *agr* and *mgr* positively regulate capsule production transcriptionally (20, 21). In *S. aureus*, virulence factors are controlled by a complex regulatory network (reviewed in references 4, 5, and 23). To further understand how capsule is regulated, we screened a transposon library and identified seven additional regulatory genes that

affected capsule promoter activity by using a reporter gene fusion system (19). One of these genes identified by this strategy, *arlR*, has been characterized further and shown to upregulate type 5 capsule mostly through an *mgr*-dependent pathway but also by an independent pathway (19). In this communication, we report the regulation of capsule by *sbcDC*. We found that *sbcDC* represses *cap5* genes through *arl* and *mgr* and that the regulatory pathway is part of the SOS regulon.

MATERIALS AND METHODS

Strains, plasmids, phage, and growth conditions. The *S. aureus* strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strain XL1-Blue was used as the host strain for plasmid construction. *S. aureus* and *E. coli* strains were routinely cultivated in Trypticase soy medium (Difco Laboratories, Detroit, MI) and Luria-Bertani medium (Difco), respectively, with appropriate antibiotic selection when necessary. Antibiotics used for selection were ampicillin, 100 µg/ml, or spectinomycin, 50 µg/ml, for *E. coli* and tetracycline, 3 µg/ml, chloramphenicol, 10 µg/ml, and erythromycin, 10 µg/ml, for *S. aureus*. Plasmids were first electroporated into *S. aureus* RN4220 by the procedure of Kraemer and Iandolo (13). Phage 52A, 80α, or φ11 was used for plasmid and chromosomal DNA transduction between *S. aureus* strains.

Plasmid construction. Plasmid pLL38 was constructed by replacing the *blaZ* gene with the *xylE* reporter gene in pLL33. Plasmid pZC3618 was constructed by ligating a 627-bp fragment containing the *cap5* promoter (19) to the *xylE* gene in pLL38 at the EcoRI-HindIII sites. To construct *sbcDC* promoter fusion plasmids, we first mapped the promoter of the *sbcDC* operon by reverse transcriptase PCR (RT-PCR). It was found that the promoter was within 362 bp upstream of the *SbcD* start codon (not shown). To construct the *sbcD* promoter fusion with *xylE*, an 872-bp fragment including 603 bp upstream of the *sbcD* start codon was amplified by PCR using primers *sbcFP1* and *sbcD2* (Table 2) and cloned into pLL38. To clone the *sbcDC* genes for complementation, a 4.8-kb PCR fragment was amplified from Newman chromosome by use of primers *sbcFP* and *sbcRP* and cloned into the BamHI-PstI sites of pZC3618 to form pZC3624. The amplified fragment, which matched completely with that of strain COL, was verified by sequencing.

Allele replacement of *sbcD*, *sbcC*, and *sbcDC* in Newman. To construct the $\Delta sbcD$, $\Delta sbcC$, and $\Delta sbcDC$ mutants from Newman, two sets of primers (Table 2) were used to amplify the upstream and downstream fragments (about 0.5 kb) of each target gene. The primer pairs were as follows: *sbcD1/sbcD2* and *sbcD3/*

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TABLE 1. Plasmids and strains

Plasmid or strain	Description of construction	Reference or source
Plasmids		
pLL28	Temperature-sensitive vector	20
pLL33	Translational <i>blaZ</i> fusion vector	19
pLL35	Transcriptional <i>blaZ</i> fusion vector	19
pLL38	Transcriptional <i>xylE</i> fusion vector	This study
pZC3618	<i>Pcap5::xylE</i> transcriptional fusion in pLL38	This study
pZC3419	<i>PsbC::xylE</i> transcriptional fusion in pLL38	This study
pZC3624	pZC3618 carrying <i>sbCD</i> operon	This study
pCL8074	<i>Pcap5/8::xylE</i> transcription fusion in pLC4	25
pAM3175	<i>Pcap5/8::blaZ</i> translational fusion in pLL33	19
pAM3176	<i>Pcap5/8::blaZ</i> transcriptional fusion in pLL35	19
pTL3562	<i>Parl-blaZ</i> transcriptional fusion in pLL35	This study
pTL3564	<i>Pagr-3::blaZ</i> transcriptional fusion in pLL35	This study
pTL3580	<i>PmgrA-blaZ</i> transcriptional fusion in pLL35	This study
pTL3573	pCL15 containing <i>arlR</i>	19
Strains		
RN4220	8325r ⁻	14
Newman	CP5 strain	T. Foster
CYL6619	Newman $\Delta sbCD::cat$	This study
CYL6620	Newman $\Delta sbC::cat$	This study
CYL6621	Newman $\Delta sbDC::cat$	This study
CYL1164	Newman $\Delta arlR$	19
CYL6851	Newman $\Delta sbDC::cat\Delta arlR$	This study

sbCD deletion, *sbC1/sbC2* and *sbC3/sbC4* for ΔsbC deletion, and *sbD1/sbD2* and *sbC3/sbC4* for $\Delta sbDC$ deletion. The amplified fragments were sequence verified and cloned into pLL28 such that the upstream and downstream fragments flanked the *cat* gene and had the same orientation as in the chromosome. The resultant plasmids were used for allele replacement as described previously (17). The mutations were verified by PCR.

RNA isolation, purification, and transcriptional analyses. Total RNAs were isolated as described previously (21). Quantification of *cap5* mRNA by real-time RT-PCR using SGcap8A1 and SGcap8A2 primers (Table 2) was performed as described previously (19). Northern hybridization was carried out as described in instructions for a Roche digoxigenin high prime DNA labeling and detection starter kit II (Roche Applied Science, Indianapolis, IN). Briefly, total RNAs were resolved in a formaldehyde agarose gel and capillary transferred to a positively charged nylon membrane (Immobilion NY+; Millipore Corp.). RNAs were immobilized by UV cross-linking at 25,000 $\mu\text{J}/\text{cm}^2$ at 254 nm using an HL-2000 Hybrilinker (UVP, Inc., Upland, CA). The DNA probes were generated by PCR and labeled using a PCR-based digoxigenin probe synthesis kit (Roche Applied Science).

UV survival test. Cultures of Newman and the $\Delta sbDC$ mutant at 4 h and 24 h were diluted 10^{-3} to 10^{-6} with Trypticase soy broth (TSB), and 100 μl of the diluted cells was plated on Trypticase soy agar plates supplied with appropriate antibiotics. Plates with the cover open were irradiated under UV light at 4,000 $\mu\text{J}/\text{cm}^2$ using an HL-2000 Hybrilinker and then incubated in the dark at 37°C overnight. Control plates were prepared similarly but without irradiation. Survival rates for each strain were calculated by dividing the CFU of the UV-treated plates with those of the control plates.

SOS induction. Overnight cultures were inoculated at a 1:100 dilution into 10 ml TSB and incubated for 2 h at 37°C with aeration. Mitomycin C or ciprofloxacin was added directly to the growing cultures at a subinhibitory level of 1 $\mu\text{g}/\text{ml}$ or 2 $\mu\text{g}/\text{ml}$, respectively. At this concentration, the growth rates of the cultures were not affected. The cultures were further incubated until specific time points for analyses.

Other tests. CP5 capsule was quantified as previously described (20). β -Lactamase (BlaZ) was assayed by the nitrocefin method as previously described (21). XylE activity was assayed as described by Zukowski et al. (31), with the following modifications. Overnight *Staphylococcus aureus* culture was diluted into 8 ml prewarmed TSB with antibiotic in 50-ml conical tubes to an optical density at 660 nm (OD_{660}) of 0.05 and incubated at 37°C with shaking at 225 rpm. At various time points, OD_{660} was measured and 1.5 ml culture was collected by centrifugation. The pellet was washed once and resuspended in 500 μl 20 mM phosphate buffer (pH 7.5). The cells were mixed with 10 μl 55 mg/ml catechol and incubated at 30°C for 10 min. The supernatant after centrifugation was measured at A_{375} . Promoter activity was expressed as the ratio of A_{375} to OD_{660} of the culture.

TABLE 2. Primers used in this research

Primer	Sequence
<i>sbCD1</i>	AATTGAATTCGACTTCTCGATTGGAAGTC
<i>sbCD2</i>	AATGCCCGGGCCTCTCTTTACCATCGTGAT
<i>sbCD3</i>	ATTAGGATCCAGCTAAAGGGTATAGACGTG
<i>sbCD4</i>	AATGCTGCAGCGGCTGTTTACCATCAGCGA
<i>sbC1</i>	AATTGAGCTCAGGATTAATGAATGAACCA
<i>sbC2</i>	ATTAGGATCCGCCATAAAAGGTCAATTTGTTGA
<i>sbC3</i>	AATTGGATCCGAAATGGAAATAGCTAGGTTAG
<i>sbC4</i>	ATTCGAATTCTCAATTTCGCTCATATGTGAA
<i>sbCFP1</i>	CGGGATCCGAAGGTGTCTCGCTGCTC
<i>sbCFP</i>	GGATCCTGGAGAATTAGGCGGCATGTTCTT
<i>sbCRP</i>	CCGCGGGCTAAGGTTGTTCTATACATTCCA
RNAIII 1	AGGAAGGAGTGATTTCATG
RNAIII 2	ACTCATCCCTTCTTCATTAC
<i>mgrA38</i>	CATATGTCTGATCAACATAATTTAAAAG
<i>mgrA39</i>	GGATCCGTTAATTTTCTTTGTTGTTTC
<i>arlR1</i>	CATATGACGCAAAATTTAATAG
<i>arlR2</i>	GGATCCTCATCGTATCACATAC
SGcap8A1	ACTAAGGGGTGACAATCCTCAG
SGcap8A2	AAGTCTTTGACACCTCATCTA

Statistical analysis. Data from reporter gene fusion analyses were analyzed by the GraphPad Prism program (San Diego, CA) using a paired Student *t* test for comparing two samples. *P* values of <0.05 were considered statistically significant.

RESULTS

The *sbCD* locus negatively affects capsule production. Previously, we screened a transposon Tn551 library of strain COL with the plasmid pCL8704 containing a *Pcap8::xylE* fusion to identify genes affecting capsule gene expression. Seven genes, including two adjacent *sbCD* and *sbC* genes, were identified by the genetic screen. Interestingly, among 17 unique Tn551 insertions, 6 were found in the *sbCD* coding region, whereas only 1 site was found in each of the other genes (19). These mutants showed increased capsule promoter activity, indicating that the *sbCD* genes negatively regulate capsule. Backcross experiments showed that the transposon insertions were responsible for the phenotype. To confirm that the two genes are involved in *cap5* gene regulation, we first constructed a deletion-insertion mutation in each gene or in both genes in the chromosome by allele replacement in strain Newman as described in Materials and Methods. All three resultant mutants had no detectable differences in growth rate compared to that of the wild type (not shown).

The mutants and the wild-type strain Newman containing the pZC3618 plasmid carrying the *Pcap5::xylE* fusion were tested for their effects on capsule promoter activity by XylE assay. As shown in Fig. 1, all of the mutations resulted in increased capsule promoter activities at 8 h and 24 h time points (all *P* values were <0.0028). At the 4 h time point, only the mutant with both the *sbCD* and *sbC* genes deleted, but not the mutants with either gene deleted, resulted in increased capsule promoter activity (*P* = 0.0085). It is not clear why the results were different at the 4 h time point. To confirm further that the *sbDC* genes are responsible for the repression of capsule promoter, we performed complementation tests by cloning a 4.8-kb fragment containing the *sbDC* genes amplified by PCR from strain Newman in pZC3618. The resultant

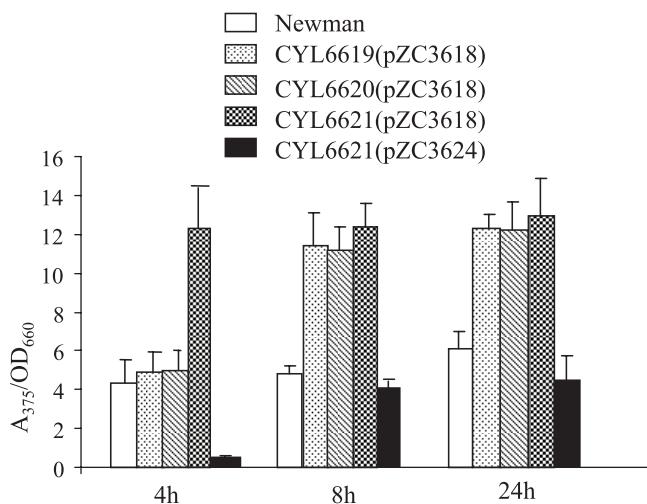


FIG. 1. XylE activities of *Pcap5::xylE* transcriptional fusion. Strains CYL6619, CYL6620, and CYL6621 are $\Delta sbcC$, $\Delta sbcD$, and $\Delta sbcDC$ deletion mutants, respectively. The complementation of CYL6619 and CYL6620 by pZC3624 (not shown) resulted in values similar to those obtained by complementation of CYL6621(pZC3624). The XylE activities are expressed as the ratio of A_{375} of the enzymatic reaction to the OD_{660} of the culture. The error bars indicate standard deviations of at least three independent experiments.

plasmid, pZC3624, was transferred to the mutants for complementation, and the resulting strains were assayed for XylE activities. The results in Fig. 1 show that the fragment was able to complement all mutations at all time points tested (all P values were <0.0019). In fact, the complementation resulted in reduced promoter activities in all mutants compared to that of the wild type, especially at 4 h ($P = 0.0027$). This could be explained by the effect of overproduction of SbcDC due to the multiple-copy plasmid used in the experiments. The much-reduced level of *cap5* promoter activities at 4 h suggests that *sbcDC* acts early in the growth phase.

To determine whether the *sbcDC* effect on the capsule promoter activity reflects the effect on capsule production, the levels of CP5 production from Newman, the $\Delta sbcDC$ mutant, and the complementation strain were assayed by immunoblotting. As shown in Fig. 2, the mutation resulted in a substantial increase in CP5 production and the mutant phenotypes were restored by the complementation. The effect was much more profound at early time points again, suggesting that *sbcDC* exerts its effect early in the growth phase. Together, these results showed that *sbcDC* negatively affected CP5 production by affecting the *cap5* promoter activity.

The *sbcDC* locus affects CP5 production mainly at the transcriptional level. The above-mentioned results suggest that *sbcDC* genes affect the transcription of the *cap5* genes. However, it is possible that they are also involved in translational regulation. To test this possibility, we analyzed the effect of *sbcDC* on translational and transcriptional *Pcap5::blaZ* fusions in Newman and the $\Delta sbcDC$ mutant. As shown in Fig. 3, the effects of $\Delta sbcDC$ mutation by transcriptional fusion assays are similar to those of the translational fusion assays, suggesting that the regulation is most likely at the transcriptional level (all P values were <0.0025).

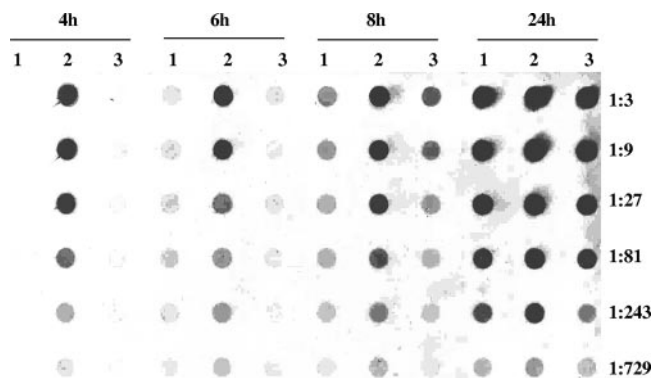


FIG. 2. CP5 assays of (lanes 1) Newman(pZC3618), (lanes 2) CYL6621(pZC3618), and (lanes 3) CYL6621(pZC3624) at the time points shown at top. Threefold serial dilutions of the samples were analyzed by Western dot blotting.

The *sbcDC* genes form an operon transcribed early in growth phase. The sequence information indicates that there are only 4 bp in the intergenic region between the *sbcD* and *sbcC* genes, suggesting that the two genes may form an operon (Fig. 4). To test this possibility, we performed Northern hybridization. We found a major band at about 4.3 kb and a faint band at around 4.8 kb (Fig. 4, leftmost lane). The size of both bands is large enough to encompass both genes, suggesting that the two genes form an operon. There were two other much smaller bands and some smearing detected, which could be the degradation products. Using individual gene probes, we also identified the larger bands (data not shown), which further supports the operonic structure. The Northern results in Fig. 4 also showed that *sbcDC* transcripts were expressed early, at 1 h, and were quickly degraded. Indeed, use of a *Psbc::xylE* fusion containing 603 bp upstream of the *sbcD* start codon revealed that the XylE activities were very high at early time points but reduced drastically thereafter (data not shown), confirming the Northern blot data.

***sbcDC* is involved in UV resistance.** The *sbcD* and *sbcC* genes in *E. coli* have been shown to encode an exonuclease involved in DNA recombination and repair upon DNA damage (10). However, the *S. aureus sbcDC* genes have not been studied. To investigate whether the *sbcDC* locus is involved in DNA recombination and repair of UV lesions, we compared the survival rates upon UV irradiation between strain Newman and the isogenic *sbcDC* mutant. We found that there was no difference between wild-type Newman and the *sbcDC* mutant when the cells were collected at 4 h. However, when the cells were collected at 24 h, the survival rate of the *sbcDC* mutant was 50.68% (standard deviation of 8.55% for five independent experiments) that of Newman, indicating that *sbcDC* genes are involved in UV resistance when the cultures are in stationary phase. It is not clear why there was no difference at the 4 h time point. Perhaps at log phase but not at stationary phase there is a redundant system for complementing *sbcDC* mutation. In *Pseudomonas putida*, a *mutT* mutation has been shown to cause a much stronger mutator phenotype in starved bacteria than in actively growing bacteria, suggesting that there is a backup system complementing the lost MutT function in the growing cells (27).

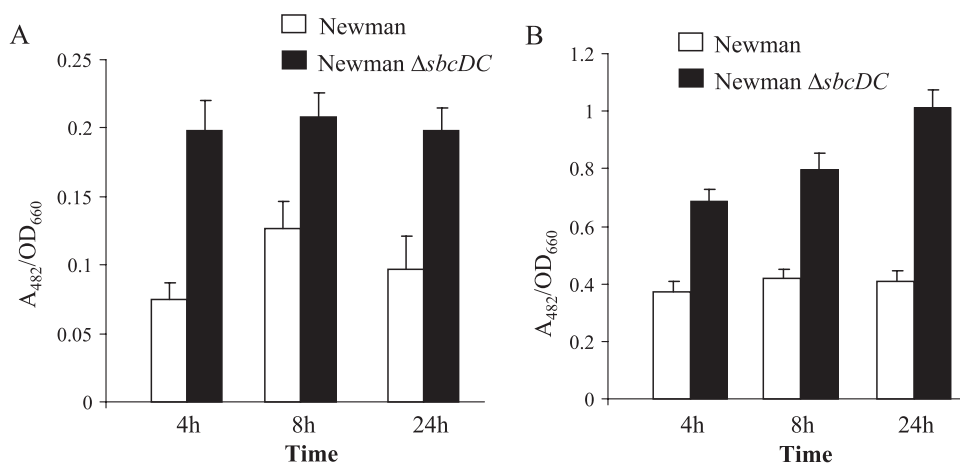


FIG. 3. Promoter activities of *Pcap5::blaZ* transcriptional fusion in plasmid pAM3176 (A) and translational fusion in plasmid pAM3175 (B) in strains Newman and CYL6621 (Newman Δ *sbcDC*) were analyzed at 4, 8, and 24 h. The *BlaZ* activities are expressed as the ratio of A_{482} of the enzymatic reaction to the OD_{660} of the culture. The error bars indicate standard deviations of at least three independent experiments.

The SOS system affects type 5 capsule through *sbcDC*. The involvement of *sbcDC* genes in UV resistance suggests that the genes could be part of the SOS regulon. Indeed, it has been reported that the *sbcD* open reading frame is preceded by a LexA box (6). Recently, microarray studies have also shown that the SOS response induces *sbcD* and *sbcC* genes (1, 6). To further test that the *sbcDC* genes are part of the SOS regulon, Newman cultures were treated with ciprofloxacin or mitomycin C, conditions that have been shown to induce an SOS response

in *S. aureus*. The expression of *sbcDC* was analyzed by Xyle reporter fusion assays. As shown in Fig. 5A, both chemicals substantially induced *sbcDC* promoter activity, indicating that the *sbcDC* genes are regulated by the SOS response. To confirm these results, we performed Northern hybridization using a DNA fragment within the *sbcDC* locus as the probe. The results in Fig. 4 showed that *sbcDC* mRNA was highly induced (Fig. 4, compare the two rightmost lanes at the 4 h time point), which is consistent with the results of the Xyle reporter assay (all *P* values were <0.0031) (Fig. 5A). To test whether SOS induction also affects CP5 production, Newman containing the *Pcap5::xyle* fusion plasmid was treated with ciprofloxacin or mitomycin C and assayed for the reporter activities. The induction of the SOS response reduced the *Pcap5* activities to about 50% at 1, 2, and 3 h time points (all *P* values were <0.0023) (Fig. 5B). Taken together, our results support the notion that *sbcDC* and its target *cap5* genes are part of the SOS regulon.

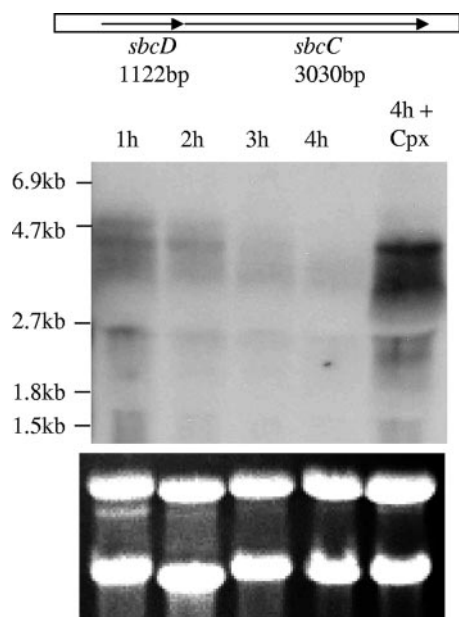


FIG. 4. Northern blot of Newman RNA samples isolated at various time points, probed with a DNA fragment containing both *sbcDC* genes. The positions of the RNA markers (Roche Applied Science) are shown on the left. The last lane indicates an RNA sample obtained from Newman incubated for 2 h in TSB at 37°C and treated with ciprofloxacin (Cpx) for an additional 2 h. The closely linked *sbcDC* genes are shown above the blot. rRNA as a loading control is shown below the blot.

***sbcDC* downregulates *cap5* genes via *arl* and *mgr*.** The similarity to other exonucleases and the ability to enhance cell survival of UV irradiation indicate that the *sbcDC* genes are most likely involved in recombination repair. Their role in capsule gene regulation is, however, not apparent. Since their role in direct gene regulation is not obvious, we considered the possibility of indirect regulation through other regulators. Several regulators have been shown to be involved in capsule gene regulation in our laboratory; these include *arlRS*, *agr*, and *mgrA* (19–21). To test this possibility, we compared the *BlaZ* reporter activities between Newman and the Δ *sbcDC* deletion mutant by using transcriptional fusion plasmids containing fusions *Parl::BlaZ*, *Pagr-3::BlaZ*, and *Pmgr::BlaZ*. As shown in Fig. 6, the promoter activities of *arlRS* and *mgrA* were increased in the Δ *sbcDC* mutation (all *P* values were <0.0075); however, the P3 promoter of *agr* (from which RNAPIII is transcribed) was not affected. To further confirm the fusion results, we employed Northern blotting. The results shown in Fig. 7 confirmed that *sbcDC* repressed *arlRS* and *mgrA* but not RNAPIII. It should be noted here that we have also performed reciprocal experiments in which *PsbcDC::blaZ* fusions were

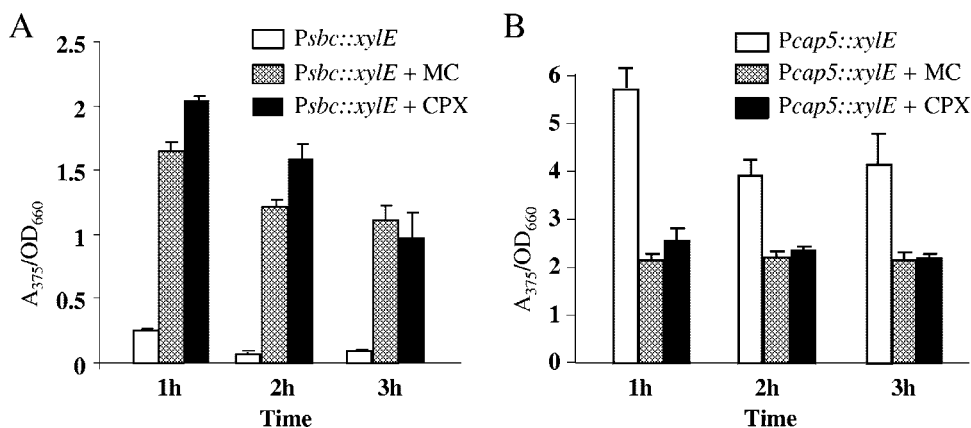


FIG. 5. Effects of mitomycin C (MC) and ciprofloxacin (CPX) on *P_sbcDC::xylE* activities in fusion plasmid pZC3419 (A) and on *P_cap5::xylE* activities in fusion plasmid pZC3618 (B). The XylE activities are expressed as the ratio of A_{375} of the enzymatic reaction to the OD_{660} of the culture. The error bars indicate standard deviations of three independent experiments.

compared in wild-type and *agr*, *mgrA*, and *arlR* mutant strains. We found that none of these regulators affected *P_sbcDC* activity (data not shown). These results suggest that *sbcDC* acts upstream of *arl* and *mgr* in capsule regulation. We have shown previously that *arl* activates the *cap5* genes mostly through *mgrA*-dependent pathways (19). The above-mentioned results therefore suggest that *sbcDC* affects capsule through the *arl-mgr* pathway. To test this hypothesis, we performed genetic epistasis assays in which *ArIR* or *SbcDC* was expressed from a multiple-copy plasmid to complement the *arlR sbcDC* double mutant. The *cap5A* gene was assayed by real-time RT-PCR. As shown in Fig. 8, we found that the double mutant could be complemented by the plasmid containing the *arlR* gene ($P = 0.0094$) but not by the plasmid containing *sbcDC*. In addition, the double $\Delta arl \Delta sbcDC$ mutant had the same phenotype as the Δarl single mutant ($P = 0.065$) but a different phenotype than the $\Delta sbcDC$ mutant ($P = 0.0007$). Taken together, these epistasis results suggest that *arl* acts downstream of *sbcDC* in the pathway.

DISCUSSION

Previously, we have shown that *sbcD* and *sbcC* were two of seven genes involved in type 5 capsule gene regulation identi-

fied by a genetic screen. In this report, we confirmed that both genes were indeed involved in CP5 regulation. We provided the evidence first by using reporter gene fusion analyses to show that mutations in the two genes caused increased *cap5* promoter activity and that the regulation was most likely at the transcriptional level. The downregulation of CP5 by *sbcDC* was then confirmed by assaying capsule production using immunoblotting and analyzing the *cap5* mRNA using real-time RT-PCR. Additionally, we showed that the mutant phenotypes could be complemented by providing, in *trans*, a DNA fragment containing only the *sbcC* and *sbcD* open reading frames.

We have shown previously that staphylococcal capsule is regulated positively by *agr*, *mgr*, and *arl*, in which *arl* acts upstream of *mgr* in strain Newman but can also regulate capsule independently from *mgr* (19–21). We now show that *sbcDC* regulated *cap5* by repressing *arl* and thus *mgr* but did not affect *agr*. This conclusion was based on genetic epistasis assays by assessing single and double mutant phenotypes of *arlR* and *sbcDC* and by complementation of the double mutant with each gene. Since we have shown previously that *arl* acts mostly through *mgrA* to regulate capsule (19), we propose that the signal transduction is transmitted through *sbcDC*, *arl*, and *mgr* (in that order) and finally to *cap5*. Based on these data, we

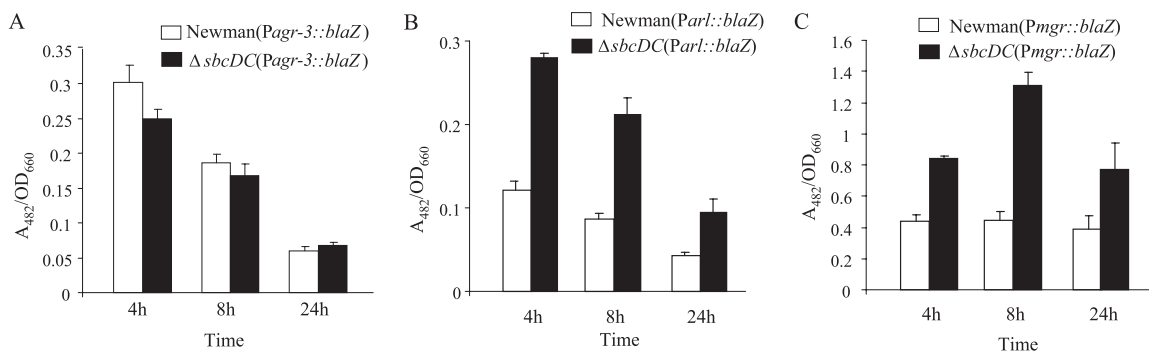


FIG. 6. Promoter activities of the *blaZ* reporter in Newman and $\Delta sbcDC$ mutant CYL6621 containing *Pagr-3::blaZ* fusion plasmid pTL3564 (A), *PParl::blaZ* fusion plasmid pTL3562 (B), or *Pmgr::blaZ* fusion plasmid pTL3580 (C). The *BlaZ* activities are expressed as the ratio of A_{482} of the enzymatic reaction to the OD_{660} of the culture. The error bars indicate standard deviations of at least three independent experiments.

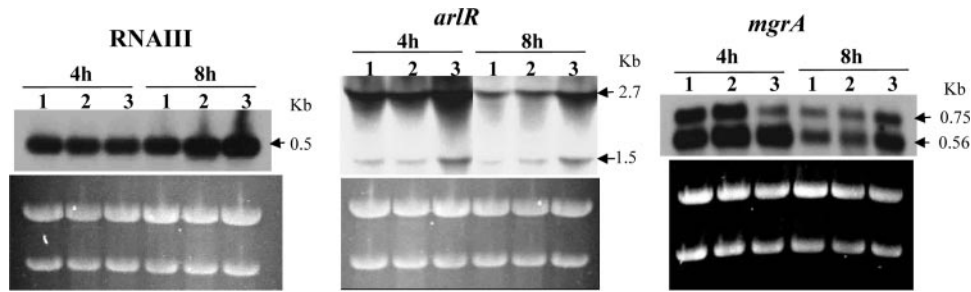


FIG. 7. Northern blots probed with DNA fragment containing RNAIII, *arlR*, or *mgrA*. RNA samples were isolated at 4 h and 8 h from (lanes 1) Newman(pTL3618), (lanes 2) Δ *sbcDC* mutant complemented strain CYL6621(pZC3624), and (lanes 3) Δ *sbcDC* mutant CYL6621(pZC3618). rRNA as a loading control is shown below each blot.

propose the putative pathway shown in Fig. 9. In this model, activated RecA induced by the SOS response causes LexA to autocleave itself, thereby derepressing the SOS regulon including SbcDC. The increased SbcDC results in reduced ArlRS, MgrA, and CP5. Our results that the *cap5* promoter activity was repressed by a subinhibitory concentration of ciprofloxacin or mitomycin C (Fig. 5B) strongly support this hypothesis. It should be noted that the pathway is not complete and is likely to involve additional regulators, particularly downstream of *sbcDC*. In fact, our preliminary studies indicated that ArlR did not bind directly to the *mgrA* promoter and MgrA did not bind to the *cap5* promoter, suggesting that additional intermediary genes may be involved in the pathway. Thus, the signaling pathway is rather lengthy even without additional players. The lengthy pathway could allow different inputs to the pathway that may reflect various needs for fine tuning capsule production in various lifestyles of *S. aureus*.

In *E. coli*, SbcD and SbcC form a complex that cleaves DNA hairpins (7, 9). It has been proposed that the protein complex could weakly bind double-stranded DNA nonspecifically and migrates to the blocked DNA end for cleavage during DNA

repair (8). In *Bacillus subtilis*, SbcC is involved in DNA inter-strand cross-link repair (22). The functions of SbcD and SbcC in other bacteria, including *S. aureus*, have not been studied. Based on homology, it is most likely that the two *S. aureus* proteins perform similar DNA repair functions. Our UV survival test supports this notion, though the effect was not observed when the experiments were performed using 4-h cultures. The involvement in DNA damage repair is also consistent with our Northern and reporter gene fusion results showing that the *sbcDC* genes were induced by ciprofloxacin and mitomycin C, indicating that the *sbcDC* genes are part of the SOS regulon, which includes genes mostly involved in DNA repair in *E. coli* (30). Two recent microarray studies (1, 6) also showed that the *sbcDC* genes were induced in the SOS response in *S. aureus*. While the involvement of *sbcDC* in DNA damage repair is expected, our finding that SbcDC acted as a repressor in capsule regulation is surprising. SbcDC has not been implicated as a transcriptional regulator in any organism. Indeed, it is difficult to envision how a DNA damage repair exonuclease can function as a transcriptional regulator. Since SbcDC can bind DNA nonspecifically, perhaps the DNA binding capability endows the protein complex to regulate gene expression. However, we showed that *sbcDC* repressed *arl*, *mgr*, and *cap5* but had no effect on *agr*, suggesting that the regulation is specific and that the nonspecific DNA binding property may not play a role in regulation. Thus, it is more likely that the SbcDC complex exerts its regulatory function not by direct specific DNA binding on the *cap5* promoter but by a posttranscriptional mechanism through other regulators. Our results showed that *arl* and *mgr* were other intermediary regulators in which *arl* functions upstream of *mgr* (Fig. 9). It has been reported previously that DNA supercoiling is involved in *arl* regulation of protein A (11). Since SbcDC cleaves DNA, which could result in relaxation of DNA supercoils, it is possible that a change in supercoiling could be the mechanism involved in the transduction of signal from *sbcDC* to *arl*. However, our results also indicate that SbcDC affects *arl* mRNA level and promoter activity, suggesting the effect is at the transcriptional level. Further studies are needed to determine whether supercoiling plays a role in the regulation and, if not, what other intermediary regulators are involved.

A recent microarray study by Cirz et al. (6) revealed that *agrB* and *saeRS* were downregulated upon the SOS response in *S. aureus*. The *agr* locus has been shown to activate the *cap5* genes (21), whereas the *sae* locus has been shown to repress the

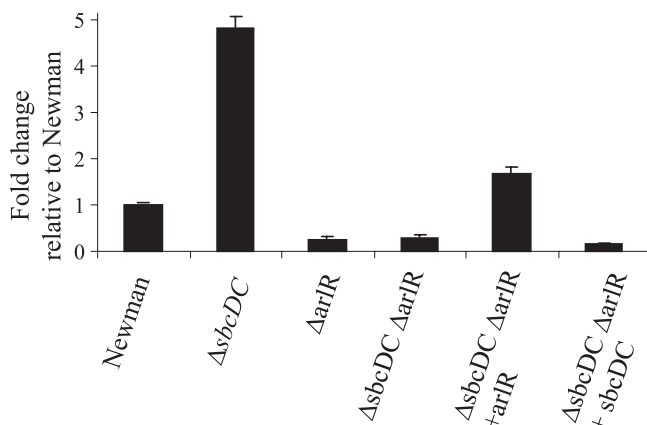


FIG. 8. Epistasis assay using relative real-time RT-PCR analysis of the *cap5* mRNA levels in the various strains indicated below the chart (Newman, Δ *sbcDC* mutant CYL6621, Δ *arlR* mutant CYL1164, Δ *sbcDC* Δ *arlR* mutant CYL6851, Δ *sbcDC* Δ *arlR* mutant CYL6851 complemented with *arlR* in pTL3287, and Δ *sbcDC* Δ *arlR* mutant CYL6851 complemented with *sbcDC* in pZC3624). The changes (*n*-fold) are expressed relative to the level for Newman, which was arbitrarily set at 1. Error bars indicate standard deviations from at least three independent experiments.

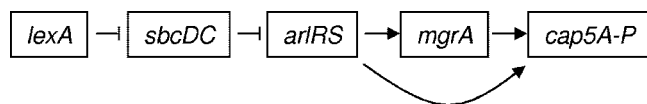


FIG. 9. Proposed regulatory pathway for *cap5* regulation by *sbcDC*. Arrows indicate positive regulation, and blocked arrows indicate negative regulation.

cap5 genes (29; unpublished data). Since our results outlined above (Fig. 7) showed that RNAPIII was not affected by *sbcDC*, the microarray results of Cirz et al. would suggest that additional pathways may be involved in capsule regulation upon the SOS response. This is not surprising since virulence gene regulation in *S. aureus* has been shown to be extremely complicated (4, 5, 23). However, it should be noted that strain 8325 was used in the microarray study. This strain is known to be defective in the *sigB* locus, which has been shown to affect capsule production and other regulatory genes (2, 15, 29). Thus, the microarray results by Cirz et al. may not be applicable to other strains, especially strains with an intact *sigB* locus, such as Newman used in our study. The different strains used in different laboratories may also explain why *arl*, *mgr*, and *cap5* genes were not detected in the two microarray studies (1, 6) that profiled SOS response genes (note that the other study by Anderson et al. [1] used the UAMS1 strain). However, the differences in growth conditions may also contribute to the discrepancies.

DNA repair function could be considered a stress response for bacteria. Capsule has been shown to be an important virulence factor for *S. aureus*, whose expression has been shown to be influenced by various environmental cues, including stress conditions (16, 24). However, regulation of capsule in response to DNA-damaging agents has not been reported. What is the biological significance for the capsule being repressed by the SOS induction? One plausible hypothesis is that capsule biosynthesis is an energy-demanding process. The cells may sense the need for saving energy in order to perform DNA repairs upon the SOS response. However, fibronectin binding protein gene *fnbB* has been shown to be repressed directly by LexA and is induced by the SOS response in the presence of ciprofloxacin (3). Since reduction of capsule would unmask the cell surface proteins, it is most likely that the *fnbB* and *cap5* genes are regulated in a coordinate fashion upon the SOS response, resulting in an increased capability of adherence for the bacteria. It is not apparent how this increase of adherence property would benefit the bacteria after DNA damage. However, it has been shown that *S. aureus* can survive intracellularly (reference 28 and references therein). Perhaps by increasing adherence capability the bacteria would promote internalization by the host cells, thereby avoiding DNA-damaging agents.

It is interesting to note that, without SOS induction, the *sbcDC* genes were expressed very early in the growth cycle but that the expression declined quickly thereafter. The early expression was detected by Northern blotting and manifested by several of our assays, showing that the mutations had the most profound effects at the early growth phase. Although SbcDC is involved in DNA repair after DNA damage, in *E. coli* or *B. subtilis* the enzyme is also involved in repairing replication errors (7, 22). Thus, it is likely that the increased expression in

the early growth phase could be due to a high DNA replication activity at this growth phase. The finding that *sbcDC* is expressed early is also consistent with our previous results showing that *cap5* and *cap8* genes are expressed highly only after the exponential growth phase (19, 21). This temporal regulation of the *cap* genes is likely due to activation by the cell density-dependent *agr* system. However, since *sbcDC* is expressed early and it represses the *cap* genes, it is tempting to speculate that *sbcDC* plays a role in the repression of the *cap* genes at the early growth phase.

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