

Regulation of α - and β -Hemolysins by the *sar* Locus of *Staphylococcus aureus*

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We recently identified a locus on the *Staphylococcus aureus* chromosome, designated *sar*, for staphylococcal accessory regulator, that is involved in the global regulation of extracellular and cell wall-associated proteins. Previous phenotypic and Southern blot analyses with Tn917 and *agr* probes indicated that this locus is distinct from *agr*, a previously described global regulator of exoproteins in *S. aureus*. To understand the mode of regulatory control of exoprotein synthesis by the *sar* locus, the *sar* genotype was transduced from the original *sar* mutant 11D2 into two prototypic *S. aureus* strains, RN6390 and RN450, with well-defined genetic backgrounds. An analysis of extracellular protein profiles by use of silver-stained sodium dodecyl sulfate gels revealed alterations in the pattern of exoprotein production in the late log-early stationary phase in the *sar* mutants in comparison with the corresponding parents. In addition, most of the phenotypic changes that occurred in the conversion from the *sar*⁺ genotype to the *sar* genotype in mutant 11D2 were also found in these mutants. Northern (RNA) blot analyses of two exoprotein transcripts (α - and β -hemolysins) from strain RN6390 and its corresponding *sar* mutant revealed downregulation of these transcripts in the mutant. Serial studies of these hemolysin transcripts at various growth intervals demonstrated that the transcriptional regulation of the hemolysin genes by the *sar* locus began during the log phase and continued into the postexponential phase. These data suggested that the *sar* locus probably regulates exoprotein genes at the transcriptional level. This mode of regulation is similar to that of exoprotein target gene transcription by *agr*.

Staphylococcus aureus is a major human pathogen that has the ability to produce a variety of extracellular and cell wall-associated proteins, many of which are postulated to be involved in pathogenesis (1). The global regulation of a number of these proteins, including α -hemolysin, β -hemolysin, coagulase, protein A, and fibronectin binding protein, is mediated by *agr*, a well-characterized global regulatory locus in *S. aureus* (8). Most of the exoproteins (including β - and δ -hemolysins) that are regulated by *agr* either are not synthesized or are synthesized at a reduced rate in *agr* mutants, while the synthesis of surface proteins is increased (8). Additional analyses of the *agr* locus indicated that it acts at the transcriptional level in regulating α -hemolysin, toxic shock syndrome toxin, and protein A (8, 16). Because of the complexities of this regulation, it has been speculated that other loci may be involved in the regulatory control of exoproteins.

We recently identified by Tn917 insertion mutagenesis an additional regulatory locus, designated *sar*, for staphylococcal accessory regulator, that is also involved in the global regulation of exoproteins and surface proteins (4). A detailed Southern blot analysis with Tn917 and *agr* probes revealed that the *sar* locus is distinct from the *agr* locus. Inactivation of the *sar* locus as a result of a single Tn917LTV1 insertion into the chromosome of a wild-type *S. aureus* isolate (strain DB) resulted in the diminished expression of β - and δ -hemolysins in an isogenic *sar* mutant (strain 11D2 with a *sar*::Tn917LTV1 mutation), while the production of serine proteases and lipase was increased (4). In contrast, the synthesis of cell wall-associated proteins (e.g., fibrinogen and fibronectin binding

proteins) was downregulated in the *sar* mutant in comparison with the parental strain. Some of these phenotypic alterations (e.g., diminished expression of selected cell wall-associated proteins) were different from those found in switching from the *agr*⁺ phenotype to the *agr* mutant phenotype.

To determine whether the *sar* locus regulates exoprotein synthesis at the transcriptional level, we transduced the *sar* genotype into two *S. aureus* strains, RN6390 and RN450, with well-described genetic backgrounds. With α - and β -hemolysins as prototypic exoproteins, Northern (RNA) blot and Western blot (immunoblot) analyses with an isogenic strain pair indicated that the *sar* locus is likely to act in *trans* at the transcriptional level.

MATERIALS AND METHODS

Bacteria, plasmids, and bacteriophages. The bacterial strains used in this study are listed in Table 1. Transducing phage ϕ 71 for parental strain DB was kindly provided by P. Pattee (Iowa State University, Ames). Phage ϕ 11 was used as a transducing phage for strain RN4220.

Media and antibiotics. CYGP and 0.3GL media (4, 14) were used for the growth of *S. aureus*, while Luria-Bertani broth was used for growing *Escherichia coli*. Antibiotics were used at the following concentrations: erythromycin, 10 μ g/ml; and ampicillin, 50 μ g/ml.

Plasmid purification. Plasmid DNA was purified from *E. coli* strains by a standard alkaline sodium dodecyl sulfate (SDS) miniprep protocol (12). To obtain DNA fragments as probes, plasmids were digested with appropriate restriction enzymes, resolved on a 0.7% Tris-acetate-EDTA (TAE) gel, and purified by use of SpinBind columns (FMC Corp., Rockland, Maine). All restriction enzymes were purchased from New England BioLabs (Beverly, Mass.).

Transduction. Phage ϕ 71 was used to produce a phage

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TABLE 1. Bacterial strains used in this study

Strain	Reference or source	Comments
<i>S. aureus</i>		
DB	4	Wild-type blood isolate that is the parent of the original <i>sar</i> mutant, 11D2
RN6390	8, 19	Laboratory strain that maintains its hemolytic pattern when continually propagated on sheep erythrocyte agar
RN450	13	Prototypic strain derived from strain NTCC 8325 cured of prophages; in contrast to strain 8325-4, this strain does not express α -hemolysin
RN4220	13	Mutant of strain 8325-4 that accepts foreign DNA
11D2	4	Isogenic mutant of strain DB with a <i>sar::Tn917LTV1</i> mutation
R	This study	Isogenic mutant of RN6390 carrying an RN6390 <i>sar::Tn917LTV1</i> mutation
A	This study	Isogenic mutant of strain RN450 carrying an RN450 <i>sar::Tn917LTV1</i> mutation
<i>E. coli</i>		
DU5384	15	Strain carrying a pBR322 plasmid with a 3-kb <i>EcoRI-HindIII</i> fragment of the α -hemolysin gene
RN6929	17	JM109 containing a pBluescript plasmid with a 2.2-kb <i>ClaI</i> fragment of the β -hemolysin gene
K440	3	Host strain (HB101) carrying pLTV1, a <i>Tn917</i> derivative

lysate of *sar* mutant strain 11D2 as previously described (4). The phage lysate was then used to infect strain RN4220 at a low multiplicity of infection (phage/recipient ratio, 1:10). Transductants were selected on 0.3GL agar with erythromycin. A ϕ 11 phage lysate of an RN4220 erythromycin-resistant transductant was prepared and used to infect strains RN6390 and RN450 to obtain transductants.

Phenotypic characterization. For phenotypic characterization, the measurements included α -, β -, and δ -hemolysin production measured on plain and cross-streaked sheep and rabbit erythrocyte agar (19), serine protease assayed by the fibrin agar method (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), lipase production measured on 1% Tween agar plates, fibronectin binding protein content assayed by 125 I-labeled fibronectin binding (6), and fibrinogen binding capacity determined by 125 I-labeled fibrinogen binding (11).

SDS-PAGE and immunoblot analyses of extracellular proteins. Equal volumes of extracellular fluid from strains RN6390 and RN450 and their corresponding *sar* mutants at mid-log, late log, and postexponential phases were harvested as previously described (19). In brief, an overnight bacterial culture grown in CYGP medium (or CYGP medium with erythromycin for the *sar* mutants) was inoculated 1:50 into 10 ml of fresh medium without antibiotics. Prior studies had established the stability of the transposon insertion when grown in this manner (4). At \approx 2, 2.75, and 6 h after inoculation, corresponding to an optical density at 650 nm (OD_{650}) of 0.7, 1.1, and 1.7, respectively, 10 ml of extracellular fluid was obtained directly from the cell culture without further volume adjustment, filtered through a 0.22- μ m-pore-size filter, dialyzed against 1 mM Tris–0.15 M NaCl (pH 8), and concentrated 50-fold with an Amicon (Danvers, Mass.) Centriprep concentrator. To evaluate the extracellular protein profiles of parental strains and their respective *sar* mutants, 2 μ l of concentrated extracellular fluid was resolved on SDS–9% polyacrylamide slab gels (10). After polyacrylamide gel electrophoresis (PAGE), the gels were stained with silver (4). To assay for the amount of α -hemolysin present, 7 μ l of concentrated fluid was immunoblotted onto nitrocellulose (22). The immunoblot was incubated with affinity-purified sheep anti- α -hemolysin antibody (Toxin Technology, Sarasota, Fla.) for 2 h at room temperature at a dilution of 1:500. A rabbit anti-sheep antibody–alkaline phosphatase conjugate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) was then added as a secondary antibody (1:1,000 dilution) for 1 h at room temperature. The reactive bands were visualized as described by Blake et al. (2).

Southern blot hybridization. *S. aureus* chromosomal DNA was prepared from lysostaphin (Applied Microbiology, New York, N.Y.)-lysed cells as previously described (4). Southern blot hybridization was performed with randomly primed samples of gel-purified DNA as probes (12). Chromosomal DNA was digested with *EcoRI* or *NcoI*, transferred to a Hybond N⁺ membrane (Amersham, Arlington Heights, Ill.), hybridized with a 32 P-labeled 1.5-kb *HindIII* fragment of *Tn917* (4) at 65°C, and washed with SSPE (1 \times SSPE is 0.18 M NaCl, 10 mM NaPO₄, and 1 mM EDTA [pH 7.7]) according to the membrane manufacturer's instructions (4). The membrane was then autoradiographed with an intensifying screening at –70°C.

Extraction of bacterial RNA. Bacterial RNA was prepared by a modification of the method described by Kornblum et al. (9). In brief, bacteria were grown in CYGP medium (or CYGP medium with erythromycin) overnight, diluted 1:75 in fresh medium, and grown to mid-log, late log, and postexponential phases, corresponding to an OD_{650} of 0.7, 1.2, and 1.7, respectively. To ensure that equal amounts of cells were available for RNA extractions, cells were adjusted to the lowest OD_{650} (i.e., 0.7) with CYGP medium at harvest. Two milliliters of these cells was quickly frozen in an ethanol-dry ice bath and then slowly thawed at 4°C. The bacteria were pelleted (8,000 \times g for 5 min at 4°C), washed with ice-cold TS buffer (20 mM Tris, 20% sucrose, 10 mM EDTA [pH 7.5]), and resuspended in 100 μ l of cold TS buffer. Lysostaphin (15 μ g) was added, and the cell mixture was incubated for 20 min at 4°C and then for 3 min at 37°C to generate protoplasts. To lyse the protoplasts, 2% SDS (100 μ l) and proteinase K (5 μ l of a stock at 10 mg/ml) were added and the mixture was incubated at room temperature for 15 min. To reduce the viscosity of the cell lysate, the sample was frozen (–70°C) and thawed (55°C) twice. The integrity of the RNA was verified by running the sample in a formaldehyde gel as described previously (12).

Northern blot hybridization. Equal volumes (7.5 μ l) of samples were electrophoresed through a 1.5% agarose–0.66 M formaldehyde gel in morpholinepropanesulfonic acid (MOPS) running buffer (20 mM MOPS, 10 mM sodium acetate, 2 mM EDTA [pH 7.0]) (12). The intensity of the 23S and 16S rRNA bands stained with ethidium bromide was verified to be equivalent among all the samples before transfer. RNA was transferred to a Hybond N membrane (Amersham) according to the manufacturer's instructions and allowed to hybridize in 50% formamide at 42°C overnight with 32 P-labeled (randomly primed) gel-purified DNA probes (12). Following hybridization, the membrane was washed twice with 2 \times SSC (1 \times SSC

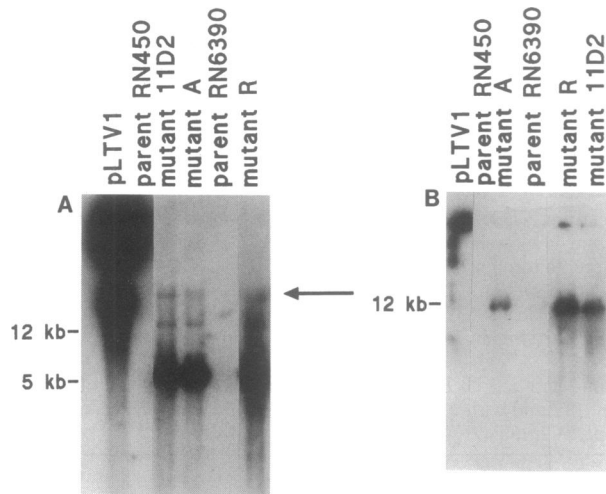


FIG. 1. Southern blots of chromosomal DNAs from strains RN6390 and RN450 and the corresponding *sar* transductants. DNA was digested with *Nco*I (A) or *Eco*RI (B) and probed with a 32 P-labeled 1.5-kb *Hind*III fragment internal to transposon Tn917. Because of the presence of a single internal *Nco*I site within transposon Tn917, there are two hybridizing fragments in the *Nco*I digests and one hybridizing fragment in the *Eco*RI digests. The arrow indicates partial digests that reacted with the probe. The positive control is plasmid pLTV1.

is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at room temperature for 10 min each time, washed once with $1 \times$ SSC–0.1% SDS at 55°C for 15 min, and finally autoradiographed.

RESULTS

Transduction of the *sar* mutant genotype. Transduction was used to transfer the *sar* mutant genotype from the original *sar* mutant 11D2 into two prototypic *S. aureus* strains, RN6390 and RN450, with well-described genetic backgrounds (13). Southern blots of chromosomal DNAs from transductants digested with either *Eco*RI or *Nco*I revealed that a single *Eco*RI fragment and two *Nco*I fragments from these transductants hybridized to a 1.5-kb *Hind*III fragment of Tn917 as a probe (Fig. 1). This hybridization pattern was analogous to that seen with *sar* mutant 11D2, suggesting that the location of the Tn917LTV1 insert in these transductants was identical to that in mutant 11D2.

Phenotypic characterization of transductants from RN6390 and RN450. As most exoproteins are secreted during the late log and postexponential phases (8), total extracellular proteins at corresponding growth intervals ($OD_{650} = 1.1$ and 1.7) from strain RN6390 and its isogenic *sar* mutant R were evaluated for protein profiles. When equivalent volumes of concentrated extracellular fluid were applied to the gel, the parental strain displayed a protein profile distinct from that of the corresponding isogenic mutant (Fig. 2). Likewise, mutant A also exhibited an extracellular protein profile that differed from that of parental strain RN450 (data not shown). The expression of individual proteins is presented in Table 2. Compared with the situation in parental strain RN6390, α -, β -, and δ -hemolysin levels were reduced in *sar* mutant R. Similarly, the production of β -hemolysin in *sar* mutant A was decreased compared with that in parental strain RN450. As α -hemolysin is not produced by strain RN450, the effect of the *sar* locus on α -hemolysin

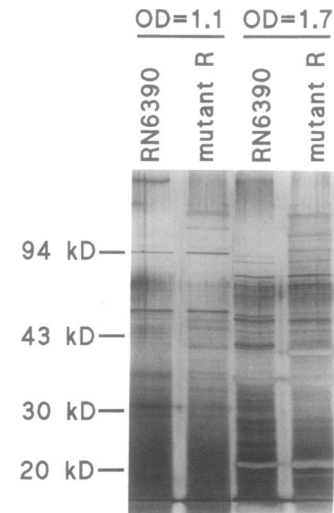


FIG. 2. Silver-stained gels of extracellular proteins (5 μ l of each concentrated extracellular fluid) from parental strain RN6390 and the corresponding isogenic *sar* mutant R. Proteins were sampled from extracellular fluid harvested during the late log ($OD_{650} = 1.1$) and postexponential ($OD_{650} = 1.7$) phases.

expression could not be determined in mutant A. In contrast, the secretion of extracellular serine proteases was increased in both mutants. The phenotypic consequence of the *sar* genotype on lipase production was more variable. More specifically, lipase production was enhanced in mutant R but remained unchanged in mutant A.

Alterations were also observed with cell-bound proteins. For instance, the expression of fibronectin binding protein was diminished in both *sar* mutants in comparison with their respective parents. Similarly, the expression of fibrinogen binding protein was markedly decreased in mutant A in comparison with parental strain RN450. However, the reduction in fibrinogen binding capacity in mutant R compared with parental strain RN6390 was not statistically significant.

With the exception of α -hemolysin, the pattern in switching from the *sar*⁺ phenotype to the *sar* phenotype is analogous to that found in an isogenic pair previously described (i.e., parent DB and *sar* mutant 11D2) (4). To further confirm the switching in α -hemolysin production, the extracellular proteins of parental strain RN6390 and isogenic *sar* mutant R at serial growth intervals were evaluated for α -hemolysin expression by Western blotting. As shown in Fig. 3, α -hemolysin was rendered undetectable in *sar* mutant R compared with parental strain RN6390 throughout the growth cycle. Of interest is the observation that protein A, which reacted with sheep antibody at a low affinity, was produced by *sar* mutant R at mid-log phase but not at late log and postexponential phases. In contrast, parental strain RN6390 did not synthesize extracellular protein A at a detectable level throughout the growth cycle.

Evidence that the *sar* locus regulates α - and β -hemolysin transcripts. As the expression of α - and β -hemolysins was downregulated in *sar* mutant R (Table 2), Northern blotting was performed to determine whether these hemolysins are regulated at the transcriptional level. As displayed in Fig. 4, the levels of transcripts of both exoproteins were reduced in mutant R in comparison with parent strain RN6390. In distinction from the α -hemolysin transcript, there were two hybridizing bands associated with the β -hemolysin transcript

TABLE 2. Phenotypic characterization of RN6390 and RN450 and their corresponding *sar* mutants

Strain	Expression of:						Fibrinogen binding protein ^b	Fibronectin binding protein ^c
	α -Hemolysin ^a	β -Hemolysin ^a	δ -Hemolysin ^a	Lipase ^a	Protease ^a			
RN6390	++	++	++	+	+	5,701 \pm 53	3,314 \pm 35	
<i>sar</i> mutant R	-	+	-	++	++	5,156 \pm 94	2,368 \pm 81	
RN450	-	+	\pm	-	+	6,138 \pm 119	23,558 \pm 398	
<i>sar</i> mutant A	-	-	-	-	++	3,462 \pm 449	2,107 \pm 402	

^a ++, strong producer; +, moderate producer; \pm , very weak producer; -, weak producer.

^b Data are presented as counts of ¹²⁵I-fibrinogen bound per minute to 10⁹ CFU and are reported as the mean \pm the standard error of the mean ($n = 4$). The reduction in fibrinogen binding of *sar* mutant A in comparison with parental strain RN450 was statistically significant ($P < 0.008$; t test).

^c Data are presented as counts of ¹²⁵I-fibronectin bound per minute to 10⁹ CFU and are reported as the mean \pm the standard error of the mean ($n = 4$). The reduction in fibronectin binding of *sar* mutant R in comparison with parental strain RN6390 was significant ($P < 0.003$; t test). The reduction in fibronectin binding of *sar* mutant A in comparison with parental strain RN450 was significant ($P < 0.0001$; t test).

(1.5 kb [broken arrow] and 2.9 kb [solid arrow]) (Fig. 4B). On the basis of the size of the previously reported β -hemolysin gene (17), it is likely that the 1.5-kb band represents the intact β -hemolysin transcript, while the larger hybridizing fragment may arise as a result of an overlapping transcript or cross-reacting transcript. Notably, other investigators also found that the intact β -hemolysin probe hybridized to at least two bands in Northern blot studies (16b). A serial analysis at different growth phases also revealed that the transcription of both hemolysins was detected at the mid-log phase and was maximal at the postexponential phase. More specifically for parental strain RN6390, there were more transcripts at an OD₆₅₀ of 0.7 than at an OD₆₅₀ of 1.1, while the levels of the transcripts were highest at an OD₆₅₀ of 1.7. Remarkably, the levels of transcripts of both α - and β -hemolysins were decreased for mutant R throughout the growth cycle.

DISCUSSION

It was shown in our previous study that a single insertion of transposon Tn917LTV1 into the *sar* locus of a wild-type clinical isolate, strain DB, resulted in a pleiotropic effect on the expression of a number of extracellular and cell wall-associated

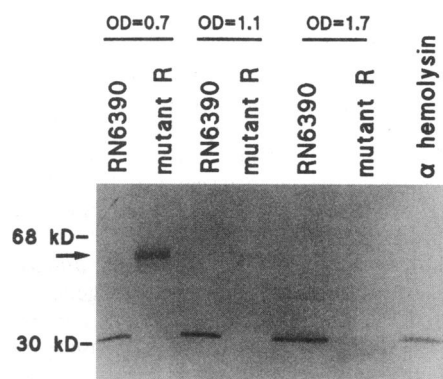


FIG. 3. Western blotting of the extracellular fluids of strain RN6390 and mutant R probed with anti- α -hemolysin antibody. Equivalent amounts of extracellular fluids obtained at serial growth intervals were applied to the lanes. The blot was developed with affinity-purified sheep anti- α -hemolysin antibody. Purified α -hemolysin (Toxin Technology) was used as a positive control. In some samples, the sheep antibody also reacted weakly with protein A present in the extracellular fluids (arrow). As the volume of extracellular fluid was not adjusted for the number of CFU present in the culture, a comparison was only valid with samples obtained at identical optical densities.

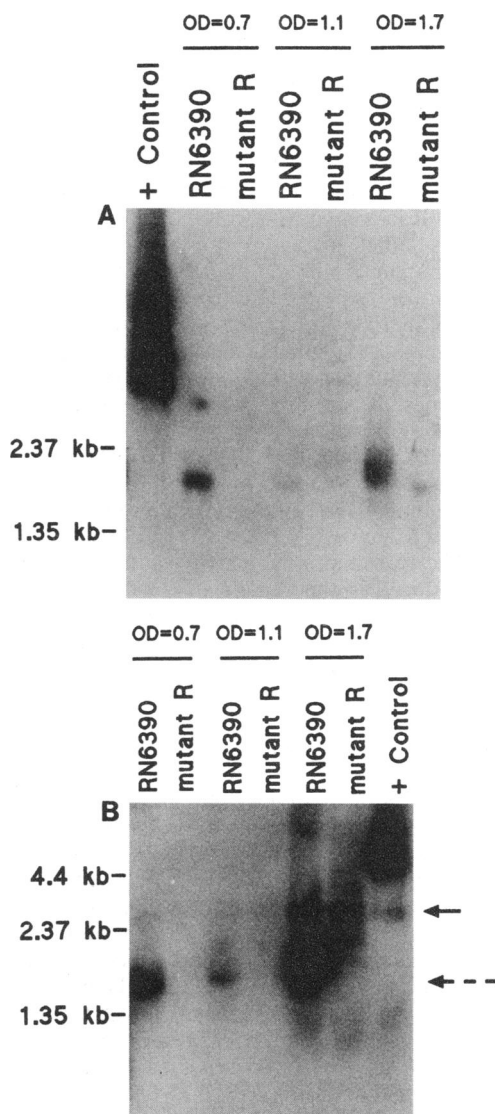


FIG. 4. Northern blots of α -hemolysin (A) and β -hemolysin (B) transcripts of parental strain RN6390 and *sar* mutant R during the growth cycle. (A) A single α -hemolysin transcript of ≈ 1.8 kb was noted. The positive control was the plasmid from *E. coli* DU5384 digested with *EcoRI-HindIII*. (B) Two bands (1.5 kb [broken arrow] and 2.9 kb [solid arrow]) were noted to hybridize with the β -hemolysin probe. The positive control was the intact plasmid from *E. coli* RN6929.

proteins in *sar* mutant 11D2 (4). The location of the Tn917LTV1 insertion in the staphylococcal chromosome was mapped to the *Sma*I-D fragment, which is in a region distinct from two regulatory loci (i.e., *agr* and *xpr*) previously described for *S. aureus* (16a, 19, 20). To understand the mode of regulatory control of exoprotein synthesis by the *sar* locus, we transduced the *sar* genotype from mutant 11D2 to two *S. aureus* strains, RN6390 and RN450, with well-defined genetic backgrounds (8, 19). The insertion of Tn917LTV1 into the *sar* locus in both mutants A and R was supported by Southern blot analysis (Fig. 1). As an additional confirmation that the *sar* locus was insertionally inactivated in both mutants A and R, we also complemented the extracellular protein profiles as well as hemolysin production in these mutants with a plasmid carrying a cloned *sar* gene (unpublished data).

With the exception of α -hemolysin, most of the phenotypic changes that occurred with the conversion of the *sar*⁺ genotype to the *sar* genotype in mutant 11D2 (4) were also found in mutants A and R (Table 2). For instance, the expression of a cell wall-associated protein, such as fibronectin binding protein, was reduced for both *sar* mutants in comparison with the parents. Similarly, both β - and δ -hemolysins were downregulated in *sar* mutants. Like that in the original *sar* mutant 11D2, the secretion of extracellular proteases was increased in mutants A and R in comparison with the parents. Likewise, lipase activity was either increased (mutant R) or remained unchanged (mutant A) in *sar* mutants. Although most of the exoproteins were expressed at lower levels in the mutants, the observation that lipase activity was increased in mutant R suggested that the augmented extracellular protease activity was unlikely to have accounted for all the observed alterations in phenotypes. In addition, the finding that some higher-molecular-weight proteins were detected in the extracellular protein profiles of mutant R but not RN6390 is also inconsistent with such a hypothesis (Fig. 2).

Northern blot analysis of the β -hemolysin gene in *sar* mutant R confirmed a decrease in the level of this transcript in this *sar* mutant. The transcriptional activity of the α -hemolysin gene was also reduced in this mutant in comparison with parental strain RN6390. These studies suggest that the *sar* locus is likely to regulate exoprotein genes at a transcriptional level. However, the possibility that the *sar* locus may alter transcript stability together with translational control cannot be entirely ruled out.

The data obtained in evaluating the levels of α - and β -transcripts at serial growth intervals revealed that the transcriptional regulation of these genes by the *sar* locus began at the mid-log phase and continued into the postexponential phase. Of interest is the observation that both α - and β -hemolysin transcript levels were higher at an OD₆₅₀ of 0.7 than at an OD₆₅₀ of 1.1 for parental strain RN6390. Given a 2-h latency period between inoculation and harvest at an OD₆₅₀ of 0.7, it is unlikely that the transcript observed at the mid-log phase was due to a carryover of the inoculum. However, the reduction in transcriptional activity from an OD₆₅₀ of 0.7 to an OD₆₅₀ of 1.1 which occurred within a 45-min interval was consistent with either mRNA decay or a decrease in the rate of transcription. Notably, both α - and β -hemolysin transcripts were at their highest level during the postexponential phase. In a previous study, Vandenesch et al. (23) also reported that the transcription of the α -hemolysin gene in an *agr*⁺ parent began at the log phase and peaked during the postexponential phase. This pattern of transcription is consistent with the hypothesis that a separate postexponential signal independent of *agr* is needed for augmented α -hemolysin transcription during the postexponential phase (23). However, the relationship of this

postexponential signal to the *sar* locus with regard to exoprotein expression remains to be elucidated.

The postexponential regulation of exoprotein genes in *S. aureus* involves at least three global regulatory systems. In addition to *sar*, the best-described regulatory element is the *agr* locus, which is composed of two divergent transcription units. One of these transcription units, RNAIII, which also encodes the 26-residue δ -hemolysin polypeptide, is required for the transcriptional control of exoprotein synthesis (7, 8, 23). Vandenesch et al. (23) had shown that the production of the α -hemolysin transcript had to be preceded by that of RNAIII. In the absence of RNAIII (i.e., *agr*), the mRNA transcriptional activity of the α -hemolysin gene was significantly diminished during the log and postexponential phases of the growth cycle (23). Thus, there appears to be a similar trend in the temporal and transcriptional control of exoprotein genes by the *agr* locus and the *sar* locus.

Another regulatory locus, designated *xpr*, was recently described by Smeltzer et al. (20). A Tn551 chromosomal insertion into the *xpr* locus in *S. aureus* S6C resulted in the reduced expression of exoproteins (lipase, enterotoxin B, α - and δ -hemolysins, protease, and nuclease) (23). In contrast to the *agr* phenotype, coagulase production was not increased in the *xpr* mutant (23). Whether the *xpr* locus regulates exoprotein production at a transcriptional or a translational level is not clear. Of particular interest is the observation that both *xpr* and *agr* mutants produce greatly reduced amounts of δ -hemolysin, which is encoded within the RNAIII transcript, the regulatory molecule of *agr*. Because of this finding and the similarities in exoprotein expression between *xpr* and *agr* mutants, it has been speculated that *xpr* and *agr* may behave as interactive regulatory genes (20).

As described in this and previous (4) studies, inactivation of the *sar* locus in three different *S. aureus* strains (strains DB, RN6390, and RN450) resulted in alterations of the expression of both extracellular and cell wall-associated proteins in the corresponding *sar* mutants. Although most of the phenotypic changes were similar among the *sar* mutants, it is also clear that host factors may play a role in gene expression. For instance, α -hemolysin was downregulated in mutant R, while it is upregulated in the original *sar* mutant 11D2. Thus, the expression of α -hemolysin in two diverse *sar* genetic backgrounds (i.e., strains DB and RN6390) may differ as a consequence of unknown host factors which can influence α -hemolysin gene expression via transcriptional and/or translational control. In support of this hypothesis is the finding by Compagnone-Post et al. (5) that *S. aureus* strains carrying single and apparently identical copies of the *agr* locus can produce different amounts of RNAIII, thereby resulting in transcriptional regulation of *agr* by a host factor(s).

The control of exoprotein synthesis in *S. aureus* is a complicated process involving several regulatory genetic elements (5, 7, 8, 18, 20, 23). Two of these determinants, *sar* and *agr*, appear to regulate exoprotein expression via transcriptional control, while the control mechanism of the third element (i.e., *xpr*) is not known. Whether these regulatory elements operate independently or in conjunction with each other (4, 20, 23) at a transcriptional level will be important to our understanding of the regulatory control of virulence determinants in *S. aureus*. More specifically, it will be of interest to know whether the transcription of *sar* is affected by *agr* and/or *xpr*. Another issue concerning *sar* regulation is whether this locus controls rates of gene transcription or mRNA decay. Future gene fusion studies would help clarify this aspect.

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