THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 52, Issue of December 24, pp. 37169–37176, 1999
Printed in U.S.A.

SarA, a Global Regulator of Virulence Determinants in *Staphylococcus aureus*, Binds to a Conserved Motif Essential for *sar*-dependent Gene Regulation*

(Received for publication, August 3, 1999, and in revised form, September 29, 1999)

Yueh-tyng Chien‡§, Adhar C. Manna‡¶, Steven J. Projan‖, and Ambrose L. Cheung‡

From the ‡Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, New York 10021 and ‖Wyeth-Ayerst Research, Lederle Laboratories, Pearl River, New York 10965

The expression of many virulence determinants in *Staphylococcus aureus* including α-hemolysin-, protein A-, and fibronectin-binding proteins is controlled by global regulatory loci such as *sar* and *agr*. In addition to controlling target gene expression via *agr* (*e.g.* α-hemolysin), the *sar* locus can also regulate target gene transcription via *agr*-independent mechanisms. In particular, we have found that SarA, the major regulatory protein encoded within *sar*, binds to a conserved sequence, homologous to the SarA-binding site on the *agr* promoter, upstream of the −35 promoter boxes of several target genes including *hla* (α-hemolysin gene), *spa* (protein A gene), *fnb* (fibronectin-binding protein genes), and *sec* (enterotoxin C gene). Deletion of the SarA recognition motif in the promoter regions of *agr* and *hla* in shuttle plasmids rendered the transcription of these genes undetectable in *agr* and *hla* mutants, respectively. Likewise, the transcription activity of *spa* (a gene normally repressed by *sar*), as measured by a XylE reporter fusion assay, became derepressed in a wild type strain containing a shuttle plasmid in which the SarA recognition site had been deleted from the *spa* promoter region. However, DNase I footprinting assays demonstrated that the SarA-binding region on the *spa* and *hla* promoter is more extensive than the predicted consensus sequence, thus raising the possibility that the consensus sequence is an activation site within a larger binding region. Because the *sar* and *agr* regulate an assortment of virulence factors in *S. aureus*, we propose, based on our data, a unifying hypothesis for virulence gene activation in *S. aureus* whereby SarA is a regulatory protein that binds to its consensus SarA recognition motif to activate (*e.g. hla*) or repress (*e.g. spa*) the transcription of *sar* target genes, thus accounting for both *agr*-dependent and *agr*-independent mode of regulation.

Staphylococcus aureus is a major cause of human infections ranging from superficial abscesses, pneumonia, and endocarditis to sepsis (1). The capability of *S. aureus* to cause a multiplicity of infections is probably attributable to the impressive array of extracellular and cell wall-associated virulence determinants produced by this organism (2). The expression of many virulence determinants in *S. aureus* is highly coordinated and is generally controlled by global regulatory elements such as *agr* (accessory gene regulator) and *sar* (staphylococcal accessory regulator) (3, 4). The regulatory elements, in turn, control the transcription of a wide variety of unlinked genes, many of which have been demonstrated to be involved in pathogenesis.

The global regulatory locus *agr* consists of two divergent transcripts, RNAII and RNAIII, initiated from two distinct promoters, P2 and P3, respectively. RNAIII is the effector molecule of the *agr* response and hence is responsible for the up-regulation of extracellular protein production and down-regulation of cell wall-associated protein synthesiss during the postexponential phase (3). The RNAII molecule, driven by the P2 promoter, encodes a four gene operon, *agrBDCA*. AgrC and AgrA correspond to the sensor and activator proteins of two component regulatory systems, respectively. Additionally, *agrD*, in concert with *agrB*, participates in the generation of an octapeptide with quorum sensing properties (5, 6). Accordingly, AgrC, upon sensing a critical extracellular concentration of the octapeptide, becomes phosphorylated and activates AgrA by a second phosphorylation step. Activated AgrA would then, presumably, stimulate the transcription of the *agr* regulatory molecule RNAIII, which ultimately interacts with target genes to modulate transcription (7, 8) and possibly translation (9).

Another regulatory locus, designated *sar*, was uncovered in our laboratory (4). Unlike *agr*, the *sar* locus activates the synthesis of both extracellular (*e.g.* hemolysins) and cell wall proteins (*e.g.* fibronectin-binding protein) in *S. aureus* (4). The *sar* locus, contained within a 1.2-kb[1] fragment, is composed of three overlapping transcripts designated sarA (0.56 kb), sarC (0.8 kb), and sarB (1.2 kb). These transcripts, all encoding the major 372-bp *sarA* open reading frame, have common 3′ ends but originate from three distinct promoters (10). Transcription and gel shift studies (11, 12) revealed that the SarA protein preferentially binds to the P2-, and to a lesser extent, to the P3-*agr* promoter region, thereby augmenting RNAII and the ensuing RNAIII transcription. RNAIII would then modulate the transcription of *sar* target genes (*e.g. hla*). More recently, we demonstrated that the SarA protein level is an important determinant of *agr* activation (13). In particular, the sequence upstream of the *sarA* gene may play a role in modulating the translation of the *sarA* gene product, the level of which correlates with *agr* expression (13). However, phenotypic and tran-

* This work was supported in part by National Institutes of Health Grants AI30061 and AI37142. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by a New York Heart Participatory Laboratory Award. Present address: Scriptgen Pharmaceuticals Inc. 610 Lincoln St., Waltham, MA 02451.

¶ Present address: Dept. of Microbiology, Dartmouth Medical School, Hanover, NH 03755.

** Recipient of the Irma T. Hirschl Career Scientist Award as well as the Genentech Established Investigator Award from the American Heart Association. To whom correspondence should be addressed. Present address: Dept. of Microbiology, Dartmouth Medical School, Hanover, NH 03755.

[1] The abbreviations used are: kb, kilobase(s); bp, base pair(s); GST, glutathione *S*-transferase; MOPS, 4-morpholinepropanesulfonic acid.

scriptional analyses suggest that the *sar* locus can also regulate target gene transcription via a SarA-dependent but *agr*-independent mechanism. Supporting this notion is the observation that the synthesis of β-hemolysin was further reduced in a double *sar/agr* mutant as compared with the single *agr* mutant (14, 15). Additionally, a recent report by Chan and Foster (16) provided evidence that SarA may up-regulate α-hemolysin production independently of *agr*. The *sar* locus, contrary to *agr,* is apparently a repressor of (V8) protease activity (16). Taken together, these data imply that SarA, the major *sar* effector molecule, may somehow interact directly with *sar* target genes (*e.g.* α hemolysin) as well as with intermediate regulatory molecules such as that of *agr* to control gene expression.

We have analyzed the SarA protein/*agr* promoter-DNA complex by DNase I footprinting assay (12). The SarA-binding site on the *agr* promoter, as mapped by this method, covers a 29-bp sequence, more proximal to P2, in the P2 and P3 interpromoter region. In this report, we demonstrate that the SarA-binding site on the *agr* promoter appears to constitute a conserved SarA recognition motif found in many of the *sar* target genes in *S. aureus* including *hla* (α-hemolysin gene), *spa* (protein A gene), and *fnb* (fibronectin-binding protein genes). This concept was supported by data from gel shift assays using purified recombinant SarA and synthetic oligonucleotides encompassing the putative SarA recognition site. Similarly, DNase I footprinting assays with *hla* and *spa* promoter fragments and purified SarA protein also uncovered binding sites encompassing the putative SarA recognition site. A deletion of the consensus binding site, as found in the promoter regions of *agr*, *hla*, and *spa*, rendered the target genes unresponsive to *sar* regulation. Because the region of SarA binding on the *hla* and *spa* promoters, as determined by footprinting assays, was wider than the consensus binding sites, our data strongly support the existence of a common "effector" site among a larger binding region to which SarA binds in *sar* target genes. We propose that the binding of SarA to a common recognition motif in target genes alters (*i.e.* activates or represses, depending on the target) the transcription of these genes in *S. aureus*, thus explaining both *agr*-dependent and *agr*-independent modes of regulation by *sar*. Because the *sar* locus controls the expression of a variety of extracellular and cell wall-associated virulence determinants in *S. aureus*, the identification of a common regulatory pathway may provide clues to the development of novel antimicrobial strategies.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—The bacterial strains and plasmids used in this study are listed in Table I. Phage φ11 was used as a generalized transducing phage for *S. aureus* strains. CYGP, 0.3GL medium (17), and tryptic soy broth were used for the growth of *S. aureus* strains, whereas LB was used for growing *Escherichia coli.* Antibiotics were used at the following concentrations: erythromycin at 5 μg/ml, chloramphenicol at 10 μg/ml, and tetracycline at 5 μg/ml for *S. aureus* and ampicillin at 50 μg/ml for *E. coli*.

Genetic Manipulations in S. aureus—Genetic constructs were first transformed by electroporation to *S. aureus* RN4220, a restriction-deficient derivative of strain 8325-4 (18). Transformants were selected on NYE agar (18) containing 10 μg/ml of chloramphenicol. For transduction, phage φ11 was used to produce a phage lysate of strain RN4220 containing various genetic constructs. The phage lysate was then used to infect *S. aureus* recipient strains as described (4). The presence of the correct plasmids was confirmed by restriction mapping. Chromosomal transduction was verified by Southern blots with gene-specific probes as described (4).

Construction and Purification of GST-SarA and SarA—The intact 372-bp *sarA* gene was amplified by polymerase chain reaction and introduced into GST vector pGEX-4T-1 (Amersham Pharmacia Biotech) as described (12). Enhanced expression of the GST-SarA construct was induced by adding isopropyl-1-thio-β-D-galactopyranoside (1 mM) to a growing culture (30 °C) at an A_{600} of 0.5 and purified as described (12). Besides the GST-SarA fusion protein, SarA was also expressed in *E. coli* BL21 containing pET14b with the 372-bp s*arA* gene. Induction by isopropyl-1-thio-β-D-galactopyranoside for the T7 RNA polymerase-based system in BL21 and purification on a His tag column were conducted following the manufacturer's instructions.

Gel Shift Analysis—Polymerase chain reaction fragments as well as complementary synthetic DNA fragments (~45 bp) containing putative SarA-binding motifs of *sar* target genes were end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (Amersham Pharmacia Biotech). DNA fragments were purified by ProbeQuant G-50 microcolumns (Amersham Pharmacia Biotech) according to the manufacturer's instructions. For gel shift assays, protein samples were mixed with ≈5,000 cpm of end-labeled double-stranded DNA fragments (0.3 ηg) in the presence of 1 μg of calf thymus DNA (Amersham Pharmacia Biotech) in a final volume of 25 μl. Incubations were carried out on ice for 30 min in 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, 5% (v/v) glycerol, 0.1 mM EDTA, and 1 mM dithiothreitol. Samples were then resolved on 5 or 8% polyacrylamide gels in 0.5× TBE buffer. Following electrophoresis, gels were dried and autoradiographed.

DNase I Footprinting—Binding reactions were performed as described for the gel mobility shift assay except that a total volume of 100 μl was used. DNase I (Roche Molecular Biochemicals) (0.01 unit) was added and incubated for 2 min at room temperature. The reaction was terminated by adding 100 μl of freshly made stop solution (50 mM Tris-HCl, pH 8.0, 2% (w/v) SDS, 10 mM EDTA, proteinase K at 0.4 μg/ml). The reaction mixture was extracted with phenol/chloroform. DNA samples were ethanol-precipitated, washed with 70% ethanol, and resuspended in loading buffer (98% deionized formamide, 10 mM EDTA, pH 8.0, 0.025% (w/v) xylene cyanol FF, and 0.025% (w/v) bromphenol blue). DNA samples were denatured at 95 °C for 3 min and run on a 6% polyacrylamide sequencing gel. Chemical cleavages at purine (A+G) residues were performed by the standard method (19).

Site-specific Deletions of sar Target Promoter Fragments—To introduce deletions of the putative SarA-binding sites within *agr*, *hla*, and *spa* promoters, site-directed mutagenesis was performed with the Stratagene Quick Change kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. The following oligonucleotides and their complements were used to delete the SarA-binding sites from plasmid templates (pALC772, pALC829, and pALC1639 for deletions in *agr*, *hla*, and *spa*, respectively): for *agr*, 5′-^{1633}TTCTTAACTGTAAATTTTTTTA-^{1654} ^{1684}AACAGTTAAGTATTTATTTCCT^{1705}-3′ (4); for *hla*, 5′-^{1253}TCT-ATTTATTAATTTACAGTAGTTA^{1277} ^{1311}ATTGATTTAATTCTAAGAT-ATTTGT^{1335}-3′^{2}; for *spa*, 5′- ^{589}AAGTTGTAAAACTTACCTTTAAA^{611} ^{634}AGTATTGCAATACATAATTCGTT^{656}-3′ (20); and for *spa* mock mutation, 5′-^{421}TTCCATTTTATTCTTAAAAATA^{443} ^{467}CCGCTTTCATTAT-AAAAAATATC^{489}-3′. After constructing the mutations, the recombinant plasmids were transformed into XL1-Blue competent cells (Stratagene). The deletion within each promoter in the vector was confirmed by DNA sequencing. DNA fragments containing the mutations were gel purified and ligated into shuttle vector pSK236 or pLC4. Electroporation of *S. aureus* RN4220 with recombinant pSK236 or pLC4 containing the mutated fragments was performed as described previously (18). Phage φ11 was used to transduce the plasmid from RN4220 into the recipient *S. aureus* strains (4). The presence of correct plasmids was confirmed by restriction mapping.

Isolation of RNA and Northern Blot Hybridization—Overnight cultures of *S. aureus* were diluted 1:50 in CYGP and grown to mid log (A_{650} = 0.7), late log (A_{650} = 1.1), and postexponential (A_{650} = 1.7) phases. The cells were pelleted and processed with a FastRNA isolation kit (BIO 101, Vista, CA) in combination with 0.1-mm-diameter sirconia-silica beads in a FastPrep reciprocating shaker (BIO 101) as described (21). 10 μg of each sample was electrophoresed through a 1.5% agarose-0.66 M formaldehyde gel in MOPS running buffer (20 mM MOPS, 10 mM sodium acetate, 2 mM EDTA, pH 7.0). Blotting of RNA onto Hybond N^{+} membranes (Amersham Pharmacia Biotech) was performed with the Turboblotter alkaline transfer system (Schleicher & Schuell). For detection of specific transcripts (*agr*, *sar*, and *hla*), gel purified DNA probes were radiolabeled with [α-^{32}P]dCTP by the random-primed method (Ready-To-Go labeling kit, Amersham Pharmacia Biotech) and hybridized under high stringency conditions (14). The blots were subsequently washed and autoradiographed.

Construction of Transcriptional Fusions—A 491-bp fragment encompassing the *spa* promoter region (see Fig. 5*A*) was amplified by polymerase chain reaction using genomic DNA of *S. aureus* strain RN6390 as the template with the following primers: upper primer, 5′-CCGGAAT-

[2] S. J. Projan, personal communication.

TABLE I
Bacterial strains and plasmids

Strains or plasmid	References	Comments
S. aureus		
RN4220	Ref. 17	A mutant of 8325–4 that accepts foreign DNA
RN6390	Ref. 3	Laboratory strain that maintains its hemolytic pattern when propagated on sheep erythrocyte agar (parental strain)
RN6911	Ref. 3	A RN6390-derived *agr* mutant in which the *agr* locus has been replaced by the *tetM* gene
DU1090	Ref. 32	8325–4 with a *hla::ermC* mutation (*i.e.* α-hemolysin mutant)
ALC488	Ref. 24	Isogenic mutant of RN6390 carrying a *sarA::ermC* mutation (*sarA* mutant)
ALC837	this study	RN6390 with *hla::ermC* mutation (α-hemolysin mutant)
ALC970	this study	ALC488 (*sarA* mutant) with pLC4
ALC1388	this study	RN6911 (*agr* mutant) with pALC1354
ALC1389	this study	RN6911 with pALC1333
ALC1525	this study	ALC837 (*hla* mutant) with pALC1335
ALC1526	this study	ALC837 with pALC1501
ALC1667	this study	ALC488 (*sarA* mutant) with pALC1640
ALC1668	this study	ALC488 with pALC1641
ALC1669	this study	ALC488 with pALC1639
ALC1794	this study	RN6390 (parental strain) with pALC1639
ALC1795	this study	RN6390 with pALC1640
ALC1796	this study	RN6390 with pALC1641
E. coli		
XL1-blue	Ref. 19	General purpose cloning strain
DH5α	Ref. 19	A cloning strain
BL21(DE3).pLys.S	Novagen	Host strain for pET14b
ALC1137	Ref. 12	DH5α containing pALC1137
ALC1237	this study	BL21(DE3).pLys.S with pALC1160
Plasmids		
pCR2.1	Invitrogen	PCR cloning vector for direct cloning of PCR fragments
pBluescript	Stratagene	A cloning vector
pET14b	Novagen	*E. coli* expression vector
pLC4	Ref. 22	Transcriptional fusion vector with a promoterless *xylE* gene
pSK236	Ref. 33	Shuttle vector containing pUC19 cloned into the *Hin*dIII site of pC194
pC194	Ref. 17	2.9-kb plasmid with a *S. aureus* origin of replication and a chloramphenicol resistance gene
pALC772	this work	pCR2.1 containing a 3.1-kb fragment encoding RNAII of *agr*
pALC829	this work	pBluescript containing a 3-kb *hla* fragment
pALC1137	Ref. 12	pGEX-4T-1 with a GST-SarA fusion containing full-length SarA
pALC1160	Ref. 12	pET14b with the intact 372-bp *sarA* gene
pALC1333	this study	pSK236 (shuttle vector) with a 3.1-kb fragment encoding RNAII of *agr*
pALC1335	this study	pSK236 with a 3-kb fragment encoding *hla*
pALC1354	this study	pSK236 with a 3.1-kb fragment encoding RNAII but lacking the 29-bp SarA binding site
pALC1501	this study	pSK236 carrying a 3-kb *hla* fragment with a 33-bp deletion in the SarA binding site
pALC1639	this study	pLC4 (transcriptional fusion vector) with an intact 491-bp *spa* promoter fragment
pALC1640	this study	pLC4 with a 490-bp *spa* promoter fragment but lacking the 22-bp SarA binding site
pALC1641	this study	pLC4 with a 490-bp *spa* promoter fragment but lacking a 26-bp AT-rich sequence upstream of the SarA binding site (mock deletion)

TC^{198}AAGACCATGCTGAACAA^{214} (*Eco*RI site underlined), and lower primer, 5′-AACGCAAGCTT^{688}CCCTGTATGTATTTGTAAAGTC^{667} (*Hin*dIII underlined) (20). The polymerase chain reaction fragment was cloned into the TA cloning vector pCR2.1 (Invitrogen, San Diego, CA). The recombinant pCR2.1 plasmid was used as the template for mutagenesis to delete the SarA-binding site as well as the control upstream AT-rich sequence (mock mutation) as described above. The *Eco*RI/*Hin*dIII fragments containing the natural or the mutated promoter region were cleaved from pCR2.1 and recloned into plasmid pLC4 (22), generating transcriptional fusions to the *xylE* reporter gene. The recombinant pLC4 plasmids were then electroporated into RN4220 and then transduced into recipient *S. aureus* strains. All transcriptional fusions and relevant constructs in different mutants are described in Table I.

Catechol 2,3-Dioxygenase Assays—For enzymatic assays, overnight cultures were diluted 1:50 in 250 ml of tryptic soy broth containing appropriate antibiotics and shaken at 37 °C and 200 rpm. After few hours of growth, 50 ml of cell culture at A_{600} of 1.7 (stationary phase) was removed and centrifuged. Following two washes in ice-cold potassium phosphate buffer (20 mM, pH 7.2), pellets were resuspended in 500 μl of 100 mM potassium phosphate buffer (pH 8.0) containing 10% acetone and 25 μg/ml of lysostaphin and incubated for 15 min at 37 °C and then iced for 5 min. Extracts were centrifuged at 20,000 × *g* for 50 min at 4 °C to pellet cellular debris. XylE (catechol 2, 3-dioxygenase) expression were assayed spectrophotometrically at 30 °C in a total volume of 3 ml of 100 mM potassium phosphate buffer (pH 8.0) containing 100 μl of cell extract and 0.2 mM catechol as described (22). The reactions were allowed to proceed for 15 min with A_{375} readings taken at 5-, 10-, and 15-min time points, with the data being presented as the average of three time points. One milliunit is equivalent to the formation of 1.0 nmol of 2-hydroxymuconic semialdehyde/min at 30 °C. Specific activity is defined as milliunit/milligram of cellular protein (22).

RESULTS

SarA Binds to a Consensus Motif Present in sar Target Genes—The SarA protein is the major regulatory molecule within the *sar* locus (13). In previous studies, we have demonstrated by gel shift and footprinting studies that the SarA protein binds to the *agr* promoter region, probably activating the global regulatory locus *agr* and the corresponding genes downstream of the *agr*-activating cascade (12). The SarA-binding site on the *agr* promoter has been mapped to a 29-bp sequence (12) in the *agr* P2-P3 interpromoter region. However, we recognize that SarA can also modulate other target genes via *agr*-independent pathways (*e.g. hla* and *fnb*) (15, 16, 23). Because of this observation, we wanted to explore whether the

agr	ATTTGTATTTAATATTTTAACATAAA	20/26
	[underlined nucleotides mapped by footprinting analysis (12)]	
hla	ATTTTTATTTAATAGTTAATTAATTG	23/26
spa	AATTATAAATATAGTTTTTAGTATTG	17/26
fnbA	ACTTGAATACAATTTATAGGTATATT	16/26
fnbB	CTTTGTATGCAATATATATGTGAGTT	19/26
sec	ATTTTCTTTTAATATTTTTTTAATTG	23/26
Consensus	ATTTgTATtTAATATTTataTAAtTg t t g	

FIG. 1. **Alignment of a putative common SarA recognition sequence from promoters of *sar* target genes.** *hla*, α-hemolysin gene; *spa*, protein A gene; *fnbA*, fibronectin-binding protein A gene; *fnbB*, fibronectin-binding protein B gene; *sec*, enterotoxin C gene. The consensus sequence was derived as follows: 4/6, capital letter; 3/6, small letter. An even distribution of nucleotides at a specific position is presented as combinations of small letters.

regions upstream of −35 promoter boxes of several *sar* target genes contain sequences homologous to the SarA-binding site on the *agr* promoter. An alignment of sequences from the promoter regions of *hla*, *spa*, *fnbA*, *fnbB*, and *sec* revealed an apparent 26-bp consensus sequence (Fig. 1) that shares homology with the SarA-binding site on the *agr* promoter (12). Because both the *S. aureus* genome and the consensus sequence are AT-rich, the specificity of such an alignment for a conserved sequence requires a rigorous confirmation. Toward that end, we synthesized ~45-bp complementary oligonucleotides encompassing the 26-bp *sar* recognition motif together with 9–11 bp of bilateral flanking sequence (Fig. 2) for each of the five genes. Using ^{32}P end-labeled oligonucleotides, we found that purified SarA was able to retard the mobility of each of these synthetic DNA fragments in gel shift assays. For illustrative purposes, only gel shift data with 46-bp *hla* and 45-bp *spa* oligonucleotides probes are shown (Fig. 2). In contrast, increasing concentrations of a 165-bp *nif*H2 promoter fragment did not bind to SarA or GST-SarA (data not shown). Similarly, an unrelated 45-bp AT-rich fragment from the promoter region of the β-hemolysin gene also did not exhibit binding activity to purified SarA protein.

To determine the SarA-binding site on target promoters more precisely, we elected to analyze two representative protein-DNA complexes (*hla* and *spa*) by DNase I footprinting. These two target genes were chosen because they represent the opposite ends of the spectrum in *sar* regulation, with up-regulation of *hla* and down-regulation of *spa* transcription by the *sar* locus. Additionally, *hla* is positively controlled by a SarA-dependent but *agr*-mediated pathway (11) as well as by direct binding of SarA to the *hla* promoter fragment as demonstrated by the gel shift assay (Fig. 2*B*). In contrast, *spa* is negatively regulated by *sar* and *agr* at the transcriptional level (24, 25); however, cross-complementation studies of an *agr* mutant with a plasmid carrying an intact *sar* locus revealed that *spa* transcription can be repressed by *sar* independent of *agr* (24).

In analyzing the DNase I footprinting data for a 235-bp *hla* promoter (nucleotides 1–80 plus 155 bp upstream of the start site), it was evident that the area protected by SarA (2–5 μg of protein) covered several regions, spanning −32 to −126 bp upstream of the transcription start site (Fig. 3*A*). Notably, the protected site encompassed the conserved SarA-binding sequence (*double underlined* in Fig. 3*A*). To assess the specificity of the binding region, we also performed DNaseI footprinting assay with a 192-bp *hla* promoter fragment (nucleotides 1–80 plus 112 bp upstream). Within the DNA region available (up to 112 bp upstream), the protected area essentially concurred

hla:ATATATAGTTAATTTTTATTTAATAGTTAATTAATTGATTTAATTC

spa: TTTAAATTTAATTATAAATATAGATTTTAGTATTGCAATACATAA

fnbA:GTTTCTGATGACTTGAATACAATTTATAGGTATATTTCAAATAATA

fnbB:TGTTCAAGAGCTTTGTATGCAATATATATGTGAGTTTCAAATAATA

sec: AATATAATTAATTTTCTTTTAATATTTTTTTAATTGAATATTTAAAG

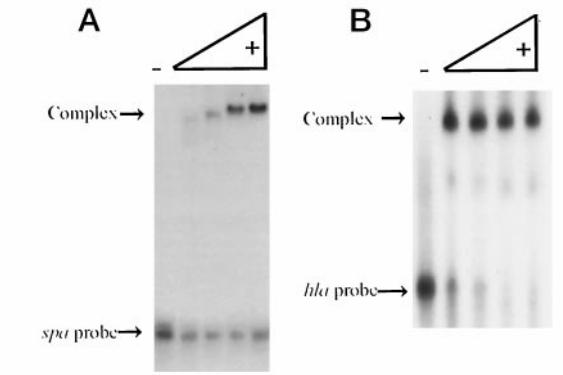


FIG. 2. **Binding of SarA to a 46-bp oligonucleotide from the *spa* promoter region (*A*) or a 45-bp oligonucleotide from the *hla* promoter region encompassing the putative SarA recognition site (*B*).** The ^{32}P end-labeled fragment was incubated with 1 μg of calf thymus DNA and 0, 1, 2, 3, or 4 μg of SarA followed by electrophoresis through a 5% polyacrylamide gel (see "Experimental Procedures"). Similar results were obtained when GST-SarA was used in place of SarA (data not shown). Binding of SarA to the *fnbA*, *fnbB*, and *sec* ds-DNA probes were also demonstrated by gel shift assays (data not shown).

with that of the longer *hla* promoter fragment (data not shown). Interestingly, as the amount of SarA was increased in the reaction, some of the nucleotides in the *hla* promoter DNA became more exposed, probably as a result of conformational changes, thus rendering these residues more susceptible to DNase I digestion and hence resulting in enhanced bands (see *arrows* in Fig. 3*A*). We also performed a DNase I footprinting assay with the *spa* promoter fragment. As with *hla*, the protected region was significantly wider (from −38 to −182 bp upstream of the transcription start) than the conserved 26-bp SarA-binding motif (Fig. 3*B*). Because of this observation with both *hla* and *spa* promoters, we speculate that the broadly protected region may constitute multiple binding sites rather than a complex conformational requirement for SarA binding. Despite the multiplicity of binding regions, it is our hypothesis that the consensus binding motif may be required for gene activation (*hla*) or suppression (*spa*) as mediated by *sar*. Deletion analyses seem to support this premise because *hla* and *spa* promoters, devoid of the consensus sequence, failed to activate the respective *hla* and *spa* transcription, whereas binding, albeit at a lower affinity, was maintained (see below).

The SarA-binding Motif Is Required for agr and hla Activation and spa Repression Mediated by the sar Locus—To assess whether the SarA-binding site identified in the *agr* promoter region *in vitro* (12) is required for *agr* activation in *S. aureus*, we introduced into the *agr* deletion mutant strain RN6911 a shuttle plasmid (pALC1354) containing a fragment encoding RNAII but lacking the 29-bp SarA-binding site (AAATGTTATTTGTATTTAATATTTTAACA, consensus region underlined). Northern analysis disclosed that the transcription of RNAII was reduced to a very low level in this strain (ALC1388) (*lane 2* in Fig. 4*A*). In contrast, the *agr* mutant strain carrying an analogous plasmid with the consensus binding motif intact (ALC1389 in *lane 3*, Fig. 4*A*) was able to express RNAII nor-

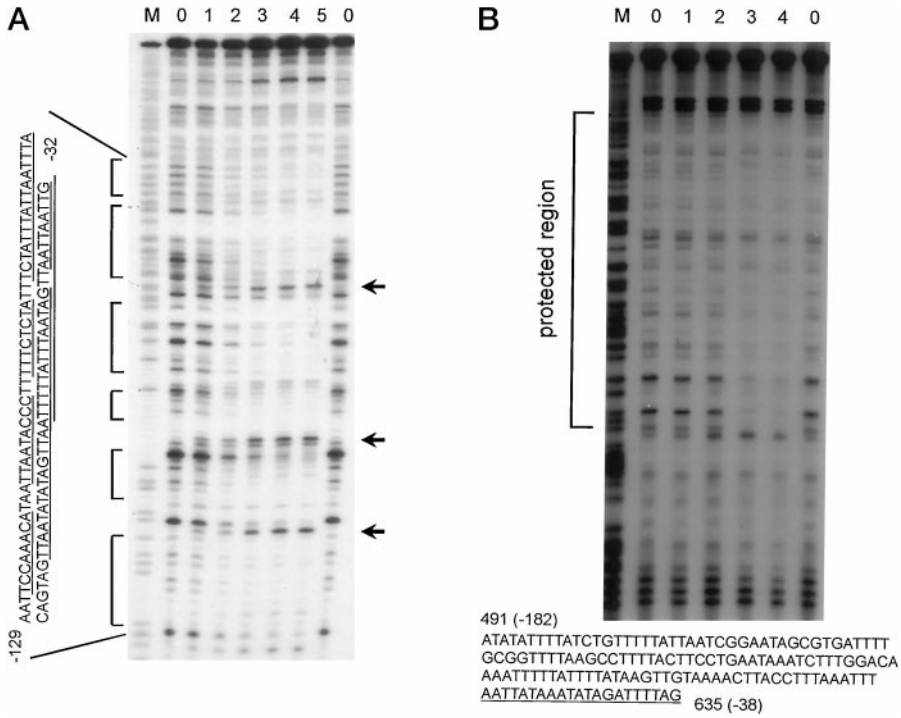


FIG. 3. **DNase I protection footprint analysis of SarA binding to the *hla* (*A*) and *spa* (*B*) promoter regions of *S. aureus*.** *A*, footprint analysis of protein binding to the *hla* promoter region. The 235-bp *hla* promoter fragment (nucleotide positions 1–80 (31) plus 155-bp upstream sequence) was end-labeled with γ-^{32}P. Labeled DNA (2 ηg) was incubated with DNase I. *Lanes 2* and *8*, no protein; *lanes 3–7*, 1, 2, 3, 4 and 5 μg of SarA. *Lane M* represents chemical cleavage at purine residues (A/G ladder). The *bracketed region* represents the protected bases. The bases that became exposed as a result of increased SarA binding and hence more susceptible to DNase I digestion are highlighted with *arrows*. The nucleotide position is given as the number of bases upstream of the transcription start. The protected region is *underlined*, with the consensus SarA-binding site *double-underlined*. *B*, footprinting analysis of SarA binding to the *spa* promoter. The 302-bp *spa* fragment was end-labeled and incubated with DNase I in the presence or absence of proteins similar to above. *Lanes 3–6* represent 1, 2, 3, and 4 μg of SarA protein. The conserved SarA-binding site is *underlined* in the protected region illustrated below.

mally, whereas the *agr* mutant alone (RN6911 in *lane 1*) did not. These data confirmed that the SarA-binding site in the *agr* P2 and P3 promoter region is required for *agr* activation in *S. aureus* cells.

To confirm the hypothesis that the SarA-binding site in the *agr* promoter region represents a conserved recognition motif required for activating or suppressing a variety of *sar* target genes, we deleted the homologous sequences within the promoter regions of *hla* and *spa,* two target genes representing extracellular and cell wall-associated virulence determinants, respectively. For the *hla* experiment, a recombinant shuttle plasmid (derived from pSK236) containing a 3-kb fragment that encodes the *hla* gene was introduced into a *hla* mutant (ALC837) of *S. aureus* to form strain ALC1525. As expected, *hla* transcription was not detected in the *hla* mutant ALC837 alone (*lane 2* in Fig. 4*B*), although it was restored by a plasmid carrying the intact *hla* gene (ALC1525) (*lane 3* in Fig. 4*B*). However, if the 33-bp SarA-binding site in the *hla* promoter region was deleted from the 3-kb fragment (ALC1526), the transcription of the *hla* gene was disrupted despite the fact that the *sar* and *agr* loci are intact in this strain. To ensure that equivalent amounts of total cellular RNA were loaded onto each lane, we also probed the same blot with a fragment encoding the HU gene the transcription of which had been found to be relatively constant during the growth cycle.[3] As displayed in Fig. 4*B*, the intensity of the HU transcript was comparable among all four samples, essentially showing that the discrepancy in *hla* transcription between lanes was not attributable to a loading artifact.

[3] Y. Chien, A. C. Manna, S. J. Projan, and A. L. Cheung, unpublished data.

Because the consensus SarA-binding motif is very AT-rich, we wanted to rule out the possibility that the binding site may be an UP element that has been shown to be the binding site for the α subunit of the RNA polymerase and is usually situated upstream of the −35 promoter boxes of target genes (26). For this reason, we deleted a major portion of the SarA-binding motif (−40 to −61 bp upstream of the transcription start) (27) from a 491-bp promoter fragment of *spa* (nucleotides 198–688) (20, 27), a gene normally repressed by the *sar* locus (Fig. 5*A*). The rationale here is that an up-regulation in *spa* transcription as a result of the deleted SarA-binding site would lessen the possibility that it is part of an UP element homologous to those found in *E. coli* (26). As a negative control, a mock deletion of an AT-rich region 142-bp upstream of the SarA-binding site (−204 to −229 bp of the transcription start site) was separately constructed. The intact and mutated *spa* promoter fragments were separately cloned into the shuttle plasmid pLC4 containing a promoterless *xylE* reporter gene (22). The recombinant plasmids were then introduced into the parental strain RN6390. In assaying activities of catechol 2,3-dioxygenase, the enzyme encoded by the *xylE* gene, the RN6390-derived clone lacking the SarA-binding site in the *spa* promoter fragment (ALC1795) expressed a high level of XylE activity (Fig. 5*B*), thus indicating a lack of repression in the absence of the SarA-binding motif. In contrast, the corresponding clone with a deletion in an unrelated AT-rich region (ALC1796) as well as that of the nonmutated control (ALC1794) exhibited reduced XylE activity as one would predict from the intact nature of *sar*, based upon the wild type genotype of this strain.[3] To confirm that the deleted SarA recognition site upstream of the *spa* transcription start (−40 to −61 bp) was indeed responsive to SarA, we introduced the plasmids constructed above into a *sar* mutant (ALC488), which

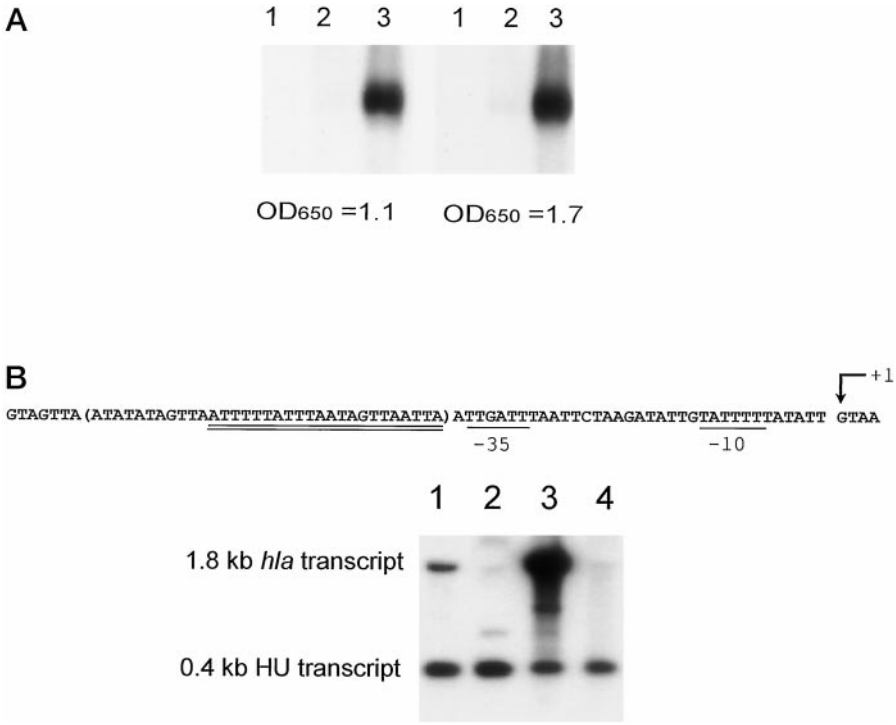


FIG. 4. *A*, Northern blots of RNAII in *agr* mutant clones carrying shuttle plasmids (pSK236 derivatives) containing fragments that encoded intact or mutated RNAII. 10 μg of RNA obtained from cells grown to A_{650} of 1.1 (late log) and 1.7 (postexponential phase) was applied to each lane. *Lane 1*, *agr* mutant RN6911; *lane 2*, *agr* mutant complemented with pSK236 containing a fragment encoding RNAII but devoid of the 29-bp SarA recognition site; *lane 3*, *agr* mutant complemented with a fragment encoding intact RNAII. *B*, Northern blots of the *hla* transcript in *hla* mutant clones containing shuttle plasmids with intact or mutated *hla* fragments. The transcription start site (labeled +1), together with the putative −10 and −35 promoter regions of the *hla* gene are show above; the deleted region corresponding to the consensus sequence (*double-underlined*) is shown in *parentheses*. 10 μg of RNA was applied to each lane. *Lane 1*, parental strain RN6390; *lane 2*, *hla* mutant of RN6390 (ALC837); *lane 3*, *hla* mutant with a recombinant shuttle plasmid pSK236 with a 3-kb *hla* fragment (ALC1525); *lane 4*, *hla* mutant with pSK236 containing a 3-kb *hla* fragment but devoid of a 33-bp SarA-binding site (ALC1526). The deleted sequence and its relationship to the transcription start (*arrow*) is shown above. The blot was also probed with a labeled fragment of the gene encoding HU. The HU transcript, stably expressed in *S. aureus*, served as an internal marker.

has been found not to produce SarA as evaluated by an immunoblot probed with anti-SarA monoclonal antibodies. As anticipated, the XylE activity of the *sar* mutant clone containing the deletion in the SarA recognition motif (ALC1667) was not repressed in the *sar* minus background. Contrary to the parental strain harboring the mock deletion (ALC1796), the analogous strain carrying the identical plasmid (pALC1641) or the nonmutated control (pALC1639) was no longer amenable to repression in the *sar* mutant (ALC1668 and ALC1669) as confirmed by elevated levels of XylE activity (Fig. 5*B*). This implies that SarA, the major *sar* regulatory molecule, is required for binding to the conserved binding motif to affect gene repression. As controls, the shuttle plasmid pLC4 alone, without any *spa* promoter sequence, did not direct expression of any XylE in either the parental or in the *sar* minus background.

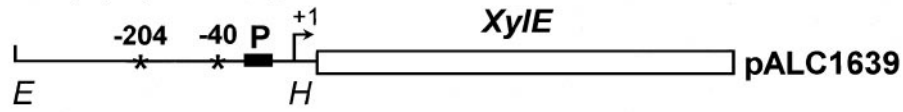
To validate the SarA recognition motif as a "SarA-responsive element" among several binding regions, we performed gel shift assays with a *spa* promoter fragment devoid of the SarA recognition motif. For positive controls, we used an intact *spa* fragment as well as a corresponding fragment with the mock deletion. In repeated experiments, we consistently found that a lesser amount of SarA was required for binding to the intact *spa* promoter fragment (Fig. 5*C*) than the mutated fragments (1 *versus* 2 μg). Despite modest differences in binding affinity, all three *spa* promoter fragments were clearly capable of binding purified SarA (Fig. 5*C*). Likewise, an *hla* promoter fragment devoid of the SarA-binding site was found to bind purified SarA, but the amount of protein required to completely retard the mutated promoter fragment (2 μg) was consistently more than its wild type counterpart (1 μg) (data not shown).

DISCUSSION

The control of expression of virulence determinants by the *sar* locus in *S. aureus* is complex, in part because the major 372-bp open reading frame (*sarA*) within *sar* is driven by a triple promoter system that is interspersed with putative regulatory elements (10–12). Because of their overlapping nature, each of these transcripts (sarA, sarC, and sarB transcripts) also includes the *sarA* coding region (10). With the promoters for the sarA and sarB transcripts being σA-dependent (active during the exponential phase) (10, 28) and that of sarC being σB-dependent (active during the postexponential phase) (28, 29), it is not surprising that *sar* transcription varies during the growth cycle. Dependent on the pattern of *sar* promoter activation, the SarA level may conceivably fluctuate (12, 28). Ultimately, the level of SarA correlates positively with the degree of *agr* expression (13).

We previously showed that the SarA protein binds to the *agr* promoter region, presumably stimulating transcription, in particular, from the *agr* P2 promoter (11). Activation of RNAII and, subsequently, RNAIII would lead to alterations in target gene expression (*e.g. hla* and *spa*), presumably by virtue of the interaction of RNAIII with the target gene at the level of transcription (8, 27) and possibly translation (9). However, phenotypic analyses indicated that SarA can also modulate target genes via an *agr*-independent mechanism. In particular, the transcription of the fibronectin-binding gene (*fnbA*) is positively regulated by *sar* via an *agr*-independent mechanism (30).[3] Likewise, supplying the *sar* locus *in trans* can repress *spa* transcription in an *agr* mutant. Additionally, recent data from

A



B

Samples	Background	mU/mg protein
ALC1795 (Δ-40 to -61)	wild type	22.9
ALC1796 (Δ-204 to -229)	wild type	1.9
ALC1794 (pALC1639)	wild type	0.9
ALC970 (pLC4 alone)	*sar* mutant	0.08
ALC1667 (Δ-40 to -61)	*sar* mutant	13.1
ALC1668 (Δ-204 to -229)	*sar* mutant	7.7
ALC1669 (pALC1639)	*sar* mutant	12.1

C

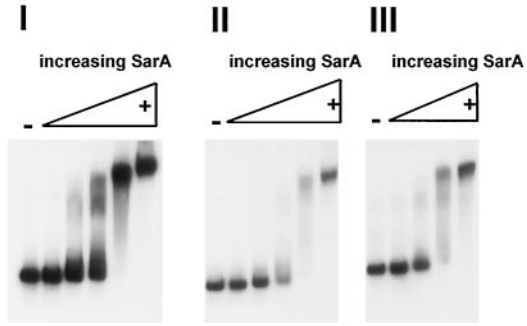


FIG. 5. **Transcriptional activity of the *spa* promoter lacking a SarA recognition site based on XylE fusion.** A schematic representation of the promoter constructs fused to *xylE* gene is presented in *A*. The results of the XylE activity for different constructs in parental strain RN6390 or its isogenic *sar* mutant are tabulated in *B*. The XylE activities are given in milliunits/mg of cellular protein. Gel shift studies of three different promoter constructs with purified SarA are given in *C*. I, II, and III represent promoter fragments as derived from ALC1794 (nonmutated control), ALC1795 (−40 to −61) and ALC1796 (−204 to −229), with the numbers indicating the deleted nucleotides upstream of the transcription start. The *left-most lanes* do not contain SarA. The amounts of SarA in subsequent lanes are as follows: *I* and *II*, 0.1, 0.2, 0.5, 1.0, and 2.0 μg; *III*, 0.1, 0.2, 0.5, and 1.0 μg. 0.5 μg of SarA can retard the nonmutated fragment as well as that of the mock mutation (*I* and *III*) but not the fragment devoid the conserved SarA-binding site.

Chan and Foster (16) also disclosed that *sar* may up-regulate *hla* transcription via both *agr*-dependent and *agr*-independent pathways. These three diverse modes of *sar*-mediated regulation of target genes (*i.e. fnb*, *spa* and *hla*) strongly imply that SarA may directly interact with target gene promoters, with or without any involvement from the *agr* gene product. In mapping the 29-bp SarA-binding site on the *agr* promoter with *in vitro* footprinting assay (12), we explored the sequence upstream of the −35 promoter boxes of several virulence determinants representative of the three putative modes of *sar*-mediated regulation via a common sequence motif. Interestingly, a consensus sequence sharing a homology with the SarA-binding site on the *agr* promoter emerged (Fig. 1). In a recent study, Chan and Foster (16) had also aligned the promoter sequence upstream of several *sar* target genes including *hla*, *hlb, tst*, *seb*, *spa*, and *spr* (V8 protease gene). However, the data of Chan and Foster are not in agreement with the sequences displayed here (Fig. 1), partly because their alignment was based solely on comparing stretches of nucleotide sequences that are extremely AT-rich (86%) and lacked any supporting biological or biochemical data. Additionally, the transcription start site of the *hla* gene is upstream of the published sequence (accession number X01645) (31),[2] thus rendering their alignment problematic. To confirm the validity of our alignment, gel shift assays of SarA and GST-SarA with ~45-bp oligonucleotide probes encompassing the putative SarA recognition motif of *spa*, *fnb*, *hla*, and *sec* were performed. Our results demonstrated that SarA did indeed bind to these probes whereas the control *nif*H2 fragment and an unrelated fragment from the β-hemolysin gene did not. Additional confirmation of the SarA recognition motif in the *hla* and *spa* promoter regions was obtained by DNase I footprinting. Surprisingly, the protected region as revealed by the footprinting assay was larger than the SarA recognition motif, thus suggesting either multiple binding sites or a complex conformational requirement for binding (discussed below).

Cognizant of the AT-rich nature of our consensus sequence (95% AT), it was important to rule out the possibility that the SarA recognition motif may be an UP element the absence of which would lead to defective binding by core RNA polymerase and hence reduce target gene transcription (26). More importantly, it will be essential to examine the role of this recognition motif *in vivo* (in the bacteria). For this reason, we chose to delete the SarA recognition motif in the promoter region of *spa*, a gene normally repressed by the *sar* locus and examine the resultant *spa* promoter activation. Using a *xylE* reporter fusion to generate quantitative data from a clone derived from the parental strain (ALC1795), we found that in the absence of the recognition motif, the transcription of *spa* in this staphylococcal strain became derepressed, thus resulting in significant up-regulation in XylE activity. This enhancement effect in *spa* transcription attributable to a lack of a SarA recognition site was completely abolished in a *S. aureus sar* mutant (ALC1667). Taken together, these data clearly indicated that the SarA recognition motif, present in a variety of *sar* target genes including *hla* and *spa*, is probably not an UP element and, in the absence of SarA or its binding motif, *sar*-mediated regulation (both up and down-regulation) will not occur.

In deleting the consensus SarA-binding site on the *agr* and *hla* promoters, we verified that this recognition motif is likely required for SarA binding and the ensuing activation of these genes in *S. aureus*. This observation was confirmed by Northern blots in which we found that the SarA recognition motif upstream of the *hla* and *agr* promoters was required for gene activation *in vivo* in the respective *hla* and *agr* mutant clones, respectively. We also found *in vitro* that a 160-bp *hla* promoter fragment lacking the SarA-binding site was still able to maintain SarA binding, albeit at a lower affinity than the 192-bp nonmutated counterpart (data not shown). Similarly, a *spa* promoter fragment devoid of the SarA recognition motif also binds to SarA with lower affinity than the intact control (Fig. 5*C*). Despite variable levels of binding *in vitro* by all promoter fragments to SarA in gel shift assays (Fig. 5*C*), only the intact *spa* promoter and its analogous counterpart lacking an unrelated AT-rich region were amenable to repression *in vivo* in a *sar*-positive strain (ALC1796) but not in a *sar* minus background (ALC1668), whereas a *spa* promoter fragment missing the SarA recognition motif was not repressible in either genetic background (see ALC1795 and ALC1667 in Fig. 5*B*). This discrepancy in binding and effector activity implies that only the conserved SarA-binding sequence represents the effector site *in vivo* among a broader binding region(s) within the promoter regions of *spa* and *hla* as determined by DNase I footprinting assay *in vitro* (Fig. 3*B*).

SarA, the major *sar* effector molecule, is thus capable of modulating the transcription of multiple target genes, thus accounting for its pleiotropic effects in *S. aureus*. Prior *in vitro* data clearly establish that SarA can bind to the *agr* promoter region to influence primarily *agr*-P2 transcription. Besides the indirect control via *agr*, the mechanism by which SarA directly up-regulates (*e.g. hla*) and down-regulates target genes (*e.g. spa*) has not been previously defined. In identifying a SarA recognition motif among the promoters of *sar* target genes, we presented a unifying hypothesis whereby SarA can modulate a variety of target genes via both *agr*-dependent and *agr*-independent pathways. The SarA recognition motif likely represents the *sar*-responsive element of the target gene. Accordingly, activation or repression of target gene promoters is dependent on the binding of SarA to the consensus binding site. In the case of *hla*, it is evident that this gene can be turned on by a double switch mechanism (via *agr* and by direct SarA binding to the *hla* promoter). Because the protected region identified by DNase I footprinting is wider than the SarA recognition motif, we propose that the promoters of target genes contain multiple bindings sites, but the SarA recognition motif by itself probably represents the effector (activation/suppression) site. Whether the expansive binding site serves as binding regions for regulatory factors other than SarA (*e.g.* RNAIII) is not clear. Depending on the threshold of activation, the level of SarA protein may ultimately determine the pattern of regulation of SarA-responsive genes in *S. aureus*. Because of the multiplicity of *sar* promoters with diverse activation requirements (*e.g.* sarC activated by SigB) (28, 29), the precise control of SarA protein levels as a result of differential *sar* promoter activation is likely to be dependent on a variety of environmental and intracellular factors. Notably, we recently identified a regulatory protein that binds to the *sar* promoter region to down-regulate sarC transcription (28).[3] Characterization of this protein and its effect on SarA expression are currently in progress.

Acknowledgment—We thank Tim Foster for providing strain DU1090.

REFERENCES

1. Boyce, J. M. (1997) in *The Staphylococci in human Diseases* (Crossley, K. B., and Archer, G. L., eds) Vol. 12, pp. 309–329, Churchill Livingston, New York
2. Projan, S. J., and Novick, R. P. (1997) in *The Molecular Basis of Pathogenicity* (Crossley, K. B., and Archer, G. L., eds) Vol. 3, pp. 55–81, Churchill Livingston, New York
3. Kornblum, J., Kreiswirth, B., Projan, S. J., Ross, H., and Novick, R. P. (1990) in *Molecular Biology of the Staphylococcus* (Novick, R. P., ed) pp. 373–402, VCH Publishers, Inc., New York
4. Cheung, A. L., Koomey, J. M., Butler, C. A., Projan, S. J., and Fischetti, V. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 6462–6466
5. Ji, G., Beavis, R. C., and Novick, R. P. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 12055–12059
6. Ji, G., Beavis, R., and Novick, R. P. (1997) *Science* **276,** 2027–2030
7. Novick, R. P., Projan, S. J., Kornblum, J., Ross, H. F., Ji, G., Kreiswirth, B., Vandenesch, F., and Moghazeh, S. (1995) *Mol. Gen. Genet.* **248,** 446–458
8. Novick, R. P., Ross, H. F., Projan, S. J., Kornblum, J., Kreiswirth, B., and Moghazeh, S. (1993) *EMBO. J.* **12,** 3967–3977
9. Morfeldt, E., Taylor, D., von Gabain, A., and Arvidson, S. (1995) *EMBO. J.* **14,** 4569–4577
10. Bayer, M. G., Heinrichs, J. H., and Cheung, A. L. (1996) *J. Bacteriol.* **178,** 4563–4570
11. Cheung, A. L., Bayer, M. G., and Heinrichs, J. H. (1997) *J. Bacteriol.* **179,** 3963–3971
12. Chien, Y., and Cheung, A. L. (1998) *J. Biol. Chem.* **273,** 2645–2652
13. Chien, Y. T., Manna, A. C., and Cheung, A. L. (1998) *Mol. Microbiol.* **31,** 991–1001
14. Cheung, A. L., and Ying, P. (1994) *J. Bacteriol.* **176,** 580–585
15. Cheung, A. L., Eberhardt, K. J., Chung, E., Yeaman, M. R., Sullam, P. M., Ramos, M., and Bayer, A. S. (1994) *J. Clin. Invest.* **94,** 1815–1822
16. Chan, P. F., and Foster, S. J. (1998) *J. Bacteriol.* **180,** 6232–6241
17. Novick, R. P. (1991) *Methods Enzymol.* **204,** 587–636
18. Schenk, S., and Laddaga, R. A. (1992) *FEMS Microbiol. Lett.* **94,** 133–138
19. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
20. Shuttleworth, H. L., Duggleby, C. J., Jones, S. A., Atkinson, T., and Minton, N. P. (1987) *Gene* (*Amst.*) **58,** 283–295
21. Cheung, A. L., Eberhardt, K., and Fischetti, V. A. (1994) *Anal. Biochem.* **222,** 511–514
22. Zukowski, M. M., Gaffney, D. G., Speck, D., Kauffman, M., Findeli, A., Wisecup, A., and Lecocq, J. P. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 1101–1105
23. Wolz, C., McDevitt, D., Foster, T. J., and Cheung, A. L. (1996) *Infect. Immun.* **64,** 3142–3147
24. Cheung, A. L., Eberhardt, K., and Heinrichs, J. H. (1997) *Infect. Immun.* 2243–2249
25. Vandenesch, F., Kornblum, J., and Novick, R. P. (1991) *J. Bacteriol.* **173,** 6313–6320
26. Ross, W., Gosink, K. K., Salomon, J., Igarashi, K., Zou, C., Ishihama, A., Severinov, K., and Gourse, R. L. (1993) *Science* **262,** 1407–1413
27. Patel, A. H., Kornblum, J., Kreiswirth, B., Novick, R., and Foster, T. J. (1992) *Gene* (*Amst.*) **114,** 25–34
28. Manna, A. C., Bayer, M. G., and Cheung, A. L. (1998) *J. Bacteriol.* **180,** 3828–3836
29. Deora, R., Tseng, T., and Misra, T. K. (1997) *J. Bacteriol.* **179,** 6355–6359
30. Cheung, A. L., and Wolz, C. (1997) *Abstracts of the 37th Annual Meeting of ICAAC, Toronto, September, 1997*, Am. Soc. Microbiol., Washington, D. C.
31. Gray, G. S., and Kehoe, M. (1984) *Infect. Immun.* **46,** 615–618
32. Patel, A. H., Nowlon, P., Weavers, E. D., and Foster, T. (1987) *Infect. Immun.* **55,** 3103–3110
33. Gaskill, M. E., and Khan, S. A. (1988) *J. Biol. Chem.* **263,** 6276–6280

SarA, a Global Regulator of Virulence Determinants in *Staphylococcus aureus*, Binds to a Conserved Motif Essential for *sar*-dependent Gene Regulation

Yueh-tyng Chien, Adhar C. Manna, Steven J. Projan and Ambrose L. Cheung

J. Biol. Chem. 1999, 274:37169-37176.
doi: 10.1074/jbc.274.52.37169